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12th European Congress of Andrology Abstract Book

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Contents

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Introduction

Abstracts of the 12th Congress of the European Academy of Andrology 19 – 21 October 2022 Barcelona, Spain

1 | WELCOME MESSAGES

1.1 | Welcome message of EAA president and LOC chair

ECA2022 represents the most relevant scientific and educational biennal event of the European Academy of Andrology (EAA). This year it will be a special edition because it coincides with the 30-year history of the EAA. Academicians and regular EAA members are going to celebrate this anniversary together with hundreds of andrologists and basic scientists coming from all over the world. As for the previous eleven editions, it will be an excellent forum for presenting cutting-edge basic/translational and clinical research in all areas of Andrology and for discussing about clinical practice related to andrological diseases. ECA2022 will also allow reinforcing our relationship with the European Society of Endocrinology (ESE), the American Society of Andrology (ASA), European Association of Urology (EAU) Section of Andrological Urology (ESAU), European Society of Human Reproduction and Embryology (ESHRE) and the newly established ANDRONET COST action. It will be the place for exchanging ideas for promoting andrology in Europe and outside the old continent and to further intensify our activities related to Male Health Awareness.

Thanks to the support of the Fundacio Puigvert, to which the Barcelona EAA center belongs, this is the second time we held ECA in Barcelona and will follow the 18th edition of the prestigious Andrology Course of Fundació Puigvert, which will take place, just prior the ECA Congress, in the morning of the 19th October. This city is full of architectural masterpieces, history and culture, and it is considered as one of the most beautiful cities of the world. We can ensure you that the capital of Catalonia offers unforgettable cultural but also culinary experiences to the visitors, and we do hope that the congress attendee will enjoy their free time after the sessions.

We are sure that this congress will be remembered not only for its exceptional scientific content but also for the exceptional venue and the pleasant Mediterranean atmosphere.

On behalf of the EAA, we are looking forward to welcome you in Barcelona,

Csilla Krausz
EAA President

Eduard Ruiz-Castane
LOC Chair

1.2 | Local organizing committee

Dr. Eduard Ruiz-Castañé (Chair)
Dr. Josvanny Sánchez Curbelo
Dr. Maurizio De Rocco
Dr. Mónica González
Dr. Joaquim Sarquella
Dr. Dorón M. Vantman
Dr. Álvaro Vives
Dr. Osvaldo Rajmil

1.3 | Message from the chairs of the program organizing committee

The Program Organizing Committee (POC) proposes an attractive, **clinical, basic as well as transitional** educational program for the 12th European Congress of Andrology, the leading scientific activity of the EAA.

Program highlights include:

- The well-established **pre-congress course** will provide an **update on male reproduction** through educational lectures by POC members.
- **State-of-the-art lectures** (some organized in collaboration with the ASA, the ESE and the ESHRE) by prominent scientists on cutting-edge research issues such as male contraception, late-onset hypogonadism, foetal germ development as well as reproductive endocrinology during puberty and sperm preservation in boys.
  - A new feature of the congress will be the inclusion of **meet the expert sessions**. Topics include discussion of new WHO as well as EAA guidelines, diagnosis/treatment of male urogenital infections biochemical assessment of androgen deficiency as well as ultrasound evaluation of the male reproductive system.
  - Another important new concept will be the introduction of virtual **eco-talks** that will feature high level international experts across the globe without the need for intercontinental traveling. Selected **eco-talks** covering issues such as clonal mosaicism in sperm, studies on RNA sequencing profiles in testicular cells from the embryo to the aging male, as well as polygenic risk scores in predicting testis cancer risks.
- Symposia that cover the whole spectrum of **basic, clinical and translational** andrology including:
- Joint sessions that highlight the very successful collaboration between the EAA and the ESAU section of the EAU.
- Presentation of the novel scientific work conducted by the young colleagues through the **Network of Young Researchers in Andrology session**, **'golden communications'** and 'selected oral communications'.
- Finally, announcement of the current research activities in andrology through **poster presentations**.

The POC does hope that you will enjoy three days full of andrology and you will return home fully updated on all the essential aspects of andrology.
We hope to see you in Barcelona!

Dirk Vanderschueren
POC Chair

Jan-Bernd Stukenborg
POC Chair

1.4 | Program organizing committee

Dirk Vanderschueren (Belgium) - co-chair
Jan-Bernd Stukenborg (Sweden) - co-chair
Herman Behre (Germany)
Jolanta Slowikowska-Hilczer (Poland)
Suks Minhas (UK)
Emmanuele Jannini (Italy)
Jorma Toppari (Finland)
Lise Aksglæde (Denmark)
Joan Palou (Spain)
Paulo Navarro-Costa (Portugal)
Ex Officio:
Csilla Krausz (Italy)
Eduard Ruiz-Castañé (Spain)

1.5 | EAA executive council

Prof. Csilla Krausz
President
FIRENZE - ITALY

Dr. Ewa Rajpert-De Meyts
Secretary
COPENHAGEN - DENMARK

Prof. Davor Ježek
Treasurer
ZAGREB - CROATIA

Prof. Andrea Isidori
Elected member
ROME - ITALY

Prof. Eduard Ruiz Castañé
Elected member
BARCELONA - SPAIN

Prof. Zsolt Kopa
Elected member
BUDAPEST - HUNGARY

Prof. Rafael Oliva
Elected member 2021-
BARCELONA - SPAIN

Prof. Jorma Toppari
Past President – Ex Officio
TURKU - FINLAND

Prof. Aleksander Giwercman
Editor, Andrology (2022–2026)
Ex Officio MALMOE/LUND
- SWEDEN

Prof. Manuela Simoni Editor,
Andrology (2017–2021) Ex Officio
MODENA - ITALY

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Scientific Program

Wednesday, 19 October 2022 - Room 1		
14:45-16:05	**Update on male reproduction - Part 1** EAA POST-GRADUATE COURSE	Moderators: Joan Palou (Spain), Jan-Bernd Stukenborg (Sweden)
14:45-15:05	PC01	**Ancient origins of male infertility** Paulo Navarro-Costa (Portugal)
15:05-15:25	PC02	**Legacy of undescended testis** Jorma Toppari (Finland)
15:25-15:45	PC03	**Genetic testing of azoospermia: what is new?** Csilla Krausz (Italy)
15:45-16:05	PC04	**Medical care for adult male DSD patients** Jolanta Slowikowska-Hilczer (Poland)
16:35-17:55	**Update on male reproduction - Part 2** EAA POST-GRADUATE COURSE	Moderators: Joan Palou (Spain), Jan-Bernd Stukenborg (Sweden)
16:35-16:55	PC05	**Update on Klinefelter syndrome** Lise Aksglaede (Denmark)
16:55-17:15	PC06	**How to deal with sexual problems in infertile couples?** Emmanuele Jannini (Italy)
17:15-17:35	PC07	**Surgical treatment in male infertility: what is new?** Suks Minhas (UK)
17:35-17:55	PC08	**Medical treatment of male infertility: what is new?** Hermann M Behre (Germany)
18:10-19:00	**Opening Ceremony with Andrology Award**	Presenters: Csilla Krausz (Italy), Eduard Ruiz-Castañé (Spain), Jan-Bernd Stukenborg (Sweden), Dirk Vanderscheuren (Belgium), Marie-Claude Hofmann (USA), Aleksander Giwercman (Sweden)
19:00-20:00	**Golden Oral Presentations**	Moderators: Ewa Rajpert-de Meyts (Denmark), Francesco Lombardo (Italy)
19:00-19:10	OC01	**Evaluation of familial cancer risk among testicular germ cell tumor patients** Viktória Rosta (Hungary/Italy)
19:10-19:20	OC02	**Identification of cellular and molecular alterations underlying two distinct types of cryptozoospermia using single cell RNA sequencing** Sara Di Persio (Germany)
19:20-19:30	OC03	**The role of unprocessed PRM2** Lena Arevalo (Germany)
19:30-19:40	OC04	**Short chain fatty acids-mediated sperm chemiotaxis: functional role of olfactory receptor 51E2 in human reproduction** Emanuela Teveroni (Italy)
Thursday, 20 October 2022 - Room 1		
08:00-08:45	**ASA/EAA Exchange Lecture**	Moderator: Jorma Toppari (Finland)
08:00-08:45	L05	**Male contraception in the 21st Century: an update** Wei Yan (USA)
09:15-10:45	**Clinical Science Session - Genetics of male infertility** ECA1	Moderators: Csilla Krausz (Italy), Lise Askglaede (Denmark)

Thursday, 20 October 2022 - Room 1		
09:15-09:40	RT01-1	**The testicular phenotypes of AZFb deletion carriers** Peter Vogt (Germany)
09:40-10:05	RT01-2	**The role of X chromosome variants in azoospermia** Antoni Riera-Escamilla (Spain)
10:05-10:30	RT01-3	**Exome analysis: time to advance the standard of care** Frank Tüttelmann (Germany)
10:30-10:40	OC05	**Mutational screening of androgen receptor gene in 8224 men of infertile couples** Maria Santa Rocca (Italy)
10:45-11:30	**Basic Science Plenary**	Moderator: Jan-Bernd Stukenborg (Sweden)
10:45-11:30	L01	**New mutations, selfish testes and human disease** Anne Goriely (UK)
13:00-14:30	**(Epi) Genetics of male reproductive function** ECA4	Moderators: Rafael Oliva (Spain), Andreas Meinhardt (Germany)
13:00-13:25	RT02-1	**Nutrition in men, fertility and offspring health: epigenetics insights** Adelheid Soubry (Belgium)
13:25-13:50	RT02-2	**Sperm-borne RNAs – the informers for future generations?** Noora Kotaja (Finland)
13:50-14:15	RT02-3	**Chromosomal fusions and 3D genome folding and recombination in the germ line** Aurora Ruiz-Herrera (Spain)
14:15-14:25	OC06	**Y chromosome loss detected by karyotyping of men with azoospermia** Jens Fedder (Denmark)
15:00-16:30	**Novo Nordisk Pharma Symposium: Obesity and Men's health** ECA6	Moderator: Eduard Ruiz Castañé (Spain)
15:00-15:05	IS01	**Welcome & Introduction** Eduard Ruiz Castañé (Spain)
15:05-15:50	IS02	**Obesity influence on sexual dysfunction, fertility & QoL** Giovanni Corona (Italy), Edudard García (Spain)
15:50-16:20	IS03	**Role of Liragluitde in the treatment of obesity (clinical experience)** Maurizio de Rocco (Spain)
16:30-17:15	**ESE-EAA Exchange Lecture**	Moderator: Manuela Simoni (Italy)
16:30-17:15	L06	**Reproductive endocrinology at the age of transition** Anders Juul (Denmark)
17:15-18:30	**EAA General Assembly**	
Thursday, 20 October 2022 - Room 2		
09:15-10:45	**Meet the expert – Diagnosis in Andrology**	Chairperson: Marij Dinkelman-Smit (the Netherlands), Mario Maggi (Italy)
09:15-10:00	RT03-1	**The contemporary role of ultrasound scan in Andrology** Francesco Lotti (Italy)
10:00-10:45	RT03-2	**How to biochemically assess androgen status?** Leen Antonio (Belgium)
13:00-14:30	**Meet the expert – Infections**	Moderators: Gerhard Haidl (Germany), Giovanni M Colpi (Switzerland)
13:00-13:45	RT04-1	**A pictorial review of genital lesions** Alvaro Vives (Spain)
13:45-14:30	RT04-2	**Impact of infections and inflammation on the male reproductive tract** Hans Christian Schuppe (Germany)
15:00-16:30	**Uro-Andrology** ECA7	Moderators: Suks Minhas (UK), Joan Palou (Spain)
15:00-15:15	RT05-1	**PSA and MRI in 2022 in the diagnosis of prostate cancer: when to avoid prostate biopsies?** Jochen Walz (Germany)

Thursday, 20 October 2022 - Room 2		
15:15-15:30	RT05-2	**Late effects of uro-oncological surgery on sexual function** Alberto Breda (Spain)
15:30-15:45	RT05-3	**Testosterone replacement in prostate cancer patients: is it safe?** Francesco Sanguedolce (Spain)
15:45-16:00	RT05-4	**Testis sparing surgery. When and why?** Suks Minhas
16:00-16:15	RT05-5	**Nerve sparing techniques in prostate and bladder cancer surgery: Do they improve preservation of sexual function?** Joan Palou (Spain)
Thursday, 20 October 2022 - Room 3		
09:15-10:45	**Clinical Science Session - Infertility/male reproduction** ECA3	Moderators: Hermann M Behre (Germany), Jolanta Slowikowska-Hilczer (Poland)
09:15-09:40	RT06-1	**Impacts of pharmaceutical exposures on male reproductive health (ECO-Talk)** Rod T. Mitchell (UK)
09:40-10:05	RT06-2	**Ageing effects on the male germline** Sandra Laurentino (Germany)
10:05-10:30	RT06-3	**Male reproductive health and COVID-19** Giulia Rastrelli (Italy)
10:30-10:40	OC07	**Testicular dysfunction in 47,XXY boys: when it all begins. A prospective study** Franz Sesti (Italy)
13:00-14:30	**Erectile dysfunction and short oral presentations** ECA14	Moderators: Emmanuele Jannini (Italy), Ahmed Mahmoud (Belgium)
13:00-13:25	RT07-1	**Erectile dysfunction in congenital hypogonadism: organic and non-organic risk factors** George Kanakis (Greece)
13:25-13:50	RT07-2	**A matter of time! - How can a two-step intracavernosal injection procedure improve the possibility to diagnosing psychological erectile dysfunction?** Daniele Santi (Italy)
13:50-14:00	OC08	**Sperm DNA and Membrane integrity test (DMI test): a novel evaluation strategy of DNA fragmentation in viable spermatozoa** Raul da Costa (Germany)
14:00-14:10	OC09	**Eunuchoid skeletal proportions in male hypogonadism: a comparative analysis of anthropometric measures between men with congenital hypogonadotropic hypogonadism (CHH) and Klinefelter Syndrome (KS)** Sara De Vincentis (Italy)
14:10-14:20	OC10	**Relevance of sperm origin in Klinefelter patients for ICSI outcome rate: large single-center experience** Hamid Kalantari (Iran)
15:00-16:30	**Sperm function and deregultation** ECA8	Moderators: Davor Jezek (Croatia), Lluís Bassas (Spain)
15:00-15:25	RT08-1	**Clonal mosaicism in sperm cells (ECO-Talk)** Martin Breuss (USA)
15:25-15:50	RT08-2	**Sperm motility – Travels without bad conscience** Timo Strünker (Germany)
15:50-16:15	RT08-3	**Sperm proteomics: A tool to unravel male reproductive function** Meritxell Jodar (Spain)
16:15-16:25	OC12	**Infertile men with unknown etiology show alterations in the abundance of specific protamine proteoforms related to sperm chromatin packaging and age** Judit Castillo (Spain)

Friday, 21 October 2022 - Room 1		
08:15-09:00	**Clinical Science Plenary**	Moderator: Dirk Vanderschueren (Belgium)
08:15-09:00	L02	**What lessons have we learned from the European Ageing Study (EMAS)?** Frederick Wu (UK)
09:00-10:30	**NYRA Session**	Moderators: Alberto de la Iglesia (Spain), Daniel Marcu (UK)
09:00-09:50	L04	**Testicular organoids: the next big thing in male fertility R&D?** Yoni Baert (Belgium)
09:50-10:05	RT09-1	**The seminal plasma microbiome of men with testicular germ cell tumours** Ailsa Maria Main (Denmark)
10:05-10:20	RT09-2	**Loss of XIST expression and the additional X-chromosome in Sertoli cells supporting focal spermatogenesis in men with Klinefelter syndrome** Sofia B. Winge (Denmark)
11:00-12:30	**Spermatogonia and its niche** ECA11	Moderators: Paulo Navarro Costa (Portugal), Judit Castillo (Spain)
11:00-11:25	RT10-1	**RNA sequencing of the human testis - from prenatal establishment to the last year in life (ECO-Talk)** Jingtao Guo (China)
11:25-11:50	RT10-2	**Germ cells and non-malignant diseases – impacts of treatments or diseases** Nina Neuhaus (Germany)
11:50-12:15	RT10-3	**Human primordial germ cell-like cell specification and progression in vitro** Joao Pedro Alves-Lopes (Sweden)
12:15-12:25	OC13	**Novel culture conditions for the improvement of the in vitro expansion of human Spermatogonial stem cells. Future stem cell therapies to restore fertility in prepuberal boys enrolled in our experimental fertility preservation program** Myriam Martin-Inaraja (Spain)
14:00-15:30	**Testicular cancer** ECA13	Moderators: Ewa Rajpert-de Meyts (Denmark), Alberto Ferlin (Italy)
14:00-14:25	RT11-1	**Polygenic risk score in predicting testis cancer risk (ECO-Talk)** Katherine L Nathanson (USA)
14:25-14:50	RT11-2	**Clinical management of testicle tumors** Andrea Isidori (Italy)
14:50-15:15	RT11-3	**Germ cell cancers – many types one origin?** Leendert Looijenga (The Netherlands)
15:15-15:25	OC14	**Risk of bilateral testicular germ cell tumors: a single-center long-term experience** Marta Tenuta (Italy)
16:00-16:45	**Translational Science Plenary**	Moderator: Jan-Bernd Stukenborg (Sweden)
16:00-16:45	L03	**How to deal with fertility preservation in boys** Herman Tournaye (Belgium)
16:45-17:15	**Closing Remarks and Closure Ceremony**	
Friday, 21 October 2022 - Room 2		
09:00-10:30	**EAA meets ESHRE** ECA10	Moderators: Stefan Schlatt (Germany), Csilla Krausz (Italy)
09:00-09:25	RT12-1	**When to introduce a new diagnostic or therapeutic tool in the andrology laboratory based on scientific evidence** Nicolás Garrido Puchault (Spain)
09:25-09:50	RT12-2	**Male reproductive health awareness initiative by ESHRE** Christopher Barratt (UK)
09:50-10:00	OC11	**In vitro somatic cell functionality as a measure of human testicular tissue quality for fertility preservation procedures** Femke Harteveld (Sweden)
10:00-10:10	Q&A	

Friday, 21 October 2022 - Room 2		
10:10-10:30	**Industry Sponsored lecture:** IS04	**Moderator: Eduard Ruiz Castañé (Spain)** Erectile dysfunction and quality of life (ECO-Talk) Scott Petterson (USA)
11:00-12:30	**Meet the expert - WHO manual and EAA Guidelines**	Moderator: Niels Jorgensen (Denmark)
11:00-11:45	RT13-1	**Debate: How should the 2021 edition of the WHO manual and the ISO 23162:2021 have relevant impact on andrological clinical care?** Lars Björndahl, Christopher Barratt (Sweden)
11:45-12:30	RT13-2	**Update on EAA guidelines** Giovanni Corona (Italy)
14:00-15:30	**Sexual medicine** ECA15	Moderators: Marrio Maggi (Italy), Osvaldo Rajmil (Spain)
14:00-14:25	RT14-1	**Post SSRI sexual dysfunction (PSSD) and Post-Finasteride syndrome (PFS)** Yacov Reisman (The Netherlands)
14:25-14:50	RT14-2	**Safety and side effects of hormonal treatment in transgenders – and what we can learn from it in for populations** Martin Den Heijer
14:50-15:15	RT14-3	**Transgender genital surgery** Jaume Masià (Spain)
15:15-15:25	OC15	**Effects of long-term GnRHa use on bone health in transgender adolescents: can a mouse model reveal novel insights for clinical practice?** Vanessa Dubois (Belgium)
Friday, 21 October 2022 - Room 3		
09:00-10:30	**Penile surgery and sexual health** ESAU Course	Moderators: Ates Kadioglu (Turkey), Eduard Ruiz-Castañé (Spain)
09:00-09:11	RT15-1	**Low-intensity shock wave treatment for erectile dysfunction** Tet Yap (UK)
09:11-09:22	RT15-2	**Sexual health after radical prostatectomy (ECO-Talk)** David Ralph
09:22-09:33	RT15-3	**Evaluation of plaque formation in Peyronie's disease: palpation vs ultrasound vs MRI** Thorsten Diemer
09:33-09:44	RT15-4	**Peyronie's surgery: choice of the appropriate graft** Carlo Bettocchi
09:44-09:55	RT15-5	**Implantation of penile prosthesis in patients with Peyronie's disease: When and to whom?** Ates Kadioglu
09:55-10:06	RT15-6	**How to avoid penile prosthesis infections?** Eduard Ruiz-Castañé (Spain)
10:06-10:18	RT15-7	**Managing the complications of the penile prosthesis implant** Ferdinando Fusco
10:18-10:30	RT15-8	**The impact of COVID-19 on male sexual health** Asif Muneer
11:00-12:30	**Onco-Andrology: an updated on diagnosis and management effects** EAA/ESAU Course	Moderators: Csilla Krausz (Italy), Nikolaos Sofikitis (Greece)
11:00-11:22	RT16-1	**Update on the molecular diagnosis of testicular cancer** Ewa Rajpert-De Meyts (Denmark)
11:22-11:45	RT16-2	**Short- and long-term effects of oncological treatments on testis function** Csilla Krausz (Italy)
11:45-12:07	RT16-3	**Fertility preservation in adult non-azoospermic or azoospermic males with testicular cancer** Thorsten Diemer

Friday, 21 October 2022 - Room 3		
12:07-12:30	RT16-4	**The importance of testicular microlithiasis for: the andrologist and the oncologist** Marij Dinkelman-Smit (The Netherlands)
14:00-15:30	**Male infertility: from the aetiology to the pharmaceutical and surgical treament** ESAU Session	Moderators: Aleksander Giwercman (Denmark), Suks Minhas (UK)
14:00-14:12	RT17-1	**Risk of death: Azoospermic males vs. non-azoospermic infertile males** Aleksander Giwercman (Sweden)
14:12-14:24	RT17-2	**Do we need PDE5 inhibitors in male infertility clinics or assisted reproductive technology laboratories?** Nikolaos Sofikitis (Greece)
14:24-14:36	RT17-3	**Sequence analysis of candidate genes for male infertility: clinical implications** Sabine Kliesch (Germany)
14:36-14:46	RT17-4	**Antioxidants decrease DNA Fragmentation Index and improve pregnancy rates in ART programs** Zsolt Kopa (Hungary)
14:46-14:56	RT17-5	**Probable detrimental effects of antioxidants; Excellence through moderation** Fotios Dimitriadis (Greece)
14:56-15:06	RT17-6	**Are there any predictive factors for successful sperm recovery in the salvage TESE/mTESE?** Paolo Capogrosso (Italy)
15:06-15:18	RT17-7	**May hormonal stimulation turn the first negative for sperm -TESE into a positive for sperm- salvage TESE/mTESE?** Suks Minhas (UK)
15:18-15:30	RT17-8	**May repair of an existing varicocele turn the first negative for sperm- TESE into a positive for sperm- salvage TESE/mTESE?** Giorgio Russo (Italy)

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ABSTRACT

Invited lectures

EAA POST-GRADUATE COURSE: UPDATE ON MALE REPRODUCTION

PC01 | Ancient origins of male infertility

Paulo Navarro-Costa
Lisbon Medical School, Lisbon, Portugal

Male germ cell development is typically regarded as divergent across species. This often serves as an argument to question if animal models can actually provide meaningful insight into human spermatogenesis. A key, and largely unexplored, aspect in this topic is the possibility that male germ cells have retained an evolutionarily conserved genetic basis. In this talk, we show that the gene expression program of animal male germ cells has an old evolutionary origin shared between vertebrate and invertebrate species. Through network analysis of spermatocyte transcriptomes, we provide evidence that old genes serve as a genetic scaffold from which complexity has evolved, and identify an ancient core module of 79 functional interactions central to the identity of a male germ cell. By genetically interfering with this program in animal models such as fruit flies (Drosophila melanogaster) and mice (Mus musculus), and correlating this information with whole exome sequencing data of infertile men, we uncover 164 previously unknown spermatogenesis genes and three new genetic causes of human infertility. Collectively, by highlighting the importance of evolutionary history on human reproductive disease, our data emphasize the usefulness of comparative biology as an ancillary tool in clinical genetics.

PC02 | Legacy of undescended testis

Jorma Toppari
University of Turku, Turku, Finland

The incidence of undescended testis in newborn boys varies from 2% to 9%, and 50–75% of these descend spontaneously during the first few months after birth (mini-puberty). Later during childhood, new cases appear when testes can ascend to cryptorchid position, which is called acquired cryptorchidism. Reasons of cryptorchidism remain unknown in most cases, although we do know many causes, such as androgen insensitivity and impaired androgen biosynthesis. Germ cell development is impaired in cryptorchid testes due to too high temperature. Early orchiopexy is recommended to prevent germ cell loss. Cryptorchidism is also associated with an increased risk of testicular cancer. Consequences of cryptorchidism appear in puberty when the testes grow to an adult size. Testes that do not descend spontaneously grow slower and end up smaller than those that descend either normally before birth or spontaneously during mini-puberty. Serum levels of FSH are higher in boys with a history of cryptorchidism than in controls, whereas inhibin B levels are lower in boys with bilateral orchiopexy than in controls. Testosterone and LH levels are not affected by cryptorchidism, indicating normal Leydig cell function. Thus, the legacy of undescended testis depends on the need of orchiopexy and whether only one or both testes are cryptorchid.

PC03 | Genetic testing of azoospermia: what is new?

Csilla Krausz
Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy

Azoospermia occurs in about 1% of men in the general population. The etiology of this condition can be divided into three major categories: (i) hypothalamic–pituitary axis dysfunction, (ii) primary quantitative spermatogenic disturbances, and (iii) urogenital duct obstruction. In all these etiological categories, we can test for known genetic factors. For instance, in patients with primary testicular failure karyotype abnormalities and Azoospermia Factor (AZF) microdeletions are routinely screened. Candidate gene mutation screening is performed in Congenital Bilateral Absence of Vas Deferens (CFTR gene) and in Congenital Hypogonadotropic Hypogonadism (35 candidate genes). Given that after a complete diagnostic workup, in about 40% of NOA the aetiology remains unknown, it is highly likely that a proportion of these cases will be of genetic origin. Given that spermatogenic process is inherently complex and >3000 genes participate in it, a high genetic heterogeneity seems to be plausible. Thanks to the widespread diffusion of next-generation sequencing (NGS)-based whole exome sequencing (WES) or gene panel sequencing a growing number of promising NOA candidate genes have been identified. Despite progress, until 2021, the number of genes, which could be included in a diagnostic gene panel for idiopathic NOA patients, was still relatively low (approx. 17 genes). A major breakthrough in androgenetics has been reached through consortia-based efforts. Thanks to the data sharing between different laboratories belonging to the International Male Infertility Genomics Consortium (IMIGC) (http://www.imigc.org), many novel genes were

discovered and previously reported candidate genes have been validated in independent cohorts. The large majority of validated genes cause maturation arrest and many of them are involved in meiotic arrest. Interestingly, some of the NOA genes are also involved in DNA repair and may represent a genetic link between NOA and higher risk for cancer development in these men. For some gene defects, the testis phenotype consistently shows pure SCOS/MA phenotypes, providing a pre-TESE prognostic value for the identified NOA-causing gene. Currently, the sole prognostic pre-TESE genetic test is based on the AZF deletion screening but, if these monogenic causes will be validated in large cohorts, their screening will complement AZF screening also as prognostic test for testicular sperm retrieval.

While the major focus in the last 25 years was the Y chromosome, thanks to a recent multicentre study of IMIGC members, we were able to shed light on the relevance of the X chromosome-linked genes in NOA. Through the sequencing of all protein-coding genes in > 2300 patients, 21 recurrently mutated, novel genes with strong relationship with NOA have been discovered and validated immediately. Screening for monogenic causes does not only allow finding the cause of NOA and in some cases to help predicting TESE success, it has also relevance for siblings. A part from its obvious relevance to the brothers, also sisters may benefit from the genetic diagnosis. In fact, there is an emerging evidence on shared genetic factors between NOA and premature ovarian insufficiency (POI). An early genetic diagnosis in the sister may allow fertility preservation through cryopreservation of oocytes.

In conclusion, the NGS era allows implementing the diagnostic work-up of azoospermic patients with targeted candidate gene screening. Genetic screening is relevant for its diagnostic value, clinical decision-making and appropriate genetic counselling.

PC04 | Medical care for adult male DSD patients

Jolanta Slowikowska-Hilczer

Department of Andrology and Reproductive Endocrinology, Medical University in Lodz, Lodz, Poland

Concerns exist with regards to the proper care of adult patients with disorders/differences of sex development (DSD). To evaluate the outcome of surgical and hormonal therapy and psychological intervention in DSD patients the European dsd-LIFE study was carried out. It was established by a multidisciplinary consortium consisting of clinical scientists in the areas of endocrinology, andrology, gynaecology, urology, surgery, psychology and ethics. The study was conducted by 14 multidisciplinary teams in Germany, France, the Netherlands, Poland, Sweden and the United Kingdom. In total, 1040 patients with DSD, aged ≥16 years, were recruited.

It was revealed that longstanding health issues other than DSD and feeling limited in daily life were reported in 51.0% and 38.6%, respectively (controls 24.5% and 13.8%). Any disorder except DSD was present in 84.3% (controls 24.6%). Males reported worse health than females. As a result of uncertainty regarding the genital aspect and/or function, and possibly impaired body image, many individuals fear intimacy and report anxiety and distress related to sexuality, resulting in a tendency to delay or avoid sexual experience. Fertility is markedly reduced in almost all forms of DSD. Adults with nonfunctional or partially functioning gonads have hypogonadism symptoms and require lifelong sex hormone replacement therapy (HRT) to promote sexual, cardiovascular and bone health as well as general wellbeing. Many patients have not been previously or fully informed of the DSD issues and options of treatment. It is concluded that multidisciplinary care is equally important in adulthood as it is in childhood. An individual approach to each DSD patient is of importance.

PC05 | Update on Klinefelter syndrome

Lise Aksglaede

Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Klinefelter syndrome is the most common sex chromosome aberration affecting approximately 1:660 newborn boys. The syndrome is characterized by the presence of an extra X chromosome (47,XXY) and a clinical presentation with hypergonadotropic hypogonadism, infertility and an increased risk of developing metabolic syndrome, cardiovascular disease and osteoporosis in adulthood. Furthermore, learning disabilities and psychosocial challenges are seen more frequently. However, the phenotypic spectrum is very wide and some patients only experience relatively mild symptoms, whereas the condition has a huge impact on physical as well as psychological health of others.

PC06 | How to deal with sexual problems in infertile couples?

Emmanuele Jannini

Chair of Endocrinology and Medical Sexology (ENDOSEX), Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy

Infertility represents a peculiar social burden affecting more than 15% of couples, provoking it a real threat to the general quality of life and to the sexual health. The medicalization (diagnosis, therapy and follow up) of the lack of fertility is frequently a challenge in term of personal and couple's involvement. In particular, while the assisted reproductive technology (ART) has allowed many infertile couples to achieve pregnancy, the therapeutic process faced by the couple bears a strong psychological stress that can affect the couple's quality of life, relationship and sexuality. Despite infertility affects both female and male sexual health, only recently the interest in the effects of ART on the couple's sexuality has grown, especially for women.

Literature largely found that intimacy and sexuality appear specifically impaired by intrusiveness of treatments and medical prescriptions. Moreover, there is a close relationship between emotional, psychological and sexual aspects, which can be integrated in the new concept of inferto-sex syndrome (ISS) that can impair the ART outcomes. Evidence demonstrates that the assessment of sexual.

A close relationship between infertility and sexuality, both in the female and male partners, was detected. ART treatments may heavily impact on the couple's psychosexual health. A couple-centred program for the integrated management of psychological and sexual dysfunction should be considered in the context of ART programs.

PC07 | Surgical treatment in male infertility: what is new?

Suks Minhas
Imperial College, London, UK

Non-obstructive azoospermia (NOA) is reported in 1% of all men and 10% of couples with male factor infertility. The standard management of NOA is testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) and the management has largely remained unchanged in the last 20 years.

Historically, mTESE has been advocated as the technique of choice for sperm retrieval in NOA, although there are no randomised studies comparing cTESE v mTESE. However, a recent, study has demonstrated that mTESE is more likely to result in successful sperm retrieval compared to TESA.

There are currently no predicative factors for successful sperm retrieval with testicular histopathology the only single reliable predictor of successful sperm recovery, although both testis size and FSH levels have also been analysed as prognostic factors for sperm retrieval, and data remains conflicting. Overall, approximately 50% of men will have sperm retrieved at the time of mTESE. The surgical retrieval rates in men with Klinefelters varies considerably in the literature and this may be accounted for by a number of confounding factors including age at surgery, surgical technique, laboratory methods and preoptimization of patients. In this context, meta-analytical data would suggest that varicocelectomy prior to TESE may improve the outcomes in men with NOA, although a recent study has suggested that hormone stimulation does not improve surgical sperm retrieval rates in men with hypergonadotrophic hypogonadism. This lecture will address these controversial areas in the management of men with NOA.

PC08 | Medical treatment of male infertility: what is new?

Hermann M. Behre
Center for Reproductive Medicine and Andrology, University Medicine Halle, Halle, Germany

Male infertility due to impairment of spermatogenesis or sperm function can be treated with good success by assisted reproductive techniques (ARTs). However, the major burden of treatment of the infertile couple is on the female side with rare but potentially severe side effects. There are also known health risks for the children born after ART. There is an urgent need for andrologists to develop and provide effective evidence-based therapies for the male when the reason for couple infertility is on the male side. In 2018, the European Academy of Andrology (EAA) published a comprehensive guideline on the management of oligo-astheno-teratozoospermia (OAT) (1). The guideline states that the EAA cannot recommend either for or against antioxidants, antiestrogens (tamoxifen or clomiphene) or aromatase inhibitors for medical treatment of male infertility because of a lack of solid evidence. Recently, a Cochrane review came essentially to the same conclusion for treatment of male infertility with antioxidants (2). In the EAA guidelines, it was also stated that treatment with FSH can be suggested with low evidence in selected men from infertile couples (normogonadotropic men with idiopathic oligozoospermia or OAT) in attempt to improve quantitative and qualitative sperm parameters and pregnancy rate. Unfortunately, no further high-quality, randomized controlled studies (RCTs) on FSH treatment of male infertility have been published since then – but at least one RCT evaluating viable pregnancies in the female partner as the primary outcome measure is ongoing according to information provided on the website ClinicalTrials.gov. Recently, potential reasons for the slow progress in development of evidence-based medical treatment of male infertility have been identified in a systematic review on the 100 largest RCT on treatments of men with infertility registered in the Cochrane Register of Controlled Trials between January 2010 and July 2021 (3). Only 36 of the 100 trials clearly defined their primary outcome, only 51 of 100 trials reported on pregnancy rate, and only 13 of 100 trials reported on live birth. Even in 2022, there is still a need for high-quality studies on medical treatment of male infertility – with the primary outcome viable intrauterine pregnancy or even better live birth.

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STATE-OF-THE-ART LECTURES

L01 | New mutations, selfish testes and human disease

Anne Goriely
University of Oxford, Oxford, United Kingdom

It is well established that advanced paternal age is associated with low sperm count/quality and infertility, but what is not always appreciated

is the impact of paternal ageing on new mutations and genetic disease in the next-generation.

We have previously described a process where some pathogenic mutations hijack the homeostatic mechanisms of spermatogenesis to their own advantage. This mechanism called 'selfish selection' was originally proposed to explain the paternal age-effect and high birth prevalence observed for some Mendelian disorders, such as Apert syndrome (FGFR2) or achondroplasia (FGFR3). It relies on principles similar to oncogenesis to explain why these mutations occur spontaneously at levels up to 1000-fold higher than the background rate. Importantly, this process emphasizes the intimate link that exists between sperm production/fertility, germline mutation rate and ageing.

In this presentation, I will describe our current understanding of so-called de novo mutations, their origin, their prevalence and importance in human disease and the impact of paternal age on their occurrence. Understanding the risks associated with delayed fatherhood should be an important factor when advising couples seeking to use ART.

L02 | What lessons have we learned from the European Ageing Study (EMAS)?

Frederick Wu
University of Manchester, Manchester, UK

Ageing is associated with multi-level alterations in the hypothalamic-pituitary-testicular (HPT) axis function, but it is not clear why/how they evolve or how they align with the common clinical scenario of symptomatic older men presenting with low or low normal testosterone (T). Whether the clinicopathological constructs of organic hypogonadism, long established in young patients, can be translated to underpin the management of the burgeoning number of middle-aged and older men being referred for possible androgen deficiency with late-onset (LOH) or functional hypogonadism (FH), is also uncertain.

I will present cross-sectional and longitudinal data from the observational cohort of >3000 men from the European Male Ageing Study (EMAS), which describe the natural history of two divergent tracks of HPT axis dysfunctional that underlie the age-related decline in T, based on the patho-physiological classification of hypogonadotrophic (secondary) or hypergondodotrophic (primary) 'hypogonadism'.

The main findings are as follows:

Unlike the female menopause, the vast majority of men in the general population do not become hypogonadal during ageing.

The symptomatic and T thresholds for identifying possible LOH were established and subsequently refined with the use of free T and longitudinal data.

Obesity is associated with the development of sexual symptoms and reversible hypothalamic/pituitary suppression (equivalent to secondary 'hypogonadism') independent of age, usually affecting middle-aged rather than elderly men. Due to the decreased SHBG, total T may be spuriously low and the use of free T is important in order to avoid over- diagnosis of hypoganadism in obese men.

In contrast, the less common equivalent of primary 'hypogonadism' found mainly in men over 70 years of age show a more severe phenotype with sexual and physical symptoms, insulin resistance and co-morbidity compatible with general health deterioration. This is compatible with the clinical entity of LOH or FH associated with poor health in ageing and potentially avoidable or deferrable for those in good health.

It is also relatively common to encounter elevated LH with normal T in ageing men - this can be considered to be a state of compensatory 'eugonadism' since they do not have definite features of androgen deficiency, but amongst them will be a small minority who eventually transitions to primary 'hypogonadism'.

Our epidemiological study cannot clearly differentiate between the co-linear symptoms of androgen deficiency and the non-specific features of ageing-related disability or prove causality of clinical features resulting from low T. Nevertheless, our observational data are consistent with results from recent RCTs of T replacement in symptomatic older men with low T.

Improved understanding of the aetiology, natural history and potential clinical significance of the age-related changes in the HPT axis can inform designs of future interventional trials and current clinical practice.

L03 | How to deal with fertility preservation in boys

Herman Tournaye
Brussels IVF, Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium

Cytotoxic therapies and/or testicular irradiation are the main cause of germ cell loss and can hamper spermatogenesis permanently. These therapies are currently not only used to treat malignant disorders, but also for benign haematological conditions that need bone-marrow transplantation. But apart from these indications, fertility preservation is becoming also more important in non-medical indications, i.e. gender dysphoria. Hence, preservation of reproductive potential has become an important quality of life issue in prepubertal boys and in adolescent and young adults (AYA). In AYA, cryopreservation of sperm is possible once both spermarche and oigarche are a fact. Besides these two prerequisites, counselling of AYA remains critical in a fertility preservation program. Prepubertal boys cannot benefit from sperm banking as active spermatogenesis is not present. As an alternative, testicular stem cell banking is being introduced into more and more clinics as a preventive measure. Yet, this strategy should still be regarded as experimental given the lack of any report on successful transplantation. Therefore, also here counselling towards both child and parents is crucial.

L04 | Testicular organoids: the next big thing in male fertility R&D?

Yoni Baert
Biology of the Testis Lab, Vrije Universiteit Brussel (VUB), University Medical Campus, Brussels, Belgium

Diseases, chemicals, and genetic alterations that effectuate male infertility remain largely understudied. Hence, a complete in-vitro physiomimetic testis model for large-scale mechanistic studies is highly needed. It would permit the discovery of workable solutions for many male infertility cases. Nowadays, in-vitro spermatogenesis in mammals is typically performed using the organ culture system. However, an important shortcoming of this system is the entrapment of cells within the boundaries of the tissue fragment, making them inaccessible for specific manipulations and turning organ cultures into an inefficient approach in an explorative set-up. In contrast, testicular organoids are 3D multicellular tissue surrogates originating from the self-assembly of customizable cell suspensions. Consequently, the TO culture system is tailorable and scalable and, therefore, an excellent male fertility R&D tool. Albeit a lack of standardization and failure to support full spermatogenesis, current TO cultures can already recapitulate the testicular architecture, grow early germ cells, and synthesize testicular hormones. The TO research area is still relatively new, yet grows at a fast pace. Given the recent achievements, advanced testis physiomimicry in TOs seems to be only a matter of time.

L05 | Male Contraception in the 21st Century: An Update

Wei Yan
National Center for Male Reproductive Epigenomics, USA

The wide usage of the female contraceptive pill has allowed women to control their own fertility and thus, has transformed human societies since its invention in the 1950s. However, the pill is hormone-based and only available for women. A male version of "the pill" would allow men to control their fertility and to participate in family planning. Despite tremendous efforts over the past four decades, the progress in developing non-hormonal male contraceptives has been very limited. Based on decades of basic research, we put forward an innovative strategy to develop non-hormonal male contraceptives, i.e., looking for a compound that targets a protein critical for the last several steps of sperm assembly because this would lead to the production of nonfunctional sperm without causing severe depletion of testicular cells. This novel idea has led to the discovery of Triptonide, a natural compound purified from a Chinese herb, as a safe, effective, and reversible male contraceptive agent in both mice and monkeys. I will share with our European colleagues how our basic research led to this discovery, and more importantly, several important lessons that we learned during the drug discovery process.

L06 | Reproductive endocrinology at the age of transition

Anders Juul
[1]*Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark;* [2]*Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*

The hypothalamo-pituitary-gonadal hormone (HPG) axis is active in different periods during a lifetime. It is very active in fetal life and the active again following its first activation in postnatal life during infancy (the so-called minipuberty). Reproductive hormones in minipuberty can be considered a diagnostic "window of opportunity" during which conditions like DSD and CHH can be diagnosed. Importantly, reproductive hormone levels during minipuberty predict adult testicular function. After minipuberty, the HPG axis is silenced and remain quiescent until it is re-activated during puberty with the well-known large interindividual variation in pubertal timing. Reproductive hormones and growth factors increase markedly during the pubertal transition, and influence linear growth, development of sexual characteristics, cognitive and metabolic function ("puberty is a transient period of mental instability, acromegalic traits and insulin resistance"). Reproductive hormones increase even after completion of linear growth and sexual maturation to ensure appropriate muscle and bone development.

SYMPOSIUM 1: CLINICAL SCIENCE SESSION - GENETICS OF MALE INFERTILITY

RT01-1 | The testicular phenotypes of AZFb deletion carriers

Peter H Vogt[1], B Deibel[1], F Kiesewetter[2], G Raabe-Meyer[3], U Bender[1], T Strowitzki[4]
[1]*Division of Reproduction Genetics, Department of Gynaecol. Endocrinology & Infertility Disorders, Women Hospital, University of Heidelberg, Germany;* [2]*Department of Andrology, University clinic of Dermatology, Erlangen, Germany;* [3]*Center of Human Genetics, Hannover, Germany;* [4]*Department of Gynaecol. Endocrinology & Infertility Disorders, Women Hospital, University of Heidelberg, Germany*

Molecular analyses of small Yq11 deletions on normal looking Y chromosomes of infertile men with 46,XY karyotype revealed interstitial microdeletions in Yq11 associated with distinct testicular histologies, i.e., disruption of spermatogenesis occurred at distinct developmental phases. In proximal Yq11, they were designated as "AZFa" when causing Sertoli cell only (SCO) syndrome; in distal Yq11 as AZFb when causing meiotic arrest (MA) and as AZFc when causing hypospermatogenesis with gradual severity including azoospermia.

Today, we know that these AZF microdeletions are found in about 13% of men with non-obstructive azoospermia and in about 7–10% of men with severe oligozoospermia. Thereby AZFc deletions are most frequent (60%) and AZFb deletions occurred in about 6–10% of azoospermic patient groups. However, these frequencies are depending on the ment's Y chromosomal haplogroups and microdeletions overlapping with AZFb and AZFc were also found on the Y chromosome of fertile men, in distinct human populations.

Genomic sequence analysis of the human Y chromosome revealed a complex organisation of large repetitive sequence blocks with high sequence homology (99,9%) especially on the long Y arm encompassing

the genomic AZFb and AZFc deletion interval (www.ncbi.nlm.nih.gov/nuccore/NC_000024.10). Molecular analyses of the so called "classical" AZFb deletions, i.e., causing meiotic arrest, revealed that "Non-Allelic Homologous Recombination (NAHR)" events within two sequence blocks: one located in the P5 palindrome in proximal AZFb, one located in the distal P1 palindrome in AZFc are causing these AZFb deletions with loss of 6.23 Mb genomic Y DNA.

They include complete deletion of six protein encoding Y genes, EIFA1Y, HSFY (2 functional copies), PRY (2 functional copies), RBMY1 (six functional copies), RPS4Y, SMCY (aka JARID1D; aka KDM5D), one copy of CDY2 and XKRY located in proximal AZFb in the P5 palindrome and additionally copies of the AZFc gene families, BPY1 (1 copy); DAZ (2 copies), CDY1 (1 copy) in the distal overlapping AZFb/c interval. "Classical" AZFb deletions overlap thus significantly (2.3 Mb) with the proximal part of AZFc deletions.

AZFb deletions diagnosed to be smaller than the genomic extension of the "classical" AZFb deletion are summarized as "non-classical" AZFb deletions. They are associated with variable testicular pathologies and some patients are even able to produce mature sperms although only in low numbers and often associated with increased dysmorphologies including the OAT syndrome. For clinical prediction of the putative testicular histology of infertile men with any AZFb deletion, it is therefore mandatory to map the molecular break sites of each diagnosed individual AZFb deletion precisely, especially in its distal overlap with the AZFc amplicons.

For this purpose, AZFc amplicon specific sequence variants (SNVs) have been developed with which it is possible to specify – separately – presence or absence of the distinct blue (b2; b3), green (g1; g2), grey1, red (r1; r2) amplicons in the overlapping AZFb/AZFc amplicons. Only AZFb deletions encompassing completely the b2, b3, grey1, g1 and r1/r2 amplicons in AZFc are associated with germ cells meiotic arrest in the patientt's testis tubules.

However, the SNV pattern of the amplicons in AZFc is rearranged in distinct Y chromosomal haplogroups. That means all SNV markers in the AZFb-AZFc overlap region on the Y chromosome of infertile men with "classical" AZFb deletions are only deleted, when this Y chromosome is related to haplogroup R1b* being the haplogroup of the Y reference sequence.

Review of the literature and more cases with "classical" and "non-classical" AZFb deletions analysed in our lab since the last 20 years suggests that the genomic Y sequence encompassing AZFb is rather variable in men with distinct Y haplogroups and "polymorphic" especially in the distal P3 palindrome and the flanking AZFb region overlapping with the proximal AZFc deletion interval (b2-b3 region). Any AZFb deletion diagnosed on the Y chromososome of infertile men with azoospermia should therefore be confirmed as "de novo" mutation event, i.e., not present on the Y chromosome of the patientt's father or fertile brother, before it is considered as putative causative agent for the patientt's testicular pathology.

AZFb deletions are also known to impair formation of the X-Y pairing process (MSCI) at midpachytene. This process of de-condensation of the complete Y chromosome in pre-meiotic germ cells with subsequent condensation of the X-Y pairing structure at meiosis is required for normal postmeiotic male fertility. It can, therefore, not be ruled out that also de-condensation of the compact chromatin domain DYZ19 in the AZFb deletion interval are functionally contributing to the controlled time course of this germ cell specific dynamics of the X-Y chromatin.

In summary, extensive amendment of the current EMQN/EAA guidelines for the identification of "classical" AZFb deletions causing meiotic arrest is required including analysis of the distal AZFb breakpoints precisely and analysis of the ment's Y chromosomal haplogroup. Questionable might be also, whether it is further recommended to predict the severe testicular pathology of meiotic arrest for azoospermic men with a diagnosed AZFb deletion without analyzing additionally the histology of a testis biopsy.

RT01-2 | The role of X chromosome variants in azoospermia

Antoni Riera-Escamilla

Andrology Department, Fundació Puigvert, Barcelona, Catalonia, Spain

Although the X Chromosome has been largely perceived as a "female" counterpart to the "male" Y chromosome, the evolutionary history of the X chromosome indicates its specialization in male fitness since it is enriched in genes expressed in early spermatogenesis. Moreover, males normally only have a single X chromosome, any loss-of-function mutations in single-copy X-chromosomal genes cannot be compensated by a normal allele. These features make X-linked genes particularly attractive for identifying novel genetic causes of spermatogenic failure. Thanks to the application of Next Generation Sequencing (NGS) the number of genes linked to male infertility is constantly growing. In this regard, in 2021, Houston et al.[1] systematically evaluated the Gene disease relationship (GDR) of >600 genes in relationship with male infertility and classified them according to their clinical evidence as limited, moderated, strong and definitive. The authors proposed that only those genes that reached at least moderate GDR evidence should be included in a diagnostic gene panel. Surprisingly, only three X-chromosome-linked genes: TEX11, AR and USP26 associated with azoospermia/oligozoospmeria, reached high enough evidence for GDR to be proposed for a diagnostic gene panel. The unexpected low number of X-chromosome-linked genes belonging to these GDR categories is likely to be due to the previous approach (targeted sequencing of candidate genes) and the relatively small cohorts of patients analyzed. We have recently concluded a multicenter study in which all X-linked protein-coding genes have been analyzed in 2,354 NOA/cryptozoospermic men.[2] Thanks to our study, seven genes reached moderate/strong GDR evidence, increasing the number of genes to be included in the diagnostic setting from three to ten. Moreover, we identified 21 novel recurrently mutated genes strongly associated and 34 moderately associated with NOA/cryptozoospermia. This study represented a significant step toward the definition of the

missing genetic etiology in idiopathic severe spermatogenic failure and significantly reduced the knowledge gap of X-linked genetic causes of azoospermia/cryptozoospermia contributing to the development of future diagnostic gene panels. Notwithstanding, due to the intrinsic limitations of NGS-based analysis, the role of multicopy and ampliconic genes in spermatogenic failure remains to be addressed in future studies.

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RT01-3 | Exome analysis: time to advance the standard of care

Frank Tüttelmann
Institute of Reproductive Genetics, University of Münster, Germany

Infertility is a common multifactorial disease and global health problem affecting 10–15% of couples. It can often be overcome by medically assisted reproduction (MAR), offering couples that fail to conceive naturally the chance for parenthood. In half of the couples, the infertility is due to male-factors. Despite advances in the past decade, in the majority of infertile men, the underlying genetic, molecular, cellular, and/or organ defect(s) still remain ill-defined or unknown altogether. Thereby, evidence-based treatment decisions for testicular sperm extraction (TESE) and MAR, estimating and counselling as to risks for both the men and their offspring, and potential preventive measures are largely precluded.

However, in recent years, the number of male infertility cases "solved" by genetic analyses, most importantly exome sequencing, increases continuously. We and others have contributed significantly to 1) identifying novel genes and causal variants (mutations), 2) functionally scrutinising them, and 3) translating findings into the clinic. Most attention is currently focussed on azoospermia, supposedly affecting up to 1% of the male population, and specific sperm defects clinically detected as morphological aberrations or decreased/completely lacking motility.

Crypto- and azoospermia (very few/no sperm in the semen) are main contributors to male factor infertility. Genetic causes for spermatogenic failure (SPGF) include Klinefelter syndrome (47,XXY) and Y-chromosomal AZF microdeletions, and CFTR mutations for obstructive azoospermia (OA). However, the majority of cases remain unexplained because monogenic causes are not analysed. The clinical distinction between azoospermia due to SPGF (non-obstructive azoospermia, NOA) and obstructive azoospermia (OA) is challenging but critical for counselling patients prior to surgical sperm retrieval procedures: men with OA have high success rates for TESE and subsequent intracytoplasmic sperm injection (ICSI), while rates for NOA patients range from virtually zero up to ~50% depending on the genotype.

One focus of the talk will be the recent results of our four years prospective exome sequencing study in crypto-/azoospermic men and the impact for predicting TESE and MAR success based on identified mutations. Another topic will be the genetic makeup of morphologically abnormal sperm and the consequences for MAR/ICSI. Lastly, a previously underestimated cause for infertility and IVF failure will be reviewed.

The conclusion is, hopefully, not surprising anymore: it is due to routinely analyse more than karyotype and AZF in infertile men!

SYMPOSIUM 2: (EPI) GENETICS OF MALE REPRODUCTIVE FUNCTION

RT02-1 | Nutrition in Men, Fertility and Offspring Health: Epigenetics Insights

Adelheid Soubry
Epigenetic Epidemiology Lab, KU Leuven University, Belgium

While it is well established that age, lifestyle, diet and environmental exposures can affect female reproductive health, little attention is paid to the male partner in preconceptional and fertility care. This omission is due to numerous factors, including cultural influences and education, the focus on women in clinical fertility care, limited male-oriented research into existing birth cohorts, and the lack of translation of animal research into clinical practice. However, it has been estimated that at least half of failed pregnancies are due to male problems. Better insights into the role played by the environment and lifestyle of men in pregnancy failure would help clinicians to better inform patients about their chances of conceiving a child, and – more importantly – to provide tailored assistance to improve male fertility.

Furthermore, if pregnancy is successful, it is unknown to what extent environmentally induced epigenetic changes from sperm – due to the paternal lifestyle or diet – may be transmitted to offspring. Animal experiments and pioneering epidemiological studies by our group and others have revealed that the epigenome is malleable and susceptible to environmental stressors. In our research studies, we found associations between male diet, obesity, and harmful environmental exposures, on the one hand, and DNA methylation differences in Imprinting Control Regions (ICRs) in sperm, on the other hand. In a birth cohort, we were able to identify ICRs in newborns that were differently methylated by paternal obesity. Based on these findings, we introduced a new concept on the Paternal Origins of Health and Disease (POHaD), where the role of the father has been suggested in disease development of his future offspring. Our findings related to this concept will be presented and discussed. If epigenetic transmissions from father to child are better understood, tailored dietary changes may positively shape the human sperm epigenetic profile and future programming of offspring health.

RT02-2 | Sperm-borne RNAs – the informers for future generations?

Noora Kotaja

Noora Kotaja, University of Turku, Finland

Differentiating male germ cells undergo dramatic changes in their gene expression during spermatogenesis. Particularly meiotic spermatocytes and postmeiotic haploid round spermatids, have exceptionally diverse transcriptomes, including only gene products supporting the unique processes taking place during and after meiosis, but also an unusually diverse set of unannotated transcripts from intergenic genomic regions. These cells produce also massive amount of PIWI-interacting RNAs (piRNAs) that are germline-specific non-coding RNAs with important functions in transposon silencing and mRNA regulation. After broad genome expression, transcription is silenced due to chromatin compaction, and translational regulation becomes prominent when transcripts need to be translationally repressed and stored for later use. Some germline intrinsic RNAs, including piRNAs, are retained in transcriptionally inactive mature spermatozoa, and sperm RNA content is still modified during epididymal transit. These dynamic changes in the germline transcriptome require accurate regulatory mechanisms to monitor the quality of transcripts and determine their fates. This is critical for not only normal spermatogenesis and fertility, but also transmission of epigenetic information about father's environmental exposures and health condition to offspring via sperm RNAs. Our studies focus on elucidating the germline-specific RNA regulatory mechanisms, particularly the functions of cytoplasmic germ granules. Furthermore, we are interested in understanding the transgenerational effects of environmental exposures on germline transcriptome.

RT02-3 | Chromosomal fusions and 3D genome folding and recombination in ther germ line

Aurora Ruiz-Herrera[1,2]

[1]*Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain;*

[2]*Genome Integrity and Instability Group, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*

The spatial folding of chromosomes inside the nucleus has regulatory effects on gene expression, yet the impact of genome reshuffling on this organization remains unclear. This is of relevance since chromosomal fusions represent the most common chromosomal rearrangement in nature (from plants to mammals), and are linked to recurrent miscarriages, infertility, and aneuploid offspring in humans. In fact, it has long been suggested that the presence of chromosomal fusions in the germ line can alter segregation patterns (the so-called interchromosomal effect). In this talk I will resume our recent results on the effect of chromosomal fusions on the higher-order chromatin organization and recombination landscapes in the germ line by combining chromosome conformation capture with single nucleotide polymorphism (SNP) genotyping and analysis of crossover events. Our results show how chromosomal fusions can alter the nuclear architecture during meiosis, including an increased rate of heterologous interactions in primary spermatocytes, and alterations in both chromosome synapsis and axis length. These disturbances in topology were associated with changes in genomic landscapes of recombination, resulting in detectable genomic footprints. Overall, chromosomal fusions impact the dynamic genome topology of germ cells in two ways: (i) altering chromosomal nuclear occupancy and synapsis, and (ii) reshaping landscapes of recombination.

SYMPOSIUM 3: MEET THE EXPERT

RT03-1 | The contemporary role of ultrasound scan in Andrology

Francesco Lotti

Italy

The aim of the present "Meet the Expert Session" is to discuss interactively about the current role of ultrasound (US) in Andrology. The discussion will take place considering the updated ultrasound (US) reference ranges of the male genital tract organs and their clinical utility in the evaluation of male reproductive and general health. In particular, the cut-offs distinguishing normal and pathologic US features, as derived from the recent European Academy of Andrology (EAA) US study on healthy, fertile men, will be discussed. In addition, the role of scrotal and transrectal US in assessing male infertility according to the more recent guidelines and evidence-based studies will be displayed. The impact of testis, epididymis and vas deferens US abnormalities as well as of varicocele and prostate-vesicular US anomalies on male reproductive health will be covered. Finally, the impact of US-derived information on the management of the infertile male / couple will be outlined. Beside reproductive issues, scrotal US (including contrast-enhanced US and sonoelastography) applications to assess scrotal pain, masses and trauma will be discussed, as well as the growing relevance of transrectal US in evaluating chronic pelvic pain. Furthermore, the role of flaccid and dynamic penile color-Doppler ultrasound to investigate erectile dysfunction, La Peyronie disease and other penile diseases will be considered. Finally, male breast US to evaluate gynecomastia, lipomastia or breast cancer will be faced.

RT03-2 | How to biochemically assess androgen status?

Leen Antonio

Clinical and Experimental Endocrinology, KULeuven, Belgium; Department of Endocrinology, University Hospitals Leuven, Belgium

Introduction

Sex steroids, such as testosterone (T) and estradiol (E2), are mainly synthesized in the testes and adrenal glands. In men, androgens are

important for many functions in the body, e.g., gonadal development, sexual function, muscle mass, muscle strength and bone mass. Hypogonadism is the combination of hypogonadal signs and symptoms and low testosterone levels. In circulation, sex steroids are bound to sex hormone-binding globulin (SHBG) and albumin with a high and low affinity respectively. Only a small fraction circulates in a non-protein bound or free form. According to the 'free hormone hypothesis', the free fraction is responsible for the biological activity of sex steroids, as only the free hormone can enter the cell and can activate its nuclear receptor.

Although serum T and E2 concentrations are very often used in the diagnosis of several endocrine disorders and follow-up of treatment, measuring sex steroids remains technically challenging.

How to measure total testosterone?

In routine clinical practice, total T concentrations are most often measured by immunoassays. Direct immunoassay methods, embedded in a multichannel, automated analyzer, are used in most clinical laboratories, as these methods are relatively cheap and allow high throughput. Unfortunately, both for T and E2, these commercially available immunoassays are susceptible for interference and lack precision and accuracy for low concentrations, such as T levels in women and E2 levels in men.

Liquid or gas chromatography-tandem mass spectrometry (LC-MS/MS or GC-MS/MS) based methods increase accuracy and sensitivity of sex steroid measurements. They can be used to overcome the limitations of immunoassays and are currently the gold standard for sex steroid measurements. Both total T and total E2 were measured by GC-MS/MS in a large number of middle-aged and elderly men who participated in the European Male Ageing Study (EMAS). This showed that a validated immunoassay method can be used to measure total T concentrations in the normal range in adult men, but the correlation between IA and GC-MS/MS declined for total T concentrations < 8 nmol/L.[1]

Free testosterone: measurement or calculation?

Although measuring total T or total E2 concentration is often sufficient for diagnosis and follow-up of endocrine disorders, the 'free hormone hypothesis' indicates that the non-protein bound or free sex steroid level plays a key role in sex steroid physiology. Therefore, the free testosterone concentration could be a better reflection of the androgenic state than the total concentration. This is especially the case in clinical situations where SHBG levels are altered, such as in ageing and obesity.[2]

However, directly measuring free T remains technically challenging.[3,4] Commercially available immunoassays for free T are inaccurate and their use is not recommended. Free T can be measured accurately by equilibrium dialysis, which is currently the gold standard, but this method is time-consuming and costly. Therefore, these methods are only available in a few reference and research laboratories. As an accurate and reliable assay to measure free T directly is not yet routinely available in clinical laboratories, calculated free T (cFT) is used to assess free T levels instead.

Several formulas to calculate free T have been described in literature. These calculations use measured concentrations of SHBG, albumin and total T to estimate free T. They are either based on the law of mass action (equilibrium-binding theory),[5–7] empirically derived equations[8–10] or derived from a dynamic allosteric model based on experimental binding data.[11] Also cFT has its shortcomings. Clearly, the accuracy of cFT results relies on accurate measurements of total T and SHBG and studies indicate that up to 25% of cFT levels are not reliable.[3] Mass action equations tend to overestimate free T in men, whereas results derived from empirical equations are method specific and transferring the equations among laboratories can be difficult.[12] Finally, for most formulae, a reference interval is not available.

Conclusion

Total T measured by a validated immunoassay is the first-line test in men with suspected hypogonadism. Calculated free T can provide additional and potentially more reliable information in patients with borderline total T and/or changes in SHBG.

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SYMPOSIUM 4: MEET THE EXPERT - INFECTIONS TO MEET THE EXPERT

RT04-1 | A pictorial review of genital lesions

Alvaro Vives

Andrology Department, Fundacio Puigvert, Barcelona, Spain

No abstract text

RT04-2 | Impact of infections and inflammation on the male reproductive tract

Hans-Christian Schuppe[1,2], Adrian Pilatz[1,2], Florian Wagenlehner[1,2]

[1]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany; [2]Hessian Centre of Reproductive Medicine, Justus Liebig University, Giessen, Germany

Infections and inflammation of the genital tract are categorized as frequent and potentially curable causes of male infertility.

Etiologically, sexually transmitted infections (STI) as well as uropathogenic bacteria ascending via the urethra are most relevant. These pathogens or their components can exert deleterious effects on male fertility, either directly via impairment of sperm integrity and function, or indirectly via inflammation-associated molecules, such as pro-inflammatory cytokines or reactive oxygen species. In addition, dysfunction of accessory glands, inflammation-induced obstruction of the seminal ducts, and deterioration of spermatogenesis should considered. Moreover, systemic infections, e.g. viral illness, may also affect male reproductive organs. In both scenarios, the induction of cellular and humoral immune responses is associated with disruption of the physiological immune regulation and the risk of irreversible tissue damage, i.e. in the testis and epididymis, and/or formation of antisperm antibodies. Notably, pathological changes seen with non-infectious (sterile) inflammation including autoimmune disease are similar to infectious or post-infectious lesions.

While acute symptomatic disease emerging as urethritis, prostatitis/prostato-vesiculitis, epididymitis/epididymo-orchitis and orchitis can be clearly diagnosed, most patients referred for fertility evaluation are asymptomatic and present with a subclinical or chronic course of infection/inflammation. Thus, diagnostic classification is complicated and relies primarily on microbiological detection of pathogens, leukocyte counts, and/or inflammatory mediators in ejaculate, prostatic secretions, and urine samples. Pathological findings are usually grouped together under the term "male accessory gland infection" (MAGI), without differentiating between infectious and non-infectious inflammatory conditions.

Prevalence data of genital tract infection/inflammation as a cause of male infertility mostly refer to the presence of MAGI and are heterogeneous due to inconsistent diagnostic criteria. While frequencies of 10–20% are reported from andrological clinics, the proportion can exceed 30% in regions with limited access to healthcare, suggesting a link between the incidence of STI and infertility. Accordingly, negative effects on semen quality including sperm function were reported in men with positive STI-PCR findings in their ejaculate. Recent studies also showed a negative association between infection with uropathogens and sperm parameters. However, conflicting results are available; thus, the potential impact of seminal bacteria on male fertility is a matter of debate. Notably, bacteriospermia (>103 cfu/ml) does not necessarily reflect genital infection because urethral commensals are frequently present in ejaculates. Concerning viruses in semen, an association between human papilloma virus and deterioration of sperm function and integrity has been reported.

The hallmark of genital tract inflammation is leukocytospermia, defined by the consensus-based WHO threshold of 106 peroxidase-positive cells per ml ejaculate. Although studies have shown a negative association between leukocyte count and sperm parameters, this issue is controversial. Oxidative damage of sperm including DNA fragmentation may occur below 106 leukocytes/ml, with macrophages as major source of pro-inflammatory cytokines. However, elevated seminal leukocyte counts do not allow prediction in terms of bacteriospermia or viruses in the ejaculate. Thus, the indication for microbiological diagnosis merely based on leukocytospermia appears questionable.

Despite the uncertainties delineated above, the risk of irreversible organ damage and persistent infertility along with infection/inflammation of the male genital tract is reflected by the sequelae of acute epididymitis/epididymo-orchitis. Persistent azoospermia was observed in 10% and oligozoospermia in another 30% of patients, even after adequate antibiotic treatment of the acute disease. Epidemiological data are lacking with regard to infertility after primary orchitis of infectious origin; however, up to 25% of testicular biopsies obtained from infertile men reveal focal inflammatory reactions. Data concerning the possible impact of chronic prostatitis on fertility are less consistent. However, recent studies and meta-analyses suggesting a negative impact on sperm parameters including DNA and chromatin integrity.

Any suspicion of an infectious or inflammatory disease in the male genital tract should prompt a systematic diagnostic evaluation and appropriate treatment. Immediate antibiotic therapy is recommended in acute symptomatic infections. In asymptomatic patients with fertility problems, antibiotic treatment should be given only when pathogenic bacteria are detected, microbiological test results can be confirmed in follow-up samples, and bacterial counts are significant. A probatory use of antibiotics, e.g. in patients with isolated signs of inflammation, or untargeted before ART, is not indicated. Non-steroidal anti-inflammatory drugs may be administered if there are signs of inflammation in the ejaculate (without pathogens or after antibiotic therapy). However, randomized controlled studies on anti-inflammatory or immune-modulatory therapies are lacking.

SYMPOSIUM 5: URO-ANDROLOGY

RT05-1 | PSA and MRI in 2022 in the diagnosis of prostate cancer: when to avoid prostate biopsies?

Jochen Walz
Germany

Recently new imaging technologies have been developed to improve the diagnosis and management of prostate cancer. Here, above all, multiparametric MRI (mpMRI) plays a major role. mpMRI can be used to improve the detection of prostate cancer by finding prostate cancer with a higher sensitivity or mpMRI can be used to rule out the need of biopsy by relying in a high negative predictive value. The later seems attractive as with such an approach unnecessary biopsies and over detection might be avoided. The presentation will critically assess the possibility to avoid biopsies based on mpMRI as a triage test in the management of patient at risk of having prostate cancer. The presentation will explore associated risks and solutions to avoid under and over detection and diagnosis.

RT05-2 | Late effects of uro-oncological surgery on sexual function

Alberto Breda
Renal Transplant Surgical Team, Urology Oncology Unit, Fundacio Puigvert, Barcelona, Spain

Sexual function has a great impact on quality of life of patients with urological malignancies. Sexual disfunction in these patients may be due to a combination of several features as the psychological aspects related to the cancer diagnosis (fear of the outcomes, fear to face complex surgeries, …), the postoperative changes in body image due to the presence of stoma or in case of demolitive external genitals surgery and the damage at the neurovascular bundles due to the surgery act, especially in pelvic surgery.

Sexual function after prostatectomy in patients with prostate cancer is widely studied but not all the studies take in consideration the long-term effects of this surgery on sexuality. A nerve sparing approach could be employed in patients with no evidence of locally advanced disease to preserve neurovascular bundles in pelvic surgery. This approach is widely employed in prostatectomy but less in cystectomy.

The employment of phosphodiesterase 5 inhibitors has been shown to improve the recovery of erectile function after prostatectomy in a recent study of Kimura et al. and the effects are maintained also after 24 months of therapy reaching almost 60% of recovery.

These findings confirm the data shown by Lee et al. that show an unexpected long-term recovery of sexual function even after 4 years from surgery.

The sexual function seems to deteriorate after 5 years from the initial treatment as stated in a recent study of Resnick et al on long term outcomes after treatment of localized prostate cancer. These findings could be explained by the physiological deteriorating in sexual function in advanced age, independently from the treatment.

The lack of erectile function recovery at 12 months should not be considered a negative outcome because as stated by Mandel et al in a recent study, the probability of functional recovery after 36 months is about 30.8% and 36.5% at 48 months.

The impact of nerve sparing approach seems to improve the sexual function outcomes also in patients who undergo radical cystectomy. In a recent systematic review and meta-analysis Xiong et al. confirmed the advantages in the nerve sparing cohort after 12 months regarding erectile function.

Other sexual sparing techniques of cystectomy (prostate sparing, seminal sparing or capsule sparing) should be proposed in very selected cases. A recent systematic review of Hernandez et al shows high recovery rate for sexual function in patient that underwent sexual sparing cystectomy, with higher rate of erectile function recovery for prostate and seminal sparing techniques, almost reaching 90% of recovery.

Sexual function is not only an organic disfunction, also psychological and emotional aspects are mean to be taken in account. This could explain the similar erectile disfunction rates found between radical prostatectomy and watchful waiting cohorts in a recent study of Johansson et al.

A recent prospective comparison by Clements et al. between continent urinary diversions and ileal conduit in quality of life after surgery showed a better sexual and erectile function with a better overall satisfaction in neobladder rather than ileal conduit cohort.

Testicular cancer could impact in several ways the sexual functions of patients. Up to 40% patients become oligo/azoospermic after orchiectomy, and usually after 2–3 years, the spermatogenesis recovers.

Retroperitoneal lymph node dissection could damage the hypogastric plexus and cause retrograde ejaculation leading to infertility and sexual discomfort. Even if nerve sparing approaches were proposed, up to 15% of patients could be affected.

The conception rate is maintained even after chemotherapy but drops after 3 cycles of PEB. Fortunately, the cryopreservation of semen could avoid the deterioration of spermatogenesis after treatments even if the rates of semen deposit are far from the optimal.

Tal et al. found that after radical prostatectomy the incidence of Peyronie's disease is 15.9% in a cohort of 1011 patients, with a median time of onset after radical prostatectomy of 13.9 months even if the pathophysiology is not yet clearly assessed.

The effects of oncological surgery on sexual function should be discussed in a multidisciplinary setting with the patients, informing them about what to expect after surgery and providing all the information on the recovery process.

RT05-3 | Testosterone replacement in prostate cancer patients: is it safe?

Francesco Sanguedolce
Spain

The relationship of testosterone and prostate cancer is a topic extensively debated but still not fully clarified. Testosterone-androgen receptor interaction is largely considered the main pathway for prostate cancer growth and spread; in fact, androgen deprivation therapy from the 40s is the spine of the systemic treatments for advanced prostate cancer.

However, in the 90s, some scholars found a significant association with a higher grade of the tumours (GS $\geq$8) and a low level of testosterone in a cohort of prostate cancer patients.

Nevertheless, some more recent evidence seems to suggest that supraphysiological androgen levels suppress prostate cancer growth in the setting of castration-resistant prostate cancer.

Nowadays, testosterone replacement therapy is widely used in patients with hypogonadism because of the multiple benefits that may provide to the affected patients, especially in terms of increasing muscular mass, tackling erectile dysfunction, preventing osteoporosis, and/or limiting cognitive impairment.

Based on the evidence nowadays available, controversies on its use are mostly arousing in the setting of patients with higher risk to develop a prostate cancer, as well as in prostate cancer patients under active surveillance or receiving radical treatments with curative intent. From one side, these patients might be considered at low risk to experience

clinically significant prostate cancer diagnosis and/or its progression, so that improvement of quality of life with TRT in case of hypogonadism should be considered. On the other side, risk of tumour misclassification and/or prostate cancer recurrence cannot be equally ignored, with TRT potentially "fuelling" prostate cancer cells.

In this lecture, the most recent and relevant evidence in literature will be presented and summarised, in order to provide the practitioners with guidance for their decision making and patients' counselling.

RT05-4 | Testis sparing surgery. When and why?

Suks Minhas
UK

No abstract text

RT05-5 | Nerve-sparing techniques in prostate and bladder cancer surgery: Do they improve preservation of sexual function?

Joan Palou
Urology Department, Fundacio Puigvert, Barcelona, Spain

Radical prostatectomy (RP) is the most frequently selected treatment choice for localized prostate cancer. Although the surgical technique has improved with the advent of laparoscopy and then robotic surgery, still has the potential to negatively affect sexual function (SF), important part related to integral component of health-related quality of life.

Population-based studies estimate that 78% to 87% of patients have erectile dysfunction following RP, an outcome associated with patient distress, dissatisfaction, and decision regret.

Radical cystectomy (RC) is the gold standard surgery for muscle invasive bladder (BC) and non-muscle invasive BC who fail to BCG. The excision of the bladder and the prostate is mandatory with a high risk of impairment of the sexual dysfunction. Since these patients are globally older and with more comorbidities, the incidence of impairment of SF is highly increased. But today is mandatory with oncologically safe technique to try to attempts a correct SF and "normal" voiding after RC. Patient age, previous sexual function and the "surgeon" are the main preoperative predictive factors.

Pelvic radical surgery in men does not mean sexual dysfunction. If oncologically safe, nerve sparing techniques have to be used in order to preserve SF as much as possible. It is not acceptable nowadays, not to attempt the perform a correct dissection of neurovascular bundles. It has to be explained also to the patients the possible alteration of orgasm.

Of all patient-reported outcomes for RP, sexual function outcomes are among the most reported and the most detrimental to quality of life. There is a wide variation in sexual function recovery associated with both patient- and surgeon-level characteristics. In the quest for a cure, we should not neglect patient-reported outcomes (e.g., sexual function) that patients care very deeply about and that directly impact quality of life. There are very few papers related to SF after RC. Curiously, from the technical point of view, it is even easier to preserve in bladder cancer.

Another important point is the continence. It has been clearly shown that neurovascular preservation improves the continence after RP. Why is it also important in RC? Because in those patients that a neobladder reconstruction is decided, there is an improvement of continence rate if a nerve sparing technique is used. The stimulation of the NV bundles increases the urethral pressure and a correct sensitivity of the urethra improves continence. Preservation of a long urethral stump improves continence which is clearly related to quality of life in patients with neobladder. In our experience, the daily continence rate is 95 % in those with correct dissection and preservation of the nerves.

It is important to know the anatomy and to be able to recognize the planes in order to refine the surgical details to preserve the NV bundles. The tips and tricks in the fine dissection and reconstruction of the pelvis structures leads to excellent functional results.

SYMPOSIUM 6: CLINICAL SCIENCE SESSION - INFERTILITY/MALE REPRODUCTION

RT06-1 | Impacts of pharmaceutical exposures on male reproductive health (ECO-Talk)

Rod T. Mitchell
[1]*Professor of Developmental Endocrinology, MRC Centre for Reproductive Health. University of Edinburgh, UK;* [2]*Consultant Paediatric Endocrinologist, Department of Paediatric Endocrinology, Royal Hospital for Children and Young People, Edinburgh, UK*

Future fertility in males is dependent on the normal development of the germ cells within the testis, which is in turn dependent on hormones and paracrine factors from the somatic populations of the testis. During fetal and prepubertal life, the testis is exposed to a variety of environmental and pharmaceutical agents, which may have potential to affect germ cell survival or differentiation [1]. This presentation will describe the testicular effects of exposure to environmental agents (e.g. phthalates, synthetic oestrogens) to which humans are frequently exposed, in addition to pharmaceuticals used during pregnancy (analgesics) or prepuberty (chemotherapeutics). The lecture will also emphasise the importance of considering the animal models used and the reliability of in-vitro models that involve rodent and human cells or tissues. The use of a human fetal or prepubertal testis xenograft system to determine human-relevance of findings from animal studies will also be discussed. Using a variety of approaches, we have shown that exposure of human fetal testis to analgesics can affect germ cell number and germ cell differentiation, whilst exposure of the prepubertal testis to several chemotherapeutics commonly used in childhood cancer can differentially affect spermatogonial populations [2,3]. This lecture will also discuss how we might be able to use this new understanding of

pharmaceutical effects on the testis to develop strategies to preserve fertility, particularly for those facing chemotherapy during childhood.

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RT06-2 | Ageing effects on the male germline

Sandra Laurentino
Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

There is a trend for increasing parental age, most obvious is industrialised societies. This has a negative impact on fertility and leads to an increase in the need for assisted reproductive techniques. The effects of reproductive ageing has so far centred mostly on the impact on the female, since males are capable of producing sperm throughout their entire adult lives. However, male reproductive ageing is associated with a wide range of changes, which should not be overlooked. Cohort studies identified effects such as endocrine alterations and negative effects on sperm parameters as common in ageing men. However, studies performed on healthy men show that these classical andrological effects are likely associated with age-associated morbidities and not with the process of ageing itself. Advanced paternal age is associated with lower fertilisation rates, a longer time to pregnancy, and an increased risk of miscarriage. Moreover, children of older fathers are at an increased risk for paternal age effect disorders, which include monogenic (e.g. Apert syndrome) and complex disorders (e.g. autism spectrum disorders). The male germline accumulates de novo mutations with age, directly due to the continuous division of spermatogonia, that maintain sperm production throughout life, and indirectly through the effects on an altered testicular somatic environment. This increase in the mutation rate cannot, however, completely explain the increase of such disorders in the offspring of older fathers. Other changes in the germline DNA were identified as likely reasons behind paternal age effect disorders. These include an increase in genomic instability and DNA damage in the male germline and genome-wide changes in the DNA methylation patterns in ageing sperm. In contrast to endocrine and sperm parameters changes, DNA alterations occur regardless of health status, contributing to reduced male fertility and poorer reproductive outcomes in older men. Whether these age-related changes to male germ cells and to the testicular micro-environment can be prevented and if interventions can be designed to correct them is currently unclear and should be explored in the future.

RT06-3 | Male reproductive health and COVID-19

Giulia Rastrelli
Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

Since the beginning of the SARS-CoV-2 outbreak, the consequences on reproductive health has raised as an important concern.
Since the first wave, the infection rate among men was observed as similar to women. Conversely, the adverse outcomes, including hospitalization, admission to Intensive Care Unit, and death are more prevalent among men. Possible explanations to the unbalanced male-to female ratio have been studied. Among these, the effects of sex hormones have been advocated. Initially, testosterone has been hypothesized deleterious because of its considerable higher serum levels in men than in women. However, the studies conducted in COVID-19 population has shown the opposite. The worst clinical outcomes are observed in the aging men and, among the older age bands, the male-to-female ratio is particularly noticeable. It is known that aging is associated with lower -rather than higher- testosterone levels. Indeed, mounting evidence shows that lower rather than higher testosterone levels are associated with adverse COVID-19 outcomes. Several explanations may be hypothesized for this observation, including that low testosterone represents an epiphenomenon of other conditions known risk factors for a worse clinical course of COVID-19. However, a direct role of this hormone in the defense from the disease could be postulated. In fact, testosterone is a sex hormone involved in the immune response regulation. In particular, testosterone is involved in the down-regulation of inflammation and immune response. Indeed, low testosterone levels are associated with excessive inflammatory and immune response leading to autoimmune disease either in animal models and in men. It is now well recognized that the most aggressive forms of COVID-19 are due to an exaggerate immune response to the virus. Therefore, a worse disease course in men with low testosterone could be explained with a deregulation of immune system.
The close association between male sex hormones and COVD-19 has led researchers to investigate further the possible consequences of COVID-19 on male health. Testosterone is responsible for both sexual function and fertility and both these aspects have been investigated in men with SARS-CoV-2 infection. Concerning fertility, conflicting evidence has been published on the presence of the virus in the semen. However, if present, the effects of SARS-CoV-2 on spermatozoa is unclear. In fact, worsening in semen quality has been documented but this is mild and often semen parameters remain within the limits of normality. Worse semen quality has been observed in subjects with worse COVID-19 course reaching higher body temperature and requiring antibiotic treatment. Therefore, it is challenging to understand whether the impairment in semen quality is due to SARS-CoV-2, the

acute febrile illness, the medications, or the change of intratesticular hormone pattern.

Sexual function after COVID-19 has been also investigated. Erectile dysfunction has been found as a long-term consequence of COVID-19 independent of other possible risk factors. Interestingly, in line with evidence showing that erectile dysfunction is a hallmark of frailty and predicts increased mortality, men with erectile dysfunction are at higher risk of developing erectile dysfunction.

Currently, studies providing a unifying overview of these different aspects of male reproductive health and COVID-19 are lacking. Low testosterone could explain most of the reproductive dysfunctions occurring in men with COVID-19. However, further studies are needed to prove this hypothesis.

SYMPOSIUM 7: ERECTILE DYSFUNCTION AND SHORT ORAL PRESENTATIONS

RT07-1 | Erectile dysfunction in congenital hypogonadism: organic and non-organic risk factors

George Kanakis
Athens Naval & VA Hospital, Athens, Greece

Erectile dysfunction (ED) is defined as the persistent or recurrent inability to achieve and maintain a penile erection adequate for satisfactory sexual activity. The aetiology of ED may be organic, psychogenic, or usually of mixed origin. Hypogonadism (HG) on the other hand, describes the failure of the testes to fulfil their secretory role concerning testosterone (T) production, spermatogenesis, or both. T has been demonstrated to affect the function of the erectile apparatus on multiple levels, while epidemiologic data show a significant overlap between HG and ED. Congenital HG is present from birth and the disorder may residue either at the testes (primary, PHG) or the level of the Hypothalamus and Pituitary gland (secondary, SHG). A third category of disorders usually included in this classification is the resistance of the peripheral tissues to the action of androgens (Androgen Insensitivity).

The most common form of PHG is Klinefelter Syndrome (KS), which is characterized by T deficiency, typically evident after the second decade of life. The incidence of ED in KS is 19%, which is higher than in age-matched controls; however, it has been correlated to advanced age and not to lower T levels. Moreover, T replacement therapy (TRT), did not improve ED in hypogonadal KS patients, despite alleviating decreased sexual desire, implying that ED in KS is mostly a result of factors other than HG, such as compromised arterial diameter and communication disorders. Cryptorchidism is another congenital disorder, which can lead to PHG and sexual dysfunction even after orchiopexy at an early age.

SHG is rare and comprises forms of isolated gonadotropin deficiency (IHH) or generalized defects of the pituitary gland. Patients typically present with pubertal failure, while ED is quite prevalent among such patients (53%). TRT does not appear to fully restore ED, despite improving depression and anxiety. The results of TRT in men with panhypopituitarism are even less impressive, implying a role for other factors such as GH and ACTH in male sexuality. The presence of a microphallus is another factor that may aggravate sexual dysfunction in these patients.

Insensitivity of the Androgen Receptor (AR) may vary, and the corresponding phenotypes represent a spectrum from complete feminization to minimal defects of virilization. Older studies assessing the sexual activity of patients with partial androgen insensitivity, who were reared as males, reported a major impairment of all parameters of sexual activity, which is worsened by compromised genital anatomy. A less pronounced effect on ED may arise from polymorphisms of the CAG repeat number of the AR. The larger the number of these repeats is, the less active the AR and the higher the risk of developing ED. Moreover, the less efficient TRT is in improving sexual function.

A heterogeneous group of congenital diseases that may be accompanied by HG and ED are iron overload disorders, such as hereditary hemochromatosis andβ-thalassemia. The cause of ED, in this case, is multifactorial, as iron deposits in the testicular and pituitary tissues evoke a mixed type of PHG and SHG, while iron also harms the penile endothelium either directly or by the ensuing hyperglycaemia due to pancreatic impairment. Similarly, sickle cell anaemia may result in a mixed form of PHG and SHG due to repeated vaso-occlusion of the hypothalamic-pituitary or testicular vessels secondary to sickling, while recurrent episodes of priapism compromise the integrity of the corpora cavernosa.

RT07-2 | A matter of time! - How can a two-step intracavernosal injection procedure improve the possibility to diagnosing psychological erectile dysfunction?

Daniele Santi
Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

Background: The recognition of the erectile dysfunction pathogenesis is essential to identify the appropriate erectile dysfunction management. As vascular erectile dysfunction could be a manifestation of a systemic arterial damage, the watershed in the erectile dysfunction diagnostic framework is the discrimination between psychological erectile dysfunction and vascular erectile dysfunction. However, reliable tools to directly diagnose psychological erectile dysfunction are currently lacking.

Objective: To identify which parameters could predict psychological erectile dysfunction. Moreover, we suggest a new intracavernosal injection procedure to optimize the erectile dysfunction diagnostic workup.

Materials and methods: A retrospective, real-world analysis was carried out including all men who underwent intracavernosal injection procedure at the Modena Andrology Unit from 2018 to 2021. A first intracavernosal injection procedure with 5 μg of prostaglandin E-1 (PGE-1) was performed. In the absence of a full drug-induced erection

(immediate or delayed), an echo-color Doppler penile evaluation after administration of PGE-1 10 μg was conducted, measuring intracavernosal blood flows, to document a possible vascular etiology. Hormonal evaluations were performed.

Results: Out of 179 enrolled patients, 70.4% showed psychological erectile dysfunction, 21.7% vascular erectile dysfunction, and 7.8% hormonal genesis. Multinomial logistic regression analysis identified absence of cardiovascular disease ($p = 0.017$), presence of spontaneous morning erections ($p = 0.018$), and normal penile erections with masturbation ($p = 0.035$) as predictors of psychological erectile dysfunction. Clinically, normal intracavernosal injection test response was detected in 86 patients and abnormal response in 93 subjects. Among the latter, 54 patients experienced a delayed response. The combination of intracavernosal injection test with late penile erections evaluation was able to diagnose psychological erectile dysfunction (sensitivity 97%, specificity 100%), avoiding unnecessary retesting.

Discussion: We propose a two-step intracavernosal injection procedure that allows to recognize psychological erectile dysfunction with a high sensitivity/specificity, saving costs and time, and limiting adverse events. Moreover, the presence of spontaneous morning erections and valid penile erections after masturbation could guide the diagnostic workup, indirectly identifying those patients deserving of a deeper evaluation of vascular health.

SYMPOSIUM 8: SPERM FUNCTION AND DEREGULTATION

RT08-1 | Clonal mosaicism in sperm cells (ECO-Talk)

Martin Breuss
Department of Pediatrics, Section of Genetics and Metabolism, University of Colorado Anschutz Medical Campus, USA

Genomic mosaicism is a naturally occurring phenomenon where some but not all cells within a tissue harbor a unique genetic variant. These mutations accumulate in every cell and reflect its developmental lineage, proliferation, and aging. Two main types of mosaicism can be distinguished: 'clonal' mosaicism which is shared among cells and arose in a common progenitor; and 'private' mosaicism which is present in only one cell and arose postmitotically. If mosaic mutations are present in the egg or sperm that form a viable embryo, they will present as germline mutations in this next generation. Thus, 'gonadal' mosaicism has the potential to affect the collective genetic variation in the human population. Importantly, they occur in offspring as de novo mutations (DNMs)—variants that are detected in a child but not the parents; these represent an important underlying cause of congenital disorders, such as autism, congenital heart disease, or epilepsy.

Despite their importance in human variation and disease, the landscape and overall features of clonal sperm mosaic mutations remained enigmatic. We identified three complementary questions to advance our knowledge of this phenomenon: 1) what is the rate of detectable clonal sperm mosaicism for DNMs across the genome? 2) How many clonal sperm mosaic mutations does a male typically carry and how do they change with time? 3) Are mutations detected in sperm in situ transmitted to offspring at expected rates? We approached all three of these through a combination of deep whole-genome sequencing (>200× sequence coverage), computational mosaicism analysis, and ultra-deep variant genotyping (>1000× sequence coverage) of primary human samples.

We first assessed the mosaicism of almost 1000 DNMs detected in 14 children in eight paternal sperm and blood samples1. Across these, approximately 2.5% presented as mosaic in the paternal sperm; this increased to 5% when only considering variants that were confirmed to be transmitted through the paternal germ line. Critically, direct assessment of sperm mosaicism allows for the stratification of familial recurrence risk into those variants with no and those with a measurable risk; this compares favorably to the common practice of using a flat population-based risk in genetic counseling.

For the determination of inter-subject, intra-subject, and age-related variation of sperm mosaicism, we assessed sperm and blood of 12 young-age (18–22 years) and 5 advanced-age (48–52 years) individuals2. We found that every male harbors, on average, up to dozens of sperm mosaic variants, which are remarkably stable over the course of a year or even decades in their number and abundance. Based on our unbiased analysis of sperm mosaicism, we further predicted that 1 in 15 males carries a likely pathogenic variant as a detectable mosaic in their sperm, and their transmission prevention would result in improved health outcomes.

Finally, we assessed the transmission of detectable clonal sperm mosaic variants—which were measured in an unbiased fashion within male sperm—to preimplantation blastocysts from three couples that underwent in vitro fertilization3. The tested variants transmitted at slightly lower but overall predictable rates. However, our results also suggested a non-uniform accumulation of mosaic mutations across sperm lineages.

Thus, clonal sperm mosaicism is present in every male, stable over the course of months to decades, and transmits to offspring based on its measured abundance in sperm cells. Together, our results provide the basis for a framework of sperm mosaicism assessment to prevent the transmission of predictable DNMs.

1. Breuss MW, Antaki D, George RD, et al. Autism risk in offspring can be assessed through quantification of male sperm mosaicism. Nat Med 2020;26:143–50.
2. Yang X, Breuss MW, Xu X, et al. Developmental and temporal characteristics of clonal sperm mosaicism. Cell 2021;184:4772–83 e15.
3. Breuss MW, Yang X, Stanley V, et al. Sperm mosaicism predicts transmission of de novo mutations to human blastocysts. bioRxiv 2022.

RT08-2 | Sperm motility – Travels without bad conscience

Timo Strünker
Germany

No abstract text

RT08-3 | Sperm proteomics: A tool to unravel male reproductive function

Meritxell Jodar, J Castillo, R Oliva
Hospital Clinic de Barcelona, Barcelona, Spain

Proteomic approaches have emerged as an invaluable tool to understand the sperm physiology and pathogenic mechanisms associated with male infertility. Currently, the application of strategies based on liquid chromatography (LC) coupled to tandem mass spectrometry (MS-MS) in the study of the whole human sperm proteome and subcellular proteome composition has resulted in high-confidence identification of almost seven thousands of non-redundant proteins. Protamines are the most abundant proteins in sperm cell but, due to their particular physical–chemical properties, cannot be identified by the standardized MS strategies. For that reason, protamines have been classically assessed by acid–urea polyacrylamide gel electrophoresis (AU-PAGE)-based methods. However, the recent incorporation of a top-down MS approach with optimized proteomic workflows and data analysis pipelines has been a milestone in the advanced study of protamines, resulting in the determination of the normal protamine profile of the male gamete which contains intact, truncated and phosphorylated proteoforms.

In addition to increasing the knowledge on sperm structure and cargo, the study of the protein composition of the male gamete has contributed to unravel past and future events related to sperm development, oocyte fertilization and preimplantation embryo development. The identification of differences on the abundance of specific sperm proteins, by comparative proteomics analyses, in subtypes of infertile patients differing on seminal parameters have revealed proteins and pathways relevant for the generation of a functional spermatozoa. The most sperm phenotype studied has been asthenozoospermia. The analysis of the protein profile of these patients, compared to controls, has shown that the most pathological mechanisms affecting sperm motility are based on disturbances in energy production and apoptosis. In addition, the study of sperm cells from infertile patients classified according to the assisted reproductive technologies (ART) outcome, including fertilization rate, embryo quality, and pregnancy rate, have revealed that the male gamete proteins may be crucial for preimplantation embryonic development. This participation of the male gamete molecular composition in post-fertilization events is supported by the integrative analysis of the human sperm, oocyte, and blastocyst proteomic and transcriptomic datasets, which has revealed a group of embryo proteins with a potential exclusive paternal origin. Additionally, it has been shown that the human sperm also contains proteins with putative epigenetic roles which could modulate gene expression in the early embryo and, finally, impact in the offspring phenotype. Since the spermatozoa are not able to produce proteins de-novo due to the blockage of the transcriptional and translational machineries, it has been proposed that protein importation from secretions of accessory sex glands and possibly other peripheral tissues during sperm maturation could be an efficient strategy to maintain the sperm proteomic profile in optimal conditions for its function at fertilization and beyond. In particular, the male gamete is able to incorporate proteins required for sperm functionality from the small extracellular vesicles (sEV) contained in the seminal plasma. These imported proteins might provide environmental epigenetic information without the need to overcome the hemato-testicular barrier.

Comparative sperm proteomic studies involving various sperm functional states have produced a large number of candidates to be fertility biomarkers with clinical utility. However, the application of proteomics in the routine clinical practice has several limitations, such as its prohibitive associated cost, due to the requirement of skilled professionals and very expensive equipment. To routinely test specific biomarkers, it would be cheaper and more feasible to develop tests based on protein microarrays, mass spectrometry selective reaction monitoring (SRM), or ELISA multiplexed. However, only small subsets of these biomarkers candidates are concordant within independent studies, hindering the translation of these results into the sperm routine clinical assessment. This limitation has been addressed by developing a novel strategy of sperm quantitative proteomic data analysis based on the establishment of stable protein-pairs, which might open up a window to its application in the personalized diagnostic of male infertility. Moreover, this new strategy is based on the analysis of proteomic data at peptide level, providing further relevant information about the presence of post-translation modifications or missense genetic variants that could be associated with male infertility.

Sperm proteomics emerge as relevant tool to provide candidates that could improve the diagnosis, prognosis and treatment of male infertility, currently limited to the analysis of semen parameters and antioxidant treatments.

SYMPOSIUM 9: NYRA SESSION

RT09-1 | The seminal plasma microbiome of men with testicular germ cell tumours

Ailsa Maria Main[1,2*], Nina Mørup[1,2*], Niels Jørgensen[1,2], Gedske Daugaard[3,4], Anders Juul[1,2,4], Kristian Almstrup[1,2,5]

[1]*The Department of Growth and Reproduction, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark;*

[2]*the International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Copenhagen, Denmark;*

[3]*The Department of Oncology, Copenhagen University Hospital, Copenhagen, Denmark;*

[4]*The Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark;*

[5]*Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Background: The human microbiome consists of the microbiota and its included elements like viruses and fungi and is estimated to

be involved in the pathogenesis of ~20% of all malignancies. Testicular germ cell tumours (TGCTs) are the most common type of malignancy in young men and arise from the precursor Germ Cell Neoplasia in Situ (GCNIS). The microbiome in semen and testicular tissue has not been thoroughly investigated in patients with TGCTs.

Objectives: To investigate the differences in the seminal plasma microbiome between men with testicular cancer or GCNIS compared with healthy controls.

Materials and methods: The study population consisted of patients with GCNIS only (n=5), TGCT (n=18), and controls (n=25) with sperm counts ranging from1.7 to 166 mio./mL. Semen samples were collected, seminal plasma isolated, and RNA purified using Trizol. Sequencing libraries were prepared using the CATS small RNA library kit from Diagenode and sequenced on an Illumina HiSeq. Using the Oasis bioinformatic pipeline, reads that did not map to human small RNAs were aligned against 2784 bacterial/archaeal and 4336 viral genomes using Kraken.

Results: We identified reads from 2172 microbiome species and the most abundant species were Alteromonas mediterranea, Falconid herpesvirus 1, and Stigmatella aurantiaca. Six species (Acaryochloris marina, Halovirus HGTV-1, Thermaerobacter marianensis, Thioalkalivibrio sp. K90mix, Burkholderia sp. YI23, and Desulfurivibrio alkaliphilus) were found at significantly (q-values below 0.05) higher levels in the seminal plasma of TGCT and GCNIS patients compared with controls. In contrast, Streptomyces phage VWB, was found at significantly lower levels in patients with TGCT and GCNIS combined compared to controls.

Discussion: The identified microbiome species in seminal plasma could be involved in the pathogenesis of TGCTs. Our study builds on small RNA sequencing, which allowed us to identify more viruses and phages than conventional 16S ribosomal sequencing but also makes it difficult to directly compare our results with existing pilot investigations.

Conclusion: To our knowledge, our study is the first to report identification of microbiome species in seminal plasma of men with TGCT and GCNIS. Confirmative studies are needed to unravel the potential involvement of seminal microbiome species in the pathogenesis of TGCTs.

RT09-2 | Loss of XIST expression and the additional X-chromosome in Sertoli cells supporting focal spermatogenesis in men with Klinefelter syndrome

Sofia B. Winge, Niels E. Skakkebaek, Lise Aksglaede, Ewa Rajpert-De Meyts, Anders Juul, Kristian Almstrup

Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Denmark

No abstract text

SYMPOSIUM 10: SPERMATOGONIA AND ITS NICHE

RT10-1 | RNA sequencing of the human testis - from prenatal establishment to the last year in life (ECO-Talk)

Jingtao Guo

State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences (CAS), China; Beijing Institute for Stem Cell and Regenerative Medicine (BISCRM), China;University of Chinese Academy of Sciences (UCAS), China

As the germline stem cells of the adult testis, spermatogonial stem cells (SSCs) must properly balance self-renewal and differentiation to maintain lifelong spermatogenesis and fertility. Adult SSCs are the culmination of a complex developmental process that begins in the embryo and continues through distinct fetal, juvenile, pubertal, and adult stages. Testis is the only organ in males where spermatogenesis takes place. It is composed of various types of germ cells as well as somatic cells which provide chemical and physical support for the germ cells. There are several unique features of the human testis and the male germline. First, although SSC is the foundation of spermatogenesis, our understanding of it largely derives from studies using rodent models. Due to the limitation of research tools and techniques, our understanding of human SSCs was still quite limited. Second, the testis structure is complex but orchestrated. The basic compartment for spermatogenesis is the seminiferous tubules in the adult testis, but such structure is not formed not until puberty. Prior work mainly focused on the physiological changes during tubule formation as puberty initiates, but the detailed molecular mechanism was lacking. Moreover, unlike women whose menstruation and ovulation stop at the age of around 50, men can maintain the ability to produce sperm for the vast majority of their lifetime. However, our understanding of how aging impact the human testis and germline was still inadequate.

Our past few years, our lab took advantage of a combination of the cutting edge molecular approaches, including single cell genomics, to address those key questions, and made several important discoveries. We identified five distinct cellular states for human SSC development in adult men, including the identification of a new SSC state called "State 0". Our follow-up studies further examined the origin of State 0 SSCs in the pre- and post-natal testis, as well as delineated how they are impacted by natural aging and possibly other concurrent factors. Alongside germline development, we also studied the specification and development of the testicular somatic cells, and their interaction with the germline. We identified a common progenitor population for all testicular somatic cells in the 6 to 7-week human embryo, and delineated accompanying molecular events as they differentiate into different lineages (such as Leygid and Sertoli) in in the pre- and post-natal testis. Overall, our work uncovers multiple key molecular events during the formation, development and aging process of human testis, which serves as a foundational dataset for the community as well as helps lay the foundation for further understanding and study of human testis.

RT10-2 | Germ cells and non-malignant diseases – impacts of treatments or diseases

Nina Neuhaus
Centre of Reproductive Medicine and Andrology, Münster, Germany

Spermatogonia are present within testicular tissues from birth until old age and give rise to more differentiated germ cells including spermatocytes and sperm from the time of puberty onwards. Spermatogonia are therefore considered the foundation of male fertility. However, 15 % of couples are considered infertile with a male factor in half the cases. A causal diagnosis can be provided solely in 28% of these men, including previous gonadotoxic treatments (radio- or chemotherapy for malignant and non-malignant diseases) or genetic disease (numerical or structural chromosomal aberration). As about 70% of men do not receive a causal diagnosis, there is an urgent need to unravel the aetiology of male infertility and cellular events affecting the stem cell compartment in the testis. Taking advantage of single cell RNA sequencing (scRNA-Seq), transcriptional profiles of human testicular cells have been generated from different types of male adult infertility samples including men with cryptozoospermia (Crypto). Moreover, to analyse the impact of disease and treatment on spermatogonia in immature boys, standardized immunohistochemical analyses have been implemented for evaluation of testicular tissues including those from boys with sickle cell disease (SCD).

Specifically, cryopreservation of testicular tissues is offered to boys with SCD before hematopoietic stem cell transplantation by specialized centers worldwide. We performed morphological analyses to assess whether hydroxyurea (HU) treatment or disease-related factors affect the quantity of spermatogonia in SCD patients. Combining clinical data of 29 boys (age range, 2.8–15.1 years) with Z-scores of spermatogonial numbers revealed that most spermatogonial numbers (n=17) were below the reference values of healthy boys. There was a correlation between the number of spermatogonia and the age at HU initiation (p = 0.029, r=0.476). Available data suggests that factors intrinsic to SCD impact spermatogonial numbers and HU exposure in early life may lead to further depletion. While the molecular profile of spermatogonia in SCD remains to be assessed, scRNA-Seq analyses of testicular tissues from adult Crypo men (n=3) and controls (n=3) has allowed to shed light on the alterations of spermatogonia in these patients. Specifically, cryptozoospermia is a severe form of male infertility in which individual sperm can only be detected in the semen sample following centrifugation. Intriguingly, within respective testicular tissues impaired germ cell differentiation was obvious in scRNA-Seq data with a reduction of germ cells from the spermatocyte state onwards. Unexpectedly, numbers of spermatogonia were comparable in Crypto and control samples. Nonetheless, we found major alterations in the Crypto spermatogonial compartment including increased numbers of the most undifferentiated spermatogonia (PIWIL4+) but reduced numbers of Adark reserve spermatogonia, characterized by tightly compacted chromatin. These findings suggest an impact of the disease on the molecular profile of spermatogonia and highlight the value of high content analyses to unravel the impact of treatments and diseases on male germ cells.

RT10-3 | Human primordial germ cell-like cell specification and progression in vitro

Joao Pedro Alves-Lopes
NORDFERTIL Research Lab Stockholm, Childhood Cancer Research Unit, J9:30, Department of Women's and Children's Health, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

Primordial germ cells (PGCs) are the precursors of sperm and eggs, which transfer genetic and epigenetic information to following generations. Human PGC (hPGC) specification happens approximately between weeks 2 and 3 after conception. After specification, hPGCs cluster in the extra-embryonic yolk sac wall in the proximity of the allantois, before their migration starts through the hindgut and dorsal mesentery into the primitive gonads. During migration and gonadal colonization, hPGCs initiate epigenetic reprogramming, including widespread DNA demethylation and chromatin reorganization. In the course of week 7–10, gonadal hPGCs start to differentiate into oogonia or pro-spermatogonia in the developing ovary or testis, respectively.

Nevertheless, the exact mechanisms that control the origin and early development of hPGCs and their epigenetic reprogramming are not yet well understood. This is in part due to technical challenges and to the lack of access to early human post-implantation embryos. To overcome this issue, several research groups developed protocols to specify hPGC-like cells (hPGCLCs) from human pluripotent stem cells (hPSCs) in order to study hPGC specification and progression in vitro. These methods paved the way to understand the mechanisms governing hPGC specification. Other studies focused on hPGCLC developmental progression by co-culturing these cells with mouse male and female embryonic gonadal cells. Although these studies provided the proof of concept for the in vitro progression of hPGCLCs, these protocols are not very efficient and require long culture periods. Several factors may contribute for the lower efficiency of the previously reported progression protocols: (1) the starting population of hPGCLCs might not be the most competent for in vitro progression, (2) the previous co-culture protocols are chimeric systems (human/mouse co-cultures), and (3) developmentally asynchronous.

The refinement of the protocols for hPGCLC specification and progression will have a great impact in the field by first increasing our knowledge on the mechanisms regulating hPGC specification and progression, and secondly, by the translation of this knowledge to future clinical applications. On the other hand, the application of such protocols to other animal species will contribute to design experimental pipelines for in vitro gametogenesis from specie-specific PSCs. This

will be of special relevance in reproductive assisted techniques for livestock and endangered species.

SYMPOSIUM 11: TESTICULAR CANCER

RT11-1 | Polygenic risk score in predicting testis cancer risk (ECO-Talk)

Katherine L Nathanson
USA

No abstract text

RT11-2 | Clinical management of testicle tumors

Andrea Isidori
Italy

No abstract text

RT11-3 | Germ cell cancers – many types one origin?

Leendert Looijenga
Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

Germ cell tumors (GCTs) comprise a heterogeneous group of neoplasms, some of them benign and some malignant. A total of seven types are currently distinguished based on various parameters, including the cell of origin, anatomical localization, histology and (epi)genetic constitution. Recognition of these subtypes is relevant for optimal diagnosis, resulting in the most effective treatment protocol including follow-up. Prevention of both under- as well as overtreatment is relevant in the context of long term quality of life. Recent investigations have resulted in identification of informative biomarkers both for tissue and liquid biopsy evaluation for malignant GCTs, in particular the seminomas/dygerminomas/germinomas and nonseminomas, with the exception of teratoma. These include the pluripotency related proteins OCT3/4 (POU5F1) and SOX2, the microRNA-371-3 family, and the methylated promotor of RASSF1A. An overview of the current status of the power and limitations of these biomarkers will be provided. Significant insights have most recently been generated regarding the underlying mechanism(s) of platin-based treatment resistance of GCTs, including a role of TP53 and a so far novel regional amplification of the short arm of chromosome 3. These results allow improvement of risk stratification of GCT patients and stimulate identification of novel treatment options for patients with refractory disease. An overview of the various relevant informative models will be provided, showing the impact of an integrated scientific approach to improve the clinical management of patients with GCTs.

SYMPOSIUM 12: EAA MEETS ESHRE

RT12-1 | When to introduce a new diagnostic or therapeutic tool in the andrology laboratory based on scientific evidence

Nicolás Garrido Puchault
Research Administration, Research/Innovation, IVIRMA Global, Valencia, Spain

Since its beginning, the field of Medically Assisted Reproduction (MAR) has been rapidly evolving thanks to the research on infertility causes, a better knowledge of both male and female reproductive physiology as well as the development of instruments, devices and technology.

So far, many infertile patients have been benefited from these developments, resulting in cases where a child is obtained where there was no hope just few years before, or the chances have been increased, leading to better cost/benefit ratio, less discomfort, risk and/or time to achieve it.

However, a remarkable proportion of patients/couples remain childless after MAR, or need several attempts to succeed, which clearly shows the need to incessantly invest in research and development to be transferred to the clinical practice and benefit them on any of the outcomes above mentioned.

Outstanding breakthroughs are very rare, and research mostly contributes to an incremental improvement, meaning that subtle ameliorations on reproductive outcomes are often derived from huge research efforts. Moreover, these frequently need a huge amount of patients to be confirmed.

We, as practitioners and scientists, are obliged to follow the principles of evidence-based medicine in our practice, and in particular, the scientific methods and good clinical practices in our research.

The development of innovative MAR treatments and technologies must be, as in other fields, conducted in a research setting with appropriate monitoring, safety precautions and preceding ethical approval.

Innovative MAR treatments can be, generally speaking, divided into two main groups: tests, and therapies. With the first, we try to predict, or at least get clinically useful inputs about the risk once we get information from a biomarker. With the second, we try to apply a determined methodology in a patient because it has been demonstrated that is better than others.

Currently, the pressure from patients and competitors to introduce new tests and therapies lead to a premature incorporation of these to the clinical routine, resulting in unnecessary economic burden for our patients, false hopes, and we cannot discard the possibility to involuntarily be harming instead of curing.

These are the cases of the otherwise called add-ons, defined as complementary tests, therapies or technologies adjacent to classic IVF treatments, with unclear or no evidence about the benefits thanks to their use.

How testing/validating diagnostic or therapeutic tools related with the andrology laboratory should be done?

First, it is key to define the outcomes evaluated. These must be aligned with the patients interests and be clinically relevant.

Second, diagnostic tests validation procedure, frequently require non-selection studies, where a biomarker, that has been on basic studies demonstrated to be involved on the main outcome evaluated, and afterwards has been shown to be different between the different outcomes' populations, should be prospectively and blindly measured, to finally define which are the predictive values associated, and ultimately demonstrate its usefulness by means of a clinical trial comparing decisions made on the basis of these biomarker results, against what the routine practice does. The need to prove a significant clinical benefit for the patients where it is applied, is mandatory.

This last part is very similar to the way a therapeutic tool should be validated.

A randomized clinical trial, where all potential confounders are controlled, and only the intervention is different between the cohorts of patients compared, is the maximum level of evidence in order to discern the clinical contribution to a meaningful outcome for the patient.

Although these are general examples, particular designs might be necessary for specific outcomes, mainly in a field as complex as MAR

So far, several sperm phenotypical and molecular markers of fertility have been described as being related with reproductive success: DNA integrity, membrane charge, apoptotic traits, hyaluronic acid receptors, platelet activating factor, and a long etc., and some can also be used to select sperm individually for using them in assisted reproduction techniques., as ultrahigh magnification, magnetic activated cell sorting, physiological ICSI, hypoosmotic swelling tests, electrophoresis, microfluidic devices, birefringence, etc.

Proper interpretation of scientific data is also a cornerstone for the improvement, including the proper conceptualization of p-values, effect's size, research design and methodology, etc…

In this lecture, we will try to cover these concepts focusing on male factor infertility and the andrology laboratory work.

RT12-2 | Male Reproductive Health Awareness initiative by ESHRE

Christopher Barratt
UK

No abstract text

SYMPOSIUM 13: MEET THE EXPERT - WHO MANUAL AND EAA GUIDELINES

RT13-1 | Debate: How should the 2021 edition of the WHO manual and the ISO 23162:2021 have relevant impact on andrological clinical care?

Lars Björndahl, Christopher Barratt
Sweden, UK

The session will give a short survey of the 6th edition of the WHO manual and the ISO standard for basic semen examination, discuss what the difference between reference limits and decision limit mean, and how scientific publications must change to ascertain that results from semen examination are reliable. All these aspects are presented in the light of improving the basis for proper andrological clinical care.

RT13-2 | Update on EAA guidelines

Giovanni Corona
Endocrinology Unit, Medical Department, Azienda Usl, Maggiore-Bellaria Hospital, Bologna, Italy

The European Academy of Andrology (EAA) guideline project started in 2015 under Prof Csilla Kraus Presidency. During the last 7 years, five guidelines have been published on Andrology including 32 authors from 11 different counties. The topics were identified within the EAA guidelines committee, and then discussed, and approved by the EAA Executive Council (EC). All the published guidelines underwent a rigorous internal review process including the EC members, the Guidelines Committee members and the EAA Center directors. In all cases clinical evidence and the grade of recommendation were based on the GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) system. The first Guideline was published on 2018 and was related to one of the most important topic in the andrology field. "The Management of oligo-astheno-teratozoospermia". In the same year, EAA published the first available guideline on "The management of bone health in the andrological outpatient clinic". Although male osteoporosis is a well-recognized clinical entity, it remains an underdiagnosed and undermanaged condition. EAA provided, for the first time, the evidence-based criteria to support male bone health investigation and treatment with a particular emphasis on the role of testosterone and testosterone replacement therapy. In 2019, another frequent andrological condition "i.e gynecomastia" was investigated and the evidence based criteria for its management provided.

The concept of functional hypogonadism was introduced by Grossmann & Matsumoto in 2017. The latter is a potentially reversible form of hypogonadism, characterized by borderline low testosterone levels mainly secondary to age-related comorbidities and metabolic derangements. In 2020, EAA published a specific guideline on the "Investigation, treatment and monitoring of functional hypogonadism in males". The latter work received the endorsement of the European Society of Endocrinology. Similar support was obtained by the first Guidelines specifically related to the Klinefelter's Syndrome, which were published in 2021. Other different topics are in the pipeline including, the cryopreservation of semen, the management of male accessory genital glands infections, the interpretation of the testicular biopsy and the management of patients with hypogonadotropic hypogonadism.

In conclusion, EAA guideline project has allowed providing based-evidence clinical recommendations on the management of different andrological topics. In addition, some important issues never

systematically analyzed were discussed. Furthermore, new topics are in the pipeline or to be discussed in the near future.

SYMPOSIUM 14: SEXUAL MEDICINE

RT14-1 | Post SSRI sexual dysfunction (PSSD) and Post-Finasteride syndrome (PFS)

Yacov Reisman
Flare-Health, Amsterdam, The Netherlands

The continued controversy regarding possible adverse effects in finasteride and SSRI users has caught the attention of clinicians, patients, and potential users alike. PSSD and PFS are conditions indicated by a mosaic of adverse effects that develop during usage and linger even after the discontinuation of the medication.

PFS and PSSD are characterized by a collection of sexual, psychological, and physical adverse effects that persist in patients previously treated with 5α-reductase inhibitors for benign prostatic hyperplasia or androgenic alopecia or SSRI for depression or anxiety. Both conditions have a variable clinical presentation with debilitating physical and psychological consequences, and have profound impact on the quality of life and sexuality of the patient, partner, and their relationship.

The pathophysiological mechanism for these conditions is still unclear as well as guidelines for treatment.

As for today the long-term safety profile of these medications remains incomplete after nearly three decades of market use. The medical community does not officially recognize these disorders due to the ambiguous symptom presentation, unconfirmed pathophysiology, and inconclusive association from the past investigations.

As a medical provider treating a patient with an indication for medication usage, one should consider the personal history of pre-existing conditions such as sexual dysfunction, infertility, and mood disorders before the prescription.

More research is needed to determine the etiology and potential mechanism of PSSD and PFS, as well as to reach an accurate diagnosis and treatment.

RT14-2 | Safety and side effects of hormonal treatment in transgenders – and what we can learn from it in for populations

Martin Den Heijer
The Netherlands

The treatment of transgender people with sex hormones was originally copied from knowledge gained from treating people with hypogonadism. The increase in the number of transgender treatments and the need for intensive monitoring because of the totally new situation has led to a lot of research, which in turn provides new insights into the treatment of people with hypogonadism and DSD. This includes effects on bone, the role of estradiol in men, effects on erythropoiesis and whether or not it is necessary to monitor liver function tests, lipids, prolactin or PSA.

RT14-3 | Transgender genital surgery

Jaume Masià
Spain

No abstract text

SYMPOSIUM 15: PENILE SURGERY AND SEXUAL HEALTH ESAU COURSE

RT15-1 | Low-intensity shock wave treatment for erectile dysfunction

Tet Yap
UK

No abstract text

RT15-2 | Sexual health after radical prostatectomy (ECO-Talk)

David Ralph
United Kingdom

Sexual dysfunction after radical prostatectomy is inevitable and occurs in every patient. Erectile dysfunction usually occurs in over 60 % of patients rising to 100% when nerve sparing is not performed. This high risk is not often relayed to the patient who are often told that a response to a PDE5i does not represent ED.

Other distressing conditions include loss of ejaculation in all, an altered orgasmic sensation and sometimes climacturia. Patients are aware of penile shortening after this operation and recent studies have shown that this may be due to the development of Peyronie's disease in up to 50% of patients. Patients should be warned of these effects and given a choice of alternative therapies.

Prevention of erectile dysfunction by rehabilitation is now common place but good evidence for this is lacking. Different regimens are used, be it daily dosing, alternate days pde5i, vacuum therapy and intracavernosal agents. The major level 1 evidence for pharmaceutical trials did not show any benefit from daily dosing over on demand treatment. The big debate is should we rehabilitate patients or just actively treat their ED early.

Vacuum therapy has had a benefit in length preservation and it would be interesting to see if it also prevents Peyronie's disease.

This lecture will address these topics with data from the trials to ask- are we giving our patients real expectations from this therapy.

RT15-3 | Evaluation of plaque formation in Peyronie's disease: palpation vs ultrasound vs MRI

Thorsten Diemer
Germany

No abstract text

RT15-4 | Peyronie's surgery: choice of the appropriate graft

Carlo Bettocchi
Italy

No abstract text

RT15-5 | Implantation of penile prosthesis in patients with Peyronie's disease: When and to whom?

Arif Kalkanli, Ates Kadioglu
Section of Andrology, Department of Urology, Istanbul Faculty of Medicine, University of Istanbul, Turkey

A surgical treatment decision should be made in Peyronie's disease with a deformity ± erectile dysfunction that prevents sexual intercourse and has entered the stable phase for at least three to six months. The aim of surgical treatment is to correct the curvature and deformity, restore the penetration ability, penile length and penile girth of the patient to pre Peyronie's disease state. Penile prosthesis implantation (PPI) with 72–100% patient satisfaction is applied alone or in combination with other reconstructive techniques in men with Peyronie's disease and erectile dysfunction unresponsive to medical treatment options (5 phosphodiesterase inhibitors, intracavernosal injections). If the curvature is exceeding 45°–60° pre-operatively, the requirement of auxiliary intervention after PPI increases up to 75–100%. Additional reconstructive method is not required in patients with residual curvature lower than 30 degrees after PPI, as the implanted prosthesis has been shown to act as an internal expander and correct the curvature over time. However, in patients with residual curvature higher than 30 degrees after PPI, shortening techniques such as manual modeling, plication, Nesbit or lengthening techniques e.g. incision with or without grafting should be applied. Manual modeling is a method based on manually bending the penis in the opposite direction of the curvature and holding it for 60–90 seconds immediately after PPI. Although the level of evidence of the modeling method is low, a success rate of 35% to 100% has been reported. Recently a novel method described as home modeling has been reported to provide straightening of curvature in 94% of patients with a residual curvature of around 30 degrees after PPI. In this method, it is recommended to do 30-second manual modeling in 20-minute cycles three times a day for six months. The technique to be used in patients with Peyronie's disease and erectile dysfunction is stratified based on penile length, degree and direction of residual curvature. In patients with sufficient penile length, shortening techniques can be used with a success rate of up to 100% (plication, Nesbit, Yachia). However, in patients with shorter penile length, high degree of curvature, ventral or ventrolateral curvature and complex deformities such as hour-glass deformity, notching and indentation, lengthening techniques such as incision with or without grafting are more appropriate. In studies reporting incision of the plaque without grafting, patient satisfaction rate has been reported as 94% to 100%. In cases where the tunical defect is more than two cm after incision, grafting should be used to prevent cicatricial contracture and prosthesis herniation. Studies using many different graft materials have been reported up to date. All grafts are classified in three groups according to their nature as autologous grafts (rectus fascia, dura, fascia lata), allografts (bovine pericardium, TachoSil, Nu-Knitl, Evarrest, porcine small intestinal submucosa) and synthetic grafts (PTFE [politetrafloroetilen, Teflon], Dacron, Gore-tex, InteXen). After PPI with incision and grafting 80–100% correction of the curvature and patient satisfaction rate have been reported. PPI in patients with Peyronie's disease and erectile dysfunction results high level of functional and anatomical success and is associated with minimal risk of complications.

RT15-6 | How to avoid penile prosthesis infections?

Eduard Ruiz-Castañé
Andrology Department, Fundació Puigvert, Barcelona, Spain

"The biggest peril in prosthetic urology is the development of an infected implant"
Steven K. Wilson, 2008

This abstract aims to explain that to carry out a penile prosthesis implant consists in different relevant steps: a preoperative evaluation before the surgery, preparation of the patient and we have to take into account that some complication may arise during the surgery and in a special intra-operative situations.

The Pre-operative evaluation when is a new case: to consider background of the patient, surgical: type of prostatectomy, Herniorrhaphy, urethral surgery and radiotherapy. Review: implant type (model), length, TIP, reservoir (type and location). Infections: time from surgery and microbiology: aggressiveness of germ, penis length and systemic inflammatory response.

It is very important to do during the pre-operative evaluation: a physical examination, a diagnostic imaging, see the penile elasticity and location size and shape of the plates (Peyronie).

Before preparing for surgery, the patient should receive comprehensive information, informed consent and in addition, doubts should be clarified to the patient as well as the therapeutic pact.

Implant infections come from bacterial contamination at the time of surgery. The incidence is between 0.6% and 8.9% (realistically ranged 1–3%). Most of the bacterial contamination (75%) is from skin organisms like *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*. The other 25% are infected with more toxic bacteria (*E. coli*, Enterococcus,

Pseudomonas) from the genitourinary tract, intestinal tract or respiratory tract. Positive culture rate of 70% in clinically uninfected penile implant repairs (Staph. epidermidis) (Montague,1987; Thomalla,1987; Henry, 2004; Mulcahy, 2008).

Conditions proven to increase risk of infection in implants: spinal cord injury (diminished or absent sensation in the genital region), diabetes mellitus (glycosylated hemoglobin levels as a predictor of infection ??), revision surgery (incidence of 13.3% in implant reparative procedures) and patients on prednisone.

The preventive strategies to avoid penile implant infections: preoperative: prophylactic antibiotics (AUA guidelines)(based on general surgery and orthopaedic literature where prosthetic devices were used), starting 1 hour before the procedure, continuing up to 24 hours after the operation, beyond 24 hours the wound is sealed and further use of AB is not needed and recommended combination: vancomycin or a cephalosporin (against Staph. organism) with gentamicin (against gram-negative rod bacteria)(Wolf et al. Best practice policy statement on urologic surgery antimicrobial prophylaxis. J Urol 2008, 179: 1379–1390).

The procedure to follow is: to shave the patient immediately before the surgery and shave minimally, scrubbing the genitalia with soap and water for 10 minutes and painting with Betadine or painting the skin with an alcohol-based product containing an antiseptic (Advagard (3M) or Triseptin) for 90 seconds. Before the surgery, urinary tract infections should be treated, placing a urethral catheter during the procedure to wash the patient with a strong soap for 3 days before, skin lesions should be eliminated before the skin preparation, no-skin-touch technique with a protective adhesive skin drape.

PER-operative: frequent forceful irrigations to the wound during the surgery Containing antibiotics (bacitracin and gentamincin) or phys. sol. the three-piece inflatable implants are available with an antibiotic coating called InhibiZone, which is a combination of rifampin and minocycline. Hydrophilic outer layer that adsorbs AB solution to the implant surface (infection rate was reduced 58% (less than 1%), double gloving the surgeon.

POST-operative. Closed suction drain for 24 hours. The incidence of hematoma was decreased in a statistically significant fashion while the incidence of infection was unchanged in patients undergoing closed suction drainage for 24 hours. Partial inflation for 24 hours, scrotal compression dressing.

Conclusions: Preoperative evaluation is essential, use appropriate surgical instruments, consensus expectations and clear decision, perform actions based on acquired or provided experience.

RT15-7 | Managing the complications of the penile prosthesis implant

Ferdinando Fusco
Italy

No abstract text

RT15-8 | The impact of COVID-19 on male sexual health

Asif Muneer
UK

No abstract text

SYMPOSIUM 16: ONCO-ANDROLOGY: AN UPDATED ON DIAGNOSIS AND MANAGEMENT EFFECTS EAA/ESAU COURSE

RT16-1 | Update on the molecular diagnosis of testicular cancer

Ewa Rajpert-De Meyts
Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

Testicular cancer comprises several different tumours, which differ in their incidence and clinical course. Malignant testicular germ cell tumours (TGCT) that are derived from germ cell neoplasia in situ (GCNIS) and comprise two main types; seminoma and nonseminoma, are of primary clinical relevance, because these tumours constitute the vast majority of all cases and occur predominantly in young men, coinciding with their peak reproductive activity. Two aspects of diagnosis are essential for the patient's management and prognosis: 1) early detection of malignancy to prevent invasive disseminated cancer with all its consequences, and 2) precise recognition of the tumour type, including the components of mixed tumours, and the assessment of the spread of the disease to make decisions concerning the prognosis and management in individual patients.

Current diagnostic and treatment-monitoring procedures are based on physical examination, including imaging (ultrasound, CT-scans), biochemical serum markers (β-hCG, AFP, LDH) and histopathological analysis of either testis biopsies or orchiectomy tissue specimens, with aid of immunohistochemical markers (e.g. OCT3/4 (POU5F1), PLAP, SOX2, AP2-γ, SALL4). Early diagnosis, at the stage of GCNIS or incipient tumour, occurs usually only in patients from high-risk groups, because there are no reliable screening methods, except the invasive testis biopsy. Immunocytological semen analysis based on two markers is highly specific but not sensitive enough for broader screening.

Among recent advances which are currently studied and can be implemented soon in clinical practice, the most promising is a novel blood test, based on the detection of GCT-specific micro-RNA (miRNA). These are small non-coding RNAs, involved in the regulation of post-transcriptional gene expression. Among several embryonic type miRNAs secreted by TGCT, the miR-371a-3p cluster has been proven the most robust in clinical trials. This test is more sensitive than the classical serum markers, and detects both seminomas and nonseminomas, except teratomas. The miR-371a-3p test is particularly useful for diagnosis of inconclusive malignancies, which are negative for classical serum tumour markers. miRNA-based tests were shown to be useful for monitoring the patients and can detect relapses, but the best results

were obtained by combining the miR-371a-3p test with classical serum markers. However, only a subset of patients with GCNIS have measurable miR-371a-3p in blood, and the assay cannot be used to detect malignant cells in semen.

No genetic diagnostic test for TGCT exists, because TGCT is a polygenic disease, without specific predisposing gene mutations. A constellation of predisposing gene variants (identified using SNPs arrays) comprises around 70 informative markers, which in the future can be used to better identify the high-risk patients, but this approach will require more studies to get closer to the clinics. For excised tumour identification, gain of chromosome region 12p, including i(12p), is pathognomonic for GCT and can distinguish TGCTs from other atypical malignancies. In addition, a few somatic (not inherited) gene mutations, including KIT, NRAS/KRAS are frequently found in the invasive TGCT. Likewise, there are marked differences in global DNA methylation and other epigenetic markers among TGCT types. Another currently studied approach is the potential use of specific hypermethylated genes in circulating cell-free DNA (cfDNA), because the increase of cfDNA is unspecific of many cancers and other diseases. Genetic and epigenetic profiles have so far a limited clinical value but have the potential to improve detection of residual teratomas and the prediction of treatment-refractory disease, especially cisplatin-resistance observed in many patients.

RT16-2 | Short- and long-term effects of oncological treatments on testis function

Csilla Krausz
Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy

Testicular germ cell tumor (TGCT) and hematological malignanices (HM) are the most frequent neoplasia in men of reproductive age. Since the large majority of these patients did not experience fatherhood at the time of diagnosis, they are strongly advised to cryopreserve their spermatozoa prior therapy. About 7–9% of patients are not eligible for this preventive measure since they are azoospermic or have extremely few and immotile spermatozoa prior cytotoxic treatment. Onco-TESE could be an option for all these patients, however in the majority of cases only TGCT patients are benefiting from this procedure, since they are undergoing orchifunicolectomy.

Although the effect of chemo/radiotherapy on testis function has been addressed by a number of studies, they are all based on relatively small study populations. Clearly the most affected testicular function is reproduction, i.e. a temporary or permanent impairment of spermatogenesis. With the current therapies, in the large majority of patients, spermatogenesis is progressively restored and after 2 years over 95% of TGCT and 75% of HM patients have oligo -or normozoospermia. While routine semen parameters are similar to the pre-therapy values, some concerns are raised about the genomic integrity of spermatozoa. Studies defining sperm chromosome constitution shows an increased aneuploidy rate in some patients even after 2 years. Sperm DNA fragmentation analysis has been performed with different methods but in general, a return to the pre-therapy values have been reported after 2 years. We performed a longitudinal study involving 116 TGCT patients for up to 3 years. We found severe DNA damage (SDD) -expressed as the SDF value > 95th percentile of fertile men- in 24% of them after 2 years and 4.8% after 3 years. The highest proportion of patients with SDD was among those treated with PEB. Therefore, the current standard indication of a 2 years interval before natural pregnancy may not be adequate for all patients. SDF may serve as a biomarker of the genotoxic effect of chemotherapy and could help in decision making concerning the best timing for natural pregnancy.

While the damage on reproductive function seems to be a short-term effect, hypogonadism usually develops after many years. Leydig cells are relatively resistant to cytotoxic therapies and hypogonadism has been reported mainly in patients who presented either borderline testosterone level prior therapy and/or received unusual high doses of chemo/radiotherapy. Young cancer survivals (especially those treated with radiotherapy for lymphoma, leukemia, brain tumor or testis cancer) are at a significant risk to develop hypogonadism in adulthood. Long-term follow-up of selected group of oncological patients with the above characteristics should be performed in order to prevent symptoms and pathological conditions associated with low testosterone levels.

RT16-3 | Fertility preservation in adult non-azoospermic or azoospermic males with testicular cancer

Thorsten Diemer
Germany

No abstract text

RT16-4 | The importance of testicular microlithiasis for: the andrologist and the oncologist

Marij Dinkelman-Smit
Department of Urology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Classic testicular microlithiasis (TM) is defined as five or more echogenic foci smaller than 3 mm per field of view on scrotal ultrasound. TM is as common as 15% in men with an indication for scrotal ultrasound because of scrotal pain, swelling, palpable mass or fertility assessment. The prevalence of TM in the general, asymptomatic, population has been evaluated in 480 Danish, 1504 American and 2179 Turkish male army recruits without testicular symptoms and was 1.1%, 5.6%, and 2.4%, respectively. A meta-analysis including 14 studies in 35578 men concluded that men with TM have a >12 fold higher incidence of testicular cancer than men without TM. Patients with infertility and TM have an

18-fold higher odds ratio for testicular germ cell tumors (TGCT). The etiology of calcified material within the seminiferous tubules is unknown, although inflammation, defective phagocytosis by Sertoli cells, rapid cell turnover, or an overactive immune response have been suggested.

The European Association of Urology (EAU) Sexual and Reproductive Health guideline recommends that TM in addition to clinical features of the testicular dysgenesis syndrome (cryptorchidism, testicular volume <12ml), contralateral or previous TGCT, disorder of sex development (DSD) and infertility warrant consultation with a urologist to consider testicular biopsy to rule out the precursor lesion of TGCT, (Germ Cell Neoplasia in Situ) GCNIS. GCNIS is a derangement of embryonic germ cells that always proceeds type II invasive TGCT. GCNIS is diagnosed on open testicular biopsy specimens with immunohistochemically examination with PLAP and OCT4. So far, liquid biopsies in blood or seminal plasma have not been able to reliable diagnose GCNIS or TGCT and cannot replace testicular biopsy as a means for early detection. However, GCNIS has an unclear lead-time to invasive TGCT. Treatment of GCNIS involves radiotherapy or preventive radical orchiectomy that may both introduce infertility and increased risk of hypogonadism.

Preventive radical orchiectomy is a 100% effective and may lead to excellent outcome in patients with pre-existent hypogonadism, testicular atrophy and non-obstructive azoospermia. Provided that lifelong testosterone replacement therapy is initiated, positive body image is restored by implanting testicular prosthesis and sperm banking is at least attempted.

The diagnosis and clinical management of an asymptomatic phenomenon like testicular microlithiasis (TM), that is associated with TGCT development, has been subject of debate for over 20 years. The benefits of the diagnosis of TM and invasive screening for GCNIS, even in patients with contralateral TGCT, present against the background of the excellent prognosis of seminoma and non-seminoma testis with overall cure rate exceeding 95%. The EAU testicular cancer guideline recommends that the risks and benefits of biopsy of the contralateral testis in patients with TGCT should be discussed with the patient. Clinical practice in Europe is highly variable, ranging from routine contralateral biopsies to never biopsy in fear of complications of the contralateral testis. The EAU testicular cancer guideline states that the low incidence of GCNIS and metachronous contralateral testicular tumours (up to 9% and approximately 2.5%, respectively), the morbidity of GCNIS treatment, and the fact that most metachronous tumours are low stage at presentation, adds to the controversy and treatment variation.

Reducing current evidence to a straightforward flowchart on how to manage TM has proven to be difficult. Managing TM with a "one fits all" strategy may expose patients to under treatment by withholding preventive treatment of GCNIS in patients at risk for TGCT or contralateral TGCT. On the other hand, patients with TM may be at risk for overtreatment when testicular biopsies are advised low-threshold. Finally, anxiety and psychological distress may occur in some men with TM exposed to inconclusive information about cancer risk, follow up hospital visits or by delegating the responsibility for the early detection of TGCT to the patient though self-examination of the testis.

Current understanding will be discussed in this lecture, as well as recommendations for shared decision making in patients and clinical management from an andrological and oncological perspective.

SYMPOSIUM 17: MALE INFERTILITY: FROM THE AETIOLOGY TO THE PHARMACEUTICAL AND SURGICAL TREATMENT ESAU SESSION

RT17-1 | Risk of death: Azoospermic males vs. non-azoospermic infertile males

Aleksander Giwercman

Deptartment of Translational Medicine, Lund University and Reproductive Medicine Centre, Skane University Hospital, Malmö, Sweden

With 15–20% of all couples having infertility problems, up to 1/5th of all males, in a relatively young age, get I contact with the health care service. Thus, management of infertility, represents a unique option for offering preventive measures for those subfertile men who are at high risk for subsequent development of diseases threatening their healthy aging and shortening their life expectancy.

There is now a substantial amount of evidence demonstrating that men with fertility problems do represent a high-risk group for early development of a number of diseases associated with aging. This is true for metabolic and cardiovascular diseases, osteoporosis as well as some cancers including prostatic malignancy. For all those diseases it appears as the level severity of infertility is positively associated with the risk of morbidity as well as mortality, thus those having non-obstructive azoospermia being a highest risk.

The pathogenetic link between subfertility, late morbidity and premature mortality is not yet completely understood. Low testosterone levels, seen in approximately 30% of all subfertile males, seem also to be predictive for same panorama of diseases as subfertility per se. Whether hypogonadism, linked to increased level of chronic inflammation, represents one of the pathogenetic mechanisms or is only an early marker of morbidity and mortality remains to be elucidated.

Having in mind that most of the pathologies associated with male subfertility are potentially preventable, it seems urgent to define which other factors, apart from the severity of impairment of sperm production, add to the subsequent risk of morbidity and mortality. Subsequently, such knowledge should be translated into clinically applicable preventive measures. This might contribute to reducing the gap in life expectancy between males and females.

Infertility management should not only be an issue of helping the couples with becoming parents but also helping the children with securing health and longevity of their fathers.

RT17-2 | Do we need PDE5 inhibitors in male infertility clinics or assisted reproductive technology laboratories?

Nikolaos Sofikitis
Greece

Several studies have indicated a therapeutic role of PDE5 inhibitors (PDE5i) in the amelioration of oligoasthenospermia in infertile males. PDE5i have a beneficial effect on the secretory function of the Leydig and Sertoli cells. The latter increases in cellular secretions result in the development of a more optimal biochemical environment within the seminiferous tubules. In addition PDE5 inhibitors have an effect on the contractility of the testicular tunica albuginea, and the prostatic secretory function. The literature suggests that the overall effect of sildenafil and vardenafil is an increased quantitative and qualitative sperm motility. Furthermore, some studies indicate that sildenafil and vardenafil influence positively the sperm ability to undergo capacitation under biochemical conditions that are known to induce the sperm capacitation process. Taking into consideration that the sperm capacitation process represents a prerequisite for the development of sperm ability to undergo a hyperactivated pattern of sperm motility and for the induction of the sperm acrosomal process, it appears that sildenafil and vardenafil may have a beneficial role in the sperm fertilizing capacity.

Clinically important are the findings concerning the positive effects of a micronutrient supplementation combined with avanafil on sperm functional assays. Considering that, the outcome of sperm functional assays (i.e., sperm hyperactivation assay and sperm hypoosmotic swelling test) significantly increases by the pharmaceutical combination treatment, it appears that combinations of the widely used micronutrient supplements nowadays with avanafil may be recommended in the therapeutic management of oligoasthenospermic males.

Much caution is necessary prior to the usage of tadalafil for the alleviation of male infertility due to its ability to inhibit in a considerable degree the PDE11.

It may be emphasized that the well-documented role of sildenafil, vardenafil, and avanafil in the therapeutic management of erectile dysfunction, offers an exciting interest for the investigation of a probable therapeutic role for these PDE5i in the management of infertile oligoasthenospermic men with erectile dysfunction using a single pharmaceutical agent for both pathophysiologies. Avanafil represents an attractive pharmaceutical agent for further testing since it increases the outcome not only of specific standard parameters of the semen analysis, but it also increases the outcome of sperm functional assays. It is well known that the standard parameters of semen analysis have not high correlation coefficients with the IVF outcome. However, in some of the investigations, positive effects of some PDE5i have been described in functional assays known to have high correlation coefficients with the final sperm fertilizing capacity. Therefore, new studies evaluating the importance of PDE5is on the therapeutic management of male infertility should have as a target to investigate the effects of PDE5i on the final sperm function. In addition, new studies evaluating the role of PDE5i in the treatment of infertile males should evaluate the effects of PDE5i on sperm DNA integrity. Any hypothetical detrimental effects of a PDE5i on sperm DNA integrity immediately preclude the therapeutic usage of the respective PDE5i.

Additional research efforts are necessary in order to recommend unequivocally the usage of sildenafil, vardenafil, or avanafil for the alleviation of male infertility.

RT17-3 | Sequence analysis of candidate genes for male infertility: clinical implications

Sabine Kliesch
Centre of Reproductive Medicine and Andrology (CeRA), University Clinic Münster, Germany; WHO Collaboration Centre, Training Centre of the European Academy of Andrology (EAA)

Genetic causes for spermatogenic failure are intensively investigated in recent years. Classical chromosomal anomalies such as Klinefeltert's syndrome, Y-chromosomal microdeletions or monogenic mutations (e.g. CFTR, TEX11 mutations) are part of clinical routine recommendations for male patients with either non-obstructive or obstructive azoospermia (Yatsenko et al. NEJM 2015, Tüttelmann et al. Med Gen 2018;30:12–20). Single nucleotide polymorphism (SNP) analysis in male infertility focused on genetic variants and its effects on regulation of spermatogenesis. Thus, T-allele polymorphisms in the FSHB-gene give rise to the assumption that FSH regulation is insufficient with potential effects on spermatogenic output in oligozoospermia and even surgical sperm retrieval rates in azoospermia (Schubert et al. JECEM 2022 doi 10.1210/clinem/dgac165, online ahead of print; Busch et al. JCEM 2019;104:2315–2324). However, the majority of genetic variants remains still unexplored. As a consequence, research efforts were prospectively bundled in recent years at our Centre of Reproductive Medicine and Andrology (CeRA), Münster, to better elucidate the monogenic contribution to severe male infertility by whole exome sequencing (WES) approaches in an interdisciplinary approach (Wyrwoll et al. Eur Urol 2022, online ahead of print).

For this study, patients with male fertility problems were prospectively recruited, clinically completely phenotyped and included in the study in case of azoospermia or cryptozoospermia (< 100,000 sperm/ml) since January 2017. Moreover, patients included in the study underwent a standardized genetic work-up to exclude chromosomal aberrations or any Y-chromosomal microdeletion. During four years of recruitment at CeRA, altogether 647 men were prospectively analysed by exome sequencing in the Institute of Reproductive Genetics, Münster. According to clinical guidelines, 60 genes and variants were analysed which had proven at least limited clinical validity.

We identified diagnostic genetic variants in 8.5% (n=55) patients. Diagnose of obstructive azoospermia was proven in 20 of these men (3.1%) and they carried mutations in CFTR or ADGRG2 genes. Testicular failure with impairment of spermatogenesis was diagnosed in 35 patients (5.4%) with non-obstructive azoospermia or severe cryptozoospermia.

In this cohort, mutations in 20 different genes were seen and at least 19 of these genes in spermatogenic failure by now reach at least moderate clinical validity. As the list of genes is constantly increasing, the list of genes cannot yet be finalized.

In summary, we could double the number of diagnostically relevant mutations by exome sequencing in azoospermic/cryptozoospermic men and found causal mutations for one in 12 men. These results will influence diagnostic work-up of patients. As most genes reveal clinical relevance, the increase in diagnostic yield will subsequently lead to therapeutic consequences, possibly influence success rates of surgical sperm retrieval in azoospermia and thus predict the chances for fatherhood (Wyrwoll MJ, Köckerling N, …..Cremers, JF, Wistuba J, Krallmann C, Kliesch S, …. Friedrich C, Tüttlemann F. Genetic Architecture of Azoospermia – time to advance the standard of care. European Urology 2022, https://doi.org/10.1016/j.eururo.2022.05.011, online ahead of print).

RT17-4 | Antioxidants decrease DNA Fragmentation Index and improve pregnancy rates in ART programs

Zsolt Kopa
Hungary

No abstract text

RT17-5 | Probable detrimental effects of antioxidants; Excellence through Moderation

Fotios Dimitriadis
Greece

Significant advances in the field of male infertility have been witnessed during the last years. Given the well-known detrimental effects of reactive oxygen species, the administration of antioxidants has emerged as a promising solution for oxidative stress (OS)-induced male infertility. Nevertheless, this perception seems largely oversimplified, and the existing literature fails to recognize a notable superiority of the excessive use of these widely available nutritional compounds. Taking into consideration that several trials have shed light on the so-called "antioxidant paradox" phenomenon, we recognize that over-the-counter consumption of such supplements might be harmful.

Current evidence reveals a positive association between certain nutraceuticals and sperm parameters, namely concentration, motility, morphology. Unfortunately, the results are rather heterogeneous and not always as expected, with the risk of overdosing remaining unequivocal. For example, administration of selenium above 70 ng/ml, results in decreased motility, and high incidence of asthenospermia. In addition, selenium levels greater than or equal to 80 ng/ml are associated with a high abortion rate which strongly supports a role for the sperm selenoprotein(s) of the outer mitochondrial membrane. Moreover, selenium at high doses has been linked with a significant reduction of sperm motility caused by a modification of thyroid hormone metabolism. An interracial variability is another important parameter to consider when a clinician suggests the administration of a nutraceutical. For example, in the Caucasian population has never been described selenium deficiency, whereas 15% of the North American population has zinc deficiency, which is linked to increased cancer risk. These findings indicate the high index of awareness needed and the apparent risk to overcome the optimal threshold.

Likewise, high doses of ascorbic acid (over 1000 μmol) decrease motility and viability and concomitantly increases lipid peroxidation. Moreover, it has been shown that daily administration of vitamin C, vitamin E, β-carotene, zinc, and selenium at certain doses increases the degree of sperm decondensation maybe due to the interfering role of vitamin C, in the opening of the interchain disulfide bridges in protamines, thus interfering with paternal gene activity during preimplantation development. Further scientific data suggest that males with moderate β-carotene intake have an increase in sperm DNA fragmentation compared with participants with low intake. One possible explanation for this deleterious effect is that autoxidation of retinoids generate superoxide which subsequently is dismutated to H_2O_2 leading ultimately to DNA damage in the presence of endogenous metals.

Given the witnessed proclivity for the broad prescription of antioxidants in the field of male infertility, various considerations have to be taken seriously into account. Current studies are remarkable for their heterogeneity as far as the ideal types and concentrations of the administered compounds are concerned. Future research should shed light on the optimal mixture of antioxidants, which will efficiently reverse OS-induced sperm dysfunction. Furthermore, the inherent nature of the antioxidant's mechanism appears as dose-dependent and host-dependent. Dosing guidelines, namely, the daily dosage of nutraceuticals, administration period, and consistent treatment regulations, are of paramount importance and should be further standardized. The lack of the above might explain why clinical studies have not demonstrated significant improvements so far.

One major problem is the lack of crucial information regarding the pre-administration bodily and seminal redox state. No universal test exists to measure the oxidative status, and no consensus on defining specific cut-off values of redox levels efficiently. Advanced sperm function tests that will efficiently define the oxidative status are needed. Every treatment approach should be individualized explicitly to control the antioxidant intake, thus avoiding the risk of overload and reductive stress. This applies in both supplementation interval and follow-up protocol.

The axiom that OS is harmful, and antioxidants will be safe and efficacious with no adverse effects appears frivolous. While initially recognized as a defense shield against OS and a promising solution in the OS-induced male infertility pathways, antioxidant supplementation lacks critical evidence and needs to be further evaluated and interpreted with caution. Irrational administration of antioxidants may disrupt the redox balance between oxidative and reductive state, leading to cellular injury and disease. Therefore, the notion that antioxidant consumption is only protective and advantageous could be regarded as misconceiving. Large-scale well-designed clinical trials that will

further assess and define the antioxidants' impact on OS-induced male infertility are certainly warranted.

RT17-6 | Are there any predictive factors for successful sperm recovery in the salvage TESE/mTESE?

Paolo Capogrosso
University of Insubria, Varese, Italy

Conventional testicular sperm extraction (cTESE) or microdissection TESE (mTESE) in patients with non-obstructive azoospermia could result in negative sperm retrieval (SR) in about 50% of cases. In this context, salvage mTESE may offer a further possibility of sperm retrieval and cryopreservation in some cases. Overall, salvage mTESE (or salvage cTESE) could be attempted after failed non-invasive procedures such as fine needle mapping (FNA) or testicular sperm aspiration (TESA), ensuring high SR rates. On the other hand, salvage mTESE after a previous failed cTESE or mTESE has been shown variable outcomes with positive SR rates ranging from 18 to 50% of cases. Small prospective and retrospective studies investigated factors associated with positive SR at salvage TESE. Data show that histological features such as hypospermatogenesis and FSH values could be associated with a higher probability of retrieving sperm at salvage surgery. Hormonal therapy before salvage TESE has been proposed to increase the probability of positive SR. Data coming from retrospective studies and case-control series show that treatment with hCG and recombinant FSH could result in a significantly higher SR rate in patients receiving hormonal treatment before salvage TESE as compared to controls. However, the current European Association Urology (EAU) Guidelines do not suggest the routine use of hormonal treatment before salvage surgery.

RT17-7 | May hormonal stimulation turn the first negative for sperm -TESE into a positive for sperm- salvage TESE/mTESE?

Suks Minhas
UK

No abstract text

RT17-8 | May repair of an existing varicocele turn the first negative for sperm- TESE into a positive for sperm- salvage TESE/mTESE?

Giorgio Russo
University of Catania, Catania, Italy

Varicocele is a highly prevalent disease in reproductive medicine practice defined as a dilatation of the veins of the pampiniform plexus [1]. Varicocele has an incidence of approximately 15% among the general population [2]; to be more precise, 35% of men with primary infertility and 70–80% of men with secondary infertility suffer from this condition [3]. Nowadays there is much evidence suggesting a putative and well-established association between varicocele and a progressive and duration-dependent decline in testicular function. The precise process by which varicocele may damage male fertility potential remains equivocal. However, different chargeable causes have been identified: elevated intrascrotal temperatures, blood reflux, oxidative stress, backflow of metabolites, seminiferous tubules hypoxia, sperm nuclear damage [4]. Varicocele Repair (VR) might have a pivotal role in the treatment of sub-fertile men who suffer from varicocele due to the high cost associated with Assisted Reproductive Technology (ART) treatment [5]. Precisely, VR seems to diminish reactive oxygen species and DNA fragmentation rates that are notoriously associated with varicocele; moreover, DNA damage has been found to be strictly related with worse ART outcomes rate. However, since the introduction of in vitro fertilization (IVF), pregnancy treatment has mostly been supplied through ART rather than treatments focused on specific male infertility causes [6]. Therefore, advancement in ART have brought the additional value of VR into question. In fact, in some cases of non-obstructive azoospermia, VR seems to improve motile sperm and spontaneous pregnancy achievement [7]. Precisely, since VR enrich semen parameters, it seems desirable to improve pregnancy rate (PR) avoiding expensive ART treatment or determine the value of VR in the era of ART [8]. If choice is possible, the employment of motile sperm from fresh sample is preferable to TESE (Testicular Sperm Extraction) preparation for ICSI since it seems to achieve higher sperm retrieval rate [9]. TESE is the process consisting of excising small portion of testis tissue in order to extract the viable sperm cells for the purpose of ICSI [10]. According to the summary analysis of the study conducted by Kirby et al., an overall increase in PR among men undergoing VR compare with untreated ones has been shown. However, when analyzing the two articles concerning men with azoospermia who underwent TESE with IVF/ICSI it is not possible to demonstrate a statistically significant difference in PR between the VR and the untreated varicocele group [11,12]. Of these two studies, the one conducted by Haydardedeoglu et al. reported a significant difference in sperm retrieval rate between men who underwent VR and untreated ones (sperm retrieval rate 60.81% and 38.46%, respectively, $p = 0.01$) [11]. Nevertheless, when combining these two studies, metanalysis showed a statistically significant improvement in PR favoring VR (OR 2.336, P=.044). A quite recent observational study had been performed by Zampieri et al. in a cohort of patients who suffer from varicocele and underwent varicocelectomy and mTESE with different timing; group 1 included patients who underwent VR 3 months before mTESE, while group 2 underwent varicocelectomy and mTESE at the same time. Authors reported that sperm retrieval rate on spermiograms performed 6 months after procedures was significantly higher in group 1 rather than in group 2 (57.8% vs. 37.5%). Similarly, sperm retrieval rate during mTESE was greater in the same cohort (57.8% vs. 25%). Finally, even though any spontaneous pregnancy had been reported in the study, also clinical fertilization rate resulted greater in group 1 (63.15% vs. 43.75%) [13]. So, in conclusion, much more evidences are

needed in order to better understand the role of VR in the context of ART.

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INDUSTRY PRESENTATIONS

IS01 | Welcome & Introduction

Eduard Ruiz Castañé

Andrology Department, Fundacio Puigvert, Barcelona, Spain

No abstract text

IS02 | Obesity influence on sexual dysfunction, fertility & QoL

Giovanni Corona[1], Edudard García[2]

[1]*Endocrinology Unit, Medical Department, Azienda Usl, Maggiore-Bellaria Hospital, Bologna, Italy;* [2]*Instituto de Urología Serrate & Ribal, Barcelona and ROC Clinic, Madrid, Spain*

Obesity represents one of the most important challenging problems of all national health care systems with an estimated worldwide prevalence by 2025, of 18% in men and higher than 20% in women. The latter feature is the result of the interaction among genetic background, environmental factors and wrong lifestyle behaviors including food abundance, and low level of physical activity. The final result is a progressive clinical condition characterized by a positive energy balance along with inflammatory, hormonal and metabolic derangements, leading to fat accumulation and organ damage, which represent the first step for the development of several preventable diseases, including cardiovascular diseases (CVD) cancer and type 2 diabetes (T2DM). Despite this evidence, the clinical awareness related to the obesity-related problems, among medical community, is still quite poor and the vast majority of general physicians still considers subjects with obesity and overweight to be lazier and more self-indulgent than people with normal weight. A large body of evidence has clearly documented a tight association between erectile dysfunction (ED) and obesity. In particular, either cross-sectional or prospective studies, have documented that patients obesity have from two to three-fold higher risk of ED when compared to non-obese subjects. Vascular damage due to obesity-associated cardiovascular risk (CV) risk factors as well as obesity-induced hypogonadism represent the main underlying pathogenetic links. The latter figure is particularly important taking in account that nowadays ED is considered an early marker of forthcoming CVD even when obesity is not associated with any complications such as dyslipidemia, arterial hypertension or T2DM. Interestingly, life-style modifications, weight loss, whatever obtained, and the optimization of the obesity associated complications can improve ED, its related CV risk as well as T levels.

The impact of obesity on male fertility is more conflicting. Data derived from preclinical and animal models suggest that obesity and its related complications can significantly impair sperm parameters. However, data derived from clinical studies are contradictory. In particular, whether obesity per sé seems not to particularly affect sperm parameters, morbid obesity (body mass index >40 kg/m2) and obesity-related complication particularly T2DM, arterial hypertension and metabolic

syndrome are more frequently associated with sperm parameters modifications. In addition, more robust data support the role of male and female obesity in the assisted reproductive techniques (ART) outcomes. Furthermore, in line to what reported for ED, emerging evidence also supports a possible association between male infertility and a decreased general health status. Hence, the same risk parameters, including wrong lifestyle behaviors and traditional CV risk factors can work together supporting the development of male infertility and, more years later ED. Both conditions if not treated and managed in the adequate manner can eventually result in higher mortality and morbidity.

Hence, adequate preventing care campaigns to reduce the impact of obesity and its related complications are urgently required. At the same time, patients seeking medical care for couple infertility or sexual dysfunction should adequately informed on the related risks. In addition, specific strategies to obtain weight loss and the optimization of the obesity-associated morbidities are mandatory.

IS03 | Role of Liragluitde in the treatment of obesity (clinical experience)

Maurizio de Rocco Spain

No abstract text

IS04 | Sponsored lecture: Erectile dysfunction and quality of life

Scott Petterson Boston Scientific, USA

No abstract text

DOI: 10.1111/andr.13274

ANDROLOGY WILEY

ABSTRACT

Oral Communications

GOLDEN COMMUNICATIONS

OC01 | Evaluation of familial cancer risk among testicular germ cell tumor patients

Viktória Rosta[1,3], Daniel Moreno-Mendoza[2], Ginevra Farnetani[1], Rebecca Passerotti[1], Matteo Vannucci[4], Josvany Sánchez-Curbelo[2], Antoni Riera-Escamilla[2], Csilla Krausz[1,2]

[1]Department of Experimental and Clinical Biomedical Sciences 'Mario Serio', University of Florence, Florence, Italy; [2]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Spain; [3]National Institute of Oncology, Budapest, Hungary; [4]University Hospital Careggi, Florence, Italy

Introduction: Testicular germ cell tumor (TGCT) is a multifactorial, polygenic, and complex disease. It is the most common malignancy of men in their reproductive ages. This neoplasm has one of the highest heritability (37–48.9%) based mainly on epidemiological and genome-wide association study data. Epidemiological studies indicate that there is an increased familial cancer risk among TGCT patients' family members, however, they are heterogeneous and, in some instances, controversial.

Objectives: To explore whether TGCT may be part of a more generalized cancer predisposition, through the estimation of the familial cancer risk among TGCT patients' relatives (first-degree and grandparents).

Materials and Methods: This is a retrospective, case-control study. Patients were recruited at the Andrology Unit of University Hospital of Careggi, Florence (Italy) and at the Andrology Department, Fundació Puigvert, Instituto de Investigaciones Biomédicas Sant Pau, Barcelona, (Spain).

A total of 1407 subjects were enrolled: 592 were affected by TGCT, 352 had oncohematological (OH) malignancies, and 463 were Spanish fertile cancer-free controls. Statistical analyses were performed using SPSS Software.

Results: We observed that both TGCT and OH patients' relatives have significantly more cancers ($p = 0.0001$ and $p = 0.0045$, respectively) than controls' relatives. No significant differences were identified comparing the TGCT cohort with the OH. We found significantly higher familial incidence of TGCT ($p = 0.01$) and OH ($p < 0.001$) malignancies, in patients affected by TGCT and OH, respectively. Moreover, we report some tumor-specific associations: among TGCT relatives, five different malignancies were seen at a significantly increased frequency, whereas in OH relatives, breast cancer was more frequently observed, as compared to control relatives ($p = 0.02$). For the first time in the literature, we assessed not only the impact of tumors versus non-tumors on familial incidence of neoplasms but also the relevance of the semen phenotypes of the cancer patients. We report a 1,57-fold higher risk (p value = 0.0048) for tumor development among family members if the subject had severe spermatogenic disturbances (azoospermia and severe oligozoospermia with TSC < 5 million). We observed significantly ($p < 0.001$) less siblings among TGCT (mean number of siblings ± SD: 1.16 ± 0.97) and OH (1.09 ± 1.08) cases in respect to controls (2.07 ± 1.47), suggesting a lower fecundity rate in the cancer patients' parents.

Conclusions: Our results show an association between TGCT, increased familial cancer incidence, and sub/infertility. In addition, our data support the previously reported higher morbidity rate, including cancer development, in men with spermatogenic defect. The biological explanation for our findings could be an overall genomic instability/DNA repair defects in the family, which is reflected in the occurrence of multiple cancers and subfertility. The increased familial risk for specific cancer types may have relevance in the clinical management of these patients and their family members.

OC02 | Identification of cellular and molecular alterations underlying two distinct types of cryptozoospermia using single cell RNA sequencing

Sara Di Persio[1], Lena Schülke[1], Tobias Tekath[2], Linda Ebbert[2], Lara Marie Siebert-Kuss[1], Nicole Terwort[1], Ina Lu[3], Gerd Meyer zu Hörste[3], Jann-Frederik Cremers[1], Joachim Wistuba[1], Sarah Sandmann[2], Corinna Friedrich[4], Frank Tüttelmann[4], Sabine Kliesch[1], Stefan Schlatt[1], Sandra Laurentino[1], Nina Neuhaus[1]

[1]Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany; [2]Institute of Medical Informatics, University of Münster, Münster, Germany; [3]Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany; [4]Institute of Reproductive Genetics, University of Münster, Münster, Germany

Infertility affects around 15% of couples globally with half of the cases due to a male factor. Despite this high incidence, the molecular and cellular mechanisms regulating (dys)function of human spermatogenesis remain poorly understood leading to just 30% of the infertile men receiving a causal diagnosis. The remaining 70% receive collectively descriptive diagnoses largely based on their semen and hormone analyses. One example are men diagnosed with cryptozoospermia (Crypto). A common feature of these patients is that spermatozoa can only be found in the sediment of semen after centrifugation, but these patients are otherwise extremely heterogeneous with regard to hormonal and histological parameters. To assess if there are sub-groups among the cryptozoospermic patient cohort, we performed cluster analysis based on semen, histological, and endocrine parameters of 132 Crypto and 160 Control patients (obstructive azoospermia). Interestingly, the clustering resulted in two Crypto groups largely based on their testicular architecture. In group 1, most seminiferous tubules contained spermatocytes as the most advanced germ cell type. In contrast, the prevalent tubular phenotypes in group 2 were Sertoli cell only (SCO) tubules and tubular shadows. We therefore termed group 1 meiotic arrest-like (MAL) and group 2 SCO-like (SCOL) Crypto. It is of note though, that about 10% of the seminiferous tubules still displayed full spermatogenesis in both groups, which is in accordance with the presence of sperm in the ejaculate and with similar sperm retrieval rates after testicular surgery in both groups. To identify potentially distinct molecular and cellular alterations between the two groups we performed single cell RNA sequencing (scRNA-seq) on four controls, two MAL and six SCOL Crypto samples, followed by histological analyses (13 samples per group). Interestingly, we identified shared alterations on the spermatogonial stem cell (SSC) compartment between the two crypto groups and the controls. Specifically, we observed a reduction in the reserve stem cells (Adark), and an increased proportion of PIWIL4+ cells in the two Crypto groups. Nonetheless, scRNA-seq analyses of over 65,000 testicular cells also revealed distinct alterations between the two groups. In MAL, but not SCOL tissues, early spermatocytes showed increased expression of histone genes and altered expression of genes related to the DNA packaging machinery. In contrast, Sertoli cells of the SCOL, but not MAL tissues, showed signs of reversion to a more immature state, as indicated by increased expression of genes involved in oxidative phosphorylation. In summary, our study revealed two potentially distinct molecular alterations associated with the two groups of Crypto patients. Furthermore, the common alterations observed in the SSC compartment may suggest a general compensatory mechanism in response to the spermatogenic impairment. Future research will address whether these two groups have distinct underlying etiologies or represent a progressive phenotype.

OC03 | The role of unprocessed PRM2

Lena Arevalo, Gina Esther Merges, Simon Schneider, Hubert Schorle

Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Germany

Protamines are small sperm-specific proteins that play an important role in packaging and protecting the paternal genome on its way to the fertilization site. The majority of mammalian species express only protamine 1(PRM1). However, primates and rodents express an additional protamine, protamine 2(PRM2). PRM2 differs from PRM1 primarily in its N-terminal domain (cP2), which is cleaved off sequentially during paternal chromatin packaging. DNA damage and infertility have been linked to alterations in this processing. However, the precise function of the cP2 domain remains unknown.

Using CRISPR/Cas9, we created mice bearing a targeted deletion of cP2. The resulting cP2 deficient males were infertile and show that cP2 deficiency causes incomplete histone-to-protamine transition. While increased histone retention cannot be observed in IHC, mass spectrometry or immunoblotting, transition proteins are not evicted from the nucleus, resulting in incomplete protamine incorporation sperm DNA degradation and infertility. Using a cP2 specific antibody we show that not all of PRM2 is processed in WT sperm, with unprocessed PRM2 accumulating in the cytoplasm and residual bodies of condensed spermatids. In vitro the cP2 sequence is able to interact with transition protein 1. This indicates that unprocessed PRM2 with its cP2 domain might be directly involved in transition protein eviction. Additionally, we find that sperm-specific histone variant H2A.L.2 retention fails in cP2 deficient sperm chromatin. Polycomb recessive complex component Pcgf5 seems to be expressed at higher levels in cP2-deficient testes and is involved in histone H2A monoubiquitination. Our ongoing investigation involves in vitro and in vivo co-IP analyses to define cP2-specific interaction partners and analyses of histone retention and modification patterns. Our results establish unprocessed PRM2 with its cP2 domain as a key element in the complex crosstalk between histones, transition proteins and protamines during sperm chromatin condensation.

OC04 | Short chain fatty acids-mediated sperm chemiotaxis: functional role of olfactory receptor 51E2 in human reproduction

Emanuela Teveroni[1], Edoardo Vergani[1], Fiorella Di Nicuolo[1], Carmine Bruno[1], Giuseppe Maulucci[2], Giada Bianchetti[2], Anna Laura Astorri[1], Giuseppe Grande[3], Iacopo Gervasoni[2], Marco De Spirito[2], Andrea Urbani[4], Federica Mancini[1], Alfredo Pontecorvi[1], Domenico Milardi[1]

[1]Dipartimento di Medicina e Chirurgia Traslazionale, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy; [2]Dipartimento di Neuroscienze, Sezione di Fisica, Università Cattolica Del Sacro Cuore, Rome, Italy; [3]Seconda Divisione di Medicina, ULSS2 Marca Trevigiana, Ospedale Regionale "Ca' Foncello", Treviso, Italy; [4]Dipartimento di Scienze Laboratoristiche ed Infettivologiche, UOC Chimica, Biochimica e Biologia Molecolare Clinica, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy

Human reproduction is a complex sequential process, which includes the journey of motile sperm through the female genital tract and the final fusion with the mature oocyte. Olfactory receptors (ORs), widely expressed in the male and female reproductive systems, and

their active ligands could be essential to chemical sperm-oocyte communication and their alteration could represent a cause of human infertility. Among ORs, OR51E2 is expressed on the extracellular membrane of the flagella, mid-piece and, with less extent, on the acrosomal cap of spermatozoa. OR51E2 has been demonstrated to bind volatile short-chain fatty acids (SCFAs) as specific ligands. SCFAs, such as propionate (PA) and acetate (AA), metabolic byproducts of anaerobic bacterial fermentation, could be the key players in a complex interplay between microbiota and human reproduction, through spermatozoa chemotaxis. Spermatozoa chemotaxis is mostly dependent on intracellular calcium release and ERK phosphorylation (pERK). ORs seem to be among the involved receptors activating this signaling pathway.

In our study, immunofluorescence analysis revealed that OR51E2 is present in the midpiece and neck of non-treated sperm cells. After an exposition of 5 mM of PA for 10 minutes, we found an enrichment of OR staining in the acrosomal compartments, that reached an average of 40% indicating that propionate is able to mediate OR51E2 acrosomal relocalization. pERK accumulation and calcium influx in a dose and time-dependent manner was found. This suggests that this ligand-receptor binding could be involved in the sperm migratory response.

To address if propionate-mediated sperm activation depends on the binding to OR51E2, seminal fluid was pretreated with the specific antibody against OR51E2. The pERK increase was impaired in the seminal fluid pre-incubated with α-OR51E2 antibody.

We wondered if the PA dependent sperm activation through OR51E2 acrosomal clusterization could be involved in sperm chemotaxis. To address this question, a modified direct swim-up assay was performed. PA and AA were added or not to the medium stratified over the seminal fluid, testing their ability to act as chemoattractants for sperm cells in comparison to BSA used as a positive control. A significant increase of migrated spermatozoa in both the PA and AA-treated samples compared to the controls, with a greater extent than BSA addition, was detected.

CASA analysis of kinematics parameters allowed the quantification of the phenomenon and documented the increase in linearity (LIN) and straightness (STR) index, related to the increase of velocity straight line (VSL), thus indicating that propionate and acetate not only promote a general migration of sperm cells but specifically a more linear orientation.

Finally, by liquid chromatography coupled to mass spectrometry we confirmed the presence of propionic and acetic acid in the cervical mucus of fertile women, showing an increase in pERK after a direct interaction between cervical mucus and sperm cells.

This study could spread a light on a possible physiological function of chemosensory receptors in successful reproduction. The present work could be useful to develop new strategies for the treatment of infertile individuals.

SELECTED ORAL COMMUNICATIONS

OC05 | Mutational screening of androgen receptor gene in 8224 men of infertile couples

Maria Santa Rocca[1], Giovanni Minervini[2], Cinzia Vinanzi[1], Alberto Bottacin[1], Carlo Foresta[3], Maria Pennuto[2,4], Alberto Ferlin[1,3]

[1]Unit of Andrology and Reproductive Medicine, University Hospital of Padova, Padova, Italy; [2]Department of Biomedical Sciences, University of Padova, Padova, Italy; [3]Department of Medicine, University of Padova, Padova, Italy; [4]Venetian Institute of Molecular Medicine, Padova, Italy

Objective: To study the frequency and type of androgen receptor gene variants in males of infertile couples. Mutations in the androgen receptor gene might be associated with infertility mainly because they cause various degree of androgen insensitivity. However, large cohort of patients have not been reported and a clear genotype-phenotype is not evident. Therefore, mutational screening of this gene is not currently recommended routinely in infertile subjects.

Design: Retrospective cohort study and functional analysis of variants.

Subjects: 8224 males of idiopathic infertile couples.

Main Outcome Measures: Mutational screening of androgen receptor gene, computational and functional analyses to correlate genotype with phenotype.

Results: We found 131 patients (1.6%) harboring 45 variants, of which 18 were novel missense variants. Patients with androgen receptor gene variants had lower sperm count ($p = 0.048$), higher testosterone concentration ($p < 0.0001$) and higher androgen sensitivity index (LH x testosterone, $p < 0.001$) compared to patients without variants. ROC analyses found testosterone ≥15.38 nmol/L and androgen sensitivity index ≥180 IU × nmol/l2 as threshold values to discriminate with good accuracy patients with androgen receptor gene variants. Patients with oligozoospermia and testosterone ≥15.38 nmol/L have a 9-fold increased risk of harboring mutations compared to patients with normal sperm count and testosterone < 15.38 nmol/L (57/1677, 3.4% vs. 13/3446, 0.4%; odds ratio OR 9.29, 95% confidence interval 5.07-17.02). Two novel variants, L595P and L791I, were identified by computational and functional studies as potentially pathogenic, causing reduced protein expression after androgen stimulation, respectively in a patient with a suggestive phenotype and in a patient without any sign on androgen insensitivity.

Conclusion: This is the largest study screening androgen receptor gene variants in men of idiopathic infertile couples. We found a prevalence of 1.6%, which increased to 3.4% in oligozoospermic subjects with testosterone ≥15.38 nmol/L. Conversely, more than 80% of men with androgen receptor gene variants had low sperm count and high testosterone levels. Although further analyses are necessary to establish the pathogenic link between some variants and the infertile phenotype, we suggest androgen receptor gene sequencing as a routine genetic test in cases of idiopathic oligozoospermia with testosterone ≥15.38 nmol/L.

OC06 | Y chromosome loss detected by karyotyping of men with azoospermia

J Fedder[1,2,3], C Fagerberg[4], MW Jørgensen[5], CH Gravholt[6,7], UB Knudsen[3], K Blach[1], A Skakkebæk[8,7]

[1]Centre of Andrology & Fertility Clinic, Odense University Hospital, Odense, Denmark; [2]Department of Clinical Research, University of Southern Denmark, Odense, Denmark; [3]Fertility Clinic, Horsens Regional Hospital, Horsens, Denmark; [4]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark; [5]Department of Clinical Genetics, Lillebælt Hospital, Vejle, Denmark; [6]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark; [7]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark; [8]Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark

Introduction: Structural abnormalities of the Y chromosome include partial or complete deletions of the Yp or Yq, isochromosome Y, isodicentric Y, or ring Y. Larger Y rearrangements such as isochromosome Y, isodicentric Y and ring Y are very frequently accompanied by mosaicism with 45,X arisen secondarily due to post-zygotic loss of the abnormal Y chromosomes.

Materials and methods: Our cohort consists of 840 unselected, non-vasectomized, azoospermic men consecutively collected during 25 years (1997-2022) in the Western half of Denmark. At least two ejaculates without sperm in raw semen, as well as in the pellet after centrifugation, were required to have the diagnosis: azoospermia. Our routine examination program included history taking, physical examination including genital examination and ultrasonography and blood sampling. Hormone analyses included follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin and thyroid stimulating hormone (TSH), inhibin-B, and anti-Müllerian hormone (AMH). Genetic analyses included standard chromosome analysis, Y microdeletion analysis and analysis of variants in the cystic fibrosis transmembrane regulator (CFTR) gene. Men with a 45,X cell line were referred to echocardiography and ultrasonography of the kidneys.

Results: Among the 840 azoospermic men, 144(17.1%) were found to have numerical or structural sex chromosome abnormalities. Ten men (1.2%) were found to have a 45,X karyotype (n = 2), or a 45X/46,XY mosaicism (n = 8) with an abnormal Y chromosome. In three men with mosaicism part of the Yq was deleted: one detected by karyotyping and further two by DNA-analysis. One man had an isodicentric Y chromosome and further two an isochromosome Y. In one man, a ring Y chromosome was detected, and in the final case 3 cell lines were found: 45,X/46,Xdel(Yq)/46,X i(Y). In addition to the men with a 45,X cell line, four had 46,X,del (Yq) and one 46,X,idic (Y) without mosaicism. In the two persons showing a pure 45,X karyotype, the SRY gene, usually located on Yp, was translocated to the chromosome 21 and chromosome 14, respectively. Y microdeletions detected by our routine DNA analysis were found in the 45,X males (n = 2), men with defective Y ± mosaicism (n = 12), 46,XX males (n = 3) and 46,XY males (n = 32), giving a total of 49 men (5.8%) with Y microdeletions.

Discussion and Conclusions: The cohort is large, containing the majority of azoospermic men from the Western half of Denmark referred for examination and fertility treatment. However, it may be suggested that some azoospermic men carrying chromosome abnormalities are not referred for fertility treatment reflecting some selection underestimating in the frequency of sex chromosome abnormalities. Identification of the etiologies of azoospermia is useful knowledge in the counselling of these men. Furthermore, identification of the sex chromosome abnormalities may contribute to elucidate the biological mechanisms causing azoospermia and the role of the sex chromosomes in sex differentiation and male fertility.

Registration: The study was approved by the Danish Patient Safety Authority (journal nr. 3-3013-2503/1) and the Danish Data Protection Agency (journal nr. 18/18147).

OC07 | Testicular dysfunction in 47,XXY boys: when it all begins. A prospective study

Franz Sesti[1], Carlotta Pozza[1], Marta Tenuta[1], Matteo Spaziani[1], Chiara Tarantino[1], Francesco Carlomagno[1], Roberto Paparella[2], Andrea Lenzi[1], Antonio Radicioni[1], Andrea M. Isidori[1], Luigi Tarani[2], Daniele Gianfrilli[1]

[1]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [2]Department of Pediatrics, Sapienza University of Rome, Rome, Italy

Introduction: Klinefelter Syndrome (KS) is the most common chromosomal disorder in men, and the most common cause of hypergonadotropic hypogonadism. The onset of puberty is accompanied by a progressive degeneration of the testicular environment. According to some authors, testicular damage begins during infancy with a gradual degeneration of germ cells, with the greatest changes occurring between mid-puberty to adulthood. However, others affirm that there is no hormonal evidence of testicular damage between mini-puberty and pre-puberty. The function of Leydig cells in Klinefelter boys is controversial: while some authors found low to normal testosterone levels in infancy and childhood, others reported normal to high values. US features have never been explored in KS boys. We aimed to describe the onset and progression of testicular dysfunction in KS through the integration of clinical, hormonal and ultrasound data.

Materials and methods: This is a 10-year prospective cohort study of consecutive KS patients aged from 7 months to 25 years followed from 2012 to 2021. We included 114 subjects with classic karyotype (47,XXY) who underwent a total of 332 clinical and US evaluations: 163 during pre-puberty, 115 during pubertal development and 54 during the transition age. We also included a cohort of 41 adult KS patients attending the endocrine outpatient clinic who had never been treated with testosterone replacement therapy. A general and genital physical examination was performed, with pubertal stage evaluation according to Tanner. Hormone blood tests (follicle stimulating hormone [FSH], luteinizing hormone [LH], total testosterone, inhibin B, 17β-estradiol, sex hormone binding globulin [SHBG]) and testicular US were performed at each evaluation. Starting from mid-puberty, a semen analysis was performed for patients who gave their consent.

Results: Sertoli and germ cell impairment is not hormonally detected before Tanner stage 4, as reflected by normal inhibin B values until stage 3 and the fall in the inhibin B/FSH ratio during Tanner stage 4; the testosterone/LH ratio peaks during Tanner stage 3 and drops significantly in stage 5($p < 0.001$), when Leydig cell deterioration begins. Impaired testosterone secretion is not an evident feature of KS boys during puberty, as reflected by the normal onset and progression of puberty observed in our KS cohort. The testicular echotexture reflects this progressive hormonal dysfunction, as it worsens during the transition age. FSH and LH have a primary role in testicular echotexture changes and chronic LH stimulation exacerbates these changes, causing the onset of Leydig cell micronodules. In childhood, testicular volume increases up to an average bilateral testicular volume of 7.7 ml (IQR 5.3-10.6) in Tanner stage 4, with subsequent degeneration and atrophy leading to an average adult volume of 4.6 ml (IQR 3.3-6.5).

Conclusions: The findings from this large prospective study of a sizable patient population contribute to our understanding of the onset of testicular dysfunction in KS, underlining the importance of testicular US in infancy and childhood, as well as in puberty and adolescents, for the optimal care of KS patients.

OC08 | Sperm DNA and Mmembrane integrity test (DMI test): a novel evaluation strategy of DNA fragmentation in viable spermatozoa

Raul da Costa, Klaus Redmann, Stefan Schlatt
University of Münster, Centre of Reproductive Medicine and Andrology (CeRA), Münster, Germany

Increased sperm DNA fragmentation has been associated with male infertility, adverse effects on fertilization, embryo development, embryo implantation and pregnancies in natural and assisted reproduction. Unfortunately, the currently available methods for evaluating this promising parameter lack specificity and predictive power in clinical settings. One of the main reasons behind these limitations is the disuse of cell viability at the moment of analysis, even though only live spermatozoa are used in assisted reproductive technologies (ART). To overcome these challenges, we recently proposed a combination of a DNA fragmentation sensitive dye and a vitality staining to simultaneously evaluate both parameters by flow cytometry. We called this co-staining DNA and Membrane Integrity test (DMI test). Using seminal samples of high quality, in our previous study, we proved that this method provides an easy, rapid and reproducible readout of the status of sperm DNA fragmentation in live spermatozoa. Therefore, in this study, we aim to appraise the clinical relevance of this approach by applying it to samples of variable quality.

Seminal samples from donors (n = 70) were used to compare the DMI test consisting of Acridine Orange (AO) and LIVE/DEAD Fixable Cell Stain (LD) against an established method for the evaluation of DNA fragmentation, the Sperm Chromatin Structure Assay (SCSA) and its DNA fragmentation index (DFI). Furthermore, we explored the relationships between DNA fragmentation evaluated and classical seminal parameters using both methods.

The samples were categorized as normal (n = 30) and abnormal (n = 40), in concordance with the WHO 2010 manual for semen analysis. In the abnormal samples, although higher DFI values were observed (25.13 ± 10.40) compared to the normal group (18.18 ± 10.23), no statistically significant differences or correlations were observed between the groups or classical semen parameters (seminal volume, sperm concentration, total count, progressive motility and normal morphology). The same observation was valid for the levels of DNA fragmentation in viable spermatozoa by the DMI test for abnormal (8.99% ± 5.01) and normal samples (9.74 ± 5.30%). However, a strong correlation was established between high DFI values and samples with low sperm vitality ($p < 0.05$). This observation was confirmed through the DMI test, which showed an association between high values of total DNA fragmentation and the non-viable fraction of spermatozoa.

The results of this study highlight the importance of evaluating cell viability while assessing the DNA status in heterogeneous samples. Specifically, our observations indicate that high DFI values provided by SCSA are not related to viable spermatozoa. On the other hand, the DMI test, despite not showing relations to classical seminal parameters, offers a more representative and detailed analysis of the live sperm fraction required in ART procedures. Therefore, our method has the potential to provide clinically relevant evidence, especially in cases of unexplained male infertility where the information provided by conventional seminal analysis is limited or irrelevant and the cause of the sperm dysfunction is not well understood.

OC09 | Eunuchoid skeletal proportions in male hypogonadism: a comparative analysis of anthropometric measures between men with congenital hypogonadotropic hypogonadism (CHH) and Klinefelter Syndrome (KS)

Sara De Vincentis[1,2,3], Alessio Bellelli[1,2], Rossella Corleto[1,2], Lucia Zirilli[2], Emanuele Santamaria[1], Antonio Granata[2], Vincenzo Rochira[1,2]
[1]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy;* [2]*Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria Policlinico di Modena, Italy;* [3]*Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy*

Background: Patients with congenital hypogonatropic hypogonadism (CHH) and Klinefelter syndrome (KS) have eunuchoid body proportions of the skeleton compared to normal male subjects, characterized by tall stature, and reduced upper-to-lower segment ratio (U/L). Vice versa, steroids exposure deeply differs between CHH and KS at puberty, with both testosterone and estradiol being very low only in CHH compared to KS. At present, a comparison of body skeletal proportions between CHH and KS is not available.

Aim: To compare anthropometric measurements of adult male CHH patients to adult KS patients.

Methods: A prospective, cross-sectional, observational study was carried out. CHH patients were subdivided into 2 subgroups according to the timing of treatment start (testosterone replacement therapy [TRT] or gonadotropins): CHH1) CHH patients who started treatment late after 18 years; CHH2) CHH patients who started treatment on time before 18. All KS patients did not start TRT before 18 since KS do not usually delay puberty. The following anthropometric measurements were collected by using a digital scale and stadiometer (Seca gmbh&co): height, weight, sitting height, total arm span. Legs length was obtained by subtracting sitting height from stature; U/L was calculated dividing sitting height for legs length.

Results: A total of 70 CHH and 45 KS age-matched patients were enrolled (mean age 33.7 ± 13.7 and 35.3 ± 13.7 years, respectively). CHH1 showed a longer arm span compared to CHH2($p = 0.001$) and KS ($p = 0.003$), and a shorter sitting height compared to KS ($p = 0.008$). On the contrary, leg's length was shorter in CHH2 compared to CHH1($p < 0.001$) and KS ($p = 0.011$). Accordingly, U/L and upper-to-height ratios were lower in CHH1 compared to CHH2($p < 0.001$) and KS ($p = 0.001$). Furthermore, the arm span-to-height ratio was higher in CHH1 compared to CHH2($p < 0.001$) and KS ($p = 0.008$).

Conclusions: Under the same definition of eunuchoid body proportions, the traditional hallmark of male hypogonadism, more fine differences are observed comparing adult CHH to KS patients. CHH patients who delayed treatment showed longer arms length and lower U/L in comparison to CHH patients receiving treatment on time at pubertal age and KS. This suggests a different mechanism involved in the eunuchoid skeleton development between CHH and KS confirming a major role for estrogen/androgen deficiency in the former (leading to disproportional growth of both legs and arms due to delay in epiphyseal closure that could benefit from on time replacement treatment) and a possible role of genetic supernumerary X in the latter, displaying a disproportional growth only at the legs site since infancy.

OC10 | Relevance of sperm origin in Klinefelter patients for ICSI outcome rate: large single-center experience

Hamid Kalantari[1], Marjan Sabbaghian[1], Maryam Mohammadi[2], Germar-Michael Pinggera[3], Giovanni M. Colpi[4], Mohammad Ali Sadighi Gilani[1]

[1]Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran; [2]Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran; [3]Department of Urology, Medical University Innsbruck, Innsbruck, Austria; [4]Andrology and IVF Unit, Next Fertility Procrea, Lugano, Switzerland

Introduction: Nowadays intracytoplasmic sperm injection (ICSI) is considered the first-line therapy for couples suffering from Klinefelter syndrome (KS), representing the most frequent type of gonosomal aneuploidy in humans with an estimated occurrence of 1 in 7 in the infertile men with non-obstructive azoospermia. Patients with 47,XXY are usually presented with azoospermia, but occasionally there are cases with spermatozoa in the ejaculate. Thus, it remains to assess the positive sperm finding rate in ejaculate as well as their role in clinical outcomes compared to TESE/ICSI procedures.

Material and Methods: This cohort study was retrospectively conducted on 763 men with genetically proven 47,XXY in our tertiary fertility referral center over 14 years. The main aim was to investigate the correlations between different sources of spermatozoa retrieval concerning clinical pregnancy (PR) and live birth (LB) rates after Intracytoplasmic sperm injection (ICSI). In group 1 were allocated only azoospermic men, whereas in group 2 severe oligoasthenoteratozoospermic (OAT) patients. The mean age in both groups was 33 ± 5 years.

Result: Totally, 21 out of 763 KS men (2.7%) had surprisingly sperm in their ejaculates, whereas the rest were classified as azoospermic. Among OAT cases, 18/21(86%) had < 1×106 sperm/ml and 3/21 had sperm count between 1 and 5 million/ml in the ejaculate. The rate of PR per number of embryo transfer (ET), per cycle, and per patient in KS patients in group 2 vs. group 1 was 27% vs. 22%, 19% vs. 16%, and 40% vs. 23%, respectively. The LB rate per ET, per cycle, and per patient amongst the two groups was 20% vs. 17%, 14% vs. 13%, and 30% vs. 18%, respectively.

Conclusion: The study provides new insights regarding the prevalence of spermatozoa in semen of KS patients. Furthermore, we demonstrated that the origin of spermatozoa did not significantly affect ICSI success rates in couples with KS, although a trend in favor of group 2 is assumable. Therefore, an accurate investigation of sperm presence in semen samples seems advocated before undergoing testicular surgery for sperm retrievals even in KS patients.

OC11 | In vitro somatic cell functionality as a measure of human testicular tissue quality for fertility preservation procedures

Joao Pedro Alves-Lopes[1], Femke Harteveld[1], Yanhua Cui[1], Magdalena Kurek[1], Halima Albalushi[1,2], Olle Söder[1], Rod T. Mitchell[3], Cecilia Petersen[1], Kirsi Jahnukainen[1,4], Jan-Bernd Stukenborg[1]

[1]NORDFERTIL Research Lab Stockholm, Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska Institutet, and Karolinska University Hospital, Solna, Sweden; [2]Sultan Qaboos University, College of Medicine and Health Sciences, Muscat, Oman; [3]MRC Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, and the Edinburgh Royal Hospital for Sick Children, Edinburgh, UK; [4]Division of Haematology-Oncology and Stem Cell Transplantation, New Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Many Nordic and Baltic paediatric medical institutes offer cryopreservation of prepubertal testicular tissue prior to treatments with

a significant risk of infertility. However, there is currently no robust method for assessing the quality of collected testicular tissue, which could lead to be better patient selection for fertility preservation programs. The goal of this study was to develop an in vitro approach for assessing the quality of prepubertal testicular tissue using somatic cell functionality. Testicular samples were collected from 64 prepubertal patients (aged 0.8 to 15.7 years) and separated into four study groups: non-treated, chemotherapy with or without alkylating drugs, and sickle cell disease (SCD) patients treated with hydroxyurea. In air-liquid interface culture conditions, testicular tissue samples were cultured for 21 days. Following tissue culture, in vitro testosterone released by Leydig cells, as well as anti-Müllerian hormone (AMH) and inhibin B secreted by Sertoli cells, were measured by ELISA in media collected after 7, 14, and 21 days. Preliminary results were based on 18 patient samples (1.6 to 13.1 years of age). AMH production was significantly diminished in SCD samples compared with testicular samples not exposed to chemotherapy. When compared to non-treated samples, samples exposed to alkylating agents had a declining production of AMH after 21 days. Testosterone secretion was comparable in non-treated and treated (with or without alkylating agents) testicular samples, but it was reduced in SCD samples over culture when compared to samples from the other three study groups. Compared to non-treated samples, inhibin B secretion was reduced in SCD and chemotherapy-treated samples. Altogether, we propose that in vitro secretion of AMH, inhibin B and testosterone in explant tissue cultures, combined with germ cell counts at the time of biopsy which have been described in previous studies, could be used as a quality indicator for testicular tissue taken for fertility preservation.

OC12 | Infertile men with unknown etiology show alterations in the abundance of specific protamine proteoforms related to sperm chromatin packaging and age

Judit Castillo[1], Marina Gay[2], Alberto de la Iglesia[1], Gianluca Arauz-Garofalo[2], Mar Vilanova[2], Juan Manuel Corral[3], Marta Guimerà[4], Marta Vilaseca[2], Meritxell Jodar[1,5], Rafael Oliva[1,5]

[1]Molecular Biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Fundació Clínic per a la Recerca Biomèdica (FCRB), Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain; [2]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain; [3]Urology Service, Andrology Unit, Nephrology and Urology Clinical Institute (ICNU), Hospital Clínic de Barcelona, Barcelona, Spain; [4]Gynecology, Obstetrics and Neonatology Clinical Institute (ICGON), Hospital Clínic de Barcelona, Barcelona, Spain; [5]Biochemistry and Molecular Genetics Service, Hospital Clínic de Barcelona, Barcelona, Spain

Protamines are considered one of the most relevant proteins in the sperm nucleus, because of their role packaging most of the DNA and protecting the paternal genome from external damage. Due to this function, protamines show unique physicochemical properties, including a small size and a high basicity. In addition, by using mass spectrometry-based approaches, our group has recently demonstrated that human sperm contain a substantial variety of protamine proteoforms, meaning molecular forms derived from the same genes and including differences on the amino acid sequence and post translational modifications. In particular, intact, truncated and phosphorylated forms were identified for protamine 1(P1), and for mature and immature components of the protamine 2(P2) family in the normal spermatozoon. These data added layers of information into the sperm chromatin composition, highlighting the importance of the protamine status not only on the accessibility of the paternal genome, but also on the sperm functionality. In fact, the imbalance between P1 and P2 protein abundance, calculated through the P1/P2 ratio, has been widely associated with cases of male infertility. However, the specific proteoform alterations that would derive into such deregulation have not yet been stablished. Furthermore, the diversity on human protamines, which cannot be fully evaluated by standard electrophoretic methods, raises the interest on deciphering whether it responds to the need of protein redundancy to ensure chromatin compaction, or whether there is any still unknown functional implication of specific protamine proteoforms. In order to deep into this question, we have applied Top Down Mass Spectrometry to identify potential quantitative alterations in the protamine proteoform profile of normozoospermic men with unexplained infertility.

Our results highlight two main variables as critical to maintain a stable protamine proteoform profile, the P1/P2 ratio and the age. A significant increase of P2 intermediate forms was detected in the group with higher P1/P2 ratio. P2 is synthesized as a precursor, which is processed by proteolysis to give rise to the P2 mature forms. Our data point to errors on P2 processing leading to an accumulation of immature forms in the sperm chromatin, which alters the P1/P2 ratio and may impact DNA integrity. In contrast, when considering the age as a factor, the specific loss of diphosphorylated P1 was observed in elderly men. Protamine phosphorylation is required for proper deposition of protamines during spermatogenesis and allows for looser binding to DNA. These results suggest that paternal age is closely related to modifications on chromatin epigenetic marking at different levels. Altogether, the evaluation of the protamine proteoform profile in males with unexplained infertility has suggested alterations on protein modifying mechanisms, rather than on protein expression, as critical factors for the proper maturity of the sperm chromatin with impact on male infertility.

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OC13 | Novel culture conditions for the improvement of the in vitro expansion of human spermatogonial stem cells. Future stem cell therapies to restore fertility in prepuberal boys enrolled in our experimental fertility preservation program

Myriam Martin-Inaraja[1,2], Lara Herrera[1,2], Clara Rodriguez[1,2], Silvia Santos[1,2], Estibaliz Solorzano[3], Ricardo Lopez-Almaraz[3], Maria Begoña Prieto[4], Roberto Matorras[4], Cristina Eguizabal[1,2]

[1]Cell Therapy, Stem Cells and Tissues Group, Basque Centre for Blood Transfusion and Human Tissues, Galdakao, Spain; [2]Biocruces Bizkaia Health Research Institute, Barakaldo, Spain; [3]Biocruces Bizkaia Health Research Institute-Cruces University Hospital, Pediatric Department, Barakaldo, Spain; [4]Biocruces Bizkaia Health Research Institute-Cruces University Hospital-Basque Country University-IVI Bilbao, Human Reproduction Unit, Barakaldo, Spain

Some of the cancer treatments and genetic syndromes, such as Klinefelter Syndrome, can cause fertility problems in adulthood in prepuberal children. Prepuberal patient do not have the option to cryopreserve the sperm so, to the date, there is no alternative to restore the fertility in the future. The aim of this experimental fertility program is to preserve testicular tissue (with Spermatogonial Stem Cells (SSCs)) to expand in vitro these stem cells for future autologous transplantation of the tissue or expanded SSCs in the adulthood. We collected and processed testicular biopsies of both adults and children (oncologic and KS). Each biopsy was divided into 3 fragments: for histological study, for clinical use and for research. We observed that adult patients, all oncologic prepuberal patients and 20% of Klinefelter Syndrome prepuberal patients express both VASA and MAGEA4 germ cell markers. Firstly, we expanded human male fetal germ cells (hPGCs) in vitro under several culture conditions. Our findings provide a 2D culture system to expand hPGCs that could be useful to study propagation to SSCs. Secondly, we pursued in vitro expansion cultures of adult SSCs using a modification of our previous culture conditions for hPGCs. We observed an increase of GPR125 + cells (3,3%) in comparison with the control culture condition (1,5%) after 28 days in culture. After these preliminary data in adult SSCs, we will use this novel culture condition for improvement of in vitro expansion of prepuberal SSCs for future cell therapy.

OC14 | Risk of bilateral testicular germ cell tumors: a single-center long-term experience

Marta Tenuta[1], Paola Mazzotta[1], Francesco Angelini[1], Franz Sesti[1], Claudio Lecis[1], Alain J. Gelibter[2], Fabio Massimo Magliocca[3], Antonella Anzuini[1], Donatella Paoli[1], Daniele Gianfrilli[1], Andrea Isidori[1], Carlotta Pozza[1]

[1]Department of Experimental Medicine, Sapienza University, Rome, Italy; [2]Oncology B Department, Sapienza University, Rome, Italy; [3]Anatomical Pathology, Sapienza University, Rome, Italy

Background: Metachronous bilateral testicular germ cell tumors (Met-TGCT) are not uncommon findings during follow-up, even over a long period of time, and risk factor are still under-explored. The aim of our study was to identify risk factors for the onset of a second tumor comparing a cohort of Met-TGCT with a cohort of patients with unilateral tumors with long follow-up, evaluating morpho-functional characteristics of the residual testicle together with histology and post-surgery treatment of the first tumor.

Materials and Methods: This is a retrospective, monocentric case-control study including TGCT patients who performed regular ultrasound (US) follow-up at the TestisUnit of Policlinico Umberto I, Rome, Italy, from 2006 to 2021. Testicular US, post-orchiectomy hormones and semen analysis were all performed in our institution. Data from serum tumor markers (STM), histological examination, post-orchiectomy treatments and follow-up time were recorded. Bilateral testicular tumors (both synchronous and metachronous) have been identified within the population. Patients with Met-TGCTs were compared with unilateral TGCT patients with a follow-up greater than the median time-to-onset of the second tumor (Uni-TGCTs), which represented our control group. The statistical analysis was carried out with non-parametric tests.

Results: The final population included 237 patients with TGCT: 155(65.4%) with pure seminomatous tumors, 82(34.6%) with non-seminomatous tumors. Among bilateral tumors, 11 patients were synchronous and 35(14.7%) Met-TGCT. In most cases (77.2%) the histological diagnosis was in agreement with the first tumor. The median of onset of the second tumor among Met-TGCTs was 54 months [IQR 28;138(range 8–211)]. Uni-TGCT group included 95 patients.

Met-TGCT showed a residual testicle with lower volume [10.4 ml (8.9;13.05) vs. 16.4 ml (12.0;19.7), $p < 0.001$], a more inhomogeneous echotexture [26/35(74.3%) vs. 47/95(49.5%), $p = 0.003$] and testicular microlithiasis (TML) [28/35(84.8%) vs. 23(24.2%), $p < 0.001$] compared to Uni-TGCT group. Met-TGCT presented higher gonadotropin values [FSH 12.1 mIU/ml (6.0;34.5) vs. 5.7 mIU/ml (2.4;9.0), $p = 0.004$; LH 6.5 mIU/ml (2.8;13.4) vs. 3.3 mIU/ml (1.7;5.3), $p = 0.014$] with similar testosterone levels ($p = 0.862$) and lower sperm concentration [6×106/ml (0.1-32) vs. 25×106/ml (9.7-52.5), $p = 0.039$]. No differences were found in the two groups for clinical history, histological features, STM and post-orchiectomy treatments. Kaplan-Meier curves confirmed that patients with contralateral testicular hypotrophy ($p = 0.028$), inhomogeneous echotexture ($p = 0.016$) and TML ($p < 0.001$) had a higher cumulative risk of development of a second tumor. Logistic regression analysis showed that the presence of TML was the best independent predictor (OR 25.23, 95% CI: 2.59-245.18, $p = 0.005$). Finally, among Met-TGCT, linear regression analysis showed that the lower was the residual testicle volume, the shorter was the time of tumor onset ($p = 0.001$).

Conclusions: We demonstrated in a monocentric wide cohort of TGCT that contralateral testicular hypotrophy, inhomogeneous echotexture and TML are important risk factors for the development of a second tumor and that lower volume of the testicle with second tumor was correlated with a shorter time to tumor onset. A complete

morpho-functional evaluation of the contralateral testis is essential to set up a personalized follow-up program for each individual patient.

OC15 | Effects of long-term GnRHa use on bone health in transgender adolescents: can a mouse model reveal novel insights for clinical practice?

Vanessa Dubois[1,2], Silvia Ciancia[2,3], Frank Claessens[1], Margarita Craen[2,3], Stefanie Doms[1], Sarah El Kharraz[1], Na Ri Kim[1], Daniel Klink[2,3,4], Dirk Vanderschueren[1,5], Martine Cools[2,3]

[1]KU Leuven, Leuven, Belgium; [2]Ghent University, Ghent, Belgium; [3]Ghent University Hospital, Ghent, Belgium; [4]Queen Paola Children Hospital, Antwerp, Belgium; [5]Leuven University Hospital, Leuven, Belgium

Background: Transgender individuals increasingly present at gender services in childhood. Consequently, to suppress pubertal development, more adolescents are long-term exposed to gonadotropin-releasing hormone analogues (GnRHa), from onset of puberty until start of gender-affirming hormones (GAH), around 16 years. Prolonged GnRHa may compromise bone health more than shorter-term treatment. Whether earlier start of GAH, when psychologically indicated, may partially restore bone health, is currently unknown. As clinical studies have ethical and practical limitations, a preclinical mouse model may provide mechanistic insight.

Methods: DXA [lumbar spine (LS), femoral neck (FN)] and pQCT (tibia 4%, 38%) were performed at start of GnRHa (Tanner stage 2–3), and at start of GAH in 16 transboys. Z-scores were calculated using references for cis-girls (Z-scoreAFAB) and cis-boys (Z-scoreAMAB). Similarly, we assessed the impact of long-term GnRHa use on bone development in a mouse model, and additionally explored the effect of earlier start of GAH. Prepubertal (4-week-old) female mice were treated with either the GnRHa Degarelix (DGX) alone, DGX at 4 weeks supplemented with testosterone at 6 weeks (early puberty), or DGX at 4 weeks supplemented with testosterone at 8 weeks (late puberty). Mice were sacrificed at adult age (16 weeks) for bone phenotyping.

Results: In transboys, mean age was 12.4(± 0.93) years at start of GnRHa and 15.8(± 0.60) years at start of GAH; mean duration of GnRHa monotherapy was 40(± 7.6) months. All bone mineral apparent density (BMAD) Z-scores decreased significantly [BMAD-LS Z-scoreAFAB from -0.25(1.76) to -1.34(1.02), $p = 0.001$; BMAD-LS Z-scoreAMAB from 0.57(1.65) to -1.21(0.83), $p < 0.001$; BMAD-FN Z-scoreAFAB from 0.26(± 0.94) to -0.37(± 0.86), $p < 0.001$; BMAD-FN Z-scoreAMAB from -0.05(± 0.98) to -0.44(± 1.05), $p = 0.033$].

In line, pQCT trabecular density at tibia 4% significantly decreased (absolute value and both Z-scores). Z-scores for pQCT cortical density at tibia 38% remained stable. Seven transboys were re-evaluated at gonadectomy, 31(± 2.6) months after addition of testosterone, showing partial restoration of trabecular bone.

In mice, DGX treatment significantly reduced femoral trabecular bone volume fraction (BV/TV) assessed by microCT from 4.03 ± 0.74% in female controls to 1.19 ± 0.34% in DGX-treated mice, $p < 0.001$. Late testosterone restored BV/TV to 7.47 ± 2.07%, $p < 0.001$. Early testosterone further increased BV/TV to 12.33 ± 1.46%, $p < 0.001$, similar to male controls (11.81 ± 2.38%). Cortical bone loss in DGX-treated mice was less pronounced and completely reversed by testosterone in both early and late groups.

Conclusions: Prolonged GnRHa use in transboy adolescents induces significant bone loss, mainly at the trabecular compartment. Early GAH start in a mouse model can restore bone volume up to male references.

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ABSTRACT

Posters

BASIC SCIENCE

P001 | Non-obstructive azoospermia as a sentinel for early diagnosis of late-onset Fanconi anemia

Antoni Riera-Escamilla[1], Daniel Moreno-Mendoza[1], Ginevra Farnetani[2], Maurizio de Rocco Ponce[1], Josvany Sanchez-Curbelo[1], Eduard Ruiz-Castañé[1], Csilla Krausz[1,2]

[1]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain; [2]Department of Biomedical, Experimental and Clinical Sciences Mario Serio, University of Florence, Florence, Ital

In the majority of cases, the clinical manifestations of Fanconi anemia (FA) appear during childhood. Nevertheless, in about 10% of patients, the diagnosis is delayed until adulthood due to slow progressive bone marrow failure. Late diagnosis may occur especially when individuals have no symptoms or present subtle findings that may be overlooked. The reason for diagnosis in these patients used to be the appearance of FA-related cancers or cancer treatment related life-threatening toxicity. Mouse models suggest that genes belonging to the FA pathway play a role in spermatogenesis in particular in primordial germ cell proliferation and meiosis. Based on the above observations, we hypothesize that defects in any of the 22 FA genes may lead to non-obstructive azoospermia in adulthood, even in the absence of overt anemia or other signs of FA. In this study, we aimed at diagnosing late-onset FA before the appearance of severe clinical complications. In particular, our main objectives were the identification and phenotypic characterization of late-onset FA cases through the genetic analysis of a highly selected group of infertile men and to provide novel knowledge on the genetic etiology of adult-onset FA. We performed targeted next-generation sequencing of the 22 FA genes in 100 patients affected by azoospermia and showing mild/borderline hematological alterations and in 78 fertile men with normal spermatogenesis and hematological values. We have identified a likely pathogenic (LP) hemizygous splicing variant in the X-chromosome linked FANCB gene, in a patient with azoospermia due to sertoli cell only and mild hematological alterations. No recessive LP variants were identified neither in patients nor in controls. Interestingly, three patients carried two heterozygous variants in two FA genes. Both were classified as variants of uncertain significance (VUS) and predicted to be pathogenic for the majority of in silico tools. The testicular biopsies of these patients ranged from sertoli cell only to incomplete meiotic arrest. A similar digenic condition was not observed in the controls. Data on DEB-induced chromosomal breakage test in leukocytes is not yet available. In conclusion, if the DEB-induced chromosomal breakage test in the FANCB mutation carrier confirms the pathogenicity of the variant, our study will provide further evidence of an expected high frequency of FA in infertile men. In that case, the screening of mutations in the FA genes in a specific group of infertile men with mild hematological parameters may have the potential to identify undiagnosed FA before the appearance of other severe clinical manifestations of the disease.

Keywords: genetics, non-obstructive azoospermia, Fanconi anemia, NGS

P002 | Steroidogenesis and androgen/estrogen signaling in in vitro matured testicular tissues of prepubertal mice

Laura Moutard, Catherine Jaeger, Céline Duparc, Estelle Louiset., Aurélie Rives-Feraille, Hervé Lefebvre, Nathalie Rives, Ludovic Dumont, Christine Rondanino

Normandie Univ, UNIROUEN, INSERM, U1239, Rouen University Hospital, Adrenal and Gonadal Pathophysiology team, Laboratory of Neuroendocrine Endocrine and Germinal Differentiation and Communication, Rouen, France

Chemotherapy has a recognized toxicity on germ cells that could lead to infertility. In order to preserve and restore the fertility of prepubertal patients, testicular biopsies are frozen/thawed and need to be matured to produce spermatozoa for assisted reproductive technology. In vitro maturation developed in mice has a poor yield. Since steroid hormones play an essential role in spermatogenesis, it appears necessary to ensure that their synthesis and mechanisms of action are not altered in in vitro cultured tissues. The aim of this project was therefore to study steroidogenesis and the androgen and estrogen signaling during in vitro maturation of prepubertal mouse testicular tissues. Histological, RT-qPCR, Western blot analyses and measurements of cholesterol and steroid hormones levels and of aromatase activity were performed. First of all, a similar number of Leydig cells (LCs) is found after 30 days of organotypic culture (D30) and at 36.5 days postpartum, the corresponding in vivo time point. However, LCs are

partially mature in vitro with a decrease in Sult1e1 and Insl3 mRNA levels (adult LC markers). The mRNA levels of Cyp11a1, Cyp17a1 and Hsd17b3 encoding steroidogenic enzymes are decreased in vitro. An increase amount of progesterone and decreased androstenedione intratesticular levels are observed at D30. Furthermore, androgen signaling is altered at D30 with decreased transcript levels of androgen target genes (Rhox5, Septin12). Moreover, the expression and activity of aromatase and estrogen signaling are impaired at D30. Addition of hCG to the medium induces an increase in androgen production but does not improve sperm yield.

P003 | Throughout first wave of spermatogenesis: transcriptomic analysis of in vitro maturation of fresh and frozen murine testicular tissues

Ludovic Dumont[1,2], Helene Lopez-Maestre[3], Frederic Chalmel[4], Laura Moutard[1,2], Aurelie Rives-Feraille[1,2,5], Christine Rondanino[1,2], Nahalie Rives[1,2,5]

[1]Université de Rouen Normandie, Inserm U1239, Rouen, France; [2]Institut de Recherche et d'Innovation Biomédicale (IRIB), France; [3]Institut Pasteur, USR 3756, Paris, France; [4]Université de Rennes, Inserm U1085, Rennes, France; [5]Centre Hospitalier Universitaire de Rouen, Rouen, France

Introduction and Objective: Cancer treatment in young boys is known to decrease male fertility. Freezing of prepubertal testicular tissue followed by a culture is an experimental strategy that may be used to preserve and restore male fertility before gonadotoxic treatment. To date, this strategy is not available in humans and is still difficult in mice. The aim of this study is to evaluate the impact of freezing and culture procedures by a high-throughput next-generation sequencing of testicular explants from an animal model allowing a complete first spermatogonial wave.

Methods: Testicular tissues from 6.5 days postpartum (jpp) mice were cultured to study (i) the impact of in vitro culture on the first wave of spermatogenesis at key stages: four days (D4), D16 and D30 (compared to corresponding in vivo controls) and (ii) the impact of freezing procedure after thawing and at the end of culture. RNAs of testicular tissues were extracted, the libraries sequenced and the gene list compared with sources of functional information to detect enriched terms.

Results: Two principal component analysis highlights three main groups: (i) 6.5 dpp, 10.5 jpp, D4; (ii) D16, 22.5 dpp, D30; and (iii) 36.5 dpp. In spite of a complete functional spermatogenesis, the culture system results in numerous differentially expressed genes (DEGs) compared to in vivo physiological conditions (8,456 DEGs). Tissues differentiate similarly up to in vitro D16 and in vivo 22.5 dpp; however, explants at D30 expressed a strong under-expression of transcripts (mainly related to steroidogenesis and insulin growth factor). Fresh and frozen tissues conditions are comparable before and after culture (only 45 DEGs).

Conclusion: This study shows that culture system, although lowing complete spermatogenesis, needs to be improved. Besides, this study confirms that testicular tissue freezing has a little impact, reinforcing the idea that the preservation of germinal tissue remains a promising strategy.

P004 | The role of piRNA-associated proteins in testicular function

Gülizar Saritaş[1,2], John Erik Nielsen[1,2], Sofia Boeg Winge[1,2], Nina Mørup[1,2], Kristian Almstrup[1,2,3]

[1]The Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark; [2]The International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark; [3]The Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Background: Spermatogenesis is a process of multiple mitotic and meiotic divisions where sperm are produced from spermatogonial stem cells in the testes and is regulated by the hypothalamic-pituitary-gonadal axis. Worldwide, ~15% of all couples struggle to conceive and the fertility rates continue to decline. Thus, more research on reproductive genetic and epigenetic factors that may reveal new causes of the increasing fertility crisis is needed. Our group has recently demonstrated that pathogenic mutations in certain piRNA-associated proteins, like PNLDC1, causes spermatogenic arrest and non-obstructive azoospermia due to a lack of mature pachytene piRNAs. In this project, we seek to further describe the role of piRNAs in male reproduction and endocrinology.

Materials and methods: From the biobank at the Department of Growth and Reproduction at the Copenhagen University Hospital (Rigshospitalet), we used archived testis biopsies from men representing normal (n = 1) or impaired spermatogenesis (n = 9). Immunohistochemistry was conducted essentially as previously described (Nielsen et al., 2019), to stain for the piRNA-associated proteins TDRKH (1:1000, Proteintech, 13528-1-AP) and PIWIL1 (1:250, CST, #2079S) proteins.

Results: In the tissue section with normal spermatogenesis, a prominent TDRKH expression was seen in spermatocytes and spermatogonia, whereas PIWIL1 was highly expressed in spermatocytes and round spermatids. In contrast, tissue sections from patients with reduced spermatogenesis displayed fewer tubules with TDRKH and PIWIL1 expression as well as a clear reduction in expression level in those tubules showing expression of either TDRKH or PIWIL1.

Conclusion: Preliminary data indicate that men with impaired spermatogenesis show lower levels of piRNA-associated proteins, albeit more data is needed to draw a conclusion.

P006 | Generation of murine testicular organoids in synthetic defined hydrogel

Yanhua Cui[1], Susana M. Chuva de Sousa Lopes[2], Jan-Bernd Stukenborg[1]

[1]*NORDFERTIL Research Lab Stockholm, Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska Institutet, and Karolinska University Hospital, Solna, Sweden;* [2]*Deptartment of Anatomy and Embryology, Leiden University Medical Center, Leiden, the Netherlands*

Prepubertal boys subjected to oncological treatments are at a higher risk of subfertility. Currently, cryopreservation of immature testicular tissue containing spermatogonial stem cells is the only way to preserve the fertility in prepubertal boys subjected to high-gonadotoxic treatments, such as total body radiation and/or high-dose chemotherapy [1]. However, today, there is no technique available to mature these stem cells into functional sperm in humans. Hence, novel functional in vitro models are required. The testicular organoid is a novel model that resembles the in vivo development of testis. Most protocols used for testicular organoids formation used Matrigel [2]. However, Matrigel is a poorly defined xenogeneic extra cellular matrix (ECM) protein. Due to existing batch-to-batch variations as well as its xenogeneic character, it has limited use for clinical application. Therefore, artificial scaffolds and defined recombinant human ECM proteins need to be developed. This project aims to develop a defined and synthetic Matrigel-alternative for the expansion and differentiation of murine testicular organoids.

Here, we used a Noviogel, which is a synthetic biomimetic hydrogel based on polyisocyanopeptides (PIC), for the generation of testicular organoids. Testes of 11 days postpartum (dpp) old C57BL/6 mice (n = 40) were digested into testicular single-cell suspensions and applied to the three-layer gradient culture system for testicular organoids formation [3]. We compared the effect of combinations of Noviogel with three different types of laminins (LN121, LN521 or LN111), on testicular organoid formation for 7 days in vitro (n = 4). Our results demonstrated that Noviogel alone is not sufficient to support testicular organoid growth. However, Noviogel supplemented with LN111, provided a supportive environment for testicular organoid formation. The effects of LN111 were superior to effects shown for LN121 and LN521. To investigate the organoids formation, the organoids were sectioned and stained with periodic Acid-Schiff (PAS) staining. Organoids cultured in Noviogel supplemented with LN111 exhibited cord-like structures, similar to in vivo control tissue samples.

Overall, our study demonstrates that primary mouse testicular cells are capable of forming testicular organoids with a compartmentalized tubular structure in a synthetic defined hydrogel-based three-dimensional model. Moreover, our results show the importance of the ECM for testicular organoid formation. This could bring valuable knowledge for the development of human testicular organoids that may further be used to develop a novel fertility restoration option for the prepubertal boys who have undergone cancer therapies.

P007 | Evaluation of the effects of the direct-acting anti-hepatitis C virus (Sofosbuvir) on the testicular structure and functions in male rats

Amr Abdelhamed[1], Walaa A. Allam[2], Sahar M Gebril[3], Nehal Nasr El Deen[1], Usama M Abdelaal[4], Mohammed Abu El-Hamd[1]

[1]*Dermatology, Venereology and Andrology Department, Faculty of Medicine, Sohag University, Sohag, Egypt;* [2]*Forensic Medicine & Clinical Toxicology Department, Faculty of Medicine, Sohag University, Sohag, Egypt;* [3]*Department of Histology, Faculty of Medicine, Sohag University, Sohag, Egypt;* [4]*Department of Internal Medicine, Faculty of Medicine, Sohag University, Sohag, Egypt*

Introduction: According to the literature, patients with hepatitis C virus (HCV) are associated with poor semen quality and hormonal disturbances. So, it will be of interest to explore if the direct-acting anti-HCV (Sofosbuvir) (SOF) has any influence on the testicular functions; especially there is no previous data about its effects in clinical studies.

Material and methods: The study included 30 adult male albino rats weighing 200-300 g. They were randomly divided into three groups: Group (1): control; Group (2) (SOF-L): rats received oral SOF 40 mg/kg/day for 12 weeks; Group (3): (SOF-H) Rats received oral SOF 80 mg/kg/day for 12 weeks. The effects of SOF on testicular functions were evaluated through testicular size; semen parameters; and hormonal profile (total testosterone, FSH, LH and prolactin). Testicular tissue was stained with H&E stain. Testicular histochemical/immunohistochemical staining was done with picrosirius red stain, alpha-smooth muscle actin (α-SMA), proliferating cell nuclear antigen (PCNA) and Caspase 3 (CAS3).

Results: The current study demonstrated a statistically significant difference of the relative testicular weight, being lower in SOF-H as compared to SOF-L ($p = 0.035$). In addition, it showed a significant reduction of both sperm concentration and motility in SOF-L and SOF-H as compared to control ($p < 0.0001$). Also, it showed pituitary hormones changes with FSH and LH were significantly reduced in both SOF-L and SOF-H as compared to control ($p = 0.001$; $p = 0.015$) respectively, with no significant changes between them. Total testosterone was significantly reduced only in SOF-L as compared to control ($p = 0.011$). Prolactin was significantly increased in SOF-L ($p = 0.012$) and decreased in SOF-H ($p = 0.036$) as compared to control.

Also, H&E stained testicular slides showed a significant reduction of seminiferous tubules diameter; germinal epithelium height; tubular differentiation index, and spermiogenesis index in both SOF-L ($p < 0.0001$) and SOH-H ($p < 0.0001$) groups as compared to control; with a more significant reduction in SOF-H as compared to SOF-L ($p < 0.0001$). Picrosirius red stain, α-SMA thickness, and CAS3 immuno-reactive germ cells showed a significant increase in both

SOF-L ($p < 0.0001$) and SOF-H ($p < 0.0001$) groups as compared to control, with a more significant increase in SOF-H as compared to SOF-L ($p < 0.0001$). However, PCNA immuno-reactive germ cells showed a significant reduction in both SOF-L ($p < 0.0001$) and SOH-H ($p < 0.0001$) groups as compared to control, with a more significant reduction in SOF-H as compared to SOF-L ($p < 0.0001$).

Conclusions: Sofosbuvir might have negative effects on the pituitary-testicular functions of the rats. These negative effects might be dose-dependent with more negative effects with a higher dose. These testicular negative effects might be mediated through the significant increase of both germ cell apoptosis, and seminiferous tubules collagen deposition, with a reduction of germ cell proliferation.

P008 | Study of positive, negative and zwitterionic liposomes on human spermatozoa

Daria Noto[1], Elena Moretti[1], Roberta Corsaro[1], Claudia Bonechi[2], Lorenzo Cangeloni[2], Cinzia Signorini[1], Giulia Collodel[1]

[1]Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy; [2]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Ital

Liposomes are vesicles consisting of single or multiple concentric lipid bilayers encapsulating an aqueous compartment and show several potential applicative uses, including basic research on spermatozoa.

Three types of rhodamine-loaded liposomes (positive and negative charged and zwitterionic) were tested in vitro on human spermatozoa to visualize their possible interaction. Fifteen semen samples were analysed following WHO guidelines (2010) and used for experiments. Empty liposomes diluted 1:10,000 were incubated for 1 hour with basal semen samples, swim-up upper and lower fractions for motility evaluation. Subsequently, basal samples and swim-up upper fractions were centrifuged and spermatozoa were used to evaluate the membrane mitochondrial potential (MMP) by JC-1, DNA status by acridine orange (AO) and acrosome integrity using Pisum Sativum Agglutinin (PSA).

Semen analysis revealed normal sperm parameters ranged from 25th to 75th centiles. After incubation with rhodamine-loaded liposomes, more than 95% of sperm were stained red, indicating a high level of adhesion or fusion, mainly in the midpiece. Regarding motility evaluation, sperm from basal samples incubated with positive and negative liposomes showed a significantly higher motility percentage than that of control and samples treated with zwitterionic liposomes ($p < 0.05$). Since spermatozoa from swim-up upper fraction are highly motile, no differences were visible among controls and swim-up selected sperm treated with liposomes. Instead, sperm from swim-up lower fraction, incubated with positive liposomes, showed a significant increased motility compared to controls and sperm treated with zwitterionic liposomes (both $p < 0.05$). Spermatozoa of both basal and swim-up upper fraction incubated with positive liposomes had a significant decreased percentage of sperm with double-stranded DNA respect to the other groups ($p < 0.01$). Sperm treated with positive liposomes showed significantly higher MMP than controls ($p < 0.05$ both in basal and swim-up selected samples) and sperm treated with zwitterionic liposomes ($p < 0.05$ in swim-up selected samples). Spermatozoa from basal and swim-up fractions showed intact acrosomes both in controls and after liposome treatments.

In conclusion, these liposomes are promising and available devices in the study of human spermatozoa. They could be studied in different situations, functionalized modifying their surface with other kind of lipids and proteins, or loading with different compounds.

P009 | Presence of Apelin in human spermatozoa and testis

Roberta Corsaro[1], Giulia Collodel[1], Daria Noto[1], Cinzia Signorini[1], Ester Sorrentino[2], Sergio Antonio Tripodi[2], Elena Moretti[1]

[1]Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy; [2]Department of Pathology Unit, AOUS Siena, Siena, Ital

Adipocytokines, a family of hormones and cytokines produced and secreted by adipose tissue, have shown regulative activities in various physiological and pathological conditions of the reproductive tissues. In particular, in male reproductive tissue, some adipocytokines have shown anti-inflammatory and anti-oxidative stress properties. Among these adipocytokines, apelin and its receptor APJ have been studying in different body systems, but a little is known about their role in male reproduction.

Semen samples of 53 individuals were investigated following WHO guidelines (2010) and apelin was dosed both in seminal fluid and sperm cells by ELISA assay. In addition, apelin localization was explored in ejaculated spermatozoa and human testicular tissue by immunofluorescence technique.

For the first time, ELISA experiments demonstrated the presence of apelin in human spermatozoa and its absence in human semen. Sperm apelin level was negatively correlated with sperm concentration ($p < 0.01$), rapid progressive motility ($p < 0.001$), slow progressive motility ($p < 0.05$), total progressive motility ($p < 0.001$), sperm vitality ($p < 0.001$) and sperm morphology ($p < 0.001$).

By grouping patients following the threshold value for normal morphology, defined by WHO (2010), group 1 with normal sperm morphology >4% showed a significant lower apelin level compared to group 2 with sperm morphology ≤4% ($p < 0.001$). In addition, immunofluorescence analysis on spermatozoa of group 1 showed a weak apelin signal in sperm tail, while sperm from group 2 expressed a strong apelin labelling in sperm tail, cytoplasmic residues and post-acrosomal sheath. In normal human testicular tissue, apelin immunolocalization was found mainly in Leydig cells, and a little amount was observed in the cytoplasm of peritubular myoid cells, Sertoli and germ cells.

It is not known whether apelin plays a protective or detrimental role in human sperm. The preliminary results of this research are promising to explore the behaviour of apelin in inflammatory conditions. The localization of APJ in human sperm and testicular tissue could help in understanding the exact role of the apelinergic system.

P010 | **Withdrawn**

P011 | **Targeting androgen receptor degradation, in spinal and bulbar muscular atrophy, through PROteolysis TArgeting Chimeras: development of an AF2 domain-oriented degradation enhancer**

Federica Pedrucci[1], Luca De Toni[1], Claudia Pappalardo[1], Marco Verona[2], Giovanni Marzaro[2], Alberto Ferlin[1]

[1]Department of Medicine, Unversity of Padova, Padova, Italy; [2]Department of Pharmaceutical Science, University of Padova, Padova, Ital

Background: Spinal and bulbar muscular atrophy (SBMA), is a rare disease characterized by the expansion of a CAG trinucleotide repeat (polyQ) in the androgen receptor gene (AR). Nuclear accumulation of ubiquitinated polyQ-AR upon ligand binding is a key step in SBMA pathogenesis, resulting in progressive lower motor neuron degeneration and muscle loss [1]. In addition to the ligand binding, the involvement of intra/intermolecular interactions with the AF-2 domain and the rectruitment of coregulators upon DNA binding are mandatory in the pathogenetic commitment of polyQ-AR [2]. Importantly, apart from the muscle phenotype, SBMA patients do not always develop clear signs of hypogonadism [3]. Anti-androgenic drugs are currently under evaluation for the treatment of SBMA but the exposure risk to the long-term hypogonadism is a matter of concern. PROteolysis TArgeting Chimeras (PROTAC) molecules are gaining interest to obtain the therapeutic knockdown of proteins of interest (POI). PROTACs structurally involve an E3-ligase moiety and a POI binder-warhead joined by a linker chain. In this study we aimed to develop a proof-of-concept panel of PROTACs targeting the AF2 domain of AR in order to enhance the specific degradation of pathogenetically committed polyQ-AR, while preserving a protective androgen sensitivity.

Methods: A screening on Protein Data Bank repository (https://www.rcsb.org/) was performed to identify suitable AF2 domain-binding warheads. Provisional AF2-PROTAC prototypes were designed on the von Hippel-Lindau (VHL)-E3 Ubiquitin Ligase binder VH032, by changing the length, in terms of carbon atoms, of a hypothetical linker chain consisting of only methylene units. In order to identify the optimal linker length, prototypes were then submitted to PRosettaC, a free web-server computational protocol for the prediction of PROTAC-induced ternary complexes [4, https://prosettac.weizmann.ac.il/]. A panel of readily synthesizable AF2-PROTAC was then generated on the basis of the reactants available on the market.

Results: 3,3',5-triiodothyroacetic acid (Triac)[5] was identified as a recognized AR ligand, suitable for the design of a AF2 domain-oriented PROTAC. Eleven AF2-PROTAC prototypes, differing for linker length (C2 to C11) and anchor site to Triac, were designed and submitted to PRosettaC. The top-score prototype, according to PRosettaC results, had a linker length of C8. Accordingly, a panel of 3 readily synthesizable AF2-PROTAC candidates was proposed.

Conclusion: AF2-PROTAC candidates will be tested on a cell model of SBMA, involving PC12 cells stably expressing highly expanded polyQ-AR [6], in order to address an efficient reduction of the ligand dependent-nuclear accumulation whilst preserving the expression of AR-responsive genes.

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P012 | **Phthalic acid esters impair sperm acrosomal reaction through the likely upstream inhibition of phospholipase A2-signaling pathwaY**

Luca De Toni[1], Ilaria Cosci[2], Stefano Dall'Acqua[3], Stefania Sut[3], Andrea Garolla[1], Alberto Ferlin[1]

[1]Department of Medicine, University of Padova, Padova, Italy; [2]Veneto Institute of Oncology IOV, Padova, Italy; [3]Department of Pharmaceutical Science, University of Padova, Padova, Ital

Background: Phthalic acid esters (PAEs) have been claimed as endocrine disruptors, influencing male fertility through the involvement of major endocrine axis [1]. However, the detection of PAEs in semen from idiopathic infertile males suggest possible direct mechanisms of cell toxicity. Interestingly, low molecular weight (LMW) PAEs have been recently recognized to bind phospholipase-A2 (PLA2) [2], whose major role in sperm function is recognized [3]. In this study we aimed to correlate sperm function with semen levels of PAEs in a group male subjects attending our center. In addition, the possible involvement of PAEs on PLA2 signaling was evaluated in vitro.

Methods: Semen samples were obtained from 100 male patients attending the Unit of Andrology and Reproductive Medicine, University Hospital of Padova, (Italy), 22 of which having a recognized history of idiopathic infertility. Semen from 12 normozoospermic healthy donors were used for in vitro assays. Acrosome reaction, triggered by A23187 Ca-ionophore, was evaluated through CD46 expression by flow cytometry. Semen level was quantified by liquid chromatography-mass spectrometry (LC-MS). Sperm accumulation of phthalates upon in vitro exposure to a standard mix of PAEs was also performed by LC-MS. The binding of PAEs to human PLA2 was modelled by a computational approach. In vitro inhibition of purified bee venom PLA2 (bvPLA2) by PAEs was assessed by commercial fluorescent substrate-based assay.

Results: Compared to fertile subjects, infertile patients showed reduced levels of acrosome reaction upon A23187 triggering. The seminal plasma of 38 out of 100 patients showed at least one PAE at a concentration greater than the level of detection. Infertile patients were more represented in the PAEs positive group (13/38) compared to the PAEs negative group (9/62, $p = 0.0266$). In vitro exposure sperms to PAEs showed an increased cell

accumulation of high molecular weight (HMW) compounds, compared to LMW ones. Computational docking procedures showed the possible binding of different PAEs to PLA2 with similar estimated stability. Accordingly, experimental inhibition assay on bvPLA2 showed similar IC50 values of PAEs, ranging from 3.98 to 5.52 nM. Finally, in vitro exposure to HMW-PAEs was associated with significant reduction of PLA2-mediated acrosome reaction which was largely restored by the incubation with PLA2-related product arachidonic acid.

Conclusion: Our data are suggestive of a novel mechanistic model of PAEs interference on sperm function, through the inhibition of PLA2-mediated signaling. According to this model, the inhibitory efficacy of the specific PAE is possibly linked to the corresponding cell accumulation.

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P013 | Concentration of bioelements and heavy metals in serum and bone tissue in aging men

Aleksandra Rył[1], Aleksandra Szylińska[1], Andrzej Bohatyrewicz[2], Alina Jurewicz[2], Iwona Rotter[1]

[1]Department of Medical Rehabilitation and Clinical Physiotherapy, Pomeranian Medical University, Szczecin, Poland; [2]Department of Orthopedics, Traumatology and Orthopedic Oncology, Pomeranian Medical University, Szczecin, Poland

Introduction: The relationship between the concentration of macro- and microelements in various tissues of the body and metabolic disorders requires in-depth research. Comparing the concentration of elements in bone and serum can provide valuable information on the relationship between these tissues in the human body. The aim of the study was to investigate the relationship between the concentration of bioelements (Zn, Cu, Fe, Cr, Mg, Se) and heavy metals (Pb) in serum and tissue and the values of VAI, LAP and BMI indicators.

Material and Methods: The study included 151 men aged 60 and 75 years qualified for hip arthroplasty. Bone tissue material was collected from patients during the arthroplasty procedure and venous blood was collected. The concentration of Mg, Zn, Cu, Cr, Fe, Se and Pb was determined in both tissues. For the purposes of carrying out the analyzes taking into account the indices of body mass and the accumulation of adipose tissue, the ROC curve was used in the first stage of the analysis to distinguish the research groups. The presence of MetS was the state variable in the analysis. The analysis showed that the cut-off point for the value of the body mass index (BMI) was 28.63 kg/m^2 ($p < 0.007$), for the visceral adiposity index (VAI) 2.27, and for the lipid accumulation product (LAP) 67.09.

Results: Analyzing the VAI index, it was shown that the Mg concentration in bone tissue was significantly higher in men with higher VAI values. Similarly, analyzing the relationship between the concentration in bone tissue and the LAP index, it was shown that both Mg concentration and Zn concentration were higher in patients with higher values in the analysis of this index. A multivariate logistic regression analysis with age adjustment was performed. It has been shown that there is a correlation between the serum Zn concentration and the VAI cut-off point. The relationship between the concentration of the examined elements in the bone tissue was observed in relation to the LAP cut-off point analysis. The value of this index was related to the concentration of Mg, Zn and Cu in bone tissue.

Conclusions: In our research, we have shown that the relationship between the concentration of selected bioelements and heavy metals in serum and in bone, and the values of VAI, LAP and BMI indicators is not the same in both analyzed tissues. Searching for simple and diagnostically easy indicators that may indicate the impact of metabolic disorders on the structure and functions of bone tissue is an important factor from the point of view of the health of the society and quick and effective prevention of bone tissue diseases. The conducted research shows that the value of the LAP index may be a good predictor of changes in the concentration of elements in the bone tissue in men. It is a metabolic index that is easy to determine on the basis of basic blood tests, therefore it deserves attention as the most reliable of the analyzed indexes.

P014 | Male infertility coexists with oxidative stress in semen and decreased sperm genomic integrity irrespective of leukocytospermia

Kamil Gill[1], Marta Grabowska[1], Tomasz Machalowski[1,2], Patryk Harasny[1,3], Mariola Marchlewicz[4], Olimpia Sipak[5], Maciej Kurpisz[6], Monika Fraczek[6], Rafal Kurzawa[7,8], Malgorzata Piasecka[1]

[1]Department of Histology and Developmental Biology, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland; [2]Department of Perinatology, Obstetrics and Gynecology, Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Police, Poland; [3]Department of Urology and Urological Oncology, Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Szczecin, Poland; [4]Department of Skin and Venereal Diseases, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Police, Poland; [5]Department of Obstetrics and Pathology of Pregnancy, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Szczecin, Poland; [6]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [7]Department of Gynecology and Reproductive Health, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland; [8]TFP Fertility Vitrolive in Szczecin, Szczecin, Poland

Because oxidative stress (OS) in semen results in low sperm quality and therefore in many cases contributes to male infertility (30–80% of cases) and can be caused by a different factors

(endo- and exogenous), our research was designed to verify the relationship between male infertility, basic semen characteristics (with respect to detailed sperm morphology), sperm DNA fragmentation (SDF), oxidation-reduction potential in semen and leukocytospermia.

Research has been carried out on ejaculated sperm cells obtained from infertile men with leukocytospermia (n = 47) or without leukocytospermia (n = 77) and men with proven fertility and without leukocytospermia (n = 80). Standard semen analysis was performed according to WHO recommendations (2010). SDF was assessed using sperm DNA dispersion test (SCD), while oxidation-reduction potential in semen, expressed as mV/106 sperm cells/mL (sORP) was evaluated by means on Male Infertility Oxidative System – MiOXSYS®.

Infertile subjects with/without leukocytospermia had significantly lower basic semen characteristics than men with proven fertility. Sperm concentration, total sperm count, sperm morphology, progressive motility and vitality were lower, in turn TZI (teratozoospermia index) and number of sperm cells with head, midpiece, tail defects and with excess residual cytoplasm was higher in both infertile groups. Furthermore, infertile men, irrespective of leukocytospermia, had significantly higher SDF (medians: 24.00% and 19.00%, respectively) and sORP (medians: 2.05 and 4.90, respectively) than fertile control group (medians: 13% [SDF] and 0.62 [sORP]). Receiver operating characteristic (ROC) analysis revealed that a threshold of >13% SDF was satisfactory predictive value (AUC = 0.733) and a threshold of >1.40 sORP was good predictive value (AUC = 0.857) for discriminating between infertile and fertile men. Moreover, the prevalence of >13% SDF and >1.40 sORP was significantly higher in both infertile leukocytospermic and non-leukocytospermic groups as compared to control group. What is important, infertile men with leukocytospermia and without leukocytospermia had almost 6-fold higher risk (OR – odds ratio) for >13% SDF and had almost 10-fold and 28-fold higher risk for >1.40 sORP respectively than fertile men. In turn, both infertile groups no differed in basic semen characteristics and SDF but differed in sORP. Unexpectedly the subjects with leukocytospermia had lower sORP. Moreover, in the latter group the prevalence of men with >1.40 sORP and OR for >1.40 sORP was lower than in group of infertile men without leukocytospermia. SDF and sORP negatively correlated with sperm number, morphology, motility and vitality, while positively with sperm head and midpiece defects. Only sORP positively correlated with sperm tail defects and with immature sperm cells with excess residual cytoplasm. There was positive correlation between SDF and sORP.

The findings indicated that: 1/ There was relationship between male infertility, SDF and OS in semen, 2/ In infertile men a clinically significant risk of SDF and risk of OS was irrespective of leukocytospermia; in study group this clinical condition no contributed to increase in SDF and sORP, 3/ Complementing the basic seminological analysis with the assessment of sperm genomic integrity and OS, as well as the introduction of antioxidant therapy should be independent of leukocytospermia.

P015 | Medium-related effects on hypothermic storage of rat testicular cells

Ahmed Reda[1], Rageia Elfageih[1], Kristín Rós Kjartansdóttir[1], Valentina Pampanini[1,2], Olle Söder[1], Jan-Bernd Stukenborg[1]

[1]*NORDFERTIL Research Lab Stockholm, Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska Institutet, and Karolinska University Hospital, Solna, Sweden;* [2]*Bambino Gesu'Childrent's Hospital, Roma, Ital*

Spermatogonial stem cells (SSCs) are responsible for maintaining spermatogenesis. Any damage to these cells, caused by genetic diseases or gonadotoxic radio- and chemotherapy doses, can lead to infertility. Fertility preservation methods, such as sperm cryopreservation, are effective techniques to preserve adult patients' fertility. However, sperm cryopreservation is not an option for prepubertal patients, since they are not able to produce sperm. Thus, testicular tissue cryopreservation is a promising technique that can lead to fertility preservation for prepubertal patients. Testicular tissue cryopreservation is only available in a few hospitals worldwide. Moreover, testicular tissue biopsied in distance hospitals requires beneficial transportation and preservation conditions to protect the biopsied tissue quality. Thus, this study aims to investigate the impact of short-term storage (up to 24 hours), hypothermic conditions (4-8°C), and selected basal culture media or balanced salt solutions on prepubertal rat testicular tissue samples. The potential effects of various conditions were monitored using morphological tissue analysis, radioimmunoassay to measure testosterone levels as well as gene expression profiles via TaqMan Low-Density Array (TLDA) card analysis of 96 genes. Seven dpp-old prepubertal rat testicular cells were stored in six different basal culture media or phosphate-buffered saline for 12 and 24 hours in hypothermic conditions, which were assessed. A variation in energetic, apoptotic, and angiogenic expression levels was observed after 12 hours, and it became more prominent after 24 hours. However, no significant differences were seen in germ and testicular somatic cells' tissue morphology and gene expression profiles. Moreover, testosterone production levels were not significantly changed in short-term storage in hypothermic conditions in any of the used basal media. In conclusion, this study shows that the transfer of testicular tissue in short-term hypothermic conditions for up to 24 hours does not affect the expression of testicular cell-specific genes; however, it has a moderate effect on the expression of certain genes specific to general cellular functions.

P016 | Strategically positioned resident immune cells shape distinct immunological landscapes along the murine epididymis

Christiane Pleuger[1,2], Dingding Ai[1,2], Minea Hoppe[1,2], Laura Winter[1,2], Daniel Bohnert[1,2], Dominik Karl[1,2], Stefan Guenther[3], Crystal Kantores[4], Slava Epelman[4], Monika Fijak[1,2], Ralf Middendorff[2,5], Kate L. Loveland[6,7], Mark P. Hedger[6,7], Sudhanshu Bhushan[1,2], Andreas Meinhardt[1,2,6]

[1]Institute of Anatomy and Cell Biology, Unit of Reproductive Biology, Justus-Liebig-University of Giessen, Giessen, Germany; [2]Hessian Center of Reproductive Medicine, Justus-Liebig-University of Giessen, Giessen, Germany; [3]ECCPS Bioinformatics and Deep Sequencing Platform, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany; [4]Toronto General Hospital Research Institute, University Health Network, Toronto, Canada; [5]Institute of Anatomy and Cell Biology, Unit of Signal Transduction, Justus-Liebig-University of Giessen, Giessen, Germany; [6]Centre of Reproductive Health, Hudson Institute of Medical Research, Clayton, Australia; [7]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash Medical Centre, Monash University, Clayton, Australia

The epididymis constitutes an important transition zone for post-testicular sperm maturation in which both immunosuppressive mechanisms to protect immunogenic spermatozoa, and immunoreactive mechanisms to resist invading pathogens, are required. Previous investigations revealed that the proximal and distal regions of the epididymis react differently towards inflammatory stimuli. While the proximal regions (initial segment, caput) remain mostly unaffected, the distal regions (corpus, cauda) are more susceptible to inflammation-associated tissue damage, which severely affects male fertility. We hypothesized that strategic positioning of resident immune cells along the organ mediates this dichotomy of immune responses. Using a mouse model of acute bacterial epididymitis (elicited by intravasal injection of uropathogenic Escherichia coli), we analyzed disease progression (1-21 days) at cellular and transcriptional levels. The distally located cauda showed a strong immune response characterized by a massive influx of neutrophils (Ly6G+) and monocyte-derived macrophages (Ly6C+CD11b+F4/80+MHC-II+, assessed by flow cytometry) with concomitant fibrosis and loss of epithelial integrity, resulting in extravasation of spermatozoa (assessed by Masson-Goldner trichrome staining). RNASeq data indicated that a switch from innate to adaptive immunity occured around day 10 post-infection within the cauda. In contrast, epithelial integrity remained intact in the caput and initial inflammatory responses were immediately resolved. To understand the physiological prerequisites that govern these distinctly different immune responses, we investigated the heterogeneity of extravascular immune cells that reside within epididymal regions by single cell RNA sequencing of CD45+ leukocytes and flow cytometry. In total, 12 distinct lymphocyte subpopulations were identified, displaying striking differences in their regional distribution The proximal regions (initial segment, caput) are predominantly populated by intra- and peri-epithelial CX3CR1hi macrophage subsets (~80% of total CD45+ cells) that express numerous homeostatic signature genes that are crucial for maintaining tissue integrity. The distal regions (corpus, cauda) are populated by a heterogenous network of different immune cell populations (monocytes [10%], macrophages [40%], conventional dendritic cells 1 and 2 [10 and 10-20% respectively], $\alpha\beta$ T cells, $\gamma\delta$ T cells [10%], NK cells [10%], B cells [2-5% of CD45+ cells]) that coexist within the intraepithelial and interstitial compartment. This indicates a potential in situ crosstalk to maintain mucosal immune homeostasis and to facilitate rapid responses towards invading pathogens. These findings suggest that resident immune cells are strategically positioned along the epididymal duct to provide different immunological milieus required to maintain tissue integrity essential for sperm maturation, and to adequately resist invading pathogens ascending via the urogenital tract. Overall, these data provide the first atlas of CD45+ leukocytes at single cell resolution within the murine epididymis that will serve as an important platform for dissecting functional roles of individual populations in epididymal immune regulation.

P017 | Novel insights of whole exome sequencing in non-obstructive azoospermia patients

Baiba Alksere[1], Agrita Puzuka[1], Linda Gailite[1], Janis Kristaps Vasiļonoks[1], Elvita Penka[1], Violeta Fodina[2,3], Juris Erenpreiss[1,2]
[1]Riga Stradins University, Riga, Latvia; [2]iVF Riga Clinic, Riga, Latvia; [3]University of Latvia, Riga, Latvia

Objectives: Male infertility is a multifactorial pathology suggested as the underlying factors in majority of cases of severe male infertility, especially non-obstructive azoospermia. The recent emergence of next-generation sequencing (NGS) offers an opportunity to analyze many genes at once and to develop various bioinformatic approaches, including variant burden investigation. Gene-based burden tests identify a set of rare variants in a given gene by comparing case and control cohorts. Another method – Gene Set Enrichment Analysis (GSEA) – analyzes gene networks, that share common regulation, biological function or chromosomal location. There is still a lack of reports on WES implementation with burden tests, gene network analysis and routine clinical diagnostics in male infertility research. The purpose of this study was to investigate the potential of exome sequencing in idiopathic azoospermia cases.

Methods: Whole exome sequencing was performed on 21 non-obstructive azoospermia patients. A gene set of previously described and novel candidate genes of azoospermia was compiled.

Samples were sequenced using the Twist Comprehensive Exome Panel. The resulting sequences were mapped against the human genome GRCh38 reference sequence using BWAMEM. Copy number variations (CNVs) were annotated using AnnotSV. Samples were analyzed and filtered with the Illumina's BaseSpace Variant Interpreter. Variants considered as pathogenic and likely pathogenic were confirmed by Sanger sequencing. Genetic burden test was performed with TRAPD. P value < 0.05 was considered significant. Protein interactions were investigated with ConsensusPathDB, STRING and CytoScape.

Results: SNV analysis detected two genetic variants of unknown significance (VUS) in genes, affecting the hypothalamic–pituitary–gonadal axis: NR5A1 and FGFR1. Clinical investigation did not demonstrate hypogonadism in the subject group. NM_004959(NR5A1):c.763C>T was interpreted at first as likely pathogenic (LP), according to ACMG guidelines. The patient's phenotype did not reflect the expected phenotype. In CNV investigation, VUS deletion in AD genes TUBG1 and TUBG2 was found (seq[GRCh38] 17q21.2(42613632-42659961)x1).

No previously described known pathogenic genetic variants were found.

Genetic variant burden was elevated in 1473 genes. 302 genes with increased loss-of-function (LoF) variant set were present in more than one sample. Variant burden of genes TKFC, DPM1, UBE2J2, MTCH2, GCLC, NPIPB11, OR2T33, POTEG was elevated in > 50% of samples. Over-representation analysis with pathway based set of genes with high variant burden demonstrated 26 pathways, half of the pathways (13) being involved in sperm development, especially sumoylation (4). Over-representation analysis with protein complex-based sets obtained 14 protein sets, all involved in DNA repair and genomic integrity. STRING interaction analysis between genes with high variant burden showed two genome instability networks.

Conclusions: Based on a preselected diagnostic gene panel, we identified four VUS in exomes, involved in hypothalamic–pituitary–gonadal axis. In patients with azoospermia, an increased burden of genetic variants is observed in interrelated genes involved in genome instability and spermatogenesis. These findings can add supporting information to the knowledge base of infertility diagnostics. WES as a routine diagnostics method in azoospermic patients calls for the further investigation.

P018 | The role of mitochondria-dependent apoptosis in human sperm damage in men exposed to genital heat stress

Monika Fraczek[1], Marta Budzinska[1], Marzena Kamieniczna[1], Lukasz Wojnar[2], Kamil Gill[3], Malgorzata Piasecka[3], Maciej Kurpisz[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Clinic of Urology and Oncological Urology, Poznan University of Medical Sciences, Poznan, Poland; [3]Department of Histology and Developmental Biology, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland

There are substantial premises that oxidative stress and apoptosis of the germ cells may be involved in the male gonad response to the genital heat stress. However, the significance of the local thermogenic factor for human ejaculated sperm has not been properly recognized in many aspects. In the present retrospective study, we investigated a link between sperm apoptosis and standard semen parameters in males exposed and not exposed to local hyperthermia. The studied cohort included 198 infertile males and volunteers 20–41 years of age. Based on the collected clinical and survey data, the men were qualified for one of the following research subgroups: professional drivers, infertile men with varicocele, infertile men not exposed to genital heat stress, and fertile men, which served as the control group. Freshly ejaculated semen samples were collected in sterile containers after 3–5 days of sexual abstinence. Within 60 minutes of ejaculation and liquefaction, standard semen analysis was performed manually according to the World Health Organization 2010 criteria. Besides, a set of classic (phosphatidylserine externalization, mitochondrial transmembrane potential, DNA fragmentation), as well as non-classic (phospholipid membrane scrambling, mitochondrial ROS generation) sperm apoptotic markers, were simultaneously investigated. In the present study, standard semen parameters including sperm density, motility, and morphology were statistically lower in all the studied subgroups compared to the control. As for other semen markers, a statistical difference was observed between all the studied subgroups and the fertile men in the percentage of sperm with damaged membrane architecture (merocyanine 540 negative cells), sperm with polarized mitochondria (JC-1 positive cells), and sperm with DNA fragmentation (TUNEL-positive cells). In turn, the sperm mitochondrial ROS generation (MitoSox Red positive cells) was significantly enhanced only in the groups exposed to genital heat stress compared to the control. A comparative analysis of the studied parameters allowed us to show some moderately strong or strong correlations of conventional semen parameters (motility, viability and/or morphology) with the percentage of merocyanine 540 negative sperm in the group of drivers and with the percentage of JC-1 positive sperm in the varicocele group. Moreover, only in the group of drivers, the percentage of MitoSox Red positive sperm was positively associated with the percentage of TUNEL-positive sperm. This study demonstrated for the first time a strong apoptosis-like phenotype initiated by increasing sperm mitochondrial ROS generation and decreasing sperm motility in men exposed to prolonged genital heat stress. These findings suggest that antioxidants may be useful as a potential treatment option for male subfertility/infertility caused by heat-induced oxidative stress.

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P019 | Haploid selection causes allele frequency divergence among sperm from within the same ejaculate

Daniel Marcu[1], Ghazal Alavioon[2], Kristian Almstrup[3], Simone Immler[1]

[1]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom; [2]Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden; [3]Department of Growth and Reproduction, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

Males produce millions of sperm in a single ejaculate, but only very few reach the fertilisation site and only one sperm ultimately fertilises the egg. The bottleneck in sperm numbers from ejaculation to fertilisation offers the opportunity for selection to act on specific sperm phenotypes and genotypes. However, to what extent sperm traits are affected by the haploid sperm genotype is currently unknown. Using in vitro assays to separate sperm within an ejaculate and likelihood ratio tests to examine allelic frequencies at heterozygous paternal sites, we show that the genetic basis of natural variation in fertile sperm changes substantially under selection. Phenotypically diverse sperm pools differed genetically across the entire genome. Our results demonstrate that sperm phenotypes are affected by their underlying genotypes and that even a short window of haploid selection may favour advantageous genotypes to be passed down to the next generation. We anticipate that sperm genotyping, and selection might be advantageous tools to improve assisted reproductive technology and

the health and quality of domesticated animals and livestock as well as overcome fertility issues in humans.

P020 | The role of Notch signaling pathway in mediating androgen-regulated cellular processes in seminiferous epithelium

Anna Hejmej, Alicja Kaminska, Sylwia Lustofin, Joanna Cyran, Malgorzata Brzoskwinia, Michal Duliban, Barbara Bilinska
Department of Endocrinology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland

The Notch pathway is a conserved cell-cell signaling mechanism mostly limited to cells that are in direct contact, such as Sertoli and germ cells in seminiferous epithelium. It was previously demonstrated that in vivo inhibition of Notch signaling in adult mouse testis increased the apoptosis of spermatocytes and induced abnormal spermatid elongation and its premature release (Murta et al. 2014; PLoS One, 9:e113365). Further, our studies showed that blockade of Notch signaling up-regulates androgen receptor (AR) expression in Sertoli cells (Kamińska et al. 2020; Andrology; 8:457-472). To get insight into the molecular mechanisms involved in these effects, we explored the interaction between Notch pathway and AR signaling in Sertoli cells and examined its role in the control of genes encoding proteins involved in (1) ectoplasmic specializations: nectin cell adhesion molecule 2 (Nectin2) and afadin (Afdn), (2) actin remodeling during spermiogenesis: actin-related protein 3 (Arp3) and epidermal growth factor receptor pathway substrate 8 (Eps8), and (3) induction of germ cell apoptosis: Fas ligand (Fasl), Fas cell surface death receptor (Fas), B cell leukemia/lymphoma 2 (Bcl2) and BCL2 associated X (Bax) in seminiferous epithelium.

The study was performed using Sertoli cell line TM4 and rat testicular explants that were exposed to testosterone, immobilized recombinant ligands for Notch receptors: Delta-like (DLL1, DLL4) and Jagged (JAG1), or Notch pathway inhibitor (DAPT). Interaction between Notch target protein, Hairy/enhancer-of-split related with YRPW motif protein 1 (HEY1), and Ar promoter was tested using chromatin immunoprecipitation. Gene expressions were determined with RT-qPCR.

HEY1 preferentially binds to E-box elements and we found mouse Ar promoter contains 3 E-boxes in a region spanning bp -1494 to -926. Chromatin immunoprecipitation followed by qPCR analysis demonstrated that HEY1 interacts with the Ar promoter in TM4 Sertoli cells. Interaction was significant compared with IgG alone and blockade of Notch pathway with DAPT resulted in decreased binding of HEY1 in comparison to the vehicle-treated cells. Next, we found that in TM4 cells the expression of Nectin2, Afdn, Arp3, and Eps8 was up-regulated by testosterone, whereas Fasl was down-regulated, which confirmed androgen-dependent regulation of these genes. Further, it was demonstrated that the expression of these genes was down-regulated by exposure to immobilized Notch ligands: Nectin2 by DLL4, Afdn by DLL1 and JAG1, Arp3 by DLL4 and JAG1, Eps8 and Fasl by DLL4 and JAG1. The addition of DAPT abolished the effects induced by the ligands, increasing expression of the genes. These regulatory mechanisms were confirmed in physiological testicular environment. In rat testicular explants the expression of Ar, Nectin2, Afdn, Arp3 and Eps8 was up-regulated following testosterone exposure and Notch pathway inhibition. In addition, DAPT increased Fasl and Fas expression as well as the Bax:Bcl2 ratio.

Taken together, our study provides novel data on the role of Notch pathway in the regulation of androgen-dependent processes in seminiferous epithelium. First, direct control of Ar expression by Notch effector HEY1 was revealed. Second, several genes involved in spermiogenesis and germ cell apoptosis were identified as targets for Notch pathway.

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P021 | Complete meiosis in rat prepubertal testicular tissue under in vitro sequential culture conditions

Justine Saulnier[1], Mathilde Soirey[1], Nasreddine Kébir[2], Nathalie Rives[1], Christine Rondanino[1]
[1]*INSERM, U1239, Adrenal and Gonadal Pathophysiology team, Laboratory of Neuroendocrine Endocrine and Germinal Differentiation and Communication, Rouen University Hospital, Rouen Normandy University, Rouen, France;* [2]*Normandie Univ, INSA Rouen Normandie, Laboratoire PBS, UMR CNRS 6270 & FR 3038, Saint-Etienne-du-Rouvray, France*

Testicular tissue cryopreservation before gonadotoxic treatments allows fertility preservation in children suffering from cancer. Fertility restoration strategies, in particular in vitro maturation of prepubertal testicular tissue, are being developed mainly in animal models. The rat, widely used in biomedical research, including in reproductive biology, is a relevant model.

The aim of the present study was to determine whether sequential two-step culture protocols can improve the efficiency of rat in vitro spermatogenesis.

Rat prepubertal testicular tissues were cultured on agarose gels with either a one-step or two-step protocol, with or without PDMS ceiling chips. The progression of spermatogenesis, the ratio of germ cells to Sertoli cells, cell proliferation, seminiferous tubule area and intratubular cell density were assessed by histological and immunohistochemical analyses. TUNEL assays and PNA lectin labelling were performed to analyse the DNA integrity and the differentiation stage of in vitro produced spermatids.

Sequential two-step protocols allowed the production of spermatids with a higher efficiency compared to the one-step culture protocol. Most of in vitro produced spermatids contained unfragmented DNA and were at an early stage of differentiation. Rare elongating spermatids could be detected in the cultured explants. Although a complete in vitro spermatogenesis could not be obtained with PDMS ceiling chips, the entry into meiosis was promoted in one-step organotypic cultures.

A complete in vitro meiosis and the beginning of the elongation phase of spermiogenesis were obtained in the rat model using sequential culture methods. Further work will be necessary to identify the culture conditions allowing the completion of spermiogenesis, before considering potential clinical applications of this procedure.

P022 | Vasovasostomy: long-term experience

Gabriel Teja, Antonio Guijarro, Rebeca Davo, Ignacio Velazquez, Paula Jimenez, Claudia Escribano, Jesus Garcia
Hospital Universitario de Móstoles, Móstoles, Madrid, Spain

Introduction and Objectives: Vasovasostomy (VV) is the gold standard technique for vasectomy reversal. According to the published series, patency and pregnancy rates are on average 90% and 50%, respectively.

The aim of this study is to analyze our VV series and compare them with the published literature.

Materials and Methods: We review 33 cases of VV procedures performed in our department between January 2001 and November 2021. In all cases, the procedure is performed with a single non-resorbable monofilament microsuture. SPSS v.28.0 was used for statistical analysis.

Results: The mean age of patients undergoing VV surgery was 43.3 years (29–62 years). The mean time to vas-recanalization was 8.8 years (1–30 years). In 32 of the 33 patients, the main reason for undergoing VV was change of partner (one remaining patient underwent surgery for post-vasectomy pain syndrome). Grade II complications of the Clavien-dindo classification were observed in four cases (three orchitis and one scrotal hematoma), no grade III or higher complications were described. Patency was achieved in 25 patients (92%). Nine of the 33 patients were lost during follow-up. After detailed analysis of the results obtained, 22 of the 24 patients followed up tried to achieve conception after VV, with a successful pregnancy rate of 41% (nine patients).

Conclusion: Our patency rate is similar to the previously published one; however, the successful pregnancy rate is lower. VV, even complex, is a widespread and a reproducible technique which can be successfully performed in centres with low prevalence of vasectomy reversal.

P023 | Retinoid signaling in Sertoli cells regulates their immunoprotective function by controlling lymphocyte apoptosis

Alicja Kaminska, Sylwia Lustofin, Malgorzata Brzoskwinia, Joanna Cyran, Anna Hejmej
Department of Endocrinology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland

Testis is an immune privileged organ, which prevents the immune responses against autoimmunogenic germ cells and inflammation. In the testis, the cells responsible for immune tolerance are mainly somatic Sertoli cells, which form the blood-testis barrier and also produce immunosuppressive factors and anti-inflammatory cytokines. It has been shown that Sertoli cells, due to the production of immunosuppressive factors, inhibit proliferation and induce the apoptosis process of lymphocytes, preventing inflammation in the testicle and maintaining testis immune tolerance. Lymphocyte apoptosis is led mostly by interaction of Sertoli cells expressing Fas ligand (FasL) with Fas-bearing lymphocytes and induction of Bcl-2-associated X protein/B-cell lymphoma 2 (Bax/Bcl-2) pathway. It has been shown that retinoic acid (RA), vitamin A derivative, blocks the ex vivo apoptosis of peripheral blood lymphocytes and thymocytes. However, the role of retinoid signaling in regulating the immune privilege of the testes remains unknown. The aim of this study was to determine whether retinoids, acting via the retinoid acid receptors (RAR) and the retinoid X receptors (RXR), control immunomodulatory functions of Sertoli cells by influencing the secretion of anti-inflammatory/pro-inflammatory factors and lymphocyte physiology.

Experiments were performed using in vitro model of co-cultures of murine Sertoli cells and T lymphocytes. Agonists and antagonists of retinoic acid receptors were used to inhibit/stimulate retinoid signaling in Sertoli cells. RT-qPCR was used for detection of the anti-inflammatory/pro-inflammatory genes expression in Sertoli cells. Next, to determine the indirect role of retinoids in the control of T cell physiology, proliferation (MTT assay) and apoptosis (TUNEL assay) was analyzed in lymphocytes co-cultured with stimulated Sertoli cells. Lastly, western blot and RT-qPCR were used for detection of the expression of apoptosis-related factors, Fas, Bax, Bcl-2, Caspase 9 and Caspase 8 in lymphocytes and FasL in Sertoli cells.

Our results demonstrate that RA inhibits the expression of immunosuppressive genes (transforming growth factor β, interleukin-10, gelactin-1 and indoleamine 2,3-dioxygenase) and enhances the expression of pro-inflammatory factors (interleukin-1, interleukin-6, interferon γ and tumor necrosis factor α) in Sertoli cells. Significant increase in viability and decrease of the apoptosis rate in lymphocytes was found after RA treatment compared with control group. Moreover, our results indicate that RA blocks lymphocyte apoptosis acting through both RAR and RXR receptors. Western blot and qPCR analysis revealed that RA acting through RXR receptor regulate apoptosis by inhibiting FasL/Fas/Caspase 8 proteins. In addition, our results showed that RA also blocks apoptosis through Bax/Bcl-2/Caspase 9 pathway in RAR-dependent manner.

Collectively, the obtained results indicate that retinoid signaling negatively regulates immunologically privileged functions of Sertoli cells, crucial for ensuring male fertility. Retinoic acid acting through both receptors (RAR and RXR) inhibits lymphocyte apoptosis, which can be related to the development of autoimmunity and inflammation and in consequence infertility.

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P024 | Ancient origins of human male infertilitY

Joana M. Almeida[1,2], Margot J. Wyrwoll[3], Daniel Sobral[4,5], Ana Novoa[1], Ana S. Leocadio[1], Joana Bom[1], Moises Mallo[1], Frank Tüttelmann[3], Paulo Navarro-Costa[1,2]

[1]Instituto Gulbenkian de Ciencia. Oeiras, Portugal; [2]Instituto de Saude Ambiental, Faculdade de Medicina, Universidade de Lisboa. Portugal; [3]Institute of Reproductive Genetics, University of Münster. Germany; [4]Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon. Caparica, Portugal; [5]UCIBIO - Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

The genetics of spermatogenesis are widely considered divergent across species. Yet, ancient biological programs like meiosis retain an evolutionarily-conserved genetic basis that can provide new insight into human reproductive disease. By screening 920 evolutionarily conserved spermatocyte genes via in vivo RNAi in the fruit fly (Drosophila melanogaster) testis, we observed that the spliceosomal component RING finger protein 113 (dRNF113) is essential for spermatogenesis. The silencing of this gene, as insect male germ cells prepared to enter meiosis (driver: bam-GAL4), resulted in male sterility and a developmental block at the spermatocyte stage, with this cell type occupying an average of 64.1% of the entire testis area (vs. 12.3% in controls).

The insect dRNF113 gene has two copies in the primate lineage, one of them (RNF113B) being testis-specific. By analysing a whole exome sequencing database consisting of 74 cases of human male meiotic arrest, we identified an infertile man, part of a consanguineous family of Middle-Eastern ancestry, with a homozygous loss-of-function (LoF) variant in RNF113B. Remarkably, the results from the testicular biopsy of this man revealed an equivalent meiotic arrest phenotype to that observed in dRNF113-silenced fruit flies. Spermatocytes were the most advanced cell stage in 89.0% of all assessed human seminiferous tubules (vs. 9.0% in controls). The expression pattern of dRNF113 and RNF113B were also largely concordant between the two species. Our single cell RNA-Seq dataset of normal human spermatogenesis indicated that RNF113B was predominantly expressed at meiotic entry, peaking at the diplotene stage of prophase I. This mirrored the localization pattern of the insect dRNF113 protein, which revealed a substantial nuclear accumulation in primary spermatocytes.

Insect dRNF113 also underwent a duplication event in the mouse lineage, independent to that that gave rise to RNF113B in humans. By generating, using the CRISPR/Cas9 system, a whole-body knockout (KO) mouse for the duplicated copy (Rnf113a2), we observed that male homozygous Rnf113a2KO mice were sterile, had visibly smaller testes than wildtype (+/+) littermate controls, and their seminiferous tubules were largely devoid of germ cells except for the rare presence of spermatogonia and spermatocytes.

In summary, by using comparative biology, we revealed a novel genetic cause of human male infertility (LoF variant in RNF113B) shared between three different species: humans, mice, and fruit flies. The retention of the key spermatogenic role of the RNF113 proteins across evolution suggests that this gene is part of the ancient molecular toolbox responsible for animal male germ cell development. Although the actual benefit of comparative biology for the identification of new genetic causes of human disease is often a contentious topic, here, we show how a merger between basic and clinical research can significantly expand our knowledge of fundamental reproductive processes.

P025 | Expression of interleukin-6 and 18 in prostate tissue with benign hyperplasia depending on the presence of metabolic syndrome

Weronika Ratajczak[1], Maria Laszczyńska[2], Aleksandra Rył[3], Anna Lubkowska[1], Olimpia Sipak[4]

[1]Department of Functional Diagnostics and Physical Medicine, Pomeranian Medical University, Szczecin, Poland; [2]State University of Applied Sciences in Koszalin, Department of Nursing, Koszalin, Poland; [3]Department of Medical Rehabilitation and Clinical Physiotherapy, Pomeranian Medical University in Szczecin, Szczecin, Poland; [4]Department of General and Gastroentereological Surgery, Pomeranian Medical University in Szczecin, Szczecin, Poland

Benign prostatic hyperplasia (BPH) is a commonly diagnosed disease among aging men. The exact mechanism of the pathophysiology and development of BPH has not yet been fully understood. However, there are many risk factors that increase the possibility of the onset and progression of BPH. These include age, metabolic syndrome (MetS), insulin resistance and hyperinsulinemia, obesity, steroid hormones, and genetic factors. There is growing evidence linking the development of BPH to the presence of inflammation. When inflamed, prostate stromal cells produce pro-inflammatory cytokines and chemokines. It is indicated that interleukins and interferon γ occurring in increased concentrations are potential mediators involved in the development of BPH. A key cytokine involved in the development of BPH is IL-17, which is practically undetectable in prostate tissue without hyperplasia. Interleukin-17 activates NFκB-mediated expression of other cytokines, including IL-6 and IL-8, which act as growth factors for glandular and stromal cells of the prostate. IL-6 is an activator for the human androgen receptor (AR), and thus regulates the expression and secretion of prostate-specific proteins. Another interleukin that is also involved in the development of BPH is interleukin-18, which is a stimulator of prostate stromal cell growth.

In our study, the immuno-expression and localization of IL-6 and IL-18 in the prostate tissue with benign hyperplasia (n = 59) were analyzed in men diagnosed with BPH with regard to MetS. The results showed that MetS significantly contributed to an increase in the percentage of cells showing the cytoplasmic expression of IL-6 in the prostate stroma. Moreover, MetS also significantly increased the percentage of cells showing high expression of interleukin-18 in the glandular part of the prostate. In addition, the relationship between

short chain fatty acids (SCFAs) and tissue immunoexpression of IL-6 and IL-18 was analyzed. SCFAs are produced in the large intestine with the participation of the intestinal microbiota during anaerobic fermentation of exogenous components, such as dietary fiber and other undigested carbohydrates from food. These acids influence immune responses not only in the intestines but also in distant tissues. Their main function is to modulate immune mechanisms and provide immunity determined by the integrity of the intestinal mucosa. Our study has provided evidence that acetic acid is associated with the tissue expression of IL-6, in both the prostate stroma and epithelium of men with BPH and coexisting MetS. In contrast, it is not related to cells showing expression of IL-18 in the prostate of men with BPH.

Disturbance of the intestinal microflora and its impact on inflammation and prostate diseases have not yet been thoroughly analyzed. Neither has been the influence of SCFAs on the development of BPH. Few publications can be found in the literature on the influence of the intestinal microflora on the prostate. They mainly concern the influence of intestinal bacteria on the synthesis of metabolites and androgens, which may contribute to the development of prostate cancer in humans.

P026 | Lipidomic profile of human sperm membrane identifies a clustering of lipids associated with spermatogenetic and reproductive function independently of lipidemia

Andrea Di Nisio[1], Luca De Toni[1], Andrea Bellussi[1], Stefano Dall'Acqua[2], Stefania Sut[2], Alberto Ferlin[1], Andrea Garolla[1], Carlo Foresta[1]

[1]Department of Medicine and Unit of Andrology and Reproduction Medicine, University of Padova, Padova, Italy; [2]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Ital

The reductions of sperm motility and/or count are among the major causes of reduced fertility or infertility in men. Lipid composition of spermatozoa is important in determining their functional characteristics, in particular on motility, capacitation and acrosome reaction. Despite some variations between different mammalian species, in general the sperm plasma membrane shares a unique lipid composition: (1) an unusually low content of cholesterol (Chol); (2) a high proportion of lipids containing a fatty acid chain attached; (3) the sn-2 position of the glycerol esterified mainly with long-chain polyunsaturated fatty acids (PUFAs); and (4) a high content of sulfogalactolipids1.

Here we performed an untargeted analysis of membrane lipid composition of sperm from 41 infertile patients (mean age = 31.3 ± 8.3 years) undergoing standardised semen analysis2 and serum lipids evaluation by liquid chromatography-mass spectrometry (LC-MS) on methanol/dichloride-methane extracts from cell pellets. Twenty-one sperm membrane lipids were unambiguously identified by mass/charge ratio (m/z) and post source decay (PSD) spectra. Sulfogalactosylglycerolipid (SGG, aka seminolipid) was the most abundant component in the membrane, in agreement with previous studies3. In addition, we observed a significant proportion of PUFAs, such as C20:4 (arachidonic acid) and C22:6, representing 12% and 25% of total PUFAs in sperm membrane, respectively. Principal component analysis was computed for subsequent statistical analysis: the 21 lipids were grouped into five principal clusters, accounting for 76% of total variance. We then included these clusters as independent variables in multilinear regression analysis with following parameters: serum total Chol, HDL, LDL and tryglicerids, sperm progressive motility, normal morphology, viability and semen concentration. Interestingly, none of sperm lipid clusters was associated with serum lipid markers. Conversely, one of the five clusters (including SGG, phosphatidic acid and phosphatydilcholine C18:1 16:0) was significantly associated with increased sperm motility, viability and concentration. A second cluster (including Chol sulphate, phosphatydilcholine C14:0 18:1 and C22:6 16:1) was associated with sperm progressive motility. To further characterize these associations, we performed Pearson's correlations analysis between the components of the two aforementioned clusters and semen parameters. We found that SGG was positively associated with sperm concentration ($p = 0.002$) and motility ($p = 0.001$), and phosphatydilcholine C22:6 16:1 was positively associated with sperm concentration ($p = 0.005$), motility ($p < 0.001$) and viability ($p = 0.011$). On the other hand, Chol sulphate was negatively associated with sperm motility ($p = 0.04$).

Altogether these results underline the important role of seminal lipids, which act independently of serum lipids levels and could rather represent an independent marker of spermatogenetic and reproductive function. Dietary PUFAs and SGG, in particular, have acknowledged antioxidant functions4 and could therefore represent the most sensitive markers of sperm quality and testicular function. SGG, the most representative component, is mostly synthetized during the mitotic phase of spermatogenesis (primary and secondary spermathocytes), and to a lesser extent in round spermatids3, thus providing a reliable proxy of the spermatogenic process.

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P027 | Transcriptional profiles of mitochondrial dynamics markers in human spermatozoa are associated with different types of spermiograms

Tamara Tomanić[1], Isidora Starovlah[1], Ana Jeremic[2], Milan Perovic[2], Tatjana Kostic[1], Silvana Andric[1]

[1]Laboratory of Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging, Centre of Excellence for Reproductive Endocrinology and Signaling, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia; [2]ART Department, Clinic for Gynecology and Obstetrics "Narodni Front", Belgrade, Serbia

Infertility has become one of the greatest health issues today, affecting millions of people worldwide, with a significant contribution of

the male factor in many reported cases. Having in mind the increasing number of unexplained cases of infertile men in the peak of the reproductive period and the lack of an accurate test for assessment of spermatozoa functionality, World Health Organization expressed the need for the development of a new prognostic/diagnostic tool for detection of male infertility. Since mitochondria play important role in spermatozoa, regulating their homeostasis and functionality, it is reasonable to presume that they could be involved in these types of abnormalities and markers of their dynamics could be used as "mitochondrial-sperm-signature," to test spermatozoa functionality. Regardless of that, little is known about mitochondrial dynamics markers in human spermatozoa. Thus, the main objective of this research was to investigate the transcriptional profile of main mitochondrial dynamics markers in spermatozoa from the population of men, diagnosed with the most common types of sperm abnormalities. For that purpose, spermatozoa obtained from men participating in the National program of in vitro fertilization, diagnosed with normozoospermia, teratozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, were separated from seminal plasma, capacitated and incubated with acrosome reaction inducer – progesterone. After RNA isolation and complementary DNA synthesis, samples were subjected to real-time PCR analysis. During quantification, all results were normalized to a normozoospermic group, used as a control and GAPDH, as a reference gene. Results showed a significant increase in the level of PPARGC1A transcript in spermatozoa from teratozoospermic group comparing to normozoospermic. Conversely, the levels of PPARGC1B and MFN1 transcripts significantly decreased. The levels of NRF2, TFAM, MFN2, OPA1, FIS1 DRP1, PINK1 and PRKN remained unchanged. Although trends of stimulation in transcription were observed for PPARGC1A, PPARGC1B and PRKN in asthenoteratozoospermic and oligoasthenoteratozoospermic group, the levels of the changes were not significant. In the same samples, the levels of transcripts of other mitochondrial dynamics markers remained unchanged. Based on the obtained results it is evident that the markers of mitochondrial dynamics in human spermatozoa exerted different patterns depending on the type of spermiogram and the most remarkable changes were observed in the teratozoospermic group. However, it is important to point out that research was conducted on a small number of the samples (3–10 individuals per group), so the results should be considered preliminary.

P028 | Nanoscale insights into the human sperm chromatin through synchrotron light small-angle X-ray scattering (SAXS): fine-tuning the current model of in vivo paternal genome packaging

Alberto de la Iglesia[1], Cristián Huck-Iriart[2], Marc Malfois[2], Marina Leiva[1], Meritxell Jodar[1,3], Marta Guimerà[4], Juan Manuel Corral[4,5], Rafael Oliva[1,3], Judit Castillo[1]

[1]Molecular Biology of Reproduction and Development Research Group, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Fundació Clínic per a la Recerca Biomèdica, Universitat de Barcelona (UB), Barcelona, Spain; [2]ALBA SYNCHROTRON, BL11 NCD-SWEET Beamline, Cerdanyola del Vallès, Spain; [3]Biochemistry and Molecular Genetics Service, Hospital Clinic, Barcelona, Spain; [4]Institute of Gynaecology, Obstetrics, and Neonatology, Hospital Clínic, Barcelona, Spain; [5]Department of Urology, IDIBAPS, Hospital Clínic, Barcelona, Spain

The sperm cell acquires a unique chromatin structure, tightly compacted and transcriptionally silent, thanks to the presence of the small and extremely basic protamines. This exceptional nuclear compaction occurs during the spermiogenesis through the synchronized process of nucleohistone-to-nucleoprotamine transition, and is essential for a proper sperm functionality. The uniqueness of this structure has attracted interest over the years, and the application of a wide range of methodologies allowed stablishing the current model of sperm chromatin packaging. In mature human sperm, an 85–95% of the DNA is packaged by protamines, while the rest remains associated with histones. Electron and atomic force microscopy revealed that protamine-DNA complexes organize into supramolecular toroidal structures. However, there is not enough evidence about how the human sperm chromatin structure behaves in solution within the context of the whole nuclear compartment.

In the present work, we apply, for the very first time, SAXS from synchrotron light to describe how is the sperm nucleus constituted in solution, in purified nuclei (n = 10) and histone-depleted nuclei (n = 11) from human control patients. Besides, we studied how different naturally impaired chromatin statuses could impact the chromatin structure, such as altered protamine ratio (P1/P2, n = 4) and double-strand DNA (dsDNA) breaks (n = 6). SAXS was performed in the BL11 NCD-SWEET beamline from the ALBA Synchrotron Light source - beam energy 12.4 keV, detector distance of 6.7 m, Pilatus 1M detector (Dectris, Switzerland), 60 frames of 0.5 s.

Remarkably, we provide evidence of in vivo fractal-like supramolecular aggregates in the human sperm chromatin, arising from well-defined small subunits (radius r ~7.5 nm) which were stable between all groups, even in histone-depleted nuclei. The slightly bigger size compared to measurements already reported of in vitro reconstituted nucleoprotamine complexes (fractal-like supramolecular aggregates from small subunits of r = 2.5 nm) could be the result of a more complex in vivo conformation of the chromatin fiber.

At the supramolecular level, patients with altered P1/P2 did not show significant differences on the supramolecular structures compared to controls. This could indicate that the protamines would have redundant functions, compensating the alteration to properly compact the sperm chromatin. Strikingly, a significant increase of the Radius of gyration (Rg) of the supramolecular aggregates was found in patients with dsDNA breaks compared to controls. Although it should be deciphered whether the dsDNA breaks are cause or consequence of this alteration, our findings reinforce the idea that genomic and chromatin integrities are closely related.

To conclude, SAXS data from human sperm nuclei in solution suggest a clearly well-defined and monodisperse structure of ~10–15 nm size, not described up-to-date, as construction brick of larger supramolecular chromatin structures. Further studies are needed to unravel what is behind these highly-specific substructures packaging the paternal DNA, as well as to achieve the best adjusted model of supramolecular aggregation.

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P029 | Correlative study between molecular techniques for the detection of oxidative stress in semen samples of infertile patients

Marina Leiva[1], Judit Castillo[1], Gennaro Lettieri[2], Alberto de la Iglesia[1], Juan Manuel Corral[3], Rafael Oliva[1,2], Meritxell Jodar[1,4]

[1]Molecular Biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Genetics Unit, Department of Biomedicine, Universitat de Barcelona (UB) Barcelona, Spain; [2]Department of Biology, University of Naples Federico II, Naples, Italy; [3]Urology Service, Andrology Unit and Clinical Institute of Nephrology and Urology (ICNU) of the Hospital Clínic de Barcelona, Barcelona, Spain; [4]Biochemistry and Molecular Genetics Service, Biomedical Diagnostic Center, Hospital Clínic de Barcelona, Barcelona, Spain

Infertility represents a global growing health problem, the causes of which remain largely unknown in most cases. It currently affects about 186 million people worldwide. Recent studies estimate that global fertility has declined by 51% in the last century and sperm quality, in particular, has worsened in the last three decades. While socio-demographic factors may explain the decline in global fertility, other studies suggest that environment and lifestyle could impact both male and female fertility in a negative way. Although the male factor is involved in approximately half of the infertile couples, the current clinical evaluation of male fertility is mainly limited to the analysis of the semen parameters. However, it only exposes obvious alterations, such as those affecting sperm production or motility. Recent studies have highlighted oxidative stress (OS) as a potential factor involved in the 30–80% of men with unexplained infertility. Nevertheless, it is still unknown which methodologies would be optimal for its evaluation.

In this work, we carried out a pilot study with the aim of comparing different methodologies to detect OS and their correlation with DNA fragmentation. These results will allow the identification of complementary parameters to the seminogram that could be useful for the study of male infertility.

In particular, we have analyzed and correlated semen quality values with the reduction-oxidation potential of the semen (with MiOXSYS system), the amount of intracellular reactive oxygen species (EROS) (by using DCFH-DA colorant and flow cytometry) and the presence of cells with single and double strand DNA fragmentation (by performing alkaline and neutral comet assay, respectively). A total of 77 idiopathic infertile patients, including oligozoospermic, asthenozoospermic and oligoasthenozoospermic males, were enrolled in the study after informed consent.

A significant positive correlation between reduction-oxidation potential and the percentage of leukocytes in the semen (Spearman: 0.4144, p-value 0.0002) has been detected. These results highlight the critical impact of no spermatogenic cells in the semen, supporting previous evidences showing that leukocytes produce several orders of magnitude more EROS than mature spermatozoa. Furthermore, reduction-oxidation potential was found positively correlated with the values obtained by the alkaline comet assay (Spearman: 0.9048, p-value 0.002).

No correlation between extracellular and intracellular EROS in the semen samples was detected, suggesting that both EROS determinations are complementary and not a substitute for one another. Moreover, despite single strand DNA fragmentation seems to be correlated with reduction-oxidation potential of semen, double strand DNA fragmentation is suggested to be independent of OS. Nevertheless, further analyses increasing the number of samples are required to confirm the correlations found in this study.

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Corresponding authors: Meritxell Jodar (meritxell.jodar@ub.edu), Rafael Oliva (roliva@ub.edu).

P030 | Evaluation of spermatogonial cell populations in male childhood cancer patients

Hajar Ali Mohammed Ba Omar[1], Jingtao Guo[2], Kirsi Jahnukainen[1,3], Jan-Bernd Stukenborg[1]

[1]NORDFERTIL Research Lab Stockholm, Department of Women's and Children's Health, Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden; [2]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences & Institute of Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China; [3]New Children's Hospital, Paediatric Research Centre, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Spermatogenesis is a continuous process that relies on spermatogonial stem cells (SSCs) and offers a male the opportunity to generate sperm until late in life. If the SSC population is not formed or destroyed, permanent infertility follows. As a result, diseases or medical treatments might affect the SSC population, thereby causing male fertility (1). Historically, SSCs were characterized by their morphology as type Adark and Apale spermatogonia (2). However, advanced research techniques like single-cell RNA-sequencing have challenged this concept (3, 4). As a result, several SSC sub-populations have been identified, with expression profiles specific to stage (0 to stage 4) SSC sub-populations.

Nevertheless, detailed studies on protein expression of these spermatogonial markers have not been evaluated in human prepubertal testicular tissue.

To study human SSC subpopulations in postnatal testes, we included testicular tissue samples of boys who participated in the NORDFERTIL fertility preservation program. Patients were diagnosed with juvenile myelomonocytic leukemia (JMML; n = 4; age range: 1.3 ± 1.1 years) or acute myeloid leukemia (AML; n = 4, age: 8.7 ± 3.9 years). None of the patients had been exposed to alkylating agents. First, genes expressed by specific spermatogonial cell subtypes were identified using published single-cell RNA sequencing data. Then, based on this data, we used immunofluorescence staining for anti-PIWIL4 (spermatogonia (spg) at state 0), anti-ID4 (spg at state 0-1), anti-GFRa1 (spg at state 1), anti-UTF1 (spg at state 0-1), anti-FGFR3 (spg at state 0-2), and anti-C-KIT (spg at state 2-4) in 4% PFA-fixed testicular tissue sections (5μm thickness) to distinguish the different SSC subtypes.

In agreement with previous published single cell RNA-sequencing data (4), we were able to identify age-dependent expression of the SSC subtypes, showing higher numbers of spermatogonia in more advanced states in the older patient group. Higher spermatogonial counts per seminiferous tubule area for all states were observed in the older AML group (PIWIL4: 0.46 ± 0.78 cells/mm^2; ID4: 0.42 ± 0.24 cells/mm^2; UTF1: 0.25 ± 0.20 cells/mm^2; GFRa1: 0.42 ± 0.77 cells/mm^2; C-KIT: 0.02 ± 0.04 cells/mm^2) compared to the juvenile JMML group group (PIWIL4: 0.20 ± 0.23 cells/mm^2; ID4: 0.29 ± 0.21 cells/mm^2; UTF1: 0.07 ± 0.15 cells/mm^2; GFRa1: 0.19 ± 0.22 cells/mm^2; FGFR3: 0.37 ± 0.27 cells/mm^2; C-KIT: 0.0 ± 0.0 cells/mm^2).

Although the data indicate an age-related effect related to the expression profile of spermatogonial markers, future research is needed to elucidate the potential impact of disease-and treatment-related effects on the spermatogonial subtypes and their functionality later in life.

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P031 | Sperm DNA fragmentation measured by the alkaline and neutral versions of the comet assay in men with normozoospermia and pathozoospermia

Irena D. Haurylchyk, Nadezhda I. Ryabokon
Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk, Belarus

Sperm DNA fragmentation (SDF) analysis by the comet assay was included in the sixth edition of the WHO manual (WHO 2020). However, while the alkaline version of the assay (pH≥13) is commonly used for the examination of human semen, the application of the neutral version of this method is somewhat limited due to insufficient data. At the same time, the neutral comet assay allows detection of DNA double-strand breaks (DSBs), which may have a higher potential for negative reproductive effects compared with DNA single-strand breaks (SSBs) or alkaline-labile sites measured by the alkaline comet. The latter also detects DSBs, but in common pool with other DNA damage mentioned, that does not allow evaluation of DSBs effects separately.

The key objectives of the present study were as follows (1) to compare the results of the alkaline and neutral comets applied to the sperm samples of men with normo- or pathozoospermia; and (2) to assess DSBs in human sperm as a diagnostic tool for male infertility.

The frozen sperm samples of patients with normozoospermia (the control group, n = 34) and pathozoospermia (the pathospermia group, n = 44) were provided by the Republican DNA Bank of a Human, Animals, Plants and Microorganisms (Minsk, Belarus). The groups were previously defined following the WHO 2010 methods and reevaluated in the recent study using the WHO 2020 recommendations. Spermatozoa were studied using the neutral version of the comet assay for the DSB detection, and the alkaline comet assay was used to detect the total number of SSBs, DSBs and alkaline-sensitive sites. Wherein, DNA damage levels were analysed by visual scoring in arbitrary units and the number of cells with damaged (fragmented) DNA was estimated as the DNA fragmentation index presented in per cent.

This study showed that significantly higher ($p < 0.0001$) levels of SDF measured by the alkaline comet, as well as DSBs, are in the pathospermia group compared with the control. The data obtained were confirmed when the most numerous subgroup with astenozoospermia (n = 23) was statistically analysed. Thus, the conducted testing using the alkaline and neutral comets allowed discriminating men with reduced fertility from the fertile ones.

The proportion of DSBs was unexpectedly high, about 50 and 44% of the total pool of DNA damage in the control and pathospermia groups respectively. A preliminary analysis of DSBs induced in vitro in spermatozoa of the control group by the well-known radiomimetic bleomycin sulphate showed that the mean level of DSBs recorded in the pathospermia group correspond to the mutagenic effect of bleomycin sulphate at a concentration of 20-30 μg/ml. These data on DSBs demonstrate a high degree of DNA disintegrity in human sperm, especially in association with reduced fertility.

In conclusion it is worthy to note that the results of this work draw attention to the fact that not only the SDF of different origin measured by the alkaline comet assay, but also DSBs analysed separately are quite common in human sperm, and are strongly associated with pathozoospermia and may be used for male infertility diagnosis after additional studies.

P032 | What's in there? Looking at immune cells in the male reproductive tract

Vishnu Kumar[1,2], Vijay Kumar Singh[3], Monika Fijak[1,2], Christiane Pleuger[1,2], Marius Rohde[3], Andreas Meinhardt[1,2], Sudhanshu Bhushan[1,2]

[1]*Institute of Anatomy and Cell Biology, Justus Liebig University of Giessen, Giessen, Germany;* [2]*The Hessian Centre of Reproductive Medicine, Justus Liebig University of Giessen, Giessen, Germany;* [3]*Department of Pediatric Hematology/Oncology, Justus Liebig University of Giessen, Giessen, German*

The male reproductive tract is comprised of the testis, epididymis, vas deferens, the accessory sex glands (seminal vesicles, prostate, ampullary, bulbourethral, and preputial glands), urethra, and penis. Infectious agents such as bacteria and viruses gain access through the urethral opening of the penis and can ascend to the more distal parts of the genitourinary (GU) tract. Leukocytes play a significant role in defence from ascending pathogens, as well as also play important functions in normal tissue homeostasis. Therefore, we aim to characterize the immune cell subsets, particularly macrophages, and their functions longitudinally throughout the male reproductive tract during development as well as under normal and inflammatory conditions in adult mice. In this regard, we used a combined approach of flow cytometry and immunofluorescence analyses.

Flow cytometry-based analysis of single-cell suspensions from healthy male reproductive organs (MRO) of 9-10 week-old C57BL/6J mice revealed that ~60% (testis), ~55% (epididymis), ~17% (vas deferens), ~3% (seminal vesicles), ~35% (prostate), ~17%(urethra), and ~3% (penis) of all CD45+ leukocytes were macrophages (F4/80+CD11b+ cells). Other myeloid cells, including monocytes and dendritic cells, were observed in all MRO; however, the proportion varies significantly between the organs. Of note, among leukocytes, B cells (CD19+) comprise the predominant populations in the lower GU tract (penis ~14%), whereas macrophages are the dominant leukocyte population in the upper GU tract (testis ~60%).

Macrophages are the sentinel immune cells of most organs, and the proportion of macrophages amongst leukocytes changes during development. Thereby, we examined the postnatal development of macrophage populations in the entire MRO. All organs contain a distinct subset of F4/80hiCD11blo macrophages at the first week of age. At the 3rd week of age, in the epididymis, vas deferens, seminal vesicles, and prostate, a second population of F4/80loCD11bhi macrophages appears and numbers progressively increase until 9-10 weeks of age. However, in the testis and penis, the second population, which appears after three weeks of age, is different to the other organs, namely F4/80hiCD11bhi. Intriguingly, the expression of MHCII increases differently in the macrophage populations with age in the various organs of the MRO. Next, we examined the heterogeneity of macrophage populations by flow cytometry based on CD206 and MHCII expression. We observed four macrophage populations, namely CD206+MHCII−, CD206+MHCII+, CD206−MHCII+, and CD206−MHCII−, with varying proportions in the MRO. In conclusion, our results indicate that the MRO contains distinct subsets of macrophage populations, likely as a reflection of fulfilling organ-specific homeostatic functions.

P033 | Oxioxidative stress and DNA fragmentation of spermatozoa in patients with cancer

Costanza Calamai[1], Oumaima Ammar[1], Viktoria Rosta[1], Csilla Krausz[1], Lisa Giovannelli[2], Monica Muratori[1]

[1]*Department of Experimental and Clinical Biomedical Sciences, "M.Serio", University of Florence, Florence, Italy;* [2]*NEUROFARBA Department, Pharmacology and Toxicology Section, University of Florence, Florence, Ital*

In recent years, it has been emerging that not only oncological therapies but also cancer itself can induce abnormal spermatogenesis. In addition, several Authors reported that occurrence of malignancy provokes also increases of sperm DNA damage, although such finding was not confirmed by others and the possible mechanisms responsible for such damage are presently unknown. This was a prospective observational study, conducted from 2018 to today, in 102 patients affected by cancer (haematological, n = 40 ; testicular, n = 62) and in 62 control subjects (male partners of infertile couples), both recruited among the population afferent to the Andrology Clinic of University of Florence for routine semen cryopreservation and semen analysis, respectively. Control subjects were normozoospermic with absence of leukocytospermia, semen viscosity, smoking habit and recent antibiotic therapies., in the recruited men, We evaluated standard semen parameters according WHO guidelines and, When semen samples was enough both sperm DNA Fragmentation (with SCD, Sperm Chromatin Dispersion) Test and oxidative stress (with a double staining with MitoSOX™ Red and LIVE/DEAD Fixable Dead Cell Green Stain, then detected by Flow Cytometry). In particular, oxidative stress was calculated as percentage of viable spermatozoa with MitoSOX™ Red labeling on total viable spermatozoa. We found poorer standard semen parameters (sperm motility, concentration and number) in cancer patients (both testicular and hematological ones) with respect to control group, whereas no difference was seen in the other tested characteristics (sperm morphology, abstinence, semen volume and pH, BMI). However, patient with testicular cancer, were younger than control subjects. No difference was observed between the two types of cancer. Regarding sDF, we found higher median values [IQR] in cancer patients (total: 22.25[17.00-25.95], n = 68; hematological: 23.00[20.13-26.38], n = 28; testicular: 21.13[16.13-25.73], n = 40) vs control subjects (12.50[8.25-14.75], n = 53); $p < 0.05$, test U di Mann-Whitney. In addition, the amount of sperm oxidative stress was dramatically higher in patients with cancer (total:38.92[24.90-58.87], n = 79; hematological: 38.85[24.98-50.77], n = 34; testicular: 38.92[20.59-63.59], n = 45) vs. control subjects (11.50[8.38-17.20], n = 62); $p < 0.05$, test U di Mann-Whitney. We also studied the occurrence of a correlation between levels of sDF and oxidative stress. We found a sharp correlation when both cancer patients and control subjects were analysed (Spearman

coefficient = 0.62, $p < 0.001$, n = 164), but such correlation was completely lost when only cancer patient were considered (Spearman coefficient = 0.10, $p > 0.05$, n = 102). This finding suggests that mechanisms different from ROS attack to DNA could explain the increase of sDF levels in cancer patients. The study did not investigate, because of scarce availability of semen samples from cancer patients, other possible mechanisms (i.e apoptosis, defects in sperm chromatin maturation, failure in DNA system repair) which could cause the observed increase of sperm DNA damage in such patients. The higher levels of both sDF and oxidative stress in cancer patients rises concern, as these subjects cryopreserve semen for using it with Assisted Reproductive Technology. Indeed, both oxidative stress and sDF represent a threat for both natural and assisted reproduction. In addition, emerging evidence suggest that oxidative stress may alter sperm epigenome, with possible consequence for embryo development.

P034 | Morphology of epididymis and expression of aquaporin 9 in epididymal duct of adult rats after long-term treatment with immunosuppressive protocols based on calcineurin inhibitors

Marta Grabowska[1], Katarzyna Michałek[2], Karolina Kędzierska-Kapuza[3], Kamil Gill[1], Tomasz Machałowski[1,5], Patryk Harasny[1,6], Andrzej Kram[4], Mariola Marchlewicz[7], Małgorzata Piasecka[1]

[1]Department of Histology and Developmental Biology, Faculty of Health Sciences, Pomeranian Medical University, Szczecin, Poland; [2]Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology, Szczecin, Poland; [3]Department of Gastroenterological Surgery and Transplantation, Central Hospital of Ministry of Internal Affairs and Administration in Warsaw, Medical Center for Postgraduate Education, Warsaw, Poland; [4]Department of Pathology, West Pomeranian Oncology Center, Szczecin, Poland; [5]Department of Perinatology, Obstetrics and Gynecology, Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Police, Poland; [6]Department of Urology and Urological Oncology, Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Szczecin, Poland; [7]Department of Dermatology and Venereology, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland

Factors that contribute to the reduction of male fertility are still being searched for. It has been revealed that calcineurin inhibitors (cyclosporin A and tacrolimus), used in patients after organ transplantation, have an adverse effect on the male reproductive system. Recently, attention is also paid to aquaporins (AQPs), which play a crucial role in the secretion/reabsorption of fluids in the transepithelial transport in the epididymal duct. Therefore, our study was designed to determine the effect of immunosuppressive treatment protocols based on calcineurin inhibitors on the morphology of epididymis and immunoexpression of AQP9 in the epididymal duct of the rat.

For 6 months (which is analogous to approximately 15 years of human life), 18 male Wistar rats (sexually mature 14 week old) received immunosuppressants: cyclosporin A (CsA), tacrolimus (FK-506), mycophenolate mofetil (MMF), and prednisone (Pre) according to the three-drug protocols used in patients after organ transplantation. Rats were divided into 3 groups (n = 6 for each group): control, CMP (CsA, MMF, Pre), and TMP (FK-506, MMF, Pre). Morphological analysis of epididymis was based on hematoxylin and eosin staining, while immunohistochemical reaction for AQP9 was performed on paraffin-embedded epididymal tissue sections. The morphometric parameters of epididymal duct and intensity of AQP9 immunoexpression were assessed by means on quantitative computer analysis (ImageScope viewer v. 11.2.0.780; Aperio Technologies, Vista, CA, USA).

In the CMP and TMP groups the wall of the epididymal duct was collapsed, which made its lumen irregular (corpus and cauda epididymis), height of epididymal epithelium was significantly lower and immunoexpression of AQP9 in the apical membrane of epididymal epithelium was decreased (caput, corpus and cauda epididymis) vs. control group. It should be highlighted that in the experimental and control groups the sperm content in the lumen of the epididymal duct was comparable and irrespective of its anatomical segment.

The long-term treatment of adult rats with immunosuppressive protocols based on calcineurin inhibitors may lead to morphological alterations in epididymis and disturbances of transepithelial fluid and solute transport in epididymal epithelium.

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P035 | Age-associated epigenetic changes in mammalian sperm: implications for offspring health and development

Oleg Sergeyev[1], Vasily Ashapkin[1], Alexander Suvorov[1,2], J. Richard Pilsner[3], Stephen A. Krawetz[4]

[1]Belozersky Institute of Physical and Chemical Biology, Lomonosov Moscow State University, Moscow, Russia; [2]Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, MA, United States; [3]C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, United States; [4]Department of Obstetrics and Gynecology, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, United States

Background: Modern reproductive behavior in most developed countries is characterized by delayed parenthood. Older gametes are generally less fertile, accumulating and compounding the effects of varied environmental exposures that are modified by lifestyle factors. Clinicians are primarily concerned with advanced maternal age, while the influence of paternal age on fertility, early development, and offspring health remains underappreciated. There is a growing trend to use assisted reproductive technologies (ART) for couples of advanced reproductive age. Thus, the number of children born from older gametes is increasing.

Objective and rationale: We review studies reporting age-associated epigenetic changes in mammals and humans in sperm, including DNA

methylation, histone modifications, and non-coding RNAs. The interplay between environment, fertility, ART, and age-related epigenetic signatures is explored. We focus on the association of sperm epigenetics on epigenetic and phenotype events in embryos and offspring.

Search Methods: Peer-reviewed original and review articles over the last two decades were selected using PubMed and the WOS. Searches were performed adopting the two groups of main terms. The first group included 'advanced paternal age', 'paternal age', 'postponed fatherhood', 'late fatherhood', 'old fatherhood' and the second group included 'sperm epigenetics', 'sperm', 'semen', 'epigenetic', 'DNA methylation', 'chromatin', 'non-coding RNA', 'assisted reproduction', 'epigenetic clock'.

Outcomes: Age is a powerful factor in humans and rodent models associated with increased de novo mutations and a modified sperm epigenome. Age affects all known epigenetic mechanisms, including DNA methylation, histone modifications, and profiles of sncRNA. While DNA methylation is the most investigated, there is a controversy in the direction that dominates the age-dependent changes of differentially hypo- or hypermethylated regions with age. Successful development of the human sperm epigenetic clock based on cross-sectional data and four different methods for DNA methylation analysis indicates that at least some CpG exhibit a linear relationship between methylation levels and age. Rodent studies show a significant overlap between genes regulated through age-dependent differentially methylated regions and genes-targeted by age-dependent sncRNA. Both age-dependent epigenetic mechanisms target gene networks enriched for embryo developmental, neurodevelopmental, growth and metabolic pathways. Thus, age-dependent changes in the sperm epigenome cannot be described as a stochastic accumulation of random "epimutations" and can be linked with autism spectrum disorders. Chemical and lifestyle exposures and ART techniques may affect epigenetic aging of sperm. Although most epigenetic modifications are erased in the early mammalian embryo, there is growing evidence that altered offspring epigenome and phenotype is linked with advanced paternal age from a father's sperm accumulating epigenetic changes with time. It has been hypothesized that age-induced changes in the sperm epigenome are profound, physiological, dynamic over the years, stable over days/months, and likely irreversible.

Wider implications: This review raises a concern of delayed fatherhood and age-associated changes in sperm's epigenome that may compromise reproductive health of fathers and transfer of altered epigenetic information to subsequent generations. Prospective studies using healthy males that consider confounders are recommended. We suggest a broader discussion focused on the regulation of father's age in natural and ART conceptions is needed.

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P036 | Constant light during maturation: a challenge to the endocrine function of the Leydig cells

Dijana Marinkovic, Marija Medar, Alisa Becin, Silvana Andric, Tatjana Kostic

Department for Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Factors influencing Leydig cell maturity and the acquisition of functional capacity are incompletely defined. Here we analyzed the constant light (LL) influence on Leydig cells' endocrine function during reproductive maturation. Rats were exposed to LL from P21 to P90. Data were collected at juvenile (P35), peri/pubertal (P42, P49), and adult (P90) stages of life. Results proved the effect of LL on rats' physiology by changing of bimodal voluntary activity pattern into free-running. Additionally, the peripheral clock in Leydig cells changed in LL condition, indicating disturbed rhythm: the positive element (Bmal1) increased in pre-/pubertal but decreased in the adult period while negative elements (Per2 and Reverba) were increased. The effects of LL were most prominent in puberty: pituitary genes encoding gonadotropic hormones (Cga, Lhb, Fshb) decreased; serum androgens and mass of testicular and sex accessory organs reduced; markers of Leydig cells maturity/differentiation (Insl3, Lhcgr) and steroidogenesis-related genes (Scarb1, Star, Cyp11a1, Cyp17a1) decreased; the steroidogenic and energetic capacity of the Leydig cell mitochondria decreased; the mtDNA copy number reduced, and mitochondrial dynamics markers changed: fusion decreased (Opa1 and Mfn2), and mitophagy increased (Pink1).

In adults, the negative effect of LL on mitochondrial function and steroidogenic capacity persists in adult Leydig cells while other parameters reached control values. Altogether, results indicate that LL slows Leydig cells' maturation by reducing the endocrine and energy capacity of cells leading to the delaying of reproductive development.

P037 | New kids on the block: testicular capsule macrophages come into plaY

Jonathan Knaus[1], Vishnu Kumar[1,2], Anika Diehm[1], Vijay K. Singh[3], Monika Fijak[1,2], Christiane Pleuger[1,2], Marius Rohde[3], Andreas Meinhardt[1,2], Sudhanshu Bhushan[1,2]

[1]Institute of Anatomy and Cell Biology, Justus Liebig University of Giessen, Giessen, Germany; [2]The Hessian Centre of Reproductive Medicine, Justus Liebig University of Giessen, Germany; [3]Department of Pediatric Hematology/Oncology, Justus Liebig University of Giessen, Giessen, German

Macrophages are sentinel cells present in almost all organs and play an essential role in tissue homeostasis, tissue repair, inflammation resolution as well as being a key player in innate immune responses. Akin to other organs, macrophages are the dominant immune cell population of the testicular parenchyma. They are supposed to play a critical role in maintaining the immune-privileged status of the testis, besides protecting against inflammatory stimuli. Until now, testicular parenchyma macrophages (TPM) were thought to be the only resident macrophage population present in the testis. However, few studies indicate the presence of macrophages also in the testicular capsule. The ontogeny, localization, phenotypes and functions of TPM are begun to be understood, however, information about testicular capsule macrophages

(TCM) is completely lacking. We hypothesize that TCM will be distinct from TPM in ontogeny, phenotype and function.

Our flow cytometry data revealed that the testicular capsule contains a heterogeneous immune cell population consisting of monocytes, macrophages, dendritic cells and T cells. However, the proportion of macrophages amongst all leukocytes in the testicular capsule is significantly lower (30%) than in the testicular parenchyma (60%). Of note, the testicular capsule harbors more dendritic cells and T cells than the parenchyma. TCM can be distinguished from TPM by their morphology as they display very long dendrites, low expression of Cx3Cr1, and CD68, while levels of Ccr2 and MHC II expression are high. During inflammatory conditions, infiltrating Ly6G+ neutrophils, the first immune cell to migrate at the site of infection, are entrapped mainly in the testicular capsule rather than the testicular parenchyma. This result suggests that entrapment of Ly6G+ neutrophils in the testis capsule is a plausible mechanism to protect the testis from a strong inflammatory reaction and thus maintains immune privilege. Moreover, our results also suggest that TCM arise from blood monocytes rather than embryonic precursors by using Ccr2−/− mice. These results indicate that the testicular capsule contains a macrophage population distinct from TPM with different ontogeny and function.

P038 | Effect of prenatal exposure to α-cypermethrin on DNA methylation patterns in rat sperm

Dajana Krsnik[1,2], Marta Himelreich Peric[1,2], Marijana Petric[3], Vedran Micek[4], Marijana Neuberg[5], Goran Kozina[5], Ana Lucic Vrdoljak[4], Ana Katusic Bojanac[1,2], Anja Katic[4]

[1]Department of Medical Biology, School of Medicine, University of Zagreb, Croatia; [2]Scientific Center of Excellence for Reproductive and Regenerative Medicine Zagreb, Croatia; [3]Department of Biology, Faculty of Science, University of Zagreb, Croatia; [4]Institute for Medical Research and Occupational Health, Zagreb, Croatia; [5]University Centre Varaždin, University North, Varazdin, Croatia

α-cypermethrin belongs to pyrethroids, one of the most used groups of insecticides that can act as endocrine disruptive chemicals (EDCs), known to impact gene expression at the epigenetic level. Testicular exposure to EDCs in foetal development may impair PGC differentiation causing decreased sperm quality and number and increased apoptosis of germ cells, as well as infertility in adults. However, such an effect has still not been established for α-cypermethrin. One of the most studied epigenetic mechanism is DNA methylation, which plays a major role in the de novo silencing of retrotransposon sequences in germ cells, as well as in the establishment of sex-specific imprinting.

In this study, we investigated the effect of prenatal exposure to α-cypermethrin on DNA methylation patterns in rat sperm of F1 offspring. Pregnant female Wistar rats were divided into 6 groups. The first three experimental groups (α-cyp1, α-cyp10 and α-cyp19) were treated per os from the 6th up to 21st gestational day with three different doses of α-cypermethrin (1, 10 and 19 mg/kg bw/day). In the same period, the positive control group (PC) was treated with diethylstilbestrol, solvent control group (SC) with corn oil and negative control group (NC) with water. DNA was isolated from the sperm of F1 generation pubertal pups. Subsequently, the methylation level of 3 different CpG sites within repetitive element LINE-1 was analysed for the assessment of global methylation by pyrosequencing method. Additionally, methylation analysis was performed for 7 different CpG sites within the differentially methylated regions (DMRs) of the imprinted genes Igf2 and H19.

The results showed no significant difference in the methylation levels of examined DNA sequences of LINE-1 between the experimental groups and the controls. Also, exposure to α-cypermethrin had no effect on the methylation levels of the examined DNA sequences of Igf2 and H19. This study implies to the possibility that prenatal exposure to α-cypermethrin does not affect the de novo DNA methylation process in the rat sperm of F1 offspring and indicates a need for further research of epigenetic changes caused by this endocrine disruptor at the genome-wide level.

P039 | Unique cytogenetic variant of Klinefelter syndrome with double Y-autosomal translocation

Vyacheslav Chernykh[1,2], Marina Minzhenkova[1], Fatima Bostanova[1], Olga Schagina[1], Nadezhda Shilova[1]

[1]Research Centre for Medical Genetics, Moscow, Russia; [2]Pirogov Russian National Research Medical University, Moscow, Russia

Actuality: Klinefelter syndrome (KS) is one of most frequent chromosomal abnormalities and commonest genetic cause of male infertility. About 85% KS patients have 47,XXY karyotype, other patients presented other mosaic and non-mosaic variants.

Materials and Methods: We reported on KS patient with unique double Y-autosomal translocation. The proband is 15 y.o. male patient (height 180 cm, weight 50 kg, normal IQ) admitted for cytogenetic examination and genetic counseling because of puberty delay. Testicular hypoplasia, hypergonadotropic hypogonadism, pituitary microadenoma and left-side varicocele were diagnosed in him. The proband was born in non-consanguineous marriage after IVF/ICSI procedure because of infertility. The father of proband is oligozoospermic man having robertsonian translocation 13;15 – 45,XY,der(13;15)(q10;q10), the mother shown normal karyotype 46,XX.

Chromosome analysis was done on cultured peripheral blood lymphocytes using by standard cytogenetic method (GTG-staining) and FISH analysis with DYX1, DYZ3, D15Z, SE13/21 and LSI UBE3A(15q11)/PML(15q24) probes. The Y chromosome loci (SRY, ZFY, AZFa,b,c) were analyzed by multiplex PCR. CAGn polymorphic locus of AR gene was evaluated in the proband and his parents to determine parental origin of the X-chromosome in the patient.

Results: Complex cytogenetic examination revealed 46,XX,der(Y)t(Y;15)(q12;q11.1),der(13)t(Y;13)(q12;p11.2) karyotype in the proband. Molecular analysis shown that the proband is SRY-positive, homozygous for CAGn polymorphic locus of AR gene (n = 24 allele), and that both X chromosomes are maternally inherited

because of X-X non-disjunction during meiosis II; no the Y chromosome microdeletion was found. Detected double Y-autosomal translocation is independent chromosomal abnormality from KS. Apparently, the der(13) and der(Y) chromosomes are resulted from abnormal meiotic recombination in the paternal meiosis between the Yq12 heterochromatic region and centromeric/pericentromeric heterochromatin of chromosomes 13 and 15, involved in robertsonian translocation in the father.

Conclusion: Rare cytogenetic variants of Klinefelter syndrome are associated with Y-autosomal translocations, originated as independent chromosome mutations.

Keywords: Klinefelter syndrome, AZF locus, AR gene, sex chromosomes, Y-autosomal translocations, robertsonian translocations.

P040 | Enhanced frequency of the L138ins variant of the CFTR gene in Russian infertile men

Vyacheslav Chernykh[1,2], Ekaterina Marnat[2], Olga Solovova[1], Tatiana Sorokina[1], Maria Shtaut[1], Anna Sedova[1], Stanislav Krasovskiy[1], Tagui Adyan[1], Anna Stepanova[1], Olga Schagina[1], Aleksandr Polaykov[1]

[1]Research Centre for Medical Genetics, Moscow, Russia; [2]Pirogov Russian National Research Medical University, Moscow, Russia

Actuality: Pathogenic variants of the CFTR gene are one of common genetic factor of male infertility, resulted to Cystic Fibrosis (CF) and CBAVD syndrome, obstructive azoospermia. L138ins (c.411_412insCTA; p.Leu138dup) variant of the CFTR gene is CF-causing mutation, which is relative common in CF patients from Slavic populations. The prevalence of this CFTR gene variant in Russian infertile men previously was not evaluated.

Materials and Methods: We examined large cohort of 6033 Russian infertile men. Genomic DNA extracted from peripheral blood lymphocytes was screened for 22 common pathogenic variants (CFTRdele2,3, 394delTT, 3944delTG, L138ins, R334W, F508del, I507del, 1677delTA, G542X, 2143delT, 2184insA, 3821delT, 3849+10kbC>T, 604insA, 621+1g>t, E92K, S1196X, W1282X N1303K, 4022insT, 4015delA, and 3272-26A>G), and IVS9Tn (IVS8Tn) polymorphic locus of the CFTR gene. Molecular analysis was performed using by AFPL, MLPA, DNA sequencing by Sanger or massive parallel sequencing methods.

Results:. Pathogenic CFTR gene variants were detected in 3.9% patients. Commonest variants were F508del and CFTRdele2,3(21kb), which present 61.0%, and 7.1% of detected CFTR mutations, respectively. A presence of two CF-caused CFTR gene variants was found in 9 (0.15%) patients. L138ins variant was detected in 17 (0.28%) patients, of them 10 individuals were heterozygous, 6 patients have two CFTR mutations (F508del/L138ins, n = 4; L138ins/L138ins, n = 1; L138ins/N1303K, n = 1), one patient have L138ins/5T genotype. Further clinical examination confirmed atypical (mild, late manifesting) Cystic Fibrosis in the patients with two CF-causing variants. Allele frequency (AF) of L138ins variant in the sample was 0.14% (in CFTR2 database – 0.00014%, RUSeq Browser – 0.04%, GNOMAD Exome and "NGSData"/Certificate of RuExAc – 0%).

Conclusion: The results show a relatively high frequency of L138ins variant among Russian infertile men. This pathogenic variant of the CFTR gene is third in frequency after the two most common mutations, F508del and CFTRdele2,3(21kb). Some infertile men with two CF-causing pathogenic variants of the CFTR gene may have undiagnosed non-severe forms of CF or CFTR-RD.

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Keywords: male infertility, CFTR gene, Cystic Fibrosis, CBAVD, azoospermia.

P041 | Use of mass spectrometry to identify proteins related to impaired sperm morphology and motility in infertile men

Shashika D. Kothalawala[1,5], Thomas Timm[2], Günter Lochnit[2], Hans-Christian Schuppe[3], Adrian Pilatz[3], Florian Wagenlehner[3], Sabine Kliesch[4], Liza O'Donnell[5], Kate Loveland[5], Daniela Fietz[1]

[1]Institute for Veterinary Anatomy, Histology and Embryology, Justus-Liebig University of Giessen, Giessen, Germany; [2]Protein Analytics, Biochemistry Institute, Justus-Liebig University of Giessen, Giessen, Germany; [3]Clinic of Urology, Pediatric Urology and Andrology, Justus-Liebig University of Giessen, Giessen Germany; [4]Centre of Reproductive Medicine and Andrology, University of Muenster, Muenster, Germany; [5]Center for Reproductive Health, Hudson Institute of Medical Research, & Dept of Molecular & Translational Sciences, Monash University, Clayton, Australia

Introduction: Genetic causes are likely involved in about 30% of male infertility cases with approximately 2000 genes specifically expressed in spermatogenesis. Next generation sequencing aided in identifying genes likely to be involved in impaired spermatogenesis and therefore male infertility. By complementing next generation sequencing approaches, mass spectrometry (MS) is a powerful tool to identify differentially expressed proteins (DEPs) between control and infertility groups. This study focused on identifying proteins in human ejaculated spermatozoa with impaired sperm morphology and motility to identify proteins associated with defects in sperm function.

Material and Methods: Human ejaculates classified as normozoospermia (NORM, n = 3) or presenting defective morphology and/or motility (asthenoteratozoospermia; AT, n = 3) were subjected to mass spectrometry (Orbitrap Eclipse). In silico analysis of DEPs were performed. Selected proteins were examined by immunohistochemistry (IHC) in testicular biopsies showing normal spermatogenesis (NSP) or spermatid arrest (SDA) phenotype.

Results: 36 proteins were significantly higher or lower in AT compared to NORM. In silico analysis identified several interesting candidate proteins of which five were selected for further investigations; of these, IPO4, ELSPBP1, and IFT57 were higher whereas CCDC105 and ACTRT2 were lower. ACTRT2, IPO4, CCDC105, IFT57 are present in testis, and IHC revealed their localisation in human germ cells with

a stronger signal for ACTRT2, IFT57, IPO4 in round/elongated spermatids. Importantly, ACTRT2 and CCDC105 were markedly lower in spermatids from SDA samples, confirming MS results. An epididymal-specific protein, ELSPBP1, identified as higher in AT, showed minimal expression in testis tissues. IHC showed nuclear localised IPO4 in all germ cells, with an enhanced cytoplasmic expression from pachytene spermatocytes to round/elongating spermatids compared to spermatogonia and leptotenes in NSP samples. Spermatogonia showed relatively low nuclear and no cytoplasmic IPO4 signal. Interestingly, IHC of SDA showed clear nuclear IPO4 signal in late germ cells, with reduced cytoplasmic expression in spermatocytes and no cytoplasmic signal in spermatogonia.

Conclusion: By mass spectrometry analysis of normal and defective ejaculated sperm, we identified 36 proteins that show quantitative changes in AT compared to NORM human sperm. These may have important functions in spermiogenesis, and altered expression levels might be linked to abnormalities in sperm function of infertile men. In particular, IPO4 is a member of the importin family involved in nuclear transport that is more abundant in AT sperm, and its differential localization in NSP and SDA patients suggests its role in protein transport could affect spermatid development. Current studies are focused on to quantify IPO4 subcellular localisation in testis sections and to characterise its localisation in sperm to understand its functional role in human spermatozoa.

P042 | DNA-FISH analysis in testicular tissue cells from prepuberal patients with Klinefelter Syndrome

Myriam Martin-Inaraja[1,2], Sara Alonso[1,2], Clara Rodriguez[1,2], Silvia Santos[1,2], Maria Iglesias[3], Maria Begoña Prieto[3,4], Roberto Matorras[3,4], Lydia Garcia-Quevedo[5], Francesca Vidal[5], Cristina Eguizabal[1,2]

[1]Cell Therapy, Stem Cells and Tissues Group, Basque Centre for Blood Transfusion and Human Tissues, Galdakao, Spain; [2]Biocruces Bizkaia Health Research Institute, Barakaldo, Spain; [3]Biocruces Bizkaia Health Research Institute-Cruces University Hospital, Pediatric Department, Barakaldo, Spain; [4]Biocruces Bizkaia Health Research Institute-Cruces University Hospital-Basque Country University-IVI Bilbao, Human Reproduction Unit, Barakaldo, Spain; [5]Universitat Autònoma de Barcelona, Cell Biology Unit-Department of Cell Biology- Physiology and Immunology, Bellaterra, Spain

Prepuberal boys with Klinefelter Syndrome usually have fertility problems in adulthood, mainly azoospermia. Due to the symptoms cause by the disease, Spermatogonial Stem Cells (SSCs) are less abundant and spermatogenesis does not occur. These patients do not have any alternative to restore the fertility in the future. When a boy has both XY and XXY cell lines, this patient is mosaic (46,XY/47,XXY). Meaning some cells could undergo spermatogenesis and generate gametes with normal sex-chromosomes number. The aim of this work is to test if KS patients diagnosed as pure can have testicular cell lines with XY chromosome number (mosaic).

We obtained samples from human prepuberal patients diagnosed with pure Klinefelter Syndrome (47,XXY) for the last 5 years. Testicular biopsy fragments are fixed for histological studies and other fragments are cryopreserved. Some fragments are used for immunofluorescence and subsequently processed by DNA-FISH to determine the sex-chromosomes content of testicular cells.

In this study, we used 10 prepuberal patients with KS and 5 prepuberal patients with other fertility problems with normal chromosome set as controls. We performed immunofluorescence to determine expression of germ cells (VASA) and SSCs (MAGEA4) markers and somatic cells markers such as Leydig cells (StAR) and Sertoli cells (SOX9). Afterwards, we perform DNA-FISH, with probes specific for chromosomes X and Y and chromosome 18 as a control.

The methodology used allows cytogenetic characterization of testicular tissue in paraffin embedded sections. Testicular mosaicism has been observed in all patients diagnosed as pure KS. We have observed a degree of mosaicism of 66-80% in SSCs, of 20-50% in Sertoli cells and of 30-50% in Leydig cells. We pursued a protocol with a good FISH efficiency that allows colocalization of previous immunocharacterized testicular cells.

We succeed to demonstrate the mosaicism of testicular cells in prepuberal patients that are diagnosed with KS. Thus, the better understanding of the SSCs with normal chromosome set (XY) could be useful for future in vitro expansion and stem cell therapies.

P043 | Brain hyperexcitability in an experimental model of chronic prostatitis/chronic pelvic pain syndrome: modulation by CO

Nikola Sutulovic[1], Aleksa Zubelic[1,2], Zeljko Grubac[1,3], Dusan Mladenovic[1], Milena Veskovic[1], Dunja Popov[4], Duto Macut[1,5], Aleksandra Rasic-Markovic[1], Olivera Stanojlovic[1], Dragan Hrncic[1]

[1]Laboratory of Neurophysiology, Institute of Medical Physiology "Richard Burian", Belgrade University Faculty of Medicine, Belgrade, Serbia; [2]Clinic of Urology, University Clinical Centre of Serbia, Belgrade, Serbia; [3]Clinic for Digestive Surgery, University Clinical Centre of Serbia, Belgrade, Serbia; [4]Clinic of Gynecology and Obstetrics 'Narodni Front', Belgrade, Serbia; [5]Clinic of Endocrinology, Diabetes and Metabolic Disease, University Clinical Centre of Serbia, Belgrade, Serbia

Introduction: The most common cause of chronic pelvic pain in male population is urological entity Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS). Besides chronical and spontaneous pain, uroandological symptoms, psychiatric comorbidities and sexual disfunction, it is also accompanied with brain hyperexcitability. Sometimes, antiepileptic medications are potent in the CP/CPPS treatment, but there is no proper investigation in experimental models. Carbon monoxide (CO), gas neurotransmitter, has been identified as anti-inflammatory and immunomodulatory agent. Experimental modulation of the endogenously synthesized CO levels can be done in vivo

by parenteral application of CO-releasing molecules (CORMs). The aim of this study was to investigate effects of parenterally administered CORM-A1 on brain excitability in rats with experimentally induced CP/CPPS.

Methods: Adult male Wistar albino rats (n = 32) were randomly divided into: CP/CPPS (n = 16, intraprostatic injection of 3% λ-carrageenan) and Sham (n = 16, 0.9% NaCl) group. Additionally, after operation, animals from both groups were treated daily by 7 consecutive days by CORM-A1 (2 mg/kg, i.p. forming the Sham-CORM and CP/CPPS-CORM groups, n = 8 in each) or its solvent, phosphate buffer (PBS, forming Sham-PBS and CP/CPPS-PBS groups, n = 8 in each). To confirm CP/CPPS development, mechanical pain thresholds in the scrotal skin were determined by electrical von Frey aesthesiometer prior to, as well as, 2, 3 and 7 days upon operation. Seventh day upon intraprostatic injection, we challenged rats with subconvulsive dose of lindane (4 mg/kg). Hereupon, we assessed rats' convulsive behavior (seizure incidence, latency and severity) and EEG manifestations (number and duration of ictal periods).

Results: Scrotal pain threshold was significantly decreased in all postoperative days in CP/CPPS-CORM and CP/CPPS-PBS animals compared to corresponding Sham-CORM and Sham-PBS animals ($p < 0.001$). In addition, CORM-A1 treatment in CP/CPPS-CORM rats led to statistically significant ($p < 0.001$) analgetic effect in all postoperative days, compared to CP/CPPS-PBS rats. Animals with prostatitis (groups: CP/CPPS-CORM and CP/CPPS-PBS) revealed significantly higher incidence ($p < 0.01$), decreased latency time ($p < 0.01$) and augmented severity ($p < 0.01$) of lindane-induced seizures compared to corresponding controls (groups: Sham-CORM and Sham-PBS). Further analysis showed that CORM-A1 treatment in CP/CPPS-CORM rats has partially reversed brain hyperexcitability and led to significantly lower incidence ($p < 0.05$), increased latency time ($p < 0.05$) and lowered severity ($p < 0.05$) of seizures, compared to the CP/CPPS-PBS rats. EEG analysis showed increased duration ($p < 0.05$) and number of ictal periods ($p < 0.01$) in CP/CPPS-CORM and CP/CPPS-PBS rats. CORM-A1 treatment has significantly reduced number of EEG ictal periods ($p < 0.05$) in CP/CPPS-PBS rats with no statistically significant influence on the duration of EEG ictal periods.

Conclusion: CO is potent modulator of brain hyperexcitablity developed in male rats with CP/CPPS.

Keywords: chronic prostatitis, chronic pelvic pain, epilepsy, lindane, CORM-A1, rats

P044 | Therapeutic effect of lipid extract of solanum lycopersicum in combination with selenium in wistar rats with benign prostatic hyperplasia

David J Arias Chavez, Patrick Mailloux Salinas, Guadalupe Bravo

Center for Research and Advanced Studies of the Instituto Politécnico Nacional; (IPN), Mexico City, Mexico

Benign prostatic hyperplasia (BPH) is the increase in the size and/or number of stromal cells and the epithelium of the prostate at a point where urinary flow is obstructed causing bladder obstruction, generating lower urinary tract symptoms (LUTS). BPH is the most frequent benign tumor in men over 60 years of age and its prevalence increases linearly with age. Its etiology is attributed to the action of dihydrotestosterone (DHT) by activating a series of cellular processes that lead to the activation of cell growth and proliferation genes. DHT is generated from testosterone by the action of 5α-reductase. The use of 5α-reductase inhibitors such as finasteride, one of the main treatments used in BPH, has reported several adverse effects due to its prolonged use, which has led to the search for safe and effective therapeutic alternatives. For decades, phytotherapy has been used as a therapeutic alternative for BPH. S. lycopersicum, commonly known as tomato, is rich in vitamins, phenolic compounds and carotenoids such as lycopene, which together have been attributed antioxidant, anti-proliferative and pro-apoptotic effects, as well as the decrease of DHT by inhibition of 5α-reductase, on the other hand, Selenium, has also been attributed antioxidant, anti-proliferative and pro-apoptotic effects in prostate tissue. Fourteen-month-old Wistar rats were divided into six groups (Control, BPH, Finasteride, Extract, Selenium and Selenium-Extract). Except for the control group, they were administered testosterone (10 mg/kg/week) subcutaneously to mimic BPH. For 30 days, finasteride (5 mg/kg/day), tomato lipid extract (5 mg/kg/day according to lycopene concentration), sodium selenite (10 μg/kg/day) were administered orally. Lipid extract in combination with Selenium had a ~60% decrease in prostate size compared to separate administration of the extract and selenium (~35%) and finasteride (~15%) and with respect to rats with BPH. In addition, the combination also improved the decrease in oxidative stress markers (Malondialdehyde and total nitrites), increased the activity of major antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione Peroxidase), while also decreasing prostatic levels of testosterone, DHT and prostate specific antigen (PSA). The combination of tomato lipid extract in combination with selenium reversed cell proliferation, increased antioxidant activity, regulated androgen and PSA levels and reduced prostate weight and size to normal values.

P045 | Withdrawn

P046 | PELP1 and its possible interactions with proteins involved in ESR-mediated signaling in human sperm cells

Izabela Skibinska[1], Mirosław Andrusiewicz[1], Magdalena Jendraszak[1], Aleksandra Zbikowska[1], Piotr Jedrzejczak[2], Malgorzata Kotwicka[1]

[1]Chair and Department of Cell Biology, Poznan University of Medical Sciences, Poznan, Poland; [2]Division of Infertility and Reproductive Endocrinology, Department of Gynecology, Obstetrics and Gynecological Oncology, Poznan University of Medical Sciences, Poznan, Poland

Purpose: A key role of estrogens in the male reproductive tract is spermatogenesis regulation and functional gamete maturation.

These events can be disrupted by the cumulative effect of endogenous and exogenous estrogens. Characterization of estrogen signaling pathway-related genes' expression in sperm cells could aid in better understanding sperm biology and quality.

Materials and Methods: In this study, we obtained samples from 119 male participants of Caucasian descent. We analyzed four genes in the sperm of men who donated semen for standard analysis. Expression levels were analyzed for estrogen receptors (ESR1 and ESR2) as well as their coregulators – proline-, glutamic acid-, and leucine-rich protein 1 (PELP1), and cellular kinase c-Src (SRC). Protein expression was confirmed with western blot and immunocytochemistry techniques.

Results: Expression of both ESRs differed in case of sperm normal and abnormal morphology and for ESR2 additionally in case of motility ($p < 0.05$). Gene expression ratios revealed significant, moderate, and negative correlations for ESR1/ESR2 and weak, negative ESR2/PELP1 correlations in the subgroup of patients with abnormal sperm values. Additionally, SRC/PELP1 were moderately and positively correlated in the subgroup with all parameters within the WHO reference range.

Conclusions: As known, ESR1 and ESR2 influence sperm biology, but our study showed that other genes are engaged in estrogen-signaling-pathway. Both PELP1 scaffolding protein and SRC kinase may be important factors influencing sperm biology via ESRs, but it would require further functional analyzes. Disrupted estrogen signaling in sperm cells may be associated with the deregulation of certain sperm cell functions.

Keywords: estrogen receptors (ESR1 and ESR2); proline-, glutamic acid-, and leucine-rich protein 1 (PELP1); proto-oncogene tyrosine-protein kinase c-Src (SRC); steroid hormones; sperm;

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P047 | Stress changed the Leydig cell's transcriptional activity depending on the diurnal time

Marija Medar, Silvana Andric, Tatjana Kostic

The University of Novi Sad, Faculty of Sciences Novi Sad, Department of Biology and Ecology, Laboratory for Chronobiology and Aging, Laboratory for Reproductive Endocrinology and Signaling, Novi Sad, Serbia

The increasing amount of data points to the circadian timing system as an essential part of processes regulating androgen homeostasis. However, the relationship between stress response, timekeeping-, and steroidogenesis-related systems is unexplored. Here, the stress-response of the testosterone-producing rat Leydig cells depending on the time of stressful events was studied. The study analyzes the effects of 3-hour immobilization (IMO) applied at different periods during the day. The IMO is performed once (1xIMO) or repeated in 10 consecutive days (10xIMO). Both types of IMO increased corticosterone and decreased testosterone blood level. However, the effect of 10xIMO occurring in the active phase on blood testosterone was less pronounced. This is related to different sensitivity to IMO-events depending on the diurnal time. Most steroidogenesis-related genes (Lhcgr, Cyp11a1, Hsd3b1/2, Cyp17a1) were down-regulated in the inactive but unchanged or even up-regulated in the active phase of the day. Both types of IMO stimulated the expression of clock elements Bmal1/BMAL1, Per1/PER1 regardless of the day's stage and reduced Rev-erba in the inactive phase. The principal-component-analysis (PCA) confirmed a major shift, for both IMO-types, in the transcription of the genes across the passive/active stage. Further, 10xIMO changed a diurnal pattern of the glucocorticoid receptor (Nr3c1/GR) expression while the observed time-dependent IMO-response of the Leydig cells correlated with different corticosterone engagements. Altogether, the Leydig cell's stress-response depends on the daytime of the stressful event, emphasizing the importance of the circadian system in supporting androgen homeostasis and male fertility.

P048 | The effect of sex steroids on RhoA/ROCK pathway in the rat distal vagina

Giulia Rastrelli[1], Ilaria Cellai[1], Paolo Comeglio[1], Sandra Filippi[2], Giulia Guarnieri[3], Serena Martinelli[1], Elisa Maseroli[1], Sarah Cipriani[1], Elena Rapizzi[1], Annamaria Morelli[3], Mario Maggi[1], Linda Vignozzi[1]

[1]Department of Experimental Clinical and Biomedical Sciences 'Mario Serio', University of Florence, Florence, Italy; [2]Department of Neurosciences, Psychology, Drug Research and Child Health; (NEUROFARBA), University of Florence, Florence, Italy; [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Ital

The RhoA/ROCK calcium-sensitizing pathway plays a role in clitoral contractility, and therefore we deemed interesting to investigate its involvement in other urogenital districts.

This study investigated the sex steroid regulation of the vagina contractility through the RhoA/ROCK pathway, using a validated animal model.

Ovariectomized Sprague-Dawley rats (OVX) were treated with 17β-estradiol (E2), testosterone (T), with (T+L) or without letrozole, and compared with intact animals. Contractility studies were performed on vaginal strips to test the effect of ROCK inhibitor Y-27632 and NO synthase inhibitor L-NAME. In vaginal tissues, ROCK1 immunolocalization was investigated, mRNA expression was analyzed by semi-quantitative RT-PCR, and RhoA membrane translocation was evaluated by Western blot (Wb) analysis. Finally, rat smooth muscle cells (rvSMCs) were isolated from distal vagina of intact animals and the quantification of RhoA inhibitory protein RhoGDI was performed by Wb after stimulation with NO-donor SNP, with or without administration of ODQ (soluble guanylate cyclase inhibitor) or KT5823 (PKGR1 inhibitor).

The results show that ROCK1 was immunolocalized in the smooth muscle bundles and in the blood vessel walls of vagina, while a weak positivity was detected in the epithelium. Y-27632 induced a dose-dependent relaxation of noradrenaline pre-contracted vaginal strips, decreased by OVX and completely restored by E2, compared to

controls, while T and T+L further decreased it, even below OVX level. Accordingly, in Wb analysis OVX significantly induced RhoA activation compared to controls, as reveled by its membrane translocation, with T decreasing it at a level significantly lower than in controls. This effect was not exerted by E2. Abolishing the NO formation via L-NAME increased Y-27632 responsiveness in the OVX+T group; L-NAME had only a partial effect in controls, whilst it did not modulate Y-27632 responsiveness in OVX. Finally, stimulation of rvSMCs with SNP significantly increased RhoGDI protein expression, an effect counteracted by ODQ and, partially, by the protein kinase inhibitor KT5823 incubation.

In conclusion, in vagina, androgen administration in OVX functionally decreases RhoA/ROCK activity by hampering RhoA membrane translocation and plays a critical role in the inhibitory mechanism of the smooth muscle distal vagina contractility. Accordingly, androgens, by inhibiting the RhoA/ROCK pathway, could positively contribute to vaginal smooth muscle relaxation, favoring the sexual intercourse.

P049 | PELP1 and SRC as key factors participating in the ESR1-mediated pathway in human testis and epididymis

Izabela Skibinska[1], Miroslaw Andrusiewicz[1], Magdalena Jendraszak[1], Aleksandra Zbikowska[1], Czeslaw Zaba[2], Malgorzata Kotwicka[1]

[1]Chair and Department of Cell Biology, Poznan University of Medical Sciences, Poznan, Poland; [2]Chair and Department of Forensic Sciences, Poznan University of Medical Sciences, Poznan, Poland

Background: The effect of estrogens on the male reproductive system is well characterized in many studies. However, as estrogens are pleiotropic hormones, their transduction signaling pathways can be modulated or modulate various cellular processes. Proline-, glutamic acid-, and leucine-rich protein 1 (PELP1) is among the proteins engaged in the estrogen social network.

Tissue samples: Twenty-six tissue samples obtained from thirteen organ donors were used in this study.

Materials and Methods: The mRNA levels of both estrogen receptors (ESR1 and ESR2) and their coregulators, including PELP1 and SRC kinase, were analyzed using quantitative PCR. The protein presence was confirmed by western blot. The cellular localization was determined with the use of immunocytochemistry.

Results: The expression of both SRC and PELP1 was significantly lower in the epididymis when compared to the testis ($p < 0.05$). Both genes were significantly, strongly, and positively correlated ($p < 0.0001$, $R = 0.78$) regardless of the tissue type.

In testis and epididymis, both gene expression levels were significant and positively correlated ($R = 0.66$, $p = 0.014$, and $R = 0.80$, $p = 0.0019$, respectively). Moreover, in the testis PELP1 level positively correlated with the ESR1 level ($R = 0.6$; $p = 0.0367$). No significant correlation was observed in the epididymis ($p > 0.05$).

Conclusion: Our study suggests a key role of both PELP1 and SRC in the ESR1-mediated pathway in human testis and epididymis

Keywords: estrogen receptors (ESR1 and ESR2); proline-, glutamic acid-, and leucine-rich protein 1 (PELP1); proto-oncogene tyrosine-protein kinase c-SRC (SRC); estrogen signal transduction coregulators; steroid hormones; human testis; human epididymis

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P050 | Characterization of lung impairment related to metabolic syndrome and protective effect of testosterone: an in vivo study in an animal model

Giulia Rastrelli[1], Paolo Comeglio[1], Sandra Filippi[2], Ilaria Cellai[1], Giulia Guarnieri[3], Sarah Cipriani[1], Elisa Maseroli[1], Annamaria Morelli[3], Linda Vignozzi[1], Mario Maggi[1]

[1]Department of Experimental Clinical and Biomedical Sciences 'Mario Serio', University of Florence, Florence, Italy; [2]Department of Neurosciences, Psychology, Drug Research and Child Health; (NEUROFARBA), University of Florence, Florence, Italy; [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Ital

Metabolic syndrome (MetS) is characterized by the co-presence of multiple physiopathological conditions including the onset, in the male population, of hypogonadotropic hypogonadism, characterized by low levels of testosterone (T) and gonadotropins.

Recent studies pointed out a correlation between MetS and decreased lung function, although the mechanism underlying this effect remains unknown. A potential cause that could contribute to the link between MetS and the decline in lung function is represented by systemic and tissue-specific inflammation induced by obesity, the paramount marker of MetS.

In order to investigate the mechanisms determining the onset of lung dysfunction, we used a non-genomic animal model of MetS, which recapitulates the main characteristics of the human Mets phenotype, obtained by exposing rabbits to a high-fat diet (HFD).

Previous studies have shown how T is able to exert powerful anti-inflammatory effects on different organs in this experimental model and the potential protective action of this hormone on MetS-induced pulmonary alterations has been investigated.

In this study, we performed functional, biomolecular and histomorphological analysis on lung samples isolated from control group (RD, regular diet, 12weeks) animals or rabbits fed with HFD (standard diet supplemented with 0.5% cholesterol and 4.0% peanut oil, 12 weeks), alone or in combination with T for the last 6 weeks (HFD + T6W group). The inflammatory and pulmonary tissue remodeling was analyzed by immunohistochemical techniques (Picrosirius Red assay for collagen deposition) and molecular analysis (semi-quantitative RT-PCR). Spirometry was used to assess the resistance to airway ventilation (pressure of the airway opening, PAO), a functional parameter related to the reduced pulmonary compliance.

At the end of treatment, the results showed the presence of a clear impairment of lung function in HFD rabbits, demonstrated by the significant increase in PAO compared to RD. Accordingly,

immunohistochemical and molecular analysis confirmed the presence of a significant increase in pro-inflammatory and pro-fibrotic status in the lungs of HFD animals.

T administration significantly improved not only some metabolic parameters but also lung ventilation, compared to the HFD group, while also counteracting pro-inflammatory macrophage activation and peribronchiolar fibrosis. The gene expression analysis of the main inflammation and fibrotic markers confirmed a positive effect of treatment with T.

This study clearly shows the validity of the experimental model employed for the development of inflammation and pulmonary fibrosis, associated with metabolic disease, and the preliminary results highlight how treatment with T is able to mediate anti-inflammatory and antifibrotic effects in this model.

P051 | Does Honeybee work for human male health? 'The effect of CAPE on erectile function'

Emine Nur Ozbek, Aybüke Bozkurt, Gunay Yetik-Anacak
Ege University, Faculty of Pharmacy, Dept. of Pharmacology, Izmir, Turkey

Erectile dysfunction (ED) is the inability to reach and maintain an erection necessary for sexual performance (1). The oxidative stress is one of the main reasons for ED (2). Hydrogen sulfide (H2S), the third gasotransmitter, have antioxidant and relaxant effects and plays a role in penile erection. H2S donors and H2S synthesis enzymes (CBS and CSE) substrate L-cysteine causes relaxation in penile corpus cavernosum dose dependently. A flavanoid isolated from honeybee propolis Caffeic acid phenethyl ester (CAPE) has also antioxidant, antienflamatuar and dose-dependent vasodilator effects in the aorta and coronary arteries just like H2S (3,4). CAPE increases expression of the CSE enzyme in fibrotic liver tissue (5). Preincubation of spermatozoa with the CAPE protects against oxidative DNA damage in vitro (6). However, the relaxant effect of CAPE in penile tissue and the role of H2S has not been investigated yet. Thus, we investigated the effect of CAPE and the role of H2S in its possible effect in erectile function and dysfunction.

Strip myograph (DMT) were used in all experiment to get contraction relaxation responses in mice strips. ANOVA was used in all statistical analyses ($n = 5$). Endothelial integrity was tested in all strips by more than 40% relaxation to ACh. CAPE and L-cystein concentration response curve were obtained in phe-precontracted (30μM) strips in the presence or absence of aminooxyacetic acid (AOAA,10mM), H2S synthesis inhibitor. CAPE (10-4-10-3M) caused concentration dependent relaxation in mice penile ($p < 0.001$) and AOAA inhibited these relaxations significantly. Beside CAPE (10 μM) increased L-cysteine-induced endogenous H2S dependent relaxation in healthy mice penile tissue ($p < 0.01$) and this augmentation is inhibited by AOAA. Thus, for the first time we showed that CAPE caused relaxation in penile tissue through H2S.

Beside the effects of endogenous H2S-induced relaxations in mice penile tissue in oxidative stress induced by pyrogallol (100μM) in control and CAPE-incubated penile strips. Pyrogallol, decreased L-cysteine-induced relaxations significantly ($p < 0.001$). CAPE ameliorated impairment of endogenous H2S dependent relaxations in oxidative stress in mice penile ($p < 0.001$) and this beneficial effect of CAPE were reversed back by AOAA. These results demonstrate that oxidative stress reduced endogenous H2S-induced relaxations and CAPE ameliorates impaired relaxations through H2S.

Our study suggest that CAPE may contribute to erectile function and could be protective effects on erectile dysfunction under oxidative stress through H2S and targeting endogenous H2S pathway may prevent ED associated with oxidative stress.

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P052 | Molecular mechanisms underlying bisphenol A-associated decline in human sperm qualitY

Joana Santiago[1], Joana Vieira Silva[1,2,3], Marketa Simkova[4], Manuel AS Santos[1], Jana Vitku[4], Margarida Fardilha[1]
[1]Institute of Biomedicine – iBiMED, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal; [2]LAQV/REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal; [3]Unit for Multidisciplinary Research in Biomedicine; (UMIB), ICBAS, University of Porto, Porto, Portugal; [4]Department of Steroids and Proteofactors, Institute of Endocrinology, Prague, Czech Republic

Every day we are exposed to environmental chemicals that affect our endocrine system commonly called endocrine disruptors, such as bisphenol A (BPA). BPA leaks from polycarbonate plastics used to line food and drink containers and seems to negatively affect male fertility. High urine and seminal plasma BPA levels have been associated with low sperm quality and production, and paternal exposure may contribute to diseases in their offspring. However, only a few studies reported the deregulation of sperm proteins or RNAs associated with this risk factor for male infertility. This work aimed to investigate the correlation between seminal plasma BPA concentration and seminal quality in Portuguese men living in the Aveiro region. Additionally, we intended to study the alterations in human sperm proteins and small non-coding RNAs to recognize potential biological markers of exposure, sperm quality decline and poor fertility to be used to diagnose and manage male infertility. To do so, 102 Portuguese men from the Aveiro region (Portugal), aged between 19 and 56 years old were included in this study. Basic semen analyses were performed

on all samples according to WHO's guidelines. To avoid contamination by somatic cells, density gradient sperm selection was performed. The levels of BPA in the seminal plasma were determined using liquid-chromatography mass spectrometry (LC-MS/MS). The proteome of 20 normozoospermic human sperm samples divided into four groups according to BPA levels in seminal plasma was evaluated by quantitative proteomic analysis. The small RNA content of 16 human sperm samples was investigated using small RNA sequencing.

In this study, BPA was detected in 88% of seminal plasma samples and the mean BPA concentration was 0.175 ± 0.133 ng/ml. Our data showed that in this study population, there was no correlation between BPA levels and the seminal parameters evaluated; however, a tendency to have a higher concentration of BPA in the seminal fluid of non-normozoospermic patients compared to normozoospermic men was observed. Proteomic analyses revealed 75 differentially expressed proteins (DEPs) between groups. Gene ontology analysis of all deregulated sperm proteins shows that protein sumoylation and translation are common biological processes affected in samples with higher levels of BPA. Transcriptomic analysis identified 5 miRNAs correlated with BPA levels some of them already associated with infertility-related phenotypes, such as asthenozoospermia, oligoasthenozoospermia and azoospermia.

In conclusion, our results showed that contrary to what has been previously observed in other studies, BPA does not have any significant effect on semen parameters in this cohort. However, the characterization of the molecular mechanisms underlying BPA-induced male infertility, by the identification of DEPs and miRNAs that may constitute potential biomarkers of exposure to this endocrine disruptor, might explain some situations of fertilization failure and abnormal embryo development associated with these men.

Keywords: Bisphenol A; seminal plasma; spermatozoa; proteome; small non-coding RNAs

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P053 | Effects of lifestyle and mercury exposure on male reproductive health: a cross-sectional studY

Magda Carvalho Henriques[1], Joana Santiago[1], Maria Teresa Herdeiro[1], António Patrício[2], Pedro Fontes Oliveira[3], Susana Loureiro[4], Margarida Fardilha[1]

[1]Department of Medical Sciences, Institute of Biomedicine; (iBiMED), University of Aveiro, Aveiro, Portugal; [2]Centro Hospitalar do Baixo Vouga, Hospital Infante D. Pedro, Aveiro, Portugal; [3]LAQV/Requimte, Department of Chemistry, University of Aveiro, Aveiro, Portugal; [4]Department of Biology, Centro de Estudos do Ambiente e do MAR; (CESAM), University of Aveiro, Aveiro, Portugal

Over the past few decades, there has been increasing evidence on the global decline in male reproductive health. Many studies have reported that lifestyle factors (e.g., alcohol, cigarette and drugs consumption) and exposure to environmental pollutants (e.g., mercury (Hg) and bisphenol A) may affect the male reproductive function and consequently, decrease the semen quality. Hg is one of the most prevalent contaminants, generated by anthropogenic activities such as industrial, pharmaceutical, and agricultural activities. Increased Hg levels were associated with male infertility or subfertility status, increased sperm DNA damage and abnormal sperm morphology and motility. Several studies showed that infertile subjects with unexplained infertility had higher levels of Hg in hair, blood, and urine than fertile ones. However, the molecular mechanisms underlying the effects of exposure to Hg and lifestyle on male fertility decline remains unknown.

Thus, the main goals of this cross-sectional study were to: i) assess male exposure to Hg in the Aveiro region using hair as non-invasive biological matrix; ii) examine the influence of variables that may contribute to Hg exposure; and iii) study the impact of Hg exposure and lifestyle on the male reproductive health. For that, the study was carried out in thirty eligible men who attended the Urology service at Centro Hospitalar do Baixo Vouga (located in Aveiro). A detailed questionnaire regarding sociodemographic, diet, lifestyle and reproductive data was completed by participants. Samples of semen, hair, and urine were collected in the normal setting of the hospital from participants. Semen samples were analysed according to the World Health Organization criteria by experienced technicians and spermatozoa extracts were performed. Total Hg (THg) levels were quantified in hair samples by atomic absorption spectrometry after thermal decomposition of the sample using the Advanced Mercury Analyzer (AMA-254, LECO). According to hair THg levels, participants were divided into three groups and urine metabolomic profiling was performed by nuclear magnetic resonance (NMR) spectrometry.

This study demonstrated Hg bioaccumulation in biological samples from participants living in the Aveiro region: 47% of all individuals had THg levels higher than Hg levels considered acceptable by United States Environmental Protection Agency (US EPA) (1000 ng/g) and 20% presented THg levels higher than Hg levels considered acceptable by WHO (2000 ng/g). Moreover, significant positive correlations between THg levels in hair and percentage of tail defects and THg levels and teratozoospermia index were found. Thus, our results yielded additional information for conducting Hg risk assessment for the male reproductive health. Further and continuous monitoring of Hg exposure and male lifestyle behaviour should be required in order to prevent possible adverse effects in male reproduction.

P054 | Deciphering the timelines of L1 retrotransposon silencing and cell cycle activity in human foetal testis

Marta Himelreich Peric[1,2], Dajana Krsnik[1,2], Tanja Lenicek[3], Nino Sincic[1,2], Floriana Bulic-Jakus[1,2], Davor Jezek[2,4], Ana Katusic Bojanac[1,2]

[1]Department of Biology, University of Zagreb School of Medicine, Zagreb, Croatia; [2]Center of Excellence for Reproductive and Regenerative Medicine,

University of Zagreb School of Medicine, Zagreb, Croatia; [3]Department of Pathology, Clinical Hospital Sisters of Mercy, Zagreb, Croatia; [4]Department of Histology and Embryology, University of Zagreb School of Medicine, Zagreb, Croatia

Epigenetic alterations in retrotransposable elements and the dysfunction in their silencing pathways in male germ cells have been recently associated with severe form of male infertility (e.g. azoospermia). In addition, possible alterations of these elements in male germ cells can be transmitted to the next generation with an effect on the offspring's phenotype. Retrotransposon silencing by DNA methylation has been extensively studied in mouse spermatogenesis, but limited achievements are observed in humans, due to the rarity of the appropriate samples.

The aim of this study was to investigate overall LINE-1 DNA methylation in human foetal testis samples which span mid- and late-gestation periods in relation to cell cycling activity, as it was predicted in rodents that DNA methylation takes place in the mitotically quiescent cells. Hence, RB1 gene methylation in human tissues along with the distribution of tumor suppressor protein pRB1 and its phosphorylated forms in relation to the proliferation marker Ki-67.

DNA methylation was studied in FFPE samples of human foetal testis using the sequencing-by-synthesis approach (e.g. pyrosequencing) which allows accurate quantification of the examined CpG sites. The protein expression was studied by immunofluorescence while also quantified on the immunohistochemically stained FFPE sections.

We observed statistically significant LINE-1 DNA methylation increase in mid-gestation period while also a slight reduction at the beginning of puberty. RB promotor region was hypomethylated until the end of pregnancy. Regarding the cell cycle, Ki-67 expression in testicular tubules showed a significant decline in the 2nd and 3rd trimester, with the highest decrease in 20-21w. Interestingly, pRB expression also decreased until the end of pregnancy, but with value oscillations. We next searched for more specific expression of RB-phosphorylated forms, as phosphorylation of its carboxyl-terminal domain at S780 and S795 directly inhibits RB association with E2F. First, we showed that the number of pRBser780-positive cells per tubule was significantly reduced in mid-gestation period (20w), but later oscillates with the increasing trend. pRBSer795-positive cells followed the similar pattern of expression as previous form, such as downtrend in the mid-gestation (20w) period.

Although more research is needed, it seems that, in humans, epigenetic silencing of L1 retrotransposon occurs during mid-gestation period and is coupled with the changes in the activity of cell cycle regulators, such as RB protein and Ki-67. More work on these events is needed to clearly define the sensitive periods of testis development in humans, whose perturbations might lead to infertility or germ cell cancer later in life.

P055 | Beta-hydroxybutyrate improves sperm motility in capacitated sperm cells

Giuseppe Grande, Luca De Toni, Claudia Pappalardo, Federica Pedrucci, Federica Finocchi, Ilaria Cosci, Alberto Ferlin, Andrea Garolla, Carlo Foresta

Department of Medicine, Unit of Andrology and Reproductive Medicine, University of Padova, Padua, Italy

Ketone bodies, D-beta-hydroxybutyrate and acetoacetate, produced by the metabolism of fatty acids, are an important energy source for many organs, especially the heart, kidney and brain. They are utilized by the body with the help of succinyl CoA transferase, which is ubiquitously expressed in various organs, including testis. Previous studies demonstrated in mouse that beta-hydroxybutyrate and acetoacetate both stimulate the motility of sperm cells.

Ketone bodies have been moreover detected in follicular fluid, thus having a putative role in fertilization, by inducing hyperactivated sperm motility. It is moreover known that beta-hydroxybutirate is reduced in follicular fluid of patients with PCOS and endometriosis.

To analyze the effect of ketone bodies on sperm motility, sperm samples have been collected by 10 fertile volunteers. Non capacitated sperm cells have been incubated with 3beta-hydroxybutyrate 4 mM. The same procedure has been performed in samples of capacitated sperm cells (BSA 3%, 3 hours). We evaluated in each sample the percentage of sperm cells with progressive motility.

No significative effects have been observed in non-capacitated sperm cells. In capacitated sperm cells, beta-hydroxybutirate induced a significant increase in sperm progressive motility.

We confirmed, for the first time in humans, previous data about the role of ketone bodies in activating sperm motility in capacitated sperm cells. These results open new perspectives in understanding the role of ketone bodies in sperm biology and in fertilization. Further clinical studies are moreover needed to analyze the effect of ketogenic diets in modifying sperm-oocyte micro-environment and improving the fertilization process, in infertility and namely in infertile patients with PCOS.

P135 | Evaluation of selected semen parameters and biomarkers of male infertility – preliminary study

Michal Kups[1,2], Kamil Gill[3], Patryk Harasny[3,4], Tomasz Machalowski[3,5], Marta Grabowska[3], Rafal Kurzawa[2,6], Malgorzata Piasecka[3]

[1]Department of Urology and Oncological Urology, Regional Specialist Hospital in Szczecin, Szczecin, Poland; [2]TFP Fertility Vitrolive in Szczecin, Szczecin, Poland; [3]Department of Histology and Developmental Biology, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland; [4]Department of Urology and Urological Oncology,

Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Szczecin, Poland; [5]Department of Perinatology, Obstetrics and Gynecology, Faculty of Medicine and Dentistry, Pomeranian Medical University in Szczecin, Police, Poland; [6]Department of Gynecology and Reproductive Health, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland

The etiopathogenesis of male infertility is a multifactorial medical problem and is correlated with many congenital and acquired defects of the urogenital tract, cancers, urogenital infections, heat stress in the scrotum, hormonal disorders, genetic abnormalities and immunological factors. It is estimated that approximately 30–50% of male infertility cases are recognized as idiopathic, very often associated with low-quality of spermatozoa. On the other hand, unexplained infertility (couples where male patients have normal basic semen parameters and female patients have normal ovulation and fallopian tube potency) is diagnosed in approximately 15% of cases Therefore, the comprehensive evaluation of male fertility status should be developed using scrotal ultrasonography (USG) and assessment of the key reproductive hormone as well as advanced seminological tests.

Our study was designed to clarify the relationship between standard semen parameters, testicular volume, levels of reproductive hormones and the fragmentation of sperm nuclear DNA (SDF) in group of infertile participants.

Patients (n = 130) were clustered as subjects: 1) with an abnormal volume (utrasonography) of at least one testis (<12 mL) or with a normal volume of testes and 2) with abnormal levels of at least one of the reproductive hormones (FSH, LH, PRL, TSH, total T – electrochemiluminescence method) or with normal hormonal profiles and 3) with high level of SDF (>30%), moderate (>15–30%) or low (≤15%) (sperm chromatin dispersion test).

In subjects with a decreased testicular volume (vs. subjects with normal testicular volume) and in subjects with abnormal levels of reproductive hormones (vs. subjects with normal levels of hormones), decreased basic semen parameters (sperm count, morphology and progressive motility) were found. Additionally, participants with abnormal testicular volume had a higher percentage of SDF (medians: 27.00% vs. 17.00%) and a higher level of FSH (medians: 8.05 mIU/mL vs. 5.29 mIU/mL). In turn, men with a high level of SDF had lower testicular volume (left testis volume – medians: 13.00 mL vs. 16.00 mL; right testis volume –medians: 12.00 mL vs. 16.00 mL) and conventional sperm parameters (sperm count, morphology, progressive motility, total motility and vitality) than men with a low level of SDF. On the other hand, there were no significant differences between men with SDF >30% and men with SDF >15–30% in any study parameters. Analysis of the Spearman's rank correlation coefficient showed negative relationship between SDF and sperm count, morphology, motility, vitality as well as with volume of the left testis. Moreover left and right testis volumes were negatively correlated with the level of FSH and positively with sperm count. Additionally, volume of left testis positively correlated with sperm progressive motility. Furthermore, the LH level was negatively correlated with sperm count.

We showed that spermatogenesis disorders coexisted with decreased testicular volume and increased FSH levels. The disorders of spermatogenesis were manifested by reduced basic sperm characteristics and a high level of sperm nuclear DNA damage. It should be highlighted that clarification of the relationship between study parameters and clinical features might help to develop new personalized strategies for therapeutic interventions.

P136 | Is response of spermatozoal mitochondrial dynamics markers to repeated stress circadian?

Isidora M Starovlah, Sava M Radovic Pletikosic, Tamara M Tomanic, Marija Lj Medar, Tatjana S Kostic, Silvana Andric

Laboratory for Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging, CeRES, DBE, Faculty of Sciences, University of Novi Sad, Serbia

In the search for the possible role of mitochondrial dynamics markers in spermatozoa adaptation, the in vivo approach was designed to mimic the situations in the human population exposed to repeated psychological stress (the most common stress in human society) at different time points (ZT3, ZT11, ZT23) during the day (24h). The hormones (stress hormone corticosterone, testosterone), the number and the functionality of spermatozoa (response to acrosome-reaction-inducer progesterone), as well as the transcriptional profiles of 22 mitochondrial dynamics and function markers and 22 signaling molecules regulating both, mitochondrial dynamics and spermatozoa number and functionality, were followed at three time-points (ZT3, ZT11, ZT23). The results show that repeated stress significantly decreased the number and functionality of spermatozoa at all time points. In the same samples, the transcriptional profiles of 91% (20/22) mitochondrial dynamics and functionality markers and 86% (19/22) signaling molecules were disturbed after repeated stress. It is important to point out that similar molecular changes in transcriptional profiles were observed at ZT3 and ZT23, but opposite at ZT11, suggesting the circadian nature of the adaptive response. The results of PCA analysis show the significant separation of repeated-stress-effects during the inactive/light and active/dark phases of the day suggesting the circadian-timing of molecular adaptations.

CLINICAL SCIENCE - FERTILITY

P058 | A complete dissection of the whole testicular parenchyma is required in most patients with non-obstructive azoospermia to obtain enough good quality testicular sperm for ICSI

Giovanni M. Colpi[1], Fabrizio Castiglioni[1], Franco Nerva[2], Cristina Campagna[1], Elisabetta M Colpi[1], Elisabetta Piatti[1], Giacomo Gazzano[3], Ettore Caroppo[4]

[1]Next Fertility Procrea, Andrology Unit, Lugano, Switzerland; [2]Next Fertility Milano, Andrology Unit, Milano, Italy; [3]Istituto Auxologico Italiano IRCCS, Pathology Unit, Milano, Italy; [4]ASL Bari, Reproductive Unit, Andrology Outpatients Clinic, PTA "F Jaia", Conversano, Ital

Due to the heterogeneous distribution of seminiferous tubules in the testes of patients with non-obstructive azoospermia (NOA), retrieving enough good quality sperm for ICSI may require a complete testicular dissection. According to the only available study in this field, sperm may be found in the testis surface in 34.2% of patients, while a deeper testicular dissection is able to provide sperm for ICSI in 28% of those without sperm in the testis surface. The present study sought to determine the probability of finding enough sperm for ICSI at the initial wide incision of the testis in another cohort of patients with NOA undergoing microdissection testicular sperm extraction (mTESE).

We retrospectively evaluated 276 patients, aged 37 (20-62) years, who underwent unilateral (86, 31.2%) or bilateral (190, 68.8%) mTESE from January 2018 through December 2021. During mTESE the entire surface of the testicular parenchyma was explored first in search for dilated seminiferous tubules: if no/ not enough sperm were retrieved, the deeper portion of the testicular parenchyma was explored. Histopathology demonstrated Sertoli-cell only syndrome in 65.6% of operated testes, while maturation arrest was found in 19.5%, hypospermatogenesis in 12.7% and hyalinosis in 2%

Sperm was retrieved in 137 patients (49.6%). Sperm were obtained at the initial wide incision in 46 out of 174 testes (26.4%) and only in patients who underwent unilateral mTESE, with a consequent probability of 16.6% in the whole cohort of patients with NOA (46/276 patients). In the remaining patients (91, 66.4% of those with SSR, 32.9% of the whole cohort), a deeper testicular dissection was required to obtain enough good quality sperm for ICSI: in some of them sperm were even found at the initial wide incision, but their number and/or quality was not sufficient for ICSI. On multivariate logistic regression, only the histopathological subcategory hypospermatogenesis was predictive of the chance of retrieving sperm from the surface of the testis (OR 3.24, 95% CI 1.37-7.69, p = 0.007).

The results of the present study suggest that most patients with NOA, particularly those with unfavorable histopathological patterns (such as Sertoli-cell only syndrome or maturation arrest), require a complete dissection of the testicular parenchyma to obtain enough good quality for ICSI. Since mTESE enables the complete exploration of the testicular parenchyma, it is to be preferred to conventional TESE, which allows the retrieval of the seminiferous tubules from the testis surface only, to retrieve sperm in patients with NOA.

P059 | Clinical risk factors for occurrence of anti-sperm antibodies: a retrospective study on over 2700 men

Chiara Castellini, Antonio Parisi, Maria Totaro, Daniele Tienforti, Francesca Di Giulio, Luca Spagnolo, Felice Francavilla, Arcangelo Barbonetti

Andrology Unit, Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Introduction: The presence of anti-spermatozoa antibodies (ASA) in the semen represents a relative cause of infertility that can become absolute when 100% of the spermatozoa are involved by the autoimmune reaction primarily due to ASA interference with sperm penetration through the cervical mucus. The latest edition of the WHO manual for the Laboratory Examination and Processing of Human Semen, unlike the previous one in 2010, no longer recommends the screening for ASA as an integral part of the routine semen analysis: direct screening tests, mixed anti-globulin reaction (MAR) and immunobead (IB) test, have been relegated among the extended examinations, to be performed only in in certain circumstances for diagnostic or research purposes. Unfortunately, there is no mention in the manual of when these tests should be executed. Sperm agglutinations may induce diagnostic suspicion, but ASA can be present without sperm agglutination. The only confirmed clinical risk condition for developing ASA is vasectomy: for a number of other putative risk factors, the evidence remains inconclusive due to discordant data and/or produced by applying unreliable methods on small series of patients.

Materials and Methods: A retrospective analysis of 2712 consecutive men who attended our university/hospital andrology clinic for the evaluation of fertility potential was carried out. Immunological screening with the IgG-MAR test had been performed on all ejaculated. Clinical data were retrieved from medical records.

Results: An IgG-MAR test positivity between 10% to 49% and ≥50% was found in 30 (1.1%) and 199 men (7.3%), respectively. Of all the possible information reported in the medical history, when compared to ASA-negative group (n = 2483), men with ≥50% IgG-MAR test positivity more frequently reported a history of ureaplasma urealyticum infection (2.5% vs. 0.6%, p = 0.009), post-pubertal parotitis (4.0% vs. 1.4%, p = 0.01), hernioplasty (7.0% vs. 2.3%, p = 0.0001) and hemorrhoidectomy (1.0% vs. 0.1, p = 0.04). At the multiple logistic regression analysis, all these variables were independently associated with IgG-ASA occurrence. A nomogram was then constructed that could predict the probability of IgG-ASA occurrence as a function of the different combinations of the four independent predictors identified at the multiple logistic regression. The variable with highest predictive power was hemorrhoidectomy surgery (OR:7.4; 95%CI: 1-46.8), followed by ureaplasma urealyticum infection (OR:4.29; 95%CI: 1.37; 11.3), hernioplasty (OR:3.2; 95%CI: 1.7-5.8) and post-pubertal parotitis (OR:2.8; 95%CI: 1.2-5.8). The internal calibration plot revealed a significant agreement degree between the predicted and observed probability.

Conclusions: The present study identified clinical conditions in which it might be appropriate to supplement standard seminal analysis with testing for IgG-ASA. Further studies are needed to clarify the pathogenetic mechanisms underlying the revealed associations and to produce an external validation of our predictive model.

P060 | Unsupervised datamining of orchiopexy surgical reports for unilateral cryptorchidism reveals unnoticed profiles of genital anomalies

Safouane Hamdi[1], Eng Virak[1], Clara Brusq[1], Elodie Prunier[1], Roger Mieusset[1], Ad Olivier Abbo[2]

[1]*DEFE, INSERM, Université de Toulouse III, CHU Toulouse, Toulouse, France;* [2]*Paediatric Surgery Department, CHU Toulouse, Université de Toulouse III, Toulouse, France*

Introduction: Cryptorchidism is the most common birth defect in newborn boys and the current standard therapy for undescended testes is inguinal or scrotal orchidopexy. This surgical procedure provides an accurate insight of anatomical variations and anomalies of the male genital tract. Analysis of cryptorchidism subphenotypes that comprise these associated anomalies may lead to a better recognition of disease heterogeneity, a deeper understanding of risk factors roles and a better further follow-up for cryptorchid boys.

Patients and Methods: A final study population of 422 boys with unilateral cryptorchidism was gathered and a set of nine data were extracted from orchiopexy reports. An unsupervised clustering was performed using the Gower distance function and the partition around medoids (available in R). We defined clusters number through silhouette analysis and reported graphical and statistical descriptions for each cluster.

Results: Median age of orchiopexy was 46.8 months. Undescended testes were 52.4% on the right side, 65.3% in the external ring, 31.4% within the inguinal canal and 3.3% in an intra-abdominal position. Amongst them, 29.1% presented a small size and 32.2% an abnormal epididymo-testis connexion. According to these variables, the Gower algorithm identified 10 different clusters of boys. Statistical and graphical analysis confirmed that these anomalies were significantly associated with higher testes' positions (i.e abdominal and intracanalar position vs external ring). However, for testes at the external ring, the frequency of anomalies was significantly higher when the peritoneal-vaginal duct remained open i.e. testis size was more often smaller and the epididymis more often disconnected (respectively 33.1% vs 13.6%, $p < 0.001$ and 33.1 vs 14.3%, $p < 0.001$). To date, this finding has not been reported and quantified. It raises the question of the link between peritoneal-vaginal duct patency, testes anomalies and migration and distinguishes an unnoticed group at risk amongst cryptorchid boys.

Conclusion: This study confirms the effectiveness of Gower algorithm to mine orchiopexy surgical data and reveals an anatomical pattern that opens news pathophysiological and clinical perspectives.

P061 | Features of Leydig cells in patients with non-obstructive azoospermia

Dinko Hauptman[1], Zoran Zimak[1], Marta Himelreich Peric[2], Tihana Maric[2], Zeljko Kastelan[1], Ana Katusic Bojanac[2,3], Davor Jezek[2,4,5]

[1]*University Hospital Zagreb, Department of Urology, Zagreb, Croatia;* [2]*Scientific Centre of Excellence, School of Medicine, University of Zagreb, Zagreb, Croatia;* [3]*University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia;* [4]*University of Zagreb, School of Medicine, Department of Histology and Embryology, Zagreb, Croatia;* [5]*University Hospital Zagreb, Department of Transfusion Medicine and Transplantation Biology, Reproductive Tissue Bank, Zagreb, Croatia*

Non-obstructive azoospermia (NOA) is a form of male infertility characterized by disorders of the testicular parenchyma and impaired spermatogenesis. This study aimed to investigate the nature of Leydig cell changes in patients with NOA, especially whether their actual proliferation occurred. The study included a total of 72 patients with azoospermia. 24 patients (control group) were diagnosed with obstructive (OB) and 48 with non-obstructive azoospermia. Qualitative histological analysis of the control group of patients (men with obstructive azoospermia and fully preserved morphology of the testicular parenchyma) and biopsies of patients with NOA showed a significant difference in the degree of preservation of spermatogenesis and morphology of Leydig cells. In the group of infertile men with NOA Leydig cells sometimes displayed an abundant cytoplasm and were organized into larger clusters. However, in some samples from patients with NOA, significant fibrosis of the interstitial compartment has been demonstrated, often with the presence of inflammatory cells (mononuclear leukocytes). The results of the stereological analysis showed that there was no increase in the number of Leydig cells; on the contrary, the comparison of the examined groups of patients showed a slight decrease in their number in the biopsy samples of patients with NOA. This decrease in the number of Leydig cells can be explained by previous inflammatory changes within the testicular interstitium that cause consequent interstitial fibrosis.

P062 | Sperm retrieval using mTESE in patients with Klinefelter syndrome and azoospermia

Jan Karol Wolski[1], Karina Gawron[1], Robert Wyban[1], Agnieszka Sobczyńska-Tomaszewska[2], Joanna Ostrowska[3]

[1]*The nOvum Fertility Clinic, Warsaw, Poland;* [2]*Medgen's Laboratory of Genetic Diagnostics, Warsaw, Poland;* [3]*Center of Pathomorphological Diagnostics, Warsaw, Poland*

Introduction: Azoospermia is caused by genetic abnormalities occur up to 25%. Klinefelter syndrome (KS) is the most frequent sex chromosomal aneuploidy, occurring in 1-2/1,000 live male births. Less than 50% of individuals with KS are diagnosed in their lifetime. KS is 13.7% in the azoospermic group. Sperm obtaining and inclusion in reproduction men with KS is still a controversial topic in the fertility clinic. The second problem is the protection of fertility in adolescents with KS, based on knowledge of present complete spermatogenesis in a younger group of patients.

Objectives: Analysis results of mTESE in patients with Klinefelter Syndrome and azoospermia.

Materials and Methods: Based on our experience (from 2012 – 467 mTESE), during 2017-2022 36 patients with KS 47.XXY / per total

321 mTESE (11.2%) had testicular biopsy mTESE after the diagnosis of azoospermia, according to EAU Guidelines. In 6 patients additional genetic abnormalities were noticed: 3 (CFTR gene) : IVS8-5T + (TG)11 / - ; IVS8(TG)13(T)5 / -; delF508 / - and 3 (karyotype): 47,XXY,9qh-,21ps+; 47,XXY[4]/46,XY[46]; 47,XXY[23]/46,XX[9]. Patients were divided into 2 groups: 1) childless couple 28/36 (77.8%), age 25-42 years (mean 33); 2) fertility protection in adolescents 8/36 (22.2%), age 16-22 years (mean 18.5). Before the mTESE, hormone therapy was given to normalize LH, FSH and testosterone levels, as a known positive correlation factors in sperm obtaining. All mTESE procedures were done as the one-day-surgery protocol, under general anesthesia, with antibiotic prophylaxis. The Leica M860 2x2 microscope was used, magnification 20-25 x. The collected specimens (3 from each testes) were placed in Bouin's fluid for standard histopathological evaluation by pathologist according to the Johnsen score (1970). At the same time, the testicular tissue was transferred to the IVF LAB for cryopreservation, for future IVF/ICSI. No significant surgical and anesthetic complications after surgery were noted.

Results: The childless couple group (28): tubules with only few spermatozoa (<5-10) – 2/28 (7.1%). In others: Sertoli Cell Only Syndrome – 19/28 (67.9%); Maturation Arrest 7/28 (25%) : spermatogonia – 2; only few (<5) spermatocytes – 1; several or many spermatocytes – 1; only few spermatids (<5-10) – 2; many spermatids – 1.

The fertility protection in adolescents group (8): tubules with sperm – 2/8 (25%): only few (1) and only few/many (1). In others: Sertoli Cell Only Syndrome – 4/8 (50%); Maturation Arrest 2/8 (25%) : only few spermatids (<5-10) – 2; many spermatids - 1.

In all cases, hyperplasia of Leydig cells with the formation of nodules (Leydigioma) were recorded, without any signs of malignancy. In 1 patient (17 years), in the left gonad intratubular pre-invasive neoplasm Germ Cell Neoplasia In Situ (GCNIS) was diagnosed and unilateral orchiectomy was done.

Conclusion: A group of patients with KS can be included in reproduction based on sperm obtaining using mTESE.

In the fertility protection adolescent group, the histological findings are better than in the group of older men from childless couple, so this is suggestion that fertility protection should be proposed immediately after the diagnosis of KS.

According to high risk of neoplasm oncological evaluation of testicular specimens should be routine.

P063 | Human spermatozoa express TNF-a receptors

Simon Göttlich[1], Said Benfadal[2], Natalija Novak[2], Gerhard Haidl[1], Yonggang Duan[3], Jean-Pierre Allam[1]

[1]Department of Andrology University Hospital Bonn, Germany; [2]Department of Dermatology and Allergy University Hospital Bonn, Germany; [3]Center of Assisted Reproduction and Embryology, The University of Hong Kong, Shenzhen Hospital, Hong Kong

Cytokine concentration such as TNF-a in human seminal plasma has been connected to male subfertility. However, only little is known about the expression of respective on spermatozoa. Thus, the aim of this study was to evaluate expression of TNF-a receptor 1 (TNFaR1) and TNF-a receptor 2 (TNFaR2) on human spermatozoa from patients referring to andrology department for semen analysis (n = 72) by immunofluorescence and flow cytometry. In this context TNFaR1 (10.88% +/- SD 5.58) and TNFR2 (30.31% +/-SD 12.34) could be detected by flow cytometry on the surface of human spermatozoa. Immunofluorescence staining demonstrated that both receptors were expressed on the mid-piece and post-acrosomal segment. TNFaR1 and TNFaR2 expression did not correlate to TNF-a concentration in seminal plasma. However, significant correlation of TNFaR1 and TNFaR2 expression could be demonstrated to spermatozoa count and concentration while TNF-a concentration in seminal plasma inversely correlated to motility. Activation of TNFaR1 and TNFaR2 on spermatozoa led to an increase of apoptosis in spermatozoa. In conclusion, human spermatozoa express TNFaR1 and TNFaR2. Its activation might be involved in altered spermatozoa function.

P064 | Withdrawn

P065 | The real-time elastography can predict sperm retrieval in non-obstructive azoospermia

Arcangelo Barbonetti[1], Chiara Castellini[1], Guido Ranieri[2], Giuseppe Calvisi[3], Maria Totaro[1], Daniele Tienforti[1], Sandro Francavilla[1]

[1]Andrology Unit, Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy; [2]Urology Unit, San Salvatore Hospital, L'Aquila, Italy; [3]Pathology Unit, San Salvatore Hospital, L'Aquila, Ital

Background: In non-obstructive azoospermia (NOA), surgical sperm retrieval (SSR) success rate of testicular sperm extraction (TESE) did not exceed 50%. Unfortunately, available "pre-surgical" parameters exhibit a poor predictive value. The real-time elastography (RTE) is an ultrasonography (US) technology assessing the mechanical elasticity of tissues. In the hypothesis that a higher elasticity could reflect a wider preserved functional testicular parenchyma, we evaluated the potential role of RTE in predicting SSR in NOA patients undergoing microdissection TESE (mTESE).

Patients and Methods: 30 consecutive infertile patients attending our Andrology Unit for azoospermia underwent mTESE. Conventional grey-scale US and RTE were performed using an Esaote MyLab alpha US machine, equipped with the software ElaXto® for RTE (Esaote S.p.A., Florence, Italy). RTE images were obtained on the largest longitudinal scan to get a representative picture of the whole parenchyma elasticity: the colour mapping scale included blue (stiff tissue), green (intermediate strain), and red (soft tissue). To quantify the percentage of different colour shades, we used the software ImageJ®, providing, for each RTE image, the green to blue ratio (G/B ratio) as a global measure of tissue elasticity.

Results: Six patients (20% of the study population) received a diagnosis of obstructive azoospermia (OA), where, as expected, mTESE resulted in successful SSR in all cases. When compared to NOA, the group with OA exhibited significantly lower levels of FSH, LH, ejaculate volume and seminal pH, as well as a significantly higher US testicular volume. In OA series, the histological confirmation of preserved spermatogenesis allowed to define the "normality features" of testicular RTE images. Quantitative analysis revealed a prevalence of green (median: 55.2%) over blue pixels (29.6%) with a G/B ratio regularly ≥1.5. In the series of 24 patients with NOA, testicular histopathology revealed different spermatogenesis disorders, included hypospermatogenesis, meiotic arrests, Sertoli Cell-Only Syndrome and scleroyalinosis. In the NOA group, 15 patients (62.5%) yielded successful SSR. They exhibited RTE images similar to those observed in OA. Whereas, in the 9 cases where mTESE yielded negative SSR, RTE images predominantly displayed centripetal, blue-colored pixels with a low G/B ratio, in no case higher than 1.4 (median: 0.78, range: 0.5-1.4). When compared to NOA patients without SSR, those yielding successful SSR exhibited RTE images with significantly lower percentages of blue pixels (42.1 ± 12.0% vs. 52.7 ± 6.6%, p = 0.01) and higher G/B ratio (1.54 ± 1.49% vs. 0.78 ± 0.26%, p = 0.02). No significant difference was found in endocrine, seminal, and conventional US parameters.

Conclusions: In our series of patients with NOA, quantitative analysis of testicular RTE images was demonstrated to be a non-invasive, easily applicable, and repeatable method to predict SSR at mTESE. In particular, a G/B ratio higher than 1.4 excluded an unsuccessful SSR.

P066 | Testosterone serum levels are related to sperm DNA fragmentation index reduction after FSH administration in males with idiopathic infertilitY

Giorgia Spaggiari[1], Monica Lispi[2,3], Drakopoulos Panagiotis[4,5,6], Francesca Caprio[7], Nicola Colacurci[7], Manuela Simoni[1,8], Daniele Santi[1,8]

[1]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Modena, Italy; [2]Merck KGaA, Global Medical Affairs Fertility, Research and Development, Darmstadt, Germany; [3]University of Modena and Reggio Emilia, Clinical and Experimental Medicine PhD Program, Modena, Italy; [4]Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium; [5]Department of Obstetrics and Gynaecology, University of Alexandria, Alexandria, Egypt; [6]In Vitro Fertilisation (IVF) Athens, Athens, Greece; [7]Department of Woman, Child and General and Specialized Surgery, University of Campania 'Luigi Vanvitelli', Naples, Italy; [8]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Ital

Background: Testicular overstimulation is the pursued therapeutic goal when exogenous follicle stimulating hormone (FSH) is empirically administered to men with idiopathic infertility. Although a robust physiological rationale supports the FSH use in male idiopathic infertility, useful biomarkers to monitor and evaluate its efficacy are still far from being detected. While pregnancy rate remains the strongest outcome in couple infertility management, the identification of reliable and possibly early biomarkers of therapeutic response to FSH in males is mandatory. Sperm DNA fragmentation (sDF) index, even if supported by many evidences, is not yet recognized as valid endpoint of hormonal treatment efficacy. Moreover, no suitable biomarkers of FSH effectiveness during the therapy are available.

Aim: The primary aim of the study was to evaluate if testosterone serum levels are related to sDF change after FSH administration. The secondary aim was to confirm the sDF index validity as biomarker of FSH administration effectiveness in male partners of infertile couples using a prospective, controlled dataset.

Methods: A retrospective post-hoc re-analysis was performed on raw data of clinical trials in which idiopathic infertile men were treated with FSH and both testosterone serum levels and sDF were reported among primary and/or secondary endpoints. Additional data regarding couple infertility history, age, anthropometric variables, FSH treatment scheme and semen parameters were included in a single dataset. Logistic regression analysis was performed using responders to FSH treatment as dependent variable and all parameters detected after FSH administration as cofactors/covariates.

Results: Three trials were included accounting for 251 patients (median age 35, 28-59 years). The comprehensive analysis confirmed the FSH beneficial effect on spermatogenesis detected in each single trial. Indeed, increasing the sample size, this re-analysis highlighted an overall significant sDF decrease ($p < 0.001$) of 20.2% of baseline value. Although sDF resulted not related to testosterone serum levels at baseline, a significant correlation was highlighted after three months of FSH treatment ($p = 0.002$). Moreover, testosterone serum levels and patients' age significantly correlated with sDF ($p = 0.006$). Dividing the cohort in responders/not responders to FSH treatment according to sDF change, the FSH effectiveness in terms of sDF improvement was related to testosterone and sex hormone binding globulin serum levels ($p = 0.003$).

Conclusion: The re-analysis of published trials data investigating FSH administration in male idiopathic infertility highlights the beneficial effect of exogenous FSH stimulation with a 20% relative decrease of baseline sDF index. In terms of sDF reduction, a 59.2% of FSH-responders was here detected. Although sDF index does not correlate with testosterone serum levels at baseline, after three months of FSH administration a significant inverse correlation appears, suggesting an association between the FSH administration-related sDF improvement and testosterone serum levels increase. This is the first time in which a communication/interaction between the two cell compartments of the testis (i.e. Sertoli and Leydig cells) can be hypothesized in response to FSH administration.

P067 | Real-world evidence of follicle stimulating hormone effectiveness in male idiopathic infertilitY

Giorgia Spaggiari[1], Marilina Romeo[1,2], Federico Nuzzo[2], Antonio R. M. Granata[1], Manuela Simoni[1,2], Daniele Santi[1,2]

[1]*Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Modena, Italy;* [2]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Ital*

Background: Exogenous follicle-stimulating hormone (FSH) administration in male idiopathic infertility showed the most convincing physiological rationale in the face of a clinical efficacy below expectations. Accordingly, it was calculated that 10 to 18 men have to be treated with FSH to achieve one pregnancy.

Aim of the study: The aim of the study is to assess the effectiveness of FSH administration in male idiopathic infertility in a real-world clinical setting.

Materials and Methods: A retrospective real-world study was carried out, including all consecutive male partners of infertile couples attending the Andrology Unit of Modena (Italy) from June 2015 to May 2022. Medical history, physical and andrological examinations, hormonal and seminal parameters, therapeutic management and pregnancy data were collected. Primary endpoints were semen parameters, while the number of pregnancies was the secondary outcome.

Results: 197 on 362 (54.4%) infertile men were treated with FSH (mean age 37.9 ± 6.1 years). After FSH administration (therapy duration 9.1 ± 7.1 months), a significant increase in sperm concentration (9.9 ± 12.2 versus 18.9 ± 38.9 million/mL, p = 0.045) was detected. Also, treatment led to a significant increase in normozoospermia (from 1.0 to 4.8%, p = 0.044) and decrease in azoospermia rate (from 9.6 to 6.5%, p = 0.044). Forty-three pregnancies were recorded (30.5%), 22 spontaneous and 21 after assisted reproduction. Dividing the cohort in FSH-responders and non-responders considering obtaining or not a pregnancy, a higher sperm concentration (15.7 ± 26.6 versus 22.2 ± 25.7 million/mL, p = 0.033) and progressive sperm motility (18.0 ± 18.2 versus 27.3 ± 11.3, p = 0.044) were found in pregnancy group.

Conclusion: Our experience suggests that FSH empirically administered to men with idiopathic infertility increases sperm concentration and leads to pregnancy in 1 of 5 patients. Although the expected limits due to a real-world data study, the number of FSH-treated patients required to achieve a pregnancy seems to be lower if compared to previously published data.

P068 | A novel diagnostic test to identify patients suffering from loss of CatSper function

Samuel Young‡[1], Christian Schiffer‡[1], Alice Wagner[1], Birgit Stallmeyer[2], Claudia Krallmann[1], Jannika Patz[1], Anton Potapenko[1], Leonnie Herrmann[1], Albrecht Röpke[2], Tim Pock[1], Tanja Sperlbaum[3], Hermann M Behre[3], Verena Nordhoff[1], Sabine Kliesch[1], Frank Tüttelmann[2], Christoph Brenker[1], Timo Strünker[1]

[1]*Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany;* [2]*Institute of Reproductive Genetics, University of Münster, Münster, Germany;* [3]*University Hospital Münster Fertility Clinic, University of Münster, Münster, German*

There is a growing body of evidence that human sperm require the activity of the sperm-specific Ca2+ channel CatSper (cation channel of sperm) to fertilize the egg.

Therefore, we developed the prototype of a novel in vitro diagnostic test to assess the function of CatSper in sperm from patients undergoing semen analysis. Using this "CatSper-Activity-Test", we identified in a cohort of 576 men seeking medical advice for suspected male infertility seven patients suffering from a loss of CatSper function. According to standard semen analysis, the CatSper-deficient patients are (with one exception) normozoospermic, feature no other pathological condition, and were diagnosed with unexplained infertility. Most notably, their sperm not only failed to fertilize the egg naturally but also upon intrauterine insemination (IUI) and in vitro fertilization (IVF), whereas intra-cytoplasmic sperm injection (ICSI) was successful. Two additional CatSper-deficient patients were identified among patients visiting our clinics for other reasons. We show that the loss of CatSper function is predominantly caused by a homozygous deletion of the CATSPER2 gene; one patient featured compound heterozygous variants of the CATSPERE gene.

The CatSper-deficient sperm from these patients were characterized using a battery of techniques, including motility analysis, electrophysiology, and Ca2+-fluorimetry. We show that CatSper-deficient sperm fail to hyperactivate and, thus, to penetrate the egg coat, explaining the failure of natural conception, IUI, and IVF. In fact, considering the total IVF failure rate of our institute, and given that each of the seven patients would have initially undergone IVF before attempting ICSI, we assume that CatSper-deficiency might account for nearly half of all male-factor IVF failures.

We conclude that loss of CatSper function is a common cause of unexplained male infertility. Affected patients are prone to experience failing cycles of medically assisted reproduction (MAR) using IUI and IVF – if the infertility is identified at all. The novel CatSper-Activity-Test faithfully identifies CatSper-deficient patients with a hands-on-time of only a few minutes and no special equipment or training required. Therefore, we envisage the CatSper-Activity-Test as a novel tool for the early diagnosis of male infertility allowing evidence-based treatment decisions in MAR, thus, sparing patients the burden of unnecessary medical and financial risks.

P069 | Epididymal organ sparing microsurgery – Adenomatoid tumors of epidydimis

Judit Vargha, Zsolt Kopa

Andrology Centre, Department of Urology, Semmelweis University, Budapest, Hungary

Introduction: The majority of scrotal tumors are malignant, whereas rare epididymal lesions are slow-growing asymptomatic scrotal masses, mostly benign in nature. In order of frequency adenomatoid tumors of mesothelial origin are the most common (73%), leiomyomas (11%) and cystadenomas (9%) can also occur.

Case presentations: Our first case is a 29-year-old male who presented with a palpable scrotal mass and negative tumor markers. Physical examination revealed a 1 cm in diameter, firm mass at the tail of the epididymis. A 12x8x12 mm diameter, fully demarcated, vascularised, echo-dense area of the right epididymis was shown by scrotal ultrasound without any testicular alteration.

The next case was a 49-year-old male with a 1cm size mass on the right side of the scrotum. Ultrasonography revealed a well-defined, vascularized, 10x7x9mm hyper-echogenic lesion on the tail of the epididymis.

In both of the cases microsurgical epididymal tumor resection was performed, where Frozen Section Examination confirmed its benign nature, thus an organ sparing approach became possible. Final histological examinations confirmed adenomatoid epididymal tumors.

Discussion: Only 1 case report in the last 5 years, and totally less than 100 cases of epididymal adenomatoid tumors have been reported in the literature.

Adenomatoid tumor is the most common paratesticular neoplasia in middle-aged males, usually incidental findings, asymptomatic and most commonly located on the tail of the epididymis.

In the case of palpable epididymal masses the correct diagnosis, fertility preservation and avoiding radical surgery have the utmost importance, especially in reproductive-aged patients.

P070 | Paternal exposure to antihypertensive treatment and perinatal characteristics of the offspring – a Swedish register studY

Joel Lindvall[1,2], Aleksander Giwercman[1,2], Emilia Ruhr[2], Angel Elenkov[1,2]

[1]Skåne University Hospital, Malmö, Sweden; [2]Lund University, Lund, Sweden

Background & Objectives: There is growing evidence showing that pharmacological agents given to women, before and after conception, may have a negative impact on their fertility and pregnancy outcome. However, such information is much more limited when it comes to the male partner. Most attention has been given to chemo- and radiotherapy, men receiving such treatment being counselled to avoid making their partner pregnant during the first 6 post-treatment months. Hypertension and antihypertensive treatment (AHT) are common even among young adults and due to delayed parenthood, in many parts of the world, one can assume the incidence of hypertension in fathers might also increase.

The objective of this study is to examine the association between paternal use of AHT and adverse outcome of the offspring.

Materials and Methods: This study was based on the use of Swedish national registries. The characteristics of all children born alive in Sweden 2006-2014 and their parents were extracted from the Swedish Medical Birth Register, which covers more than 99% of all births in Sweden. The data on prescribed medication given to fathers was derived from the Swedish National Prescribed Drug Register. A total number 885,730 children were included. In this cohort a total of 14,886 (1.7%) fathers had dispensed AHT at least one time during the 6 months prior to conception. The odds ratios (OR) of low birth weight (LBW), small for gestation age (SGA), prematurity, low Apgar score (LAS) and major malformations were analyzed using binary logistic regression. The model was adjusted for parental age and educational level, maternal BMI, smoking-status and parity, and the use of intracytoplasmic sperm injection.

Results: Children to fathers using AHT were more likely to have a LBW (adjusted odds ratio [aOR] 1.14, 95% CI 1.06 to 1.24) and to be SGA (aOR 1.18, 95% CI 1.06 to 1.31) compared to children of fathers without AHT. Children to fathers prescribed only angiotensin-converting enzyme inhibitors (ACEi) were more likely to be with LBW (aOR 1.20, 95% CI 1.00 to 1.44). Children to fathers who were prescribed only beta blockers (BB) were more likely to be SGA (aOR 1.28, 95% CI 1.07 to 1.54).

Conclusions: According to our results there are a higher risk for adverse outcome among children to fathers using AHT in the 6 months prior to conception. This is the first study to establish a link between AHT and adverse outcome of the offspring. However, we cannot differentiate weather the association is due to the AHT or underlying disease. Thus, further research is needed to clarify the causation before clinical recommendations can be given to prospective fathers using AHT.

P071 | Correlation of reductive stress with conventional semen parameters in a Greek population cohort

Koumasi Dimitra[1], Kanakis Georgios[2], Karagiannidis Lazaros[3], Markogiannaki Maria Melina[1], Evgeni Evangelini[1]

[1]Cryogonia Cryopreservation Bank Athens, Greece; [2]Department of Endocrinology and Assisted Reproduction Unit, Naval & VA Hospital of Athens Athens, Greece; [3]Reproductive Gynecologist Athens, Greece

Introduction: Approximately 15% of male infertility cases cannot be etiologically accounted for solely based on conventional semen analysis. Semen redox status imbalance has been recently proposed as a possible cause in these cases, either due to the excessive presence of reactive oxygen species (ROS) or the excessive presence of antioxidants, a condition described as reductive stress. Reductive stress can lead to infertility through various mechanisms, such as the complete depletion of oxidizing agents or tissue damage, which eventually results in increased ROS production, a phenomenon described as the "Antioxidant Paradox".

Purpose: Our study aimed to explore the presence of reductive stress in samples from Greek infertile men and investigate possible associations between the levels of redox potential and conventional semen parameters, as well as lifestyle factors.

Materials-Methods: The levels of redox potential were assessed by the MiOXSYS method in samples provided by 395 men of an age range between 18 and 60 years (median: 40, average: 40.6), over a period of 4.5 years (2017-2022). For all the subjects, conventional semen

analysis was performed, according to the WHO manual (WHO, 2010 & 2021) and the ESHRE-NAFA recommendations. The subjects' fertility history was also recorded.

Results: 5.06% of the samples exhibited reductive stress, in contrast to 51.8% showing oxidative stress. 43.04% of the examined samples were evaluated at the state of redox balance (values within the reference range, i.e. <1.34 mV/106/ml spermatozoa).

Men with reductive stress were between 31 and 51 years (median: 43, average: 41.2). 35% of these subjects reported smoking on a daily basis by conventional cigarettes, electronic or vaporizer. 65% reported alcohol consumption 1-4 times per week. 10% of the patients had been on therapy with tamoxifen before the examination, 5% with antibiotics and 5% had used muscle relaxants.

In almost all of the above cases, at least one basic semen parameter was found below the WHO reference limits. Only one sample exhibited all conventional parameters within the reference ranges. Typical morphology was found below the reference limits in all but one of the examined samples (5%), while sperm motility and concentration were found below the reference limits in 69% and 55% of the cases, respectively. Also, 40% of these subjects had previously used antioxidant supplementation.

None of these men reported the achievement of paternity by the time of examination. Interestingly, 40% of them had undergone one or more failed ART attempts, ending to absence of pregnancy or miscarriage.

Conclusions: Although oxidative stress is the most studied condition related to sperm redox imbalance, reductive stress may also constitute an etiological factor of male fertility, with negative repercussions on the basic seminal profile. Excessive use of antioxidants can lead to the impairment of oxidative mechanisms which are important for sperm function. Thus, baseline sperm redox levels must be taken into account before antioxidant administration in the context of the therapeutic management of male infertility.

P072 | Hormone levels, semen quality and sperm function in unselected young men from Lodz, a city in central Poland

Renata Walczak-Jedrzejowska[1], Radoslaw Zajdel[2], Joanna Jurewicz[3], Wojciech Hanke[3], Jolanta Slowikowska-Hilczer[1]

[1]Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Poland; [2]Department of Medical Informatics and Statistics, Medical University of Lodz, Poland; [3]Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz, Poland

In 1992, Carlsen et al. published a meta-analysis of 61 studies that showed a decrease in sperm concentration in male semen over the past 50 years. Since then, many prospective studies have been conducted around the world to assess the fertility status of young men from the general population, which revealed the presence of geographical differences in semen quality. Thus, the aim of the study was to analyze the semen quality and hormonal profile in young men from general population, inhabitants of Lodz, central Poland.

The study was conducted over a period spanning from December 2015 until March 2018 and 288 men aged 18-30 took part in it. Each participant underwent a physical examination, anthropometric measurements, and provided semen and blood samples. Additionally, participants completed questionnaires on demographic data, current health status, past diseases and lifestyle factors. Semen was tested using the manual method according to the WHO recommendations 2010. Apart from basic semen analysis sperm chromatin dispersion test (SCD) was performed to determine sperm DNA fragmentation and sperm hyaluronan binding test (HBA) – to determined sperm maturity. The concentration of FSH, LH, testosterone, estradiol was determined by the chemiluminescent method, while inhibin B (InhB) by the microimmunoenzymatic method.

Previously diagnosed diseases, not related to reproductive system, were reported by 15% of men. They were mainly allergies (4%), asthma, thyroid disease and hypertension (2% each). The varicocele was observed in 9% of men and 2.8% reported childhood cryptorchidism. Seventeen percent of participants smoked cigarettes, and 52% consumed alcohol 1-3 times a week. In the last 3 months prior to the study, 44% of participants were taking medications unrelated to the treatment of their underlying disease, 30% were taking vitamins and supplements, 11% - sports nutrition products, 12% - drugs, and 1% - anabolic androgenic steroids. Two percent of the respondents were underweight, 26% were overweight and 5% were obese (1st and 2nd degree).

The vast majority of participants have normal hormone levels. The FSH level was above 7,0 mIU/mL in 2,4% of men while 3,4% of them had it below 1,0 mIU/mL. The LH level was above 8 mIU/mL in 1,7% of participants and below 1,0 mIU/mL in 0,7%. Inhibin B level below 100 pg/mL and testosterone below 8 nmol/L were observed in 6,9% and 1,7% of men, respectively. Only 1 men had estradiol level above 220 pmol/L. According to the WHO 2021 reference values, oligozoospermia was found in 14%, asthenozoospermia - in 6% and teratozoospermia - in 40% of participants. Overall, in 46% of men, at least one of the above parameters was suboptimal. Additionally, 24% of them had abnormal HBA results, and although only 8% had an index of sperm DNA fragmentation above 30%, 38% had it between 15-30%.

The obtained results indicate that a quite high percentage of young men from the general population present suboptimal semen and sperm quality

P073 | New profil of Globozoospermia: Sperm parameters, cytogenetic and molecular investigations

MB. Ben Khedher, A. Achile, S. Dimassi, S. Mougou, A. Saad M. Gribaa, S. Ibala-Romdhane

Laboratory of Cytogenetics, Molecular Genetics and Reproductive Biology. Farhat Hached Hospital, Sousse, Tunisia

Introduction: Total globozoospermia is a rare and severe form of teratozoospermia characterized by round-headed spermatozoa lacking an acrosome. Two genes (DPY19L2 and SPATA16) are known to be

associated with total globozoospermia. It was reported in the literature that mutations in these gene influence the acrosome formation during spermiogenesis, whereas the meiosis was not disturbed. Recently a new SPATA16 mutation was identified in Tunisian patients. This mutation was shown to be related with a specific phenotype of total globozoospermia. In this work, we described the sperm parameters, sperm DNA fragmentation, molecular and cytogenetic findings of this new globozoospermia phenotype.

Patients and Methods: Five unrelated Tunisian globozoospermic patients were included. They were recruited during routine infertility treatment at Farhat Hached University Hospital's Laboratory of Human Cytogenetics, Molecular Genetics, and Reproductive Biology (Sousse, Tunisia).Semen parameters were assessed according to the World Health Organization guidelines. Sperm morphology was assessed according to David's modified classification (Auger, Eustache, & David, 2000). Sperm DNA fragmentation was evaluated by TUNEL assay. Karyotyping and Yq chromosome microdeletion were performed. Mutation screening was assessed by polymerase chain reaction (PCR) and Sanger sequencing of the SPATA 16.

Results: Semen analysis revealed severe oligozoospermia (sperm concentrations averaging 3.2 106/ml) and a complete absence of sperm motility (akinozoospermia). Morphology examination indicated total globozoospermia consisting of 100% round headed acrosomeless spermatozoa with a significant presence of double or multipleheaded (35%) and multitailed (25,2%) spermatozoa associated to high index of multiple abnormalities (MAI) with an average of 3,58. They all showed DNA fragmentation indexes higher than 30%, indicating that their spermatic DNA had been altered. All of the patients had normal somatic karyotypes and no Y microdeletions were found. For the molecular study, two patients were found to be homozygous for the new SPATA16 exon 2 deletion; screening for other mutations cases is ongoing.

Conclusion: In this study, we report a particular morphological sperm defect (double-/ multi-headed and multi-tailed spermatozoa) frequently observed in five globozoospermic Tunisian patients. Two of them, were found homozygous for the rare and newly identified SPATA16 exon 2 deletion. The results confirm again the pathogenicity of SPATA16 mutations in globozoospermia and this anomaly could lead to an abnormal meiosis, explaining this particular phenotype of total globozoospermia.

Keywords: globozoospermia, multiple round-headed, multi-tailed spermatozoa, SPATA16.

P074 | Macrocephalic spermatozoa syndrome: study of a cohort of Tunisian patients

MB Ben Khedher, H. Ouedraogo, S. Dimassi, S. Mougou, A. Saad, M. Gribaa, S. Ibala-Romdhane

Laboratory of Cytogenetics, Molecular Genetics and Reproductive Biology. Farhat Hached Hospital, Sousse, Tunisia

Introduction: Macrocephalic spermatozoa syndrome has been described as a rare phenotype of severe male infertility. Different AURKC mutations severely affecting the protein function have been identified and associated to the typical phenotype form. The homozygous c.144delC mutation has been identified as the most frequent variant causing macrozoospermia in North African patients. It's frequency of 1/50 was established on individuals from the Maghrebian general population. We reported, in this study, the spermiological and molecular characteristics of patient's cohort with this syndrome.

Patients and Methods: Thirty-seven patients were diagnosed with Macrocephalic spermatozoa syndrome during a fifteen years period in the Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology, Farhat Hached University Hospital (Sousse, Tunisia). Sperm analysis was performed in accordance with the World Health Organization recommendations. Sperm DNA fragmentation was evaluated by TUNEL assay. Karyotyping and Y-chromosome micro-deletions were also performed. Genomic DNA was extracted from peripheral blood and PCR was performed to amplify the seven exons of AURKC gene. Sequencing analysis was carried out using the Big Dye Terminator v3.1 sequencing kit and an ABI 310 Genetic Analyzer (Applied Biosystems).

Results: The patients' average age was 36.6 years and all suffered from primary infertility with a mean duration of 5.3 years. Twelve patients were issued from a consanguineous marriage (31.6%). A spermocytogram indicated on average of 100% atypical forms, with 84.5% macrocephalic and irregular heads, 93.3% abnormal acrosomes, and 42.9% multiple flagella which correspond to the typical phenotype form. The multiple anomaly index (MAI) is extremely high, with a mean value of 3.52. This trait is almost often accompanied with oligoasthenozoospermia. The fragmentation index was evaluated in ten patients, and all of them had an index higher than 30%, indicating an altered spermatic DNA. All patients had a normal somatic karyotype and none of them was positive for Y-chromosome microdeletions. Molecular analysis of AURKC gene revealed that 34 (91.89%) out of the 37 patients studied were homozygous for the c.144delC mutation at exon 3 of the gene. One patient (2.7 %) was homozygous for the Y248X mutation at exon 6 of the same gene, and two patients had neither of these mutations.

Conclusion: Macrocephalic sperm syndrome is a rare morphological defect commonly encountered in North Africans. The Maghrebian mutation c.144delC in the homozygous form of the Aurora kinase C gene would be the most major cause of this condition. This highlights the necessity of AURKC molecular analysis for macrozoospermic patients in reducing unnecessary ICSI attempts.

Keywords: infertility, teratozoospermia, macrocephalic spermatozoa, AURKC mutations.

P075 | Whole Exome Analysis in idiopathic non-obstructive azoospermia: identification of a novel candidate gene involved in meiosis

Matilde Spinelli[1], Antoni Riera-Escamilla[2], Ginevra Farnetani[1], Daniel Moreno-Mendoza[2], Eduard Ruiz-Castañé[2], Csilla Krausz[1]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain

Background: The most severe form of male infertility is non-Obstructive Azoospermia(NOA). In a relatively high proportion of NOA men (40%) the etiology cannot be established with the currently available diagnostic tools; and this condition is termed as "idiopathic" NOA. Whole Exome Sequencing (WES) allowing the simultaneous analysis of all exons belonging to approximately 20,000 protein-coding genes of the human genome, has contributed to the identification of many novel monogenic causes of azoospermia, mainly due to meiotic arrest.

Objective: To identify novel genetic causes of NOA through the sequencing of all protein coding genes.

Methods: DNA was extracted from peripheral blood leukocytes of 31 NOA men. WES was carried out using the Agilent SureSelect_V6 kit and the Illumina NovaSeq6000. After standard quality check, variants in the coding/splicing sequences were filtered for Minor Allele Frequency (MAF $\leq$ 0,01) and for an in-house Pathogenic Index (PI) based on six in silico prediction tools. Predicted to be pathogenic variants according to our in-house PI were selected according to a recessive model and further classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Those predicted to be Benign or Likely Benign were excluded. Finally, the expression profile in the human testis, the reproductive phenotype in the knock out (KO) mice and data available in the literature was evaluated for each gene to determine their potential role in spermatogenesis.

Results: We have identified a homozygous Likely Pathogenic variant in MEI1 (MEI1:c.3741del) in a patient with meiotic arrest (TESE negative). It is a known NOA gene that encodes a protein required for meiotic synapsis of chromosomes and the formation of DNA double-strand breaks in spermatocytes. The mouse model recapitulates the human phenotype and both male and female mice are infertile due to meiotic arrest. We have identified a homozygous missense variant, classified as Hot VUS, in SMC1B (c.1712T>G) in an other patient affected by meiotic arrest (TESE negative). This gene encodes a testis-enriched protein which participates in the formation and maintenance of the cohesin complex and DNA recombination during meiosis. Similarly to MEI1, both male and female KO mice are infertile due to meiotic arrest. We calculated the gene-disease relationship (GDR) of the two genes: MEI1 reaches a score of 14 (strong GDR), whereas SMC1B reaches a score of 4 (limited GDR).

Conclusions: Through WES we identified a plausible genetic cause in 6.5% of patients. Both mutation carriers were affected by meiotic arrest confirming the high diagnostic yield in this particular testis phenotype. We have confirmed the role of MEI1 in NOA and for the first time in the literature, we propose SMC1B as a novel candidate gene. Both gene defects were associated with TESE negative outcome implying a potential prognostic value for testicular sperm retrieval. Wider implications of our finding is the discovery of a novel human meiotic gene (SMC1B) potentially involved both in NOA and premature ovarian failure.

P076 | High prevalence of HPV infection in young males and in subjects with risk factors: the need for a universal vaccination in males

Giuseppe Grande, Carlo Foresta, Luca De Toni, Ilaria Coisci, Alberto Ferlin, Andrea Garolla

Unit of Andrology and Reproductive Medicine & Centre for Male Gamete Cryopreservation, Department of Medicine, University of Padova, Padova, Italy

The human papillomavirus (HPV) infection is very common among men and women across all geographic, racial, and socioeconomic areas. The role of HPV in the etiology of cancer at different genital sites is well documented, and growing evidence suggests that the virus also acts in the causation of oral cancer. In men, the presence of HPV has been well documented in the anal region, perineal area, scrotum, glans, penile shaft, urethra, and even semen. Furthermore, its persistence at these sites has been related both to male factor infertility and to cancer development at different genital and nongenital areas. Our previous studies described the possible role of HPV in male infertility. In July 2021 ESHRE published the guidelines for medically assisted reproduction in patients with a viral infection or disease, analyzing the impact of the most frequent viral infections on reproductive health in couples candidate to ART, including HPV. These guidelines referred for the prevalence of the infection at 24% in <25 years old women.

The aim of the present study was to evaluate the prevalence of human papillomavirus (HPV) in a population of unselected young males and in a population of males with risk factors for HPV.

150 male subjects, aged 18-25 y.o., participating to a surveillance programme conducted by our centre, have been enrolled for this study. We moreover enrolled 450 patients 18-45 y.o. with risk factors for HPV infection (partner with diagnosed HPV infection, history of HPV infection or HPV-induced lesions, infertility, unprotected sexual intercourses with different partners).

In all subjects we performed polymerase chain reaction and fluorescence in situ hybridization (FISH) for HPV detection. We moreover performed immunofluorescence for HPV 16-L1 and immunoglobulins (IgA, IgG, and IgM) determination. Flat penile lesions (FPL) were detected by penoscopy.

We observed a high prevalence of HPV infection in unselected young males: condylomas have been observed in 4.7%, HPV-associated FPL in 7.3%, seminal detection of HPV-DNA in 38.6% and anti-HPV antibodies in 27.3% of the studied population.

In male subjects with risk factors the prevalence raised to 94%: HPV-DNA was detected in 36.6%, Ab anti-HPV in 30.7%, HPV-associated FPL in 7.8% and condylomas in 18.9%.

In many cases we reported the presence of high-risk HPV genotypes or genotypes included in HPV vaccines.

Our data underline the high prevalence of HPV in an unselected population of young males and the precence of HPV in almost all subjects belonging to a population with risk factors, thus underlining the importance of HPV vaccination as a pivotal strategy aimed to prevent both infertility and cancer.

P077 | Microsurgery of the vas deferens as a first step for spermatozoal recovery in non-obstructed azoospermic men

Fotios Dimitriadis[1], Aris Kaltsas[2], Athanasios Zachariou[2], Chara Tsiampali[2], Athanasios Papatsoris[3], Panagiota Tsounapi[4], Atsushi Takenaka[4], Nikolaos Sofikitis[2]

[1]Aristotle University of Thessaloniki, 1st Department of Urology, Thessaloniki, Greece; [2]University of Ioannina, Department of Urology, Ioannina, Greece; [3]2nd Department of Urology, School of Medicine, Sismanoglio General Hospital, Athens Greece; [4]University of Tottori, Department of Urology, Tottori, Yonago, Japan

Introduction & Objective: To investigate whether a subpopulation of non-obstructed azoospermic (NOA) men are positive for the presence of spermatozoa in vas deferens (VD) and evaluate the fertilizing capacity of spermatozoa recovered from the VD.

Patients and Methods: Ninety-nine NOA-men underwent microscopic testicular biopsy and microsurgical aspiration of fluid from the VD. Testicular tissue was minced and evaluated for the presence of spermatozoa. Another piece of testicular tissue was processed for hematoxylin-eosin (H-E) stain. Fluid aspirated from the VD was also microscopically observed. Forty-eight men were positive for testicular spermatozoa. Nine men were positive for spermatozoa in the lumen of VD. Frozen thawed testicular spermatozoa or VD-spermatozoa from the latter nine men were injected into approximately half of the oocytes of each one respective female partner. Fertilization rates and embryonic development rates after ooplasmic injections of spermatozoa (VD-spermatozoa vs testicular spermatozoa) and oocyte culture procedures were compared.

Results: A percentage of NOA-men equal to 48% or 9% were positive for testicular spermatozoa or VD-spermatozoa, respectively. Among NOA-men positive for testicular spermatozoa, a percentage equal to 18% were positive for VD-spermatozoa. There were no significant differences in the percentage of fertilized oocytes and embryonic development rate after ooplasmic injections of testicular spermatozoa vs VD-spermatozoa ($p > 0.05$; chi-square test was used). Among four NOA-men with late maturation arrest (i.e., arrest at the round spermatid stage), three NOA-men were positive for spermatozoa in the testis and the VD.

Conclusions: Spermatozoa from the VD can be recovered from NOA-men avoiding the complications of testicular surgery. The latter spermatozoa have acceptable fertilizing capacity. In addition, ooplasmic injections of VD-spermatozoa can trigger early embryonic development. Seventy-five percent of NOA-men with arrest at the round spermatid stage in H-E stain were positive for spermatozoa in VD.

P078 | Spermatological parameters inY chromosome mosaic patients with and without AZF deletions

Maria Shtaut[1], Ludmila Dobrodeeva[1], Svetlana Ermolaeva[1], Anna Sedova[1], Tatyana Sorokina[1], Lubovy Kurilo[1], Nataliya Oparina[2], Nadezhda Shilova[1], Aleksandr Polyakov[1], Vyacheslav Chernykh[1,3]

[1]Research Centre for Medical Genetics, Moscow, Russia; [2]Moscow Regional Research and Clinical Institute 'MONIKI', Moscow, Russia; [3]Pirogov Russian National Research Medical University, Moscow, Russia

Sex chromosome abnormalities and the Y chromosome microdeletions in the AZF locus are common genetic causes of male infertility. The effect of several genetic anomalies on spermatogenesis has not been sufficiently investigated. The aim of the study was a comparative analysis of semen parameters in Y chromosome mosaics with and without AZF deletions.

Material and Methods: Patients were examined for fertility disorders, identified genetic abnormalities, or due to pregnancy planning. Examined sample consist 16 men with Y chromosome mosaicism identified by cytogenetic study. Chromosome analysis was performed using by standard cytogenetic examination. Fluorescence in situ hybridization (FISH) was performed to identify/verify mosaicism and determine the structural Y chromosome anomaly. Y chromosome microdeletions were detected by multiplex PCR with primers for loci SRY и ZFX/ZFY (Yp11.3); sY84, sY86, sY615 (AZFa), sY127, sY134 (AZFb), sY254, sY255 (AZFc). The semen examination was carried out with the recommendations of the WHO Guidelines.

Results: Examined cohort were divided into two groups: group I – patients with AZF deletions: AZFb+c, n = 7 and AZFc(b2/b4), n = 1 (n = 8); group II – without AZF deletions (n = 8). Cytogenetically identifiable unbalanced Y chromosome abnormalities were found in 13 patients, including 5 of 8 patients of group I, and all patients of group II. There was no statistically significant difference between in the groups for the average age (33,1 ± 11,8 y.o. and 32,0 ± 3,8 y.o.), ejaculate volume, pH and ejaculate viscosity. A higher concentration of spermatozoa was found in patients without AZF deletions (group I – 15.9 ± 31.0 mln/ml, group II – 0.003 ± 0.009 mln/ml; p = 0.026). Prominent difference was found between groups in the structure of pathozoospermia: various forms of pathozoospermia were revealed in group I (azoospermia, n = 3; oligoastenotheratozoospermia, n = 3; asthenoteratozoospermia, n = 2); in group II shown only severe forms of pathozoospermia (azoospermia, n = 7; severe oligozoospermia, n = 1), as well as in the frequency of oligospermia – 37.5% and 12.5%, respectively. Both mosaics with normal sperm counts showed no unbalanced abnormalities and AZF deletions, low percentage (%) of cells without the Y chromosome.

Conclusions: Patients with Y chromosome mosaicism have high frequency structural Y chromosome abnormalities and AZF deletions. Unbalanced cytogenetic Y chromosome rearrangements and pathogenic Yq11.2 microdeletions are characterized by a severe degree of spermatogenesis disorder in male patients with Y chromosome mosaicism (azoospermia and extremely severe oligozoospermia). In men with Y chromosome mosaicism the preservation of fertility is possible in absence of unbalanced rearrangements (ring (Y), Yp isochromosomes, and dicentric Yq and Yp chromosomes, terminal Yq11.2 deletions), dramatically disrupting meiosis and "severe" types of AZF deletions, especially AZFb+c and AZFa+b+c deletions, as well as severe disorders of gonadal development.

P079 | Immune cell infiltration of testicular germ cell tumors – the possible role of T cell subsets

Rashidul Islam[1,2], Miriam Figura[1,3], Jannis Heyer[1,3], Florian Dittmar[3], Adrian Pilatz[3], Katja Hartmann[1], Christiane Pleuger[4], Monika Fijak[4], Sabine Kliesch[5], Florian Wagenlehner[3], Mark Hedger[2], Bruce Loveland[6], Kate Loveland[2,7], Daniela Fietz[1], Hans-Christian Schuppe[3]

[1]Department of Veterinary Anatomy, Histology and Embryology, Justus Liebig University Giessen, Giessen, Germany; [2]Centre for Reproductive Health, Hudson Institute for Medical Research, Clayton, Victoria, Australia; [3]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University Giessen, Giessen, Germany; [4]Institute of Anatomy and Cell Biology, Justus Liebig University, Giessen, Germany; [5]Centre of Reproductive Medicine and Andrology, University of Muenster, Muenster, Germany; [6]Burnet Institute, Melbourne, Australia; [7]School of Clinical Sciences, Monash University, Clayton, Victoria, Australia

Introduction: Testicular germ cell tumors (TGCT) can present as seminoma (SE) and non-seminoma, e.g. embryonic carcinoma (EC). Tumor development, progression, and prognosis are poorly understood, but infiltrating immune cells and associated cytokines/chemokines are involved. Of note, T cells represent the major component of tumor infiltrating lymphocytes (TIL) in TGCT, however the presence of rare subtypes, such as regulatory (Treg) and follicular helper T (Tfh) cells has not been studied.

Material and Methods: As part of an ongoing prospective study including a comprehensive clinical database collected since 2017, testicular tissue was obtained from 91 patients aged 18-69 years undergoing orchiectomy due to TGCT. To identify key cell and molecular components, we analyzed tissue samples from different areas of tumor (tu)-bearing and contralateral testes. Analysis of infiltration density and distribution of immune cells including TILs was performed using immunohistochemistry (IHC) in specimens from patients with SE (n = 47), EC (≥ 50%; n = 16), and GCNIS-derived TGCT (n = 13) then compared to specimens with normal spermatogenesis (n = 10). In addition, immune cells were analyzed by flow cytometry (FC) using fresh human testis samples (n = 24) from different areas of tumor-bearing and contralateral testes.

Results: General histopathology of all patients showed considerable heterogeneity. Semi-quantitative scoring of IHC revealed immune cell infiltrates mainly comprised of T cells and macrophages with an increasing trend from tumor-distant to tumor-central biopsy sites. Interestingly, T cells, including Treg and Tfh cells, were more abundant in SE compared to all other groups as shown by IHC. FC analysis confirmed the highest abundance of T cells and respective subsets within the tumor in SE compared to other localizations. Two-way hierarchical cluster analysis revealed a high homogeneity of the immune cell infiltrates within the SE group with no marked differences between localized and metastatic TGCTs.

Conclusion: This cohort study demonstrates the complexity and interindividual variability of TIL and provides suggestive evidence for the possible importance of rarer T cell subtypes in the immune environment of TGCT. Future experiments will interrogate Treg and Tfh functions to identify novel prognostic factors and/or immune-therapeutic concepts for human TGCT.

P080 | Developing a non-invasive predictive model for sperm presence in testicular tissue of men with azoospermia

Ana Planinic[1,2], Sinisa Skokic[3], Davor Jezek[1,2]

[1]Department of Histology and Embryology, School of Medicine University of Zagreb, Zagreb, Croatia; [2]Scientific Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia; [3]Croatian Institute for Brain Research, School of Medicine University of Zagreb, Zagreb, Croatia

Azoospermia is a condition where there are no spermatozoa in the ejaculate and men who wish to have children undergo testicular sperm extraction (TESE). The success rate of TESE in nonobstructive azoospermia (NOA) is only around 50%. Histological analysis is the only method used for predicting sperm retrieval. The shortcomings are that histology in NOA is heterogeneous and the method is invasive. Magnetic resonance imaging (MRI), on the other hand, can provide whole testis imaging and parameter mapping and is also a non-invasive method. The aim of this study was to assess which parameters obtained with ex-vivo MRI could be useful in predicting testicular histology. Thirty-five samples of testicular tissue were obtained via TESE and all of them underwent 7T MRI, specifically diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and magnetic resonance spectroscopy (MRS). Images were analyzed in ImageJ while the concentrations of metabolites were determined using the QUEST algorithm of the jMRUI software and tetramethylsilane (TMS) as a reference. Samples were then histologically processed, analyzed, and divided into groups based on their mean Johnsen score: JS<2, JS≥2, JS≤4, 4<js

P081 | Primary infertility is a risk factor for female sexual dysfunction: A case-control studY

Athanasios Zachariou[1], Vagia Sapouna[2], Aris Kaltsas[1], Ioannis Giannakis[1], Ioannis Champilomatis[1], Minas Paschopoulos[3], Andreas Karagiannis[1], Fotios Dimitriadis[4], Panagiota Tsounapi[5], Athanasios Papatsoris[6], Atsushi Takenaka[5], Nikolaos Sofikitis[1]

[1]Department of Urology, University of Ioannina, Ioannina, Greece; [2]Physical Medicine and Rehabilitation Centre EU PRATTEIN, Volos, Greece; [3]Department of Obstetrics and Gynaecology, University Hospital of Ioannina, Ioannina, Greece; [4]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [5]Department of Urology, Tottori University, Yonago, Japan; [6]Department of Urology, Sismanoglio General Hospital, University of Athens, School of Medicine, Athens, Greece

Objective: Current female sexual dysfunction diagnostic criteria require an assessment of both symptoms and symptom-associated distress. Female sexual dysfunction can lead to infertility due to decreased intercourse frequency. On the other hand, primary infertility is a stressful condition that affects female life's mental, physical, social, and personal aspects. It is a cause of wasting time and money and

background of marital disruption and divorce. There is limited information about the direct impact of infertility on female sexual function and especially on female sexual distress.

This study aimed to evaluate the effect of primary infertility on female sexual function using both the FSFI and FSDS-R questionnaires, according to the female sexual dysfunction definition.

Materials and Methods: A hundred and sixty infertile women with primary infertility and 160 healthy female controls within 18-45 years were included. None of them received treatment for IUI, IVF, or ovulation induction. All women were assessed with FSFI-Gr, FSDS-R, and a short questionnaire about the frequency of sexual intercourse and sex-life satisfaction using the Likert visual scale. Based on the data features, suitable statistical analyses were performed, including percentage, Chi-square test, student t-test and Pearson's correlation test.

Results: There were no observed significant differences in the general characteristics of the two groups. Using both questionnaires, FSFI and FSDS-R, 32 women in the infertility group (29.09%) and 13 females in the control group (11.82%) presented sexual dysfunction. In all individual domains and total FSFI scores, the infertility group showed statistically significantly lower scores ($p < 0.05$) than the control group. Also, the frequency of intercourse (infertility group vs control group, mean value ± SD: 3.52 ± 1.05 vs. 4.61 ± 1.27) and the sex life satisfaction visual scale (2.46 ± 0.32 vs. 3.54 ± 0.42) were statistically significantly lower in infertile women group.

Conclusion: Women with primary infertility are at considerable risk of sexual dysfunction based on the FSFI and FSDS-R questionnaires. The infertile person loses self-esteem by repeatedly attempting to achieve the desired goal (having a baby) but failing to make it. The problem can be significantly worse when the female has been highly successful in other areas of life. Furthermore, there is a natural or feared loss of marital relationships and relationships with family and friends.

P082 | Long term effect of cytotoxic treatment on sperm DNA fragmentation in patients with testicular cancer

Ginevra Farnetani[1], Maria Grazia Fino[2], Francesca Cioppi[1], Matteo Vannucci[2], Viktoria Rosta[1], Antoni Riera-Escamilla[3], Elena Casamonti[1], Leila Turki[1], Lara Tamburrino[4], Monica Muratori[1], Csilla Krausz[1]

[1]*Department of Biomedical, Experimental and Clinical Sciences 'Mario Serio', University of Florence, Florence, Italy;* [2]*University Hospital Careggi, Florence, Italy;* [3]*Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain;* [4]*Department of Experimental and Clinical Medicine, University of Florence, Florence, Ital*

Background: Testicular Germ Cell Tumor (TGCT) is the most frequent malignancy in men of reproductive age. It is a highly curable disease and treatment is based on carboplatin (CP), cisplatin-etoposide-bleomicyn (PEB) or radiotherapy (RT). The damaging effect of these therapies on sperm DNA after the first year of treatment is well established. Concerning their effect after 2 years (T2), the literature is controversial; most of the studies observe a return of sperm DNA fragmentation (SDF) values to baseline at T2, while few studies report higher SDF at T2 in respect to T0 with about 20-30% of patients above the normal limit at T2. Data on long term follow-up is scarce and based on small cohorts. It remains an open question what would be the optimal SDF value and time interval for safe conception after oncological treatments.

Objective: To evaluate the level and the persistence of sperm DNA damage after antineoplastic treatment in a longitudinal survey up to 3 years from the end of oncological therapy.

Methods: SDF of 116 TGCT patients was evaluated through TUNEL assay coupled with Flow Cytometry before the beginning of cancer therapy (T0, n = 68) and after 2 (T2, n = 77) and 3 (T3, n = 63) years from the end of it. Patients were divided into three groups according to the type of treatment used: CP (n = 32), PEB (n = 57) or RT (n = 16). For 25/116 patients SDF data were available for all the three time points (paired longitudinal cohort) allowing the comparison of matched samples.

79 fertile normozoospermic men served as controls; the SDF value corresponding to the 95th percentile of the control group (SDF = 50%) was considered as a threshold for severe DNA damage (SDD). Data are expressed as medians and interquartile ranges.

Results: Total sperm count at T0 (43x106 [13.5-110] and T2 (76,5x106 [8-186] was not significantly different. At T2 SDF values were higher than T0, reaching statistical significance only in the CP group (29.2% [20.1-33] at T0 vs. 37.9% [25.7-49.1] at T2, $p < 0.05$); at T3 SDF values returned to baseline in all the three treatment groups (CP 26.6% [18.6-31.2]; PEB 30.3% [20.2-38.9]; RT group (30% [20.3-38.4]). Concerning the presence of SDD, there was a relatively high percentage of patients (24.4%) at T2 with SDF>50%, which was significantly higher ($p < 0.05$) than what observed at T0 (10.3%); at T3, the percentage of patients who still exhibited SDD decreased to 4.8%. The highest proportion of patients with SDD is among those treated with PEB at all the three time of observation.

The above results were confirmed in the paired longitudinal cohort.

Conclusions: Our study shows that antineoplastic therapies may have prolonged effects on the sperm genome. At T2 SDD might still be present in 20-30% of patients depending on the type of treatment. Therefore, the current standard indication of a 2 years interval before natural pregnancy may not be adequate for all patients. We propose SDF as a biomarker of the genotoxic effect of chemotherapy and we advise to personalize the follow-up period of TGCT patients based on this parameter.

P083 | Testicular germ cell tumor and familial history of cancer: the role of DNA repair genes

Ginevra Farnetani[1], Viktoria Rosta[1], Antoni Riera-Escamilla[2], Daniel Moreno-Mendoza[2], Matteo Vannucci[3], Maurizio De Rocco Ponce[2], Eduard Ruiz-Castañé[2], Csilla Krausz[1,2]

[1]*Department of Experimental and Clinical Biomedical Sciences 'Mario Serio', University of Florence, Florence, Italy;* [2]*Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain;* [3]*University Hospital Careggi, Florence, Ital*

Background: Testicular Germ Cell Tumor (TGCT) is a multifactorial and polygenic neoplasia. Despite epidemiological and Genome-Wide Association Studies highlighted its high inheritability and the increased familial cancer risk among TGCT patients' relatives, the genetic basis for TGCT remains unclear. Germline mutations in DNA repair genes have been reported in several autosomal dominant cancer predisposition syndromes, such as Lynch syndrome (LS) and Hereditary Breast and Ovarian Cancer syndrome (HBOCS). Recently, TGCT has been linked with LS, but no association with other cancer syndromes has been proposed so far.

Objective: To elucidate the role of DNA repair genes in the etiology of TGCT in the context of familial history of multiple cancers.

Methods: DNA was extracted from peripheral blood lymphocytes of 63 TGCT patients with more than one family member suffering from different cancer types. WES was carried out using the Agilent SureSelect_V6 kit and the Illumina NovaSeq6000. After standard quality check, variants in the coding sequences and splice-sites were filtered for Minor Allele Frequency (MAF $\leq$ 0,01) and for an in-house Pathogenic Index based on six in silico prediction tools. Genes carrying variants have been crossed with a list of 547 genes involved in DNA repair mechanisms obtained from Gene Ontology. Variants were classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines and those having "Pathogenic" (P) or "Likely Pathogenic" (LP) verdict were selected. Further bioinformatic analysis included: expression profile, cancer databases, animal models, and literature search. Selected variants were validated by Sanger sequencing.

Results: We identified 29 heterozygous and 1 hemizygous LP and P variant in 28 DNA repair genes in 25/63 patients (40%). 10/30 heterozygous variants were located in genes already known to be associated with several cancer types present in the family members of the probands. We have identified the following variants in genes associated with: i) HBOCS (ERCC3:c.2065-2A>C; FANCC:c.455dup POLK:c.1577A>G; BRIP1:c.1941G>C; and DCLRE1C:c.1903dup); ii) LS (MSH6:c.2906_2907del, MLH3:c.3232_3237del); iii) colorectal cancer (MUTYH:c.1640del) iv) breast cancer (MMS19:c.346C>); v) leukemia (SETMAR:c.401A>T).

Conclusions: P and LP variants in DNA repair genes are present in 40% of patients in our highly selected cohort, suggesting their potential role in the pathogenesis of TGCT. Our findings provide further evidence for the link between TGCT and LS and proposes novel associations with HBOCS and other type of cancers. Additional experiments are ongoing in order to confirm the causality of these variants.

P084 | Sperm retrieval rate in Klinefelter syndrome - Hungarian data, case series report

Aniko Bata, Zsolt Kopa

Andrology Centre, Semmelweis University, Department of Urology, Budapest, Hungary

Introduction: Klinefelter syndrome is the most common numerical sex chromosome abnormality, characterized by small but firm testes, gynecomastia, hypergonadotropic hypogonadism and azoospermia in the vast majority of the cases. Testicular sperm extraction combined with intracytoplasmic sperm injection represents a chance for azoospermic men with KS to father a child. The aim of our study is to evaluate the microsurgical sperm retrieval rate of Klinefelter's syndrome patients in our Andrology Center.

Methods: Sixty-seven KS patients were enrolled in a retrospective study from May 2009 to May 2022. WHO semen analysis, age, endocrine parameters (FSH, LH, testosterone, Inhibin B and AMH), and scrotal ultrasound findings were analyzed. Surgical sperm retrieval was performed by the same surgeon using the microdissection technique. Samples were examined on-site and during cryopreservation.

Results: A total of 67 KS patients' clinical data was processed. After the semen analysis, in cases of 65 patients (97 %) non-obstructive azoospermia, while in 2 cases (3%) cryptozoospermia was detected. Only 27/67 (40%) of azoospermic patients requested the offered microdissection TESE. The mean age of the patients, undergone and denied surgery was similar (31.4 nd 31.7 years, respectively). The mean FSH and LH level was 32.8 IU/l and 16.1 IU/l, respectively, mean total testosterone was 8.0 nmol/l. The main Inhibin B and AMH were 39.4 pg/ml and 0.6 ng/ml, respectively.

Positive sperm retrieval was found in 9 patients (33.3%). The mean age in the positive and negative SRR patient group was 25.3 versus 31.4 years. Mean FSH differed between the positive and negative groups 28.4 vs. 35.4 IU/l., LH 13.5 vs. 17.7 IU/l., testosterone 10.7 vs. 6.7 IU/l., and Inhibin B 21.3 vs. < 12 pg/ml.

Conclusion: SRR in Klinefelter syndrome was found 50% by the latest meta-analysis, although in the most recent international data report SRR was between 20 and 30%. Our data approaches the latest European rates.

P085 | Obstructive azoospermia as first presentation for Von Hippel Lindau disease

R Scafa[1], M Merico[1], A Garolla[1], YH Zhu[1], PF Palego[1], Nicola Caretta[1], A Mambro Di[1], R Selice[1], C Foresta[1], I Piva[1], U Valente[1], S Zovato[2], Fernando S Watutantrige[2], AM Ferrara[2], M Mancini[3], M Iafrate[3], G Novara[3], F Dal Moro[3], A Ferlin[1]

[1]Andrology Unit Department of Medicine, University of Padova, Padova, Italy; [2]Familial Cancer Clinic and Oncoendocrinology, Veneto Institute of Oncology, IOV-IRCCS, Padova, Italy; [3]Urology Clinic, Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Ital

Introduction: Von Hippel-Lindau disease (VHL) is one of the most common inherited neoplasia syndromes and is characterized by highly vascular tumors of the eyes, brain, and spine, as well as benign and malignant tumors and/or cysts of the kidneys, adrenal medullae and sympathetic paraganglia, endolymphatic sac, epididymis, and broad ligament. This disorder is caused by highly penetrant mutations of VHL gene (3p25.3).

Presentation: We report a case of an infertile /azoospermic 38 years old man, seeking diagnostic investigations and eventually sperm cryopreservation. The semen analysis showed an ejaculate pH 7.2, a volume of 2cc and azoospermia confirmed after centrifugation.

In his medical history the only relevant data was the findings of bilateral epididymal cysts when he was 18 years old. On physical examination we highlighted abdominal obesity, absence of gynecomastia, normotrophic testis associated with bilateral epididymal hard mass. We performed scrotum ultrasonography which identified a epididymis bilateral enlargement due to several cysts (right maximum diameter 14 mm). To further investigate this condition the patient underwent a scrotal and pelvic MRI with contrast that confirmed the presence of multiple epididymis cysts. Biochemical tests showed negative testicular cancer biomarkers and a subclinical slight normogonadotropic hypogonadism. On June 26, 2020 the patient underwent bilateral biopsy of testis (with sperm cryopreservation), tunica vaginalis'eversion and partial enucleation of epididymal cysts bilaterally. Histology concluded for bilateral papillary cystadenoma of the epididymis.

Given the rarity of epididymis bilateral papillary cystadenoma, in order to exclude the presence of Von Hippel-Lindau disease the patient underwent abdominal ultrasound and ophthalmology evaluation.

These medical examinations showed multiple pancreatic cysts with maximum diameter of 60mm at the pancreatic head, kidney cysts and at the middle third of the right kidney a solid mass with 2 peripheral calcific spots. These findings were confirmed at MRI evaluation; one of the pancreatic cystis (because of the reduction of DWI) and the renal cystic mass (33 mm maximum diameter with solid content) were highly suspicious for malignancy. Partial nephrectomy was performed and the histology confirmed the diagnosis of a clear cell renal cell carcinoma. Ophthalmologic analysis showed retinal capillary hemangioblastomas. Further evaluations for pancreatic lesions are ongoing.

The patient had been tested positive for VHL gene mutations (c.464-1G/A;t.?). Screening for pheochromocytoma was negative.

Discussion: to our knowledge this is the first report of a male infertile/azoospermic condition leading to a previously unknown VHL syndrome, presenting with life-threatening renal, retinal and pancreatic lesions.

P086 | The results of comprehensive genetic examination of azoospermic and severe oligozoospermic Russian men

Olga Solovova[1], N.V. Oparina[1], O.P. Ryzhkova[2], T.M. Sorokina[2], Vyacheslav Chernykh[2]

[1]Moscow Regional Research and Clinical institute (MONIKI), Moscow, Russian Federation; [2]Research Centre for Medical Genetics, Moscow, Russian Federation

Introduction: Male infertility is commonly characterized by severe pathozoospermia, especially azoospermia and severe oligozoospermia. These forms are often caused by genetic defects, and very heterogeneous. There are numerous copy number variations (CNVs) and pathogenic variants in genes associated with male infertility, therefore it's detection requires the use of complex approach and genomic methods.

Materials and Methods: The sample included 200 patients with male infertility associated with azoospermia (n = 172) or severe oligozoospermia (n = 28). The age of the patients ranged from 18 to 48 (30.5 ± 5.7) years. We performed clinical and genetic examination, karyotype analysis, FISH-analysis on peripheral blood lymphocytes and buccal epithelium cells using centromeric probes for X- and Y-chromosomes (DXZ1, DYZ3), detecting of Y-chromosome microdeletions (locus AZF), analysis of common pathogenic variants and polymorphic IVS9Tn locus of the CFTR gene. 42 patients underwent whole-exome sequencing (WES) on the Illumina NextSeq500 device (Illumina Inc., USA).

Results: Karyotype abnormalities were found in 52 (26.8%) patients: Klinefelter syndrome (47,XXY; n = 34), dysomy Y (47,XYY; n = 6), 46,XX-male syndrome (n = 3), balanced autosomal abnormalities (n = 9). FISH-analysis shown no hidden gonosomal mosaicism in patients with the sex chromosomes aneuploidy. Complete AZF deletions were detected in 16 (8%) patients: AZFb (n = 3), AZFc(b2/b4) (n = 8), AZFb+c (n = 5). Monogenic forms of male infertility were diagnosed in 24 (12%) patients, among them: Congenital Hypogonadotropic Hypogonadism (CHH)/Kallmann syndrome in 15 (7.5%) patients, cystic fibrosis in 6 (3%) men and CBAVD syndrome in 3 (1.5%) men. Thus, genetically determined forms of fertility disorders were found in 92 (46%) patients, including 75 (37.5%) azoospermic men and 19 (67.8%) oligozoospermic men. A group of 42 patients (idiopathic non-obstructive azoospermia – NOA, n = 40; severe oligozoospermia, n = 2) underwent WES. Variants of the nucleotide sequence were detected in 18 (42.8%) patients in 29 genes (EP300, AR, MCM8, ANOS1, CYP21A2, CFTR, CATSPER1, LHCGR, TEX15, GNGT1, DNAH11, DNAH17, FSIP2, FSHB, DUSP6, HS6ST1, SMAD3, TEX14, VWA2, FSIP2, CFAP44, MEIOB, GNRHR, RNF216, QRICH2, PLCZ1, CYP11B1, KMT2D, SLC4A1) involved in the spermatogenesis. In one patient, a heterozygous 7q21.3 microdeletion, capturing gene GNGT1, was detected. GNGT1 gene is involved in controlling the migration of primordial germ cells (PGCs) to genital ridges. Another patient was found to have 410 bp-microdeletion capturing the DUSP6 gene (12q21.33), pathogenic variants in which lead to the development of CHH, type 19. However, this CNV was no detected by the reference method (MLPA). Some detected heterozygous variants in the genes responsible for autosomal recessive forms of male infertility, and also did not combine with clinical and spermatological picture. The identified variants in the genes require segregation and functional studies to confirm their pathogenicity. The remaining 24 out of 42 patients (57.1%) did not have genetic variants associated with severe forms of pathozoospermia. However, this does not exclude genetic factors of male infertility and requires further genetic examination.

Conclusion: Comprehensive genetic approach and using genomic methods in examination of infertile patients increase the effectiveness of the diagnosis of genetically determined pathozoospermia.

P087 | Chromosome 8 And Non-Obstructive Azoospermia: a report of two cases

R. Kooli[1], S. Dimassi[1], A. Benzarti[1], M. Gribaa[1], S. Rzouga[2], A. Hidoussi[3], A. Saad[1], S. Ibala-Romdhane[1]

[1]Laboratory of Cytogenetics, Molecular Biology and Reproductive Biology, Farhat Hached University Teaching Hospital, Sousse, Tunisia; [2]Urological surgeon in private sector, Mahdia; [3]Urological surgeon in private sector, Sousse, Tunisia

Introduction: Non obstructive azoospermia (NOA) can be caused by several genetic factors, including chromosomal abnormalities, Y chromosome microdeletions and gene mutations. Cytogenetic anomalies, especially structural chromosomal aberrations were found in 10-15% of azoospermic males. Nonetheless, new research has shed light on the impaction of autosome gene unbalanced translocations in spermatogenesis. In this study, we report genetic exploration by karyotype, Fluorescence in-situ hybridization (FISH) and molecular analysis of chromosome Y of two patients addressed for exploration of their azoospermia.

Patients and Methods: Two cases of azoospermic patients were addressed to the laboratory of Human Cytogenetics, Molecular Genetics, and Reproductive Biology (Sousse, Tunisia) for genetic testing.

For both patients, physical examination was normal. Follicle stimulating hormone levels in the blood were increased for both patients while plasma level of testosterone was reduced for patient P2. Ultrasound showed a bilateral testicular hypotrophy for patient P1 and unilateral hypotrophy for patient P2. Analyses of sperm revealed non obstructive azoospermia (NOA). Chromosomal analysis was obtained from cultured blood lymphocytes using R-banding technique. FISH analysis was carried out with commercial probes to characterize chromosome rearrangements. Multiplex Ligation Probe Amplification was used to determine the chromosome Y microdeletion.

Results: Karyotype revealed that all metaphase cells, for P1, had a 45,X,der(8)t(Y;8) formula. FISH analysis using SRY gene-specific probe localized on Yp11.31 showed a positive signal on the short arm of chromosome 8. Signals for the SHOX gene localized on Yp11.32 and for the Y centromere (DYZ3) were detected on the short arm of chromosome 8. For patient P2, the karyotype revealed an apparently balanced reciprocal translocation t(8;14)(p12;q12). Investigations of AZF region microdeletions revealed in P1 the loss of the long arm of the Y chromosome involving AZF and Yq heterochromatin regions.

Discussion: Azoospermia in patients with structural chromosome abnormalities can be explained by the fact that spermatogenesis failure was caused by chromosome mis-segregation during meiosis. For patient P1, the causative factor of male infertility and spermatogenetic failure in Y-autosome translocation may be caused by the disruption, loss of AZF loci by the translocation breakpoint or by a positional effect. For patient P2, recent research have identified genes at 8p12 such as KAL2, TEX15, and NRG1 as being involved in spermatogenesis and their loss in unbalanced translocations can result in azoospermia. Also, a position effect of unknown spermatogenesis regulatory gene(s) at 14q12 could explain NOA for P2 moreover, further investigations are needed.

Conclusion: Our study, discussing two new cases of a rare (Y;8) and (8;14) unbalanced translocations in NOA, highlights the necessity of genetic testing in NOA before any investigation, as well as the need for future research in order to improve genetic counselling in male infertility therapy.

P088 | The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: clinical, seminal and biochemical characteristics

Francesco Lotti[1], Francesca Frizza[1], Giancarlo Balercia[2], Arcangelo Barbonetti[3], Hermann M. Behre[4], Aldo E. Calogero[5], Jann-Frederik Cremers[6], Felice Francavilla[3], Andrea M. Isidori[7], Sabine Kliesch[6], Sandro La Vignera[5], Andrea Lenzi[7], Marios Marcou[4], Adrian Pilatz[8], Olev Poolamets[9], Margus Punab[9], Maria Fernanda Peraza Godoy[10], Osvaldo Rajmil[10], Gianmaria Salvio[2], Osama Shaeer[11], Wolfgang Weidner[8], Elisa Maseroli[1], Sarah Cipriani[1], Elisabetta Baldi[1], Selene Degl'Innocenti[1], Giovanna Danza[12], Anna Lucia Caldini[13], Alessandro Terreni[13], Luca Boni[14], Csilla Krausz[1], Mario Maggi[12]

[1]Andrology Unit, University of Florence, Florence, Italy; [2]University of Marche, Ancona, Italy; [3]University of L'Aquila, L'Aquila, Italy; [4]Center for Reproductive Medicine and Andrology, Halle, Germany; [5]University of Catania, Catania, Italy; [6]Münster University Hospital, Münster, Germany; [7]Sapienza University of Rome, Rome, Italy; [8]Justus Liebig University, Giessen, Germany; [9]Tartu University Hospital, Tartu, Estonia; [10]Fundació Puigvert, Barcelona, Spain; [11]Cairo University, Cairo, Egypt; [12]Endocrinology Unit, University of Florence, Florence, Italy; [13]Careggi Hospital, Florence, Italy; [14]Toscano Cancer Institute, Florence, Ital

Background: Infertility affects 7-12% of men and its etiology is unknown in half of cases. To fill this gap, use of the male genital tract colour-Doppler ultrasound (MGT-CDUS) has progressively expanded. However, MGT-CDUS still suffers from lack of standardization. Hence, the European Academy of Andrology (EAA) has promoted a multicenter study ("EAA ultrasound study") to assess MGT-CDUS characteristics of healthy-fertile men to obtain normative parameters.

Objectives: To report (a) the development and methodology of the "EAA ultrasound study", (b) the clinical characteristics of the cohort of healthy-fertile men and (c) the correlations of both fertility history and seminal features with clinical parameters.

Methods: A cohort of 248 healthy-fertile men (35.3 $\pm$ 5.9 years) was studied. All subjects were asked to undergo, within the same day, clinical, biochemical, seminal evaluation and MGT-CDUS before and after ejaculation.

Results: The clinical, seminal and biochemical characteritics of the cohort have been evaluated. The seminal characteristics were consistent with those reported by the WHO (2010) for the 50th and 5th centile for fertile men. Normozoospermia was observed in 79.6% of men, while normal sperm vitality was present in almost the entire sample. Time to pregnancy (TTP) was 3.0 [1.0-6.0] months. TTP was

negatively correlated with sperm vitality (Adj.r = -0.310,p = 0.011), but not with other seminal, clinical or biochemical parameters. Sperm vitality and normal morphology were positively associated with fT3 and fT4 levels, respectively (Adj.r = 0.244,p < 0.05 and Adj.r = 0.232,p = 0.002). Sperm concetration and total count were negatively associated with FSH levels and positively, along with progressive motility, with mean testis volume (TV). Mean TV was 20.4 ± 4.0 ml and the lower reference values for right and left testes were 15.0 and 14.0 ml. Mean TV was negatively associated with gonadotrophins levels and pulse pressure. Varicocele was found in 33% of men.

Conclusions: The cohort studied confirms the WHO data for all semen parameters and represents a reference with which to assess MGT-CDUS normative parameters.

P089 | The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: scrotal ultrasound reference ranges and associations with clinical, seminal and biochemical characteristics

Francesco Lotti[1], Francesca Frizza[1], Giancarlo Balercia[2], Arcangelo Barbonetti[3], Hermann M. Behre[4], Aldo E. Calogero[5], Jann-Frederik Cremers[6], Felice Francavilla[3], Andrea M. Isidori[7], Sabine Kliesch[6], Sandro La Vignera[5], Andrea Lenzi[7], Marios Marcou[4], Adrian Pilatz[8], Olev Poolamets[9], Margus Punab[9], Maria Fernanda Peraza Godoy[10], Osvaldo Rajmil[10], Gianmaria Salvio[2], Osama Shaeer[11], Wolfgang Weidner[8], Elisa Maseroli[1], Sarah Cipriani[1], Elisabetta Baldi[1], Selene Degl'Innocenti[1], Giovanna Danza[12], Anna Lucia Caldini[13], Alessandro Terreni[13], Luca Boni[14], Csilla Krausz[1], Mario Maggi[12]

[1]Andrology Unit, University of Florence, Florence, Italy; [2]University of Marche, Ancona, Italy; [3]University of L'Aquila, L'Aquila, Italy; [4]Center for Reproductive Medicine and Andrology, Halle, Germany; [5]University of Catania, Catania, Italy; [6]Münster University Hospital, Münster, Germany; [7]Sapienza University of Rome, Rome, Italy; [8]Justus Liebig University, Giessen, Germany; [9]Tartu University Hospital, Tartu, Estonia; [10]Fundació Puigvert, Barcelona, Spain; [11]Cairo University, Cairo, Egypt; [12]Endocrinology Unit, University of Florence, Florence, Italy; [13]Careggi Hospital, Florence, Italy; [14]Toscano Cancer Institute, Florence, Ital

Background: Scrotal colour-Doppler ultrasound (CDUS) still suffers from lack of standardization. Hence, the European Academy of Andrology (EAA) has promoted a multicenter study to assess the CDUS characteristics of healthy-fertile men (HFM) to obtain normative parameters.

Objectives: To report and discuss the scrotal organs CDUS reference ranges and characteristics in HFM and their associations with clinical, seminal and biochemical parameters.

Methods: A cohort of 248 HFM (35.3 ± 5.9 years) was studied, evaluating, on the same day, clinical, biochemical, seminal and scrotal CDUS following Standard Operating Procedures.

Results: The CDUS reference range and characteristics of the scrotal organs of HFM have been evaluated. CDUS showed a higher accuracy than physical examination in detecting scrotal abnormalities. Prader orchidometer (PO)- and US-measured testicular volume (TV) were closely related. The US-assessed TV with the ellipsoid formula showed the best correlation with the PO-TV. The mean TV of HFM was ~17 ml. The lowest reference limit for right and left testis was 12 and 11 ml, thresholds defining testicular hypotrophy. The highest reference limit for epididymal head, tail and vas deferens was 12, 6 and 4.5 mm, respectively. Mean TV was associated positively with sperm concentration and total count and negatively with gonadotrophins levels and pulse pressure. Subjects with testicular inhomogeneity or calcifications showed lower sperm vitality and concentration, respectively, than the rest of the sample. Sperm normal morphology and progressive motility were positively associated with epididymal head size/vascularization and vas deferens size, respectively. Increased epididymis and vas deferens sizes were associated with MAR test positivity. Decreased epididymal tail homogeneity/vascularization were positively associated with waistline, which was negatively associated with intratesticular vascularization. CDUS-varicocele was detected in 37.2% of men and was not associated with seminal or hormonal parameters. Scrotal CDUS parameters were not associated with time to pregnancy, number of children, history of miscarriage.

Conclusions: The present findings will help in better understanding male infertility pathophysiology, improving its management.

P090 | Extended ultrasound of the male urogenital tract

Stanislav Tjagur[1,2], Margus Punab[1,2,3]

[1]Andrology Centre, Tartu University Hospital, Tartu, Estonia; [2]Institute of Clinical Medicine, Faculty of Medicine, University of Tartu, Tartu, Estonia; [3]6th clinical division, Tartu University Hospital, Tartu, Estonia

Background: The andrological ultrasonography including transrectal and testicular sonography is largely unstandardized, and consensus on assessment and classification of several parameters is still lacking. Recently, the European Academy of Andrology (EAA) proposed the reference values for scrotal ultrasound. However, the reference values for prostate and seminal vesicles (SV) using transrectal ultrasound (TRUS) are still missing. The aim of this study was to describe the ultrasonographic differences in testicular, seminal ducts and prostatic-vesicular region among well-defined andrological patients groups.

Methods: During the period 03.06.2019–26.05.2022, fifty four (54) outpatient males passed the extended urogenital tract sonography for four different causes (16 patients with idiopathic azoospermia [FSH range 3.3–29.9 U/L, LH range 2.9–15.2 U/L]; 21 patients with oligo-astheno-teratozoosmpermia (OAT) [FSH range 2.0–22.7 U/L, LH range 2.5–11.6 U/L]; 8 patients with haematospermia; 9 patients with pelvic pain exacerbating after ejaculation;) in Andrology Centre of Tartu University Hospital, Tartu, Estonia. All the study participants were investigated by one clinician (S. T.), who has trained for ultrasound in the frame of EAA Ultrasound School. The ultrasound investigations consisted of scrotal ultrasound, pre- and post-ejaculatory TRUS. All the ultrasonographic studies were performed using the following ultrasound machines: FlexFocus 400 (BK Medical®) or bkSpecto

(BK Medical®). For scrotal ultrasound, a high-frequency linear probe (7-15 MHz) was used. For transrectal ultrasound, a 5-10 MHz endocavital biplane probe or 4-14 MHz endocavital triplane probe was used.

Results: The mean age of patients was 37.7 years (range 19.8–62.2); median sexual abstinence time was 4.0 days (range 1.5–14.0).

We found that testicular tissue's findings (testicular tissue inhomogeneity grade ≥1 and/or testicular micocalcifications) presented more frequently in azoospermic and OAT patients (37.5% and 52.4%, respectively).

Seminal tract findings (ectasia of rete testis; epididymal calcifications, body and tail cysts; ectasia of epididymis; changes in proximal ductus deferens; findings in ductus ejaculatorius (dilatation, cysts, microcalcification); unilateral absence of SV; calcifications of distal ductus deferens; unilateral absence of distal ductus deference) were highest among haematospermic patients (50%). Male accessory gland findings (sclerosis of SV; cysts or calcifications in SV; low volume of SV [pre-ejaculatory volume below 15 ml]; small prostate volume [<15 ml pre-ejaculatorily]; median cyst in prostate) and potentially normal US findings (prostate calcifications, epididymal head cysts) presented over 80% in all the four groups.

Median (range) pre-/post-ejaculatory volume of left SV was 15.9 ml (3.6–80.1)/10.2 ml (2.5–73.8). Median (range) pre-/post-ejaculatory volume of right SV was 16.9 ml (3.7–81.7)/11.3 ml (3.6–74.3). Median (range) ejection fraction of left and right SV was 23.1% (-52.2 – 69.1), and 32.1% (-137.5–81.1), respectively.

Median (range) pre-/post-ejaculatory prostate volume was 21.3 ml (13.6–37.1)/21.4 ml (12.5–37.1). Median (range) prostate volume change post-ejaculation was 1.7% (-23.8–24.8).

Conclusion: Our study confirms high value of testicular and TRUS examination in case of all the different study groups investigated in our study. However, real clinical value of extended ultrasound investigations, which include pre- and post-ejaculatory ultrasound examination, should be proved in large-scale studies.

P091 | The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: prostate-vesicular transrectal ultrasound reference ranges and associations with clinical, seminal and biochemical characteristics

Francesco Lotti[1], Francesca Frizza[1], Giancarlo Balercia[2], Arcangelo Barbonetti[3], Hermann M. Behre[4], Aldo E. Calogero[5], Jann-Frederik Cremers[6], Felice Francavilla[3], Andrea M. Isidori[7], Sabine Kliesch[6], Sandro La Vignera[5], Andrea Lenzi[7], Marios Marcou[4], Adrian Pilatz[8], Olev Poolamets[9], Margus Punab[9], Maria Fernanda Peraza Godoy[10], Claudia Quintian[10], Osvaldo Rajmil[10], Gianmaria Salvio[2], Osama Shaeer[11], Wolfgang Weidner[8], Elisa Maseroli[1], Sarah Cipriani[1], Elisabetta Baldi[1], Selene Degl'Innocenti[1], Giovanna Danza[12], Anna Lucia Caldini[13], Alessandro Terreni[13], Luca Boni[14], Csilla Krausz[1], Mario Maggi[12]

[1]Andrology Unit, University of Florence, Florence, Italy; [2]University of Marche, Ancona, Italy; [3]University of L'Aquila, L'Aquila, Italy; [4]Center for Reproductive Medicine and Andrology, Halle, Germany; [5]University of Catania, Catania, Italy; [6]Münster University Hospital, Münster, Germany; [7]Sapienza University of Rome, Rome, Italy; [8]Justus Liebig University, Giessen, Germany; [9]Tartu University Hospital, Tartu, Estonia; [10]Fundació Puigvert, Barcelona, Spain; [11]Cairo University, Cairo, Egypt; [12]Endocrinology Unit, University of Florence, Florence, Italy; [13]Careggi Hospital, Florence, Italy; [14]Toscano Cancer Institute, Florence, Ital

Background: Transrectal ultrasound (TRUS) parameters are not standardized, especially in men of reproductive age. Hence, the European Academy of Andrology (EAA) promoted a multicenter study to assess the TRUS characteristics of healthy-fertile men (HFM) to establish normative parameters.

Objectives: To report and discuss the prostate and seminal vesicles (SV) reference ranges and characteristics in HFM and their associations with clinical, seminal, biochemical parameters.

Methods: One hundred eighty-eight men (35.6 ± 6.0 years) from a cohort of 248 HFM were studied, evaluating, on the same day, clinical, biochemical, seminal, TRUS parameters following Standard Operating Procedures.

Results: TRUS reference ranges and characteristics of the prostate and SV of HFM have been evaluated. The mean PV was ~25 mL. PV lower and upper limits were 15 and 35 mL, defining prostate hypotrophy and enlargement, respectively. PV was positively associated with age, waistline, current smoking (but not with testosterone levels), seminal volume (and negatively with seminal pH), prostate inhomogeneity, macrocalcifications, calcification size and prostate arterial parameters, SV volume before and after ejaculation, deferential and epididymal size. Prostate calcifications and inhomogeneity were frequent, while midline prostatic cysts were rare and small. Ejaculatory duct abnormalities were absent. Periprostatic venous plexus size was positively associated with prostate calcifications, SV volume and arterial peak systolic velocity. Lower and upper limits of SV anterior-posterior diameter after ejaculation were 6 and 16 mm, defining SV hypotrophy or dilation, respectively. SV total volume before ejaculation and delta SV total volume (DSTV) positively correlated with ejaculate volume, and DSTV correlated positively with sperm progressive motility. SV total volume after ejaculation was associated negatively with SV ejection fraction and positively with distal ampullas size. SV US abnormalities were rare. No association between TRUS and time to pregnancy, number of children or history of miscarriage was observed.

Conclusions: The present findings will help in better understanding male infertility pathophysiology and the meaning of specific TRUS findings.

P094 | Which seminal, clinical and hormonal adrological parameters predict a pregnancy with live birth?

Francesco Lotti[1], Beatrice Gasperetti[1], Mario Maggi[2]

[1]Andrology Unit, University of Florence, Florence, Italy; [2]Endocrinology Unit, University of Florence, Florence, Ital

Background: Infertility affects 8-12% of men of reproductive age. Although a)scientific literature reports several clinical parameters likely associated with male infertility/fertility, b)previous studies report gonadotropins and testosterone, thresholds suggestive of abnormal/normal testicular function and c)values of several seminal parameters > 5° centile of their distribution reported by the WHO Manual suggest a higher probability to be fertile, so far thresholds of clinical, seminal and biochemical parameters predicting a pregnancy with live birth are not available.

Objectives: To assess differences in clinical, seminal and biochemical characteristics of males of infertile couples (MIC) and males of fertile couples (MFC) and to identify clinical, seminal and biochemical predictors of a pregnancy with live birth evaluating several parameters in MIC and MFC with live birth.

Methods: We evaluated 604 MIC (37.5 ± 7.0 years) and 115 healthy MFC with live birth (36.6 ± 5.3 years). We compared the clinical, seminal and biochemical characteristics of the two groups. Subsequently, for clinical, seminal and biochemical variables with continuous distribution we performed iterative ROC curves to identify parameters predictive for a pregnancy with live birth, reporting the threshold with the best sensibility and specificity predicting a pregnancy with live birth. Then, dichotomous variables related to the thresholds found for each parameter were used as independent variables in iterative binary logistic regression analyses adjusted for confounders (male and female partner age), to evaluate the adjusted risk ("odds ratio", OR) to be a man of a fertile couple (dependent variable), i.e. to induce a pregnancy with live birth. Accordingly, clinical, seminal and biochemical dichotomous variables have been used as independent variables in iterative binary logistic regression analyses adjusted for confounders to evaluate the adjusted risk ("odds ratio", OR) to induce a pregnancy with live birth (dependent variable).

Results: MFC and MIC, as well as their female partners, showed a similar age. MCF had a lower prevalence of cryptorchidism and mumps history, less general and adrological physical examination abnormalities, better seminal and hormonal parameters, better sexual function, lower urinary tract symptoms and psychopathological traits compared with MIC. Female partner age < 34.5 years old, mild alcohol consumption, absence of cryptorchidism and mups history; mean blood pressure < 93 mmHg, mean testis volume (Prader) ≥ 20 ml, absence of epididymal dilation, absence of bilateral agenesis of vas deferens; sperm concentration ≥ 40 * 106/ml, sperm total count ≥ 128 40 * 106/ejaculate, progressive motility ≥ 52%, normal morphology ≥ 5%, seminal pH ≥ 7.5; FSH < 3.8 U/L, LH < 3.2 U/L, total testosterone ≥ 17 nmol/l, calculated free testosterone ≥ 0.335 nmol/l, SHBG ≥ 31 nmol/l; absence of erectile dysfunction (IIEF-15-EFD ≥ 26) and MHQ total score ≤ 20 were predictive for a pregnancy with live birth.

Conclusions: This study reports for the first time thresholds related to clinical, seminal and biochemical parameters predictive for a pregnancy with live birth. The application of these thresholds can be useful in the clinical practice to evaluate if a man consulting to evaluate his fertility potential, shows characteristics predictive for a pregnancy with live birth.

P095 | Fertility alterations of males with non-obstructive azoospermia due to hypogonadotropic hypogonadism after gonadotropin treatment

Athanasios Zachariou[1], Aris Kaltsas[1], Ioannis Giannakis[1], Minas Paschopoulos[2], Andreas Karagiannis[1], Fotios Dimitriadis[3], Panagiota Tsounapi[4], Athanasios Papatsoris[5], Atsushi Takenaka[4], Nikolaos Sofikitis[1]

[1]Department of Urology, University Hospital of Ioannina, Ioannina, Greece; [2]Department of Obstetrics and Gynaecology, University Hospital of Ioannina, Ioannina, Greece; [3]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [4]Department of Urology, Tottori University, Yonago, Japan; [5]Department of Urology, Sismanoglio General Hospital, University of Athens, School of Medicine, Athens, Greece

Introduction: Hypogonadotropic hypogonadism (HH) is a rare cause of non-obstructive azoospermia (NOA). Treatment recommendations include GhRHa and gonadotropins for at least 12 months. In case of poor treatment results and persisting azoospermia, surgical spermatozoa extraction from the testes is the suggested solution. The present study aims to evaluate the fertility results of NOA males undergoing treatment for HH in the Urology Department of Ioannina University.

Methods: The present study includes twenty-five NOA males suffering from HH. Patients underwent hormonal evaluation and double sperm centrifugation to establish azoospermia and HH. After the recommended treatment period, sperm diagram results, sperm retrieval rates, pregnancy rate and live births were assessed.

Results: Twenty-five males suffering from NOA received treatment with gonadotropins for a median time of 13.1 months. After that period, 10 NOA males presented with spermatozoa (40%). The 15 azoospermic men followed a micro-TESE procedure to retrieve spermatozoa from testicles. This group of males underwent a gonadotropins treatment program for 14.1 months (range 6-21 months). In 13 men (86.7%), the micro-TESE process resulted in the presence of spermatozoa. After 12 cycles of ICSI with spermatozoa from testicles, we had 6 (50%) successful pregnancies and five live births (including two twins) and one abortion after nine weeks of pregnancy.

Conclusions: A significant number of males suffering from NOA due to HH will produce spermatozoa. The azoospermic NOA males with HH, after treatment, can retrieve spermatozoa with new surgical techniques and proceed to live births.

P096 | Is non-morbid obesity the cause of idiopathic male infertility?

Victoria Ghenciu[1], Ion Dumbraveanu[1], Iurii Arian[1], Stela Bacinschi-Gheorghita[2], Emil Ceban[1]

[1]'Nicolae Testemiteanu' State University of Medicine and Pharmacy, Chisinau, Republic of Moldova; [2]Republican Clinical Hospital, Chisinau, Republic of Moldova

Introduction: After performing standard andrological tests, in about 40% of cases, male infertility has no obvious cause and is considered idiopathic. The role of obesity in male infertility in the absence of other morbid hormonal or cardiovascular changes is not fully elucidated and continues to be controversial.

Purpose: Determining the incidence of non-morbid obesity in male infertility and the results of management.

Material and Methods: The study included 207 men with male infertility who considered themselves healthy, with a BMI of less than 40, and standard andrological examination (medical history, physical examination, semen analysis, sex hormone profile, testis ultrasound) found no causes of infertility but confirmed alterations in semen analysis. Additional tests included: Lipid profile (Cholesterol, LDL, HDL Triglycerides), 25-OH-Vit.D and thyroid hormones.

Results: In 61 (29%) patients BMI was less than 25, including BMI up to 20 in 12 (6%) men, 78 (38%) patients were overweight and 68 (33%) had grade I-II obesity. Patients were divided into 3 groups. The total amount of sperm was 15.6 (± 4.5) mln - group 1, 17.1 (± 5.6) mln - group 2 and 14.2 (± 3.1) mln - group 3. The average progressive motility in groups was 13%, 16% and 11.4%, and the average normal morphology was 3.4%, 4.8%, 2.8% respective. Azoospermia or OAT was detected in 7 patients (3 men in group 1 and 2 men in groups 2 and 3). Hypothyroidism was founded in 3 patients in group 3 and 1 in group 1. Hyperthyroidism founded in 1patient in group 1. In group 3, 55 (81%) patients had increased cholesterol and/or its fractions and 13 (19%) patients had normal lipid profiles. In group 1, 7 (11%) patients had increased lipid profiles. We administered antioxidant treatment to 196 patients. In patients from groups 2 and 3, we advised lowering BMI by changing the lifestyle and diet for a period of 6 months. Repeated evaluation at 6 months showed improvement in lipid profile in 22 (28%) patients in group 2 and 32 (48%) patients in group 3, and improvement in sperm analysis in 21 (34%) patients in group 1, 32 (48%) patients in group 2 and 39 (57%) patients in group 3. Vitamin D deficiency was determined in about 80% of cases, with no differences within the groups.

Discussions and conclusions: We cannot say for sure that MI considered idiopathic is more common in obese patients, but only 23% of patients had a healthy BMI (18.5-24.9). We did not report significant differences between the total sperm count, mobility or normal morphology in patients in different groups, but there is a tendency of decreased motility and normal morphology in patients with non-morbid obesity. BMI and lipid profile can be important parameters in the complex assessment of male infertility.

Antioxidant treatment, concomitant with BMI reduction, showed an improvement in spermogram parameters at a 6-month interval in all patients, but more obviously in those with initial non-morbid obesity (BMI 30-39).

P097 | Negative correlation between serum Vitamin D level and semen quality in fertile men

Iurii Arian[1], Ion Dumbraveanu[1], Victoria Ghenciu[1], Stela Bacinschi-Gheorghita[2], Emil Ceban[1]

[1]*'Nicolae Testemiteanu' State University of Medicine and Pharmacy, Chisinau, Republic of Moldova;* [2]*Republican Clinical Hospital, Chisinau, Republic of Moldova*

Introduction: Recent studies suggest that low serum vitamin D may be associated with low semen quality and male infertility. The relationship between Vit D and semen parameters gained even more importance after it was reported that vitamin D supplementation altered granulosa cell gene expression. In this context, the possible relationship between vitamin D and semen parameters has been discussed by many researchers recently, but the results were significant in some studies and no relationship could be shown in others.

Aim: The aim of the study was to investigate the serum level of vitamin D and semen parameters in proven fertile men to see if there is any correlation that confirms the role of vitamin D in reproduction.

Material and Methods: Thirty-one men with a history of conceiving a pregnancy in the last 12 months were included in the study. The participant's ages ranged from 21 to 39 years, with a mean age of 32.54 years old. Each participant was tested for serum vitamin D and semen parameters following three-day abstinence. Serum vitamin D levels were assessed by the immunoassay method, measurement range - 5.3 - 133 ng/mL, analytical sensitivity - 2.81 ng/mL.

Results and conclusions: Vitamin D levels were found to range from 12.1 to 40.2 ng/ml, with an average of 23.7 ng/ml. In almost 82.31 %, we detected vitamin D insufficiency (20-30 ng/ml). The semen volume with a mean of 3.2 ml, sperm count 64.34 mln/mL, sperm total motility of 56.10 %, progressive motility of 41.32%, sperm normal morphology - 12.0 %. Almost 87.41% with completely normal sperm parameters according to WHO manual, 5th edition. Individually was observed that there is no positive correlation between sperm parameters and Vitamin D levels, participants with lower Vitamin D levels were found to the higher sperm parameters, and those with low semen quality with better vitamin D levels. However, there is no cause-and-effect relationship, and there is a need for further research in this area to understand whether vitamin D supplementation can improve the fertility rate.

P098 | Hypogonadotrop hypogonadic fathers – a single centre experience

Tamas Takacs[1,2], Anett Szabo[2], Zsolt Kopa[2]

[1]*Semmelweis University, Dept. of Gynecology and Obstetrics, Centre of Assisted Reproduction, Budapest, Hungary;* [2]*Semmelweis University, Dept. of Urology, Andrology Centre, Budapest, Hungar*

Introduction: Hypogonadotropic hypogonadism is a relatively rare, congenital or acquired malfunction of the pituitary gland, characterised by low gonadotropin and consequently low testosterone levels and male infertility. Gonadotropin substitution is a potentially effective treatment to induce/restore fertility within 3-24 months.

Material and Methods: Between January 2012 and December 2021, 49 patients were diagnosed with hypogonadotropic hypogonadism. Classical sperm parameters, hormone levels and fertilisation

success rates were analysed retrospectively. Combined human chorionic gonadotropin (hCG) and recombinant follicle stimulating hormone (rFSH) treatment was initiated and tailored individually. Follow up was performed every 12 weeks.

Results: Thirty-seven patients (75.5%) were diagnosed with congenital disease (isolated form, Kallmann syndrome, panhypopytuarism, pituitary hypoplasia, and 21-hydroxylase deficiency in 28, 3, 3, 2 and 1 cases, respectively). Twelve patients (24.5%) suffered from the acquired form of the disease (previous pituitary macroadenoma surgery, anabolic steroid use, and recent diagnosis of pituitary craniopharyngeoma in 7, 4 and 1 cases, respectively). Baseline FSH and LH levels were below 1.5 IU/L (mean: 0.61 IU/L, range: 0.1-1.47 IU/L) and 1.95 IU/L (mean: 0.62 IU/L, range: 0.01- 1.9 IU/L), respectively. Nine out of the 49 patients underwent testosterone replacement therapy previously. In the untreated group of 40 patients, 80% (32/40) showed low testosterone levels (below 8 nmol/L, mean: 3.15 nmol/L), 20% (8/40) of patients' testosterone levels were in the grey zone (between 8-12 nmol/L, mean: 10.18 nmol/L).

The drop out rate was 38.8% (19 patients), a total of 30 patients' data could be collected for the therapeutic database. Azoospermia was found in 70% of the enrolled patients (21/30). Cryptozoospermia, oligo-astheno-teratozoospermia and astheno-teratozoospermia was diagnosed in 3 (10%), 5 (16.7%) and 1(3.3%) cases, respectively.

During the treatment period spermatogenic improvement occured in 83.3% of the cases (25/30).

Eighteen of the azoospermic patients (85.7%) had improved (average timespan until the appearance of sperm cells in the ejaculate took 7 months), 9.52% became normozoospermic. All three continuously azoospermic patients underwent microsurgical testicular sperm extraction, with a 100% sperm retrieval rate. Eight patients of the non-azoospermic group (88.9%) showed a significant spermatogenic improvement, 33.3% became normozoospermic.

Seven patients (23.3%) achieved spontaneous pregnancy (a total of 10 newborns). In the remaining 23 patients 10 cycles of ART resulted in five pregnancies therefore for the 30 treated patients 15 children were born during the study period.

Conclusion: Gonadotropin replacement is an effective method to treat hypogonadotropic hypogonadism to achieve fertility. In our series 12 of 30 patients achieved fatherhood (in three cases multiple times), and sperm cells could be harvested in all azoospermic patients after treatment (surgically in three cases) in an average of 7 months of the therapy. The high success rate of the treatment underlines the importance of appropriate diagnostic process, and the early recognition of hypergonadotropic hypogonadism.

P099 | Morphostructural characterization of the testis in a large cohort of men living in highly polluted areas of Campania Region in south Italy: a focus on cadmium exposure

Francesco Garifalos[1], Cristina De Angelis[1], Aldo DI Nunzio[2], Davide Menafra[1], Michele Castoro[1], Nunzia Verde[1], Giacomo Galdiero[1], Mariangela Piscopo[1], Claudia Pivonello[1], Renata Simona Auriemma[1], Paolo Chiodini[3], Marco Trifuoggi[2], Annamaria Colao[1,4], Rosario Pivonello[1,4]

[1]Università Federico II di Napoli, Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Naples, Italy; [2]Università degli Studi di Napoli Federico II, Dipartimento di Scienze Chimiche, Naples, Italy; [3]University of Campania "Luigi Vanvitelli", Medical Statistics Unit, Naples, Italy; [4]Unesco Chair for Health Education and Sustainable Development, Università Federico II di Napoli, Naples, Ital

Campania Region has been facing waste management crisis since 1980, characterized by urban, toxic and industrial waste illegal disposal, burying and incineration. Cadmium (Cd) is consistently shown to affect male reproductive function by multiple mechanisms, mostly elucidated in experimental models. The aim of the current single-center, observational, cross-sectional cohort study was to evaluate the prevalence of testis morphostructural alterations in a large cohort of men living in 3 municipalities of Campania Region (Acerra, Afragola, Giugliano) belonging to the high-environmental impact area "Land of Fires", by addressing the potential association with seminal Cd (sCd) levels. Study cohort included 465 males (age range: 14-50 yrs; mean: 29.5 ± 7.23 yrs). Morphostructural testis characteristics were assessed by ultrasound and sCd determination was performed in 385 samples by inductively coupled plasma-mass spectrometry. Prevalences of testis morphostructural alterations, unilateral or bilateral, included varicocele (35.4%), hydrocele (34.8%), parenchymal structure inhomogeneity (19%), hypotrophy (14.6%), microlithiasis (2.5%), solid lesions >5 mm (0.2%). Participants with detectable sCd levels (N = 128) displayed significantly reduced mean testicular volume (16.56 ± 4.68 vs. 17.66 ± 4.34; p = 0.0153) and higher prevalence of hypotrophy (21% vs. 10%; p = 0.0059) and varicocele I-V° grade (47.5% vs. 29.5%; p = 0.0008), but not clinically relevant varicocele III-V grade (18% vs. 11%, p = 0.09), together with a slightly higher prevalence of parenchymal structure inhomogeneity (25.8% vs. 16.7%; p = 0.059), compared to participants with undetectable sCd levels (N = 257). Furthermore, a significant difference in mean testicular volume was detected when comparing participants with sCd levels above (N = 49) and below median value (N = 79) and undetectable sCd levels, respectively (14.88 ± 3.79 vs. 17.22 ± 5.03 vs. 17.66 ± 4.34; p < 0.001). sCd level was persistently correlated with mean testicular volume after correction for the presence of clinically relevant varicocele (r = -0.185; p = 0.001). sCd levels was identified as the best predictor of mean testicular volume in linear regression analysis performed by setting sCd, smoking habit, age and BMI as independent variables. ROC curve analysis highlighted that a sCd level > 0.76 μg/L correctly identified testicular hypotrophy with a 60% sensibility and 70% specificity. In conclusion, the current study demonstrated for the first time, in a large cohort of adult males living in high-environmental impact areas of Campania Region, an inverse relationship between sCd levels and mean testicular volume and prevalence of varicocele, independently from age, BMI and smoking habit, therefore further strengthening the concept of gonadal toxicity exerted by Cd.

P100 | Reproductive function in healthy men living in the "Land of Fires" of the Campania region: focus on three highly contaminated municipalities and on the role of cadmium as a potential harmful factor for semen qualitY

Cristina de Angelis[1], Giacomo Galdiero[1], Antonella Giarra[2], Francesco Garifalos[1], Davide Menafra[1], Mariangela Piscopo[1], Nunzia Verde[1], Michele Castoro[1], Claudia Pivonello[1], Renata Simona Auriemma[1], Paolo Chiodini[3], Marco Trifuoggi[2], Annamaria Colao[1,4], Rosario Pivonello[1,4]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Università Federico II, Naples, Italy; [2]Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Naples, Italy; [3]Medical Statistics Unit, University of Campania "Luigi Vanvitelli", Naples, Italy; [4]Unesco Chair for Health Education and Sustainable Development, Federico II University, Naples, Ital

During the last 30 years, Campania Region has been characterized by waste management crisis, resulting in the largely documented illegal disposal of urban, toxic and industrial waste, and diffuse practice of illegal waste burning. Chronic exposure to environmental pollutants has been shown to adversely affect male reproductive function and semen quality; the heavy metal cadmium (Cd) is a human carcinogen ubiquitous in the environment consistently shown to affect male reproductive function by endocrine and non-endocrine actions. Testis is particularly susceptible to Cd poisoning, and in experimental studies in animals and human spermatozoa Cd exerted reproductive toxicity, mediated by structural damage to testis vasculature and blood-testis barrier, inflammation, Sertoli and Leydig cells cytotoxicity, oxidative stress, epigenetic actions, and disturbance of the hypothalamus-pituitary gonadal axis. No large investigations have been performed addressing semen quality of young men living within the high-environmental impact area "Land of Fires" (LF) and/or the potential implication of non-occupational exposure to heavy metals, particularly Cd. Within a population-based awareness and screening campaign for male infertility in Campania Region, the current study enrolled a large cohort (N = 493) of healthy men (mean age: 24 yrs) with at least 10 years of residence in 3 high-environmental impact municipalities (Acerra, Afragola, Giugliano in Campania; AAG) belonging to LF, for comparison with 410 age-matched men living in low-environmental impact municipalities (Other areas, OA)(ARPAC D.L.136/2013). No "a priori" exclusion criteria were applied concerning andrological profile. Anamnesis, clinical and andrological examination, testis ultrasound, and 2 semen analyses (WHO 2010) were performed. Serum and semen concentrations of 23 trace elements were determined by ICP-MS. Participants from AAG had significantly lower sperm concentration ($p < 0.001$), count ($p < 0.001$) and normal sperm morphology ($p = 0,035$), compared to participants from OA; consistently, the prevalence of normozoospermia was significantly lower ($p = 0.0022$) and that of oligozoospermia was significantly higher ($p = 0.0017$) in AAG. Participants from AAG were grouped as being normozoospermic (N = 328) or having seminal parameters below reference range (N = 165); no difference was detected in serum trace elements concentrations between groups, whereas seminal Cd concentration was significantly higher ($p = 0.031$) in the pathological seminal parameters group. Moreover, seminal Cd concentration was negatively correlated to sperm concentration ($r = -0.211$; $p = 0.017$) and count ($r = -0.177$; $p = 0.045$). Since seminal Cd concentration was frequently below the limit of detection (LoD = 0.20 μg/L) across AAG cohort, participants were grouped as having seminal Cd concentration below (N = 364) or above (N = 129) LoD. Participants with detectable seminal Cd concentration had significantly reduced sperm count ($p = 0.028$) and normal sperm morphology ($p = 0.036$), compared to those with undetectable concentration. Compared to AAG, none of the participants from OA had detectable seminal Cd levels (0% vs. 26.2%; $p < 0.0001$). The current study supports a detrimental effect of the specific environmental exposure pattern of AAG polluted municipalities on semen quality, and higher seminal Cd levels were associated to worse seminal parameters, suggesting that cumulative Cd exposure might exert reproductive toxicity at environmentally relevant levels of exposure. Further studies including the objective assessment of Cd burden in environmental matrices are encouraged to corroborate the impact of Cd exposure on the risk of male subfertility/infertility.

P101 | Improvement of serum 17-alpha hydroxyprogesterone after varicocelectomy correlates with significant improvement in sperm count

Mohamed W Ragab[1], Mohamed Ragab[1], Ashraf Zidane[1], Tarek Ramzy[2], Mohamed Abbas[1]

[1]Department of Andrology, Cairo University, Egypt; [2]Department of Chemical Pathology, Cairo University, Egypt

Serum 17 alpha hydroxy progesterone (17 OHP) has been hypothesized to reflect intra-testicular testosterone (ITT) level. In this study, we aimed to evaluate the correlation between changes of serum 17-OHP after microsurgical varicocelectomy and improvement of semen parameters in infertile men with palpable varicocele and eligible for operation. This prospective study was conducted between March 2021 and December 2021 in Cairo University hospital. We included 45 men with palpable bilateral varicocele and abnormal semen parameters. Conventional semen parameters, Serum gonadotropins, total testosterone (TT) and serum 17 OHP were measured before and three months after varicocelectomy. According to the distribution of data, comparison between data before and after operation was performed using Wilcoxon Sign Ranks test. Spearman's rho correlation coefficient was used to correlate between variables. Sperm concentration improved significantly from 8.36 ± 5.04 million/ml to 12.52 ± 8.42 million/ml after 3 months following microsurgical varicocelectomy ($p = 0.001$), with normalization of concentration in 15/45 (33%) patients. Total motility did not improve significantly ($p = 0.9$) but progressive motility improved significantly from 8.62 ± 8.74% to 16.24 ± 14.45% ($p = 0.001$). Abnormal forms were insignificantly declined from 89.09 ± 16.3% to 87.64 ± 17.38% ($p = 0.075$). Serum

17 OHP and 17 OHP/TT improved significantly from 1.21 ± 0.45 ng/ml and 0.26 ± 0.09 to 1.42 ± 0.76 ng/ml and 0.3 ± 0.16 (p = 0.013, p = 0.004), respectively, while serum TT did not improve significantly (p = 0.167). A significant correlation was found between improvement in sperm concentration and improvement in both serum 17 OHP and 17 OHP/TT ratio (p = 0.001, p = 004).

Improvement of serum 17 OHP - a serum biomarker that reflects ITT-after varicocelectomy correlates with improvement in sperm count. This finding may explain one of the mechanisms of improvement of male fertility potential following varicocelectomy.

P102 | Decrease in sperm motility between one and three hours after ejaculation: association with the Hypo-Osmotic Swelling Test

E Arapogianni[1], G Mintziori[1], DG Goulis[1], E Evgeni[2]

[1]Unit of Reproductive Endocrinology, 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece, European Academy of Andrology Training Center in Thessaloniki, Greece; [2]Cryogonia Cryopreservation Bank, Athens, Greece

Background: The Hypo-Osmotic Swelling (HOS) Test has been associated with the membrane integrity of spermatozoa, as it determines the sperm's membrane ability to maintain equilibrium between the sperm cell and its environment. It is used as an alternative to dye exclusion, especially when choosing spermatozoa for intracytoplasmic sperm injection (ICSI).

Aim: To assess the decrease in sperm motility over time (one and three hours after ejaculation) and to correlate sperm motility with membrane integrity using the HOS test.

Methods: Semen samples from 103 men with couple infertility (normozoospermia, n = 8; dyspermia, n = 95), aged 20 to 66 years, underwent conventional semen analysis. The effect of time (one and three hours after ejaculation) on three categories of sperm motility [rapid progressive (RP), total progressive (TP), total motile (TM)] was assessed.

Results: A significant decrease was observed in RP, TP and TM three hours after ejaculation compared with the baseline values (p < 0.001 for all categories). Sperm concentration, total sperm count, normal morphology and the HOS test results were positively correlated with RP, TP and TM three hours after ejaculation (p < 0.001 for all categories). In contrast, the abnormal sperm forms (head and tail) were negatively correlated with RP, TP and TM three hours after ejaculation (p < 0.001 for all categories). The HOS test result explained 51%, 68% and 79% of the decrease in RP, TP and TM, respectively, three hours after the ejaculation.

Conclusions: The decrease in sperm motility between one and three hours after ejaculation is significant and depends on several semen parameters. The HOS test may help in predicting the semen quality three hours after ejaculation, which might be of clinical importance in cases of assisted reproductive technique (ART) procedures.

P103 | Sperm static oxido-reduction potential in men with infertility and fertile men

G Mintziori[1], E Arapogianni[1], D Koumasi[2], G Kanakis[3], DG Goulis[1], L Evgeni[2]

[1]Unit of Reproductive Endocrinology, 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece, European Academy of Andrology Training Center in Thessaloniki, Greece; [2]Cryogonia Cryopreservation Bank, Athens, Greece; [3]Department of Endocrinology and Assisted Reproduction Unit, Naval and Veteran Affairs Hospital, Athens, Greece

Introduction: Oxidative stress, alongside metabolic and psychological stress, has been suggested to affect semen quality and male reproductive potential detrimentally. Though the concept has been widely accepted, technologies that can be applied to quantify this effect are still under investigation. Male Infertility Oxidative System (MiOXSYS) has been suggested to be used in everyday clinical practice.

Methods: This study was of case-control design, involving 358 men with infertility and 60 fertile controls. All participants were assessed for all conventional sperm parameters according to the WHO 5th and 6th editions (2010 and 2021) recommendations. Sperm samples were collected by masturbation following 2–7 days of abstinence. Sperm static oxido-reduction potential (sORP) and DNA fragmentation were assessed in all men with infertility and fertile controls, as well as sperm chromatin decondensation.

Results: sORP was detected to be higher in men with infertility compared with fertile controls [median (interquartile range) 2 (5) vs. 1 (1) mV/10ˆ6 sperm/ml, respectively, p = 0.027]. DNA fragmentation was also higher in men with infertility compared with fertile controls [26 (22) vs. 18 (17)%, p < 0.001]. Sperm chromatin decondensation was similar between the two groups (p = 0.4). sORP was negatively correlated with classic sperm parameters such as sperm concentration and normal morphology (Spearman's rho -0.783 and -0.394, respectively, p < 0.001 for both), positively with DNA fragmentation (Spearman's rho 0.370, p < 0,001) and marginally with sperm chromatin decondensation (Spearman's rho 0.215, p = 0.051).

Conclusion: MiOXSYS technology can evaluate the oxido-reduction potential in semen samples from men with infertility providing additional information for their optimal management.

P104 | Inhibin-B and FSH are hood indicators of spermatogenesis but not the best indicators of fertilitY

Katarzyna Jankowska[1], Natalia Suszczewicz[2], Michał Rabijewski[3], Piotr Dudek[1], Wojciech Zgliczynski[1], Radosław B. Maksym[3]

[1]Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland; [2]Department of Mini-invasive and Endoscopic Gynecology, Military Institute of Medicine, Legionowo, Poland; [3]Department of Reproductive Health, Centre of Postgraduate Medical Education, Warsaw, Poland

Biochemical markers of spermatogenesis and fertility assessment are important in the practical management of infertile males and the determination of an individual's prognosis. We performed an analysis on 100 males with a male infertility factor. The following study inclusion parameters were analyzed: seminogram, FSH, LH, testosterone, estradiol, prolactin, TSH, and inhibin B concentrations. The patients were subsequently treated by reproductive endocrinologists in accordance with AUA/ASRM and EAU guidelines. The reproductive status was evaluated over a period of 3 years. We found a strong correlation of sperm count with inhibin B ($r = 0.74$, $p < 0.001$) and FSH concentration levels ($r = -0.46$, $p < 0.001$). Among 95 patients at follow-up, pregnancies occurred for 59 of their partners (48 spontaneous, 5 after IVF–ET, and 6 after IUI). Thirty-six patients remained childless despite the therapy. Sperm count and inhibin B level were the best predictors of natural fertilization (ROC AUC: 0.86 and 0.84; cut-off: 2.7 mln/mL and 45 pg/mL). Although inhibin B and FSH can be used to evaluate spermatogenesis and fertility, the initial sperm concentration appeared to be the best predictor of success. Pregnancy was achieved in a surprisingly large proportion of patients with a very low concentration of inhibin B and a low initial sperm count. It is noteworthy that 81% of the pregnancies were achieved without medically assisted reproduction.

P105 | Is oxidative stress evaluated in viable human spermatozoa a marker of good semen quality?

Giulia Traini[1], Lara Tamburrino[2], Linda Vignozzi[1,3], Elisabetta Baldi[2,3], Sara Marchiani[1]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [3]Andrology, Women's Endocrinology and Gender Incongruence Unit, Careggi University Hospital, Florence, Ital

Background: Oxidative stress (OS) is defined as unbalance between reactive oxygen species (ROS) production and antioxidant defences. Low levels of ROS are necessary for physiological sperm functions, whereas high levels impair fertility damaging membranes, proteins and DNA. Previous studies, performed by using different methods and probes for OS evaluation in semen or in spermatozoa, highlighted the negative role of ROS on sperm functions. However, such studies were not conclusive because of the small number of included subjects and of high variability in the cohorts. In the present study, we used two probes, CellROX® Orange Reagent and Dihydroethidium (DHE), which reveal different ROS in viable spermatozoa, to evaluate the association between ROS and standard semen parameters and DNA fragmentation.

Methods: Sperm ROS were evaluated by two fluorescent probes revealed by flow cytometry: CellROX® Orange, that reveals hydrogen peroxide, and DHE that shows distinct specificity toward both superoxide anion and hydrogen peroxide. Sperm DNA fragmentation (by TUNEL/PI method), sperm kinematic parameters and hyperactivated motility (by C.A.S.A. system) were concomitantly assessed. Phosphatidylserine membrane exposure was evaluated by Annexin V.

Results: To demonstrate that the two probes were sensitive to an induction of ROS in spermatozoa, we used Tert-Butyl hydroperoxide (TBHP, an analog of hydrogen peroxide) for CellROX® Orange and Menadione (which generates superoxide) for DHE. After incubation with TBHP for 30 min at 37°C, an increase of the percentage of positivity CellROX® Orange spermatozoa was observed. Similarly, when Menadione was added to spermatozoa for 30 min at room temperature, a noticeable increase in DHE positivity was revealed. We evaluated ROS levels in 121 semen samples, and we found that ROS in viable spermatozoa, assessed with the two probes, were positively associated with motility, morphology and number and negatively with DNA fragmentation. In unviable spermatozoa, ROS levels were negatively associated with semen quality. The positive correlations between ROS in viable spermatozoa and semen parameters, above described, indicate that the oxidative status revealed by the two probes is related to a better sperm quality. To further investigate this possibility, we double labelled spermatozoa with CellROX® Orange and Annexin V, a marker of early signs of apoptosis. We found that only 2.8% of Annexin V positive spermatozoa was also positive for CellROX® Orange, indicating that the probe mostly identifies spermatozoa without apoptotic features. To further verify that CellROX® Orange identifies spermatozoa with better quality, we evaluated CellROX® positivity in swim-up selected spermatozoa finding significantly higher levels of CellRox® Orange positivity respect to unselected samples.

Conclusion: Our results suggest that ROS detection with CellRox® Orange and DHE identifies viable spermatozoa with better performance.

P138 | Which sperm parameter limits could really guide the clinical decision in assisted reproduction?

Leonardo Dalla Valentina[1,2], Giorgia Spaggiari[1], Daria Morini[3], Beatrice Melli[3,4], Lorenzo Aguzzoli[3], Maria Teresa Villani[3], Manuela Simoni[1,2], Daniele Santi[1]

[1]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Ospedale Civile of Baggiovara, Modena, Italy; [2]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [3]Department of Obstetrics and Gynaecology, Fertility Centre, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; [4]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy

Background: The predictive role of sperm motility and morphology was recently detected in a large sample of more than 20000 assisted reproductive technology (ART) fresh cycles. However, the complete ART procedure consisted in both fresh and frozen-embryos transfers and only a comprehensive evaluation of the entire process could really confirm if these parameters really predict the ART success.

Aim of the study: To identify predictive parameters of ART success, applying a real-world data analysis (RWD) on the entire ART path, combining fresh and frozen cycles.

Materials and methods: A retrospective RWD analysis was performed, enrolling all couples attending a single ART centre from 2008 to 2021. The analysis included both fresh and frozen cycles, and both in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) procedures. Primary endpoints were strong ART outcomes, i.e. biochemical and clinical pregnancies and live birth rates (LBR).

Results: Fresh cycles success (considering LBR) was predicted by female age (OR: 1.04 [1.02-1.06]), injected oocytes (0.96 [0.93-0.99]), embryo number (0.79 [0.75-0.83]) and progressive sperm motility (0.98 [0.97-0.99]). On the contrary, frozen cycles outcomes were predicted only by sperm motility (0.97 [0.95-0.99]). This prediction was confirmed in IVF but not in ICSI cycles.

Conclusions: Both female and male's parameters predicted the ART success considering entire path. However, frozen cycles success was predicted only by progressive sperm motility, suggesting that the potential amelioration of this male parameter is relevant to improve ART success. Those couples expected to obtain the highest embryos number after fertilization (low female age and better semen parameters) will have more attempts with frozen cycles and thus would benefit of a potential treatment focused to improve sperm parameters.

P139 | Diagnostic and management challenges of Leydig cell tumor in infertile man: a case report

Radvile Dobrovolskyte[1], Jonas Ceponis[1,2], Birute Zilaitiene[1,2]

[1]Lithuanian University of Health Sciences, Medical Academy, Kaunas, Lithuania; [2]Lithuanian University of Health Sciences, Institute of Endocrinology, Kaunas, Lithuania

Introduction: Increasing referrals for testicular imaging during infertility investigation have led to an increase in findings of Leydig-cell tumors (LCTs). These tumors account for only 1–3% of testicular neoplasms. In general occur unilaterally, with only 3% of cases found bilaterally. LCTs are strongly associated with male infertility. Up to 20 percent of Leydig cell tumors in adults are classified as malignant based predominantly upon large size, vascular invasion and increased mitotic activity. The differential diagnosis for a Leydig cell tumor also includes adrenal testicular rest tumors that are found in men with congenital adrenal hyperplasia.

The diagnostic and treatment pathways can be challenging, because there are no evidence-based guidelines for the clinical management of LCT.

Case: 33-years-old male patient was referred to endocrinologist's due to infertility, lasting for approximately one year with a normal sexual life.

After clarification of past medical history testicular tumors emerged. In 2019, the patient was consulted by the urologist, ultrasound revealed lesions in the testicles on both sides. It was decided to remove the right testicle on suspicion of a malignant process. Histopathological features were characteristic to testicular tumor associated with congenital adrenal hyperplasia/testicular adrenal rest tumors.

In the Department of Endocrinology, additional investigation due to infertility was performed, elevated 17-OH-progesterone (11,4 nmol/l), elevated follicle-stimulating hormone (FSH) 23,4 U/l and slightly decreased testosterone (11,6 nmol/l) were determined. A routine semen test indicated azoospermia. Repeated ultrasound revealed 1,1x0,7 and 0,9x0,7 cm diameter hypoechogenic lesion in the left testicle (an approximate volume of the testicle was 20ml). Other laboratory tests were within normal limits (CEA 1,8 mcg/l, AFP 0,83 kU/l, βHCG 0,6 U/l, LDH 189 U/l, LH 8,84 U/l, SHBG 29 nmol/l, inhibin B 47,19 ng/l). Depending on the patient tests results, congenital adrenal hyperplasia (CAH) was suspected. Cosyntropin stimulation test, CYP21A2 gene mutation test and steroid profiling were performed. After analysis of laboratory tests, CAH was excluded. Remaining unclear diagnosis was decided to perform biopsy of the testicle. Histopathological analysis confirmed Leydig cell tumor with low proliferation index. Furthermore, paraffin samples with the right testicle postoperative material were re-examined, LCTs were confirmed bilaterally.

The patient was discussed at multidisciplinary team meeting, cause of azoospermia was confirmed and it was decided to continue active surveillance (testicular ultrasound twice-yearly, chest-abdomen-pelvis CT yearly) and refer patient to fertility and reproductive medicine center for mTESE (depending on the concentration of inhibin B, the chance of ongoing spermatogenesis remains).

Conclusion: Due to its low incidence, the management of these small tumors is still a challenge for urologists, endocrinologist, surgeons, and pathologists. The management options for these lesions include radical orchiectomy, active surveillance and testis-sparing surgery. If the initial work-up is without pathological findings and no other risk factors for malignancy are detected, active surveillance appears a safe option, once the diagnosis is ascertained using the latest imaging approaches.

P140 | Translational medicine in Andrology

Anett Szabo[1,2], Julia Acs[1], Teodora Filipov[2,3], Attila Forintos[3], Szilard Vancsa[2], Peter Hegyi[2], Peter Nyirady[1], Zsolt Kopa[1]

[1]Semmelweis University, Department of Urology, Budapest, Hungary; [2]Semmelweis University, Center for Translational Medicine, Budapest, Hungary; [3]Semmelweis University, Department of Interventional Radiology, Budapest, Hungary

The goal of translational medicine is to conduct research the results of which can be used directly at the bedside of the patient. This is how we chose the currently hot topic in andrology – sperm DNA fragmentation (SDF) and the risk factors associated with it.

Sperm DNA fragmentation is a marker of fertility, and it shows how many sperm cells have fragmented DNA. There are several factors influencing SDF – both modifiable and non-modifiable ones.

To get a clear picture of all risk factors involved, we conducted our search in the 3 main databases (PubMed, Embase and Central), which yielded almost 27.000 articles and we could extract data from over 200

of them. We were able to summarize the following and many other risk factors as part of our meta-analysis: regarding associated health conditions, the presence of varicocele compared with its absence increases SDF by more than 10%. Abnormal glucose tolerance compared with normal glucose tolerance has an even greater impact – almost 14%. From infections, HPV had close to no effect (0.16%) on SDF, the presence of Chlamydia had a moderate (4.87%), but statistically insignificant effect, whereas the presence of STIs had a moderate (5.54%), but statistically significant effect. Regarding tumors, testicular malignancies had moderate effect on SDF, slightly below 10%, Hodgkin-lymphomas had statistically significant, but clinically mild effect with an increase in SDF by less, than 4%, non-Hodgkin lymphomas had a higher – over 6% – increase, but statistically insignificant effect. Lymphomas in general, result in a moderate increase of 5.19%, whereas leukemias mean an increase of 6.07%, but a statistically insignificant one.

Regarding lifestyle factors, when comparing normal BMI with the other categories, only underweight vs. normal BMI came out to be statistically significant, but neither of the three comparisons (regarding WHO BMI categories) were clinically significant, though it was still obvious that as BMI increased, SDF also increased. The difference in SDF for alcohol consumption did not come out to be statistically significant either, but a dose-dependency could be observed when comparing heavy drinkers vs. abstainers and moderate drinkers vs. abstainers. Smoking also showed a dose dependency: the heavy smokers vs. non-smokers comparison came out to be both statistically and clinically significant though, with a difference of almost 10% of SDF.

In accordance with previously suggested short ejaculatory abstinence period, we looked at different abstinence times, but none of the results suggested that it would be beneficial to increase (or decrease) ejaculatory frequency.

Another risk factor I would highlight is age, for which we found that when considering SDF only, there's a more drastic increase after the age of 50 with a mean difference of 12.58%.

Our results contradict some of the currently popular "beliefs" regarding the risk factors' effect on SDF, like short ejaculatory abstinence time or the importance of HPV on SDF, but many other studies, especially the establishment of registries would be necessary to clarify the exact effects of all the risk factors.

P141 | The effect of fasting on semen parameters of hypofertile men

Ines Boughzala[1,2], Amira Sallem[1,2], Manel Boussabbeh[1], Rim Kooli[1], Samira Ibala[3], Meriem Mehdi[1,2]

[1]Laboratory of Cytogenetics and Reproduction Biology, Fattouma Bourguiba University Teaching Hospital, Monastir, Tunisia; [2]Laboratory of Histology, Embryology and Cytogenetic (LR 18 ES 40), Faculty of Medicine, University of Monastir, Tunisia; [3]Laboratory of Cytogenetics, Molecular Biology and Reproductive Biology, Farhat Hached University Teaching Hospital, Sousse, Tunisia

Introduction: Fasting is likely to be a therapeutic way to cure or at least to help to cure some pathologies as obesity, endocrinologic disorders and even cancers. As an example of fasting, Ramadhan, a holy month, during which moslems abstain from eating, drinking, smoking, and sexual intercourse. The period of Ramadhan fast occurs during the day for 14 hours per day, and the subsequent 10 hours period is open for consumption of food, drink, and sexual intercourse. The aim of this study was to determine the potential impact of Ramadhan fasting on sperm parameters.

Patients and methods: This retrospective, monocentric study was conducted on 50 patients, who were addressed to the Laboratory of Cytogenetics and Reproduction Biology (Monastir, Tunisia) for semen analysis during the year of 2020 and 2021. Two sperm analysis were conducted before and after Ramadhan covering the spermatogenesis period (3 months). Semen parameters were assessed and interpreted according to the WHO 2021 guidelines.

Results: The median age was of 35±5.24 years, the sexual abstinence delay was similar before the first and the second analysis (3.06±0.52 and 3.12±0.64 days, respectively).

We have shown that the mean total sperm motility decreased after the fasting period, from 32.2% to 28% (p= 0.020). A significant increase in the percentage of dead spermatozoa, which was 24.8% before fasting and were 29.5% after fasting (p= 0.024) was pointed out. The multiple abnormalities index was also higher after fasting rising from 1.81 to 1.89 (p= 0.046).

Comparing semen abnormalities, we have shown that the percentage of asthenozoospermia decreased from 74.5% to 90.2% (p= 0.006) and the percentage of hypospermia increased from 9.8% to 11.8% (p< 0.001).

Conclusion: Our study treats a non-explored aspect of fasting which obviously impacts male fertility. Contrary to the beneficial effect of fasting in many diseases, we have shown through the current study that fasting may impair semen quality, impacting vitality, motility, and morphology.

Keywords: Male infertility; semen parameters, fasting.

P142 | A strange case of spermatogenesis in a 48,XXYY male presenting with secondary infertility: a case report

Francesco Angelini, Matteo Spaziani, Paola Mazzotta, Davide Ferrari, Emilia Sbardella, Andrea M Isidori

Department of Experimental Medicine, Sapienza University of Rome, Italy

Background: Sex chromosome aneuploidies are defined by the loss or gain of one or more sex chromosomes. 48,XXYY syndrome is a very rare syndrome of males (up to 1:20,000 live births), phenotypically similar to Klinefelter Syndrome, but with a more severe clinic. Clinically, it is characterized by tall stature, testicular dysfunction associated with infertility and reduced testosterone production, cognitive, affective and social interaction deficits, global development delay and increased risk of congenital defects.

Case report: a 36-years-old man presented to our andrological outpatient clinic for secondary infertility. Patient reported a history of orchidopexy for right cryptorchidism at 6 years old and former thrombophlebitis. Upon physical examination our patient's height was 184 cm and he weighed 120 kg (Body Mass Index = 35.5 kg/m2); external genitalia were normal, except for small bilateral testes.

At scrotal ultrasound we found testicular hypotrophy with right dysmetria (right volume 6.2 mL, left volume 9 mL). Both testes were characterized by markedly and diffusely inhomogeneous echotexture due to the presence of different echogenicity areas, with increased vascularization. Semen analysis revealed oligospermia (total sperm count: 5 x 106), reduced motility (15% progressive and 5% dyskinetic), teratozoospermia (atypic 98%).

Hormonal assessment revealed a low testosterone (9.6 nmol/L), with elevated FSH (15.15 mUI/mL) and high LH (9.45 mUI/mL). Thyroid assessment and prolactin levels were within the normal range. A molecular study to detect Y chromosome microdeletion was performed using the Multiplex Oligo-Azoospermia Kit1 and the result showed no deletion in any of the Y chromosomes. Therefore, a karyotype was performed and showed a 48,XXYY male karyotype due to the presence of a duplicated X chromosome and a small acrocentric chromosome, identified as part of the short arm of the Y chromosome: 48,XXY + mar.arr [GRCh38] Yp11.31p11.2(311030_6212.305)x2.

Conclusion: Major aneuploidies were initially considered a variant of Klinefelter syndrome but nowadays it are widely accepted as distinct clinical and genetic syndromes. A review of the literature showed that all 48,XXYY patients are usually azoospermic. To the best of our knowledge, this is the first case of a 48,XXYY patient with spermatogenesis, probably due to the extra part of the short arm of the Y chromosome.

CLINICAL SCIENCE - OTHER

P107 | The effect of FSH on glucose metabolism in young healthy males

Berk Hazir[1], Angel Elenkov[1,2], Aleksander Giwercman[1,2], Yvonne Lundberg Giwercman[1]

[1]Department of Translational Medicine, Lund University, Sweden; [2]Reproductive Medicine Centre, Skane University Hospital Malmo, Sweden

Introduction: Follicle-stimulating hormone (FSH) has important effects on gonadal function, but it has been suggested that FSH also can have extra-gonadal effects with high levels impacting impairment of glucose metabolism.

Materials and Methods: Sixty-three healthy men aged 19-32 years had fasting blood samples taken at three-time points/visits (V1, V2, and V3). Gonadotropin releasing hormone (GnRH) antagonist was administered to all subjects at V1. One group (n = 16) was randomized to 300 IU recombinant FSH (rFSH) subcutaneously 3 times/week for five weeks. The rest (n = 47) served as controls not receiving rFSH. The V2 samples were obtained three weeks after V1, and at the same time point, 1000 mg testosterone undecanoate was administered intramuscularly. V3 samples were collected two weeks thereafter. Thus, men who received rFSH had increased FSH levels at V2 and V3, whereas controls had close to zero. Both groups had testosterone at castration level at V2, but slightly increased concentration of this hormone at V3. At all-time points, serum levels of lipids, glucose, and insulin were also measured. Moreover, the homeostatic model assessment of insulin resistance (HOMA-IR) and the triglyceride-glucose index (TyG) used for the prediction of type 2 diabetes mellitus and metabolic syndrome were calculated. TyG was calculated as ln (fasting triglyceride [mg/dL] × fasting glucose [mg/dL]/2). V2 and V3 levels were expressed as percentages of V1, and, using the Mann-Whitney test, the metabolic markers were compared between the two groups.

Results: At V2, the group receiving rFSH had statistically significantly higher glucose (medians: 104% vs. 98%, p = 0.003), and higher TyG levels (medians: 103% vs. 99%, p = 0.011) compared to controls. For all other comparisons, no statistically significant differences were found.

Conclusion: Increased FSH levels in the low testosterone milieu were associated with increased glucose levels and TyG. FSH may hence have a negative effect on glucose metabolism.

P108 | Cardiometabolic indices predict hypogonadism in male patients with type 2 diabetes

Nicola Caretta[1], Paolo Facondo[2], Silvia Mereu[3], Andrea Delbarba[4], Maria C. Crepaldi[5], Monica Vedovato[5], Angelo Avogaro[5], Alberto Ferlin[6]

[1]Unit of Andrology and Reproductive Medicine, University Hospital of Padova, Padova, Italy; [2]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [3]Department of Medicine, University of Padova, Padova, Italy; [4]Unit of Endocrinology and Metabolism, ASST Spedali Civili, Brescia, Italy; [5]Departmento of Medicine, Division of Metabolic Diseases, University of Padova, Padova, Italy; [6]Department of Medicine, Unit of Andrology and Reproductive Medicine, University of Padova, Padova, Ital

Objective: To evaluate, in men with uncomplicated type 2 diabetes, the association of cardiometabolic indices [Visceral Adiposity Index (VAI), Triglyceride Glucose Index (TyG), and Lipid Accumulation Product (LAP)] with testosterone (T) plasma levels, and to assess their predictive cut-off values in identifying hypogonadism.

Research Design and Methods: Two hundred and sixty-five consecutive men aged 40-70 years with type 2 diabetes performed a clinical andrological evaluation, including IIEF (International Index of Erectile Function), IPSS (International Prostatic Symptoms Score), and AMSS (Aging Male Symptoms Scale) questionnaires. Metabolic parameters, total T (TT), and luteinizing hormone were determined. The association between the different variables was performed by multivariate analysis, and ROC (Receiver Operating Characteristic) curves were used to identify cut-off values of cardiometabolic indices in predicting low testosterone (TT <12 nmol/l).

Results: VAI, TyG, and LAP were all negatively associated with TT levels. The prevalence of hypogonadism in men in the fourth quartiles of VAI, TyG, and LAP was 70-75% compared to 10-17% in men in the first quartiles ($p < 0.001$). The sensitivity and specificity of the three cardiometabolic indices in predicting TT <12 nmol/l were significantly higher concerning BMI, waist circumference, lipid profile, glycemia, HbA1c, and AMSS. Cut off values of VAI ≥3.985, TyG ≥4.925, and LAP ≥51.645 predict low T with good sensitivity (74-78%) and specificity (71-73%).

Conclusions: This is the first study evaluating the association of VAI, TyG, and LAP with hypogonadism in men with type 2 diabetes. Alterations in these indices should direct the patients to andrological evaluation.

P109 | The effect of testosterone treatment on bone mineral density in Klinefelter syndrome

Stien Willems[1], Karel David[2,3], Brigitte Decallonne[2,3], Philip Marcq[2], Leen Antonio[2,3], Dirk Vanderschueren[2,3]

[1]Faculty of Medicine, University of Leuven, Leuven, Belgium; [2]Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium; [3]Laboratory of Clinical and Experimental Medicine, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium

Background: Although Klinefelter syndrome (KS) is the most frequent sex-hormone disorder, there is ongoing uncertainty about the often-associated sex-hormone deficiency, its impact on common comorbidities, and therefore about adequate prevention and treatment. In this study, we focus on bone loss, reported to occur in over 40% of KS patients.

Objectives: This single-center retrospective cohort study in a tertiary hospital assessed the effect of treatment with or without testosterone replacement therapy (TRT) on evolution of bone mineral density (BMD) in KS patients.

Methods: A total of 52 KS patients were included and divided into 3 groups, according to TRT use. BMD was measured by dual-energy X-ray absorptiometry (DXA) and expressed as T-scores.

Results: We observed significant gain in BMD T-score at the lumbar spine (0.58 ± 0.60, $p = 0.003$; mean gain of 0.62% areal BMD per year) and total femur T-score (0.24 ± 0.39, $p = 0.041$; mean gain of 0.25% areal BMD per year) after start of TRT. Compared to untreated patients, a significant difference in evolution was seen at the lumbar level ($+0.58 \pm 0.60$ vs. -0.14 ± 0.42, $p = 0.007$).

In untreated patients without measured hypogonadism, a loss of BMD (-0.27 ± 0.37, $p = 0.029$; mean loss of 0.49% areal BMD per year) at the femoral neck was measured. This decline was equal to the predicted loss, seen in the general male population, and can thus be seen as clinically irrelevant.

Conclusion: TRT results in BMD gain, especially at the lumbar spine (0.62% per year), in patients with KS with clear hypogonadism before start of treatment. However, the effect remains limited, and patients who were not treated with TRT because of sufficient endogenous testosterone levels, did not suffer from substantial bone loss during follow-up. The need for TRT in maintaining bone health in KS may not be universal and should be evaluated on an individual basis according to the grade of sex steroid deficiency.

P110 | Introducing CAVERNOSAL BANDING: a new intraoperative strategy for significant weakening/loss of albuginea following multiple penile prosthesis surgeries

Edoardo Pescatori[1], Andrea Loreto[1], Davide Dall'Orco[2]

[1]Andrology Service, Villa Verde, Reggio Emilia, Italy; [2]TEGEA, Boston Scientific, Bologna, Ital

We present an original technique to reinforce penile tunica albuginea during prosthetic surgery, that we deem appropriate in cases of significant weakening and/or loss of albuginea, particularly following multiple penile prosthesis surgeries. We named this technique "cavernosal banding".

Case report: A 52 y.o. white male presented with a malfunctioning inflatable penile implant: upon activation no erection developed, while the scrotum enlarged. He had ad an overall of four former surgeries with a penoscrotal approach: a first inflatable implant and three replacements. MRI disclosed a bilateral extrusion of the cylinders at the likely area of former corporotomies for cylinders insertion, with aneurysm in the right side, and kinking in the left one.

Replacement surgery: we used a penoscrotal incision. In the extrusion area there was bilaterally a wide loss of albuginea, with weakened tissue in the adjacent areas.

CAVERNOSAL BANDING technique: We performed in the involved area a complete circumferential isolation of the albuginea of both corpora, i.e. isolating the corpora: dorsally from Buck's fascia, inclusive of the neurovascular bundle, and ventrally from urethra. We also managed to develop some tissue planes to cover the albuginea loss in the area of the former corporotomies. All the components of the old device were removed, Henry's stepwise antibiotic protocol was applied, and a new device (AMS 700CX 24 cm with bilaterally 5 cm rear tips) inserted. The following were measured: circumference of isolated corpora in tumescence state (12 cm), and distance between the limits of the two isolated circumferences -proximal and distal- (5 cm). A 12x5 cm rectangle of Tutopatch® (scaffold of bovine pericardium) was then fashioned. When closing the albuginea losses of the former corporotomies, care was taken to proximally incise the tissue to reach healthy albuginea, to let the device connecting tubes exit there. Cavernosal banding was achieved transferring the fashioned Tutopatch® rectangle around the isolated albuginea of the two corpora cavernosa, with its smooth surface against the albuginea. The two short sides were sutured together and to the ventral albuginea, beneath the urethra. Following, anchoring stitches were applied to fix the patch to the albuginea, to hold the patch in site, flat. Final steps: closure in layers, suction drain kept overnight, device left deflated.

P111 | miR-30b as a biomarker for male pubertal onset

Nina Mørup[1,2], Rytis Stakaitis[1,2,3], Ailsa Maria Main[1,2], Ieva Golubickaite[1,2,4], Casper Hagen[1,2], Anders Juul[1,2,5], Kristian Almstrup[1,2,6]

[1]The Department of Growth and Reproduction, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark; [2]The International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health, Rigshospitalet, Copenhagen, Denmark; [3]The Laboratory of Molecular Neurooncology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania; [4]The Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania; [5]The Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen; [6]The Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen

Background: Puberty marks the transition from childhood to adulthood, where full reproductive capacity is attained. For boys, a testicular volume of ≥ 4 mL is considered a hallmark of pubertal onset. Essential for pubertal onset is the activation and release of gonadotropin-releasing hormone (GnRH) from the hypothalamic neurons. In the rodent hypothalamus, the expression of Makorin ring finger protein 3 (MKRN3) decreases before the activation of the Hypothalamic-pituitary-gonadal (HPG) axis. In children, circulating levels of MKRN3 decrease during the peripubertal transition, although with high inter -and intra-individual variation. microRNAs have been shown to be involved in the reactivation of hypothalamic GnRH production and thus regulate pubertal timing in rodents. Specifically, miR-30b seems to directly influence MKRN3 expression – and in boys, circulating levels of miR-30b increase during induced puberty. In this study, we measured the levels of miR-30b in serum from boys during pubertal development to reveal its potential as a novel biomarker for pubertal onset.

Materials and Methods: Forty-six boys from the longitudinal part of the Copenhagen Puberty Study were included. All boys underwent multiple clinical examinations including estimation of testis size by palpation. Age at pubertal onset was defined as the age between the last examination with a testis volume < 4 ml and the first examination with a testis volume ≥ 4 ml. The boys were between the age of 7.6 and 11.8 years when the first blood samples were collected. miR-30b was measured in serum by RT-qPCR. Thirty-nine boys had miR-30b levels measured in three consecutive samples (pre-, peri-, and post-pubertal onset) and seven boys had miR-30b levels measured in ten consecutive samples (4-5 pre-, 1 peri-, and 4-5 post-pubertal onset).

Results: There was a significant increase in circulating miR-30b levels when the post-pubertal samples were compared with the pre- and peri-pubertal samples; median pre: 6.77×10-9, peri: 7.18×10-9, and post: 1.58×10-8, $p = 1.21\times10\text{-}5$ and $p = 6.0\times10\text{-}4$, respectively. There was great inter-individual variation in miR-30b levels during pubertal development (CV: 1.1) and we were unable to define a specific threshold of miR-30b levels for pubertal onset. The circulating concentrations of miR-30b and MKRN3 were not correlated (R2: 0.03).

Conclusion: Serum miR-30b increases during the pubertal transition supporting its role as a key activator of the HPG axis but is unlikely to serve as a novel clinical biomarker for pubertal onset.

P112 | Comparison of triglyceride-glucose (TyG) index and homeostatic model assessment of insulin resistance (HOMA-ir) index in prediction of hypogonadism

Berk Hazir[1], Andrea Salonia[2,3], Aleksander Giwercman[1,4], Angel Elenkov[1,4]

[1]Department of Translational Medicine, Lund University, Sweden; [2]Division of Experimental Oncology/Unit of Urology, URI, IRCCS Ospedale San Raffaele, Milan, Italy; [3]University Vita-Salute San Raffaele, Milan, Italy; [4]Reproductive Medicine Centre, Skane University Hospital Malmo, Sweden

Introduction: Insulin resistance (IR) plays an important pathologic role in metabolic syndrome (MetS), which is associated with male hypogonadism. Homeostatic model assessment of insulin resistance (HOMA-IR) is a well-established marker of decreased insulin sensitivity. Recently, a new marker, the triglyceride-glucose index (TyG), calculated as ln (fasting triglyceride [mg/dL] × fasting glucose [mg/dL]/2), was suggested to be a cheaper and a reliable surrogate marker to detect IR. Our aim was to compare the TyG index with HOMA-IR in the prediction of male hypogonadism.

Material-Method: The data on 192 subfertile patients (18-50 years; sperm concentration <20 x 106/ml) and population-based matched controls (n = 199) collected during the years 2009-2012 were evaluated retrospectively. Sperm concentration of 20 x 106/mL was used as cut off since, at the start of the study, this level was considered as lowest level of normozoospermia by World Health Organization. Half of these subjects (72 subfertile men and 122 controls) were re-investigated 5 to 10 years later. The patients receiving any hormonal therapy were excluded. Primarily, using ROC analysis, we defined cut off values of HOMA-IR and TyG for prediction of MetS at re-examination. Using these cut-off values, in a logistic regression model, we tested the association between normal/abnormal HOMA-IR or TyG and +/- hypogonadism. Hypogonadism was defined as fasting, morning serum testosterone below 12 nmol/L.

Results: In ROC curve analysis, for prediction of future incident MetS, HOMA-IR had slightly higher AUC values than TyG (AUC: 0.886, $p < 0.001$ vs. 0.816, $p < 0.001$). The optimal diagnostic cut-off values for HOMA-IR and TyG were 2.68 and 8.60, respectively. Moreover, in binary logistic regression analysis the ORs for having hypogonadism were 4.93 (95% CI: 2.77 – 8.76; $p < 0.001$) for high values of HOMA-IR and 2.19 (95% CI: 1.25 - 3.824; $p = 0.006$) for high TyG, respectively.

Conclusion: Both high HOMA-IR and high TyG are significantly associated with the risk of hypogonadism. This association seems to be stronger for HOMA-IR than for TyG. However, being not user-friendly and expensive to calculate limits HOMA-IR usage in clinical practice.

P113 | Education degree as a predictor of major adverse cardiovascular events in men suffering from erectile dysfunction

Dimitri Yannas[1], Elena Zago[1], Elena Cavallini[1], Tommaso Todisco[1], Giovanni Corona[2], Linda Vignozzi[1,3], Mario Maggi[3,4], Giulia Rastrelli[1]
[1]Andrology, Women's Endocrinology and Gender Incongruence Unit, Careggi Teaching Hospital, Department of Experimental and Clinical Biomedical Sciences - University of Florence, Florence, Italy; [2]Endocrinology Section, Maggiore Hospital, Bologna, Italy; [3]Istituto Nazionale Biostrutture e Biosistemi, Rome, Italy; [4]Endocrinology Unit – Careggi Teaching Hospita, Department of Experimental and Clinical Biomedical Sciences - University of Florence, Florence, Ital

Background: The level of education has been recognized as a CV risk factor; nevertheless, it is often neglected in CV risk stratification.
Aim: To evaluate, in men seeking medical care for ED, whether the level of education is associated with cardio-metabolic risk factors, sexual and psychological features and if it could predict the occurrence of forthcoming major adverse CV events (MACE).
Methods: A consecutive series of 3733 men (aged 49.8 ± 13.7 years) attending an andrology outpatient clinic for ED was studied. A subset of 956 patients was included in a longitudinal retrospective study with a mean follow-up 3.9 ± 2.4 years. Patients were categorized according to the education level (higher, upper secondary, lower secondary, and primary).
Outcomes: Several clinical, biochemical and instrumental (penile color Doppler ultrasound; PCDU) parameters were evaluated. In the longitudinal study, incidence of MACE was assessed.
Results: As compared with men with university degree, those with lower education had an increased frequency of moderate-severe ED (OR = 1.21 [0.99;1.48], 1.41 [1.14;1.73], 1.70 [1.26;2.30] for upper secondary, lower secondary and primary school, respectively) and reduced flaccid peak systolic velocity at PCDU. Men with a lower level of education had a worse metabolic profile, including higher waist circumference, glucose and glycosylated hemoglobin. Accordingly, patients with a lower level of education had a greater probability of suffering from metabolic syndrome (OR = 1.38 [1.06;1.79], 1.73 [1.34;2.24], 1.72 [1.24;2.37] for upper secondary, lower secondary and primary school, respectively) and were more likely to have personal history of previous CV events. Subjects with primary education had a higher estimated 10-year CV risk according to Progetto CUORE score (mean difference from university degree group = 1.94 [1.01;2.87]%). When considering patients included in the longitudinal evaluations, those with higher level of education had a significantly lower incidence of MACEs. The protective role of higher education was confirmed even after adjusting for confounders (HR = 2.14 [1.24-3.69]).
Conclusion: In subjects consulting for ED, lower level of education is associated with a more severe ED of atherogenic pathogenesis and with a worse cardio-metabolic profile. In addition, a lower level of education predicts forthcoming MACEs. Education level should be considered as a costless but valuable information in the assessment of CV risk in patients suffering from ED.

P114 | Testosterone does not affect lower urinary tract symptoms while improving markers of prostatitis in men with benign prostatic hyperplasia: a randomized clinical trial

Giulia Rastrelli[1], Sarah Cipriani[1], Francesco Lotti[1], Ilaria Cellai[1], Paolo Comeglio[1], Sandra Filippi[2], Valentina Boddi[1], Pier Andrea Della Camera[3], Raffaella Santi[4], Luca Boni[5], Gabriella Nesi[6], Sergio Serni[3], Mauro Gacci[3], Mario Maggi[1,7], Linda Vignozzi[1,7]
[1]Department of Experimental Clinical and Biomedical Sciences 'Mario Serio', University of Florence, Florence, Italy; [2]Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Department of Neuroscience, Psychology, Pharmacology and Child Health, University of Florence, Florence, Italy; [3]Urology Unit, Azienda Ospedaliera Universitaria Careggi, Florence, Italy; [4]Pathological Anatomy Unit, Careggi University Hospital, Florence, Italy; [5]Unit of Clinical Epidemiology, IRCCS Policlinico San Martino, Genoa, Italy; [6]Department of Health Sciences, University of Florence, Florence, Italy; [7]Istituto Nazionale Biostrutture e Biosistemi, Rome, Ital

Purpose: Benign Prostatic Hyperplasia (BPH) is a result of prostate inflammation, frequently occurring in metabolic syndrome (MetS). Low testosterone is common in MetS. A randomized clinical trial was designed to evaluate if 24 weeks of testosterone therapy (TTh) in BPH men with MetS and low testosterone improve urinary symptoms and prostate inflammation.
Methods: One-hundred-twenty men with MetS waitlisted for BPH surgery were enrolled. They were categorized into normal testosterone (TT ≥ 12 nmol/L and cFT ≥ 225 pmol/L; n = 48) and testosterone deficient (TD) (TT < 12 nmol/L and/or cFT < 225 pmol/L; n = 72) then randomized to testosterone gel 2% (5 g/daily) or placebo for 24 weeks. At baseline and follow-up, questionnaires for urinary symptoms and trans-rectal ultrasound were performed. Prostate tissue was collected for molecular and histopathological analyses.
Results: No differences in the improvement of urinary symptoms were found between TTh and placebo (OR [95% CI] 0.96 [0.39; 2.37]). In TD + TTh, increase in prostate but not adenoma volume was observed (2.64 mL [0.07; 5.20] and 1.82 mL [- 0.46; 0.41], respectively). Ultrasound markers of inflammation were improved. In a subset of 61 men, a hyper-expression of several pro-inflammatory genes was found in TD + placebo when compared with normal testosterone. TTh was able to counteract this effect. For 80 men, the inflammatory infiltrate was higher in TD + placebo than in normal testosterone (0.8 points [0.2; 1.4]) and TD + TTh men (0.9 points [0.2; 1.5]).
Conclusions: Twenty-four weeks of TTh in TD men with BPH and MetS improves ultrasound, molecular and histological proxies of prostate inflammation. This does not result in symptom improvement.

P115 | Biochemical predictors of structural hypothalamus-pituitary abnormalities detected by magnetic resonance imaging in men with secondary hypogonadism

Sarah Cipriani[1], Tommaso Todisco[1], Nicola Ghiandai[1], Linda Vignozzi[1,2], Giovanni Corona[3], Mario Maggi[1,2,4], Giulia Rastrelli[1]
[1]Andrology, Women's Endocrinology and Gender Incongruence Unit, Department of Experimental Clinical and Biomedical Sciences 'Mario Serio', Careggi Hospital, University of Florence, Florence, Italy; [2]I.N.B.B., Istituto Nazionale Biostrutture e Biosistemi, Rome, Italy; [3]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy; [4]Endocrinology Unit, Department of Experimental Clinical and Biomedical Sciences 'Mario Serio', Careggi Hospital, University of Florence, Florence, Ital

Purpose: Organic conditions underlying secondary hypogonadism (SH) may be ascertained by magnetic resonance imaging (MRI) of the hypothalamic-pituitary region that could not be systematically proposed to each patient. Based upon limited evidence, the Endocrine Society (ES) guidelines suggest total testosterone (T) < 5.2 nmol/L to identify patients eligible for MRI. The study aims to identify markers and their best threshold value predicting pathological MRI findings in men with SH.
Methods: A consecutive series of 609 men seeking medical care for sexual dysfunction and with SH (total T < 10.5 nmol/L and LH ≤ 9.4 U/L) was retrospectively evaluated. An independent cohort of 50 men with SH was used as validation sample. 126 men in the exploratory sample and the whole validation sample underwent MRI.
Results: In the exploratory sample, patients with pathological MRI findings (n = 46) had significantly lower total T, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prostate specific antigen (PSA) than men with normal MRI (n = 80). Receiver Operating Characteristics analysis showed that total T, LH, FSH and PSA are accurate in identifying men with pathologic MRI (accuracy: 0.62-0.68, all p < 0.05). The Youden index was used to detect the value with the best performance, corresponding to total T 6.1 nmol/L, LH 1.9 U/L, FSH 4.2 U/L and PSA 0.58 ng/mL. In the validation cohort, only total T ≤ 6.1 nmol/L and LH ≤ 1.9 U/L were confirmed as significant predictors of pathologic MRI.
Conclusion: In men with SH, total T ≤ 6.1 nmol/L or LH ≤ 1.9 U/L should arise the suspect of hypothalamus/pituitary structural abnormalities, deserving MRI evaluation.

P116 | Clomiphene citrate for male hypogonadism

Manou Huijben, Tycho Lock, Vincent de Kemp, Jack Beck, Laetitia de Kort, Jetske van Breda
[1]University Medical Center Utrecht, Utrecht, The Netherlands; [2]St Antonius Hospital, Nieuwegein, The Netherlands

Background: Hypogonadism is a worldwide problem among men causing sexual, physical and mental problems. Testosterone therapy is the first-choice treatment for male hypogonadism, with several side-effect i.e. subfertility. Clomiphene citrate (CC) is an alternative off-label therapy for hypogonadal men especially for those with an active or future child wish. There is scarce literature in usage of CC for men with hypogonadism. The aim of this retrospective study was to evaluate the effectiveness and safety of CC for hypogonadal males.
Methods: In this single-centre study, men treated with CC for hypogonadism were evaluated retrospectively. Primary outcome was hormonal evaluation including total testosterone (TT), free testosterone (FT), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Secondary outcomes were hypogonadal symptoms, metabolic and lipid parameters, hemoglobin (Hb), hematocrit (Ht), prostate specific antigen (PSA), side-effects, reversed TT response, the effect of a physician-initiated proof stop and potential predictors for biochemical and/or clinical response.
Results: In total, 153 hypogonadal men were treated with CC. Mean TT, FT, LH, and FSH increased during treatment. TT increased from 9 to 16 nmol/L, with a biochemical increase in 89% of the patients. Increased level of TT persisted after eight years of treatment. With CC treatment, 74% of the patients experienced hypogonadal symptom improvement. LH at the lower normal range before CC treatment was predictive for better TT response. During CC therapy, few side-effects were reported and no clinical important changes in PSA, Hb and Ht were found.
Conclusion: CC seems to be an effective therapy on short- and long-term, improving both clinical symptoms and biochemical markers of male hypogonadism with few side-effects and good safety aspects.
Keywords: Clomiphene citrate, male hypogonadism, testosterone deficiency syndrome

P117 | Reproductive Parameters and Bone Mineral Density in Men from infertile couples – first clinical data from ReproUnion Biobank and Infertility Cohort (RUBIC)

Angel Elenkov[1,2], Niels Jorgensen[3], Laerke Priskorn[3], Pontus Djärf[1], Aleksander Giwercman[1,2]
[1]Department of Translational Medicine, Lund University, Malmö, Sweden; [2]Reproductive Medicine Center, Skåne University Hospital, Malmö, Sweden; [3]Department of Growth and Reproduction, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark

Objective: Male infertility is associated with shorter life expectancy and higher risk of developing various non-communicable diseases. Research has indicated associations between subfertility and testosterone deficiency which, to a certain degree, might serve as a possible mediator between impaired fertility and co-morbidity. Low levels of sex hormones may have a negative effect on bone mineralization. If untreated, testosterone deficiency leads to decreasing bone mineral density (BMD). However, the studies showing the association between total testosterone (T) and BMD often focused on men in their 60s and 70s. Thus, possible preventive measures would be of relatively limited success at this time point. Bone health among younger men is scarcely studied. Previous study has shown decreased BMD to be associated with hypogonadism in a small group of young sub fertile men. We

therefore aimed to investigate the associations between reproductive parameters and BMD among men from couples referred due to couple infertility.

Methods: The presented study is a cross sectional analysis including the first 192 Swedish men recruited in RUBIC (ReproUnion Biobank and Infertility Cohort): A binational clinical foundation to study risk factors, life course, and treatment of infertility and infertility-related morbidity. In short, men from couples referred for infertility underwent routine clinical investigation and counselling, filled in an extensive questionnaire, and delivered biospecimens including blood for hormone and biochemistry analyses as well as biobanking. All participants also underwent dual x-ray absorptiometry scanning (DXA). Statistical analysis was performed utilizing linear and logistic regression.

Results: Positive correlation was detected between z-score at the lumbar spine (L1-L4) and total testosterone (R = 0.233, p = 0.001), free testosterone (R = 0.214, p = 0.003), inhibin B (R = 0.194, p = 0.009), SHBG (R = 0.191, p = 0.008) and sperm concentration (R = 0.148, p = 0.045). BMD at the hip correlated only with free T (R = 0.173, p = 0.017). The men having z-score below -1 at L1-L4 were more likely to have T levels below 8 nmol/l (OR = 11.05, 95% CI: 1.26-96.7, p = 0.03), as well as free testosterone below 0.23 nmol/l (OR = 4.35, 95% CI: 1.53-12.40, p = 0.006).

Conclusion: This study indicates that lower BMD at the level of hip and lumbar spine in men is associated with negative alterations in reproductive parameters such as total and free T, sperm count, SHBG and inhibin B. Considering the burden of osteoporotic fractures on life quality and morbidity, early detection and application of preventive measures would be of great importance. Men referred for fertility examination having low testosterone levels and decreased sperm concentration might benefit from BMD evaluation and follow-up.

P118 | Pharmacodynamics and safety of human recombinant luteinising hormone (LH) in hypogonadotropic hypogonadal men: a new ongoing multicenter studY

Chiara Furini[1,2], Giorgia Spaggiari[1,2], Francesco Costantino[1,2], Leonardo Dalla Valentina[1,2], Cristina de Angelis[6], Emanuele Ferrante[4], Francesco Garifalos[6], Rita Indirli[4,5], Francesco Lombardo[3], Giovanna Mantovani[4,5], Davide Menafra[6], Micaela Notarianni[3], Francesco Pallotti[3], Rosario Pivonello[6], Marilina Romeo[1,2], Manuela Simoni[1,2], Daniele Santi[1,2]

[1]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Ospedale Civile of Baggiovara, Modena, Italy; [2]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [3]Laboratory of Seminology and Sperm Bank 'Loredana Gandini', Department of Experimental Medicine, 'Sapienza' University of Rome, Rome, Italy; [4]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [5]Department of Clinical Sciences and Community Health, University of Milan; [6]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Università Federico II di Napoli, Naples, Ital

State of the art: In pharmacology, human chorionic gonadotropin (hCG) is considered equivalent to luteinising hormone (LH) since both act on the same receptor. Thus, when testicular function needs to be clinically restored (i.e. in case of hypogonadotropic hypogonadism [HH]), hCG is used instead of LH. However, growing evidences showed LH and hCG activate different molecular pathways and offer different outcomes in women undergoing assisted reproduction. The different action between LH and hCG is still not evaluated in men.

Aim of the study: To assess the pharmacodynamics of recombinant LH in HH men, comparing recombinant LH (Luveris®) to the gold standard approach, i.e. hCG (Gonasi HP®).

Study design: A multicenter, longitudinal, randomized, open label, phase II, 'non-inferiority' clinical trial was designed. Endpoints will be testosterone serum levels and drug safety. 32 men with acquired HH will be enrolled and randomized (1:1) to study group treated with Luveris or to control group treated with Gonasi. In both groups, increasing drug doses will be administered (75, 150, 225, 300 IU daily for LH and 500, 1000, 1500 and 2000 IU two times weekly for hCG). Both treatments will be performed for 8 weeks, during which the patient will be evaluated twice weekly, followed by a 4-week washout period. Testosterone and its metabolite will be evaluated at the end of study using the gold standard mass spectrometry methodology.

Expected results: We expect to describe for the first time the pharmacodynamics of both LH and hCG chronically administered in men, creating a dose-response curve for both compounds. Moreover the clinical hCG dosage is still empirical, thus we will recognize the best treatment regimen to restore normal testosterone levels in HH, for both LH and hCG treatment. This study is preliminary to further studies assessing LH in hypogonadal and/or infertile patients.

Current study state: The study started in March 2022 and we currently enrolled 3 HH patients.

P119 | An overview on the biochemical assessment of male andrological status using sex hormone-binding globulin and total and free testosterone in European clinical laboratories

Nick Narinx[1,2], Karel David[1,3], Joeri Walravens[4], Gido Snaterse[4], Pieter Vermeersch[2], Tom Fiers[5], Bruno Lapauw[4,6], Dirk Vanderschueren[1,3], Leen Antonio[1,3]

[1]Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, KU Leuven, Belgium; [2]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium; [3]Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium; [4]Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium; [5]Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium; [6]Department of Endocrinology, Ghent University Hospital, Ghent, Belgium

Background: Male hypogonadism (HG) manifests by one or more signs and symptoms of androgen deficiency. However, prior to starting testosterone replacement therapy (TRT) in patients affected by HG, biochemical confirmation of deficient gonadal production of testosterone (T) is necessary. Despite the recommendation in all clinical practice guidelines of assessing circulating total T levels, to date there is no agreement on differentiating low from normal circulating total T. Several studies have demonstrated the potential added value of free T in context of HG. The Endocrine Society (ES) suggests adding free T as an extra biochemical marker when total T is near the lower limit of normal or in men that have a condition altering sex hormone-binding globulin (SHBG) concentrations.

Surveys conducted in clinical laboratories in the United States of America, the United Kingdom and the Republic of Ireland uncovered that inconsistencies exist between guidelines on HG and clinical practice. These studies highlighted a high variability in the methodology on biochemical assessment of male andrological status. Different assay types for total and free T are used, lacking standardization and harmonization between laboratories, resulting in divergent reference values for total and free T. As diagnosis of HG and prescription of TRT is highly dependent on biochemical assessment, and TRT is not without risk of adverse events, harmonization of laboratory practice concerning total and free T is strongly recommended.

Objective: The purpose of our study is to map the diagnostic landscape concerning methodology on biochemical assessment of male andrological status (total T, free T and SHBG) throughout Europe.

Methods: A web-based survey will be issued to European clinical laboratories via several platforms (national and European organizations for clinical laboratories, the European andrology network Andronet, European academy of Andrology…). The survey will contain questions on patient sampling, analytical methodology, reference ranges and post-analytical phase of SHBG and total, free and bioavailable T. Distribution of the survey is scheduled for the 1st of June 2022. Responses will be analyzed in a descriptive way.

Results: As the survey has not been launched yet, results are not available at the time of abstract submission. Responses are expected to come in through the course of June and July. By October we will have collected and analyzed these data.

P120 | Sex steroid deficiency in men with chronic kidney disease is characterized by low T/LH ratio, suggesting testicular failure

Karel David[1,2], N Narinx[1,3], V Sommers[4], Henriette de Loor[5], Leen Antonio[1,2], Dirk Vanderschueren[1,2], Frank Claessens[4], Brigitte Decallonne[1,2], P Evenepoel[5,6]

[1]Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, KU Leuven, Leuven, Belgium; [2]Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium; [3]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium; [4]Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, Leuven, Belgium; [5]Nephrology and Renal Transplantation Research Group, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium; [6]Department of Nephrology, University Hospitals Leuven, Leuven, Belgium

Introduction: Sex steroid levels have been reported to be decreased in male patients with chronic kidney disease (CKD), parallel to the severity of the kidney dysfunction. These studies, at large, used immunoassays, a method that is inferior to gold standard chromatography measurements and, in addition, failed to give insights into the underlying pathophysiology as they lacked data on estradiol and gonadotropin levels.

Objective: Comprehensive mapping of the gonadal status in male CKD patients not yet on dialysis and age- and BMI-matched controls, using gold standard techniques.

Methods: We performed a case-control study in 121 male CKD patients (age 65.0 y, BMI 26.8 kg/m2), matched (1:1) with non-CKD controls for age and BMI. We divided CKD patients into 3 categories based on KDIGO classification: CKD 1-2 (n = 24), CKD 3 (n = 42) and CKD 4-5 (n = 55). We measured total testosterone (T) and estradiol (E2) using liquid chromatography tandem mass-spectrometry. Albumin, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin levels were measured on the Roche Cobas 8000 platform. Free T levels were calculated via Vermeulen formula.

Results: CKD patients showed lower total T levels (421.0 ng/dL [14.1-1350.0] vs. 541.0 ng/dL [15.5-985.0]; $p < 0.0001$) and lower free T levels (7.4 ng/dL +/-3.2 vs. 9.3 ng/dL +/-2.7; $p < 0.0001$) as compared to non-CKD counterparts. SHBG and E2 levels, conversely, were comparable in cases and controls. Prolactin levels were only increased in CKD 4-5 patients as compared to controls. Finally, LH and FSH levels were higher in CKD patients (9.1 IU/L [2.4-102.6] vs. 5.3 IU/L [0.3-25.5]; $p < 0.0001$ and 7.8 IU/L [1.4-118.0] vs. 5.6 IU/L [1.7-71.6]; $p < 0.0001$ respectively). Consequently, the T/LH ratio, a marker of testicular function, was markedly depressed in CKD (1.73 [0.03-5.57] vs. 3.98 [0.50-10.51]; $p < 0.0001$). In the CKD cohort, regression analysis identified eGFR and age as independent determinants of T/LH ratio (R2 0.2988).

Conclusion: Male patients with CKD not yet on dialysis show low total and free T levels, confirming CKD as a risk factor for male hypogonadism. The high levels of gonadotropins and low T/LH ratio point to testicular failure as the underlying pathophysiological mechanism.

P121 | Sex steroids are poorly related to erectile dysfunction (ED) in young to middle-aged People living with HIV (PLWH)

Sara De Vincentis[1,2,3], Maria Chiara Decaroli[1,2], Giulia Tartaro[1,2], Chiara Diazzi[2], Flaminia Fanelli[4], Marco Mezzullo[4], Enrica Baraldi[5], Simonetta Tagliavini[5], Uberto Pagotto[4], Giovanni Guaraldi[6], Vincenzo Rochira[1,2]

[1]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria Policlinico

di Modena, Italy; [3]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy; [4]Endocrinology Unit and Center for Applied Biomedical Research (CRBA), Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy; [5]Department of Laboratory Medicine and Anatomy Pathology, Azienda USL of Modena, Italy; [6]Multidisciplinary Metabolic Clinic, Unit of Infectious Diseases, University of Modena and Reggio Emilia, Ital

Background: ED is the most common sexual problem in male PLWH, occurring earlier and more frequently in comparison to age-matched HIV-uninfected men. Although several HIV-related factors (e.g. duration of infection, antiretroviral drugs) rather than classical risk factors (sex steroids) have been identified as causative factors for ED in HIV, the impact of each factor has not been well established so far.

Aim: To investigate the contribution of sex steroids and other etiopathogenetic components in ED development in a cohort of young to middle-aged HIV-infected men.

Methods: A prospective, cross-sectional, observational study was conducted enrolling HIV-infected men aged <50 years on receiving antiretroviral therapy (ART). The validated questionnaires International Index of Erectile Function (IIEF-15, IIEF-5) and Structured Interview of Erectile Dysfunction (SIEDY) were used to assess sexual function; particularly, ED was defined as a score ≤25 at the specific domain of IIEF-15 (3), <22 at IIEF-5 (4) and <2 at SIEDY appendix A (question 1a+2) (5). IIEF-15 version specific for homosexual subjects was not used since the translation in Italian is still to be validated. Psychological component of ED was explored using scale 3 of SIEDY survey. Serum total testosterone (TT), estradiol (E2) and dihydrotestosterone (DHT) were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), while free testosterone (cFT) was calculated by Vermeulen equation. Lifestyle habits, use of phosphodiesterase type 5 inhibitors (PDE5-i), sexual orientation and morbidity of patients were recorded.

Results: A total of 313 consecutive HIV-infected men were enrolled (median age 47.0 years [25.2-50.5]; median duration of HIV-infection 16.2 years [1.1-35.4]). At bivariate regression analysis, IIEF-15 score was inversely related to years of HIV-infection ($p = 0.002$), SIEDY scale 1 ($p = 0.014$) and scale 3 ($p = 0.42$); no significant relation was found between ED and sex steroids. At multivariate analysis only SIEDY scale 3 was included in the model. A robust linear regression was found between IIEF-15 score and IIEF-5 score ($p < 0.0001$; R2 0.546) as well as between IIEF-15 and SIEDY Appendix A ($p < 0.0001$; R2 0.123). According to IIEF-15, 187 patients (59.7%) had ED, with more severe ED degree in the last decade of age ($p = 0.003$). ED was more prevalent among homosexual than heterosexual patients ($p = 0.003$); no difference was found considering smoke, BMI, diabetes and cardiovascular diseases. Only 18.7% ($n = 35$) of patients affected by ED reported the use of i-PDE5.

Conclusions: In PLWH ED seems to be not strongly influenced by patients' gonadal status and other classical risk factors non-related to HIV. Conversely, the psychogenic sphere, assessed by SIEDY scale 3, is found to be more deeply associated to the onset of ED in PLWH, further highlighting the contribution of peculiar factors related to HIV distress (e.g., fear of virus transmission, changes in body image, and stigma). IIEF-15, IIEF-5 and SIEDY Appendix A scores correlate each other, suggesting that these tools are equally reliable in recognizing ED patients even in PLWH. Despite the high prevalence of ED, only few patients report the use of PDE5-i revealing that sexual issues tend to go unaddressed in the clinical HIV management.

P122 | Effects of Follicle-Stimulating Hormone (FSH) on Non-Reproductive Organs of Young Men

Angel Elenkov[1,2], Yvonne Lundberg Giwercman[2], Aleksander Giwercman[1,2]

[1]Reproductive Medicine Centre, Skane University Hospital Malmö, Malmö, Sweden; [2]Department of Translational Medicine, Lund University, Malmö, Sweden

Objective: Expression of follicle-stimulating hormone FSH (FSH) receptor has been reported in many extra-gonadal tissues, raising the question of non-reproductive effects of FSH. Increasing usage of FSH in treatment of male infertility warrants deeper knowledge of possible harmful off-target effects of FSH.

Methods: In total 33 healthy men were included. At the first visit (V1), all received a s.c. injection of gonadotropin-releasing hormone (GnRH) antagonist and subsequently, 16 men were randomized to recombinant FSH (300 IU 3 times/week) for 5 weeks. N = 17 served as controls. Blood samples were taken at V1, after 3 weeks (V2) and after 5 weeks (V3), when the study ended. At V2, all subject received 1000 mg testosterone undecanoate i.m. At V2 and V3 relative levels of a standard set of bio- and inflammatory markers were compared between the groups using Mann-Whitney test adjusted for multiple testing. Additionally, in order to increase statistical power, a sub analysis was performed including 31 men to the control group from a previous study with similar protocol except the administration of FSH.

Levels of following markers were measured at all three time points: P-testosterone, P-sex hormone binding globulin (SHBG), P-follicle stimulating hormone (FSH) and P-luteinizing hormone (LH). Furthermore, P-thyroid-stimulating hormone, P-C-reactive protein (CRP), P-Sodium, P-Potassium, P-Creatinine, P-Albumin, P-Calcium, P-Urea, P-Magnesium, P-Troponin T, P-creatine kinase, P-aspartate aminotransferase (ASAT), P-alanine aminotransferase (ALAT), P-alkaline phosphatase (ALP), P-Gamma-Glutamyl Transferase (GT), P-Cholesterol, P-Triglycerides, P-Apolipoprotein A1, Apolipoprotein B incl. ratio, B-Haemoglobin, B-Leukocytes, B-Thrombocytes and Blood Differential Test. These biomarkers were chosen because they mirror the function of different non-reproductive organs.

Results: As compared to controls, the FSH treated men had higher levels of SHBG ($p = 0.024$) and albumin ($p = 0.027$) at V2, and lower levels of alanine aminotransferase ($p = 0.026$) and magnesium ($p = 0.028$) at V3. However, none of the results remained statistically significant after Bonferroni correction ($p > 0.0011$). The sub analysis with the extended cohort did not change the results.

Conclusions: When FSH was given in standard therapeutic doses to young men for five weeks, it had no significant effects on selected parameters monitoring the function of non-reproductive organs. Therefore, FSH treatment can be considered safe in otherwise healthy young men, being candidates for infertility treatment with FSH.

P123 | Pathophysiology and molecular insight of testicular germ cell tumor

Vishma Pratap Sur[1], Ondrej Simoník[1], Eliska Vacurova[2], Zuzana Kratka[3], Radomir Kren[4], Pavel Skrobanek[5], Jiri Neuzil[2,6], Tomas Hradec[7], Libor Zamecnik[7], Tomas Buchler[5], Katerina Komrskova[1,8]

[1]Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [2]Laboratory of Molecular Therapy, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [3]Laboratory of Immunology, IVF Clinic GENNET, Prague Czech Republic; [4]Laboratory of embryology, IVF Clinic GENNET, Prague, Czech Republic; [5]Department of Oncology, First Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic; [6]School of Pharmacy and Medical Science, Griffith University, Southport, QLD, Australia; [7]Department of Urology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic; [8]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Aims: This study aims to address sperm reproductive parameters and testis germ cell pathologies related to testicular germ cell tumour (TGCT) from both experimental and clinical angles. The goal is to deliver novel diagnostic strategies for the evaluation of sperm samples collected from TGCT men prior to surgery and after the therapeutic intervention, to monitor and compare the quality of fertility parameters and recommend the first choice of cryopreserved semen sample for assisted reproduction.

Methods: A combination of Ki67, a marker of cell proliferation was used for TGCT proliferation assessment accompanied by hematoxylin-eosin stain based histopathological assessment. Assessment of tumour progression and sperm quality parameters such as acrosome integrity (PNA lectin), DNA integrity (TUNEL), motility (CASA) and apoptosis (ApoFlowEx) were addressed. Assessment of testicular and sperm mitochondrial activity was evaluated by oroboros O2k based on oxygen consumption by complex I and complex II and complex IV and OXPHOS by seahorse. The 2-photon FLIM was performed to assess NADH lifetime in mitochondria of sperm samples and TGCT tissue. Gene regulation was assessed by qPCR to understand the genomic expression patterns and roles. Ultrastructure of sperm was analysed by Cryo-SEM and Cryo-TEM to assess the sperm morphology in TGCT patients for the first time. Epigenetic profiling and analysis of sperm and testicular germ cell epigenome was addressed through monitoring of H3K9ac, H3K36me3 and H3K27me3 patterns.

Results: Sperm analyses of acrosome integrity and sperm motility provide a detailed analysis of impaired sperm function which correlates with elevated DNA fragmentation. Sperm parameters correlate with the severity of the TGCT tumour assessed by Ki67 marker. Sperm morphology and motility is highly affected. Mitochondrial respiration and ATP generation in TGCT sperm are lower than for normospermic sperm. Oxygen consumption by mitochondrial complex I, complex II, and complex IV was estimated and revealed that CI CII and CIV oxygen consumption is higher in tumour compared to normal tissue. High heterogeneity in the TGCT tissue for NAD(P)H is discovered, and patients' specific unique lifetimes ranging between 0-5 ns, indicate differences in respiration metabolism. Various important genes related with spermatogenesis and germ cell development are down-regulated. The epigenetic profile of sperm is severely altered and a multi-parametric correlation of TGCT sperm pathologies is needed.

Conclusions: A ki67 proliferation marker is a promising tool for an assessment of the severity of TGCT. Histone modification assessment in testicular tissue and sperm shows differences between tumour and non-tumour tissue. Total and progressive sperm motility by CASA points to the best sperm quality in the sample prior to 1st and 2nd chemotherapy. Assessment of acrosome damage is crucial for the prediction of optimal IVF method and correlates with DNA fragmentation. The altered mitochondrial activity in TGCT patients' sperm and testicular tissue compared to sperm of healthy donors and non-tumour tissue points to the potential use of mitochondrial markers as a diagnostic tool for reproductive parameters of TGCT affected men.

P124 | Impact of the cancer treatment received and the disease itself on the quality of the human (pre)pubertal testicular tissue prior to testicular tissue freezing

Aurelie Feraille[1], Agnes Liard[2], Michael Bubenheim[3], Anne Laure Barbotin[4], Sandrine Giscard d'Estaing[5], Sophie Mirallie[6], Amelie Ancelle[7], Christophe Roux[8], Florence Brugnon[9], Victoria Greze[10], Myriam Daudin[11], Genevieve Willson-Plat[12], Remi Dubois[13], Louis Sibert[14], Pascale Schneider[15], Nathalie Rives[1]

[1]Normandie Univ, UNIROUEN, EA 4308 "Gametogenesis and Gamete Quality", Rouen University Hospital, Biology of Reproduction-CECOS Laboratory, Rouen, France; [2]Normandie Univ, UNIROUEN, Rouen University Hospital, Department of Child Surgery, Rouen, France; [3]Normandie Univ, UNIROUEN, Rouen University Hospital, DRCI, Rouen, France; [4]Institut de Biologie de la Reproduction – Spermiologie – CECOS de Lille, CHU de Lille, EA 4308 "Gametogenesis and Gamete Quality", Lille, France; [5]Laboratoire de Biologie de la Reproduction - CECOS de Lyon, CHU de Lyon, Lyon, France; [6]Laboratoire de Biologie de la Reproduction - CECOS de Nantes, CHU de Nantes, Nantes, France; [7]Laboratoire de Biologie de la Reproduction - CECOS de Caen, CHU de Caen, Caen, France; [8]Laboratoire de Biologie de la Reproduction - CECOS de Besancon, CHU de Besancon, Besancon, France; [9]Laboratoire de Biologie de la Reproduction - CECOS d'Auvergne, CHU de Clermont-Ferrand, Clermont-Ferrand, France; [10]Service d'Hematologie Oncologie Pediatrique, CHU de Clermont-Ferrand, Clermont-Ferrand, France; [11]CECOS Midi-Pyrenees, University Hospital of Toulouse, Hopital Paule de Viguier, Toulouse, France; [12]Service d'Hematologie Oncologie Pediatrique, University Hospital of Toulouse, Hopital des Enfants, Toulouse, France; [13]Departement de Chirurgie Pediatrique

viscerale, CHU de Lyon, Lyon, France; [14]Normandie Univ, UNIROUEN, Rouen University Hospital, Department of Urology and Andrology, Rouen, France; [15]Normandie Univ, UNIROUEN, Rouen University Hospital, Department of Pediatric Hematology and Oncology, Rouen, France

Background: Testicular tissue freezing (TTF) is proposed for fertility preservation to (pre)pubertal boys with cancer before highly gonadotoxic treatment. Studies accurately comparing human (pre)pubertal testicular tissue quality before freezing and after thawing are exceptional. No study has reported this approach in a systematic manner and routine care.

Objectives: To assess the impact of a control slow freezing (CSF) protocol on testicular tissue architecture and integrity of (pre)pubertal boys after thawing.

Materials and Methods: (Pre)pubertal boys (n = 87) with cancer from 8 Reproductive Biology Laboratories of the French CECOS network benefited from TTF before hematopoietic stem cell transplantation. Seminiferous tubule cryodamage was determined histologically by scoring morphological alterations and by quantifying intratubular spermatogonia and the expression of DNA replication and repair marker in frozen-thawed testicular fragments.

Results: A significant increase in nuclear and epithelial score alterations was observed after thawing ($p < 0.0001$). The global lesional score remained lower than 1.5 and comparable to fresh testicular tissue. The number of intratubular spermatogonia and the expression of DNA replication and repair marker in spermatogonia and Sertoli cells did not vary significantly after thawing. These data showed the good preservation of the seminiferous tubule integrity and architecture after thawing, as previously reported in our studies performed in prepubertal mice and rats.

Discussion: The current study reports, for the first time, the development of a semi-quantitative analysis of cryodamage in human (pre)pubertal testicular tissue, using a rapid and useful tool that can be proposed in routine care to develop an internal and external quality control for TTF. This tool can also be used when changing one or several parameters of the freezing-thawing procedure.

Conclusion: CSF protocol without seeding maintains the seminiferous tubule architecture and integrity, the concentration of spermatogonia and the expression of DNA replication and repair marker in spermatogonia and Sertoli cells after thawing.

P125 | Osteoporosis in men with hypogonadism due to Apo A-I Leu75Pro amyloidosis under long-term testosterone replacement therapY

Paolo Facondo[1], Andrea Delbarba[2], Letizia Chiara Pezzaioli[1], Alberto Ferlin[3], Carlo Cappelli[1,2]

[1]University of Brescia, Department of Clinical and Experimental Sciences, Brescia, Italy; [2]Unit of Endocrinology and Metabolism, ASST Spedali Civili, Brescia, Italy; [3]Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padua, Ital

Introduction: Apo A-I Leu75Pro amyloidosis is a rare form of systemic hereditary amyloidosis that finds a peculiar prevalence in the province of Brescia (northern Italy). The hallmark and earliest involvement of this disease is the testicular impairment, characterized by hypogonadism and macrorchidism; renal and hepatic late onset involvement is the other characteristics.

Objective: To evaluate for the first time the prevalence of osteopenia, osteoporosis and vertebral fractures in a large cohort of men with hypogonadism due to Apo A-I Leu75Pro amyloidosis under long term testosterone replacement therapy (TRT).

Methods: Cross-sectional retrospective study of 50 men >50 years (median age 64.5 years) with hypogonadism under TRT due to this amyloidosis form, who performed andrological examination, gonadal and bone biochemical assay, and a lumbar and femoral Dual-Energy X-ray Absorptiometry (DXA) exam.

Results: At a median of 7.5 years from the testicular impairment and start of TRT, lumbar or femoral osteopenia and osteoporosis were found in 54.0% and 10.0% of patients, respectively. Of the 34 men who performed a morphometric assay, 14.7% (n = 5) had a vertebral fracture. With respect to patients with normal Bone Mineral Density (BMD), men with osteopenia or osteoporosis were significantly older, had a lower Body Mass Index (BMI), higher sex hormone binding globuline (SHBG) before the start of TRT, and had a more frequent renal involvement. Osteopenia-osteoporosis was significantly more frequent in men with renal (22/29, 75.9%), hepatic (16/20, 80.0%) or renal and hepatic involvement (15/19, 78.9%) and in patients with macrorchidism (16/23, 69.6%, $p = 0.008$). In addition, increasing the organ disease involvement, without different TRT dosage, serum levels of total testosterone (TT), calculated free testosterone (cFT) and hematocrit were significantly lower, while luteinizing hormone (LH) was higher.

The presence of osteopenia-osteoporosis was correlated with age ($r = 0.29$, $p = 0.042$), BMI ($r = -0.34$, $p = 0.013$), renal involvement ($r = 0.29$, $p = 0.041$) and SHBG levels before TRT start (0.46, $p = 0.005$). Among 5 men with lumbar fracture, 3 had osteopenia, 1 osteoporosis, and 1 normal BMD. All patients with lumbar fracture showed (in addition to testicular impairment) renal and hepatic involvement, and 3 had macrorchidism.

Conclusion: Among men with hypogonadism due to Apo A-I Leu75Pro amyloidosis, at a median follow up of 7.5 years of TRT we found a prevalence of osteopenia, osteoporosis and vertebral fractures of 54%, 10% and ~ 15%, respectively. Osteopenia-osteoporosis was mainly found in older patients with systemic disease involvement. In the latter, it has been identified biochemical signs of inadequate TRT compensation, which may further impaired bone health.

P126 | The strength of the pelvic floor muscles improves female sexual function and reduces sexual distress

Athanasios Zachariou[1], Vagia Sapouna[2], Aris Kaltsas[1], Ioannis Giannakis[1], Erriketi Douvli[1], Athanasios Paliouras[1], Minas Paschopoulos[3], Andreas Karagiannis[1], Fotios Dimitriadis[4], Panagiota

Tsounapi[5], Athanasios Papatsoris[6], Atsushi Takenaka[5], Nikolaos Sofikitis[1]

[1]Department of Urology, University of Ioannina, Ioannina, Greece; [2]Physical Medicine and Rehabilitation Centre EU PRATTEIN, Volos, Greece; [3]Department of Obstetrics and Gynaecology, University Hospital of Ioannina, Ioannina, Greece; [4]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [5]Department of Urology, Tottori University, Yonago, Japan; [6]Department of Urology, Sismanoglio General Hospital, University of Athens, School of Medicine, Athens, Greece

Objective: Substantial proof demonstrates that pelvic floor muscle training (PFMT) improves pelvic floor muscles' strength (PFMS) and enhances stress urinary incontinence and female sexual function. Most of these studies included incontinent women; therefore, it is uncertain if the improvement of urinary incontinence burden or the more robust pelvic floor contributed to a better sexual function.

The study examines pelvic floor muscle strength and sexual function or sexual distress in healthy nulliparous women. It is one of the few evaluating sexual distress and pelvic floor muscle function.

Materials and Methods: Sixty sexually active women aged 24-39 years participated in this cross-sectional study. All women completed the FSFI questionnaire, validated in Greek, and the FSDS-R questionnaire. Two experienced urologist examiners assessed pelvic floor muscle strength using the Modified Oxford Grading Scale and the Peritron manometer. Women comprised two groups using the median values of Peritron manometer measures of the total population. Group A included 31 females with weak pelvic floor contraction (< 43,8 cm H20), and Group B included 29 females with a strong pelvic floor (>43,9 cm H20). The FSFI and FSDS-R results were interpreted with Mann-Whitney U tests and the Modified Oxford Grading Scale and Peritron manometer values with Spearman's correlation.

Results: There was no significant difference between the two groups concerning the participants' demographic characteristics. Women with strong pelvic floor (Group B) displayed higher statistically significant results ($p < 0.05$) in desire (Group A:3.27+0.14 vs. Group B:3.99+0.19), arousal (4.17+0.51 vs. 4.92+0.38), orgasm (4.20+0.33 vs. 5.29+0.64), satisfaction (4.34+0.35 vs. 5.51+0.67), lubrication (4.19+0.42 vs. 5.23+0.43), total FSFI score (26.91+1.01 vs. 30.94+1.17) and FSDS-R score (9.13+5.12 vs. 7.75+4.91). There was no statistically significant difference in the pain domain. There was also a strong correlation between PFMS evaluated with Modified Oxford Grading Scale, and PFMS assessed with Peritron manometer values ($r = 0.69$).

Conclusions: Female sexual function is a multidimensional phenomenon associated with psychological, biological, physiological, socio-cultural, and interpersonal factors. Women with a robust pelvic floor scored significantly higher in the FSFI questionnaire and exhibited lower sexual distress. PFMS is an essential factor concerning better female sexual function and lower sexual distress.

P127 | Patients who need psychological support during andrological investigations

Rim Kooli[1], Amira Sallem[1,2], Dhekra Chebil[3], Manel Bousabbeh[1], Ines Boughzala[1,2], Samira Ibala[4], Meriem Mehdi[1,2]

[1]Laboratory of Cytogenetics and Reproductive Biology, Fattouma Bourguiba University Teaching Hospital, Monastir, Tunisia; [2]Laboratory of Histology, Embryology and Cytogenetic (LR 18 ES 40), Faculty of Medicine, University of Monastir, Tunisia; [3]Faculty of Medicine of Sousse, University of Sousse, Tunisia; [4]Laboratory of Cytogenetics, Molecular Biology and Reproductive Biology, Farhat Hached University Teaching Hospital, Sousse, Tunisia

Introduction: Performing andrological investigations and especially semen analysis could be perceived as the cause of lower self-esteem feelings in patients dealing with infertility issues. Literature data on the topic are scarce in the Tunisian population.

Objective: This study aimed to assess levels of anxiety and depression among men undergoing andrological investigations and to identify their associated factors.

Methods: We conducted a cross-sectional study in the Laboratory of Cytogenetics and Reproductive Biology of Fattouma Bourguiba University Teaching Hospital (Monastir, Tunisia) between August 30th, 2020, and March 16th, 2021. Were included in the current study patients addressed to the laboratory for semen quality assessment as well as infertility management. All patients who had a previously diagnosed psychological disorder, those who presented severe depression and/or anxiety symptoms or had a stressful life event were excluded. Anxiety and depression levels were assessed using the valid Arab version of the HAD (Hospital Anxiety and Depression) scale. Semen parameters were analyzed and interpreted according to 2021 WHO (World Health Organization) guidelines.

Results: A total of 282 patients were included in the current study. The mean HAD-D (depression) and HAD-A (anxiety) scores were of 6.56 ± 3.07 (IIQ [4-8]) and 7.94 ± 3.73 (IIQ[5-10]) respectively. Univariate analysis showed that patients having two or more comorbidities were nearly five times more likely to be anxious than those without or with only one comorbidity (ORc = 4.71; p = 0.007). Furthermore, single patients were about four times more anxious than those in couple having primary or secondary infertility (ORc = 3.85; p = 0.027). With regards to semen parameters, patients having hypospermia were more than two times anxious compared with those with normal semen volume (ORc = 2.33; p = 0.034). As for depression, we observed that patients with an infertility history lasting for a year or more have a nine times greater risk of depression (ORc = 9.848; p = 0.007). With regards to semen parameters, patients exhibiting two or more semen abnormalities were two times and half more depressed (ORc = 2.478; p = 0.036).

The multivariate analysis has shown that anxiety level was associated with comorbidities (ORa = 3.74; p = 0.046) and that depression symptoms were associated to single marital status (ORa = 7.20, p = 0.035), professional exposure (ORa = 2.35; p = 0.044) as well as urogenital history (ORa = 4.9; p = 0.023). A positive correlation between

HAD-A and HAD-D scores was shown (R = 0.44; p < 0.001). HAD-A was also correlated to the patient age (R = -0.14, p = 0.015).

Conclusion: We pointed out through the current study the associated factors with anxiety and depression in patients performing andrological investigations to precociously identify those who need psychological counseling and hence to better manage infertility issues.

P128 | Total Osteocalcin Levels are Independently Associated with Worse Testicular Function and a Higher Degree of HPG Axis Activation in Klinefelter Syndrome

Francesco Carlomagno, Matteo Spaziani, Alessia Aureli, Chiara Tarantino, Marta Tenuta, Franz Sesti, Daniele Gianfrilli, Andrea Lenzi, Andrea M. Isidori

Department of Experimental Medicine, Sapienza University of Rome, Italy

Context: Osteocalcin (OCN) is an osteoblast-produced polypeptide, emerging as the core element of the bone-testicular axis toghether with its undercarboxylated form (uOCN), proposed to increase testosterone (Te) levels in healthy men, by binding the Gpcr6a receptor on Leydig cells and modulating the GnRH pulse frequency and amplitude. However little is known with regards to its role in pubertal development and male hypogonadism.

Objective and Design: We investigated OCN concentrations in 47,XXY men affected by Klinefelter syndrome (KS), a model of adult hypergonadotropic hypogonadism, in a retrospective longitudinal study between 2007 and 2021 at an academic referral center.

Patients and Methods: 254 KS subjects, divided into the following groups: 1) pre-pubertal (n = 48, from 1 year of age until Tanner stage II), 2) pubertal (n = 46, Tanner stages II through V, <18 years), and 3) adults (n = 160, Tanner stage V, ≥18 years). All (pre-)pubertal patients were Te-naïve. Adult patients were categorized as: 1) eugonadal (total Te > 10.4 nmol/L; n = 47), 2) hypogonadal (Te < 10.4 nmol/L; n = 39), and 3) receiving testosterone replacement therapy (TRT) (n = 74). Data are presented as means ± SD, were tested with Brown-Forsythe and Welch ANOVA tests for unequal variances, corrected for multiple comparisons (Dunnett T3), and with partial correlations, after bootstrapping on 2000 samples.

Main outcomes: Total serum OCN (tOCN), hypothalamic-pituitary-gonadal (HPG) axis hormones (LH, FSH, total Te, 11β-estradiol, SHBG), and derived indexes.

Results: tOCN levels varied throughout the life span, with a mean of 85.9 ± 30.4 ng/mL in pre-pubertal infants, peaking at 130.0 ± 77.2 ng/mL in pubertal children (p = 0.243 vs. pre-pubertal) and then declining to 22.9 ± 9.0 ng/mL in adults (p < 0.001 vs. pre-pubertal and pubertal). In (pre-)pubertal boys no correlation with HPG axis hormones was found. When comparing adult KS, tOCN values were highest in eugonadal (26.5 ± 10.4 ng/mL), slightly lower in hypogonadal (24.5 ± 8.1 ng/mL, p = 0.268 vs eugonadal) and significantly lower in TRT subjects (20.4 ± 8.0 ng/mL, p = 0.008 vs. eugonadal and = 0.013 vs. hypogonadal). In adults, tOCN correlated with both LH (r = 0.23, p = 0.017) and FSH levels (r = 0.28, p = 0.004). These significancies were maintained after the exclusion of subjects on TRT. Surprisingly, adjusting for age and BMI confirmed previous findings and revealed significant inverse correlations with total Te (r = -0.44, p = 0.004), calculated free Te (r = -0.37, p = 0.016), the Te/LH (r = -0.40, p = 0.010) and the calculated free Te/LH ratios (r = -0.33, p = 0.031).

Conclusions: In an experimental model of hypergonadotropic hypogonadism, tOCN showed no association with gonadal function during normal pre-puberty and pubertal development. In adults, tOCN levels were unexpectedly associated with worse testicular function and a higher degree of HPG stimulation.

P129 | The dose-effect of supernumerary X chromosomes on clinical, metabolic and cardiac outcomes of male subjects

Chiara Tarantino, Matteo Spaziani, Francesco Carlomagno, Beatrice Mastrostefano, Ludovica Vincenzi, Christopher Nardi, Emilia Sbardella, Daniele Gianfrilli, Andrea M. Isidori

Department of Experimental Medicine, Sapienza University of Rome, Italy

Introduction: High grade aneuploidies of sexual chromosomes with a male phenotype (HGAs) are exceedingly rare conditions. For long HGAs were considered as complex variants of Klinefelter syndrome (KS), until recent evidence prompted to reconsider the two as distinct conditions. We first illustrated the endocrine and metabolic differences between the two clinical conditions demonstrating the more premature gonadal failure of HGAs compared to KS and their worse metabolic profile. Previous works highlight how HGAs patients exhibit a poorer outcome as the number of X increase, but comprehensive studies are lacking, and little is known of the mechanism involved.

Objective of the study: to investigate the impact of the number of supernumerary X chromosomes on clinical, hormonal, metabolic and echocardiographic features of male subjects with HGAs.

Subjects and Methods: We compared 23 HGAs and 46 KS subjects according to the number of supernumerary X chromosomes: 56 subjects with two supernumerary X (47,XXY and 48,XXYY), 5 with three supernumerary X (48,XXXY and 49,XXXYY) and 8 with four supernumerary X (49, XXXXY). Clinical, hormonal, metabolic and echocardiographic parameters were analysed.

Results: The increase in the number of extra-Xs was associated with a progressive decrease of ultrasonographic bitesticular volume (p < 0.001); total testosterone (p = 0.01); DHEAS (p < 0.001); Δ4-androstenedione (p = 0.001); fT4 (p = 0.01). Conversely, a progressive linear increase in ACTH levels was found (p = 0.02). OGTT 2-hour insulin levels and HOMA-index were also higher in patients with ≥ 2 supernumerary X chromosomes, without a progressive trend (respectively p = 0.047 and p = 0.01). Right and left ventricular end-diastolic diameters were decreased (p = 0.016 and p = 0.001, respectively). Cardiac ejection fraction was also found associated with the extra-X, but without an apparent "dose-dependent" trend (p < 0.001): we found out that the addition of more than one extra X chromosome does not result in further worsening of cardiac output.

Conclusions: The increase in supernumerary X chromosomes is associated with a "dose-dependent" progressive impairment in steroidogenic function, insulin resistance and worse cardiac performance, namely the higher the number of X the higher the impairment of clinical, metabolic and cardiac outcomes.

P130 | A difficult case of Burned-out testicular tumors: can orchiectomy be avoided?

Valentina Di Vito, Marta Tenuta, Ilaria Bonaventura, Paola Mazzotta, Marianna Minnetti, Alessandra Tomaselli, Alain Gelibter, Andrea Isidori, Carlotta Pozza
Policlinico Umberto I, Roma, Italy

Background: A "burned-out" tumor (BOT) is a rare clinical entity which describes the presence of distant metastases with the complete regression of the primary testicular lesion. We report the complex management of a case of BOT in a young male.

Case report: A 22-year-old male presented to ER with acute low back pain, severe vomiting and sweating. In past medical history: bilateral orchidopexy for cryptorchidism. Abdominal CT scan displayed two retroperitoneal masses of 35 and 20 mm. The patient was discharged with suspicion of paraganglioma and referred to our Department only 6 months after. In the suspicion of testicular tumor metastasis, a scrotal US was performed, showing multiple calcifications within the left testis. Serum tumor markers (STM) were increased: β-hCG 663 mIU/ml (<1.5), αFP 8 ng/ml (<5). A new CT scan showed a dimensional doubling of retroperitoneal masses. BOT was therefore suspected, and the case was handled in a multidisciplinary unit (TestisUnit). Retroperitoneal biopsy confirmed the diagnosis of metastatic embryonal testicular carcinoma. The patient underwent first-line chemotherapy (BEP protocol, three cycles). Post-treatment 18F-FDG-PET confirmed hypermetabolic activity in the left para-aortic site lesion and showed an unexpected additional uptake in the right, healthy testis (SUV max 3.7). STM were negative and a new US showed no focal lesions on both testicles. Retroperitoneal lymph node dissection was performed and a single left para-aortic lymph node (3.5 x 2.9 cm), site of focal residual neoplastic repetitions, was found. Post-surgery 18F-FDG PET confirmed a diffuse right testicular uptake (SUV max 3.4). Again, no intratesticular mass was visible. Because of the SUV reduction, the absence of suspicious lesions within the right testicle and negative STM, the board scheduled a strict testicular US follow-up together with repetition of STM and a PET re-evaluation after 6 months. US and STM remained stable, but 18F-FDG PET showed an increase in the intensity of uptake of the tracer at the right testicle (SUV max 5.6).

Considering the non-concordance of PET and US, a false positive testicular uptake, due to testicular cellular hyperactivity, was hypothesized. Therefore, the multidisciplinary board decided to temporarily suppress pituitary-gonadal axis with injectable testosterone undecanoate, subject to the patient's informed consent. After three injections (performed at 0, 6 and 8 weeks), the 18F-FDG PET showed no areas of pathological uptake in both testes. Follow-up was continued with regular testicular US along with STM (every 3 months) and six-monthly CT, which remained negative. Hormonal assessment showed biochemical eugonadism with a progressive increase of gonadotropins within the normal range after discontinuation of Testosterone.

Conclusion: Scrotal US is an essential part of the work-up of male retroperitoneal mass. In this difficult case functional imaging would probably have led to a not necessarily bilateral orchiectomy. The suppression of pituitary-gonadal axis avoided this option in our patients, and opens the debate on whether orchiectomy is actually necessary in these conditions.

P131 | The bidirectional relationship between testosterone and metabolic disorders: testosterone deficiency as an early marker of cardiovascular risk in young men

Davide Menafra[1], Cristina de Angelis[1], Francesco Garifalos[1], Michele Castoro[1], Nunzia Verde[1], Mariangela Piscopo[1], Giacomo Galdiero[1], Claudia Pivonello[1], Renata Simona Auriemma[1], Paolo Chiodini[2], Annamaria Colao[1,3], Rosario Pivonello[1,3]
[1]*Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Federico II University, Naples, Italy;* [2]*Dipartimento di Medicina di Preciosione, Unità di Statisticat, University of Campania "Luigi Vanvitelli", Naples, Italy;* [3]*Unesco Chair for Health Education and Sustainable, Development, Federico II University, Naples, Ital*

In the last years, an increasing incidence of cardiovascular diseases (CVD) has been reported in young adults (18-45 years), probably accounted by the significant increase in CV risk (CVR) factors. Observational and interventional studies, mainly focused on middle-aged and elderly men, demonstrated that metabolic CVR (mCVR) factors and CVD manifestations are common in hypogonadal men and, conversely, testosterone deficiency is highly prevalent in metabolic disorders; the lack of similar robust studies in younger adults requires focused investigation. The current single centre, observational, cross-sectional study aimed at better defining the mutual relationship between androgenic status and the prevalence of mCVR factors in a large cohort of 720 young adult (18-35 yrs) men, subjected to physical examination and fasting morning venous blood sampling for the assessment of anthropometric, metabolic and hormonal parameters. Body weight, BMI and waist circumference (WC) significantly decreased across total testosterone (TT) ($p < 0.0001$), SHBG ($p < 0.01$; $p < 0.01$; $p < 0.0001$) and calculated free testosterone (cFT) ($p < 0.05$) tertiles, whereas systolic blood pressure (SBP) and triglycerides (TG) levels significantly decreased across TT ($p < 0.05$; $p < 0.01$) and SHBG ($p < 0.05$; $p < 0.0001$) tertiles, and diastolic blood pressure (DBP) across SHBG ($p < 0.05$) tertiles. Spearman correlation analysis revealed a negative association of TT, SHBG and cFT with BMI ($r = -0.204$; $p < 0.001$)($r = -0.165$; $p < 0.05$)($r = -0.132$; $p < 0.05$), and of TT and SHBG with WC ($r = -0.234$; $p < 0.001$)($r = -0.225$; $p < 0.01$), SBP ($r = -0.112$; $p < 0.05$)($r = -0.142$; $p < 0.05$) and TG ($r = -0.017$; $p < 0.01$)($r = -0.204$; $p < 0.001$), whereas a positive association of TT and SHBG with

HDL-cholesterol (r = 0.167; p < 0.01)(r = 0.251; p < 0.001) was demonstrated. In multiple linear regression analysis in models adjusted for age, BMI and WC, TT and SHBG were strong independent predictors of serum HDL-cholesterol levels (β = 0.151; p < 0.01)(β = 0.186; p < 0.01), and SHBG was an independent predictor of SBP and DBP (β = -0.177; p < 0.05)(p = 0,008; β = -0,204). Lastly, in the subgroup of men with hypotestosteronemia (TT ≤ 12.1 nM), the prevalence of normal weight was significantly lower (p < 0.05) and that of obesity (p = 0.0003), visceral obesity (WC >102 cm)(p = 0.059), hypertension (p = 0.173) and metabolic syndrome (p = 0.457) was significantly higher, compared to normal-testosterone subgroup. Consistently, in the subgroup of overweight/obese men, serum TT levels were significantly lower (p < 0.01) and the prevalence of hypotestosteronemia was significantly higher (p < 0.01), compared to normal-weight subgroup. The current study demonstrated that in young adult men a bidirectional relationship between testosterone deficiency and metabolic disorders exists, and that a worse androgenic status is associated to a worse cardiometabolic profile, and might represent a strong early predictor of mCVR factors, potentially associated to the onset of future CVD.

P132 | Cardiovascular risk and metabolic profile of transgender people: a retrospective, observational studY

Nunzia Verde[1], Rosario Ferrigno[1], Cristina de Angelis[1], Stefano Zarrilli[1], Chiara Abagnale[1], Francesca D'Ambrosio[1], Andrea Tessito[1], Francesco Garifalos[1], Davide Menafra[1], Michele Castoro[1], Alessandra Veneri Delli[1], Roberto Vitelli[2], Renata Simona Auriemma[1], Annamaria Colao[1,3], Rosario Pivonello[1,3]

[1]Surgical, Clinical Medicine Department, Section of Endocrinology, Andrology, Reproductive, Sexual Medicine Unit (FERTISEXCARES), University of Naples Federico II, Naples, Italy; [2]Neuroscience, Reproductive, Odontostomatologic Science Department, Section of Clinical Psychology, University of Naples Federico II, Naples, Italy; [3]Unesco Chair for Health Education, Sustainable Development, Federico II University, Naples, Ital

Cardiovascular (CV) and metabolic wellbeing of transgender (T*) people assuming hormonal transitional therapy or undergone gender-affirming surgery has been recently a matter of debate.The aim of the current study was to retrospectively evaluate the CV risk and the metabolic profile of a cohort of T* people, at their last available follow-up visit. 155 T*people(54 T*women, 101 T*men, mean age:29.3 ± 9.3 yrs) were enrolled, having assumed hormonal transitional therapy for at least six months, in particular 13.5% had also undergone major gender-affirming surgery (i.e. gonadectomy), whereas 86.5% exclusively performed hormonal transitional therapy or hormonal transitional therapy and secondary gender-affirming surgery(breast augmentation and aestheticsurgery for T*women, mastectomy for T*men). Considering T*women, 81.5% assumed estradiol gel(mean dose:1.1 ± 0.4mg/day), whereas the remaining 18.5% assumed oral estradiol (mean dose:2.2 ± 0.6mg/day); moreover, 77.8% assumed cyproterone acetate(mean dose:54.2 ± 37.4 mg/day). Considering T*men, 58.4% assumed transdermal testosterone(mean dose:24.4 ± 7.9mg/day), whereas 41.6% assumed injective testosterone esters(mean dose:9 ± 2mg/day). The PROCAM score was calculated to assess 10-year CV risk for each subject. Data regarding metabolic assessments, including body mass index (BMI), systolic (SBP) and diastolic (DBP) blood pressure, total, LDL, and HDL cholesterol, triglycerides, and glycemia;hormonal profile (FSH,LH,17b-estradiol,testosterone,prolactin levels),smoke, family history for CV events, current hormonal treatment, duration of treatment, and eventual gender-affirming surgery were collected. Overall, the mean PROCAM score for T*people was 18.7, accounting for a CV risk <1%. In T*men, PROCAM scores (p = 0.002) and the prevalence of CV risk 1-2% (p = 0.008)were significantly higher, while the prevalence of CV risk <1% significantly lower compared with T*women(p < 0.001). Moreover, PROCAM score was positively correlated with testosterone gel dosage in T*men(r = 0.304p = 0.02). T* men had significantly higher SBP(p = 0.001), DBP(p = 0.001), total(p < 0.001), LDL(p < 0.001), and HDL(p = 0.016) cholesterol, and triglycerides levels (p = 0.001), as well as a significantly higher prevalence of arterial hypertension(p = 0.048), hypercholesterolemia(p = 0.005) and hypertriglyceridemia(p = 0.05) compared with T*women. Moreover, T*people undergone gender-affirming surgery had significantly higher BMI(p = 0.033), DBP(p = 0.004), total(p = 0.001) and HDL cholesterol(p = 0.012), triglycerides(p < 0.001) and glycemia levels(p = 0.015) compared with those who did not. In the overall population, age was positively correlated with BMI(r = 0.224p = 0.005), SBP(r = 0.232p = 0.004) DBP(r = 0.277p < 0.001 and with total(r = 0.512p < 0.001), LDL(r = 0.444p < 0.001), HDL(r = 0.192p = 0.018) cholesterol, triglycerides(r = 0.298p < 0.001)and glycemia(r = 0.309p < 0.001);hormonal treatment duration was positively correlated with LDL(r = 0.227p = 0.005)and total(r = 0.320p < 0.001) cholesterol and triglycerides(r = 0.231p = 0.004). In T*men, gel testosterone dosage was positively correlated with triglycerides(r = 0.334p = 0.01),injective testosterone dosage was negatively correlated with BMI(r = -0.380p = 0.013)and 17b-estradiol levels were negatively correlated with total cholesterol(r = -0.263p = 0.008) and triglycerides levels(r = -0.257p = 0.011). The current study demonstrated a low CV risk and a normal metabolic profile in T* people under hormonal replacement therapy for at least six months, although T* men experienced a higher CV risk and a worse metabolic profile compared to T* women, as T* men assuming higher dosages of transdermal testosterone.

P133 | Covid-19 pandemic and two-month mass quarantine in Italy: impact on psychological distress in men with Klinefelter syndrome

Cristina de Angelis[1], Rita Indirli[2,3], Rosita A. Condorelli[4], Sara De Vincentis[5,6], Walter Vena[7], Raffaella Carotenuto[8], Marco Bonomi[9,10], Andrea Delbarba[11], Daniele Gianfrilli[12], Francesco Garifalos[1], Dimitri Yannas[13], Angelo Cignarelli[14], Emanuele Ferrante[2,3], Aldo E. Calogero[4], Vincenzo Rochira[5,6], Andrea Garolla[15], Andrea M.

Isidori[12], Giovanni Corona[16], Emmanuele A. Jannini[17], Rosario Pivonello[1,18]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile; (FERTISEXCARES), Università Federico II di Napoli, Naples, Italy; [2]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milano, Italy; [3]Department of Clinical Sciences and Community Health, University of Milan, Italy; [4]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy; [5]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [6]Azienda Ospedaliero-Universitaria di Modena, Ospedale Civile di Baggiovara, Modena, Italy; [7]Endocrinology, Diabetology and Andrology Unit, Humanitas Clinical and Research Center IRCCS, Rozzano, Milan, Italy; [8]Department of Advanced Medical and Surgical Sciences, University of Campania 'Luigi Vanvitelli', Endocrinology units, Naples Italy; [9]Dipartimento di Biotecnologie Mediche e Medicina Traslazionale – Università degli Studi di Milano; [10]IRCCS Istituto Auxologico Italiano - Dipartimento di Medicina Endocrino-Metabolica e Lab di Ricerche Endocrino-Metaboliche; [11]Unit of Endocrinology and Metabolism, Department of Medicine, ASST Spedali Civili Brescia; [12]Department of Experimental Medicine, Sapienza University, Rome, Italy; [13]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy; [14]Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari, Italy; [15]Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padova, Italy; [16]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy; [17]Chair of Endocrinology and Medical Sexology; (ENDOSEX), Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy; [18]Staff of Unesco Chair for Health Education and Sustainable Development, Federico II University, Naples, Ital

Since December 2019, worldwide population has been facing the rapid spread of Severe Acute Respiratory Syndrome-Coronavirus2, causing the highly transmissible Covid-19 global pandemic, requiring restrictive measures to reduce contagion, including extensive mass quarantine, lasting in Italy for two months (March 8th-May 16th). A severe psychological burden of Covid-19 pandemic and mass-quarantine has been reported in the general population, likely determined by forced confinement, social deprivation, freedom limitation and uncertain future. Men with Klinefelter Syndrome (KS) display an increased risk of psychological/psychiatric disorders compared to the general population, possibly representing the underlying cause of social anxiety and impulsivity, often resulting in social challenging interactions/withdrawal. It has been argued as to whether psychological disorders are associated with alterations of brain regions deputed to emotional control, to genetics, or to hormonal cues (i.e. hypogonadism); conflicting data exist on the effects of testosterone replacement therapy (TRT). The current multicentre case—control study aimed at evaluating the occurrence and severity of Covid-19 pandemic- and two-months mass quarantine-related psychological distress in 148 KS aged 40.3 ± 12.9 years compared to 148 age-matched non-KS from the general population, through questionnaires anonymously and telematically administered during the last three weeks of Italian mass quarantine for the evaluation of perceived stress (PSS), anxiety (GAD-7), depression (PHQ-9), ego-resiliency (ER89-R) and psychological wellbeing (PWB). KS and non-KS did not differ for age, marital, cohabitation and working status, and going out from home frequency during the Covid-19 mass quarantine. KS had significantly higher mean scores in PSS ($p = 0.003$), GAD-7 ($p = 0.000$) and PHQ-9 ($p = 0.001$) scales. Moreover, in KS: a significantly lower prevalence of low ($p = 0.0261$) and higher prevalence of moderate ($p = 0.0357$) perceived stress severity; a significantly lower prevalence of minimal ($p < 0.0001$) and higher prevalence of moderate ($p = 0.0008$) anxiety severity; a significantly lower prevalence of none-minimal ($p = 0.0270$) and higher prevalence of moderately severe ($p = 0.0180$) depression. Assessed outcomes did not correlate with age, TRT or age at TRT initiation in KS. KS diagnosed before age 18 years (30.41%) had significantly lower mean scores in PWB scale, compared to KS diagnosed later in life ($p = 0.049$). Noteworthy, KS diagnosed before age 18 years vs after age 40 years, displayed a significantly lower ability to adapt and react to traumatic or stressful experiences (optimal regulation ER89-R subscale: $p = 0.014$), a lower independence and self-determination (autonomy PWB subscale: $p = 0.014$), and a lower ability to have a satisfying relationship with others (positive relations with others PWB subscale: $p = 0.047$). KS with one or more (67.57%) vs without concurrent disorders/comorbidities (cryptorchidism, gynecomastia, obesity, diabetes, osteopenia, osteoporosis, cardiovascular and neurological complications) had significantly lower mean scores in optimal regulation ($p = 0.041$). The current multicentre case—control study demonstrated for the first time that KS experienced significantly higher and more severe levels of Covid-19- and mass quarantine-related perceived stress, anxiety and depressive symptoms, and a significantly lower positive attitude toward the self and self-past life, vs non-KS. A lower age-related maturity at diagnosis, a longer awareness of the syndrome, and concurrent disorders/comorbidities might all contribute to psychological burden and therefore influence the personal attitude in challenging situations.

P134 | The effect of the new 75 mg orodispersible film of Sildenafil on erection and sexual quality of life: insights from an observational studY

Andrea Sansone[1], Valeria Frangione[2], Arturo Lanzarotti[2], Emmanuele A Jannini[1]

[1]Chair of Endocrinology and Medical Sexology (ENDOSEX), University of Rome Tor Vergata, Italy; [2]IBSA Institut Biochimique SA, Pambio-Noranco, Switzerland

Introduction: The newly devised orodispersible film (ODF) of Sildenafil is the first phosphodiesterase type 5 inhibitor (PDE5i) in a 75 mg dosage. This intermediate dosage and the particular properties of the ODF formulation can improve the clinical management of erectile

dysfunction (ED) patients. We investigated the effects of the sildenafil ODF 75 mg dose on both sexual quality of life and erectile function based on the results from an observational study in daily practice in Italy.

Materials and Methods: This was a post-hoc analysis of results from an observational study carried out in six centers in Italy and performed on a real-life population of men coming for a complaint of ED and treated in accordance with the individual investigators' normal clinical practice. All subjects were asked to take the prescribed dosage of Sildenafil ODF at inclusion (Visit 1) and to return for a control visit (Visit 2) to confirm or adapt the prescribed dose after a minimum of 4 weeks. An End of study Control visit (Visit 3) was performed after additional 4 weeks. Patients were encouraged to attempt sexual intercourse using the drug on at least 8 occasions during the period between visits. Erectile function was assessed by the International Index of Erectile Function – Erectile Function Domain (IIEF-EF); sexual quality of life was measured using the sexual QoL instrument for men (SQoL-M).

Results: Among the 36 subjects initially recruited at visit 1 for the 75 mg dose, three patients dropped out of the study at visit 2 and two dropped out at visit 3. None of the subjects withdrew due to treatment inefficacy or serious adverse events. At visit 2, the mean IIEF-EF scores significantly increased ($\Delta = 7.97 \pm 4.71$, $p < 0.0001$) in the overall population; likewise, SQoL-M also increased significantly ($\Delta = 10.76 \pm 10.46$, $p < 0.0001$). At visit 3, IIEF-EF and SQoL-M scores were still significantly improved compared to baseline ($\Delta = 10.64 \pm 7.01$, $p < 0.0001$ and $\Delta = 18.15 \pm 12.32$, $p < 0.0001$, respectively). By using ANCOVA, we found no significant effect for age, BMI, history of previous use of PDE5i, presence of metabolic comorbidities, and smoking habits on IIEF-EF and SQoL-M scores at both visits 2 and 3.

Conclusion: The new 75 mg ODF Sildenafil formulation is a safe and effective treatment for erectile dysfunction, significantly improving both erectile function and sexual quality of life in patients undergoing treatment. Such improvement is not transient and limited to the first weeks of treatment, but rather more stable and reliable over time. Additionally, factors known to affect sexual health, including age, BMI, smoking status, metabolic comorbidities, and symptoms duration, resulted in significantly different outcomes for treatment efficacy, suggesting that the treatment with this intermediate dose is equally viable for all patients on average. The use of the ODF formulation for sildenafil represents a novel opportunity to take care of ED patients.

P137 | Nocturnal penile tumescence test in patients with erectile dysfunction associates with penile and systemic vascular disease

Maurizio De Rocco Ponce[1], Josvany R. Sanchez Curbelo[1], Guillem Abad Carratala[2], Claudia F. Quintian Schwieters[1], Josefa Martinez Barcina[1], Eduard Ruiz-Castane[1]

[1]*Andrology Department, Fundacio Puigvert, Instituto de Investigaciones Biomedicas Sant Pau (IIB-Sant Pau), Universitat Autonoma de Barcelona, Barcelona, Spain;*[2]*Hospital General Universitario de Castellon, Castellon de la Plana, Spain*

Background: Erectile dysfunction (ED) is the most frequent male sexual dysfunction along with premature ejaculation. Moreover, its prevalence is increasing worldwide due to ageing of the population and spreading of risk factors like obesity, diabetes, hypercholesterolemia, hypertension, etc. Also, it represents an important cardiovascular risk marker because of its demonstrated tight association with cardiovascular disease (CVD). We dispose of many diagnostic tools to assess ED and a proper workup includes the anamnesis, a physical exploration, blood tests for hormonal and metabolic evaluation, and ED grading through validated questionnaires such as the International Index of Erectile Function (IIEF).

One of the instrumental tests we may indicate is the nocturnal penile tumescence (NPT) test or "Rigiscan". This test measures the spontaneous erections overnight and helps discriminating between organic and psychological ED. However, this is a highly specific but low sensitive test since a positive (normal) result can roll out an organic ED and suggest a psychological etiology while a negative result cannot confirm or exclude an organic condition.

Aim: In this context the aim of this study is to investigate the predictive role of Rigiscan in discriminating patients with a organic ED and the presence of vascular disease in patients with ED.

Methods: We conducted a prospective study in patient 25 patient who underwent a Rigiscan test as part of ED assessment. Each patient had blood tests for hormonal and metabolic analysis, a measurement of the carotid intima-media thickness (cIMT), a brachial flow-mediated vasodilation test (FMD), and a penile color doppler ultrasound (PCDU) to assess penile hemodynamics.

Results: Our patients had a mean age of 47.9 ± 13.7 years, with a mean IIEF score of 9.0 ± 6.5. Among them 13 (54.2%) were active smokers, 5 (20.8%) were diabetics, 8 (32%) had dyslipidemia, and 4 (16.7%) had hypertension. Eleven patients (44%) had a normal Rigiscan while 14(56%) had a pathological result.

We found that patient with a pathological Rigiscan presented with a cluster of impaired vascular function at different levels. In particular, they had statistically lower peak systolic velocity (PSV) in the PCDU (52.5 vs 80.2 cm/s $p = 0.014$) and a higher prevalence of arterial insufficiency (42.9% vs 0.0% $p = 0.020$). Moreover, in patients with negative Rigiscan, there was a higher cIMT (0.9 vs 0.7 mm) and lower response to FMD (7.7% vs 9.6%) with a more frequent diagnose of impaired FMD (63.6% vs 46.2%) but without reaching a statistically significance.

Conclusions: We can conclude that a negative Rigiscan is indicative of altered penile hemodynamics and may suggests the presence of systemic vascular disease. Therefore, while a positive Rigiscan excludes organic ED, a negative Rigiscan should be considered a cardiovascular risk marker and induce the clinician to address the cardiovascular risk in that patient. More studies with larger population are warranted to confirm and further investigated these findings.

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