ANDROLOGY

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WELCOME

On behalf of the European Academy of Andrology (EAA), we look forward to your participation at the 8th European Congress of Andrology (ECA 2014) that will be held in Barcelona from 15th to 17th October, 2014. We hope it will be a memorable event for all members of the EAA and all professionals engaged in this field who decided to join us.

The excellent scientific programme provides a forum for specialists to encourage discussion about the latest developments in male reproduction, onco-andrology, andro-genetics, male and female sexual dysfunction, sexual transmitted diseases and some aspects of andrological surgery. The programme includes plenary lectures and offers a multidisciplinary platform for furthering our understanding of men's sexual and reproductive health. We are confident that the meeting will be of major scientific interest.

As in the previous edition, ECA 2014, together with the European School of Urology (ESU) and the European Society of Andrological Urology (ESAU), has organized three courses focusing on the surgical aspects of NOA, varicocele and Peyronie disease. In addition, on October 15th a Post-graduate Course dealing with 'Standard care and diagnostic procedures in Andrology' will take place.

We are warmly welcoming you to Barcelona!

Osvaldo Rajmil
Chair of the LOC
(Local Organising Committee)

Csilla Krausz
Chair of the POC
(Programme Organising Committee)

Andrology is the meeting point of many medical and veterinary specialties. It is also a home for those who work in the field of stem cells and reproductive biology. We face many challenges in male reproductive health, such as infertility, lack of male contraceptives, male sexual problems, secondary effects of cancer treatment and many more. We need a wide front of researchers and clinicians who tackle the andrological problems in good collaboration. European Academy of Andrology wants to give a forum for this action, and European Congress of Andrology in Barcelona will be the place to meet this year. Local organizers and the programme organizing committee have created a wonderful opportunity for us to come together to push andrology forward with great science and good spirit. On behalf of the European Academy of Andrology I want to welcome you to join us in Barcelona.

Jorma Toppari
President of the European
Academy of Andrology

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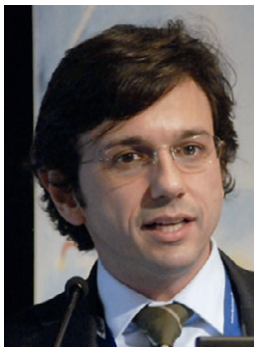

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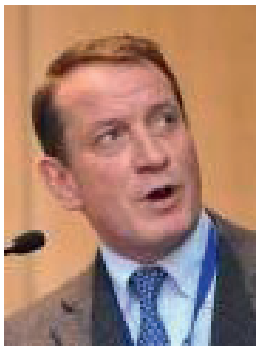

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ANDROLOGY
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8th EUROPEAN CONGRESS OF ANDROLOGY

PRE-CONGRESS EVENTS

15 October 2014

Post-Graduate Course: Standard Care in Andrology
Chairpersons: C. Krausz (Italy) and O. Rajmil (Spain)

09:00–09:20	**Semen parameters: what is the normal range?** *N. Jorgensen (Denmark)*
09:20–09:40	**Semen analyses according to 2010 WHO guidelines: merits and limits** *J. Auger (France)*
09:40–09:50	Discussion
09:50–10:10	**Imaging of the male reproductive tract: towards standardization** *F. Lotti (Italy)*
10:10–10:30	**Novel imaging modalities for testicular tumors** *A. Isidori (Italy)*
10:30–10:40	Discussion
10:40–11:00	Coffee break
11:00–11:20	**Genetic testing: the new EAA/EMQN Guidelines for AZF deletions** *C. Krausz (Italy)*
11:20–11:40	**Genetic testing: karyotype anomalies and sperm FISH analysis** *R. Oliva and M. Codina (Spain)*
11:40–12:20	**Pre-ART standard diagnostic work up of:** **- The Azoospermic man** *L. Bassas (Spain)* **- The Oligozoospermic man** *J. L. Ballesca (Spain)*
12:20–12:30	Discussion
12:30–12:50	**Testosterone measurement: what is the normal range?** *F. Wu (UK)*
12:50–13:10	**Standard diagnostic work up in erectile and ejaculatory dysfunction** *S. Arver (Sweden)*
13:10–13:20	Discussion
13:20–13:30	**Closing Remarks**

AMS Symposium
Chair: E. Ruiz-Castañé (Spain)

09:00–09:10	**Welcoming remarks** *E. Ruiz-Castañé (Spain)*
09:10–09:30	**Last generation on penile prostheses** *J.I. Martínez (Spain)*
09:30–09:50	**Strategies to avoid complications** *J. Sarquella (Spain)*
09:50–10:20	**Penile implant techniques. Tips and tricks** *C. Bettocchi (Italy)*
10:20–10:30	Coffee break
10:30–11:10	**Other contingencies. Options** *E. LLedó (Spain)*
11:10–11:40	**Surgical options in La Peyronie Disease** *O. Shaeer (Egypt)*
11:40–12:30	**Panel discussion and closing remarks**

ECA MAIN PROGRAMME

15 October 2014

15:15–16:15	**WELCOME CEREMONY**
16:15–17:00	**Plenary I:** *Chairperson: J. Toppari (Finland)* **Testicular cancer: fate or bad luck.** *E. Rajpert-De Meyts (Denmark)*
	PARALLEL SESSIONS **ECA I: Sexual development and gender identity** *Chairpersons: G. Forti (Italy) and G. Haidl (Germany)*
17:00–17:30	**Sex differentiation and the brain: relation to gender identity, sexual orientation** *D. Swaab (The Netherlands)*
17:30–18:00	**Hormonal treatment in gender dysphoria.** *G. T'Sjoen (Belgium)*
18:00–18:30	**Long term outcome of gender dysphoria** *C. Dhejne (Sweden)*
	ECA selected oral I: Golden presentations *Chairpersons: A. Meinhardt (Germany) and O.Rajmil (Spain)*
17:00–17:20	**Mutations in an X-Linked gene are a common cause of meiotic arrest and azoospermia.** *A.N. Yatsenko (USA)*
17:20–17:40	**Differential effects of perinatal, prepubertal or conception to adulthood exposures to a low dose mixture of genistein and vinclozolin on the genital tract and testicular gene expression in the exposed generation and the unexposed progeny.** *F. Eustache (France)*
17:40–18:00	**Evaluation of intratesticular perfusion by color-coded duplex sonography (CCDS) in TESE-patients with azoospermia: a prospective study.** *B. Altinkilic (Germany)*
18:00–18:20	**Association between endogenous testosterone (T) levels, sexual dysfunction and PDE-5 inhibitor (PDE5i) use in the registry of hypogonadism in men (RHYME).** *G. Rastrelli (Italy)*
	Bayer Symposium **Cardiovascular Risk: a Concern for the Andrologist?** *Chairperson: M. Maggi (Italy)*
17:00–17:30	**Effects of long-term testosterone treatment in obese men with and without type 2 diabetes.** *F. Saad (Germany)*
17:30–18:00	**Testosterone and cardiovascular risk – what is the evidence?** *M. Maggi, (Italy)*
18:00–18:30	**Erectile Dysfunction as a cardiovascular risk marker and predictor of poor quality of life.** *E. García–Cruz (Spain)*
18:30–19:30	**POSTER GUIDED TOUR:** *Chairpersons: L. Bassas (Spain), C. Boitani (Italy), G. Corona (Italy), J. Erenpreiss (Latvia), M. Gonzalez - Fernandez (Spain), Zs. Kopa (Hungary), H.C. Schuppe (Germany), O. Shaeer (Egypt) and J. Slowikowska-Hilzer (Poland)*
19:30–21:00	**WELCOME RECEPTION**

16 October 2014

09:00–9:45	**Plenary II:** *Chairperson:* E. Baldi (Italy) **ASA Exchange Lecture: From the Epydidimis to the Egg.** *P. Cuasnicu (Argentina)*

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09:45–10:15	Coffee break
	PARALLEL SESSIONS
	ECA 2. Male Sexuality
	Chairpersons: H. Behre (Germany) and G. Papp (Hungary)
10:15–10:45	**PDE5i or testosterone: which is the first in andrological clinical practice.**
	E. Jannini (Italy)
10:45–11:15	**A new view on lifelong premature ejaculation: erectio praecox, ejaculatio praecox and detumescentia praecox**
	M.D. Waldinger (The Netherlands)
11:15–11:45	**Hypersexuality.**
	K. Görts Öberg (Sweden)
	ECA Selected Oral II: Spermatology
	Chairpersons: D. Goulis (Greece) and *S. Francavilla (Italy)*
10:15–10:30	**Characterization and kinetic of the human spermatogonial compartment**.
	E Vicini (Italy)
10:30–10:45	**Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity and oxidative stress.**
	E. Baldi (Italy)
10:45–11:00	**Routine use of flow cytometry for counting human spermatozoa and round cells: lessons from a 5-year experience representing more than 20.000 analyses.**
	J. Auger (France)
11:00–11:15	**Influences of age, days of abstinence, and BMI on results of semen analysis from Swedish men of sub-fertile couples**.
	J.N. Flanagan (Sweden)
11:15–11:30	**Glycation adducts in sperm and activin regulation of inflammation induced by diabetes.**
	C. Maresch (Germany)
	EAA/ESAU/ESU Course: Sperm Retrieval in non-obstructive Azoospermia.
	Chairperson: E. Ruiz-Castañé (Spain)
10:15–10:45	**Clinical diagnostics and prognostic factors for sperm retrieval.**
	Zs. Kopa (Hungary)
10:45–11:15	**Surgical treatment of Klinefelter patients.**
	H. Tournaye (Belgium)
11:15–11:45	**Surgical treatment (M-TESE).**
	W. Weidner (Germany)
11:45–12:45	**POSTER GUIDED TOUR**
	Chairpersons: Chairs: L. Bassas (Spain), C. Boitani (Italy), G. Colpi (Italy), J. Ramalho-Santos (Portugal), A. Giwercman (Sweden), O. Shaeer (Egypt) and *J. Erenpreiss (Latvia)*
12:45–14:00	**LUNCH**
	Menarini Symposium
	Understanding premature ejaculation. A new approach to the anatomy, neurophysiology and treatment
	Chairperson: A. Fernández-Lozano (Spain)
12:45–13:05	**The role of anatomy on premature ejaculation.**
	F. Giuliano (France)
13:05–13:25	**A different look at the neurophysiology.**
	M. Waldinger (The Netherlands)
13:25–13:45	**What's new on premature ejaculation treatment?**
	A . Fernández Lozano (Spain)
13:45–14:00	Discussion
	PARALLEL SESSIONS
	ECA 3. Prostate: news and views
	Chairpersons: J. Palou (Spain) and M. Maggi (Italy)
14:00–14:30	**Associations between prostate pathologies and sexual dysfunction**
	F. Wagenlehner (Germany)
14:30–15:00	**Benign Prostatic hyperplasia: a new metabolic syndrome-related disease.**
	L. Vignozzi (Italy)

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15:00–15:50	**Face to face on prostate cancer screening: PSA versus novel biomarkers.** *M. Roobol (The Netherlands)* and *M. Lazzeri (Italy)*
	ECA 4. Bone Testis cross talk *Chairperson: N. Joergensen (Denmark)*
14:00–14:30	**Vitamin D and male reproduction.** *M. Blomberg Jensen (Denmark)*
14:30–15:00	**Testis versus bone.** *A. Ferlin (Italy)*
	ECA Selected Oral III: Reproductive Health *Chairpersons: M. Punab (Estonia)* and *G. Balercia (Italy)*
15:00–15:15	**Sperm DNA damages after chemotherapy or radiotherapy for testicular cancer.** *N. Rives (France)*
15:15–15:30	**Prenatal phthalate exposure and reproductive indices in adolescent males.** *J. Axelsson (Sweden)*
15:30–15:45	**Does reproductive tract inflammation mediate reproductive dysfunction in males with the metabolic syndrome?** *K. Leisegang (South Africa)*
	ESU/ESAU/EAA Course: Peyronie's Disease *Chairperson: E.Meuleman (the Netherlands)*
14:00–14:30	**Pathophysiology and diagnostic management.** *F. Fusco (Italy)*
14:30–15:00	**Drug treatment and other non-operative therapy.** *D. Ralph (UK)*
15:00–15:30	**Surgical treatment.** *E. Meuleman (The Netherlands)*
15:50–16:00	**"Andrology" Award Ceremony** *Chaipersons: E. Rajpert De Meyts (Denmark) and D. Carrell (USA)* **"Hormone suppression with GnRH antagonist promotes spermatogenic recovery from transplanted spermatogonial stem cells in irradiated cynomolgus monkeys"** *M.L. Meistrich (USA)*
16:00–16:35	**Plenary III** *Chairperson: C. Krausz (Italy)* **The bright side of the male gamete: the telomere length** *A. Aviv (USA)*
16:35–16:55	Coffee break
	PARALLEL SESSIONS **ECA 5. Imaging in Andrology** *Chairperson: A. Isidori (Italy)*
16:55–17:30	**Imaging of the male genital tract: 2014 update.** *F. Lotti (Italy)*
17:30–18:10	**Pictorial review of cutaneous genital lesions.** *A. Vives (Spain)*
	ECA 6. From basic to Clinical Andrology *Chairpersons: R. Oliva (Spain)* and *G. Mieusset (France)*
16:55–17:25	**Profiling human sperm: from functional studies to omics analyses, and back again.** *J. Ramalho-Santos (Portugal)*
17:25–17:55	**Intra-testicular cellular dynamics and regulation of Leydig cell function.** *P. O'Shaughnessy (UK)*
17:55–18:10	*Selected Oral presentation:* **Serum delta4 pathway steroids profiling by isotopic diluition-liquid chromatography-mass spectrometry (ID-LC-MS/MS) after human chorionic gonadotropin (HCG) stimulation in men with klinefelter's syndrome (KS) and in eugonadic controls (ec).** *S. Belli (Italy)*
	ECA Selected Oral IV: Infertility *Chairs: K. Kula (Poland)* and *J. Heràcek (Czech Republic)*
16:55–17:10	**Inhibin B as a marker of success after varicocelectomy in severe oligozoospermia.** *A. Khelaia (Georgia)*

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17:10–17:25	**Motile sperm count, DNA fragmentation and mitochondrial membrane potential are not useful in predicting intrauterine insemination for mixed indications: a prospective study.** *B. Zorn (Slovenia)*
17:25–17:40	**Subcapsular orchiectomy as an alternative method for identification and isolation of testicular sperm from men with Klinefelter's syndrome.** *J. Fedder (Denmark)*
17:40–17:55	**Gene expression analysis of human sperm needs differential normalizers.** *M. Barragan (Spain)*
17:55–18:10	**Evaluation of testicular biopsies from infertile men: correlations between histopathology, protamine mRNA expression, and sperm retrieval** *H-C Schuppe (Germany)*
18:10–19:30	**EAA GENERAL ASSEMBLY**
20:30–23:00	**CONFERENCE DINNER**

17 October 2014

	PARALLEL SESSIONS
	ECA 7. Androgens and Hypogonadism
	Chairs: S. Arver (Sweden) and F. Wu (UK)
8:45–9:15	**Abuse of Androgens.** *E. Nieschlag (Germany)*
9:15–9:45	**The vulnerable uremic man - role of hypogonadism.** *P. Stenvinkel (Sweden)*
9:45–10:00	*Selected Oral presentation:* **Novel proteomic biomarkers of androgen deficiency from seminal plasma profiling using high-resolution mass spectrometry.** *G. Grande (Italy)*
10:00–10:15	*Selected Oral presentation:* **Testosterone supplementation and sexual function: a meta-analysis study.** *G. Corona (Italy)*
	ECA 8. Session of the INYRMF
	Chairs: C. Chianese (Italy) and *J. Castillo (Spain)*
8:45–9:15	**Pharmacogenetics in male infertility: what about personalised FSH-treatment in 2014?** *F. Tuettelmann (Germany)*
9:15–9:30	*Selected Oral presentation:* **High-resolution profiling of novel transcriptional events during human spermatogenesis.** *F. Chalmel (France)*
9:30–9:45	*Selected Oral presentation:* **Why are some human sperm immotile? A differential proteomics approach.** *C. Paiva (Spain)*
9:45–10:00	*Selected Oral presentation:* **Expression of functional histamine H4 receptors in murine and human Leydig tumor cells.** *C. Mondillo (Argentina)*
10:00–10:15	*Selected Oral presentation:* **Differential activation of inflammatory pathways in testicular macrophages provides a rationale for their subdued inflammatory capacity.** *S. Bhushan (Germany)*
	ESU/ESAU/EAA Course: Varicocele
	Chairperson: W. Weidner (Germany)
8:45–9:15	**Role in infertility and clinical diagnosis.** *N. Sofikitis (Greece)*
9:15–9:45	**Surgical therapy.** *G. Dohle (The Netherlands)*

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9:45–10:15	**Sclerozation and embolization.** *C. Bettocchi (Italy)*
	PARALLEL SESSIONS
	ECA 9. Infertility I: Drugs and Reproductive Health *Chair: J. Auger (France)* and *M. Simoni (Italy)*
10:15–10:40	**The headache of analgesics during pregnancy and the fetal reproductive system: how and why.** *B. Jégou (France)*
10:40–11:05	**Common drug intake and human spermatogenesis** *A. Giwercman (Sweden)*
11:05–11:30	**Late consequences of childhood and adolescence cancer treatment** *G. Dohle (The Netherlands)*
	ECA 10. Infertility II: Genetics and Epigenetics *Chairpersons: A. Calogero (Italy) and Y. Giwercman (Sweden)*
10:15–10:40	**The sperm epigenome.** *D. Carrell (USA)*
10:40–11:05	**Sperm nuclear proteome and its epigenetic potential.** *J. Castillo (Spain)*
11:05–11:20	*Selected Oral presentation:* **X chromosome-linked CNVs in male infertility: discovery of overall duplication load and recurrent, patient-specific gains with potential clinical relevance.** *C. Chianese (Italy)*
11:20–11:35	*Selected Oral presentation:* **Impairment of DAZ-AZFc gene expression in postmeiotic human male germ cells of men with severe hypospermatogenesis.** *P.H. Vogt (Germany)*
	ECA Selected Oral V: Sexual Dysfunction *Chair: P. Gutiérrez (Spain) and K. Shaeer (Egypt)*
10:15–10:30	**Development of secondary hypogonadism in aging men: risk factors and clinical picture: longitudinal results from the European Male Aging Study (EMAS).** *G. Rastrelli (Italy/UK)*
10:30–10:45	**Mechanism of estrogen- or antiandrogen-induced penile maldevelopment.** *H. Goyal (USA)*
10:45–11:00	**Tadalafil ameliorates metabolic syndrome-induced alterations in visceral adipose tissue and liver: an experimental study in the rabbit.** *L. Vignozzi (Italy)*
11:00–11:15	**Sub-albuginean adipocyte accumulation is associated with erectile dysfunction: first clinical evidence and pathophysiological implications.** *J.I. Vinay (Chile)*
11:15–11:30	**Penile enlargement and augmentation: 411 cases.** *M. Rosselló Gayá (Spain)*
11:40–12:15	**BRUNCH**
12:15–13:00	**Plenary 4.** *Chair: S. Schlatt (Germany)* **Spermatogonial stem cell preservation and transplantation anno 2014: what every clinician should know.** *H.Tournaye (Belgium)*
13:00–13:15	**Closing Remarks**

ANDROLOGY

ABSTRACTS ISSN: 2047-2919

INVITED SPEAKER ABSTRACTS

INVITED TALKS: POST-GRADUATE COURSE

IOP1

Imaging of the male reproductive tract: towards standardization

F. LOTTI[1], S. ARVER[2], G. BALERCIA[3], A. BARBONETTI[4], H. M. BEHRE[5], A. E. CALOGERO[6], S. FRANCAVILLA[4], A. GIOIA[3], A. M. ISIDORI[7], N. JØRGENSEN[8], S. KLIESCH[9], K. KULA[10], S. LA VIGNERA[6], A. LENZI[7], M. MARCOU[5], U. PAASCH[11], A. PILATZ[12], O. POOLAMETS[13], M. PUNAB[13], C. QUINTIAN[14], O. RAJMIL[14], W. WEIDNER[12] AND M. MAGGI[1]

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Even if medical care of the man suffering of involuntary childlessness is growing faster, in andrology, diagnostic and therapeutic measures have not reached yet a critical mass to ensure a reasonable understanding of the underlying problem and the consequent, evidence-based, treatment. Nowadays, scrotal and transrectal imaging of the male genital tract (MGT) have greatly helped in deciphering anatomy, physiology and pathology of male infertility. However, sonographic imaging of MGT still suffers from lack of standardization and often tends to produce subjective and vague diagnosis. In addition, scrotal and transrectal colour-Doppler ultrasound (CDUS) in relation to male fertility has not resulted in clear cut-offs, discriminating normal or pathological CDUS parameters. Finally, the association between CDUS findings and male infertility/semen parameters has been poorly studied. This is the reason why the European Academy of Andrology (EAA) promoted an ongoing multicentre study aimed at investigating the CDUS features of the entire MGT in healthy, fertile men, in order to obtain 'normative' parameters for both scrotal and transrectal CDUS. The project has been discussed in Florence on October 20th, 2012 (practical investigator meeting) and in Berlin on November 29th, 2012, during the European Congress of Andrology (protocol discussion). So far, approval from Ethical Committee has been submitted in several Centers and already obtained in some of them. The characteristics of the study are available at http://www.andrologyacademy.net/studies.php. The aim of the study is: primary, to evaluate the CDUS features of the scrotal and prostate-vesicular regions in healthy fertile men; secondary: to correlate CDUS findings with clinical, seminal and biochemical parameters evaluated during the same CDUS session. The study is designed as a prospective, cohort, multicentric, international, observational study. 14 EAA Centers participate in the study (Ancona, Italy; Barcelona, Spain; Catania, Italy; Copenhagen, Denmark; Florence, Italy; Giessen, Germany; Halle-Saale, Germany; L'Aquila, Italy; Leipzig, Germany; Lodz, Poland; Muenster, Germany; Rome, Italy; Stockholm, Sweden; Tartu, Estonia). The Florence EAA Center is coordinating the study. The study will enroll 200 adult healthy, fertile subjects. The main inclusions criteria concern males aged ≥18 years, without serious organic disease, partners of a pregnant woman in the second or third trimesters of pregnancy or men who fathered a child during the last year, following natural conception. All patients enrolled will undergo, during the same day, the following procedures: (i) Blood samples for the determination of LH, FSH, PRL, total testosterone, SHBG, TSH, FT3, FT4, PSA, glycaemia, insulin levels, total cholesterol, HDL, triglycerides, 17 βestradiol. Biochemical analyses will be performed in a central lab at the end of the study. (ii) Personal and medical history assessment. (iii) Physical examination. (iv) Scrotal and transrectal CDUS evaluation before and after ejaculation. (v) Semen analysis, performed according to the World Health Organization, 2010. New knowledge generated by our multicenter research consortium will create a step change in defining the impact of MGT-CDUS on reproductive medicine. Standardization of MGT-CDUS parameters in a healthy, fertile population will lead to the improvement of our diagnostic skills in identifying etiological factors of male infertility.

IOP2

Genetic testing: the new EAA/EMQN Guidelines for AZF deletions

C. KRAUSZ[1], L. HOEFSLOOT[2], M. SIMONI[3] AND F. TÜTTELMANN[4]

[1]Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy; [2]Department of Clinical Genetics, Erasmus MC, University Medical Center Rotterdam, the Netherlands; [3]Department of Biomedicine, Metabolism and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [4]Institute of Human Genetics, University of Münster, Münster, Germany

The molecular diagnosis of Y-chromosomal microdeletions is a common routine genetic test which is part of the

diagnostic workup of azoospermic and severe oligozoospermic men. Since 1999, the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have been actively involved in supporting the improvement of the quality of the diagnostic assays by publication of the laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions and by offering external quality assessment trials. The latest version of the Guidelines has been published in 2014 (Krausz *et al.*, *Andrology* 2014, 1: 5–19). The original basic protocol based on two multiplex polymerase chain reactions remains fully valid and appropriate for accurate diagnosis of complete AZF deletions and it requires only a minor modification in populations with a specific Y chromosome background. Screening for isolated AZF gene specific deletions is still not recommended since they are extremely rare and have been reported only in the AZFa region. Deletions of the *USP9Y* gene is associated with a heterogeneous semen phenotype, ranging from azoospermia to normozoospermia. The screening for gr/gr deletion, a significant risk factor for impaired sperm production, is under debate but it can be performed in selected populations for which robust and consistent data with risk estimate are available (at present Italian, Spanish, Dutch and Chinese). In the light of novel data on genotype–phenotype correlations, the extension analysis for the AZFa and AZFb deletions is now routinely recommended. Concerning the importance of the Quality Control Scheme, between 2000 and 2012 the number of participating laboratories almost tripled from 57 to 148. Recurrent interpretation problems still arise owing to laboratories using an unnecessary high number of markers, which are specifically included in commercially available kits. An excessively high number of markers do not improve the sensitivity of the test, may even complicate the interpretation of the results therefore they are not recommended.

The analyses of AZF deletions should be performed according to the novel EAA/EMQN guidelines. The established external quality control scheme is a successful tool to improve the performance of participating laboratories and has demonstrated an improvement on reporting practice and decreasing diagnostic error rates.

IOP3

Genetic testing: karyotype anomalies and sperm FISH analysis

R. OLIVA AND M. CODINA

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Karyotype anomalies are present in 0.6% of the general population and are an important cause of male infertility. The frequency of karyotype anomalies in men presenting fertility problems is 2%, but this frequency increases proportionally to the severity of the infertility (6% oligozoospermia, 14% non-obstructive azoospermia). Karyotype anomalies may be either numerical (aneuploidy) or structural. Non-mosaic aneuploidies represent 4% of the clinical pregnancies, and result from the formation of a zygote in which one of the gametes, either the male or the female one, was aneuploid. Aneuploid gametes originate from the non-disjunction of autosomal or sex chromosomes either in the first or the second meiotic division. About 10% of the autosomal and 50% of sex chromosomes aneuploidies are of paternal origin. All fertile men have a frequency of aneuploid sperm cells of 4.5%. This frequency increases by 2-4 fold in men presenting an altered seminogram, in those fathers of aneuploid children and in cases of recurrent abortions. Individuals with Klinefelter syndrome (47, XXY) may be fertile, however they show a 10 fold increase of sperm cells carrying an extra sex chromosome. These higher frequencies of aneuploid sperm cells result in a higher risk of generating aneuploid embryos. The presence of a structural chromosome anomaly in a karyotype such as a reciprocal translocation, a Roberstonian translocation or an inversion, may also impair the correct segregation of the reorganized chromosomes in the meiotic divisions, as well as of other chromosomes (interchromosomal effect), and produce a high number of unbalanced and/or aneuploid sperm cells. Carriers of reciprocal translocations and inversions produce up to 50% unbalanced sperm cells, and carriers of Robertsonian translocation up to 15%. (Tempest *et al.*, Syst Biol Reprod Med, 2011, 57: 93–101; Templado *et al.*, Mol Hum Reprod, 2013,19: 634–643; Piomboni *et al.*, Adv Exp Med Biol. 2014;791: 27–5).

The chromosomal content of a sperm cell can be characterised by applying the sperm FISH analysis. It consists of hybridising specific fluorescent labelled DNA probes onto previosuly fixed and decondensed sperm cells. The combination of chromosome specific DNA probes allows the characterisation of several chromosomes at the same time. In the clinics, the chromosomes analysed to study sperm aneuploidy are chromsomes 13, 18, 21, X and Y, since these are the unique aneuplodies compatible with life. However, other chromosomes may also be studied. For carriers of chromosome structural reorganizations, a case-specific DNA probe set is designed and used to analyse the frequency of normal or balanced vs unbalanced sperm cells.

Assisted reproductive techiques (ART) are offered to infertile men, either with normal or abnormal karyotypes. The analysis of the frequency of sperm cells with abnormal chromosomal content in these infertile men, and the consequent risk to generate embryos with chromosomal unbalances, is important to offer an adequate genetic counseling to the couple to improve the success of the ART and implantation rates.

IOP4

Pre-ART standard diagnostic work up of: the azoospermic man

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Approximately 10% of men consulting for couple infertility show azoospermia, defined as the absence of sperm in the ejaculate. Since this condition represents an extreme phenotype leading to permanent sterility, many guidelines focus on the diagnosis and treatment of azoospermia. Although centrifugation of semen is usually recommended, extensive examination of the sample seems is equally effective, and maintains the motility of any residual sperm found. Some characteristics of the ejaculate can give important clues to underlying the cause of azoospermia. Low volume (hypospermia) may suggest hypoandrogenism, and hypospermia plus low pH and fructose indicates distal obstruction of genital tract, including congenital bilateral absence of vas deferens (CBAVD).

Clinical assessment: The initial evaluation of the azoospermic male requires a medical history, physical examination and hormone analysis. A comprehensive survey of conditions capable of producing azoospermia may be better obtained by structured questionnaires covering somatic and pubertal development, past fertility history, sexual function, genitourinary diseases and surgery, systemic diseases, drugs and genotoxic agents, as well as a family history. Physical inspection should include signs of delayed or incomplete androgenization, differentiation and maturation of genitalia, inguinal region and scrotal contents. Assessment of testis size and consistency, epididymis, palpation of vas deferens and spermatic cord is mandatory. Digital rectal examination may reveal abnormalities of inner genital structures.

Investigations: FSH, LH and testosterone levels, will help to know the physiological regulation of the hypothalamo-pituitary-gonadal axis, and to establish a classification of derangements (at pre-testicular, testicular or post-testicular level) causing azoospermia. In selected cases, scrotal or transrectal ultrasonography, vasography, seminal tract washout, and abdominal and cranial imaging studies can be performed to identify or confirm morphological defects.

Genetic screening: Genetic factors have a significant contribution as a cause of azoospermia, and should be considered in the genetic and reproductive counselling of the couples before deciding the treatment. Pretesticular factors (idiopathic hypogonadotropic hypogonadism, Kallmann syndrome) include a cluster of genes (KAL-1, FGFR1, PROK2, FGF8, KiSS1/GPR54 and others) that can be mutated in 30–40% of patients. Primary testicular dysfunction is a consequence of sex chromosome hyperploidy (Klinefelter's syndrome) in 15% of azoospermic men, and Y chromosome microdeletions of the AZF regions (a,b and c) account for up to 5% of them. Among the genetic causes of post-testicular azoospermia, mutations of the CFTR gene are responsible for a mild form of cystic fibrosis and CBAVD. Routine kits of CFTR mutations should be expanded to include specific infertility mutations.

Testis biopsy and sperm extraction: The combination of clinical, analytical, imaging and genetic tools leads to the diagnosis of most azoospermic patients. Diagnostic testicular biopsy may be considered to distinguish obstructive and non-obstructive in men with normal-sized testes and a normal hormone profile. At the same time, testicular sperm extraction (TESE) and cryopreservation can be done for future assisted reproductive treatment. Both fresh and frozen gametes are equally effective in the majority of patients. In men with extreme testicular failure (small testes, high FSH) microscopically-guided TESE can offer higher chances of finding sperm, which can be fresh-used with simultaneous oocyte retrieval, or cryopreserved using special micro-carriers.

IOP5

Pre-ART standard diagnostic work up of: the oligozoospermic man

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Reproductive difficulty in humans is a disease that in developed societies has increased in incidence in recent decades and this rise is consequent a variety of factors, including economical, social, environmental, medical, among others.

There is no doubt that in most situations, especially in the olizozoospermic males, conjugal sterility is the consequence of a subfertility of each of its members, but the man's fertility is assessed numerically, which makes it easier to quantify.

Obviously this goal could be reached easily if we know the root of the cause or the etiology of the decrease in the number of sperm cells produced. But this can be complex and in fact more than 25% of male patients never reach a diagnosis, which reduces the choices for the treatment and its effectiveness.

Andrologic assessment is essential and any clinical situation should be based on a detailed medical history and a gonadal and secondary sexual traits examination. Hormonal determinations of FSH and inhibine B are very important in the possible therapeutic approach for these patients and should be consistent with the seminogram, clinical examination and hormonal levels. When this does not happen should be further research should be done.

A large number of the male infertility cases are for a genetic cause. It is necessary to study these genetic etiologies, which will help to establish a prognosis and to avoid genetic risks in their offspring. In the cases of very severe oligozoospermia it is advisable to perform a molecular study of the Y chromosome to rule out potential microdeletions.

High levels of FSH and low inhibine B allow establishing an unfavorable prognosis to any medical treatment, either hormonal or surgical, even in the presence of an evident clinic varicocele, since this raise reflects a possible reduction in the number of spermatogonia.

In the patients in which a treatment is not recommended, either medical or surgical, or after the failure of these, the assisted reproductive technologies (ART) offer a very valuable resource. The ART aim at facilitating the interaction

of the male and the female gametes and therefore to allow the generation of a zygote, achieving a proper fertilization.
Homologous intrauterine insemination is the easier and cheapest technique, but its performance is usually poor, even being very strict in their indications. We do not recommend it in woman over 38, and preferably the limit should be set in 35 years. A relatively sufficient seminal quality is also required, with a lower limit of hyperactive motile sperm of 3 million after the selective capacitation, but with an optimal number of 5 millions. However, it is not recommended to make 3–4 attempts after which it is advised to perform an in vitro fertilization (IVF).
The conventional IVF is a procedure that is in addition to being therapeutic it also can be diagnostic. It allows observing the interaction between the gametes and to detected defects at fertilization, while assessing the resulting embryo quality. The intracytoplasmic sperm injection (ICSI) is a variant of the IVF. ICSI is used when very low sperm counts are present or with a prior unexplained failure of fertilization in IVF.
Present proteomic, genomic and other molecular studies of sperm are opening promising horizons in the detection of novel diagnostic and prognostic biomarkers 'which have the potential to further help these males.

IOP6

Standard diagnostic work up in erectile and ejaculatory dysfunction

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Erectile dysfunction and premature ejaculation are the most prevalent sexual dysfunctions in males.
Erectile dysfunction is defined as inability to obtain and maintain an erection sufficient for sexual activity which often means penetrating intercourse. Important ethological factors include vascular function and conditions that compromise this, endocrine status, CNS function and integrity of nerve supply to the penis (efferent as well as afferent) and integrity of the cavernous tissue. Sexual history should include current and previous sexual relationships, emotional status and if possible partners sexual health. Previous consultations or own initiatives (including use of remedies, prescription drugs, devices etc.) and onset, duration and consistency of ED. Rigidity and duration of rigidity with sexual stimulation with partner, masturbation and the presence of morning erections. The erection harness score can be used to classify rigidity. Clear analysis of arousal, ejaculation and orgasm in order to identify related problems. Depression should be evaluated evt. use of a depression questionnaires. The International Index of Erectile Function (IEEF) is a validated instrument that assess sexual domains.
Laboratory testing assess etiological factors and include: blood-sugar (HbA1c), lipid profile, and Serum testosterone (morning in fasting state). Other lab tests if needed based on medical history.
Physical examination focuses on genitourinary, endocrine, cardio-vascular and neurological function. Blood pressure (if not known) is important to measure, palpation of penis (Peyronie's), testis (size and consistency) and in men >40 years prostate (nodules, inflammation, enlargement).
Cardiovascular disease (CVD) and ED share many risk factors and ED is common in men with CVD and should not be overlooked.
Premature ejaculation is not very well understood and no specific underlying disorders have been identified. Diagnosis of PE suffers from lack of consensus and there are four different definitions. All definitions include time to ejaculation (latency after penetration), inability to control ejaculation and distress. PE can be classified into life-long with an onset from the first sexual experience or acquired where PE has developed gradually or with a sudden onset. Latency time seem shorter in the primary form. There are also two other PE syndromes; natural variable PE with variable occurrence of rapid ejaculation (a normal variation in sexual performance) and Premature – like ejaculatory dysfunction which is a perceived PE while the latency time fall in the normal range.
Diagnostic work-up is based on medical history and a self-reported latency time persistently <1–2 min. Laboratory work up should include thyroid and gonadal hormones, other assessments based on specific findings. It is important to assess the presence or absence of ED as some mend with ED develop PE, supposedly due to increased anxiety. There are no specific physical examinations suggested but urinary tract infections and Peronei's disease should be taken into consideration. A multidimensional medical and sexual history taking with assessment of latency time, perceived control, distress and interpersonal difficulties approach constitutes the diagnostic platform.

References

Jern *et al*. J Sex Med 2012; 9: 866–72; Mulhall *et al*. J Sex Med 2007 4: 1626–34; Rosen *et al*. Urology 1997 49: 822–30; Waldinger *et al*. Int J Impot Res 2004; 16: 369-81; Whooley *et al*, J Gen Intern Med 1997 12: 439–45.

INVITED TALKS: ECA MAIN PROGRAM

PLENARY 1

IOP7

Testicular cancer: fate or bad luck

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Testicular cancer is primarily manifested as germ cell tumours (TGCT), occurs predominantly in young adult men, and is considered a developmental disease. The most commonly accepted hypothesis concerning the pathogenesis of TGCT postulates a three-hit scenario. First, a disruption of the cross-communication between developing germ cells and their somatic niche leads to the differentiation arrest of germ cells at the stage of gonocyte, which retains a high expression of pluripotency genes. Among the disrupted pathways, the androgen signalling, the SRY function, the TGF-beta pathways and the sex-dimorphic mitosis-meiosis switch have been identified. Secondly, the surviving arrested gonocytes rapidly proliferate after puberty, gradually acquire genomic changes and transform into malignant testicular carcinoma in situ (CIS). Thirdly, CIS progresses to invasive tumours, either seminoma or one of the heterogeneous types of nonseminoma. The aetiology of TGCTs remains to be elucidated. The rising incidence of testicular cancer and other phenotypes related to testicular dysgenesis (TDS) points at environmental/lifestyle factors that target gonadal development. Among external factors that may increase risk of testicular cancer, only a few have been implicated, e.g. exposure to some endocrine disrupters (organochlorine pesticides and polychlorinated biphenyls), cannabis smoking, heat and fire fighting. On the other hand, testicular cancer has a strong hereditary component, with substantial familial risk. Recent genome-wide and targeted studies identified a group of genes strongly associated with a risk of TGCT. The susceptibility genes nearly exclusively operate within pathways regulating sex differentiation and germ cell development/survival (DMRT1, KITLG, BAK1, SPRY4), sex steroid hormone action (ESR2, CYP19A1, CYP3A4) as well as some epigenetic modifications. Genomic variation and modulation by epigenetic factors combined with different level of exposure to environmental factors may explain the individual- and population-level differences in the prevalence of TGCT.

ECA 1. SEXUAL DEVELOPMENT AND GENDER IDENTITY

IOP8

Sex differentation and the brain: relation to gender identity, sexual orientation

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Functional sex differences are expressed from early childhood onwards, e.g. in our interest, playing behaviour and drawings. Sex differences in cognition, reproduction, gender identity (the feeling to be male or female), sexual orientation, paedophilia and the incidence of neurological and psychiatric disorders are thought to be based upon structural and functional sex differences in the brain. Many of such sex differences have now been described also in the human brain. The field is becoming extra complex by the presence of splice variants of estrogen receptors and the local production of steroid hormones (neurosteroids) in the human brain. This means that sex differences may be expected in many functions in all stages of life, in heath as well as in disease.

The sex differences in the brain arise during development by an interaction of sex hormones and the developing neurons, although direct genetic effects are probably also involved. Factors influencing structural en functional sex differences in the brain are genetic factors like mutations or polymorphisms in the sex hormone receptors, abnormal prenatal hormone levels and compounds that interact with the action of sex hormones on the brain during early development such as anticonvulsants, DES and environmental endocrine disrupters. An influence of postnatal social factors on gender identity or sexual orientation has so far not been established. In rodents, masculinisation of the brain in development is due to estrogens that are formed by aromatization of testosterone. Sexual differentiation of the human brain in relation to gender identity and sexual orientation takes place by direct effects of testosterone as is clear from people with mutations in the androgen receptor, estrogen receptor or in the aromatase gene.

In transsexual people we observed a reversal of the sex difference in the central nucleus of the Bed Nucleus of the Stria terminalis (BSTc). The size, type of innervation and neuron number agreed with their gender identity and not with their genetic sex. Data of persons with abnormal sex hormone levels show that the sex reversal of the BSTc can not be explained by alterations in adult hormone levels. Also the volume of the hypothalamic nucleus, the INAH3, was two times larger in control males than in females and contained 2.3 times as many cells. MtF transsexual people had an INAH3 volume and neuron number similar to that of the control females. A group of castrated men was included as a control for the effect of changes in adult hormone levels.

Various brain differences related to sexual orientation and paedophilia have now also been reported. In addition, there are sex differences present in the way the brain ages and in Alzheimer neuropathology (Swaab, 2014, Random House, USA, Penguin, UK).

IOP9

Hormonal treatment in gender dysphoria

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Gender dysphoria (GD) is a condition in which a person experiences discongruency between their assigned sex and what they feel their genderidentity is. A person with gender dysphoria experiences persistently uncomfortable feelings about their birth gender (Diagnostic and Statistical Manual of Mental Disorders, Fifth edition (DSM-5) (American Psychiatric Association). During the 'real life experience' hormonal treatment starts and applicants are required to live socially in the desired gender role before irreversible surgical reassignment is considered. Cross sex hormonal treatment is desired by transsexual persons to help them successfully live as a member of their identified gender. It is clear that both in adults and adolescents the decision for starting hormonal treatment in transssexualism is not to be made by the endocrinologist. The mental health professionals (psychiatrists and/or psychologists), by preference working in a multidisciplinary Gender team, will guide these persons to make an informed decision about hormonal treatment. Eligibility criteria and readiness as described by WPATH's Standards of Care for GID-7th version, should be evaluated. The goal of treatment in female-to-male transsexual persons is to induce virilization and to stop menses. The prinicipal hormone treatment is a testosterone preparation. In male-to-female transsexual persons oestrogen and anti-androgen treatment is provided. Treatment regimens are currently not standardised and include various forms of oestrogens, progestins, and/or (anti-) androgens as reported by different clinical centres. So far, no randomized intervention trials are available so treatment is largely experience-based.

The European Network for the Investigation of Gender Incongruence (ENIGI) aims at a thorough biopsychosocial description of a large number of transgender clients, both before and during hormonal intervention. It is timely that transgendercare evolves from experience- to evidence-based. Appropriate care for transgender persons will lead to better outcome and should avoid unnecessary psychological pain, health risks (e.g. secondary psychiatric conditions or suicide), or self medication with inherent greater risk of complications. Gender variance (only role change, or only hormonal intervention), transgender care in adolescents (by means of puberty blocking) and attempts to guarantee fertility are developing scientific field (G. T'Sjoen *et al*. Eur J Endocrinol 2013).

IOP10

Long term outcome of gender dysphoria

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Gender identity is the personal sense of being a female or male or a gender non confirming identity. Gender incongruence; denotes the discrepancy between the gender identity and a persons' sex assigned at birth. Gender dysphoria denotes the distress caused by the gender incongruence. For some people, the level of distress meets criteria of Transsexualism, ICD-10 (WHO 1992), or Gender Dysphoria, DSM-5 (APA 2013). The prevalence of transsexualism is unknown and estimates varies from 1/3000 to 1/30 000. The aetiology of transsexualism remains unknown. Atypical levels of perinatal sex steroids during a critical period of brain development has been suggested, and there are unsubstantiated claims of psycho-social factors (Burke 2014, Berglund 2008, Swaab 2007). The international Standards of Care for transgender individuals include gender confirming treatment (GCT) with cross-sex hormones, hair removal, speech therapy and surgical corrections to aid changes of primary and secondary sex characteristics, and a change of juridical gender (WPATH 2011). The primary aim of interventions is to ease gender dysphoria and create more congruence. A meta-analysis shows 78–80% improvement of gender dysphoria, psychological/ psychiatric symptoms and quality of life after GCT. The incidence of suicide attempt rates do decreased after sex reassignment but remain higher than the reference population (Murad 2010). Later studies confirms treatment related amelioration of gender dysphoria. (Johansson 2010, Wierckx 2011, Gooren 2011, Gorin-Lazard 2012, 2013, Gomez-Gil 2012, Heylens 2014).The elevated suicide and depression rates are also confirmed and seem to be correlated to minority stress and gender related abuse (Blosnich 2011, Nuttbrock 2010, 2013, Clements-Nolle 2006, Bockting 2013, Hoffman 2014). Oestrogen treatment is safe providing use of parentral estradiol and avoiding derivatives. There is an increased mortality in transwoman related to non-hormonal-related causes such as suicide, AIDS, cardiovascular diseases and drug abuse (Elamin 2010, Asscheman 2011, Dhejne 2011, Wierckx 2014, Gooren 2014). The study by Dhejne et al also disclosed an elevated mortality in transmen.

Most follow-up studies suffer from short observation time, high drop-out rates, selections bias and lack of control group. Therefor we conducted a population-based matched cohort study using Swedish registries during 1973–2003. The overall mortality was higher during follow-up (adjusted HR [aHR]) 2.8; 95% CI 1.8–4.3); particularly death from suicide (aHR 19.1; 95% CI 5.8–62.9. If separated depending on when sex reassignment was performed, during the period 1973–1988 or 1989–2003, the overall mortality didn't reach statistical significance for the period 1989–2003. Mortality due to cardiovascular disease was moderately increased among the sex-reassigned, whereas there was no difference in risk for malignancies. Sex-reassigned persons had an increased risk for suicide attempts (aHR 4.9; 95% CI 2.9–8.5) and psychiatric inpatient care (aHR 2.8; 95% CI 2.0–3.9) compared to controls.

Conclusion: Individuals with transsexualism, after sex reassignment, have a higher risks for psychiatric morbidity, suicidal behavior, and premature death than the general population. These suggests that gender confirmation treatment alone isn't sufficient and prompt for improved and continued access to psychiatric and somatic care for this group.

PLENARY 2

ASA EXCHANGE LECTURE

IOP11

From the epydidimis to the egg

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Fertilization is a key process to the development of a new individual. However, the molecular mechanisms underlying sperm-egg interaction still remain to be elucidated. For the past twenty five years, our laboratory has been dedicated to underpin the molecular mechanisms involved in mammalian sperm-egg interaction using CRISP proteins as model molecules. Epididymal protein CRISP1, identified by our laboratory, is the first described member of the evolutionarily conserved CRISP (Cystein-Rich Secretory Protein) family with members present mainly in the male reproductive tract. Substantial evidence from our laboratory obtained using in vitro assays and knockout models shows that epididymal CRISP1 associates with the sperm surface during maturation and participates in both sperm-zona pellucida interaction and gamete fusion through its binding to complementary sites in the egg. These observations can be extended to human as judged by our findings showing that the human homologue of the rodent protein (hCRISP1) is also involved in both stages of fertilization. Interestingly, recent results using knockout mice revealed that CRISP1 is also expressed along the female tract including the cumulus cells that surround the egg and that this female CRISP1 protein plays an important role in cumulus penetration by modulating the functional status of sperm. Together, our observations indicate that CRISP proteins escort both the male and the female gamete and are multifunctional proteins playing key roles during the fertilization process. We believe these results not only contribute to a better mechanistic understanding of sperm-egg interaction but also support CRISP proteins as excellent targets for future research on infertility and contraception.

ECA 2. MALE SEXUALITY

IOP12

PDE5i or testosterone: which is the first in andrological clinical practice

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In general clinical practice, the initial choice of treatment is one of the most controversial issues. Different backgrounds may dramatically influence early management of the patient. Surgeons, for example, may find it less time consuming and more effective to remove unhealthy tissue as early as possible. In contrast, internists usually prefer to give patients the chance to recover with the help of pharmacological treatments before attempting more invasive therapies. Finally, psychologists may argue that the first approach to a symptom or a disease should be based on *vis medicatrix naturae* and on the ability of talking therapy to induce self-cure. Despite the excellent production of guidelines and standard operating procedures by the involved scientific societis, this never-ending debate is also ongoing vigorously in the field of sexual medicine.

Science, experience and and clinical reasoning suggest: (i) all (not just some) patients with erectile dysfunction (ED) should be evaluated for testosterone levels before any therapy. Although evaluation of the endocrine profile has been suggested solely in patients with comorbid hypoactive sexual desire, this is a mistake for two reasons. Sexual thoughts and fantasies are lost in very deep (and relatively rare) hypogonadism, that is generally associated with several physical signs and symptoms that affect general well-being. In contrast, although deserving of medical attention and treatment, late onset hypogonadism (LOH) is not always characterized by such low testosterone concentrations. (ii) Hypogonadism should be first treated with hormone replacement. Many patients may recover from sexual symptoms with testosterone alone, without the need for PDE5i. Furthermore, while PDE5is should be considered a symptomatic treatment, testosterone replacement in the hypogonadal subject is a typical etiologic therapy, directly addressing the cause of the disease. In fact, testosterone cures the disease, while a PDE5i cures the symptom. Physicians generally agree that etiologic therapies should be preferred over symptomatic therapies. (iii) It is good clinical practice to proceed from mono- to multiple therapy. Hence, it should also be considered good medical practice to use testosterone alone initially, adding a PDE5i only in patients whose hypogonadism is resolved but whose ED still remains. Finally (iv), several reports demonstrate that the enzyme PDE5 is under testosterone control and that normal testosterone levels are needed for PDE5is to be fully effective. If this is true, why use a PDE5i before testosterone levels have been normalized?

In conclusion, the answer to the question 'which first' is controversial in almost all male sexual dysfunctions and even more so in female sexual dysfunctions, which are both less studied and have fewer therapeutic options. Intuition, experience and available evidence should guide the choice of which treatment to use first. This decision is

highly critical in influencing the therapeutic outcome as well as the patient's and couple's adherence.

IOP13

A new view on lifelong premature ejaculation: erectio praecox, ejaculatio praecox and detumescentia praecox

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Animal research and human pharmacological research of lifelong premature ejaculation (PE) has much contributed to a better understanding of the role of the central and peripheral nervous system in mediating ejaculation. Research of genetic polymorphisms in men with lifelong PE and research of the validity of the classification of PE into four PE subtypes has provided better insight into lifelong PE and its distinction from the three other PE subtypes. Nevertheless, a number of symptoms of lifelong PE and its treatment by SSRIs are still not well understood. It will be argued that lifelong PE is not only characterized by early ejaculations (ejaculatio praecox), a diminished control over ejaculation, and negative personal consequences, but also by early erections (erectio praecox) and an immediate detumescence of the penis after ejaculation (detumescentia praecox) as symptoms of a (sub)acute hypertonic or hypererotic physical state when making love. It is postulated that facilitated erection, facilitated ejaculation and facilitated penile detumescence are associated with centrally and peripherally increased oxytocin release. Research into these three praecox characteristics of the (sub)acute hypererotic state will contribute to a better phenomenological description of and to a better neurobiological understanding of lifelong PE and its delineation to the three other PE subtypes.

Reference: Waldinger MD. Ejaculatio praecox, erectio praecox, and detumescentia praecox as symptoms of a hypertonic state in lifelong premature ejaculation: a new hypothesis. Pharmacol Biochem Behav 2014; 121: 189–94.

IOP14

Hypersexuality

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Background: Hypersexual Disorder; HD was proposed as a new diagnostic category for the DSM-5, but rejected. However, in clinical settings patients are acknowledged that suffer from compulsive masturbation, excessive pornography-use or in repeated initiations of short-term sexual relationships. According to the formerly proposed HD diagnostic criteria, excessive sexual behaviors can be seen as attempts to ameliorate negative mood-states like stress, depression or anxiety.

Objectives: To apply the psychometric properties of the hypersexual disorder screening inventory (HDSI), and describe differences in hypersexual behaviour (specifiers) between women and men and further, to describe the distributions of paraphilia in this sample.

Method: Data were gathered from men and women, self-identified as hypersexual, recruited by advertisement in a large circulating newspaper and responded through online administration of the Hypersexual Disorder Screening Inventory (HDSI), (APA: s suggested diagnosis for DSM5), and indicators of sexual paraphilia (self-reported sexual interest, SSI following APA: s DSM-IV-TR), MADRS and Core-OM. Sixty-four men and 16 women, aged 19–61 perceived themselves as hypersexual. The participants agreed to take part in a study on hypersexual behaviour and were offered assessment for possible participation in treatment for hypersexual disorder.

Results: Fifty percent of the sample fulfilled the diagnostic criteria's for Hypersexual Disorder (HD) according to HDSI (men 48% and women 62%). The vast majority (97%), of HD-men reported uncontrolled pornography-consumption while 50% of the women reported this. Most prevalent hypersexual behaviour among women was sex with consenting adults (90 and 83%, clinical and sub-clinical group respectively). In the whole sample 92% had at least mild depressive symptoms during the last three days. Those who fulfilled the criteria for HD reported significantly ($p < 0.05$) lower psychiatric well-being compared to those who did not fulfill criteria and for the whole sample women were significantly ($p < 0.05$) more distressed than men. Among all respondents 80% self-reported some kind of paraphilic interest, voyuerism and exhibitionism most commonly reported (45 and 21% respectively), followed by fetishistic (19%) and pedophilic interest (13%). Two-thirds of those with hypersexual disorder had at least one sexual paraphilic interest, significantly more common ($p < 0.05$) among those with a hypersexual disorder than in the non-disorder group.

Conclusion: In clinical settings clinicians seeing patients with Hypersexual Disorder should assess sexual paraphilic behaviour, psychiatric co-morbidity and acknowledge gender differences.

ECA 3. PROSTATE: NEWS AND VIEWS

IOP15

Associations between prostate pathologies and sexual dysfunction

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Prostate pathologies comprise benign prostatic hyperplasia, prostate cancer and the prostatitis syndrome. All three of them are frequent diseases, sometimes affecting men in their reproductive age. Most patients are however afflicted by the negative implications on sexual function. The andrological implications are heterogenous and described as decreased male fertility, sexual dysfunctions and endocrinological alterations. Sexual dysfunction includes different components such as erectile, ejaculatory, orgasmic and sexual desire dysfunctions. There is also a significant negative impact of medical treatments in these entities on male sexual function.

In benign prostatic hyperplasia an association to erectile dysfunction has been noted since several years. Common pathways in this development amenable to targeted medication are currently investigated. This is also reflected in the recent evaluation of PDE-5 inhibitors for treatment of benign prostatic hyperplasia. Medical treatment in benign prostatic hyperplasia, such as alpha-blockers and 5-alpha reductase inhibitors have been shown to be significantly associated with ejaculatory dysfunction.

Erectile function is one of the trifecta outcomes of curative treatment strategies in prostate cancer. Erectile dysfunction is therefore also one major cause of reduced quality of life after prostatic surgery for prostate cancer. Hormone ablative treatments severly affect male sexual health, amongst others.

In the prostatitis syndrome impaired sperm quality parameters have been demonstrated. Functional sperm disorders, such as acrosomal malfunction have also been attributed to chronic prostatitis syndromes, probably due to the formation of reactive oxygen species. Sexual dysfunction in chronic prostatitis is a very frequent phenomenon and adds to the number of positive symptom phenotypes and correlates therefore with increasing symptom scores in patients with chronic prostatitis syndromes. The prevalence of impaired sexual desire ranges from 6 to 70%, of erectile dysfunction from 23 to 40%, of premature ejaculation from 10 to 67%, of painful ejaculation from 37 to 65% in studies assessing patients with chronic prostatitis syndrome. Orgasmic dysfunction has not been specifically assessed in studies. However, prospective interventional studies on the role of sexual dysfunctions are missing, as up to now sexual dysfunction has not been addressed as outcome variables in most interventional studies in prostatitis syndrome.

In summary benign prostatic hyperplasia, prostate cancer and the prostatitis syndrome are frequent disease entities and are also frequently associated with sexual dysfunctions, which has only more recently been addressed by researchers and practicing urologists as important disease and treatment outcome variables.

IOP16

Benign Prostatic hyperplasia: a new metabolic syndrome-related disease

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People are living longer and, in some parts of the world, healthier lives. In 2006, almost 500 million people worldwide were 65 and older. By 2030, that total is projected to increase to 1 billion—1 in every 8 of the earth's inhabitants. Significantly, the most rapid increases in the 65 and older population are occurring in developing countries, which will see a jump of 140% by 2030. Hence, we must proactively face the health issues of the elderly. BPH/LUTS represent significant bother among aging men; they were historically considered as a 'normal' consequence of the aging process and, as such, their negative effects on men's well-being only dealt with through medical or surgical intervention. This view has been challenged in the last decade and now BPH/LUTS are seen more as preventable than inexorable ailments of the elderly.

Evidence presented indicates that several modifiable metabolic factors play a role in the determinism or progression of LUTS/BPH. A constellation of hormonal, inflammatory and metabolic derangements might have a permissive and /or causative role in determining BPH. MetS, and in particular dyslipidaemia and hyperinsulinaemia, could induce or maintain an inflammatory state within the prostate that could even be exacerbated by a relative hyperestrogenism or by androgen deficiency, medical conditions often associated to MetS and in particular to increased waist circumference (WC). An overt, or even a subclinical, bacterial or viral infection could induce a prostatic inflammation (first hit) that could be auto-sustained or exacerbated by the presence of an altered metabolism and in particular by hypercholesterolemia (second hit). Hypogonadism and/or hyperestrogenism could act as a third hit, favouring the maintaining of these inflammatory state, the overexpression of lipoprotein receptor (LOX-1) and the relative prostatic reaction. The combined action of all the three hit, or even of two of them, may result in overexpression of TLR, transformation of prostatic cells in antigen presenting cells and activation of resident human (PALT or chemoattracted CD4+ T lymphocytes (Th1/Th17) ending in overproduction of growth factors which, in turn, will induce prostate remodelling and further prostate enlargement.

BPH/LUTS may be therefore viewed as a complex disorder that also involves a metabolic component that may begin early in the life of the male, and, although asymptomatic, it is likely detectable even in the early stages of the disease. The mechanisms underpinning the relationship between MetS and prostate inflammation are likely to be similar in young and old men but chronic exposure to elevated inflammation, along with low T/high 17βE2, may contribute to BPH in the long term. Preventing the development of the disease even from the asymptomatic phase should be the basis for designing a resilient program of elderly healthcare. Analysis of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort has clearly demonstrated that the adverse CV effects of

having MetS on coronary heart disease could be substantially reduced or nullified by increasing physical activity. Although the three hit hypothesis is attractive, intervention studies are needed in order to prove that treating dyslipidaemia early on will result in halting further progression of prostate enlargement and LUTS. Several epidemiological studies support this view also for BPH/LUTS; intervention studies are urgently needed.

IOP17

Face to face on prostate cancer screening: PSA vs. novel biomarkers

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The European Randomized study of Screening for Prostate Cancer (ERSPC, www.erspc-media.org) has shown that with systematic PSA based screening as compared to a situation where PSA testing is uncommon, suffering from metastatic prostate cancer (PC) and disease specific mortality can be reduced with app 40–20% respectively. This is good news. However, the coinciding unnecessary testing (i.e. prostate biopsies in men who are actually at low risk having PC) and over diagnosis (diagnosis of a PC that without PSA screening would never become apparent) are important drawbacks of PSA based screening (Schroder *et al*. N Engl J Med 2012; 366: 981–90; Schroder *et al*. Eur Urol 2012; 62: 745–52).

The mainstay of PC early detection, i.e. the PSA test is however not PC specific. As a result, many studies focus on risk stratification with the goal to improve the identification of those men that are at risk of having a potentially life threatening PC who may be cured by early detection and treatment (Roobol *et al*. Nat Rev Urol 2013; 10: 38–48). Several studies have shown that PSA can be predictive for potentially aggressive/lethal PC decades before the actual diagnosis. Used in this way PSA can risk stratify men at younger age to being low risk to reduce (omit) future testing. On the other hand the frequency of future testing can be increased for those considered at high risk (Vickers *et al*. World J Urol 2012; 30: 131–5).

PSA based risk stratification (i.e. predicting the current chance of a biopsy detectable PC) mainly focusses on including other clinical parameters like e.g. the outcome of a digital rectal examination (DRE) in the decision for further assessment. PSA is closely related to the volume of the prostate (except of course in situations like e.g. prostatitis). This means that risk stratification on the basis of the PSA test should entail inclusion of information on prostate volume. This can be done in several ways. The most obvious way is to measure prostate volume with the use of a trans rectal ultrasound (TRUS) and correct the elevated PSA level with the measured prostate volume (PSA density). Nomograms including next to e.g. age and DRE outcome the TRUS measured prostate volume outperform predictions based on PSA alone or with DRE (Roobol, *et al*. Eur Urol 2010; 57: 79–85) (Roobol *et al*. World J Urol. 2012 2: 149–55).

Potential drawback of such an approach is that an invasive procedure is needed before the decision to biopsy or not. This can be avoided by estimating the volume of the prostate on the basis of a DRE. This relatively rough estimate is still capable to increase predictive capability and circumvents the need of an invasive procedure before biopsy. PSA and volume based nomograms can be improved by adding more relevant information like PSA sub forms or other biomarkers. This has been done, however the added value, maintaining high sensitivity is in general limited (Vedder *et al*. Eur Urol, 2014; in press; Ankerst *et al*. J Urol. 2008 180: 1303–8).

The serum PSA test is well-known by its users. The assay is well developed, stable and in addition it is cheap, with high quality test results available within hours. The poor predictive capability in the decision to perform a biopsy or not can be improved by a correction for prostate volume. This so-called PSA density is one of the strongest predictive factors, not only in predicting biopsy outcome, but also in predicting the nature of the prostate cancer (Steyerberg *et al*. J Urol 2007 177:107–12).

IOP18

Face to face on prostate cancer screening: PSA vs. novel biomarkers

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Prostate specific antigen (PSA) is recognized as an organ specific marker, with low specificity and sensitivity in discriminating prostate cancer (PCa) from other benign conditions, such as prostatic hyperplasia or chronic prostatitis. Thus, if there is the clinical suspect, PCa diagnosis cannot be done without prostate biopsy. [-2]proPSA (p2PSA), a precursor of PSA, has been investigated as a new marker to accurately detect PCa. A growing body of data is now available about the clinical validity and utility of p2PSA and its derivatives, p2PSA/fPSA (%p2PSA) and Prostate Health Index (PHI). In order to make the point on the current status of PHI, a systematic search of PubMed and Scopus electronic databases was performed in accordance with the PRISMA statement (http://www.prisma-statement.org/), considering the time period from January 1990 to January 2014 and looking for the following search terms: pro-prostate specific antigen, proenzyme PSA, proPSA, [-2]proPSA, p2PSA, prostate health index and PHI. To date, 115 studies were published, but only 35 were finally considered for the qualitative analysis. These studies suggest that p2PSA is the most cancer-specific form of PSA, being preferentially expressed in PCa tissue and being significantly elevated in serum of men with PCa. It is now evident that p2PSA, %p2PSA and PHI measurements improve the specificity of the available tests (PSA and derivatives) in detecting PCa. It has been showed in different populations such as men with a tPSA between 2 and 10 ng/mL, obese patients (BMI > 30) and men younger than 60 years. Moreover increasing PHI levels seem to correlate with more aggressive diseases. Some studies compared p2PSA and derivatives with other new biomarkers, finding that the firsts were significantly more accurate. Indeed their implementation in clinical practice has the potential to significantly increase physicians ability to detect PCa and avoid unnecessary biopsies, having also an effective impact on costs.

ECA 4. BONE TESTIS CROSS TALK

IOP19

Vitamin D and male reproduction

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Vitamin D is a versatile signalling molecule with a well-established role in the regulation of calcium homeostasis and bone health. The spectrum of vitamin D target organs has expanded and the reproductive role is highlighted by expression of the vitamin D receptor (VDR) and vitamin D metabolizing enzymes in the gonads, reproductive tract and human spermatozoa. The expression level of VDR and CYP24A1 in human spermatozoa serves as a positive predictive marker of semen quality and VDR mediates a non-genomic increase in intracellular calcium concentration that induces sperm motility. Interestingly, functional animal models showed that vitamin D is important for estrogen signalling and sperm motility, while cross-sectional human studies supported the positive association between serum 25-hydroxyvitamin D level and sperm motility in both fertile and infertile men. Vitamin D is also a suggested regulator of insulin secretion, aromatase and AMH and may thus exert its influence on gonadal function indirectly through these endocrine factors. Expression of VDR and enzymes that metabolize vitamin D in fetal testis indicates a yet unknown role during development, which may be extrapolated from invasive testicular germ cell tumours where 1α,25-dihydroxyvitamin D induces a mesodermal differentiation of the pluripotent testicular cancer cells. Taken together, vitamin D signalling has a positive effect on semen quality, increases estrogen responsiveness and may induce differentiation of germ cells. Future studies are needed to determine when 1α,25-dihydroxyvitamin D acts in a paracrine manner and whether systemic changes, which are subject to pharmacological modulation, could influence male reproductive function.

IOP20

Testis versus bone

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Until a few years ago, the only connection between testis and bone was the well-known action of testosterone on skeletal growth and bone mass accrual and, consequently, the role of hypogonadism in causing low bone mass and osteoporosis. This view is reflected by the common opinion, also highlighted in clinical guidelines, that the diagnosis of male hypogonadism in general and in men with osteoporosis in particular can be made solely by the determination of testosterone levels. Hypogonadism is a common secondary cause of male osteoporosis. Nevertheless, men with mild testicular dysfunction are also at increased risk of osteopenia and osteoporosis, and testosterone replacement therapy alone does not completely restore bone mass in men with hypogonadism and osteoporosis. Basic science and clinical findings in the past few years now provide new information on the crosstalk between testis and bone function and could contribute to defining an improved clinical approach to the biochemical diagnosis and therapeutic management of both hypogonadism and male osteoporosis. This story seems to be focused on the functions of the Leydig cells of the testis. Other than being responsible for steroidogenesis and production of testosterone, the function of these cells is fundamental to bone health in at least two other ways: Leydig cells produce insulin-like 3 (INSL3), which has a role in osteoblast function, and they contribute to 25-hydroxylation of vitamin D.

INSL3 is a peptide hormone produced under the long-term regulatory effects of LH and it is increasingly used as a marker of Leydig cell function, as an alternative or in addition to testosterone. In men and mice, INSL3 regulates bone metabolism by modulating the activity of osteoblasts. Interestingly, low levels of INSL3 are observed in many conditions characterized by disturbed Leydig cell function, such as infertility, obesity and Klinefelter syndrome, as well as in ageing.

Leydig cells express the CYP2R1 gene, which encodes the major enzyme involved in 25-hydroxylation of vitamin D. Population studies and studies form our group showed an association between the levels of testosterone and 25-hydroxyvitamin D in men and demonstrated that the expression of CYP2R1 is regulated by LH and reduced also in the presence of testicular dysfunction.

Therefore, impaired INSL3 production and CYP2R1 expression (which leads to low levels of 25-hydroxyvitamin D) might contribute to increase the risk of low BMD, osteopenia and osteoporosis. Interestingly, INSL3 and 25-hydroxyvitamin D levels are lower than normal not only in cases of overt hypogonadism but also in cases of subclinical hypogonadism (normal testosterone, high LH), a condition that is also associated with risk of low BMD. These data suggest that INSL3 and 25-hydroxyvitamin D levels might be additional markers of Leydig cell function (other than testosterone), and that the influence of testicular function on bone metabolism is more intricate than was recognized until a few years ago.

'ANDROLOGY' AWARD CEREMONY

IOP21

Hormone suppression with GnRH antagonist promotes spermatogenic recovery from transplanted spermatogonial stem cells in irradiated cynomolgus monkeys

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Hormone suppression given before or after cytotoxic treatment stimulates spermatogenesis from endogenous and transplanted spermatogonial stem cells (SSC) and restores fertility in rodents. To test whether the combination of hormone suppression and transplantation could enhance the recovery of spermatogenesis in primates, we irradiated (7 Gy) the testes of 12 adult cynomolgus monkeys and treated 6 of them with GnRH-antagonist (GnRH-ant) for 8 weeks. At the end of this treatment, we transfected cryopreserved testicular cells with GFP-lentivirus and autologously transplanted them back into one of the testes. There was a moderate acceleration of endogenous spermatogenic recovery by GnRH-ant treatment alone. Although transplantation alone after irradiation did not significantly increase the percentage of tubules containing differentiated germ cells (tubule differentiation index; TDI), lentiviral DNA from the transplanted cells was detected in the sperm of one radiation-only monkey. However, the combination of transplantation and GnRH-ant clearly stimulated spermatogenic recovery in testes of GnRH-ant-treated monkeys receiving transplantation as evidenced by several observations: (i) significant increases in the volume and weight of the testes compared to contralateral sham-transplanted testes and/or to the transplanted testes of the radiation-only monkeys; (ii) increases in TDI compared to the transplanted testes of radiation-only monkeys; (iii) detection of lentiviral sequences in the sperm or testes of five of the GnRH-ant–treated monkeys; and (iv) significantly higher sperm counts than in the radiation-only monkeys. Thus spermatogonial transplantation in conjunction with hormone suppression enhances spermatogenic recovery in primates and may be a useful tool to restore fertility in men after cancer treatment.

PLENARY 3.

IOP22

The bright side of the aging male gamete: the telomere length

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The age of fatherhood is rising in modern societies. This trend has considerable ramifications with respect to the health outcomes of offspring of older fathers. The harmful, although relatively rare, disorders attributed to the effect of older paternal age are well known and have received considerable medical and public attention. Less known, however, is the following: older men are more likely than their younger counterparts to father a child with long telomeres, as expressed in leukocyte telomere length. This observation suggests that offspring of older fathers might live longer and display resistance to atherosclerotic cardiovascular disease, given that short leukocyte telomere length is associated with diminished longevity and atherosclerosis in adults. (The potential trade-off, however, might be increased risk to some types of cancer associated with inherently long leukocyte telomere length, e.g. melanoma). Although the underlying mechanisms of the effect of older paternal age on the offspring's leukocyte telomere length are not well understood, they have been attributed to the fact that the DNA of sperm of older men is altered in a way that makes their telomeres longer, perhaps due to increased telomerase activity in the male germ stem cells. Thus, while telomere length undergoes progressive age-dependent attrition in somatic tissues, it apparently becomes longer in the male germ line. The longer telomeres in sperm are then inherited by the offspring based on ordinary Mendelian principles. Our recent work points to an age-dependent germ stem cell selection as a potential explanation for the longer telomeres in sperm of older men.

ECA 5. IMAGING IN ANDROLOGY

IOP23

Imaging of the male genital tract, 2014 update

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With the newer advances in technology, in terms of image quality and resolution, color-Doppler ultrasound (CDUS) is widely used in the assessment of male reproductive problems, male genital tract (MGT) abnormalities, male accessory gland infection/inflammation, scrotal or chronic pelvic pain, testicular or prostate malignancy. Hence, CDUS imaging of MGT has a relevant impact on reproductive and overall man's health. Scrotal CDUS has been commonly used for several years, and only recently transrectal US (TRUS) has assumed growing importance extending the examination to the prostate-vesicular region. Physical examination of the scrotal region is usually informative enough in the work-up of the male patient in everyday clinical practice. However, scrotal CDUS plays an independent role when physical examination is unreliable (i.e. large hydrocele, inguinal testis, enlarged epididymis) or testicular, epididymal, deferential or pampiniform plexus characteristics must be defined carefully. CDUS is useful in localizing inguinal but not intraabdominal testes and its role is debated in preoperative planning. CDUS may detect signs of testicular damage or testicular dysgenesis (i.e. nonhomogeneous testicular architecture, microcalcifications, cryptorchidism), often related to an impaired spermatogenesis and to a higher risk of malignancy. In addition, CDUS reveals testicular lesions suggestive of malignancy. Although histology remains the only certain diagnostic tool, new imaging techniques, such as contrast-enhanced ultrasound (CEUS) and tissue elastography have improved the characterization of testicular lesions. CDUS may indicate characteristics suggestive of testicular damage in non-obstructive azoospermia, however, it shows poor utility in surgical sperm extraction decision making, since the latter is performed even when small testes or karyotype abnormalities are found. In addition, it is not predictive of sperm retrieval in spermatogenic arrest-associated non-obstructive azoospermia. A decreased testis vascularization is characteristic of testicular torsion, whereas hyperemia is often observed in epididymo-orchitis or in some malignant conditions (i.e. lymphoma, leukemia). Regarding varicocele, its clinical management is mainly based on physical examination. However, CDUS is useful in assessing venous reflux, when palpation is unreliable and/or in detecting recurrence/persistence after surgery. Epididymis head and/or tail dilation is suggestive of MGT obstruction or inflammation and both are related, along with echo-texture abnormalities, to impaired sperm parameters. Scrotal and transrectal US are useful in detecting congenital uni- or bilateral absence of vas deferens (CBAVD), which may be associated with epididymis, seminal vesicles (SV), or kidney abnormalities/agenesis, and suggest more specific examinations (CFTR gene evaluation, urinary tract ultrasound evaluation, surgical sperm extraction). TRUS plays a key role in assessing obstructive azoospermia, detecting distal CBAVD or anomalies related to ejaculatory ducts obstruction, such as ejaculatory duct abnormalities, prostate median cysts or SV enlargement/emptying impairment. TRUS findings lead to operational decision making, such as testicular sperm extraction in the case of CBAVD, cyst aspiration in the case of a large prostatic median cyst, and surgical treatment if ejaculatory duct abnormalities are observed. TRUS may reveal prostate volume reduction (suggestive of hypogonadism) or enlargement, which can be related to aging or even metabolic abnormalities. Finally, TRUS may reveal prostate and SV CDUS abnormalities suggestive of inflammation or SV stasis.

ECA 6. FROM BASIC TO CLINICAL ANDROLOGY

IOP24

Profiling human sperm: from functional studies to omics analyses, and back again

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The human ejaculate is a very heterogeneous biological mixture, containing subpopulations of sperm with distinct properties, and functional abilities. Several properties are needed for a functional mammalian sperm to achieve fertilization, including a morphologically normal structure, motility, mitochondrial activity or the ability to undergo important events such as capacitation and the acrosome reaction, for example. Discrete levels of reactive oxygen species (ROS) have also been proposed as players in the activation of the male gamete. Distinct strategies have been developed to characterize the most functional human gametes within the ejaculate, either by comparing samples from men with clearly distinct spermiogram parameters (Normozospermic vs. Asthenozoospermic samples, for example), or by isolating specific sperm subpopulations with distinct functional properties from a single sample. The latter can be achieved by using classic semen preparations techniques, such as gradient centrifugation and swim-up; or by employing fluorescence assisted flow cytometry coupled with cell sorting (FACS) using a variety of probes that monitor distinct functional sperm parameters. We have focused on several ways to monitor mitochondrial activity using fluorescence microscopy and fluorescence-activated cell sorting in order to determine if mitochondrial function could be employed as a criterion to obtain more functional sperm from a given ejaculate. We first determined that mitochondrial activity correlated with the quality of distinct human samples, from healthy donors to patients with decreased semen quality. Furthermore, using FACS to separate sperm with more active and less active mitochondria we found that this was also true within samples. Indeed, sperm with more active mitochondria defined a more functional subpopulation, which contained more capacitated and acrosome intact cells, sperm with lower chromatin damage, and, crucially, sperm more able to decondense and participate in early development using both chemical induction and injection into mature bovine oocytes. Furthermore, cell sorting using mitochondrial activity produced a more functional sperm subpopulation than classic swim-up. In addition we have also shown that human ejaculates are heterogeneous in terms of mitochondria-specific reactive oxygen species (mROS) production, and that the sperm subpopulation producing the lowest amount of mROS represent the most functional subset of male gametes within the ejaculate. Importantly this subpopulation was clearly more prevalent in samples that gave rise to pregnancies following Assisted Reproduction. Mitochondrial activity and mROS production seem therefore to be able to identify the most functional sperm. But what are the molecular mechanisms underlying these results, and can their study result in new diagnostic tools or therapeutic targets? To address these issues we are currently performing collaborative proteomic and metabolomic studies, by comparing human sperm samples and/or subpopulations with distinct functional abilities. This integrated omics analyses approach will help determine if distinct proteomic characteristics or metabolic pathways can be used to obtain a more detailed signature of human sperm functionality.

IOP25

Intra-testicular cellular dynamics and regulation of Leydig cell function

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Two generations of Leydig cells develop sequentially during fetal and early post-natal life in the mouse. The fetal Leydig cells arise soon after initial testis differentiation and are responsible for masculinisation of the fetus through secretion of testosterone. In the rodent these cells can function normally throughout fetal life in the absence of luteinising hormone (LH) suggesting that they may show high levels of intrinsic activity, although a number of other factors have been shown to stimulate cell activity. The adult Leydig cell population develops in the mouse in early post-natal life and is responsible for the pubertal rise in circulating testosterone leading to development of the secondary male sex characteristics, male fertility and male reproductive behaviour. This Leydig cell population is dependent on LH for activity but the role of other testicular cell types, and particularly the Sertoli cells, in maintaining Leydig cell function is largely unknown. In order to study interactions between the Sertoli cells and Leydig cells we have generated a transgenic mouse model expressing the diphtheria toxin receptor (DTR) on the Sertoli cells. Injection of diphtheria toxin (DTX) at any stage of post-natal development will ablate the Sertoli cells and allow the effects on testis function to be determined. In this model, ablation of the Sertoli cells on day 2 of post-natal life had no marked effect on fetal Leydig cell function providing further evidence that these cells will function largely autonomously. There was, however, a failure of adult Leydig cell development in these animals except in the region of the rete testis where Sertoli-like cells persisted. Ablation of the Sertoli cells with DTX in the adult animal led to a 75% decrease in Leydig cell number 90 days after treatment although there was no further loss of cells up to 1 year later. The decrease in Leydig cell number was associated with an increase in Cleaved Caspase 3 immunolocalisation in the interstitial tissue suggesting that cell loss was through apoptosis. This data shows that in the adult animal the Sertoli cells are essential for maintenance of the adult Leydig cell population. Interestingly, the Leydig cells remaining after Sertoli cell ablation were clustered around the rete testis and in the subcapsular region of the testis. Survival of Leydig cells around the rete testis may be due to the presence of Sertoli-like cells in

this region but it is not clear why sub-capsular Leydig cells should behave differently to other Leydig cells in the parenchyma of the testis. The Leydig cells remaining after Sertoli cell ablation remain active and can maintain circulating basal testosterone levels although pulsatile testosterone surges seen in the normal animal are absent. This data shows that Sertoli cells are essential for both development and maintenance of the adult Leydig cell population although the factors involved remain to be identified.

ECA 7. ANDROGENS AND HYPOGONADISM

IOP26

Abuse of androgens

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Through the ages athletes have tried to enhance their physical and mental performance by natural products and drugs. After the synthesis of testosterone in 1935 and its introduction into clinical use shortly thereafter, testosterone was also abused by athletes and taken for other non-medical purposes. The first documented (ab)use of testosterone in sports was by rowing teams in 1952 which precipitated sports associations to issue the first rather rigid anti-doping guidelines concerning androgens. However, the illicit use of testosterone and the then new class of anabolic androgenic steroids (AAS) was further fueled by the pharmaceutical industry's search for AAS for therapeutic purposes. While AAS have largely disappeared from regulated medicine registers, they flourish on the black and internet markets, supplied by dubious producers in various countries. Some of these AAS are hidden in over-the-counter supplements without proper declaration. Testosterone is still strongly favoured in competitive athletics, and by body-builders and is used in amateur and recreational sports. However, several other AAS, so-called designer steroids and SARMs are among the popular performance-enhancing drugs. Many of these have not undergone proper toxicological and clinical testing and are thus potentially dangerous, while those tested for clinical use are often consumed at extremely high doses and in irrational combinations with different AAS and other substances and thus may also become toxic.

Due to the illicit nature of AAS abuse and due to ethical restrictions to expose volunteers to potentially toxic regimen, controlled clinical trials of the short and long-term undesired side-effects of AAS are lacking, thus making it difficult to establish a causal relationship between AAS abuse and assumed sequelae. In addition, when medical problems occur, AAS abusers only reluctantly admit to drug consumption. Thus the real nature of the disorder may long remain masked; in unclear clinical situations the possibility of AAS abuse should be taken into consideration.

Because of the negative feedback in the regulation of the hypothalamic-pituitary-gonadal axis, AAS can cause reversible suppression of spermatogenesis, testicular atrophy and infertility. Should spermatogenesis not recover after AAS abuse, a pre-existing fertility disorder may have resurfaced. AAS frequently cause gynecomastia and acne. Most serious effects on the liver such as cholestasis, peliosis, adenomas and hepatic coma arise from 17-alpha-alkylated AAS. AAS-induced polycythemia and increased coagulation may cause thrombosis and stroke. Hypertrophy of the left ventricle, arrhythmias, coronary heart disease and myocardial infarction can be consequences of high-dose AAS doping. Under abuse AAS-dependence may

develop, sometimes in combination with alcohol and other drug addictions. Pre-existing aggressiveness may be intensified. Considering this yet incomplete list of side effects it must be kept in mind that these occur under exceedingly high doses of AAS and should not be confused with therapeutic effects of testosterone replacement in hypogonadal patients.
Finally, as AAS abuse in sports of all kinds will continue as long as the Olympic motto reads. 'Faster, higher, stronger', the mission of the World Anti-Doping Agency (WADA) will not only be to detect unfair competition, but also to protect individuals from harmful side effects of doping.

IOP27

The vulnerable uremic man - role of hypogonadism

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The epidemics of cardiovascular disease, obesity, diabetes, HIV and cancer have all received much attention from the public, media and policymakers. By contrast, chronic kidney disease (CKD) has remained largely a 'silent' epidemic. This is unfortunate because early diagnosis of renal disease based on proteinuria and/or reduced estimated glomerular filtration rate could enable early intervention to reduce the high risks of cardiovascular events, end-stage renal disease (ESRD) and death that are associated with CKD. Given the global increase in the incidence of the leading causes of CKD - hypertension, obesity and diabetes mellitus - better disease management and prevention planning are needed, as effective strategies are available to slow the progression of CKD and reduce cardiovascular risk. Testosterone deficiency or hypogonadism is a common finding in men with CKD, to a great extent a consequence of the failing kidney per se. However, some drugs commonly used in these patients may also affect testosterone levels. Although testosterone restoration in hypogonadism is common practice among endocrinologists there is currently little awareness of this condition among nephrologists, and in many cases, testosterone deficiency remains unscreened and untreated. In this presentation our current understanding of the role of testosterone deficiency at the crossroad of cardiometabolic complications of patients with CKD will be discussed. Pathways discussed include, among others, the plausible role of testosterone deficiency in the development of anaemia and ESA hyporesponsiveness, muscle catabolism, endothelial dysfunction, cognitive dysfunction, decreased libido, cardiovascular disease and mortality. As there are limited sources to guide decision-making, existing testosterone replacement therapy studies in the context of CKD as well as considerations for side and adverse effects will be discussed. In this talk I will make a case for consideration of screening and better management of hypogonadism in men undergoing dialysis and present an ongoing randomized placebo-controlled study on the effects of testosterone supplementation in male dialysis pts.

ECA 8. SESSION OF THE INYRMF

IOP28

Pharmacogenetics in male infertility: what about personalised FSH-treatment in 2014?

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FSH treatment to improve spermatogenesis in infertile men with oligo- or even azoospermia has long been hypothesised to be a beneficial and tried in a number of studies with ambiguous outcomes. While some men seemed to respond well with increased sperm counts and sometimes also improved motility and morphology, other did not.
Then, a single nucleotide polymorphism (SNP) in the FSH-receptor gene (FSHR, 2039A>G, rs6166) was shown to be associated with reproductive parameters in women. The G-allele is associated with higher serum FSH concentrations, duration of the menstrual cycle, follicular growth dynamics and response to ovarian stimulation. Therefore, this SNP was studied repeatedly also in men. However, most of these were case-control studies not looking at specific parameters and, indeed, did not yield clear results as would have been expected from the female data.
More recently, another SNP, located in the FSHB promoter (211G>T, rs10835638) was found to be associated with lower serum FSH levels and oligozoospermia in males. This was immediately replicated in other populations and, meanwhile, also data in women provides evidence for effects on female reproductive parameters. Our group then performed the first study analysing both SNPs in a large group of >1200 well-characterised men with couple infertility. We confirmed that the FSHB 211G>T T-allele had significant dosage effects for FSH, LH and bi-testicular volume. In contrast, the FSHR 2039A>G G-allele by itself exhibited non-significant trends for associations with higher FSH and reduced testicular volumes. However, in a combined model taking both SNPs into account, FSHR 2039A>G significantly modulated the more dominant effect of FSHB 211G>T on serum FSH and testicular volume among the T-allele carriers. Very recently, we showed that the FSHB 211G>T variant even attenuates serum FSH levels in the supraphysiological gonadotropin setting of Klinefelter syndrome. Analysing our patient clientele, we estimate that about 10% of men with couple infertility - exhibiting oligozoospermia but normal FSH levels - would benefit from FSH treatment.
In conclusion, the SNPs in the FSHB and FSHR genes have significant impact on reproductive parameters in both sexes and the combinatory effects of variants in hormone and receptor are an unparalleled example in endocrinology. Oligozoospermic patients carrying unfavourable variants affecting FSH action may benefit from FSH treatment and women undergoing IVF may receive tailored ovarian stimulation in the future. The already available - albeit currently sparse - data on tailored treatment with FSH will also be reviewed in the talk.

ECA 9. INFERTILITY I: DRUGS AND REPRODUCTIVE HEALTH

IOP29

The headache of analgesics during pregnancy and the fetal reproductive system: how and why

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More than half of the pregnant women in the Western world report taking mild analgesics. Epidemiological studies suggest associations between the use of analgesics during pregnancy and increased risks of reproductive disorders in the male offspring. Furthermore, some of these mild analgesics exert potent anti-androgenic effects in the male rat.

Based on this information, we have undertaken a series of experiments in order to understand whether or not mild analgesics have the ability to directly affect the male endocrine system.

We used an in vitro system based on the culture of human fetal testes exposed or not to paracetamol, its metabolite AM404, aspirin, and indomethacin at 10^{-4} to 10^{-7} M. Human fetal testes were from pregnant women after induced abortion. Indomethacin and aspirin stimulated testosterone production, particularly by the younger testes (8–9 GW vs. 10–12 GW). Paracetamol and AM404 decreased insulin-like factor 3 levels. Aspirin stimulated AMH production. PGE2 levels were inhibited by paracetamol and aspirin in the 7–12 GW testes and by indomethacin but only in 7–9.86 GW testes. We concluded that analgesics at concentrations relevant to human exposure cause endocrine disturbances in the fetal testis. The analgesic-induced inhibition of INSL3 may be the mechanism by which analgesics increase the risk of cryptorchidism (Mazaud-Guittot *et al*, JCEM 98, E1757-E1767, 2013).

Adult human testis explants were also cultured with 10^{-4} or 10^{-5} M paracetamol, aspirin or indomethacin for 24–48 h. Testes were obtained from prostate cancer patients. The production of testosterone and insulin-like factor 3 by Leydig cells was altered by exposure to all these drugs. Inhibin B production by Sertoli cells was marginally affected by aspirin only. Our experiments also revealed that mild analgesics display direct anti-prostaglandin activity, which varied depending on the drug used, the dose and the duration of exposure. Nevertheless, associations between the alteration of the PG and testosterone profiles were not systematically observed, suggesting that a combination of mechanisms of endocrine disruption is at play (Albert *et al*, Human Reprod 28, 1890–1898, 2013).

Overall we believe that caution concerning the consumption of mild analgesics during pregnancy and by men should be strengthened, particularly for the latter in high-risk population subgroups such as elite athletes.

IOP30

Common drug intake and human spermatogenesis

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Infertility is a common disease affecting approximately 15–20% of all couples. Since this condition is so widespread and as we witness a significant increase in the age of males and female having a wish of parenthood, it can be expected that an increasing proportion of men with infertility problems will be on concomitant medication for some other disease. Thus, the question will arise whether the drug intake can be a cause or a contributing factor to the fertility problem of the couple.

Medication may affect male fertility by a direct effect on germ cells or their supporting cells. However, other effects related to fertility may be exerted through the hypothalamo-pituitary-gonadal axis, erectile or ejaculatory function, and also impact on libido.

The question regarding possible interaction between medicine intake and fertility is often asked by the patient, but quite often the information regarding possible negative effect of a compound on male reproductive capacity is scarce or completely lacking. A classic example is sulphalsalazine, a drug previously extensively used in treatment of inflammatory bowel disease as ulcerous colitis and Crohn's disease. It took decennia before the link between use of this compound and risk of oligozoospermia and even azoospermia was discovered.

An important aspect of the issue of drug intake in relation to male fertility is timing of the exposure. It has been hypothesized that the early foetal life is the most vulnerable period as considers the effect of external toxicants on male reproductive system. Thus, it is not only the patient's own consumption of the pharmaceuticals but even his mother's medicine intake which may be of relevance.

However, there are some well-known examples of commonly used drugs known to have a negative impact on male fertility. Opiates suppress the pituitary function and can, thereby, cause reduction of gonadotropin secretion. The same is true for testosterone, prescribed to men with androgen deficiency, which may be associated with poor testicular function including subfertility. The widely used SSRI compounds, can lead to impairment of ejaculation and can, thereby, also affect fertility. Finasteride, an alpha 5-reductase inhibitor can not only affect ejaculation and erectile function but was also reported to cause reduction is sperm counts.

The list of compounds shown to have a negative effect on male reproductive function is long and will probably be growing. It means that each physician taking care of infertile couples should ask the patients about concomitant medication. However, some drugs may also have beneficial effects. E.g. a recent Cochrane review concluded that antioxidant supplementation in subfertile males might improve the outcomes of live birth and pregnancy rate for couples undergoing assisted reproduction.

IOP31

Late consequences of childhood and adolescence cancer treatment

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Abstract: Survival of childhood cancer has increased dramatically the last two decades: more than 75% of young cancer patients become long term survivors, mainly due to improved cancer treatment protocols. In addition, quality of life has become an important issue for these patients. Unfortunately, many of them face long term consequences of cancer treatment, such as infertility, increased risk for cardiovascular diseases, metabolic syndrome and second malignancies.

Fertility in cancer survivors: The negative effect of cytotoxic drugs on spermatogenesis has been extensively investigated: most harmful drugs are nitrogen mustard derivates and alkylating drugs (Lee SH *et al*, Ann Pediatr Endocrinol Metab. 2013;18:168-72). Combination chemotherapy, like the MOPP-regimen administered for Hodgkin's disease, has a high chance of sterilizing the patient. Other multi-drug regimens applied in young cancer patients, such as ABVD for Hodgkin's disease and BEP for TGCTs have a lower risk of permanent infertility. Some cancer treatment are associated with a high risk of infertility: whole body irradiation for leukemia and chemotherapy for soft tissue sarcoma's cause sterility in many cases. Also, the prepubertal period is not a silent period for testicular development and cancer treatment at this age will have a pronounced effect on the gonads.

Radiation therapy usually causes more permanent damage to the germ cells, even at doses higher that 4 Gy. Routinely, the gonads will be protected against irradiation damage: exceptions are whole body irradiation before bone-marrow transplantation and irradiation in case of tumor involvement of the testis.

Cardiovascular diseases and metabolic syndrome: Cardiovascular diseases are the main risk factor for young cancer survivors (Steingart *et al*. Semin Oncol 2013; 40: 690–708). A majority of them will have some form of cardiomyopathy, especially those treated with anthracyclines and after radiation therapy of the chest. Total cholesterol is often elevated in cancer survivors. Obesity, hypertension and type 2 diabetes (metabolic syndrome) is much more frequent in cancer survivors.

Second malignancies: The risk for developing new malignancies in childhood cancer survivors is 2 fold higher compared to the general population. There is a strong relation with radiation therapy and with alkylating chemotherapy. These second malignant neoplasms (Travis *et al* Health Phys 2014; 106(2): 229–46) are mainly solid tumors and may occur at younger age compared to the general population. Surveillance is recommended to begin at the age of 35 or 10 years after radiation therapy.

Summary: Men and women treated for cancer at early age run a high risk of developing a chronic health condition, that might even be life-threatening. Surveillance of cancer survivors is warranted and should start early.

ECA 10. INFERTILITY II: GENETICS AND EPIGENETICS

IOP32

The sperm epigenome

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Sperm are unique compared to all of the other cells produced in a human's body in numerous aspects, including their unique packaging of DNA. During spermiogenesis, up to 95% of the genome is re-packaged in sperm-specific nuclear proteins termed 'protamines', that facilitates compaction of the chromatin. Interestingly, it is now apparent that the protamine replacement is not random, but rather takes place in a manner that maintains an elegant sperm epigenome that is established initially in stem cells. The sperm epigenome includes retention of 'bivalently marked' histones and DNA demethylation at loci associated with embryogenesis, microRNAs, and imprinted genes, which suggests a role in poising the sperm genome for early embryogenesis. This hypothesis has been strengthened by studies demonstrating sperm epigenetic abnormalities in a high proportion of IVF patients with severely altered embryogenesis. This talk will highlight recent data that supports an association of altered sperm DNA methylation with poor IVF outcome, and possibly can lead to a diagnostic assay. Additionally, the role of epigenetic abnormalities as a result of environmental exposures will be discussed, including the effects of smoking on sperm DNA methylation. Lastly, the role of advanced paternal age on the sperm epigenome will be discussed, highlighting recent data that suggest consistent changes in an aging male that may be causative of the elevated risk previously reported for neuropsychiatric disorders in offspring of fathers with advanced age.

IOP33

Sperm nuclear proteome and its epigenetic potential

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It is increasingly becoming clear that although the most classical idea about mature sperm cell function is to transmit the paternal genetic message encoded in the DNA to the oocyte, the male germ cell also contributes to the fertilized egg with epigenetic information. Importantly, the vast majority of the sperm DNA is silenced by its association with protamines, while genes potentially needed in the initial stages of development are packaged by histones in an opener structure. This fact reveals a non random gene distribution between both chromatin domains,

which represent a form of epigenetic marking. But so far little attention has been devoted to other sperm chromatin associated proteins that, in addition to histones and protamines, may also be involved in a regulatory role. A total of 581 nuclear proteins have been described in the human sperm cell so far, including a great number of zinc finger- and bromodomain- containing proteins, transcription factors, histones and histone-modifiers and other DNA-related proteins. More than a half of these sperm nucleoproteins are involved in potential epigenetic activities as chromosome and chromatin organization, protein-DNA complex assembly, DNA packaging, gene expression, transcription, chromatin modification and histone modification. In addition, common trends have been showed in the sperm chromatin protein composition of different mammalian species. Therefore, the mammalian sperm cell may deliver to the offspring a rich combination of histone variants, transcription factors, chromatin-associated and chromatin-modifying proteins which could constitute additional layers of epigenetic information involved in transcription regulation of paternal genes after fertilization.

PLENARY 4

IOP 34

Spermatogonial stem cell preservation and transplantation anno 2014: what every clinician should know

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Spermatogenesis is the process in which male gametes are being produced from testicular stem cells. Hence, any substantial loss of testicular stem cells implies a decline in fertility. Testicular stem cells are lost during ageing, however, substantial loss is mainly caused by both chemo- and radiotherapy or because of specific genetic disorders, e.g; 47, XXY Klinefelter's syndrome or Y-q deletions.

Fertility preservation strategies may be proposed to patients who subject to testicular stem cell loss. In adult men undergoing gonadotoxic treatments, sperm freezing can be proposed as an efficient preventive strategy.

Since spermatogenesis starts only at puberty, the only spermatogenic cells that are present in the seminiferous tubules before the onset of puberty are the spermatogonial stem cells. Therefore fertility preservation in prepubertal boys lies within the preservation of their spermatogonial stem cells. While this approach should be considered as experimental, three options for preservation are currently under research: the spermatogonial stem cell transplantation, the grafting of testicular tissue pieces and the *in vitro* proliferation and/or maturation of spermatogonia. Each technique has of course its (dis)advantages, but each one of them might eventually find its way to the clinic. Testicular tissue grafting seems to be the most promising tool for fertility restoration in young boys. However, testicular tissue from cancer patients, e.g. leukemic patients, may be contain malignant cells. Reintroduction of malignant cells into an otherwise cured patient must be avoided. Therefore, grafting can only be proposed in cases of non-malignant diseases, e.g. sickle-cell anemia requiring bone marrow transplantation. For cancer patients at risk of testicular malignant contamination, the tissue needs to be digested and decontaminated before any spermatogonial stem cell transplantation (SSCT) can be performed. In-vitro spermatogenesis is an alternative to preserve male fertility. The in-vitro generated spermatozoa could be used for intracytoplasmic sperm injection. When SSCT or SSC culture becomes available for clinical use, efficient protocols for the cryopreservation of SSCs and testicular tissue will be of great benefit.

The derivation of male gametes from stem cells, albeit under research in rodents, is even further away from any clinical application.

Preservation in 47, XXY Klinefelter patients is even more experimental because stem cells may be sex chromosomal disomic and the niche cells may be compromised by sclerosis and atrophy of the seminiferous tubules.

Data from the literature indicate that existing semen-depots are infrequently used, this because of recovery of fertility potential with paternity, because of death, because good sperm quality was regained or because the patient did not want children. Patients should thus receive updated information about potential benefits and risks before embarking for cryobanking, either of spermatozoa or testicular stem cells.

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SELECTED ORAL PRESENTATIONS

ECA SELECTED ORAL I: GOLDEN PRESENTATIONS

OP1

Mutations in an X-linked gene are a common cause of meiotic arrest and azoospermia

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Background: The genetic causes of non-obstructive azoospermia (AZ) are unknown in the majority of infertile men.
Aim: To investigate the genomic imbalances responsible for AZ we carried out a genome-wide copy number variation study. One variant was identified in an X-linked gene. It encodes a protein critical for male germ cell meiotic DNA recombination and knockout male mice exhibit AZ with meiotic arrest.
Methods: We performed array comparative genomic hybridization (aCGH) in 15 AZ patients. Mutation screening was carried out via direct Sanger sequencing of the gene in 289 AZ patients of diverse ethnicity and 384 controls.
Results: We identified a 99-kb hemizygous deletion of three exons in an AZ patient. Our subsequent mutation screening found 6 novel mutations in 288 AZ patients (7/289, 2.4% total): three novel splicing mutations, two novel missense mutations, and one identical genomic deletion. These mutations were absent in 384 normozoospermic controls ($p < 0.002$). Importantly, five mutations were found in 15.2% (5/33) of AZ patients diagnosed with meiotic arrest, resembling the deficient mouse phenotype. Immunohistochemical analysis showed specific cytoplasmic expression in late spermatocytes as well as in round and elongated spermatids in normal human testes. In contrast, testes from AZ patients with mutations showed meiotic arrest and absence of expression.
Conclusion: Mutations in this X-chromosomal gene are a frequent cause of meiotic arrest and azoospermia in humans.

OP2

Differential effects of perinatal, prepubertal or conception to adulthood exposures to a low dose mixture of genistein and vinclozolin on the genital tract and testicular gene expression in the exposed generation and the unexposed progeny

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Background: The effects on the genital tract and the male reproductive function of exposure to low doses of xenoestrogens and/or antiandrogens as a function of the pre and postnatal developmental periods have rarely been studied jointly.
Aim: The present study was designed to study this question and to investigate a possible reproductive impact in the unexposed male progeny.
Methods: F1 rats were exposed from conception to lactation (GL), from puberty to adulthood (PA) and, from conception to adulthood (CA) to 1 mg/kg/day genistein, a relevant dose for human diet and/or 1 mg/kg/day vinclozolin, a dose lower than the NOAEL. For the three exposure conditions, adults were mated with unexposed females and male offspring (F2) was studied at adulthood. A number of reproductive endpoints was evaluated. The study of the testicular transcriptome in adults was undertaken jointly.
Results: Among the endpoints modified (detailed in poster) GL did not modified sperm production in F1 and F2 whereas PA and CA decreased it to about 2/3 and to 1/2 of controls respectively. It is the combination of the mixture and lifelong exposure that led to the largest number of endpoints significantly changed. The 6 exposure modalities (GL, PA and CA and g, v and gv) were found to differentially impact the expression of testicular genes in both F1 and unexposed F2.
Conclusion: Pre and postnatal chronic exposures to low doses of a phytoestrogen and an antiandrogenic food contaminant affect differentially the male reproductive system and testis gene expression. These conditions impacted differently the testis transcriptome of the unexposed progeny.

OP3

Evaluation of intratesticular perfusion by color-coded duplex sonography (CCDS) in TESE-patients with azoospermia: a prospective study

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Background: Scrotal ultrasonography and CCDS have a key role for a non-invasive investigation of the testis.
Aim: Our objective was to assess whether CCDS might predict the outcome of testicular sperm retrieval in patients with azoospermia. We also evaluated sonographic alterations of the testicular structure before and after the TESE procedere.
Methods: Sixty-one patients were enrolled prospectively: 12 with obstructive (OA) and 49 with non-obstructive azoospermia (NOA). 24 of 49 patients in the NOA group had negative surgical sperm retrieval. Testicular volume, hormonal parameters, and sonographic findings were compared before and after TESE. CCDS was performed at three different locations of the testis, the peak systolic value (PSV) was measured in all regions pre- and postoperatively. Testicular histology was investigated in all patients.
Results: Spermatogenesis score count was significantly correlated with the intratesticular PSV in the corresponding sonographic position. PSV of the OA patients was significantly higher than in men with negative surgical sperm retrieval in the NOA group. Testicular volume and epididymal head cross diameter were significantly higher in OA patients. Testicular volume decreased postoperatively in the follow-up after 6 weeks. FSH and LH increased in the follow-up, testosterone levels decreased. Overall, the PSV was significantly increased in all patients 24 h after surgery, with normalization after 6 weeks.
Conclusion: CCDS reveals differences in patients with OA and NOA. It is also valuable to assess pathological changes in the follow-up after surgery. Since intratesticular PSV is correlated with testicular histology, the assessment of PSV might help to identify those patients with successful sperm retrieval.

OP4

Association between endogenous testosterone (T) levels, sexual dysfunction and PDE-5 inhibitor (PDE5i) use in the registry of hypogonadism in men (RHYME)

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Background: The relationship between sub-normal T levels with low desire and erectile dysfunction (ED) has not been confirmed in untreated hypogonadal patients and the impact of PDE5i use is unknown.
Aim: To describe the prevalence of sexual dysfunction (SD), its association with T levels and possible influence of PDE5i on this relationship.
Methods: RHYME is a multi-center registry of 999 hypogonadal men (mean age 59.1 ± 10.5 years and mean T 9.5 ± 1.6 nmol/L), naïve to androgen treatment, from 25 sites in 6 European countries (DE/ES/IT/NL/SE/UK). ED, low desire, and PDE5i use were assessed by medical record review and, in sexually active patients ($N = 752$), also by IIEF sexual desire (SD) and erectile function (EF) domains.
Results: Of the total sample, 64.7% had low desire and 81.0% ED. Subjects with low desire had significantly lower T than those without (9.3 vs. 10.0 nmol/L; $p = 0.03$). In the total sample, 76.4% was sexually active and had higher T relative to inactive men (9.9 vs. 8.5 nmol/L; $p < 0.001$). Based on IIEF-EF in sexually active men, 34.8, 31.1 and 34.1% had severe, moderate or no ED. While ED men did not have lower T overall, those treated with PDE5i had significantly higher T than untreated men (11.0 vs. 9.1 nmol/L; $p < 0.0001$). PDE5i use were highest in Spain (44.2%) and lowest in Italy (9.4%), and were more than twice as high in urology (35.9%) than endocrinology or general medicine (16.1%) practices.
Conclusion: SD and PDE5i use are frequent in European hypogonadal men before starting treatment. PDE5i use was associated with higher T in ED men whereas low desire and sexual inactivity were associated with the lowest levels of T.

ECA SELECTED ORAL II: SPERMATOLOGY

OP5

Characterization and kinetic of the human spermatogonial compartment

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Background: The spermatogonial compartment, including the stem cells, are at the foundation of spermatogenesis. In humans, this compartment includes two types of type A spermatogonia (Adark and Apale), and one generation of type B spermatogonia. However, only scant data are available on the kinetic of spermatogonial proliferation and self-renewal. Until recently only 6 germ cell associations were described in human spermatogenesis but recently we have re-classified it and now we can distinguish 12 epithelial stages, similar to most laboratory mammals including monkeys (Muciaccia *et al.*, BOR 2013).
Aim: Our aim is to investigate the proliferation and self-renewal of human spermatogonia within the frame of the novel classification of the spermatogenic stages.
Methods: Testicular biopsies samples were obtained from heart beating organ donors. For the immunohistochemical detection experiments sections were processed as previously described (Muciaccia *et al.*, BOR 2013). For BrdU or EdU incorporation experiments, intact tubules were processed as described (Grisanti *et al.*, Stem cells 2009).
Results: We have analyzed the stage distribution of spermatogonial mitotic divisions by means of PHH3 (mitotic marker) IHC detection in nine different donors. Three peaks of mitosis are evident at stage II, IV and IX–X. We are currently investigating the molecular phenotype of dividing cells. By short-term incorporation of EdU in intact seminiferous tubules we are studying the clonal size of mitotic and meiotic germ cells.
Conclusion: These data will allow to develop a scheme describing the kinetic of spermatogonial proliferation and self-renewal in relation to the 12 stages of the spermatogenic cycle.

OP6

Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity and oxidative stress

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Background: Sperm DNA fragmentation (sDF) represents a threat for human reproduction. The causes of origin of sDF are still unclear, even if apoptosis, oxidative assault and defects in chromatin maturation may be responsible for the onset of DNA damage.
Aim: We directly evaluated signs of apoptosis, oxidative stress and sperm maturation in DNA fragmented spermatozoa
Methods: Using multicolor flow cytometry and sperm sorting, we detected sDF and oxidative damage (8-hydroxy, 2′-deoxyguanosine, 8-OHdG, and malondialdehyde, MDA), apoptosis (caspase activity and cleaved Poly ADP-ribose polymerase, cPARP) and sperm immaturity (creatine phosphokinase, CK, and excess of residual histones).
Results: Apoptosis resulted highly associated to sDF, being caspase activity and cPARP present in 82.6 ± 9.1% and 53.5 ± 16.4%, of sperm with DNA breaks vs. 16.2 ± 11.3% ($p < 0.0001$) and 21.7 ± 15.2% ($p < 0.010$) of non-fragmented. Chromatin immaturity was significantly higher in flow cytometry sorted sperm with sDF (74.8 ± 17.5% vs. 37.3 ± 16.6 %, $p < 0.005$), and mostly overlapping caspase activity. Oxidative damage was not associated to sDF in the total sperm population, however, in live sperm, 8-OHdG, MDA and caspase activity were clearly associated to sDF, suggesting a role of oxidative stress in generating sDF by inducing an apoptotic mechanism.
Conclusion: This is the first investigation on the origin of sDF directly evaluating the co-localization of the signs of the hypothesised mechanisms with DNA breaks at single cell level. Results indicate that the main pathway leading to sperm DNA breaks is a process of apoptosis triggered by testicular conditions (including impairment of chromatin maturation) and during the transit in the male genital tract by oxidative stress.

OP7

Routine use of flow cytometry for counting human spermatozoa and round cells: lessons from a 5-year experience representing more than 20 000 analyzes

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Background: Following a study of our group (Eustache *et al.*, 2001) demonstrating the feasibility of counting human sperm by flow cytometry (FCM), we have had the opportunity to implement this approach for routine practice, from the year 2009.
Aim: The objective of this study is to report our concrete experience with our FCM method applied routinely to native semen samples and sperm preparations in an andrology laboratory and a sperm bank having a significant level of activity.
Methods: A native semen sample or a sperm preparation, a solution of fluorescent beads having a known concentration and propidium iodide for DNA staining are well mixed in predetermined volumes. Spermatozoa are detected according to gating on size, granularity and DNA staining. Spermatozoa and 1n/2n round cells concentrations are calculated from the ratio of detected events to beads count and concentration.
Results: The validation study on 250 semen samples of various qualities reflecting routine activity showed a high

level of agreement between FCM and haemocytometry. The method is fast (automatic passage using a carousel) and highly reproducible (CV ~3%). The only limit where FCM is not used is azoospermia. Several examples of the advantages of the approach for the diagnosis and the prognosis will be presented in the poster.

Conclusion: In conclusion, this study demonstrates that human sperm concentration can be easily and accurately assessed by FCM with the advantage of counting precisely the round cells populations in the same passage. Further developments integrating the characterization and routine count of leukocytes are ongoing.

OP8

Influences of age, days of abstinence, and BMI on results of semen analysis from Swedish men of sub-fertile couples

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Background/Aim: The aim of this study was to evaluate if age, days of abstinence (Abs), and Body Mass Index (BMI) had any influence on the results of semen analysis from 3105 Swedish men of sub-fertile couples.

Methods: We evaluated results of semen analysis performed on men following WHO 2010 and ESHRE guidelines for semen volume, sperm concentration, total sperm number, male accessory gland markers, morphology and motility. Linear regression analysis was performed to assess if age, Abs, or BMI had any significant affects on any of the sperm parameters.

Results: Age decreased sperm volume due to decreased secretory contributions from the seminal vesicles, without changes in secretions from the prostate and the epididymis. Age decreased sperm motility and impaired sperm morphology but did not affect sperm number. Abs increased from 2 to 4 days had increased semen volume, prostatic and epididymal secretory contributions but showed no effect on seminal vesicular contribution. Abs also increased total sperm number and sperm concentration, regardless of an increased volume, and impaired sperm morphology but not sperm motility. Semen volume decreased with increased BMI. Obese males with BMI = 30 compared to non-obese (BMI < 30) had significantly lower total sperm number, sperm concentration, semen volume, and higher impaired sperm morphology.

Conclusion: For evaluation of semen analysis results, it is critically important to control for the duration of Abs. Aging men had decreased secretory capacity of the seminal vesicles. Age also had effects on sperm morphology and motility. Obese men had lower sperm volume and sperm production and negative effects on sperm morphology.

OP9

Glycation Adducts in sperm and activin regulation of inflammation induced by diabetes

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Background: The underlying mechanisms of reproductive dysfunction in type I diabetic men are not well understood. The TGF-β superfamily cytokine, activin A, is widely expressed in various inflammatory conditions, such as diabetes mellitus, and plays several key roles in the regulation of testicular function, particularly spermatogenesis. Furthermore its production by testicular Sertoli cells is increased by inflammatory mediators.

Aim: Our aim was to identify the regulatory functions of activin and its binding protein, follistatin, in the context of diabetes-induced low-grade inflammation.

Methods: Quantitative (q) polymerase chain reaction (PCR) was used to evaluate the expression of Inhba (the gene encoding activin A subunits), as well as the genes for follistatin (FST) and the activin receptor subunits, in the male mouse testis. Activin A protein expression was detected by immunohistochemistry. Sertoli cell culture of the WL-3 cell line was used to evaluate the impact of glucose on Activin A expression.

Results: Here we show that Activin ßA subunit mRNA is significantly upregulated in a type I (Ins2Akita) diabetes related mouse model in an age dependent manner. This effect could be due to an increase of Activin within Sertoli cells as shown by in vitro experiments in WL-3 cells. Expression of Activin ßA subunit Protein was proven to be present in Sertoli cells, interstitial macrophages and peritubular cells. Contrarily mRNA expression of Follistatin in total as well as of its two Isoforms 288 and 315 was not significantly affected by diabetic conditions.

Conclusion: These results indicate a role for activin A within diabetes-induced male infertility.

ECA SELECTED ORAL III: REPRODUCTIVE HEALTH

OP10

Sperm DNA damages after chemotherapy or radiotherapy for testicular cancer

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Background: An increase of testicular cancer (TC) incidence has been observed over the past 40 years. The long-term survival rate has improved with advances in medical therapy.
Aim: The main of objective of our study was to prospectively evaluate sperm characteristics and DNA alterations before and after TC treatment.
Methods: 53 TC patients were included in a multicenter prospective study and performed semen samples before TC treatment and 3, 6, 12, and 24 months after the end of treatment. Sperm DNA quality (chromatin condensation, DNA fragmentation), number and relative telomere length were assessed.
Results: 26 patients were treated by radiotherapy, 28 patients by BEP regimen. Sperm count recovered pre-treatment value at 12 months for patients given less than 2 BEP cycles, and at 24 months after radiotherapy or more than 2 BEP cycles. Sperm chromosome abnormalities were higher 6 and 12 months after chemotherapy, 3 and 6 months after radiotherapy. About 50% of patients did not recover their pre- treatment rate. Sperm chromatin condensation was altered 6 months after radiotherapy. Sperm DNA fragmentation did not vary after the end of treatment. Telomere number increased at 12 and 24 months after radiotherapy but did not vary after chemotherapy. The relative telomere length was higher 12 months after chemo- or radiotherapy.
Conclusion: Quantitative and qualitative sperm characteristics decreased significantly after treatment, with the lowest values at 3 and 6 months. Sperm nuclear status recovered pre-treatment values 12 months after two BEP cycles, after 12 months when more than two cycles of BEP or radiotherapy were used.

OP11

Prenatal phthalate exposure and reproductive indices in adolescent males

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Background: Prenatal exposure to phthalates is suggested to negatively impact male reproductive function, but human data are lacking.
Aim: To study associations between prenatal exposure to diethylhexyl phthalate (DEHP) and diisononyl phthalate (DiNP), and reproductive parameters of adolescent men
Methods: Using regression models adjusted for abstinence time and own and parental smoking, we studied associations between DEHP/DiNP metabolites in maternal sera from early pregnancy, and testicular size, semen quality and reproductive hormones in 112 men, 17–20 years old, from the general population.
Results: Men in the highest exposure tertile of one DiNP metabolite [mono-4-methyl-7-carboxyheptyl phthalate] compared with men in the lowest tertile had 4.3 mL (95% CI: 0.89, 7.6 mL; $p < 0.001$) lower testicular volume, 30% (95% CI: 3.6, 63%; $p = 0.024$) higher follicle-stimulating hormone and 0.87 mL (95% CI: 0.28, 1.5 mL; $p = 0.004$) lower semen volume. Those in the highest tertile of one DEHP metabolite [mono-(2-ethyl-5-hydroxylhexyl) phthalate] had 0.70 mL (95% CI: 0.090, 1.3 mL; $p = 0.025$) lower semen volume compared with men in lowest tertile. Two DiNP metabolites [mono-(4-methyl-7- hydroxyloctyl) phthalate and mono-(4-methyl-7-oxooctyl) phthalate] were linearly associated with luteinizing hormone ($p < 0.01$).
Conclusion: Prenatal exposure to DEHP or DiNP seemed associated with lower volumes of semen and testicles, and with altered reproductive hormones.

OP12

Does reproductive tract inflammation mediate reproductive dysfunction in males with the metabolic syndrome?

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Background: Metabolic syndrome (MetS), a constellation of various metabolic risk factors, is associated with low-grade systemic inflammation and potential reproductive dysfunction in males. Cytokines, known mediators of the hypothalamic-pituitary-testes axis, are present in human semen and modulate male reproduction and fertility potential in physiological concentrations. Increased seminal cytokines are associated with reproductive dysfunction in genital tract infections, however, the impact of systemic inflammation requires investigation.
Aim: To determine the impact of MetS related systemic inflammation in male reproductive tract inflammation and reproductive dysfunction.

Methods: Males recruited from the general public underwent comprehensive clinical assessments with serum and semen samples were obtained. Important exclusions included systemic or reproductive inflammatory disorders and infections. In addition to parameters required for MetS diagnosis and serum CRP, serum and seminal fluid was assessed for TNFa, IL1ß, IL6, IL8 (cytokines) and leptin.Semen analysis was performed according to WHO (2010) recommendations.
Results: Participants diagnosed with MetS (n = 44) had significantly increased serum and seminal cytokine and leptin concentrations compared to healthy controls (n = 34). This was associated with significantly reduced sperm concentration, motility, vitality, mitochondrial function and DNA integrity, with no effect on morphology. Serum and seminal cytokines and leptin correlated positively, as did cytokines and serum CRP.
Conclusion: MetS males have reproductive tract inflammation, in the absence of local infection, associated with reduced fertility parameters. Immune mechanisms may be associated with reduced male fertility in MetS, and serum CRP may predict seminal cytokine concentrations. These novel findings warrant further immunological investigation into male reproductive health in the setting of MetS.

ECA 6: FROM BASIC TO CLINICAL ANDROLOGY

OP13

Serum Delta4 pathway steroids profiling by isotopic dilution-liquid chromatography-mass spectrometry (ID-LC-MS/MS) after human chorionic gonadotropin (HCG) stimulation in men with Klinefelter's syndrome (KS) and in eugonadic controls (EC)

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Background: KS is associated with primary hypogonadism. Only few and old studies are available in the literature about stimulated serum testicular steroid profile. Recent studies demonstrate greater intratesticular testosterone (ITT) concentrations in KS than in controls.
Aim: Identify any enzymatic blocks as cause of hypogonadism in KS.
Methods: 13 KS patients (mean 34 ± 8 years) not receiving testosterone replacement therapy and 12 EC (mean 32 ± 8 years) were enrolled. Hormonal assays by ID-LC-MS/MS were performed at baseline and for 5 days after intramuscolar injection of HCG, 5000 IU.
Results: HCG rised significantly in the serum of both KS and EC (ANOVA, $p < 0.001$), without significant difference between the two groups (t-test, $p = 0.715$). All Delta4 pathway steroids showed a significant increase after HCG stimulation (RM-ANOVA): progesterone (P) ($p < 0.001$), 17OH-progesterone (17OHP) ($p < 0.001$), androstenedione (A) ($p = 0.049$ in SK and $p < 0.001$ in EU), testosterone (T) ($p < 0.001$). The 17OHP/p ratio ($p < 0.001$) and T/A ratio ($p = 0.003$ in KS and $p < 0.001$ in EC) rised significantly after HCG. Comparison of response to HCG between patients and controls (t-test) resulted significant for T ($p = 0.001$), 17OHP/P ($p = 0.005$) and T/A ratios ($p = 0.002$).
Conclusion: Leydig cells of KS patients are able to respond to HCG, by increasing T production, less than in EC. Different trend in the two groups of 17OHP/P and T/A ratios suggests a less effective enzymatic activity of P450c17/POR and 17ßHSD3 in KS than in EC. This study supports a disturbed steroidogenesis in Leydig cells of KS. A contemporary evaluation by ID-LC-MS/MS of both serum and ITT levels after HCG could definitively clarify pathophysiology of hypogonadism in KS.

ECA SELECTED ORAL IV: INFERTILITY

OP14

Inhibin B as a marker of success after varicocelectomy in severe oligospermia

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Background: Severe oligospermia is a dramatic reason of infertility.
Aim: The role of varicocelectomy and to find the predictive markers of spermatogenesis improvement.
Methods: 37 infertile men (mean age 30.7 years) with left side varicocele grade II–III and severe oligospermia (sperm concentration <5 M/mL). At the same time in all these patients we checked the level of Inhibin B in serum, as a hypothetic predictive marker, which ranged from <10 to 203.77 ng/L In all patients left subinguinal varicocelectomy was done, control semen analysis was assessed after 12 months.
Results: Baseline semen analysis changed from 2.47 M/mL (range 1.23–4.75 M/mL) till 4.37 M/mL (range 1.02–9.57 M/mL) ($p = 0.07$). Statistically significant improvement in semen analysis was achieved only in group of 14 patients – from 3.17 M/mL (range 2.37–4.75 M/mL) till 6.84 M/mL (range 3.89–9.57 M/mL) ($p = 0.0001$). Surprisingly all these men had higher levels of Inhibin B > 95 ng/L. In this group, after 12 months the average level of Inhibin B in serum increased till 143 ng/mL (baseline level 129 ng/mL). In men with low Inhibin B level (<95 ng/L) we didn't find significant improvement in sperm count and Inhibin B level.
Conclusion: The role of varicocelectomy in severe oligospermia is controversial. As we can see, in spite of the small number of recruited patients (because of strict inclusion criteria), semen improvement after varicocelectomy is limited. Inhibin B can be used as a predictive marker of spermatogenesis improvement.

OP15

Motile sperm count, DNA fragmentation, and mitochondrial membrane potential are not useful in predicting intrauterine insemination for mixed indications: a prospective study

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Background: Combined with ovarian stimulation, homologous IUI is a widely used assisted reproductive technology, often proposed as a first-line treatment. However, studies report variable pregnancy rates (PR) (6–20%) and a lot of outcome predictors in often small retrospective series.
Aim: The aim of this prospective study was to determine whether clinical data and sperm characteristics influence IUI outcome.
Methods: Eighty-seven couples were enrolled consecutively and 98 IUI cycles performed. The analysis of native sperm was performed 3 to 6 months before IUI using 2010 WHO criteria. There were 43 couples with female infertility factors, 15 couples with female and male factors, 16 couples with male factor and 24 couples with unexplained infertility.
Ovaries were stimulated with clomiphene citrate ($n = 6$), gonadotropins ($n = 14$) and letrozole ($n = 78$). Before IUI sperm was prepared with density gradient centrifugation. Mitochondrial membrane potential (MMP) was measured by means of DiOC6 staining and the percentage of sperm with fragmented DNA was assessed by the TUNEL method.
Results: PR per cycle was 10.0%. No relationship was observed between IUI outcome and type of infertility, mode of stimulation and sperm quality at IUI: motile sperm count, DNA fragmentation and MMP.
Conclusion: In this prospective study of couples with mixed indications for IUI we have not found that type of infertility, type of ovarian stimulation and sperm quality at IUI had influence on outcome.
The explanation may be the same patient's large variation of sperm concentration, MMP and DNA fragmentation and the relatively small number of cycles performed which consequently obstructed the expression of small influence factors.

OP16

Subcapsular orchiectomy as an alternative method for identification and isolation of testicular sperm from men with Klinefelter's syndrome

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Background: Men with Klinefelter's syndrome (KS) are often diagnosed and treated in fertility clinics.
Aim: The aim of this study was to optimize the chance to find and retrieve vital testicular sperm.
Methods: Thirteen azoospermic KS men were included in this ongoing study. Unilateral subcapsular orchiectomy was performed. General anaesthesia was given when requested by the patient. Twenty mL of lidocain (5 mg/mL) was injected around the funicle and under the scrotal skin (2 mL). All testis tissue was removed through a longitudinal cut, and the Tunica albuginea, Tunica vaginalis and skin were closed separately. Testis tissue was dissected mechanically and enzymatically with the purpose to identify sperm. Surplus testis tissue was cut into small pieces and cryopreserved or used for histological examination.
Results: Twelve KS had a non-mosaic 47,XXY karyotype, while one had a (47,XXY/46,XX/46,XY) mosaicism. Four KS (31%) had a history of cryptorchidism. Motile sperm were

found in 4 (31%), and in two cases clinical pregnancies were obtained. The mean age was significantly lower in KS in whom sperm were detected compared to KS without testicular sperm (27.5 years vs. 32.9 years; $p = 0.03$). Presence of testicular sperm was not significantly associated to height ($p = 0.41$), weight ($p = 0.07$), BMI ($p = 0.16$), testis volume ($p = 0.37$), or FSH ($p = 0.09$).

Conclusion: The new treatment approach makes it much easier to retrieve testicular sperm from men with KS. Additionally, since a whole testis is removed, the chance to have sperm enough for cryopreservation and several fertility treatments increases. The procedure seems ethically acceptable since most KS need androgen substitution anyway.

OP17

Gene expression analysis of human sperm needs differential normalizers

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Background: Although infertility is often of male origin, there are still no proven molecular markers of sperm quality. The search for gene expression markers is partially hindered by the lack of a recognized set of reference genes to normalise qPCR data across studies. Moreover, the effect of cryopreservation and RNA isolation on these genes is unknown.

Aim: The aim of this study was to define a set of sperm reference genes for different handling and RNA isolation methods.

Methods: Twelve normozoospermic individuals were included in this study. From each, a semen sample was split into 2, 1 aliquot was cryopreserved by slow freezing and the other analyzed fresh. For each of the 24 aliquots, total RNA was extracted with either phenol-free or phenol-based methods, treated with amplification grade DNaseI, and 1 μg RNA was reverse transcribed to cDNA. Twenty putative housekeeping genes (HKGs) were analyzed and their expression stability across samples estimated by qPCR. Three different algorithms (geNorm, BestKeeper and NormFinder) were applied to qPCR data.

Results: We found that HKGs such as GAPDH or ACTB, useful in other biological contexts, cannot be used for human sperm. It is possible to compare gene expression from fresh and cryopreserved sperm samples using the same isolation method, while RNA isolation technique alters the array of expressed genes. The most appropriate HKGs for qPCR analysis are RPLP1, RPL13A, RPLP2 and PPIA.

Conclusion: Both RNA isolation methods and sample handling are critical when gene expression experiments are performed, and should be taken into account while planning a study.

OP18

Evaluation of testicular biopsies from infertile men: correlations between histopathology, protamine mRNA expression, and sperm retrieval

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Background: In patients with azoospermia, detailed clinical work-up does not allow definitively predicting the presence of elongated spermatids in the testis. Hence, histological evaluation of testicular biopsies remains a key investigation. Moreover, protamines (Prm) in haploid germ cells have been proposed as molecular biomarker for successful testicular sperm extraction (TESE).

Aim: To investigate associations between testicular histology, sperm retrieval, and Prm expression in a systematic approach.

Methods: Bilateral, multi-focal testicular biopsies were obtained from 135 azoospermic patients (aged 19–54 years). Routine histological specimens were used for semi-quantitative assessment of spermatogenesis (score count; percentage of tubules revealing elongated spermatids). Corresponding snap-frozen tissue samples were processed for RT-qPCR with primers for Prm1 and Prm2. Specimens cryopreserved under 'therapeutic' conditions were subjected to a TESE trial.

Results: Histological score count evaluation correlated with testicular volume, serum FSH, and TESE trial results ($p < 0.001$). Similarly, Prm1/2-mRNA contents were significantly associated with score counts ($p < 0.001$). In Sertoli-cell-only syndrome, cycle numbers (Ct) for Prm1 [Prm2] were 32.5 (11.5-39.1) [32.6 (13.4-39.7)], whereas in specimens showing hypo- or normal spermatogenesis Ct values were 22 (11–37.2) [22.2 (12.8–38.25)] and 19.5 (12.3–37.1) [19.8 (13–36.5)], resp. ROC analysis revealed a Ct value of 27.8 for Prm1 and 28.9 for Prm2 to predict successful sperm retrieval (84% sensitivity/specificity). Moreover, Prm1 and Prm2 were correlated with TESE trial results (both $p < 0.001$).

Conclusion: Our results confirm the high prognostic value of a histological score count evaluation of spermatogenesis with regard to successful TESE. Moreover, Prm1/2-mRNA contents reflecting the presence of spermatids can be useful as additional diagnostic tool.

ECA 7: ANDROGENS AND HYPOGONADISM

OP19

Novel proteomic biomarkers of androgen deficiency from seminal plasma profiling using high-resolution mass spectrometry

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Background: Seminal plasma contains proteins secreted by testis, epididymis and male accessory glands and involved in successful fertilization. The function of epididymis, prostate and seminal vescicles is dependent upon the presence of androgenic stimuli.
Aim: To clarify the effect of pituitay-testis axes on on accessory glands, we applied high resolution mass spectrometry on humans seminal samples upon patients affected by secundary hypogonadism before and after testosterone replacement therapy (TRT).
Methods: We studied 10 fertile subjects and 20 hypogonadic patients. Ten patients were evaluated after 6 months of TRT. Seminal proteomic analysis was performed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module and coupled with an LTQ Orbitrap XL hybrid mass spectrometer. Bioinformatic tools were used to annotate the panel of androgen-dependent proteins according to GO annotations and to identify novel putative interactomic networks.
Results: Stringent criteria permitted the identification of a significative lower number of proteins in patients compared with normogonadal men. 14 proteins were present in controls, absent in hypogonadic patients and were identified after TRT. GO annotation analysis revealed that binding and enzymatic activities are mainly deficient in male hypogonadism. Seven differentially expressed proteins can fall into one large protein-protein interaction network, which directly involves the androgen receptor.
Conclusion: A high resolution proteomic approach was used for the first time to describe the seminal protein panel for severe male hypogonadism. These proteins represent putative physiological in vivo targets for androgen deficiency and might explain the association between infertility and male hypogonadism.

OP20

Testosterone supplementation and sexual function: a meta-analysis study

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Background: The role of testosterone supplementation (TS) as a treatment for male sexual dysfunction remains questionable.
Aim: To meta-analyse the effect of TS on male sexual function and its synergism with the use of phosphodiesterase type 5 (PDE5i).
Methods: An extensive Medline Embase and Cochrane search was performed. All randomized controlled trials (RCTs) comparing the effect of TS vs. placebo or the effect of TS as add on to PDE5ì s on sexual function were included.
Results: Out of 1702 retrieved articles, 41 were included in the study. TS is able to significantly ameliorate erectile function and to improve other aspects of male sexual response in hypogonadal patients. However, the presence of possible publication bias was detected. After applying 'trim and fill' method, the positive effect of TS on erectile function and libido components retained significance only in RCTs partially or completed supported from pharmaceutical companies (confidence interval [0.04–0.53] and [0.12; 0.52], respectively). In addition, we also report that TS could be associated with an improvement in PDE5i outcome. These results were not confirmed in placebo-controlled studies. The majority of them, however, included mixed eugonadal/hypogonadal subjects.
Conclusion: TS plays positive effects on male sexual function in hypogonadal subjects. The role of TS is uncertain in men who are not clearly hypogonadal. The apparent difference between industry-supported and independent studies could depend on trial design more than on publication bias. New RCTs exploring the effect of TS in selected cases of PDE5i failure who persistently retain low T levels are advisable.

ECA 8: SESSION OF THE INYRMF

OP21

High-resolution profiling of novel transcriptional events during human spermatogenesis

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Background: Advances in sequencing technologies make it possible to explore transcriptomes in unprecedented detail and to identify numerous novel transcriptionally active unannotated genomic loci (NUTs). The resulting uncharacterized transcriptional products are usually grouped into a heterogeneous class of transcripts, termed non-coding RNAs (ncRNAs) as they are likely not to be translated into proteins. While several recent studies provide global overview of the rodent testicular expression program using RNA-seq, no comprehensive survey and characterization of novel unannotated loci expressed in human testicular cells has been undertaken.
Aim: To identify novel transcriptional events corresponding to potential new actors of human spermatogenesis.
Methods: We performed paired-end high-throughput sequencing with RNAs (RiboZero kit, Invitrogen) from highly enriched preparations of five human testicular cell types.
Results: We identified 15′353 differentially expressed transcripts subsequently classified into 15 expression patterns. About 54.0 and 44.4% of the assembled transcripts were identified as known isoforms or novel isoforms of annotated loci, respectively, among which 14% corresponded to long non- coding RNAs (lncRNAs). Importantly, 378 NUTs located either within the intergenic streches (~3%), in the antisense (~24%) or within the intronic regions (~73%) of annotated genes were uncovered.
Conclusion: Our analysis has yielded the most comprehensive genome-wide insight into the transcriptome of human testicular cells during spermatogenesis. This human dataset is currently compared to those obtained in rodents to detect NUTs and lncRNAs with a conserved expression pattern across mammalian species. Our study represents a high-quality resource of novel loci expressed in testis that may play important roles during spermatogenesis and in male fertility.

OP22

Why are some human sperm immotile? A differential proteomics approach

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Background: The ability of sperm to move forward is crucial to accomplish in vivo fertilization and reduced motility (asthenozoospermia) is a common cause of male infertility. However, the mechanisms that regulate sperm motility are not fully understood.
Aim: Quantitatively compare the proteome of human sperm samples with different motility using cutting- edge proteomic techniques that had never been used before at this level.
Methods: Ten human sperm samples with different percentages of progressive motility (5 normozoospermic: mean ± SEM = 70.4 ± 0.81%; 5 asthenozoospermic: 18.7 ± 4.33%; $p = 0.008$), and with no differences in the percentage of live cells ($p = 0.093$), were compared. Proteins were labelled using 6-plex Tandem Mass Tag Reagents (Thermo Scientific TMT™) and analyzed by LC-MS/MS.
Results: Over a thousand proteins were identified and 80 differentially expressed proteins were detected (ratios astheno/normo ranging from 0.537 to 2.491). The differential proteins were categorized according to the information available at the Uniprot Knowledgebase and GOterms and cellular pathway enrichment analyses were performed. The larger group of altered proteins in asthenozoospermic samples is related to metabolism and energy production (26%). Interestingly, our results revealed that many of these proteins are post-glycolytic enzymes, supporting the idea that other metabolic pathways also contribute to fuel and regulate sperm motility. Proteins related to tail structure (6%), signal transduction (8%) and intracellular trafficking (6%) were also altered in asthenozoospermic samples, suggesting other possible players in this mechanism.
Conclusion: Altogether, these outcomes open new insights to understand the molecular basis of human sperm motility. Work supported by PI13/00699 and EU-FP7-PEOPLE-2011-ITN289880 to RO.

OP23

Expression of functional histamine H4 receptors in murine and human Leydig tumor cells

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Background: Histamine is known to modulate testicular steroidogenesis through H1 and H2 receptors in Leydig cells (LC), and a recent study suggests that it may regulate LC proliferation under pathological conditions. The H4 receptor (H4R), discovered thirteen years ago, is considered a promising drug target for allergy, inflammation, autoimmune disorders and cancer. Although there is indirect evidence of its presence within the testis, neither its specific localization nor its role has been established.
Aim: To examine H4R expression and function in LC.
Methods: We assessed H4R expression in MA-10 Leydig tumor cells (LTC) by immunofluorescence, and evaluated the effects of two H4R agonists on steroidogenesis and proliferation. We also studied H4R immunoexpression in eleven normal human (h) testes of four age groups (Gr): Gr1 (neonatal, $n = 2$), Gr2 (postnatal activation, $n = 4$), Gr3(pre-pubertal, $n = 3$) and Gr4 (pubertal, $n = 2$), and in two hLC tumors (3.92 and 6.0 years-old).
Results: We found that MA-10 LTC express H4R, and that its activation leads to inhibition of LH/hCG- induced cAMP production, StAR expression and steroid synthesis. Moreover, we observed decreased MA-10 cell proliferation after a 24-h H4R agonist treatment. We also found H4R to be expressed in R2C LTC. Finally, We detected H4R in 2/2 hLC tumors, but only in hLC of 3 normal testes, corresponding to Gr1, Gr2, and Gr4. No H4R was found in other testicular cells.
Conclusion: This study constitutes the first important step into understanding the physiological and/or pathological significance of H4R expression within the testis, and should be taken into consideration in the rational design of H4R agonists for therapeutic purposes.

OP24

Differential activation of inflammatory pathways in testicular macrophages provides a rationale for their subdued inflammatory capacity

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Background: Spermatogenic cells express cell-specific molecules that have the potential to be seen as 'foreign' by the immune system. In respect to the time difference between their appearance in puberty and the editing of the lymphocyte repertoire around birth, local adaptations of the immune system coined immune privilege are required to confer protection from auto-attack. Testicular macrophages (TM) play an important role in maintaining testicular immune privilege and display reduced pro-inflammatory capacity compared to other tissue-specific macrophages.
Aim: Investigate the molecular mechanism of subdued inflammatory response of testicular macrophages.
Methods: Western blot, QT-PCR, ELISA.
Results: We present evidence that TM have a lower constitutive expression of Toll like receptor pathway specific genes compared to peritoneal macrophages (PM). Moreover, in TM stimulated with lipopolysaccharide (LPS) the classical pro-inflammatory NF-κB signaling pathway is blocked due to lack of IκBα ubiquitination and, hence, degradation. Instead, challenge of TM with LPS or poly I:C induces MAP kinase, AP-1 and CREB signaling pathways which leads to production of pro-inflammatory cytokines such as TNF-α, though at much lower level than in PM. Pretreatment of TM with highly specific inhibitors for MAP kinases p38 and ERK1/2 suppress activation of AP-1 and CREB signaling pathways and attenuates LPS induced TNF-α secretion.
Conclusion: Our results suggest that TM maintain testicular immune privilege by inhibiting NF-κB signaling by impairing IκBα ubiquitination and a general reduction of TLR cascade gene expression. However, TM do maintain some capacity for innate immune responses through AP-1 and CREB signaling pathways.

ECA 10 INFERTILITY II: GENETICS AND EPIGENETICS

OP25

X chromosome-linked CNVs in male infertility: discovery of overall duplication load and recurrent, patient-specific gains with potential clinical relevance

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Background: Spermatogenesis is a highly complex process involving several thousand genes, only a minority of which have been studied in infertile men. In a previous study, we identified a number of Copy Number Variants (CNVs) by high-resolution array-Comparative Genomic Hybridization (a-CGH) analysis of the X chromosome, including 16 patient-specific X chromosome-linked gains.
Aim: Five gains (DUP1A, DUP5, DUP20, DUP26, DUP40) were selected for further analysis to evaluate their clinical significance.
Methods: The copy number state of the five selected loci was analyzed by quantitative-PCR on a total of276 idiopathic infertile patients and 327 controls in a conventional case-control setting (199 subjects belonged to the previous a-CGH study). For one interesting locus (intersecting DUP1A) additional 338 subjects were analyzed.
Results: All gains were confirmed as patient-specific and the difference in duplication load between patients and controls is significant (p = 1.65E-4). Two CNVs are private variants, whereas 3 are found recurrently and exclusively in patients. These CNVs include, or are nearby, genes with testis-specific expression. DUP1A, mapping to the PAR1, is found at the highest frequency (1.4%) that was significantly different from controls (0%) (p = 0.047 after Bonferroni correction).
Conclusion: DUP1A may cause spermatogenic failure due to the fact that this CNV fully duplicates a long non-coding RNA (LINC00685) that potentially acts as a negative regulator of the PPP2R3B gene, which is highly expressed in the testis. This study allowed the identification of novel spermatogenesis candidate genes linked to the 5 CNVs and the discovery of the first recurrent, X-linked gain with potential clinical relevance.

OP26

Impairment of DAZ-AZFc gene expression in postmeiotic human male germ cells of men with severe hypospermatogenesis

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Background: DAZ (Deleted in AZoospermia) genes are located in the AZFc deletion interval of the long arm of the human Y chromosome. AZFc deletions are known as most frequent genetic mutation event causing azoospermia and severe oligozoospermia. DAZ proteins are germ cell specific RNA binding proteins with function in the translational control of phase specific germ cell transcripts.
Aim: Functional analysis of distinct DAZ gene copies during postmeiotic human male germ cell development in men with azoospermia and severe oligozoospermia.
Methods: Collection of 150 testicular tissue samples from TESE patients displaying with different pathologies, to isolate DNA, RNA, and proteins. RT-PCR expression assays, immunoblotting and immunohistochemical staining assays for the analysis of DAZ genes expressed only in postmeiotic germ cells. Focus on DAZ1/DAZ2 gene copies to identify their distinct transcript and protein expression in men with severe hypospermatogenesis including severe oligozoospermia
Results: Expression of DAZ was found to be diagnostic for the presence of postmeiotic germ cells. Specific lengths of the DAZ RT-PCR amplification products revealed dominant expression for DAZ1 in testis tissue with normal spermatogenesis and specific expression of DAZ2 in sperm tails. Testicular tissue samples of men with severe disruptions in postmeiotic germ cell development displayed reproducible impairment of this DAZ gene expression pattern. Men with severe oligozoospermia display a significant reduction of DAZ2 protein expression in sperm tails.
Conclusion: DAZ proteins seem to be involved in the translational control of late postmeiotic germ cell transcripts functional for human sperm maturation and probably encoding distinct sperm tail proteins.

ECA SELECTED ORAL V: SEXUAL DYSFUNCTION

OP27

Development of secondary hypogonadism in aging men: risk factors and clinical picture: longitudinal results from the European Male Aging Study (EMAS)

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Background: Secondary (s) is the most common form of hypogonadism (HG). However, risk factors for its development and the associated clinical picture is unclear.
Aim: To identify factors which predispose eugonadal men (EUG) (T = 10.5 nmol/L, LH = 9.4 U/L) to develop sHG (T < 10.5 nmol/L, LH = 9.4 U/L) and to characterize symptoms associated with the development of sHG.
Methods: EMAS is a prospective cohort survey of 2736 community-dwelling men aged 40–79 year followed for 4.3 years. Subjects were classified according to their gonadal status into persistently (p) EUG (EUG at baseline and follow-up) and incident (i) sHG (EUG at baseline, sHG at follow-up).
Results: Gonadal status at baseline and follow-up was definable for 2006 subjects. For the purpose of this study, we considered 1671 subjects (83.3%) that were pEUG and 141 (7.0%) that were isHG. Risk factors for isHG were baseline overweight (BMI = 25 and <30 kg/m^2: odds ratio [OR] = 1.94 [1.14–3.28]; $p = 0.014$]), obesity (BMI = 30 kg/m^2: OR = 3.53 [2.01–6.22]; $p < 0.0001$) and presence of at least one comorbidity (OR = 1.52 [1.03–2.25]; $p = 0.034$). After adjusting for confounders, isHG men had a significantly higher risk of developing/worsening three sexual symptoms [low libido, erectile dysfunction (ED) and impaired spontaneous erections]. Besides lowering T levels, incidence/worsening ED was also associated with increasing BMI and presence of comorbidities at baseline or follow-up.
Conclusion: Obesity and comorbidities are risk factors for isHG. isHG is associated with incidence/worsening of sexual symptoms. Amongst them, low libido and decreased spontaneous erections are robustly related to isHG, independently from obesity and/or comorbidities. Although ED is also associated with isHG, it is also linked to obesity and comorbidities.

OP28

Mechanism of estrogen- or anti androgen-induced penile maldevelopment

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Background: Estrogen and/or anti-androgen exposure perinatally adversely affects reproductive tract development, but the mechanism remains unclear.
Aim: Determine mechanism of estrogen- or anti-androgen-induced penile development.
Methods: One-day-old rat pups were treated daily, subcutaneously, with estrogen or GnRH antagonist (GnRH-A) for 1–6 days. Tissues were collected at age day 7 and adulthood and examined for various parameters.
Results: Estrogen treatment induced penile mal-development characterized by loss of smooth muscle cells and sinusoids and accumulation of fat cells. These effects were dose-dependent (10 μg/kg/day or higher) and occurred in rats treated before 12 days of age, but not after. Microarray, Q-RT-PCR, Western blots, and/or immunohistochemistry showed significant reductions in Myh11 and Acta2 (smooth muscle cell markers), and up-regulation in Ppar gamma (adipocyte marker) and Esr1. Estrogen receptor (ESR) antagonist ICI 182 780 or androgen receptor (AR) agonist dihydrotestosterone (DHT) co-administration mitigated estrogen-induced effects. GnRH-A treatment induced penile abnormalities similar to those of estrogens, and DHT co-administration mitigated them. Both estrogen and GnRH-A treatments caused 70–90% reductions in the neonatal testosterone surge and steroidogenic enzymes in the testis.
Conclusion: Exposure of neonatal rats to estrogen or anti-androgen induces similar penile abnormalities, possibly mediated via ESR and/or AR pathways. It is hypothesized that the neonatal testosterone surge is essential for normal histogenesis of the penis; and that a reduction in the surge resulting from estrogen or anti-androgen exposure reprograms penile stromal cells such that biomarkers for smooth muscle cell differentiation are down-regulated and those for adipogenesis are up-regulated and consequently leading to penile mal-development (Support: NIH grants 5SC1ES019355, RCMI-5-G12RR03059).

OP29

Tadalafil ameliorates metabolic syndrome-induced alterations in visceral adipose tissue and liver: an experimental study in the rabbit

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Background: Development of 'brown-like' adipocytes within white visceral adipose tissue (VAT) has potential antiobesity effects. Genetic manipulation of cGMP formation suggests a role for this pathway in preadipocytes (PAD) commitment towards a brown phenotype.

Aim: To investigate the effect of the PDE5 inhibitor, tadalafil, on metabolic syndrome (MetS)-induced VAT dysfunction.

Methods: We studied the adipogenic capacity of VAT preadipocytes (rPADs) isolated from rabbits fed a high-fat diet (HFD), with or w/o tadalafil. Rabbits fed a regular diet were our controls.

Results: In HFD-induced rabbit model of MetS, in-vivo treatment with tadalafil (by increasing cGMP signaling), completely normalized HFD-induced increase of VAT mass and morphological alterations (adipocyte hypertrophy and hypoxia), triglycerides levels. Tadalafil also significantly increased brown adipocyte marker, UCP1, expression in VAT. HFD-induced increase in circulating level and liver expression of TNFa were also decreased by in-vivo tadalafil dosing. Comparative gene expression analysis in rPADs demonstrated that in vivo tadalafil dosing dramatically increased the expression of genes related to brown-differentiation (UCP1, TMEM26, PGC 1ß, BMP4, CIDEA) and mitochondrial biogenesis (TFAM, NRF1) in rPAD, whilst genes related to white adipocytes differentiation (HOXC9) were significantly reduced. Also in-vitro treatment with tadalafil, in HFD-rPAD induced the expression of genes related to brown differentiation (UCP1, TMEM26). By using transmission electron microscopic images analysis in rPAD, we found that HFD-induced mitochondrial morphological abnormalities (reduced size, loss of mitochondrial cristae, an increased matrix density) were all counteracted by in-vitro tadalafil dosing.

Conclusion: Tadalafil dosing ameliorates MetS-induced alterations by inducing a brown-like phenotype in VAT.

OP30

Sub-albuginean adipocyte accumulation is associated with erectile dysfunction: first clinical evidence and pathophysiological implications

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Background: Obesity and metabolic syndrome related androgen alterations are thought to play a pivotal role in the pathophysiology of erectile dysfunction. The role of androgens in the differentiation of sub- albulginean cells into trabecular smooth muscle has been poorly studied. Animal studies have shown that androgen alterations produce erectile dysfunction associated with replacement of normal smooth muscle by adipocytes, in the penile sub-albuginean region.

Aim: The aim of this study is to determine the presence of adipocyte accumulation under penile tunica albuginea in a group of refractory erectile dysfunction patients.

Methods: International multicenter case-control study. 17 erectile dysfunction patients (case group) and 14 potent patients with Peyronie's disease (control group) underwent penile prosthesis implantation and curvature correction surgeries, respectively. In both groups, sub-albuginean tissue samples were taken within the operative time. Groups were compared in terms of clinical characteristics, co-morbidities and presence of sub-albuginean adipocyte accumulation.

Results: Of the 17 patients in the case group, 11 presented cavernous fat cell accumulation, while only 1 patient in the control group presented this finding ($p < 0.05$). A significant association ($p < 0.05$) was found between adipocyte accumulation and erectile dysfunction (OR 24, 95% CI 2.48–229.36).

Conclusion: This is the first human study to report an association between erectile dysfunction and penile sub-albuginean fat accumulation. Metabolic syndrome-related conditions could cause disruption in androgen homeostasis, leading to adipocyte accumulation. Venous leakage secondary to accumulation of fat under tunica albuginea could be an important element in the pathophysiology of erectile dysfunction, especially in metabolic syndrome patients that do not respond to medical therapy.

OP31

Penile enlargement and augmentation: 411 cases

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Background: Increase in patients who require and/or desire penile lengthening and augmentation, has become

more evident throughout the last decade. Management of these patients was at first questioned because of the overall management of the procedures and the poor results of the surgical techniques. Fortunately over the years different surgical procedures have demonstrated to obtain acceptable results if patient selection criteriaisadequate.

Aim: We aim to present our series of 411 cases performed between January 1998 and December 2013. Focusing on the patient selection criteria, diagnostic methodology, surgical technique, post operative follow-up and overall management of this kind of patients.

Methods: Statistical analysis of our 411 cases, with explanation of the selection criteria, pre and post surgical measurement methodology and patient satisfaction rates as well as partner satisfaction rates were measured.

Results: Over 10 000 patients were seen by at three reference centers who had as initial demand penile lengthening or augmentation techniques. Less than 5% of these patients underwent any kind of surgical or mechanical treatment. Different cohorts where proposed depending on the treatment option followed. Satisfaction rates where in the range of 55–73% for patients and between 57 and 80% for partners.

Conclusion: Selection criteria in these patients are mandatory in order to obtain acceptable results. Multidisciplinary management is essential in order to avoid complications. Protocolisation of the technique increases the chances of obtaining good results and allows better management of complications.

BAYER INDUSTRY SYMPOSIUM CARDIOVASCULAR RISK: A CONCERN FOR THE ANDROLOGIST?

OP32

Effects of long-term testosterone treatment in obese hypogonadal men with and without type 2 diabetes

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Obesity is a worldwide epidemic which has reached a prevalence of more than 30% of the adult population in the US and more than 40% in some of the Gulf countries. Epidemiological studies consistently show that obesity is negatively associated with testosterone. Changes in body weight have a much greater impact on testosterone levels than age. Adipose tissue is considered the largest endocrine organ producing a number of substances such as estrogens, leptin, and inflammatory cytokines that suppress testosterone production. Low testosterone, however, leads to further accumulation of fat mass. This situation represents a vicious circle.

In an ongoing registry study of 340 hypogonadal men (total testosterone ≤12 nmol/L and presence of hypogonadal symptoms) being treated with testosterone in a urology office in Northern Germany, only 7% had normal weight (BMI < 25 kg/m^2) at baseline, 23% were overweight (BMI 25–29.9 kg/m^2) and 70% obese (BMI ≥ 30 kg/m^2). The same has been observed for waist circumference (WC) with 4% having a WC < 94 cm, 31% of 94–101.9 cm, and 65% ≥102 cm at baseline. This distribution seems representative for a mixed hypogonadal population as other registries show similar proportions of overweight and (central) obesity.

In controlled studies, testosterone treatment in hypogonadal men has consistently been demonstrated to reduce fat mass and increase lean mass. However, effects on weight were inconsistent with short-term studies suggesting moderate changes in weight in either direction. Most studies resulted in a reduction of waist circumference. Weight loss was only seen in very few studies, for instance, a weight loss of 4.3 kg within 30 weeks of testosterone treatment in men with metabolic syndrome (Kalinchenko *et al*. 2010).

Only when long-term studies with injectable testosterone undecanoate (TU) with a duration of 5 years and longer were published in 2013 and 2014, a new phenomenon became apparent, i.e. substantial and sustained weight loss. In a controlled study by Aversa's group, 5-year treatment with TU in men with metabolic syndrome resulted in progressive weight loss of 15.4 kg with a parallel decrease in WC of 9.6 cm (Francomano *et al*. 2014). In observational registry studies, patients lost 15.4 kg (Saad *et al*. 2013), 11.1 kg (Yassin & Doros 2013) and 14.3 kg (Zitzmann 2014). In none of these studies, patients regained weight during the observation time.

As was recently published in a meta-analysis on TU, the magnitude of effects depends on baseline BMI, duration of treatment, and age with younger patients responding in

a more pronounced manner than older men do (Corona *et al*. 2014).

In a subgroup analysis in 156 obese hypogonadal men with type 2 diabetes (T2DM), 6 years of treatment with TU resulted in not only profound weight loss but also a progressive and sustained reduction of glycosylated haemoglobin (HbA_{1c}) by 1.93% at the end of the observation period (Haider *et al*. 2014). Results of controlled trials in hypogonadal men with T2DM have been either positive or neutral, depending on duration of treatment, baseline diabetic control, and the preparation of testosterone that was administered.

It can be hypothesised that increase in lean mass and basal metabolic rate as well as behavioural changes are important factors in achieving weight loss and improvement in diabetic control by testosterone treatment, but also an impressive number of basic mechanisms have been revealed that are regulated by testosterone.

In conclusion, testosterone may have the potential to decrease obesity and improve T2DM in hypogonadal men.

OP33

Testosterone and cardiovascular risk: what is the evidence?

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Recent reports have significantly halted the enthusiasm regarding androgen boosting, suggesting that testosterone supplementation (TS) increases cardiovascular (CV) events. In order to overcome some of the limitations of the current evidence, we performed an updated systematic review and meta-analysis of all placebo-controlled randomized clinical trials (RCTs) on the effect of TS on related CV -problems. Out of 2747 retrieved articles, 75 were analyzed, including 3016 and 2448 patients in TS and placebo groups, respectively, and a mean duration of 34 weeks. Our analyses, performed on the largest number of studies collected so far, indicate that TS is not related to any increase in CV risk, even when composite or single adverse events were considered. In RCTs performed in subjects with metabolic derangements a protective effect of TS on CV risk was observed. Our data do not support a causal role between TS and adverse CV events. Our results are in agreement with a large body of literature from the last twenty years supporting TS of hypogonadal men as a valuable strategy in improving a patient's metabolic profile, reducing body fat and increasing lean muscle mass, which would ultimately reduce the risk of heart disease.

OP34

Erectile Dysfunction as a cardiovascular risk marker and predictor of poor quality of life

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Erectile dysfunction (ED) is one of the most common sexual dysfunctions in men. In the Massachusetts Male Aging Study, 52% of men aged 40–70 years were reported to have ED (Feldman HA *et al*. J Urol 1994; 151: 54–61). In a multicentre, cross-sectional, observational study in Spain with 1278 patients, ED was diagnosed at a screening visit in 74.3% of the subjects (Martínez Jabaloyas JM. J Sex Med 2014; 11: 2083–2091). On the other hand, several epidemiological studies have shown that the sexual function of women is affected in multiple domains when ED in their partner is present, especially in the aspects of arousal, orgasm, sexual satisfaction, and sexual pain.

Effective treatment of ED has resulted in improvements in men's erectile function (EF) and in multiple aspects of the sexual function of their untreated female partners as well as in the sexual quality of life of the couple (Jiann B-P, Su C-C, Tsai J-Y. J Sex Med 2013; 10: 420–429).

Organic ED is the manifestation of a vascular disease in smaller arteries and can give a two to three years early warning of an incident myocardial infarction. ED carries a 50% additional risk of coronary events, a level comparable to moderate smoking or positive first degree relative family history. ED can be considered a better predictor of coronary risk in type 2 diabetes than HbA_{1c}, hypertension, micro-albuminuria, or hyperlipidaemia (Hackett GI. BMJ 2008; 337: a2166). The above-mentioned new evidence suggests that organic ED is analogous to endothelial dysfunction, a known precursor to atherosclerosis in terms of molecular mechanisms and underlying risk factors.In other words, ED is associated with the same underlying risk factors as systemic atherosclerosis. ED and endothelial dysfunction are both mediated by a reduction of NO; the molecular basis of this reduction is virtually identical for erectile dysfunction and endothelial dysfunction (Ganz P. Am J Cardiol 2005;96 [suppl]:8M–12M).As mentioned, ED and cardiovascular disease (CVD) should be regarded as two different manifestations of the same systemic disorder. In Spain, ED is underdiagnosed (Prieto R et al. Atlas study; Rev Int Androl 2014, in preparation) and, for this very reason, it is important to highlight the message to all physicians who treat these patients to consider ED as an early marker of symptomatic CVD. Patients with ED without known comorbidities should be carefully examined for cardiovascular risk factors and diseases (Gandaglia G *et al.*: Eur Urol 2014; 65: 968-978).

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POSTERS

POSTER SESSION: ANDRO ONCOLOGY

P1

A potential tumour suppressor role of polymerase-1 and transcript release factor (PTRF) in prostate cancer

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Background: Prostate cancer remains a significant health issue for men world-wide. In the plasma membrane, 'cave-like' structures, called caveolae are present. Within these structures, the caveolae associated protein, polymerase-1 and transcript release factor (PTRF), is reported to be involved in the formation of caveolae. Expression of PTRF is lost in prostate cancer cell lines and tissues but it is unclear whether the loss of this gene will promote the progression of prostate cancer.
Aim: This study investigates the role of PTRF in normal prostate epithelial cells using siRNA.
Methods: Cells were treated with 120 nM of PTRF siRNA and control cells received scrambled siRNA. Seventy 2 h post transfection, PTRF expression was quantified using western analysis. MTT assays were used to measure cell proliferation and transmission electron microscopy was used to determine the presence of caveolae. Cell migration and invasion were also investigated.
Results: Treatment with PTRF siRNA significantly reduced PTRF expression and this was accompanied by a reduction in the number of caveolae ($p < 0.05$). Increased cell proliferation ($p < 0.05$), migration ($p < 0.05$) and invasion ($p < 0.01$) were observed in PTRF siRNA treated cells compared with controls. Furthermore, upregulation of expression of caveolin-1 but not caveolin-2 was observed.
Conclusion: These data confirm the role of PTRF in the regulation of caveolae formation. They also suggest that a reduction in PTRF expression and/ or the accompanying decrease in caveolae may result in the cells developing a malignant phenotype.

P2

Effect of aqueous cissampelos capensis extract on the prostate cancer cell line LNCaP

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Background: Prostate cancer and its metastases remain a problem among ageing men. Cissampelos capensis is a widely used medicinal plant in South Africa and has been found to be a rich source of different alkaloids, which can inhibit reactive oxygen species, cancer cell cytotoxicity and metastasis.
Aim: Since no scientifically documented information concerning the effect of *C. capensis* towards prostate cancer exists, this study aimed to investigate these aspects using the prostate cancer LNCaP cell line.
Methods: LNCaP cells were cultured under standard conditions and exposed to different concentrations (0, 0.001, 0.01, 0.1, 1, 10, 100, 1000 μg/mL) of an aqueous extract of *C. capensis* rhizomes for 24 and 96 h, respectively. Cell viability DNA fragmentation and collagenase activity were determined using standard methods. In addition, ROS and RNS inhibition was determined.
Results: Cell viability showed an initial dose-dependent increase indicating cellular stress. At higher concentrations (>0.1 μg/mL) adaptation and subsequent cell death followed after 96 h of exposure. Similarly, a significant ($p < 0.0001$) dose-dependent increase in DNA fragmentation after 24 h of exposure was found, followed by an absence of DNA fragmentation, excluding the two highest concentrations, after 96 h. These findings may either indicate antagonistic effects between the alkaloids or the development of resistance. *C. capensis* was able to significantly (inhibit collagenase activity, ROS production and RNS production.
Conclusion: *C. capensis* has rather effective cancer preventing than anti-proliferative properties. It may also prove to be an effective means of sensitising cells to lower doses of conventional anti-cancer drugs.

P3

The influence of selected immunosuppressants on the rat prostate

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Background: Immunosuppressants are used in order to reduce the risk of rejection of vascularized transplanted organs. However, their use may lead to adverse changes. Immunosuppressants can cause the excessive generation of reactive oxygen species, which are known to damage the cellular components and intensify the processes of apoptosis.
Aim: The aim of the study is to evaluate the effect of immunosuppressants on morphology, cytoskeleton proteins, intensity of apoptosis and PCNA in rat prostate.
Methods: The research was performed on 48 adult male Wistar rats, which were divided into a control and seven experimental groups. In the latter, the animals were being orally fed with pharmaceutical forms of drugs such as Encorton, Tacrolimus, Rapamycin, Cyclosporin A and Mycophenolate mofetil. Afterwards, the rats were killed, and the dorsal, lateral and ventral lobes of the prostates were obtained. In order to make a morphological evaluation, the sections of the prostates were stained by means of standard methods. Immunohistochemical reactions were

performed to the immunolocalization and immunoexpression of PCNA, cytoskeleton proteins (cytokeratin, desmin, vimentin), and histochemical reaction TUNEL to detect apoptosis.
Results: The immunosuppressants administered to the animals in different schemes caused differentiated glandular epithelial hyperplasia and stronger immunoexpression of PCNA. In most groups, stronger immunolocalization and immunoexpression of selected cytoskeleton proteins of three lobes of rat prostate were observed, as well as the highest degree of intensity of apoptosis in the ventral lobe.
Conclusion: Epithelial hyperplasia, stronger immunoexpression of cytoskeleton proteins, stronger immunoexpression of PCNA and numerous TUNEL-positive cells may indicate to the adverse influence of immunosuppressants.

P4

Effect of eurycoma longifolia (Tongkat Ali) on the prostate cancer cell line LNCaP

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Background: Eurycoma longfolia (Tongkat Ali, TA) is widely distributed in South-East Asia. Reportedly, TA extracts have cytotoxic, anti-proliferative and aphrodisiac properties. Although, there are studies on the cytotoxicity effects on MCF-7, HepG2 or HeLa cells, little is known pertaining the prostate.
Aim: This study investigated the effects of TA (Physta) on LNCaP cells and prostate specific antigen (PSA) production.
Methods: LNCaP cells were exposed to 0.0025, 0.025, 2.5, 25 and 250 μg/mL TA (Physta) for 24 and 96 h with and without testosterone. The following parameters were tested: morphology, cell viability (MTT), testosterone concentration, annexin V-CY3 binding, DNA fragmentation (TUNEL), caspase 3/7 activity and PSA production.
Results: Exposure of LNCaP cells to TA resulted in cellular detachment and death via apoptosis after treatment for 24 and 96 h at higher dosages (25 and 250 μg/mL). Significant inhibitory effects on testosterone-stimulated cell proliferation were seen at TA concentrations as low as 0.0025 μg/mL TA. At higher concentrations (>25 μg/mL), for all testosterone dosages, a decreasing trend in proliferation was found. All concentrations of TA showed a significant dose-dependent increase in apoptosis including DNA fragmentation. The relative caspase 3/7 activation showed significant ($p = 0.0043$) activation at 250 μg/mL TA. Relative PSA production resulted only in a 5% increase with no difference at all doses indicating that the decline in PSA concentration is due to LNCaP cells dying.
Conclusion: This study opens perspectives on the use of TA (Physta) in the treatment of aging male symptoms, prostate cancer prevention or as additional treatment to standard prostate cancer therapy.

P5

Hypogonadism in testicular cancer patients associated with risk factors of atherosclerosis

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Background: Testicular cancer (TC) is curable in 95% of patients, however long term risk of cardiovascular disease is increased.
Aim: To investigate whether hypogonadism (HG) in TC survivors is associated with early marker of atherosclerosis ABI (Ankle brachial index) and risk factors of cardiovascular diseases hypertension (HT) and insulin resistance.
Methods: Ninety two TC patients (median age at diagnose:30,8 years; mean follow-up: 9.2 years), and equal number age-matched controls .Serum (fasting, morning) was analysed for total testosterone (TT), LH, glucose and insulin. HOMAir:Glukos X Insulin)/22.5 mmol/L (normal = 2.0). HG:TT <10 nmol/L and/or LH>10 IU/L and/or androgen replacement. ABI: systolic (BP at the ankle/BP at the arm), (normal = 1.4). HT: systolic BP >140 mmHg or diastolic BP >90 mmHg. TC patients vs. controls as well as HG vs. eugonadal TC patients were compared using logistic regression (OR for abnormal value) and linear regression, adjusted for age and smoking.
Results: The ABI was significantly higher in TC patients compared to controls (mean diff 0.05, 95% CI; 0.01–0.09, $p = 0.008$). HG TC patients had increased OR of HT (OR= 4.8, 95% CI 1.1–22, $p = 0.04$) and borderline increased OR of abnormal HOMAir (OR = 3.4, 95% CI 0.04–12, $p = 0.06$). HOMAir was significantly higher in HG TC men as compared to eugonadal (Mean diff = 0.70, 95% CI 0.12–1.5, $p = 0.02$).
Conclusion: Increased ABI in TC survivors is an early sign of higher risk of atherosclerosis and may be associated with a more pronounced risk profile in hypogonadal patients

P6

Semen parameters in cancer patients before cytotoxic treatment

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Background: With increasing cure rate of young patients with cancer, quality of life after treatment has become a concern. It is known that neoplasms in general and testicular cancer in particular are accompanied by changes in semen characteristics.
Aim: To characterize cancer patients' semen parameters before cytotoxic treatment and to check if testicular tumors behave differently than other tumours.

Methods: Review of spermograms' results performed between January 2010 and March 2014, at the time of sperm harvest for cryopreservation, in oncological patients and prior to cytotoxic therapy. Statistical analysis was performed using SPSS V20.0
Results: 124 patients were selected with a mean age of 27.7 years. Semen was collected after an average 3.7 days of abstinence. 65 patients had testicular germ cell tumour (GCT) and 59 had various forms of cancer, most frequently haematological. 18.5 % of patients had hypospermia. As for sperm concentration, 7.3% had azoospermia and 27.4% oligozoospermia. For motility and morphology, 87.9% of patients had asthenozoospermia and 11.3% were teratozoospermic. Comparing semen parameters values and classification categories between GCT and other tumours, we found no main differences between these two groups. They only differed in immotility percentage ($p = 0.049$) and morphology ($p = 0.026$).
Conclusion: We found marked changes in cancer patients' semen parameters before cytotoxic treatment but no differences between GCT and other neoplasms

P7

Can effective routine HPV screen in men be possible?

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Background: Difficult approach for routine screening has been limited the early detection of persisting human papilloma virus (HPV) infection in male patients.
Aim: The objective is to develop a novel procedure for routine HPV screen in men?
Methods: Participants were selected from the Changhua County, Taiwan who visited the urology clinics. Male patients of age greater than 45 years with either first visit or follow up for benign prostate hypertrophy were selected. Digital rectal examination (DRE) was performed with gentle prostate massage before beginning stream urine collection. Standard in-house HPV PCR was assessed using the urine sample. A week later, urine was again collected but without digital maneuver and assessed with same PCR protocols?
Results: ?Test were performed for 4 subjects in one clinic, and urine samples 1 and 3 collected after prostate maneuver showed that both samples had the positive beta-lactin (DNA) and positive HPV markers, and that samples 2 and 4 were negative for both DNA and HPV markers. A week later, only subjects 2 and 3 returned to the clinic and urine samples were once collected without prostate maneuver and both urine samples showed negative result in beta-lactin DNA markers?
Conclusion: In our preliminary test using urine sample after a digital prostate maneuver, 2 out of 4 patients showed both positive DNA and HPV markers. As CDC of the U.S. announced that there was no effective method for HPV detection in male patients, we suggest that our study method could be used for HPV detection in male patients.

P8

The transrectal ultrasonography of prostate in men with congenital hypogonadism treated by long term testosterone replacement therapy

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Background: Long-term studies assessing the benefits and risks of testosterone replacement therapy in men, but little information is available regarding its effects on prostate tissue.
Aim: The aim of the study was to evaluate prostate transrectal ultrasonography findings in men with congenital hypogonadism treated by long term testosterone replacement therapy.
Methods: We have gradually included 31 men with congenital hypogonadism in period of 2001-2011. The average follow-up was 7.3 years (2 months–10.8 years). We have used Sustanon 250 i.m. every 3 weeks or Nebido i.m. every 3 months for continual testosterone replacement therapy. We performed to all patients the transrectal ultrasonography of prostate and seminal vesicles by biplanar rectal probe every 6 months.
Results: During the transrectal ultrasonography we observed in 22 (71.0 %) patients changes in prostatic tissue. In case of 12 patients were diagnosed asymptomatic prostatic cysts, in 9 patients prostatolithiasis and in 5 patients changes in echogenity of prostatic tissue. In 2 patients was found simultaneous occurrence of prostatic cyst and prostolithiasis, in further 2 patients simultaneous occurrence of hyperechogenic prostatic lesion and prostatolithiasis. The above described findings were diagnosed in 5 patients in the treatment lasting from 3 to 5 years, for the other 17 men with hormone replacement therapy longer than 5 years.
Conclusion: The study presents long term results of complex treatment in patients with disorders of sexual development. The interdisciplinary cooperation of andrologist and endocrinologist may significantly contribute to clarify an impact of testosterone replacement therapy on prostate development.

P9

Prevalence of testicular carcinoma in situ in Chile: research in high-risk population

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Background: An increased incidence of testicular cancer worldwide has been described. Testicular carcinoma in situ (CIS) is a pathological precursor of invasive cancer. In Chile, reports of testicular CiS are erratic.

Aim: We investigated the prevalence of CiS in the contralateral testis of men with invasive germ cell tumor. In a previous report (CAU Congress 2000) 0% incidence of testicular CiS was found. We developed a second serie of patients evaluated with greater precision histopathological diagnostic techniques.
Methods: In 78 patients with unilateral testicular cancer assisted at our institution, contralateral testis surgical biopsy was done at the time of radical orchiectomy. The specimens underwent inmunohystochemical analysis at the Department of Pathology, University of Granada, Spain for detection of placental alkaline phosphatase and OCT3-4.
Results: Only 1 sample showed focal presence of intratubular neoplastic germ cells positive for PLAP and OCT3-4, meaning a prevalence of 1.2% for this pathologic condition. Five additional patients showed other significant pathological findings: microcalcifications (4), and epithelioid granuloma (1). All six patients have coursed without events within a 7 years follow-up.
Conclusion: This results reinforce the findings of our previous research: low prevalence of testicular CiS in Chilean men, even in this high risk group. Possible explanations for these results could be (i) reduced exposure to endocrine disruptors, (ii) the size of the sample obtained, (iii) normal contralateral testes volume. We conclude contralateral biopsy is not routinely necessary in our country while remaining healthy testicle is of normal volume.

P10

Oestrogens in men: a misty in the male biochemical universe

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Background: To add some information about the physiology of oestrogens in men.
Aim: Attempting stimulate more interest and research in this controversial issue.
Methods: 16 men, aged 50–90, suffering an advanced prostatic carcinoma and submitted to orchiectomy, went to a serial follow-up of their plasmatic oestradiol levels. The follow-up ranged between 12 and 220 months.
Results: In 11/16 men, the plasma estradiol levels maintain the normal range along the follow-up.
Conclusion: Osteopenia/osteoporosis are prevalent conditions in aging male, especially in hypogonadic males as the men in our studied group. We propose oestradiol plasma levels serial determinations in their follow-up as an useful tool for setting adequate timing to seek and treat this pathological condition

P11

The role of magnetic resonance imaging in the identification of Leydig cell tumors of the testis: a large cohort prospective study

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Background: The use of testicular ultrasound (US) in the work-up for infertility has led to the incidental detection of an increasing number of small, asymptomatic, non-palpable masses. US is considered insufficient to discriminate benign from malignant neoplasms.
Aim: To evaluate the diagnostic accuracy and MRI features of Leydig cell tumors of the testis.
Methods: 115 patients with non-palpable lesions were consecutively enrolled in a prospective study. All patients underwent contrast-enhanced-ultrasonography (CEUS) and 50/115 also had gadolinium enhanced MRI (1.5-Tesla). MRI features and differential diagnosis of Leydigiomas from malignant tumors were analyzed.
Results: All lesions were = 1.5 cm. Malignant tumors were found in 23/50 patients while 27 had a benign lesion, and of these 19 were leydigiomas. Groups were compared by odds ratio (OR) and the frequency of MRI features by chi-squared and Fisher's exact tests. The parameters best discriminating leydigiomas from malignancies were well-defined margins (OR 2.054, $p = 0.004$) and very-low signal intensity on T2- weighted sequences (OR 2.769, $p = 0.001$). Leydigiomas showed marked hypervascular pattern with early peak enhancement (x^2 22.380, $p < 0.001$) and late and low wash-out (x^2 28.246, $p < 0.001$) when compared with malignant tumors, characterized by progressive enhancement and a continuous increase in signal intensity throughout time (x^2 21.314, $p < 0.001$). The sensitivity and specificity of MRI in identifying leydigiomas were 89 and 95%, respectively; the accuracy vs. histological diagnosis was 93%.
Conclusion: Testicular leydigiomas are more frequent than previously suspected and present peculiar MRI features. MRI could be used as an additional tool in the differential diagnosis of non-palpable testicular lesions.

P12

Elastosonography of the testis in the differential diagnosis of scrotal lesions: a qualitative and quantitative study

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Background: Testicular elastosonography (RTE) has recently proved useful in detecting testicular malignancies. Aigner (2012) reported 100% sensitivity of RTE in detecting neoplasms, but none has ever performed quantitative analysis.

Aim: The aim of this study was to evaluate RTE diagnostic ability in the characterization of testicular lesions using a qualitative (stiffness score, SS) and quantitative assessment (average mean strain, SR).
Methods: We retrospectively analysed 58 men with histologically proven testicular lesions, who underwent US and RTE evaluation. SS and SR were analysed in blind using dedicated software.
Results: Overall, 30 lesions were benign and 28 malignant. According to SS, 15.5% of lesions were assigned a score of 1 (homogeneously green), 41.4% a score of 2 (green and blue mix) while 43.1% a score of 3 (entirely blue). 62.5% of SS = 2 lesions were benign, while 64% of lesions with a SS = 3 were malignant (?2 3.441, $p = 0.064$, sensitivity 60% and specificity 70%). When comparing neoplastic vs. non-neoplastic lesions, we found that none of the non-neoplastic lesion presented a score of 3 (?2 6.031, $p = 0.016$, sensitivity 50% and specificity 100%). For the quantitative assessment, the mean SR was 0.67 ± 0.23 for malignant and 0.79 ± 0.31 for benign lesions ($p = 0.143$), while 0.71 ± 0.24 for neoplastic and 0.90 ± 0.45 for non-neoplastic lesions ($p = 0.257$).
Conclusion: The qualitative SS was found significantly different when comparing neoplastic vs. non- neoplastic lesions, but within the neoplastic group failed to discriminate between benign and malignant lesions. Quantitative SR calculation seems not to improve the diagnostic accuracy of testicular elastosonography, although confirmation in a larger cohort is needed.

P13

Evaluation of oxidative stress parameters in patients with prostate carcinoma treated with radiotherapy and androgen deprivation therapy

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Background: With the great survival improvement in patients with prostate cancer, long-term complications are becoming more relevant, especially cardiovascular risk, which is also influenced by androgens levels and oxidative stress (OS). We previously demonstrated low antioxidant levels in hypogonadism and their increase with replacement therapy.
Aim: To evaluate the relationship between OS and androgens levels in prostate cancer.
Methods: We enrolled 32 patients, 56–79 years, treated by radiotherapy and antiandrogen therapy (bicalutamide), studying: metabolic parameters (glycemia, total HDL LDL cholesterol, triglycerides, uric acid, albumin), hormones (testosterone, LH, FSH, estradiol, dihydrotestosterone, SHBG, IGF-1, PRL, FT3, FT4, TSH, insulin), OS parameters (total antioxidant capacity and the lipophilic antioxidant Coenzyme Q10).
TAC was evaluated using the system H202-metmyoglobin and chromogen ABTS, whose radical cation is spectroscopically evidenciated. The latency time (LAG, sec) in the appearance of radical ABTS is proportional to antioxidants content of sample. Coenzyme Q10 (CoQ10) was measured by electrochemical method, which allows to determine its reduced and oxidized form.
Results: 12 patients, previously treated with LHRH analogues, were still hypotestosteronemic (mean $\pm$ SD 1.36 ± 1.38 ng/mL), while in the other patients testosterone exhibited normal values (6.52 ± 2.35 ng/mL). Metabolic parameters were markedly worse in hypo-T vs. normo-T (HOMA index 5.01 ± 5.32 vs. 2.14 ± 1.43). OS parameters were markedly worse in hypotestosteronemic patients (LAG 70 ± 3.54 vs. 94.67 ± 16.04; total CoQ10 0.48 ± 0.19 vs. 0.61 ± 0.18 μg/mL; %CoQ10 ox/CoQ10 tot 0.13 ± 0.06 vs. 0.08 ± 0.07 sec).
Conclusion: These preliminary data suggest that in patients with persistent iatrogenic hypogonadism, oxidative stress is increased. This can damage cardiovascular system and reduce the survival of these kind of patients.

P14

Feasibility of testicular tissue preservation in pre- and post-pubertal boys

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Background: Cancer treatment has adverse side effects on male gonad functions and fertility preservation should be proposed before cancer treatment.
Aim: The aim of the study was to evaluate the feasibility of testicular tissue preservation (TTP) and the quality of post-thaw testicular tissue in pre- and post-pubertal males.
Methods: Age, urogenital history, clinical and biological data, histological diagnosis were assessed for each patient. A bilateral testicular biopsy was performed. Testicular tissue was frozen using a controlled slow freezing protocol. Integrity and structural modifications of post-thaw testicular tissue were evaluated on paraffin-embedded tissue sections.
Results: 80 patients, aged between 12 months and 35 years were included in our study: Malignant disease accounted for 72% of patients. Patients less than 20 years with malignant disease underwent bone narrow transplantation after TTP. TTP was proposed in post-pubertal boys because of sperm cryopreservation failure or severe sperm alterations. Only one haematoma occurred after TTP. After thawing, nuclear and epithelial alterations of seminiferous tubules remained very low. Spermatogonia concentration was significantly decreased in patients with lymphoblastic acute leukemia and lymphoma. Interstitial tissue fibrosis and thick basal lamina were more frequently observed in myeloblastic acute leukemia.
Conclusion: TTP is a feasible and suitable procedure for fertility preservation both in pre-and post- pubertal males. A slow freezing protocol maintains a good architecture of

seminiferous cords. However, the treatment received before TTP even if considered as a low level toxicity may have a negative impact on spermatogonia number and survival.

P15

Testicular cancer awareness: does the gender makes difference?

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Background: Testicular Cancer (TC) is one of the most common cancers among young male adults. The increasing incidence worldwide should concern doctors and patients. Several authors advocate that increasing awareness in target population prevent late-stage diagnosis and negative outcomes.

Aim: This study intended to evaluate the awareness of TC in an academic population comparing male and female population.

Methods: Online survey in academic setting concerning TC awareness and Testicular Self-Examination (TSE) pratice patterns and importance.

Results: A total of 815 participants (507 male and 308 female) answer the questions. The majority of participants, M-78.7% vs. F-89.3% ($p < 0.0001$) answered they had knowledge about TC. Only 76 (15%) male participants and 77 (25%) female ($p < 0.001$) participants answered correctly to the question about most frequent age of diagnosis. Most of participants knew the most frequent symptom (M-51.9% vs. F-57.8%) (ns).

In the question if they had ever hear aboutTSE, 38.3% male and 50.3% female answered afirmatively. Most of male participants (77.5%) had never performed TSE and only 8.7% did it monthly. Both gender considered it important and the majority of female participants were motivated to inform their male friends/partners to do it.

Conclusion: This is the first study to compare awareness of TC in both male and female populations. Our respondents showed a low level of knowledge and awareness to this condition.

Despite women are not affected by this disease, this group of female participants showed a comparable level of awareness to TC and could act as an important vehicle of information and motivation to men.

POSTER SESSION: GENETICS AND EPIGENETICS

P16

Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella

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Background: Ten to fifteen percent of couples are confronted with infertility and a male factor is involved in approximately half the cases.
Aim: A genetic etiology is likely in most cases yet only few genes have been formally correlated with male infertility.
Methods: Homozygosity mapping was carried out on a cohort of 20 North African individuals, including 18 index cases, presenting with primary infertility resulting from impaired sperm motility caused by a mosaic of multiple morphological abnormalities of the flagella (MMAF) including absent, short, coiled, bent, and irregular flagella.
Results: Five unrelated subjects out of 18 (28%) carried a homozygous variant in DNAH1, which encodes an inner dynein heavy chain and is expressed in testis. RT-PCR immunostaining, and electronic microscopy were carried out on samples from one of the subjects with a mutation located on a donor splice site. Neither the transcript nor the protein was observed in this individual, confirming the pathogenicity of this variant. A general axonemal disorganization including mislocalization of the microtubule doublets and loss of the inner dynein arms was observed.
Conclusion: Although DNAH1 is also expressed in other ciliated cells, infertility was the only symptom of primary ciliary dyskinesia observed in affected subjects, suggesting that DNAH1 function in cilium is not as critical as in sperm flagellum.

P17

The distribution of FSH and FSHR related polymorphisms in Danish testicular cancer patients (preliminary results)

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Background: Testicular carcinoma in situ (CIS) cells are precursors of testicular germ cell cancer (TGCC). CIS cells occur due to a neoplastic transformation of fetal germ cells caused by a niche- disturbances affecting the Sertoli cells. Several studies have reported association between the promoter polymorphism in the B-subunit of the Follicle Stimulating Hormone (FSHB) (−211 G/T) and reduced reproductive function in men. In addition, both sensitivity and transcriptional activity of the FSH receptor (FSHR) have been linked to two different FSHR polymorphisms, e.g. 2039 G/A and -29G/A, respectively.
Aim: To examine if these three genetic variants have a possible association with the development of TGCC. We investigated the frequency and distribution of the FSHB and two FSHR polymorphisms in patients with TGCC and healthy Danish controls.
Methods: 328 patients diagnosed with TGCC (seminoma: $N = 170$, non-seminoma and mixed: $N = 129$, unknown: $N = 29$) were retrospectively included. The control group consisted of 888 young men from the general Danish population. Genotyping was done by competitive allele-specific quantitative PCR (rs10835638, rs6166 and rs1394205).
Results: The distribution of the genotype frequencies of the three polymorphisms did not differ significantly alone or in combination between the TGCC patients and controls. Nor did it differ when comparing the distributions between the patients with semiomas and non-seminomas. Age at diagnosis was not influences by the three genotypes.
Conclusion: Our preliminary result indicates that the three genetic variants that modulates FSH-action do not have an individual or combined role in the development of TGCC in the Danish population. Further detailed analysis is needed.

P18

'Breaking' news from spermatids

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Background: Spermiogenesis is characterized by major alterations in chromatin structure. Using a combination of pulse-field gel analysis, comet assays and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), our group has established that the chromatin remodeling process is characterized by a transient surge in DNA strand breaks in the whole population of spermatids.
Aim: Since haploid cells cannot rely on homologous recombination for templated DNA repair, we hypothesized that the process may be genetically unstable and largely responsible for the observed male de novo mutations bias
Methods: We designed a flow cytometry sorting (FACS) scheme and generated three highly-purified populations of spermatids. A new DNA strand breaks immunoprecipitation method to capture the free 3′OH was used and libraries were prepared for next generation sequencing (NGS).
Results: Multiplex NGS yielded approximately 220 million 40 bp paired-end reads from a single Illumina HiSeq2000 lane. Using a peak-calling bioinformatics tool, we narrowed down 100–750 potential DNA breaks hotspots depending on the cell population and are currently validating these hotspots by qPCR. Dataset analysis from the elongating spermatids population library, revealed that a large fraction (61%) of the potential hotspots are located in the vicinity of genes extending from promoter to 3′UTR. In addition, a significant part of these potential hotspots occurs within coding exons showing 4-times enrichment compared to the normal genome distribution of exons.
Conclusion: We therefore provided the first genome-wide map of the endogenous DNA strand breaks in spermatids pointing to a newly found male-driven mechanism for the potential induction of de novo polymorphism.

P19

Testicular MicroRNA profiling in infertile patients with spermatogenic impairment

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Background: Spermatogenesis depends on precise, developmental stage- and germ cell type-specific gene expression. MicroRNAs (miRNAs) act as potent posttranscriptional suppressors. Our group postulate that the altered expression pattern of essential genes in impaired spermatogenesis could be due to differential expression of miRNAs in germ cells.
Aim: The aim of our study was to analyse differential miRNA expression levels in testicular biopsies from men with impaired and conserved spermatogenesis.
Methods: Testicular biopsies from 6 men with secretory azoospermia [3 of them presenting Sertoli cell only-syndrome (SCO) and the other 3 meiotic arrest (MA)], as well as 3 men with conserved spermatogenesis (CS) as control group were studied. We analyzed the expression levels of 623 mature miRNAs by RT-qPCR using Panel I and II v2 miRCURY LNA Universal RT miRNA (Exiqon) and the Lightcycler 480 (Roche). The relative quantitative method of 2-ddCp was used to calculate the relative quantification (RQ) miRNA expression values. Statistical analysis of the miRNA RQ data was performed using the t-Student test.
Results: The presence of 421 miRNAs (Cp value < 38) was confirmed in the CS human testicular samples. We found 217 miRNAs that showed significant differences in expression in SCO and/or MA groups ($p < 0.05$). In detail, 123 miRNAs were upregulated and 88 were downregulated in the SCO-CS comparison. From the downregulated miRNAs, 70 miRNAs were absent in SCO (Cp value > 38). In MA samples 9 miRNAs were found upregulated and 17 miRNAs downregulated when compared to CS.
Conclusion: Our results revealed several differentially expressed miRNAs in infertile patients with impaired spermatogenesis.

P20

Aberrant sperm DNA methylation patterns in unexplained infertility

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Background: DNA methylation has critical roles during germ cell maturation and gametogenesis, and alteration of this epigenetic process has been described in male infertility related to spermatogenic impairment and poor quality of semen. However, little is known about sperm DNA methylation pattern in men with unexplained infertility.
Aim: The aim of our study was to perform a genome-wide analysis of DNA methylation in sperm samples from normozoospermic fertile and infertile men.
Methods: Semen samples from 32 normozospermic infertile patients and 18 fertile men as controls were collected. Spermatozoa were isolated and DNA extracted (Wizard genomic extraction kit, Promega). Microarray-based DNA methylation profiling and specific analysis of genomic region and CpG island status was performed with Illumina Infinium HD Human Methylation 450K BeadChip and global DNA methylation status was quantified using the Methylamp global DNA Methylation quantification Kit (Epigentek). DNA methylation patterns of specific repetitive sequences were analyzed by bisulfite pyrosequencing.
Results: We have identified almost 4,000 CpGs showing aberrant DNA methylation pattern, and more importantly, the differentially methylated CpGs were significantly associated with specifically methylated CpG sites in sperm. In physiological conditions DNA methylated sequences were lower in sperm than in somatic cells, not only at specific loci but also at several repetitive sequences (LINE-1, Alu Yb8, NBL2, D4Z4). Notably, sperm samples of infertile patients showed significantly lower Alu Yb8 DNA methylation levels than controls.
Conclusion: These results provide a promising basis for future research.

P21

Analysis of meiotic segregation, sperm DNA fragmentation and screening for DPY19L2 gene in Tunisian globozoospermic patients

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Background: Globozoospermia is a rare but severe form of teratozoospermia. Lack of acrosome and round-headed spermatozoa are its main characteristics. Homozygous deletion of the whole DPY19L2 gene was identified as the most frequent alteration causing this defect. Abnormalities in nuclear structure can be associated to globozoospermia that could compromise embryonic development in case of use of medically assisted procreation.
Aim: To evaluate the levels of DNA fragmentation and aneuploidy rate and estimate the contribution of DPY19L2 deletion in 16 globozoospermic patients consulting for primary infertility.
Methods: The semen samples were analyzed using FISH and TUNEL assay. We performed PCR and screening for DPY19L2 gene.
Results: The mean DNA fragmentation index was significantly higher in patients compared to the control group (39.62 ± 5.87 vs. 10.41 ± 3.77). We also found a significant increase in the total sperm aneuploidy rate in patients compared to the controls ($3.67 \pm 0.60\%$ vs. $1.48 \pm 0.22\%$). We found a significant increase in the mean disomy rate for sex chromosomes (1.30 ± 0.22 vs. 1.06 ± 0.24) and chromosome 18 (1.75 ± 0.56 vs. 0.16 ± 0.07) and diploidy

rate (0.40 ± 0.18 vs. 0.13 ± 0.08). Molecular analysis of DPY19L2 gene showed that 9 patients (64.3%) were homozygous for the deletion, 2 (14.3%) were homozygous for the non-synonymous mutation (p.R298C) in exon8 and no DPY19L2 mutations was identified for 3 (21.4%) patients.
Conclusion: We confirm in this study that analyzing chromosomal aneuploidies and sperm DNA fragmentation associated to a molecular diagnosis for globozoospermic patients is of great importance, it would have an important impact for adopting the best course of treatment for these patients.

P22

Idiopathic recurrent miscarriage (RM): spermatic DNA anomalies

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Background: Semen analysis is usually used as the first step to evaluate the male factor infertility. However, a failure of the conventional semen parameters to predict this paternal effect indicates that hidden anomalies lie at the sperm DNA level or at the chromatin level.
Aim: To examine the possible relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters and RM.
Methods: Transversal study including 20 men whose partners had two or more unexplained RM and 20 fertile men as a control group. Semen samples were analyzed according to the WHO guidelines. Sperm DNA fragmentation was detected with TUNEL assay. Sperm chromatin condensation was evaluated with aniline blue staining and Fluorescence in situ hybridization was used for the aneuploidy analysis.
Results: Both motility (30.23% vs. 46.5%) and normal morphology (54.20% vs. 74.82%) were significantly lower in the RM group.
The index of fragmented DNA was significantly increased in the RM group (17.13 ± 11.6) compared to controls (10.25 ± 3.83) ($p = 0.01$).
The rate of abnormal chromatin condensation was significantly higher in the RM group (22.8 ± 8.7) compared to controls (11.8 ± 5.7%) ($p < 0.001$).
There was a significantly higher aneuploidy rate among the RM group compared to controls especially for gonosomal disomy and diploidy ($p < 0.001$).
Conclusion: Our results indicated that male partners of patients suffering from recurrent miscarriage had a higher rate of aneuploidy, sperm DNA fragmentation and chromatin anomalies. Their evaluation with TUNEL technique and aniline blue staining could be considered as part of the semen analysis for the assessment of male factor in couples with unexplained recurrent abortion.

P23

AZF midrodeletions screening in infertile men of the Portuguese population

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Background: Analysis of genetic conditions associated with male infertility is, at present days, restricted to chromosome analysis, AZF Y-chromosome microdeletions screening, and to patients with hypogonadotrophic hypogonadism or with congenital absence of the vas deferens. Among different populations AZF microdeletions can explain 10–15% of the infertile phenotype of azoospermic men and 2–5% of oligozoospermic men.
Aim: Here we present the results of AZF deletions screening performed in a selected group of infertile Portuguese men with idiopathic non-obstructive azoospermia or with oligozoospermia (men with other causes of male infertility, endocrinological alterations, varicocele, criptorquidism, professional risk factors, autosomal chromosomal abnormalities, were excluded for this study).
Methods: Analysis was performed by Multiplex-PCR using specific STS for the three AZF regions. Microdeletion breakpoints were confirmed using a second multiplex-PCR. We analysed 865 infertile men (270 azospermic and 595 oligozoospermic with [spermatozoa] $<5 \times 106$/mL) and 300 DNA samples obtained from fertile men of the Portuguese population.
Results: While AZF microdeletions were found in 27 azoospermic (10.0%) and in 22 oligozoospermic men (3.7%), in fertile men no microdeletions were detected. Microdeletions identified in azoospermic men: AZFc, 9 (3.3%); AZFb, 4 (1.5%); AZFa, 1 (0.4%); AZFc+b, 10 (3.7%); AZFc+b+a, 3 (1.1%); and in oligozoospermic: AZFc, 22 (3.7%).
Conclusion: Our results demonstrate that AZF microdeletions are frequent among males with the infertile phenotype described above. The regions absent have prognostic value for the clinical decision and patients treatment. Genetic counselling is recommended to all patients with AZFdel. AZFcdel will be obligatorily transmitted to all male offspring by ICSI, which will seriously impair their fertility.

P24

Genome-wide association study reveals two new susceptibility loci for testicular germ cell tumor

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Background: Epidemiological studies support the existence of a genetic component contributing to susceptibility of testicular germ cell tumor (TGCT), and to date 18 risk loci have been reported from GWA studies in UK and US populations. The incidence of TGCT is especially high in Western countries and Norway has one of the highest incidence rate worldwide, twice as high as in the neighboring country Sweden.

Aim: A GWA study was performed in order to gain novel insight in the genetics underlying TGCT in Scandinavian populations.

Methods: Genotyping: 596 577 SNPs in 1326 cases and 6687 controls from Norway and Sweden were performed using the Illumina HumanOmniExpress bead-chip. After excluding known susceptibility loci, 34 SNPs from 17 regions were selected for replication in an independent sample set of 806 case-parent triads and 313 cases and 313 controls. Genotyping in the replication stage was performed on a MassARRAY iPLEX system. Statistical analysis: Initially we assessed associations using a Cochran–Armitage trend test, and finally a fixed effect meta-analysis of the results from both the screening and replication stage.

Results: We observed genome-wide significant association for rs7501939 on chromosome 17q12 (per allele OR = 1.29 (95% CI = 1.19–1.40), p = 1.1x10e-9) and rs2195987 on chromosome 19p12 (OR = 1.31 (95% CI = 1.19–1.45), $p = 3.2\times10e{-}8$). The variant rs7501939 on chromosome 17q12 is located in the HNF1B gene, encoding a member of the homeodomain-containing superfamily of transcription factors, and has previously been reported to be associated with prostate cancer.

Conclusion: We identified two new TGCT susceptibility loci in Scandinavian populations, which should be followed up as promising functional candidate genes.

P25

PGS by array-CGH increases embryo implantation rate in patients with pathological sperm fish

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Background: Aneuploidy in sperm is related with disrupted spermatogenesis and high aneuploidy rates are correlated with poor IVF results and previous aneuploid conceptions. It is also established that PGS by array-CGH is a valid technique to discard aneuploidy in oocytes, embryos and blastocysts.

Aim: To evaluate if PGS by array-CGH improves embryo implantation rate in couples with pathological values of sperm aneuploidy diagnosed by FISH.

Methods: Retrospective cohort study of the 69 cases performed in our centre during 2012 and 2013, where ICSI was carried out in couples with a previous diagnosis of pathological sperm FISH. Forty eight couples decided to perform PGS/array-CGH (Group A), whereas 21 decided not to perform PGS (Group B). PGS was performed on day-3 and euploid embryos were transferred on day-5. Array-CGH was performed by BACs-based microarrays (BlueGnome protocol).

Results: In Group A, implantation rate was 39.6% whereas in Group B was 11.5%. The Student's t-test shows a statistically difference (p value = 0.033). Clinical pregnancy rate per transfer in Group A was 45.2% and in Group B, 21.4% (p = 0.052). Homogeneity between groups was evaluated with Student's t-test and no statistical differences were observed in relation with patient's age (35.3 vs. 35.9), oocytes retrieved (8.6 vs. 8.7), zygotes (4.9 vs. 4.4) and number of embryos transferred (1.8 vs. 1.6).

Conclusion: Our results suggest that the selection of euploid embryos by PGS based on array-CGH, could improve embryo implantation rate and pregnancy rate in patients with a previous diagnosis of pathological sperm FISH.

P26

New insights into the function of sperm retained histones

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Background: We previously demonstrated that the nuclear form of Glutathione Peroxidase 4 (nGPx4) has a peculiar distribution in sperm head and is required for proper paternal chromatin decondensation at fertilization. While protamines are the major component responsible for sperm chromatin packaging, a small amount of histones is also retained, the relevant role of which has been recently highlighted in influencing early embryo development

Aim: We hypothesized that paternal histone modifications are implicated in the process of sperm chromatin disassembly in the zygote.

Methods: Addressing this issue in nGPx4 KO mice as experimental model.

Results: We first assessed the presence of acetylated histones in cauda epididymal sperm from WT mouse and by immunofluorescence we were then able to detect hyperacetylated histone H4 in mature sperm partially decondensed by treatment with glutathione and heparin. Western blot analysis of hyperacetylated histone H4 and histone H3 acetylated at K9 and K14 showed significant higher amounts of modified histones in nGPx4 KO sperm

compared to WT sperm. When WT sperm chromatin status was analyzed in zona pellucida free oocytes fertilized in vitro in the presence of trichostatin (TSA), a faster decondensation kinetics was observed compared to untreated sperm. Having a higher content of acetylated histones, nGPx4 KO sperm did not show any decondensation following TSA treatment. In addition the analysis of DNA methylation pattern of the paternally imprinted gene Igf2/H19 showed a significant hypomethylation in KO sperm compared to WT ones.
Conclusion: These findings reveal a link between acetylated histones retained in sperm and paternal chromatin remodeling after fertilization.

P27

A quantitative-PCR assay for the screening of DPY19L2 copy number variations (CNVs) in patients with globozoospermia

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Background: Globozoospermia is a rare cause of infertility characterized by the absence/hypoplasia of the acrosome. The most frequent genetic defect is a deletion of approximately 200Kb removing the DPY19L2 gene, either in homozygosis (detectable by ±PCR) or in heterozygosis (detectable by deletion junction fragment amplification, DJFA). Two flanking Low Copy Repeats cause deletion through Non- Allelic Homologous Recombination.
Aim: To design a qPCR assay defining DPY19L2 copy number in globozoospermic patients.
Methods: Eight globozoospermic patients and two controls were analyzed by standard ±PCR and DJFA analysis. Exploration on the DPY19L2 locus in the Database of Genomic Variants showed a threefold increase in duplications compared to deletions, and that several deletions might have a different breakpoint undetectable by standard DJFA. Therefore, we developed a qPCR assay able to identify these deletions based on the amplification of the 5′UTR-Exon1 region in DPY19L2 in parallel with exon 10 of the HAL gene as the reference. Due to the high homology of DPY19L2 with different genomic regions, primers design for reliable results required a thorough bioinformatic analysis.
Results: ±PCR and DJFA identified the deletion in homozygosis in one patient and in heterozygosis in another. qPCR confirmed these results and revealed that the remaining six patients had normal DPY19L2 dosage. New deletion types or duplications were not detected in our small study population.
Conclusion: Our qPCR assay could be used as a first step for diagnostic testing of globozoospermia. Moreover, it allows detection of DPY19L2 duplications, which have not yet been explored in relation to male infertility.

P28

Clinical and genetic features of a large cohort of patients with congenital hypogonadotropic hypogonadism

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Background: Deficiency of the GnRH secretion/action is the causal event of congenital hypogonadotropic hypogonadism (CHH). CHH could be associated with a normal or defective sense of smell, respectively identifying the normosmic CHH (nCHH) or the Kallmann's syndrome (KS). CHH is clinically characterized by delayed/absent puberty and infertility and its pathogenesis present a strong genetic component.
Aim: We created an Italian network aiming to collect and characterize a large national CHH patients series in order to eventually improve their management.
Methods: We collected 405 cases, 169 KS and 236 nCHH, analyzing their genetic and clinical profile.
Results: The number of familial cases was similar (32.3% KS and 19.6% nCHH), but the prevalence of associated malformations (midline defects, renal agenesis) or cryptorchidism was higher in KS (17.9 or 49% vs. 6.9 or 18.4% in nCHH, respectively). Genetic analysis allowed the identification of contributing genetic defects in about 1/3 of the patients, with a major involvement of KAL-1 (7%), FGFR1 (14%) and PROKR2 (9%) and a rare involvement of other candidate genes. Variants were detected on a single allele in 85.2% of the cases, whereas biallelic variations, affecting either GnRHR or PROKR2 genes, were found in 4.5% and digenic defects in the remaining 10.2%.
Conclusion: In conclusion, although accumulating evidences indicate the existence of common pathogenic mechanisms for KS and nCHH, the combination of CHH with associated malformations or cryptorchidism appears more frequent in KS. Our analyses indicate that the pathogenesis of KS/nCHH is still largely unknown, but multiple gene defects may be seen in both categories.

P29

Further insights into the phenotypic expression of T222P variant of RXFP2 gene

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Background: Genetic variants of INSL3 and RXFP2 are the strongest candidate genetic factors in the still largely unknown etiology of non-syndromic cryptorchidism. All genetic variants detected in cryptorchid patients, including the T222P of RXFP2, are heterozygous and were also found in subjects with normal testis descent. A novel candidate gene for cryptorchidism in mice, PTGDS, has been described and barely explored in human so far.
Aim: To investigate, for the first time, about a possible oligogenic etiology of non-syndromic cryptorchidism in T222P mutation carriers.
Methods: We sequenced the full coding region and intron-exon boundaries of the RXFP2, INSL3 and PTGDS genes in 9 Spanish subjects (6 cryptorchid and 3 non-cryptorchid) carrying the T222P variant in heterozygosis.
Results: We found a total of 12 variants in the three genes: eight synonymous (RXFP2, INSL3 and PTGDS); one in the 3′ UTR of PTGDS; two missense mutations (INSL3, RXFP2) both bioinformatically predicted as tolerated; one in frame insertion at the acceptor splice site of intron 13 of RXFP2. All variants were present in both cryptochid and non-cryptorchid T222P carriers. The mean number of variations/genome was almost identical in the two groups (12.6 vs. 12.3).
Conclusion: None of the identified variants in the three candidate genes is likely to contribute to the etiology of non-syndromic cryptorchidism either alone or acting sinergically with the T222P variant. Similarly, the different phenotypic expression of T222P variant is not related to the SNP load in the three genes.

P30

A case of 46,XY disorder of sex development diagnosed prenatally

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46,XY disorder of sex development is characterized by a 46,XY karyotype, ambiguous genitalia with mild to severe penoscrotal hypospadias with or without chordee, dysgenetic testes, reduced to no sperm production, and müllerian structures that range from absent to presence of a fully developed uterus and fallopian tubes. Several gene mutations or genomic deletions and duplications have been associated with the condition. Deletions of the distal 9p region have previously been described as causal in rare cases of ambiguous genitalia, with clinical manifestations ranging from male external genitalia with cryptorchidism and undescended testes to 46,XY sex reversal 4 (OMIM #154230) with complete gonadal dysgenesis, female external genitalia and uterus. DMRT1, which encodes a male-specific transcriptional regulator and is a key factor involved in sex determination and differentiation in other species, is considered the most likely causative gene for disorders of sexual development seen with 9p deletions.
29-year old gravida was referred to our institution in the 31st week of her second pregnancy due to ambiguous genitalia detected on fetal ultrasound. Our patient and her partner were both healthy and the family history was unremarkable. Her first pregnancy had ended in miscarriage at 5 weeks gestation. Her second pregnancy had been uneventful, with normal results on nuchal translucency screening test. Fetal morphology scan showed no structural abnormalities but the genitalia could not be clearly visualized. Additional scans indicated the fetus was male but at 31 weeks gestation genitalia were shown to be ambiguous with a small penis or an enlarged clitoris and a bifid scrotum with visible gonads.
Our patient underwent amniocentesis with subsequent fetal karyotyping. The fetus was shown to have a normal male karyotype (46,XY). In addition, molecular karyotyping was performed on amniotic fluid and showed a microdeletion of the 9p24.3 region (arr[hg19] 9p24.3(220,253-1,999,170)x1) encompassing DOCK8, KANK1, DMRT1, DMRT2 and DMRT3 genes. FISH analysis confirmed the hemizygosity for the 9p region in the fetus. Parental studies for subtelomeric regions using MLPA method showed that the fetus inherited 9p24.3 deletion from the mother.
The parents underwent extensive genetic counseling and decided to continue the pregnancy. At 40 weeks gestation, our patient gave birth to a baby with a micropenis and a bifid scrotum that was otherwise healthy. A multidisciplinary team including a paediatric endocrinologist and paediatric urologist undertook postnatal management.
Our case highlights the importance of prenatal genetic testing which should include molecular karyotyping in cases of ambiguous genitalia. Detection of a rare microdeletion which is a likely cause of ambiguous genitalia but is not usually associated with developmental delay was very important for subsequent pregnancy management.

P31

Comprehensive study of three cases of macrozoospermia caused by AURKC mutatios

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Background: Macrozoospermia is a rare cause of infertility characterized by large-headed polyploid multi-tailed spermatozoa in the ejaculate. AURKC is the major candidate gene for this phenotype given its function in male meiotic cytokinesis. To date, five mutations in the AURKC gene were reported in this pathology.

Aim: To perform a thorough genetic characterization of three cases of macrozoospermia.

Methods: Two Moroccan and one Spanish macrozoospermic patients underwent a detailed genetic analysis including: (i) AURKC mutation screening; (ii) sperm FISH for chromosomal anomalies; (iii) sperm DNA fragmentation; (iv) microsatellite instability analysis.

Results: Sperm morphology evaluation showed 100% of combined anomalies with sperm count between 17-64 millions/ejaculate. All patients were homozygous for two AURKC mutations: (i) c.144delC (Moroccan patients), (ii) c.744C>G (Spanish patient). Chromosomal anomalies (mainly tetraploidy) ranged from 89.6 to 100%. No microsatellite instability was observed in spermatozoa, whereas sperm DNA fragmentation resulted to be increased (54.3% vs. 34% normal value). In one Moroccan patient two unsuccessful ICSI cycles were performed (no embryos generated). One couple underwent ART with sperm donor and one is attending ICSI with pre-implantation genetic screening (PGS).

Conclusion: This study confirms that AURKC mutation screening in macrozoospermic men is useful for both diagnostic and prognostic purposes. For the first time we show that microsatellite instability does not occur in macrocephalic spermatozoa. Negative ICSI outcome is likely to be related to the high rate of sperm chromosomal anomalies, however our data also shows a relatively high DNA fragmentation rate as an additional consequence of the mutation.

POSTER SESSION: INFERTILITY BASIC RESEARCH

P32

The effects of hydro-alcoholic extract of fennel (*Foeniculum vulgare* L.) seed on structure of testis in male rats

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Background: Overpopulation is one of the serious problems in the modern societies. Due to the adverse effects of synthetic drugs, contraceptive effects of herbal plants have been considered.
Aim: The aim of this study was to determine the effects of hydro alcoholic extract of fennel on structure of testis and number of sexual cells in male rats.
Methods: In this study 40 male Wistar rats were divided to five groups of eight rats each. Animals in control group received 1 mL of distilled water and experimental groups received 1 mL of hydro alcoholic extract of fennel at doses of 35, 70, 140 and 280 mg/kg for 60 days by gavage. At the end, animals were anesthetized, caudal part of the right epididymis was used for sperm counting. After fixation of testis, tissue sections were prepared and studied microscopically for the evaluation of histological changes. The results were analyzed using SPSS15 software and one-way analysis test.
Results: The results showed that the number of spermatogonia and sertoli cells decreased significantly in doses of 140 and 280 mg/kg ($p < 0.05$). The number of primary spermatocyte and sperm count decreased significantly in the experimental groups when compared to control group ($p < 0.05$). Furthermore thickening of basement membrane, cell apoptosis and irregular arrangement of germinal epithelium were observed in the experimental groups compared to the control group.
Conclusion: Hydro alcoholic extract of Fennel seed in used doses could reduce reproductive activity in male rats and has anti-fertility activity
Keywords: Foeniculum Vulgare Mill, testis, rat.

P33

Role of NLRP3 in an experimental model of testicular ischemia and reperfusion in mice

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Background: Multi-protein complexes called in?ammasomes have recently been identi?ed and shown to contribute to cell death in tissue injury. In?ammasomes are composed of one of several leucine-rich repeat receptors (NLRs) including NLRP1, NLRP3, NLRC4 and AIM2. NLRP3 is currently the most fully characterized in?ammasome and it is triggered by a variety of stimuli, including infection, metabolic dysregulation and tissue damage. Testicular torsion leads to tissue degeneration and usually requires emergency surgical intervention to allow reperfusion of the affected testis. The testis produces several in?ammatory cytokines, including IL-1beta. Furthermore, testicular-ischemia and reperfusion results in exaggerated production of reactive oxygen species and triggers the apoptosis machinery.
Aim: To better understand the role of NLRP3 during testicular-ischemia/reperfusion (TI/R), we investigated the molecular mechanism underlying the effects of inflammasome in KO mice during TI/R.
Methods: KO (Nlrp3tm1bhk) and wild-type (WT: C57Bl6) animals underwent 1 h testicular-ischemia followed by 1 and 7 days reperfusion. The mice were killed with an overdose of pentobarbital sodium and bilateral orchidectomies were performed.
Caspase-3 expression, a histological examination and TUNEL assay were performed.
Results: Following 1 and 7 days reperfusion there was an increase of caspase-3, a marked histological damage, an increase in the activity of TUNEL and an altered spermatogenesis in WT mice. KO mice inhibited caspase-3, decreased histological damage, reduced the activity of TUNEL as well as spermatogenic activity in testes subjected to I/R.
Conclusion: Our data suggest that the inhibition of the NLRP3 might have a therapeutic role for the management of patients with unilateral testicular torsion.

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Regulation of adherens junction dynamics in the rat testis: an in vivo study using an androgen withdrawal model

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Background: In the seminiferous epithelium, the restructuring of adherens junctions (AJs) plays a crucial role in the spermatogenesis. This eventis dependent on proteins kinase activity.

Aim: In order to better understand the function and regulation of AJs, expression of N-cadherin, ß- catenin, and c-Src kinase were evaluated in control rats and those exposed to the anti-androgen flutamide.
Methods: Flutamide (50 mg/kg bw) or vehicle injections were administered to 82-days old Wistar rats every day in six doses. Testes were collected from 90-days old rats. Expression of relative transcript levels for N-cadherin and ß-catenin were detected by qRT-PCR. Western blot analyses were performed to measure AJs and c-Src protein levels. Both analyses were worked out qualitatively, quantitatively, and further, validated by *U*-Mann–Whitney test. Additionally, localization of AJs proteins was immunohistochemically analyzed. To verify the AJs proteins and c-Src association in the rat testis co- immunoprecipitation and immunofluorescence staining were performed.
Results: In flutamide-treated rats statistically significant increase in N-cadherin expression ($p < 0.05$) was detected at both mRNA and protein levels. Immunohistochemical data revealed changes in N-cadherin and ß-catenin localization after flutamide treatment. Immunofluorescence showed down-regulation of N- cadherin/c-Src complexes. Additionally, co-immunoprecipitation revealed down-regulation of N- cadherin/c-Src, N-cadherin/ß-catenin, and ß-catenin/c-Src complexes following flutamide exposure.
Conclusion: Concerning our results, it seems likely that flutamide, by blocking the androgen action, is responsible for altered expression of AJs proteins and their co-localization with c-Src. The results indicate that restructuring of AJs can be modified by androgen-dependent activation of protein kinase.
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Broad actions of vitamin D in human testicular cells

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Background: Vitamin D is a steroid hormone involved in calcium homeostasis, but also in a number of pleiotropic actions. The vitamin D receptor (VDR), vitamin D hormones and vitamin D metabolizing enzymes are widely distributed throughout the body.
Aim: Our aim was to investigate effects of vitamin D on gene expression in a primary human testicular cell culture model.
Methods: Human testicular cells were isolated mechanically and enzymatically from testis tissue of brain dead donors. Testicular cell cultures were treated with various concentrations of 1.25(OH)2D3 for 16 h and RNA isolated. Changes on mRNA level before and after vitamin D addition were studied using microarrays. Highly regulated genes were selected and confirmed by RT-qPCR. After statistical analysis, candidate genes were analysed using Partek and IPA (Ingenuity Pathway Analysis) software.
Results: Sixty-three genes were significantly up and down-regulated. Highly significantly regulated genes were e.g. alkaline phosphatase (ALPL), CD14 molecule (CD14), calmin (CLMN), aromatase (CYP19A1), 1,25-dihydroxyvitamin D-24-hydroxylase (CYP24A1), dipeptidyl-peptidase (DPP4), insulin-like growth factor 1 (IGF-1), krüppel-like factor 4 (KLF4), methylenetetrahydrofolate reductase (MTHFR), sodium-dependent glutamate/aspartate transporter 3 (SLC1A1), six-transmembrane epithelial antigen of prostate 4 (STEAP4), and transmembrane protein 37 (TMEM37).
Conclusion: Besides well-known effects of 1,25(OH)2D3 on calcium homeostasis, we were able to show that 1,25 (OH)2D3 is involved in many other physiological functions in humans, including immune response, endocrine and reproductive as well as skeletal pathways demonstrating a broad spectrum of interaction of vitamin D with organ systems including testes.

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Human sperm removal by leukocytes: preliminary microscopic analysis

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Background: The mechanisms of leukocyte-sperm interactions are not finally elucidated. It is known, that in addition to traditional mechanisms of phagocytosis and degranulation, immune cells can also form extracellular traps (ETs) to ensnare and kill pathogens as well as sperm cells. However, there is a lack of information concerning the extracellular traps particularly in relation to human sperm.
Aim: The aim of this research was to describe the morphological characteristics of the sperm elimination.
Methods: The study was performed on ejaculated spermatozoa from normozoospermic men with leukocytospermia (in vivo experiment) or without leukocytospermia (in vitro experiment) using natural stimulator of the immune system – uropathogenic Escherichia coli O75:HNT.
Results: Scanning electron and light microscopy demonstrated adhesion of the E. coli and immune cells to the spermatozoa and agglutination of male gametes by the bacteria. Apparent immobilization of multiple sperm, and their removal by active leukocytes mediated by traditional phagocytosis and sperm entrapment (extracellular, unique networks with granular components), were observed in both experiments. Very interestingly, individual immune

cells phagocytized sperm cells and generated ETs simultaneously. At the early stage of spermiophagy, direct cell-to-cell attachment or contact via leukocytic processes was observed; however, at the final stage, engulfed sperm heads were visible in the cytoplasm of phagocytes. Moreover, leukocytes assembled and created a cluster-like structure that functioned as a centers of phagocytosis.
Conclusion: The morphological data obtained suggest that human sperm cells can stimulate and activate leukocytes triggering simultaneously traditional phagocytosis and the formation of ETs, which finally culminate in extensive sperm elimination.

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ER stress is involved in metabolic syndrome-induced male infertility

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Background: The metabolic syndrome (MetS) is a disease cluster of obesity, hypertension, dyslipidaemia and diabetes resulting from nutritional overflow. In recent years, obesity, a cardinal feature of MetS, has garnered much attention due to its association with male factor infertility. It was reported that obese men exhibit a higher incidence of infertility in association with metabolic disturbances and hormonal dysregulation compared to normal weight men.
Aim: Thus, we are interested to investigate the potential relationship between MetS and male factor infertility.
Methods: histomorphological analysis [hematoxylin and eosin (H&E) staining], hormone measurement, immunohistochemistry, sperm biology analysis, and gene expression analysis [real-time quantitative polymerase chain reaction (qPCR)].
Results: Db/db mice became morbidly obese and exhibit impaired reproductive function at 8 weeks of age. Declining sperm density and increased percentage of immotile sperm were evident in the testes of 24-week db/db mice. Spermatogenesis appeared to be normal, but reduced InSL3 positivity was observed, indicating lack of the adult Leydig cells. Low mRNA expression of steroidogenic enzymes like steroidogenic acute regulatory protein (STAR) was detected. With qPCR, we identified several endoplasmic reticulum (ER) stress-related genes that were expressed at a significantly higher level in db/db mice than in controls at all time points, among these were activating transcription factor 3 (Atf3), activating transcription factor 4 (Atf4), ER DnaJ homolog 4 (Erdj4).
Conclusion: The results of the current study show that ER stress in testis may play a role in the obese male mice infertility via affecting Leydig cell function.

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VASA mRNA detection in contrast to immunochemistry with a poly- and monoclonal antibody is specific for germ cells in the male urogenital tract

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Background: VASA protein (VASA or DDX4) is reported to be specifically expressed in cells belonging to the germ cell lineage, both in male and female. Spermatocytes show the highest protein level of VASA and therefore the strongest immunohistochemical staining.
VASA immunohistochemistry has not been used on semen to detect germ cells, which could be an informative marker in the context of male infertility.
Aim: To verify the specificity of VASA immunochemistry for semen analysis, we performed a detailed VASA immunohistochemistry analysis on the different anatomical parts of the urogenital tract.
Methods: The different anatomical parts of the urogenital tract, from multiple individuals, from pyelocalicial system to urethra and from tubuli seminifiri to urethra prostatica, were stained with immunohistochemistry using both a polyclonal and monoclonal VASA antibody. In addition, to confirm possible expression, fresh frozen samples of vesicula seminalis, testis and semen were investigated for mRNA-VASA using qRT-PCR.
Results: The polyclonal antibody against VASA stained bladder, vesicula seminalis, (germ cells in) testis as well as semen. The monoclonal VASA antibody, showed a similar-like pattern, although bladder remained negative.
mRNA-VASA qRT-PCR resulted in a positive finding in germ cell containing testis and sperm, but not in vesicular seminalis.
Conclusion: Polyclonal and monoclonal VASA antibodies stain testicular germ cells, as well as epithelial cells of the vesicula seminalis. mRNA-VASA expression was specifically detected in testis and semen. These data indicate that mRNA-VASA detection is a better germ cell marker for semen analysis than immunochemistry.

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Hormonal modulation of androgen receptor density in developing testes vs. initiation of spermatogenesis

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Background: In newborn rats Sertoli cell androgen receptor (AR) expression is weak and becomes more apparent around postnatal day 20 what correspond to first completion of meiosis. This may indicate minor role of androgen for first spermatogonial development, the steps preceding meiosis.

Aim: In this study we attempted to modify AR expression, measured quantitatively by AR optical density (ARopd) in Sertoli and peritubular cells of infantile rats' testes during first spermatogonial development.
Methods: Pups were treated with GnRH-antagonist alone (Ant) or in combination with sex steroids between postnatal day 5th and 15th and autopsied on day 16th. AR was identified immunohistochemically, ARpod was analysed semiquantitatively using ImageJ. Quantitative analysis of seminiferous epithelium was also performed.
Results: Androgen deficiency following Ant alone or Ant co-administered with estradiol benzoate decreased ARopd to 31 or 21% of control value (C) in Sertoli cells and to 46 or 37% in peritubular cells. This was associated with increased number of type A spermatogonia to 200% of C and their decreased differentiation to further steps (70% of C). Contrary, co-administration of Ant with testosterone propionate increased ARopd in Sertoli cells to 150% of C, reduced number of type A spermatogonia to 70% of C, but maintained their differentiation normal. Combined administration of EB and TP to Ant- treated rats normalized both ARopd and spermatogonial development.
Conclusion: AR expression in newborn rats' testes may be a subject of hormonal manipulations. Lower AR expression may facilitate initial germ cell multiplication, whereas its normalized expression may assure first differentiation of spermatogonia. Grant-UMED 503/1-089-03/503-01.

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The roles of ERp57 and surface thiol/disulphide exchange in spermatozoa-zona pellucida binding

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Background: A spermatozoon acquires the fertilization capacity by a process termed capacitation. Capacitated spermatozoa initiate fertilization by binding to the zona pellucida (ZP). Defective spermatozoa-ZP binding is a major cause of male subfertility. Despite the importance of spermatozoa-ZP interaction, its regulatory mechanisms are unclear. Accumulated evidence suggested that sperm ZP- receptor is a composite structure requiring coordinated action of different proteins that are assembled into a complex during capacitation.
Aim: By using native-gel electrophoresis, we have identified a ZP-binding complex on the capacitated human spermatozoa. Protein disulphide isomerase family A, member 3 (ERp57) which is important in mediating cell surface protein reduction, is subsequently identified as one of components. Thus, we hypothesize that sperm surface thiol/disulphide exchange mediated by ERp57 plays an important role in sperm-ZP interactions.
Methods: The sperm surface thiol was localized and quantified by a membrane-impermeable thiol- reactive reagent, 3-(N-maleimidylpropionyl)biocytin. The immunoreactivities of ERp57 and ZP-binding capacity were determined by immuno-staining and hemizona binding respectively.
Results: Our results showed that sperm surface thiol content can be induced by capacitation. Consistently, up-regulation of the sperm surface thiol content in vitro stimulates ZP-binding capacity of uncapacitated spermatozoa. Sperm surface thiol and ERp57 immunoreactivity are further demonstrated to be localized at the head region of capacitated spermatozoa, a region responsible for ZP-binding. In addition, treatment with ERp57 inhibitors suppresses the ZP-binding capacity of spermatozoa.
Conclusion: Our studies provide evidence that sperm surface ERp57 is involved in human fertilization. Continued investigation of the area will provide considerable understanding of the regulation of fertilization.

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Soy isoflavones and oxidoreductive balance in rat's epididymis

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Background: Soy isoflavones can mimic the action of estrogens. Their influence on the organism is discussed because of their additional potential antioxidant activity. Enviromental chemicals may enhance oxidative stress. Other hand, the relevant concentration of ROS is essential e.g. for sperm fertilization processes. Effects of soy isoflavones on oxidoreductive balance in male reproductive system is unclear.
Aim: Therefore, the aim of this study was to investigate the effect of these substances on the morphology of the epididymis and activity of antioxidant enzymes: GPX, GR and concentration of GSH in homogenates of rat's epididymis.
Methods: In order to this, females during pregnancy and lactation, and then male offsprings until reaching sexual maturity, were given daidzein and genistein (2 mg/kg bw/d and 20 mg/kg bw/d, p.o).
Results: In control rats we showed significantly higher activity of GPX in cauda compared with caput epididymis. However, exposure of animals on soy isoflavones caused a loss of that difference. The activity of GR after exposure on soy isoflavones at medium dose was decreased in caput, while within the cauda of the epididymis, there was a substantial increase in the activity of the GR compared to control group. Content of GSH in the homogenates of caput and cauda of epididymis was similar in all groups. We did not observe effect of supplementation with soy isoflavones at both doses on epididymis histology.
Conclusion: Therefore, we conclude, that exposure to soy isoflavones in low and medium doses during prenatal period until reaching sexual maturity can affect oxidoreductive balance in rat's epididymis.

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Effect of semen selection on DNA fragmentation in total and live sperm population

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Background: The effect of procedures to select sperm on DNA integrity is controversial.
Aim: We investigated the effect of density gradient centrifugation (DGC) on sperm DNA Fragmentation (sDF).
Methods: sDF was detected by TUNEL/PI.
Results: We found that after selection sDF decreased (from 22.7 ± 15.4 to 12.1 ± 11%) in 38/64 samples and increased (from 27.7 ± 14.2 to 57.3 ± 26%) in 26/64. Further, we found a positive correlation between the values of sDF before and after DGC (r = 0.49; p <0.0001). As selection deletes dead/immotile cells and most fragmented sperm are not viable/not motile, the change in the level of sDF during the selective procedures, depends also on the elimination of dead/immotile sperm. Hence, we investigated the effect of selection on sDF as detected in live sperm (live-sDF), comparing DGC, indirect Swim Up (iSU) and direct (dSU). By DGC, iSU and dSU, live-sDF increased in, respectively, 11/18 (from 18 ± 15 to 62 ± 29%), 7/10 (from 15 ± 12 to 39 ± 22 % to) and 6/9 (from 16 ± 12 to 47 ± 23%) whereas decreased in 7/18 (from 22 ± 20 to 8 ± 6%), in 3/10 (from 38 ± 30 to 14 ± 7%) and 3/9 (from 18 ± 9 to 16 ± 3%).
Conclusion: It seems that sDF developing during selection in live sperm is not caused by the selective procedures per sè, but rather by a damage induced by oxidative stress in samples susceptible to DNA damage. Although SU is less stressing than DGC, it did not ameliorate the effect of selection on live-sDF suggesting that the exposure of susceptible sperm to the athmosphere O2 is enough to provoke the increase of DNA damage.

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Sperm characteristics in an animal model of metabolic syndrome

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Background: The association between metabolic syndrome (MetS) and infertility is unclear.
Aim: We analyzed epididymal sperm characteristics in rabbits fed with high fat diet (HFD), that developed the main characteristics of MetS (high glycaemia, cholesterol, triglycerides, arterial pressure) and become hypo-hypo (high estrogens and low testosterone) (Filippi *et al*. 2009).
Methods: Sperm parameters were evaluated by microscopy and CASA (motility parameters), gene expression by RT-PCR and acrosome reaction by FITC-labelled-Lectin and fluorescence microscopy.
Results: HFD decreased progressive motility, impaired morphology and augmented parameters of hyperactivated motility (HA). MetS features were negatively correlated with motility and morphology. HFD rabbits showed altered expression of testis genes linked to fibrosis and of inflammation. Expression of such genes was related with MetS features and sperm motility and morphology. HFD also altered epididymal expression of genes of aquaporins, involved in fluid reabsorption. Expression of aquaporins correlated with MetS features and sperm motility. A group of HFD rabbits was treated with Tamoxifen, used in clinical practice to treat male infertility. Tamoxifen restored sperm total motility, but further decreased sperm morphology and increased HA. Finally, we evaluated acrosome reaction in response to progesterone: sperm of HFD rabbits did not respond to the steroid and Tamoxifen did not restore such activity.
Conclusion: Our data suggest that MetS affects sperm functions by inducing fibrosis and inflammation in testis and by altering fluid reabsorption in epididymis. Impaired acrosome reaction and premature HA highlight possible alterations in sperm fertilizing ability of HFD rabbits. In our model, Tamoxifen did not ameliorate HFD effects.

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Effect of varicocelectomy on sperm DNA fragmentation and sperm quality

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Background: Varicocele is one of the major and most frequent causes of poor quality sperm leading many men to infertility. Among the patients with this anatomic anomaly, have been observed a high sperm DNA fragmentation. The varicocelectomy has been suggested as a possible tool used for infertility treatments.
Aim: To evaluate sperm DNA fragmentation and seminal quality of varicocele patients and controls, as well to verify if the effects of surgical correction in the varicocele group for these characteristics.
Methods: Was performed the DNA fragmentation with Halosperm G2 kits and spermogram in men with varicocele (n = 9) and controls (n = 4). In the varicocele group, three men who did varicocelectomy were compared to those who have not been submitted to the procedure (n = 6), before and after a period of nine months.
Results: This result was observed a significant difference on sperm DNA fragmentation between the affected group (62 ± 0.29%) and controls (25± 0.07) (p = 0.03). The patients of the varicocele group that underwent surgery not showed differences in DNA fragmentation and seminal quality, although there was a harsh drop of sperm vitality in the group that was not submitted to the correction.

Conclusion: Men with varicocele presented higher sperm DNA damages. Despite no differences with the varicocelectomy, it seems to improve sperm quality. However, an increasing of sample size is necessary to confirm the results.

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Association between body mass index and reproductive characteristics in men with unknown fertility status

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Background: Obesity has a negative effect on female fertility, and alterations in testosterone, sex hormone binding globulin (SHBG), oestradiol and inhibin B are observed in overweight and obese men. Consequences of male obesity on anti-Müllerian hormone (AMH) levels are less explored, but recently a negative correlation was shown. How semen quality is influenced by a high BMI is still unclear, as conflicting results are reported.

Aim: To investigate the association between BMI and reproductive hormones and semen parameters in men with unknown fertility status.

Methods: 161 male participants were recruited. Blood samples were drawn before 10 a.m. for analyses of testosterone, SHBG, oestradiol, FSH, LH, inhibin B and AMH. Semen analysis was performed according to WHO recommendations. Associations between reproductive parameters and BMI were analysed by multiple linear regression (*indicates log-transformed data).

Results: Mean age was 35.5 years (22–58) and median BMI was 29 kg/m^2 (range 18–62 kg/m^2). 73 % of participants were overweight or obese. BMI was negatively associated with testosterone (B = −0.383, $p < 0.001$), SHBG (B = −0.562, $p < 0.001$), inhibin B (B = −3.234, $p < 0.001$) and AMH (B = −0.010*, $p < 0.001$*). FSH and LH were unchanged, while oestradiol increased with high BMI (B = 0.001, $p < 0.001$). An inverse relationship was found between BMI and sperm parameters; total count pr. ejaculate (B = −0.181*, $p = 0.009$*), concentration (B = −0.070*, $p = 0.040$*), progressive motility (B = 0.679, $p = 0.006$) and morphology (B = −0.075, $p < 0.001$).

Conclusion: In addition to well-known effects on sex hormones, we found that BMI was negatively correlated with AMH and semen characteristics.

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In vitro sperm production from prepubertal testis in mice: fresh, slow freezed and vitrified tissues

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Background: Cancer treatment during childhood impairs male fertility by spermatogonia depletion. Cryopreservation of testicular tissue was a strategy for fertility preservation before gonadotoxic treatments.

Aim: Therefore, the aim of this study was to investigate which technique between control slow freezing (CSF) or solid surface vitrification (SSV) could be an effective strategy regarding the integrity and the spermatogenesis yield of in vitro prepubertal mouse testicular tissue.

Methods: Post partum (dpp) 6.5-days mice testes were cryopreserved by CSF and SSV then cultured during 30 days (D30). 36.5 dpp and D30 fresh tissue (FT) were used as controls. Histological analyses were evaluated by light microscopy. Cellular proliferation (PCNA), differentiation between germ and Sertoli cells (Tra98) and presence of round spermatids (CREM) were evaluated by immunohistochemistry. Presence of spermatozoa (a-tubulin) was evaluated by immunofluorescence. Testosterone was measured by radioimmunoassay.

Results: For CSF and SSV, freezing caused serious alterations on testicular tissue at D0. However, SSV presented results closer to FT than CSF. Moreover, after culture (D30), only the ratio germ/Sertoli cells of testicular explants was significantly higher in CSF than in SSV ($p = 0.016$). Indeed, there was a greater survival of Sertoli cells in SSV. In addition, testosterone levels showed a better survival of Leydig cells in SSV. Finally, these parameters allowed a better sperm yield in SSV than in CSF ($p = 0.03$).

Conclusion: SSV appears to be a promising approach, representing an alternative strategy to CSF in the emerging field of immature testicular tissue cryopreservation and cryobanking with in vitro sperm production at D30.

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In experimental epididymo-orchitis uropathogenic *E. Coli* determine damage by controlling host cell death pathways

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Background: Bacterial epididymo-orchitis is the main cause of infectious fertility impairment in men with the severity differing according to the involved pathogen. Infections of the genitourinary tract with uropathogenic *E. coli* (UPEC) result in long term impairment of semen parameters. Bacterial virulence factors can control cell

death pathways in infected tissues: apoptosis is bactericidal, whereas necrosis facilitates bacterial dissemination and transmission.
Aim: To investigate the molecular mechanism how UPEC manipulate sertoli cells (SC) death pathways.
Methods: qPCR, Chip, Westren blot, immunofluorescence.
Results: In isolated rat SC, the UPEC virulence factor alpha-hemolysin can activate the transcription factor FOXO, which is mainly sequestered in the cytoplasm by AKT dependent phosphorylation. Activation of FOXO1 and FOXO3 was documented by dephosphorylation following the inactivation of AKT in SC. BIM (Bcl2-Interacting Mediator of Cell Death) is a proapoptotic protein which can directly activate BAX or BAK to induce apoptosis. FOXO can bind to the BIM promoter region to upregulate BIM expression. After UPEC infection FOXO was localized in the nucleus and shows increased DNA- binding activity in SC. Nonetheless, no change in the expression of BIM was observed. Mechanistically BIM expression seems to be epigenetically silenced by a decrease in histone 4 acetylation, but not by any change in DNA methylation status.
Conclusion: These results suggest UPEC can epigenetically silence BIM expression, a molecular switch which drives SC in necrosis, but prevents apoptosis. The resulting inflammatory response boosts germ cell death and prevents phagocytes from locating and eliminating the dead cells and bacteria by phagocytosis, a means for the pathogens to persist.

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Antioxidative potential of zinc in infertile patients with leukocytospermia

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Background: There are several causes leading to male infertility, such as oxidative stress due to leukocytospermia or nutritional insufficiency of trace elements such as zinc.
Aim: This study was undertaken to evaluate the impact of seminal leukocytes on sperm DNA integrity and to assess whether in vitro zinc supplementation can improve sperm quality.
Methods: Our study included 72 men divided into three groups according the level of leukocytospermia (L): group A (L = 0; $n = 20$), group B ($0 < L < 0.09$ M/mL; $n = 17$); group C ($0.1 < L < 0.99$ M/mL; $n = 21$) and group D (L = 1 M/mL; $n = 14$). All semen samples were analysed for DNA fragmentation using TUNEL assay. Thirty one semen samples were selected to study the effectiveness of zinc when incubated with sperm at a concentration of 6 mmol/L during 2 h. Reactive oxygen species (ROS) production was investigated using nitrobleu tetrazolium (NBT) staining. Seminal plasma total antioxidant capacity was measured by TAS Randox reagents.
Results: DNA fragmentation index (DFI) was significantly higher in group D compared to the other groups ($p < 0.001$) and significantly decreased after zinc incubation ($p = 0.032$). Samples from group D had the highest ROS level. ROS production was significantly lower after zinc supplementation ($p < 0.001$). ROS level and DNA fragmentation were positively correlated ($p = 0.003$; $r = 0.52$). Total antioxidant capacity was higher in group D compared to the other groups and rised from 1.41 ± 0.62 mmol/L to 1.9 ± 0.33 mmol/L ($p = 0.01$) after zinc supplementation.
Conclusion: Zinc ameliorates sperm quality in patients with leukocytospermia due to its strong antioxidant property.

P49

The effects of soybean phosphatidylcholine supplement on human sperm progressive motility: a pilot study

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Background: The egg yolk-based extenders contributed significantly to advances in human sperm cryopreservation. The use of soybean lecithin, that formulation contains 10% of phospholipid present in egg yolk, has recently been investigated. In a preliminary test conducted in our laboratory, purified soybean phosphatidylcholine lipids (soy PC) added to cryoprotectant medium has been found to support superior postthaw outcomes compared with egg yolk alone (Patent N° BR1020130192139/2013).
Aim: The aim of this study was to evaluate the effects of soy-PC lipids added to TEST yolk-based medium on sperm progressive motility.
Methods: Experimental study. Semen samples (one ejaculate from each of 5 consenting study participants) were subjected to routine semen analysis both before and after cryopreservation using TEST yolk buffer (Irvine Scientific) supplemented with soy PC at 1% or 3% (w/v). Semen and cryoprotectants combined to a final 1:1 v/v were loaded into 2.0-mL cryovials and cooled by rapid freezing method. Samples were thawed at room temperature and maintained at 37 °C for semen analysis. The results were analyzed by Student's t test at the $p < 0.05$ level.
Results: Sperm progressive motility of pre-freeze (fresh) and postthawed samples were 48.1% (fresh), 24.4% (TEST-yolk), 34.6 (TEST yolk PC + 1%) and 41.6% (TEST yolk PC + 3%). There was a statistically significant increase in

sperm motility from a mean of 24.4–41.6% in soy PC- supplemented medium at 3%.
Conclusion: The use of purified phospholipids can be a viable alternative as a supplement to the freezing medium to improve human sperm motility.

P50

In vitro effect of zinc on total antioxidant status and lipid peroxidation of ejaculated human spermatozoa

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Background: Spermatozoa are extremely vulnerable to oxidative stress caused by the unbalance between concentrations of reactive oxygen species and antioxidant scavenging systems present inside the male reproductive tract.
Aim: Main aims of the present study were to assess the influence of zinc in vitro incubation on total antioxidant status and lipid peroxidation in asthenoteratospermic and normospermic men.
Methods: Our study included 24 semen samples obtained from asthenoteratozoospermic (n = 14) and normozoospermic men (n = 10). After performing a semen analysis, each sample was aliquoted into two samples, the first sample was incubated with 6 mmol/L of zinc while the second was used as control. The two samples were then incubated at 37° for 2 h. Total antioxidant status (TAS) of seminal plasma was mesured using Randox Kit. Lipid peroxidation was detected by the dosage of MDA level in seminal plasma.
Results: Total antioxidant status was significanty lower in asthenoteratospermic group than normospermic group (1.84 ± 0.43 mmol/L vs 1.3 ± 0.66 mmol/L; p = 0.047). It significantly rised after zinc incubation (from 1.38 ± 0.65 mmol/L to 1.96 ± 0.28 mmol/L). MDA levels showed no significant increase in asthenoteratospermic group compared with normospermic group (p = 0.154). There was no significant difference in the rate of MDA after zinc supplementation (respectively 0.196 ± 0.177 nmol/L and 0.15 ± 0.099 nmol/L; p = 0.28).
Conclusion: Zinc seems to have a direct protective effect on human spermatozoa by preventing the decrease of total antioxidant status during in vitro incubation.

P51

Tissue analysis and ultra-high resolution for detection of sperms

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Background: Surgical sperm retrieval in spermatogenesis disorders is a challenging surgery, when in several locations must be found viable sperms in testicle by using testicular tissue extraction and subsequent microscopic evaluation.
Aim: The aim of the work was to verify the possibility to obtain information on the locations of viable sperms in testicle even before surgery.
Methods: We chose two methods: ultrasonography with ultra-high resolution and tissue analysis - histoscanning.
Result: We examined 9 patients with nonobstructive azoopermia. Before incision of testicle by ultrasonography detection using BK Medical Profocus 800 Ultraview we gained by linear probe at a frequency of 18 MHz image of testicular tissue in which we evaluated the suspected locations. The image of the cut was taken by scientific applications Advanced Medical Diagnostics (Waterloo) for examination of computer analysis - histoscanning. We used algorithms for the examination of prostate tissue, because algorithms for testicular examination are not available. As a control, we used the data from a surgery, where we incised the testicle with the identical cut as from ultrasonography and by repeated retrieval under microscopic control we got a map with locations of viable sperms. While the ultrasonography examination at 18 MHz locations matched in 76 % with findings from surgery, the histoscanning was not successful, probably because algorithms for the prostate cancer diagnosis are not sensitive to the testicular examination.
Conclusion: The examination of testicle with ultra-high resolution is of some importance in the detection of sperm locations, for more accurate assessment we assume a study with elastography.

P52

Sperm telomeres located in fragmented and non-fragmented DNA regions show similar telomere length

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Background: Telomeres are made up of DNA repeats and associated proteins, their main function is to protect chromosome ends from degradation and fusion. During meiosis, telomeres anchor chromosomes to inner nuclear membrane facilitating pairing and recombination. On the other hand, sperm DNA fragmentation is related to male

infertility, and different effects have been reported if single or double stranded DNA damage occurs.
Aim: The purpose of our study is to compare the telomere length of telomeres located on areas with fragmented DNA and areas of non-fragmented DNA using a novel methodology that combines the sperm Comet assay and Peptide Nucleic Acid Fluorescent In Situ Hybridisation (PNA-FISH).
Methods: Sperm Comet assay allows distinguishing both single and double stranded DNA fragmentation depending if alkaline or neutral electrophoresis is performed, respectively. PNA-FISH shows quantitative fluorescence intensity, which is related to telomere length. The combination of these two techniques allow quantification of the relative length of telomeres located on fragmented DNA regions (Comet tail) or non-fragmented DNA regions (Comet halo).
Relative telomere length from 25 fragmented Comets was analysed using TFL-Telo Software.
Results: No statistical differences were found between the lengths of telomeres located on fragmented and non-fragmented regions ($p > 0.05$) neither in alkaline Comet (816 ± 374 and 1005 ± 768, respectively) nor neutral Comet (816 ± 695 and 1026 ± 810, respectively).
Conclusion: The methodological combination of Comet assay and PNA-FISH allows evaluating the telomeres located on fragmented DNA regions. Our results show that the presence of DNA breaks in telomeric or subtelomeric regions do not impair telomere length on fragmented DNA sperm cells.

P53

Effect of Cissampelos capensis rhizome extract on human spermatozoa in vitro

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Background: In South Africa, Cissampelos capensis, is commonly known by the Afrikaans name 'dawidjies' or 'dawidjieswortel'. *C. capensis* is the most important and best known medicinal plant of the family Menispermaceae used by the Khoisan and other rural communities in the western regions of South Africa. Among numerous other ailments, it is traditionally taken to treat male fertility problems.
Yet, no studies have investigated the effects of this plant or its extracts on human spermatozoa.
Aim: The aim of the study was to investigate the effects of *C. capensis* extracts on sperm function.
Methods: Semen samples of 35 patients and 45 donors were collected and washed with Human tubular fluid medium supplemented with bovine serum albumin. Spermatozoa were incubated with different concentrations of *C. capensis* (0.05, 0.5, 5, 50, 200 μg/mL) for 1 h at 37 °C. A sample without addition of *C. capensis* served as control. The sperm functional parameters analyzed were motility, vitality, acrosome reaction, reactive oxygen species (ROS), capacitation, annexin V-binding, nuclear DNA fragmentation and mitochondrial membrane potential (MMP).
Results: Results show that viability, annexin V-positivity and MMP were not affected. However, the percentages of ROS-positive, TUNEL-positive, capacitated and hyperactivated spermatozoa increased significantly (ANOVA: $p < 0.0001$) in a dose-dependent manner.
Conclusion: It is concluded that the alkaloids present in the extract of *C. capensis* rhizomes triggered sperm intrinsic superoxide production leading to sperm capacitation and DNA fragmentation.

P54

Cytokines and insulin modulate steroidogenesis in TM3 Leydig cells: implications for metabolic syndrome

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Background: Obesity and metabolic syndrome (MetS) in males is associated with hypogonadism, low grade systemic inflammation and insulin resistance (IR). Furthermore, decreased progesterone has recently been associated with MetS in males, indicating a steroidogenesis collapse.
Aim: As cytokines and insulin modulate the hypothalamic-pituitary-testes axis, the relationship between MetS related inflammation and IR requires further investigation.
Methods: hCG stimulated TM3 Leydig cells were exposed to various concentrations of TNFa, IL1ß, IL6 and IL8 (0.1, 1, 10 and 100 ng/ml) and insulin (0.01, 0.1, 1 and 10 ng/ml) in optimal cell culture conditions. Cell viability, protein, testosterone and progesterone concentrations were assessed.
Results: TNFa significantly decreased all parameters in a dose dependent relationship. IL1ß and IL6 had a subtle but significant negative effect on cell viability, protein and testosterone concentrations, with a marked significant decrease in progesterone at all concentrations investigated. IL8 showed a dose dependent increase in cell viability, no significant effect on protein and testosterone concentrations, but a significant decrease in progesterone concentrations. Insulin significantly increased cell viability, protein and testosterone concentrations in a dose dependent relationship, however, progesterone concentrations were significantly decreased for all concentrations in a dose dependent relationship.
Conclusion: The inflammatory cytokines studied negatively influence cell function and steroidogenesis in Leydig cells. Insulin increased testosterone and decreased progesterone in this insulin sensitive model, and induction of IR in TM3 cells requires investigation. Mechanisms of immunological and endocrine mediators of steroidogenesis collapse in males with systemic inflammation may provide novel therapeutic targets for reproductive complaints and MetS associated consequences.

P55

Osteocalcin is associated with low testosterone concentrations but not oligo-astheno-teratozoospermia in men with infertility

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Background: It has been demonstrated that osteocalcin (Ocn) regulates testosterone (T) production in males. In mice, it was shown that Ocn promotes male fertility by stimulating T synthesis in Leydig cells, whereas in human, Ocn is associated with total T concentration, in the general population and in patients with bone disease.
Aim: The aim of this study was to investigate whether Ocn concentration is associated with the fertility status.
Methods: Thirty infertile Caucasian men (34.9 ± 1.2 years) were divided into two subgroups: men with normal T < 250 ng/dL (n = 15) or men with low T = 250 ng/dL (n = 15). Ten healthy Caucasian men from the general population (age 34.5 ± 2.1 years, p = 0.851 vs. infertile) were used as controls. Clinical, serum hormonal (FSH, LH, prolactin, T, inhibin-B, antimüllerian hormone, Ocn), and semen parameters were determined.
Results: Osteocalcin levels were statistically significant higher in infertile men with normal-T levels compared to infertile ones with low-T levels. When all men with normal-T levels (infertile and fertile) were analyzed as a single group, Ocn concentration was significantly higher compared with infertile group with low-T levels (12.46 ± 0.88 and 7.39 ± 1.52 ng/mL, respectively, p = 0.010). When all studied men were analyzed as a single group, there was no correlation between Ocn concentration and direct (sperm concentration) or indirect (FSH, testis volume, Inh-B, AMH) indices of spermatogenesis. On the contrary, serum Ocn concentration was positively correlated with serum T (r = 0.442, p = 0.004) and interestingly with prolactin concentration (r = 0.385, p = 0.021).
Conclusion: Osteocalcin is associated with T but not with the male fertility status.

P56

Mitochondrial activity and reactive oxygen species production define human sperm quality

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Background: Human ejaculates are heterogeneous, comprised of different subpopulations with discreet functional attributes. This complexity is lost in analyses on bulk samples. Reactive oxygen species(ROS) are mostly produced in mitochondria, and are implicated both in aiding sperm function and in causing gamete dysfunction and death, depending on amounts and timing of production.
Aim: In this study our aim was to to determine the existence of distinct subpopulations in human sperm, based on ROS content, and to study their relevance in terms of sperm function.
Methods: We applied the mitochondria-specific superoxide fluorescent probe MitoSOX™ Red (MitoSOX) to detect mitochondria-specific reactive oxygen species production in human sperm samples using flow cytometry, and functionally analyzed the resulting subpopulations in terms of functional markers, and their relationship to ART outcomes.
Results: We show that human ejaculates are heterogeneous in terms of ROS production, with three subpopulations clearly detectable, comprised of sperm that produce increasing amounts of mROS. The sperm subpopulation producing the lowest amount of mROS represented the most functional subset, as it was correlated with the highest amount of live and non-apoptotic sperm, and increased both in samples with better semen parameters, and in samples processed to select for higher quality sperm. Importantly the MitoSOX- subpopulation was clearly more prevalent in samples that gave rise to pregnancies, regardless of other characteristics.
Conclusion: Our work describes discreet human sperm heterogeneity at the ROS level, but also suggests that ROS may represent a strategy to both evaluate sperm samples, and isolate the most functional gametes for Assisted Reproduction.

P57

Poster cancelled

P58

Environmental levels of bisphenol A, genistein and vinclozolin feminize digit length ratios in male rats: towards a new sensitive indicator of prenatal endocrine disruption and its impact in the progeny

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Background: Second to fourth digit length ratio (2D:4D) is associated with various physiological, pathological conditions and some behavioral traits. The mechanisms determining the modulation of digit length ratios depend on both the prenatal levels of androgen to estrogens together with a different amount of their receptors especially in the developing fourth digit.
Aim: Here we aimed to test the hypothesis that low environmental doses endocrine disruptors may modify digit

length ratios in prenatally exposed rats as well as in their unexposed progeny.
Methods: We studied the effect on adult digit lengths and ratios of low doses Bisphenol A (BPA), genistein and vinclozolin in combination, the associations BPA/genistein, BPA/vinclozolin and, BPA/genistein/vinclozolin in male rats prenatally exposed (F1) as well as in their male unexposed progeny (F2). Radiographies were made for both left and right forepaws and 2D to 5D proximal to distal phalanx distances were measured using a standardized procedure based on semi-automatic image analysis. Moreover, we tested the possible association with anogenital distance.
Results: Exposure to environmental levels of BPA, genistein and vinclozolin was found to significantly change the digit lengths resulting in feminized digit length ratios, in comparison to the controls, especially, 2D:4D of the right forepaw. In addition, 2D:4D was also found significantly feminized in F2. Digit ratio and relative anogenital distance were not found associated.
Conclusion: Environmental levels of estrogenic and/or antiandrogenic compounds alone or in combination markedly influence digit length ratios making this measurement a highly sensitive indicator of low dose prenatal endocrine disruption.

P59

Neo-natal, pre, post-pubertal and adult modifications of the male reproductive axis and testicular gene expression after a continuous dietary exposure to mixtures of endocrine active substances

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Background: The reproductive impact of mixtures of endocrine active substances (EAS) remains poorly known.
Aim: To study the testicular transcriptome and various reproductive endpoints at critical developmental steps in rats chronically exposed to EAS mixtures.
Methods: Male rats orally exposed from conception to adulthood to bisphenol A (B, 5 μg/kg/day) alone and in mixture with genistein (G, 1 mg/kg/day) and/or vinclozolin (V, 10 μg/kg/day) were studied neonatally, prepubertally, postpubertally and as adults using various reproductive endpoints and testicular mRNA expression profiles followed by the integrative search of the functions modified using the Ingenuity software.
Results: Conventional reproductive endpoints were weakly affected by the various mixtures. In contrast, the testicular transcriptome was significantly impacted by B and all EAS mixtures in the neonatal period with very significant changes in networks of genes involved in the development and functions of the reproductive and endocrine systems. The impact of GV exposure on these systems dwindled gradually from birth until adulthood despite the continued exposure. Instead, BG, BV and BGV exposures from birth until adulthood increased significantly the modifications of expression of the numerous genes involved in the functions of the reproductive and endocrine systems. Of note, the male unexposed offspring of fathers exposed to EAS mixtures exhibited significantly delayed puberty and decreased sperm production.
Conclusion: Pre and postnatal exposure to EAS mixtures at low environmental concentrations seriously affect testicular gene expression without marked changes of the reproductive phenotype. The change of several phenotype traits in the unexposed progeny suggested epigenetic modifications in the germline requiring further studies.

P60

Considerable correlations between sperm chromatin integrity and sperm specific mRNA transcripts (PRM1, PRM2 & TNP2) in male partner of couples with recurrent pregnancy loss

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Background: 40% of reasons are idiopathic in women who experience recurrent pregnancy loss (RPL). Studies focused on female factors and male factors are ignored.
Aim: Findings in this research tracked the role of long-live sperm mRANs in early stage of embryo development and emphasize the importance of transcriptome of mature sperm in RPL.
Methods: Sperm were obtained from 51 male of RPL and 30 controls, attending to the Avicenna Infertility Clinic. Aniline Blue and Chromomycin A3 Staining were done to determine the replacement of the histones by protamines and assessment the sperm protamination respectively. QReal-time PCR was performed on RNAs.
Results: Significantly diminished PRM2 transcripts was seen in men involved RPL when compared to controls ($p < 0.001$). In contrast to PRM2, TNP2 mRNA quantity showed an inverse results, in a way that TNP2 transcripts significantly was higher in RPLs ($p < 0.05$). Then, statistical correlation tests between results of sperm chromatin damaging analysis (CMA3/AB staining) and the level of transcripts from PRM1 and PRM2 genes, revealed a significant negative correlation in RPLs. Means, decrease the mRNA copy numbers of protamines significantly increases the sperm DNA fragmentation.
Conclusion: Analysis of sperm DNA fragmentation is a potentially valuable method for explaining the paternal origin of RPL. Transcript quantification of protamines which are presented only in spermatozoa, and not detected in oocyte with involvement to chromatin

packaging and prohibition of DNA-damaging may be useful for prognosis in early embryogenesis and preventing of RPL. These data affirm the clinical indication for the evaluation of sperm DNA-damage prior to RPL treatment.

P61

Catsper calcium channels and human sperm functional parameters

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Background: CatSper is sperm-specific calcium channel activated by Progesterone (P) in human spermatozoa (Strunker *et al.*, 2011). KO mice for CatSper are infertile because lack of hyperactivated motility (HA). The role of CatSper in HA of human sperm is less clear. e demonstrated (Tamburrino *et al.*, 2014) that CatSper-1 is localized in the principal piece of sperm tail and highly expressed in swim up selected spermatozoa vs unselected. Two CatSper blockers inhibited several parameters related to progressive, but not to HA.
Aim: In view of the lack of specificity and the toxic effect of these inhibitors we decided to directly investigate the relationship between CatSper expression and some sperm functional parameters.
Methods: CatSper expression was evaluated by flow cytometry.
Results: We observed that the percentage of CatSper-1 expressing sperm is significantly lower in asthenozoospermic respect to normozoospermic men (mean ± SD: 54.4 ± 16.4% vs 72.04 ± 15.7%, $p = 0.001$) and there is a significant positive correlation between CatSper-1 expression and progressive motility ($r = 0.387$, $p < 0.001$, $n = 56$). CatSper-1 is also correlated to the percentage of HA ($r = 0.5$, $p < 0.001$, $n = 28$) and of HA parameters (VCL, ALH and negatively with LIN). A trend towards a positive correlation between CatSper-1 expression and basal [Ca2+]i was also observed in preliminary experiments ($n = 9$). Conversely, no correlation was found between CatSper-1 expression and basal/P-induced acrosome reacted sperm.
Conclusion: Our data suggest that CatSper expression is directly involved in HA and establishing [Ca2+]i levels in human sperm. CatSper is a possible molecular target for the development of novel therapeutic strategies for male infertility as well as for male-directed contraception.

P62

SUMO1 is related with DNA damage in human sperm

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Background: Sumoylation is a post-translational modification regulating many cellular processes. We demonstrated that SUMO1-ylation in mostly live human sperm is related to impaired motility suggesting an involvement in infertility (Marchiani *et al.* 2011). SUMO and sumoylation machinery are often located at DNA breaks (DSBs), including in mouse spermatocytes.
Aim: We investigated the relationship between sumoylation and sperm DNA fragmentation (SDF).
Methods: SDF (TUNEL) and sumoylation (immunofluorescence) were evaluated by flow cytometric method and multiparametric analysis. Confocal microscopy and immunoprecipitation/western were used for evaluation of Topoisomerase sumoylation.
Results: The percentage of SUMO1-ylated and DNA-fragmented sperm are correlated ($r = 0.4$, $p < 0.02$, $n = 37$) and most sumoylated sperm shows DSBs (70.6 ± 23.1%, $n = 3$). The two signals mostly localized in the nucleus. These results indicate that sumoylation may mostly mark live sperm with DSB. To further investigate association between SDF and sumoylation, we evaluated SUMO1 after stress conditions that induce SDF: freezing and thawing and treatment with H2O2 (5 mM, 2 h). We found that both SDF and SUMO1 levels increased after freezing and thawing (mean ± SD fold-increase, SDF: 189.4 ± 75.5% $p = 0.002$; SUMO: 242.5 ± 234.0%, $p = 0.02$, $n = 6$) and after H2O2 treatment (SDF: 122.4 ± 75.2%, $p = 0.001$; SUMO: 28.1 ± 28.2, $p = 0.04$, $n = 8$). Interestingly, we also demonstrated that Topoisomerase IIa, involved in introducing/repairing DSBs during spermiogenesis, is a substrate of SUMO1 in human sperm and co- localization of the enzyme with SUMO1 significantly increased after freezing and thawing stress (32.9 ± 6.1% vs. 50.7 ± 10.6%, $p = 0.04$).
Conclusion: These data indicate that sumoylation pathways are active and may increase in sperm following stress. We hypothesize an involvement of SUMO pathways in formation/repair of DSBs.

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EcoFoodFertility - environmental and food impact assessment on male reproductive function (EU research project proposal)

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Background: Over the past 60 years, several studies show a reduction of sperm concentration in men of many industrialized countries. Although environmental factors (pollution, stress, dietary lifestyle) may have a role in the reduction of quali-quantitative semen parameters, nevertheless, the causal effect of environmental factors on male fertility is still obscure.
Aim: The project 'EcoFoodFertility' aims at investigating the relation between the bioaccumulation of chemical

contaminants in blood and semen with sperm disorders and food intolerances in a cohort of men of different European countries.
Methods: In this observational cross-sectional multicenter study, 1200 healthy men (age 20–40 years, no- smoking, no exposed to occupational hazard, no-drinking), living in specific areas defined at high and low indices of environmental pressure, will be recruited from four European countries (Italy, Spain, Greece, Czech Republic), will measure the levels in the semen and in the blood of heavy metals, polycyclic aromatic hydrocarbons, Dioxins, PCBs and nanoparticles and it will correlate with conventional semen parameters,redox state, and with the index of DNA sperm fragmentation. For blood
lymphocytes will be made karyotyping and chromosomal stability by cytogenetic tests, will also measure the AntiTga and cytokines for gluten sensitive and celiac disease.
Results: In progress.
Conclusion: It is expected that the study will provide: (i) knowledge of the state of bioaccumulation of environmental contaminants in homogeneous populations in areas with different environmental pressure index; (ii) most reliable biomarker of cellular stress associated with bioaccumulation of environmental contaminants; (iii) the potential therapeutic role of innovative dietary formulations in a selected number of participants.

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Sperm protamine content and testis histology in a mouse model of intermittent hypoxia

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Background: Oxidative stress is present in sperm cells of infertile patients and is associated with DNA damage (Aitken and De Iuliis, 2010, Mol Hum Reprod; 16: 3–13) and decreased sperm motility (Domínguez *et al.*, 2007, Cytometry A; 71A: 1011–8). Moreover, a mouse model for intermittent hypoxia and oxidative stress showed a reduction in sperm motility (Torres *et al.*, 2014, Sleep, in press). Finally, sperm DNA damage correlates with changes in protamine content (Castillo *et al.*, 2011, J Androl; 32(3): 324–32).
Aim: To gain insight into the previously detected changes in mice subjected to intermittent hypoxia we aimed at measuring whether changes in protamine content in the mature sperm cell and in the histological organization of the testis could also be detected.
Methods: Adult male mice (n = 40) were exposed to either intermittent hypoxic (n = 20) or normoxic (n = 20; control) air conditions for six weeks. A subset (n = 10) from each group were then exposed to normal oxygen conditions for the following six weeks. Cauda spermatozoa were analyzed for concentration, viability and protamine ratio. Testes were fixed in paraformaldehyde and sectioned for histological assessment of spermatogenesis.
Results: Intermittent hypoxia did not alter sperm concentration, viability or protamine ratio. Moreover, all stages of spermatogenesis were seen among groups and further demonstrated that intermittent hypoxia did not alter endpoints associated with male fertility.
Conclusion: Despite no differences were observed, future studies will quantify sperm DNA oxidation levels and evaluate the presence of differential proteins within the sperm proteome that could provide clues into the fertility mechanisms involved in the mouse intermittent hypoxia model.
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Improvement of sperm quality in hyper viscous semen following DNase I treatment

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Background: Semen hyperviscosity can lead to male infertility and impairs sperm motility.
Aim: The improvement of spermatozoan motility.
Methods: Taking into consideration that neutrophils release chromatin in order to trap bacteria, we used DNase-I in order to improve spermatozoan motility.
Results: Following a fifteen minute treatment of semen with high viscosity, the motility of (a and b) spermatozoa increased to a statistically significant degree ($p < 0.0001$). The above treatment was also accompanied by a significant increase in the percentage of normal spermatozoa, from 5.468 to 7.25%, $p = 0.0076$, while at the same time, head and neck abnormalities decreased from 81.75 to 74.937% ($p = 0.0001$) and from 22.002 to 19.343% ($p = 0.0066$), respectively. Note that DNase treatment of semen with normal viscosity, had no effect on sperm motility. Comparison between sperm samples that underwent density gradient centrifugation following DNase I treatment, to those collected after density gradient treatment alone, showed that in the first case the results were more spectacular. In particular, the respective increase in (a) motility was 10.27 times in the first case versus 4.242 times in the second. Furthermore, PR movement in the first group increased from 32 to 76% of spermatozoa (2.375-fold) in comparison to a 1.776-fold improvement in the second group (from 40.866 to 72.666% of spermatozoa). The evaluation of each preparation in terms of yield (the percentage of final PR spermatozoa in relation to the initial number) revealed that the combined approach resulted in 29.782 vs. 18.519 with density treatment alone ($p = 0.0055$).
Conclusion: DNase-I treatment results in a spectacular improvement of spermatozoan motility.

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Method for intracellular detection of infectious pathogens in sperm cells

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Background: Viral presence in sperm is a cause of infertility. Viral factors as a cause of male subfertility have not greatly concerned the medical community up until now. On the contrary, a correlation between high numbers of Natural Killer lymphocytes (NK) in the blood of women with a history of subfertility and/or miscarriages, and the presence of subclinical herpes viremia (HSV1-2, EBV, CMV, HHV6 and HHV7) has been described. Observation of miscarriage material revealed that NK mostly infiltrated at the implantation site, while the blood NK levels in a portion of these women were normal. This can be explained if the embryos in these cases were by themselves antigenic due to the presence of viral (at least herpes-viral) antigens, originating from the male through the sperm cells, causing woman's NK respone.
Aim: The detection of intra-spermatozoan pathogens.
Methods: A method of intracellular detection of pathogens has been developed. The sperm cells are fixed, permeabilized and DNA digestion is accomplished with DNase I. Incubation with antibody against each pathogen is followed by incubation with a fluorescent conjugated secondary antibody. The samples are acquired in a flow cytometry apparatus and analyzed with suitable software.
Results: Results so far, show that a significant percentage of samples taken from infertile men were found to be infected by intracellular chlamydia and/or viruses. Especially in men infected with Chlamydia trachomatis, microbial load fell or the infection disappeared following antibiotic treatment and also improve their TERATOZOOSPERMIA Index (TZI).
Conclusion: The intra-spermatozoan pathogens can be detected and their treatment with antobiotics improve TZI.

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Contractile cell function and sperm on the move: time-lapse imaging

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Background: Contractile activity of testicular peritubular cells and the smooth muscle layer of the epididymal duct is crucial for maintaining male fertility. Sperm transport needs to be well orchestrated to ensure transport of spermatozoa thereby enabling their maturation, acquisition of motility and fertilizing capacity.
Aim: cGMP-related signaling mediates smooth muscle relaxation and can be enhanced e.g. by the PDE5 inhibitor sildenafil. Contractile cells in testis and epididymis express components of the cGMP pathway and are therefore susceptible to cGMP and sildenafil effects.
Methods: We developed a time-lapse imaging approach to visualize and assess contractions of seminiferous tubules and the epididymal duct as well as sperm transport in a near-physiological setting and to study cGMP-related effects.
Results: Rat seminiferous tubules showed an irregular contraction pattern of the tubular wall which could be transformed into a characteristic frequency spectrum by Fourier analysis. cGMP signaling and sildenafil shifted the frequency spectrum towards the slower frequencies.
In contrast, the rat epididymal duct showed a regular pattern of phasic contractions in all regions that resulted in movement of intraluminal contents. cGMP signaling and sildenafil slowed down contractile frequency.
Human seminiferous tubules, different to the rat, showed very slow peristaltic contractions eliciting sperm transport. These contractions seem to be absent in case of disturbed spermatogenesis and fibrotic changes.
Conclusion: Time-lapse imaging is a feasible technique that allows to study and visualize contractile cell function and sperm transport in testis and epididymis under near-physiological conditions. Moreover, the influence of various drugs and signaling pathways can be assessed using the time-lapse imaging approach.

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Influence of oxygen tension on human sperm function

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Background: The production of ROS in semen has been associated with a reduction in sperm motility, a decreased ability form spermatozoa-oocyte fusion and a diminished fertility in vitro, as well as in vivo. It is important in clinical terms to prevent the generation of ROS in sperm preparations in order to prevent the potential oxidative stress undergo by spermatozoa during course of in vitro fertilization (IVF).
Aim: As it has been shown previously that the oxygen tension in the incubation medium greatly influences the amount of ROS involved, the purpose of this work was to study the influence of tension oxygen (5, 10 and 20%) used during sperm capacitation on phosphorylation tyrosine protein, spontaneous acrosome reaction and fragmentation DNA.
Methods: Phosphorylation tyrosine protein by PY20 Immunostaining; acrosome reaction by immunostaining with Pisum sativum agglutinin lectin (FITC-PSA) and Sperm DNA fragmentation was evaluated by TUNEL. The cells sperm were observed with fluorescence microscopy.
Results: In the spermatozoa capacitated under an atmosphere of 20% O_2 observed levels significantly higher in tyrosine phosphorylation protein (PY20 labelled),

percentages of acrosome reacted (immunostaining with Pisum sativum agglutinin lectin) and DNA fragmentation (TUNEL assay), than in those capacitated sperm in gas phase of 10% O2 and 5% O_2.
Conclusion: We conclude that the use of a low oxygen tension (5% O_2) might improve spermatozoa competence during IVF programmes.

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Ultrastructure of human sperm cephalic vacuoles

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Background: High-resolution analysis of sperm morphology has become an interest area in male infertility evaluation. The presence of sperm vacuoles seemed relatively common in the sperm heads from both fertile and infertile men. However, the existence of vacuoles larger in size (which occupy between 13 and 50% of the sperm head's surface area) is relatively contradictory. Moreover, the precise origin of sperm vacuoles remains unknown.
Aim: Here, we analyzed the sperm morphology from four normozoospermic subjects using Transmission Electron Microscopy (TEM) in order to better define the ultrastructure of human sperm cephalic vacuoles.
Methods: Liquefied, fresh semen samples were evaluated according to the World Health Organization 2010 guidelines and then prepared for study by TEM.
Results: Intranuclear large vacuoles were recorded in 27% of the 580 studied sperm sections. In relation to vacuole origin, we observed that these vacuoles are invaginations of nuclear envelope. In addition, vacuoles with electron dense inclusion with membranes were detected in some sections. Finally, TEM analyses confirm that the high percentage of vacuoles is located in the anterior part of sperm heads.
Conclusion: Our findings questioned the hypothesis that vacuoles formation are related to acrosomal or plasma membrane residues.

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Cytomorphological evaluation of semen analysis in infertile patients with and without varicocele: a case- control study

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Background: Cytomorphological semen analysis is an essential part of the diagnostic work-up for male infertility. Apart from the assessment of sperm morphology, it should include the qualitative evaluation of cellular elements other than spermatozoa
Aim: Our aim was to study the characteristic changes in sperm morphology and other semen cellular components in infertile patients with varicocele.
Methods: The study included seventy cases divided into three groups. Group 1: infertile patients with varicocele, Group 2: infertile patients without varicocele and Group 3: fertile men. Semen analysis and round cells concentrations were analyzed. Differentiation between immature germ cells and inflammatory round cells was done by peroxidase test and cytological basis.
Results: Small, tapered and amorphous head percentages were higher in group 1 than in group 2. The percentages of midpiece abnormalities, bent neck and thick irregular neck defects were higher in group1 compared to other groups. The number of round cells in semen was lower in group 3 compared to groups 1 and 2. There were significant negative correlations between percentages of all types of tail abnormalities and sperm progressive motility in infertile patients, the strongest correlation was with irregular tail ($r = 0.71$, $p < 0.001$). There was a significant difference in the number of spermatogenic cells in semen between group1, group 2, and the control group ($p = 0.014, 0.018$,) respectively.
Conclusion: Varicocele affects mainly sperm head and midpiece morphology. No specific sperm tail defect has prevalence in infertile patients with varicocele. Increase in spermatogenic and peroxidase positive cells can occur in infertile patients due to other factors causing infertility.

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Seminal *Helicobacter pylori* treatment improves weak sperm motility in infertile infected men: a pilot study

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Background: *Helicobacter Pylori* (*H. Pylori*), one of the causes of chronic gastritis, was demonstrated in some extra-gastric disorders. Lately, the possibility of H Pylori involvement in male infertility gained attention.
Aim: This study aimed to assess the effect of treatment of seminal H Pylori in infertile men with weak sperm motility.
Methods: The study was conducted on 223 infertile men with weak sperm motility that was subjected to history taking, clinical examination, semen analysis and estimation of H Pylori IgA antibodies in their seminal fluid. Infected men were subjected to triple drug treatment; Omeprazole 20 mg, Tinidazole 500 mg and Clarithromycin 250 mg twice/day for 2 weeks. Semen analysis as well as H Pylori IgA antibodies was estimated after three months.
Results: In all, 22/223 men (9.87%) demonstrated H Pylori IgA antibodies in their seminal plasma. After treatment, mean seminal *H. Pylori* Ig A antibodies levels dropped from 1.55 ± 0.4 to 0.52 ± 0.26 concomitant with improved sperm motility. *H. Pylori* IgA antibodies demonstrated significant negative correlation with sperm motility grade A, sperm motility grade B, normal sperm morphology, non-significant correlation with sperm motility grade C, significant positive correlation with sperm motility grade D.
Conclusion: *H. Pylori* treatment improved weak sperm motility in infertile infected men. Also, seminal H pylori infection could be added as a cause to be searched for in male infertility.

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Blue staining of sperm tail as a marker of epididymal dysfunction in asthenozoospermic infertile men

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Background: Epididymal maturation of spermatozoa is essential for their fertilizing capacity and for the development of progressive motility. Epididymal dysfunction was found to be associated with increased percent of abnormally blue-stained sperm flagella in morphologically normal sperms. The determination of atypically stained flagella might be helpful in the diagnosis of epididymal diseases.
Aim: To evaluate the importance of blue stained sperm tail % as a marker of defective sperm maturation and its relation to abnormal sperm tail and midpiece morphology.
Methods: Fifty infertile patients and 20 healthy control subjects were included in this study. The samples were subjected to semen analysis, Papanicolau staining, determination of seminal neural alpha glucosidase level. Serum FSH, LH and testosterone were measured.
Results: The percentage of spermatozoa exhibiting an abnormally blue-stained tail was higher in the infertile group than the control group ($p = 0.000$). There were significant negative correlations between blue-stained sperm tail percent and total sperm motility, seminal a-glucosidase and serum testosterone. There were also significant positive correlations between % of blue-stained sperm tails and immotile sperms %, with % of morphologically abnormal forms % ($r = 0.538$, $p = 0.000$), while, it showed negative correlation with % of normal forms ($r = -0.63$, $p = 0.000$). Blue stained sperm tails were correlated positively with tail morphologic abnormalities and with % of a thick midpiece.
Conclusion: Our results point to the importance of assessment of blue stained spermatozoa in the evaluation of infertile males to detect the potential presence of an

epididymal factor influencing the maturation and motility of spermatozoa in asthenozoospermic patients.

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Evaluation of sperm DNA damage in men from infertile Saudi couples

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Background: Recent worldwide reports suggest a decline in semen quality in men with increasing percentage of male factor abnormalities in infertile couples. The pattern of abnormalities may be different from one part of the world to another, and this is probably multifactorial.
Aim: To review the pattern of sperm DNA damage in men screened for infertility in Saudi Arabia in order to establish prevalence and help future identification of responsible etiologies and possible treatments.
Methods: A retrospective study of semen analysis and sperm DNA damage using the Sperm Chromatin Dispersion (SCD) technique of men screened for infertility in dedicated infertility clinic, King Khalid University Hospital, Riyadh, Saudi Arabia between 1st of January 2009 to end of December 2011.
Results: A total of 405 male semen and SCD analysis reports were reviewed. 133 male subjects demonstrate normal semen parameters, while 251 had one or more abnormal semen parameters. Saudi male partners demonstrated increasing level of DFI in general (25.4, range = 5–97), though men with normal semen parameters had a significantly lower DFI (17.5, range = 5–32) than men with abnormal semen parameters (49, range = 21–97, $p < 0.001$). 66.6% of the study group had a DFI >30%.
Conclusion: This study demonstrates a high percentage of sperm DNA damage in our environment, more obvious in men with sub-optimal semen parameters. Thus, more studies are needed to address possible etiologies and treatments in order to improve fertility rates.

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Androgen receptor gene CAG tract polymorphism in infertile men

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Background: To date, no concrete evidence about the pathogenetic role of androgen receptor CAG repeat polymorphism (AR-CAG) in male infertility has been obtained.
Aim: This study aims to evaluate the role of AR-CAG in men with idiopathic infertility (IM) or with cryptorchidism (CryM) and Y –chromosomal microdeletions (YM).
Methods: One hundred and ten IM [90 oligoospermic (IOM) and 20 azoospermic men (IAM)], 19 CryM and 10 YM were included. Sixty-one healthy fertile man (FM) served as the control group.
Serum testosterone (T), FSH, LH and Inhibin B (IB) levels were measured as well as Y microdeletions and AR-CAG were assessed.
Results: AR-CAG were significantly longer in IOM ($p < 0.05$), CryM ($p < 0.05$) and YM ($p < 0.001$) than in FM. When the AR-CAG were subdivided in three subgroups according to the CAG length assessed in FM [the middle one with 19–21 triplets (n II) and the ends that are the <25% (15–18 triplets; n I) and >75% inter-quartiles (22–28; n III), respectively], there was a statistically significant difference among FM and IM ($p = <0.0005$). Moreover, the inter quartile n III was associated with lower levels of IB both in IOM ($p = <0.01$) and IAM ($p = <0.05$), while, when FM and IM were gathered in a single group, both the inter-quartiles I and III were associated with significantly reduced sperm count, lower testis volume and IB levels ($p < 0.01$).
Conclusion: The shortest tract and the longest one of AR-CAG may negatively affect the spermatogenesis in fertile and infertile men.

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Fine structure of Leydig cells in patients with non-obstructive azoospermia

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Background: Within the testis, Leydig cells play important role in maintianing spermatogenesis by producing testosterone and a number of paracrine factors that act on seminiferous epithelium and myoid cells of the lamina propria.
Aim: The aim of our study was to investigate the fine structure of Leydig cells in testicular biopsies of men with non-obstructive azoospermia (NOA).
Methods: Methods employed included histological analysis on semi- and ultrathin sections, immunohistochemistry, morphometry, and hormone analysis in the blood serum.
Results: Leydig cells in NOA patients displayed a kind of a mosaic picture across the same bioptic sample: both normal and damaged Leydig cells with pronounced vacuolisation and various intensity of expression of testosterone have been observed. Vacuoles seemed to have a unit membrane and content with a low electron density, apart from the thin area along the rim of the vacuole. The majority of cisternae of smooth endoplasmic reticulum were wider than normal, although the same cell could have areas of the cytoplasm with normal cisternae as well.

Leydig cells of NOA biopsies displayed a kind of mixed expression of testosterone. Some of the cells had normal intensity of the signal, whereas some demonstrated a weak and irregular expression of testosterone. The irregular and inhomogeneous signal was especially present in samples where many Leydig cells had vacuoles in their cytoplasm. Stereological analysis indicated a significant increase in volume density of vacuolated Leydig cells.
Conclusion: The continuous gonadotropin overstimulation of Leydig cells could result in the damage of steroidogenesis and deficit of testosterone in situ.

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Usefulness of a phenotypic morphologic check-up for diagnosis and treatment of infertile azoospermic men

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Background: Most studies of infertile azoospermic patients report azoospermia as non obstructive (NOA) or obstructive (OA). However, in each testis spermatogenesis can be affected independently of the other one (congenital defects, acquired pathologies); the same for each excurrent duct (ED: epididymis, vas deferens, ejaculatory duct). Moreover, on each side, testis and ED may be independently affected.
Aim: To improve the phenotypic classification of azoospermia on evidence-based medicine for a better management of infertile males and couples.
Methods: Descriptive data from Materials and methods in articles from literature as well as data for our own institution were used.
Results: At least six phenotypic classes of azoospermia are present in infertile men (Table). Using only two classes (OA/NOA) induces negative consequences for diagnosis and treatment; e.g.: Classification of azoospermia as NOA is not evidence-based when: - using indirect signs of impaired spermatogenesis (e.g. high FSH levels, low testes volume) which are not proofs of patency of ED; - using negative indirect signs of bilateral obstruction/absence of ED (e.g. normal semen volume, absence of CF mutations) which induces missing obstruction or non genetic absence of ED.
Besides, to not diagnose a double azoospermia (NOA + OA) or a mixed predominant NOA (NOA+ one side obstructed) negatively impacts results of any treatment aiming at restore spermatogenesis.
Conclusion: Excurrent ducts phenotypic status - based on past histories, clinical examination, seminal plasma markers and ultrasonic evaluation (scrotal, transrectal) - is required as part of an evidence-based etiological diagnosis of azoospermia for the most appropriate treatment.

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Analysis of surgical sperm retrieval, pregnancy outcome and waiting times in azoospermic men in a new service provided by IVF clinicians

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Background: Surgical sperm retrieval was previously performed by the urologist in our hospital. We have recently introduced this service by IVF clinicians after adequate training.
Aim: To evaluate the rates of sperm retrieval and subsequent pregnancy outcome in men with azoospermia by fledgling IVF consultants and to minimize waiting times for the men for an andrology consult.
Methods: This was a retrospective clinical analysis in an IVF unit. Twenty one azoospermic men consented for surgical sperm retrieval after appropriate evaluation of their partners. Twelve men were diagnosed with obstructive azoospermia and nine men with non-obstructive azoospermia. We analysed the sperm retrieval rate, appointment time and clinical pregnancy rate.
Results: Sperms were successfully retrieved in 83.3 and 66.7% of men with obstructive and non- obstructive azoospermia respectively. In the obstructive azoospermia group, a pregnancy rate of 50% was achieved as compared to 0% in the non-obstructive group. Although the number of patients included in the study was small, it is similar to the rates as published by major studies. Most of the appointments to see the men were scheduled quickly.
Conclusion: These results were comparable with published literature. The surgical technique for sperm retrieval can be acquired within a short period of intensive training. Having surgical skills for sperm retrieval allows an IVF clinician to provide one-stop-shop service to the couple and minimises waiting times for the male partner. Furthermore, the couples can be adequately counselled about all variable parameters which translate to a successful pregnancy before an individualised management plan is made.

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The virtual slide: a new efficient tool for training and quality control when assessing human sperm morphology

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Background: Wide range of results in andrology analysis.
Aim: Semen analysis requires adapted and relevant approaches for measuring the skill of the biologists and technicians. In the present study we introduced the methodology of the virtual slide commonly used in pathology

for a common assessment of sperm morphology by different laboratories through an internet connection.
Methods: A semen smear was prepared and Shorr-stained following WHO guidelines (2010). Then, the slide was scanned on all contiguous fields using the software driving both the x-y motorized stage and the z-autofocus of an Olympus station dedicated to the building of virtual slides. One hundred sperm with contrasted outlines and textures were visually selected, the system allocating an overlaid number to each to ensure that all observers analyze the same sperm. The selected spermatozoa were assessed using the modified classification of David (Auger, 2010) by a panel of experienced biologists from the Biologie Prospective (BP) Quality Control Steering Committee (BPSC) gathered for this purpose. Then, it was proposed to each BP adherent to assess the same spermatozoa thanks to the web connection facility.
Results: Four hundred and seventy BP adherents have participated in this pilot study and all succeeded in classifying the selected sperm having thus the possibility to compare their results to those of the BPSC.
Conclusion: From these preliminary results (exposed in detail in the poster), we concluded that the virtual slide is a useful tool for the andrology laboratory, both for training and for quality control. Further developments integrating the WHO 2010 criteria are ongoing

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Analitycal characteristics in men with primary (PH), secondary (SH) and late onset (LO) hypogonadism compared to a control group of healthy volunteers (V)

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Background: It has been proposed the need to define the testosterone (T) standard level in the general population through asssays that allow a correct diagnostic, in combination with at least one clinical symptom, of PH, SH & LO.
Aim: To compare the hormonal levels of the PH, SH & LO with V.
Methods: A comparative & descriptive study of PH (10), SH (10), LO (10) & V(10). Data of age, BMI, Laboratory assays & reference range: LH & FSH-IRMA [0.5–10.0 mUI/mL] & [2.2–10.0]; total T (TT) chemolu, [6.0–27.0 nmol/L]; Free T(FT)-RIA [5.6–27.0 pmol/mL].
Results: Mean (SD): Age V 22.18 (4.1), PH 56.5 (17.0), SH 41.8 (15.5), LO 62.3 (5.2) ($p < 0.000$); BMI: V 23% (3.7), PH 29.77% (3.4), SH 29.57% (3.8), LO 29.77% (3.8) ($p < 0.000$); LH V 3.39 (1.3), PH 21.29 (11.3), SH 1.15 (1.1), LO 7.27 (6.4) (p 0.002); FSH: V 2.83 (1.38), PH 40.25 (8.2), SH 1.93 (1.8), LO 15.07 (16.1) (p 0.177); TT: V 21.16 (4.1), PH 4.51 (2.7), SH 6.94 (6.1), LO 9.31 (1.5) ($p < 0.000$); FT: V 16.36 (3.3), PH 2.59 (1.7), SH 4.63 (4.9), LO 5.30 (1.3) ($p < 0.000$). Correlations: a significant difference betwween age and BMI of V & the other groups ($p < 0.000$); LH of V with PH ($p < 0.000$), PH & SH ($p < 0.000$), PH & LO (p 0.004); FSH with V & PH ($p < 0.000$), PH & LO (p 0.006); TT, V & SH, PH & LO (p 0.000); FT, V & PH, SH (p 0.000).
Conclusion: We found a significant difference between the groups. It is necessary a study of our referral population to diagnose correctely the Hypogonadism.

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Comprehensive investigation in patients affected by globozoospermia

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Background: Globozoospermia is a rare (<0.1% of infertile patients) but severe form of teratozoospermia causing male infertility due to the presence of round-headed acrosomeless spermatozoa associated to high rates of fertilization failure and embryo loss.
Aim: This study aims at a comprehensive phenotypic/genetic characterization of subjects suffering this condition.
Methods: A total of 8 infertile patients displaying >90% of globozoospermic spermatozoa was subjected to: (i) genetic analysis of the DPY19L2 gene, the most frequently mutated gene in globozoospermia, and for SPACA1, a novel candidate gene reported to be associated to globozoospermia in knock-out mice models; (ii) fluorescent in situ hybridization (FISH) analysis in spermatozoa; (iii) transmission electron microscopy (TEM) examination; (iv) microsatellite instability (MSI) analysis to evaluate genomic instability; (v) TUNEL assay for sperm DNA fragmentation (DF) assessment; (vi) investigation on ART (assisted reproductive technology) history/outcome.
Results: One patient carried the homozygous DPY19L2 deletion and one was heterozygous, but not carrying any other variant. SPACA1 was never mutated, suggesting a minor contribution of this gene in human globozoospermia. The most consistent sperm chromosome alteration was a higher diploidy rate. Sperm DF mean value ±SE was above the reference values (51.7 ± 5.3% vs. 35 ± 1.6%). We did not observe microsatellite instability between leucocyte and sperm DNA in any of the patients. As for ART treatment, only one patient with 97% globozoospermia obtained pregnancy after two cycles of ICSI.
Conclusion: This study provides a comprehensive picture of globozoospermia that, for the first time, gathers together both detailed phenotypic and novel molecular genetic data.

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Correlation of leukocytes and round cells concentration in human semen with semen parameters

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Background: Association of leukocyte counts and leukocytospermia with semen parameters and male infertility is still under examination.
Aim: Aim of the study was to find association between the presence of leukocytes and round cells with certain parameters of human semen.
Methods: Semen examination according to WHO guidelines (2010) was performed in 480 men. Data was grouped in partially overlapping subsets: (A) sperm concentration = 1 × 10^6/mL, (B) sperm concentration = 15 × 10^6/mL, (C) total sperm count = 39 × 10^6/ejaculate, and (D) total sperm count <39 × 10^6/ejaculate. In each subset, samples with leukocytes <1 × 10^6/mL (LN) were compared with samples with leukocytes = 1 × 10^6/mL (LE), while samples with round cells <5 × 10^6 (RCN) were compared with samples with round cells =5 × 10^6 (RCE).
Results: Sperm concentration and total sperm count were lower in the LE than LN group, but higher in the RCE than RCN group. The progressive motility and vitality of sperms were lower in the RCE than in RCN group. The percentage of morphologically normal sperm was higher in the LE than in LN group. Leukocyte concentration correlated negatively with sperm concentration or total sperm count in subsets A-C. Round cell concentration correlated positively with sperm concentration or total sperm count in subsets A-D, negatively with sperm vitality in subsets A-C and sperm progresive motility in subsets B-C. The percentage of normal sperm forms was positively correlated with leukocyte concentration in subsets A and D.
Conclusion: Both round cells and leukocytes presence is related to semen parameters. Presence of leukocytes and round cells is an important parameter in semen analysis.
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Interplay between adrenal and reproductive hormones

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Background: The endocrine interplay between testicles and andrenal glands is poorly understood. Secretion of adrenal cortical hormones is stimulated by ACTH, however, LH-receptors are present in the adrenal cortex (Pabon JE *et al.* 1 J Clin Endocrinol Metab 1996 Jun ;81(6): 2397–400).
Aim: We report preliminary results of a pilot study aiming to describe the possible associations between adrenal hormones and pituitary-testicular hormones.
Methods: 395 participants from a study of young Danish men(Jorgensen N *et al.*. BMJ Open 2012;2(4)) had a blood sample drawn in the morning for assessment of LH, testosterone (T), SHBG, FSH, inhibin-B, DHEA, DHEAS, androstenedione (Adione), 17-hydroxyprogeste¬rone(17-OH-P), and cortisol.
Results: Positive correlations with LH was found for 17-OH-P (Pearson's $R = 0.26$, $p < 0.0001$), DHEAS ($R = 0.12$, $p = 0.002$), and Adion ($R = 0.15$, $p = 0.003$).
Positive correlations with T was found for 17-OH-P ($R = 0.4$, $p < 0.0001$) and Adion ($R = 0.3$, $p < 0.0001$). Inhibin-B was positively correlated with cortisol ($R = 0.15$, $p = 0.02$), DHEA ($R = 0.16$, $p = 0.002$), and DHEAS ($R = 0.11$, $p = 0.04$).
SHBG correlated negatively with DHEAS ($R = -0.14$, $p = 0.006$). FSH did not correlate with adrenal hormones.
Expected correlations between the pituitary-testicular hormones were found.
Conclusion: Positive correlations between adrenal hormones and LH, testosterone, and inhibin-B were observed. These data may indicate that men with decreased Leydig cell capacity and increased LH levels may have an elevated adrenal activity. This ought to be further explored.

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Prenatal ethyparaben (EtP) exposure is associated with reduced penile width in 273 boys

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Background: The results of experimental studies on effects of parabens have been inconsistant. The majority of studies in rats and mice found adverse effects of butylparaben (BuP) on reproductive system such as increase in progesterone levels and decrease in testosterone levels, in spermatid counts, sperm motility and decrease in weight of reproductive organs while others could not confirm those findings. One human study has shown an association between BuP exposure and sperm DNA damage.
Aim: Examine the effects of prenatal paraben exposure on reproductive organs and growth.
Methods: We included 514 women from Odense child cohort (January 2010–December 2012) who gave birth to singleton child of Caucasian origin. Prenatal paraben exposure was assumed by paraben concentrations in maternal urine samples, collected in gestational week 28. 6 different parabens were analysed by TurboFlow-LC-MS/MS method. Association between maternal urinal paraben concentrations and AGD short, AGD long, penile width and birth outcome (birth weight, birth length, head circumference, abdominal circumference, weight for gestational age and gestational age at birth) were examined.
Results: Women in the highest EtP tertile (>2.73 ng/mL) gave birth to boys with significantly smaller penile width [−0.43 mm (95% CI: −0.79 to −0.07; p-trend = 0.02)]. No significant association between paraben exposure and

AGD or the other birth outcomes was found. Furthermore, the total urinary paraben excretion expressed as nmol/ml was also not associated to any of the outcome parameters.
Conclusion: The observed significant association between maternal EtP exposure and penile width in the boys may be a chance finding due to mass significance.

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Effect of bisphenol A on male reproductive function

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Background: Endocrine disruptors, such as bisphenol A (BPA), have been associated with male reproductive dysfunction. However, very few studies have assessed BPA concentrations according to the cause of male infertility.
Aim: The aim of this prospective, case-control study was to investigate the plasma BPA concentrations in infertile men taking into account the cause of infertility.
Methods: Fifty-five subfertile men (mean age ± SEM 35.4 ± 0.8 years), diagnosed as having idiopathic non-obstructive azoospermia (n = 23), cryptorchidism (n = 12) or varicocele (n = 20), by means of history, physical examination, hormonal investigation (FSH, LH, total testosterone, SHBG, AMH, inhibin B), spermiogram and ultrasound imaging, were included in the study. Twenty-five control men of proven fertility (age 30.7 ± 1.1 years) were also studied. Plasma BPA concentrations were measured in all men.
Results: BPA was detected in all infertile and control men. There was no difference in BPA concentrations between infertile and control men [median (IQR) 0.19 (0.45) vs. 0.18 (0.28) ng/mL, p = 0.689] or among the cause of infertility [idiopathic azoospermia 0.30 (0.69), cryptorchidism 0.12 (0.39), varicocele 0.17 (0.23) ng/mL, p = 0.316]. However, the highest levels of BPA were observed in the infertility group, though the difference was not significant (20% of the controls and 43% had BPA above the upper quartile, x^2 = 0.85, p = 0.356). A negative correlation was observed between BPA concentrations and AMH (r = −0.320, $p < 0.01$) in the whole group of subjects.
Conclusion: Although male infertility cannot be attributed to exposure to BPA, high concentrations of BPA may be related to infertility.

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A-glucosidase concentrations in seminal plasma of men with asthenospermia: a pilot study

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Background: The neutral alpha-glucosidase (a-Glu) is an androgen-depended enzyme, whose concentrations in the seminal plasma are considered a good indicator of the epididymidal function.
Aim: The main aim of this pilot study was to test the hypothesis that men with infertility and asthenospermia only exhibit lower concentrations of seminal plasma a-Glu, indicative of epididydimal dysfunction, compared with men with infertility and disturbances in all semen parameters and fertile men.
Methods: In 33 men with infertility and 32 fertile controls [age (mean ± standard error) 36.9 ± 0.6 years] a series of spermiograms was performed. Semen volume, sperm concentration, progressive motility and morphology were analyzed according to the WHO protocol. Concentrations of neutral a-Glu were measured by the photometric method.
Results: Fertile men had similar seminal a-Glu concentrations (n = 32, 18.5 ± 3.1 mg/mL) compared to men with asthenospermia only (n = 4, 26.2 ± 14.0 mg/dL, p = 0.467 vs. fertile) and higher concentrations compared to men with oligo-astheno-teratospermia (n = 29, 11.3 ± 3.5 mg/dL, p = 0.030 vs. fertile, p = 0.081 vs. asthenospermia only).
Conclusion: These results do not confirm the hypothesis that isolated asthenospermia is a result of epididymal dysfunction. On the contrary, a-Glu appears to be decreased in men with infertility and more deteriorated semen parameters, such as oligo-astheno-teratospermia. Further studies are needed in order to reveal the association between epididymal dysfunction and semen quality.

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Assessment of chromatin maturity by aniline blue assay in patients with leukocytospermia

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Background: Leukocytospermia is considered as one of the most contributing causes of male infertility. Several studies have reported a deleterious effect of leukocytes on sperm concentration, motility, and normal morphology. They have also demonstrated that patients with leukocytospermia have a significantly higher DNA fragmentation

index (DFI) than healthy fertile men. One of the possible mechanisms of DNA fragmentation that deserves to be studied is the aberrant chromatin packaging during spermatogenesis.
Aim: This study set out to investigate nuclear chromatin quality in patients with leukocytospermia.
Methods: The study groups included 50 patient with leukocytospermia and 30 fertile donors who acted as a control group. Semen analysis was performed according to World Health Organization criteria and sperm chromatin integrity by aniline blue assay.
Results: Our results clearly suggest that leukocytospermia severely affect semen parameters. Patients showed a significant decrease in both total and progressive motility compared to the control group. The rate of abnormal sperm morphology was greater in leukocytospermic samples when compared to the control group. As expected there was a significant difference between the two groups with regard to aniline blue staining: leukocytospermic patients had significantly more spermatozoa with impaired chromatin condensation than fertile donors ($p < 0.01$).
Conclusion: Leukocytospermia affects not only standard semen parameters or sperm DNA integrity but also chromatin condensation that would be possible to routinely evaluate by an easy, inexpensive and accurate test: aniline blue staining. This method may be useful in selecting a proper sperm with un¬damaged chromatin for ART.

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Sperm DNA integrity, chromatin condensation and aneuploidy in men with total non syndromic teratospermia

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Background: DNA integrity is an objective marker of sperm function. Infertile men including those with teratospermia manifest various nuclear alterations such as abnormal chromatin structure, DNA fragmentation and aneuploidy. The impact of total non syndromic teratospermia prevailing on head abnormalities has not been studied extensively.
Aim: The purpose of our study was to evaluate sperm DNA fragmentation, chromatin integrity and aneuploidy in patients with total non syndromic teratospermia.
Methods: Our study includes 30 patients with non syndromic total teratospermia and 23 fertile men with normal semen profiles who acted as controls. Semen samples were analyzed according to the World Health Organization criteria. Sperm DNA fragmentation was assessed by TUNEL assay, chromatin condensation by aniline blue staining and chromosome abnormalities by fluorescence in situ hybridization (FISH) with probes specific to chromosomes X, Y and 18.
Results: Semen analysis shows total teratozoospermia predominant on head abnormalities. The mean DNA fragmentation index (DFI) and the rate of aniline blue-reacted spermatozoa was significantly higher in patients compared to the control group ($p < 0.001$). The results of aneuploidy frequencies showed a significant difference between both groups especially for gonosomal disomies.
Conclusion: Our data indicate that men with non syndromic teratospermia have an impaired nuclear sperm quality as well as chromosomal aberrations focusing mainly on gonosomes which obviously affects the fertilizing sperm power and useful for selecting assisted reproduction techniques (ART) for patients.

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Leydig cell function associated with sperm counts in infertile men

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Background: Reduced Leydig cell function has been shown in infertile men (JCEM, 2004; 89(7): 3161–3167), however, the potential association to sperm counts were not described.
Aim: To investigate possible associations between serum levels of Leydig cell related hormones and sperm counts.
Methods: We included 987 infertile men. Linear regression models were used.
Results: The sperm concentration (SC) and total sperm counts (TSC) ranged from 0 to 275 mill/mL and 0–738 mill, respectively, with medians of 6.9 mill/mL and 24 mill. The median SC and TSC in the lowest quartile of men were 0.002 mill/mL and 0.1 mill, and in the highest 34 mill/mL and 90 mill. Trend analyses showed negative associations between LH and both sperm counts (SC, $p = 0.001$; TSC, $p < 0.0005$). LH levels were 53% (95% CI: 22–90%) and 41% (95% CI: 18–69%) higher in the lowest SC and TSC quartiles compared to the highest. For Testosterone/LH-ratio (T/LH) trend analyses showed lower ratios for lower sperm counts (SC, $p = 0.002$; TSC, $p < 0.0005$). T/LH were 36% (95% CI: 17–50%) and 30% (95% CI: 15–436%) lower in lowest SC and TSC quartile vs. the highest. Neither total testosterone nor calculated free androgen index were statistically associated with sperm counts, although there was a tendency of increasing testosterone with increasing sperm counts.
Conclusion: Our results are preliminary as collection of data is still ongoing, and more detailed statistical analyses have to be included. The current results suggest a direct association between low sperm count and compensated Leydig cell failure (cLCF). cLCF may develop into hypergonadotropic hypogonadism with increasing age, and low sperm counts may be an early warning.

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Diagnostic assesment and reproductive treatment of infertile patients with ejaculatory abnormalities

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Background: Infertile patients showing hypospermia or aspermia are often facing diagnostic challenges to ascertain the definition, etiology and management of their infertility.
Aim: To review the diagnostic procedures and results of treatment of infertile men with suspected ejaculatory abnormalities.
Methods: A retrospective survey was done in 56 patients consulting for couple infertility and showing aspermia with conserved orgasm (69%), severe hypospermia (17%), and other qualitative defects of orgasm (14%). Mean age was 37 y/o, range 26–56. Sperm parameters were analyzed in semen specimens (when available), and post-orgasm urine (POU) after alkaline water load. Transrectal ultrasonogram and testicular sperm extraction (TESE) were performed in selected patients.
Results: Retrograde ejaculation (RE) was confirmed in 22 cases, due to diabetic neuropathy (23.2%), surgical damage of retroperitoneal or pelvic nerves (26.8%), neurological diseases (12.5%), distal obstruction of genital tract (12.5%), psychogenic origin (3.6%), and other undetermined causes (7.1%). Ejaculated sperm concentration in partial RE was 22.7 ± 45 ×106/mL. Sperm concentration in alkalinized POU was 5.8 ± 4.9 ×106/mL. Percent progressive motility in baseline semen and POU was 37.3 ± 28 and 48 ± 22.85 respectively. All patients who underwent TESE had positive sperm recovery (0.162 ± 0.14 ×106/100 mg of wet weight tissue). Treatment with intrauterine inseminations was done in 7 couples, and in vitro fertilization in 52 couples (75 cycles), with full term delivery rate of 14 and 39% respectively.

Conclusion: A combination of diagnostic techniques can be used to classify most ejaculatory disturbances producing male infertility, and the reproductive results of couples with these conditions are reassuring.

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Gonadotropin treatment to induce spermatogenesis and fertility in hypogonadotropic hypogonadism: retrospective survey of a hospital based cohort

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Background: Hypogonadotropic hypogonadism (HH) is a condition of male infertility that can be rationally treated, yet the clinical outcome is influenced by a number of variables.
Aim: To characterize the results of treatment and potential predictors of efficacy in men with HH.
Methods: Retrospective analysis of 62 men diagnosed of HH, treated with gonadotropins to produce maturation of the testes (n = 18) pregnancy (n = 40), or both (n = 4) with a total of 78 courses of treatment (CTx).
Results: Prepubertal HH was diagnosed in 42 men, and postpubertal HH in 20. Testicular maturation was induced in 22 CTx using combined hCG plus FSH therapy (age 23.6 ± 6.4 y/o). Baseline bitesticular volumen (BTV) was 8.9 ± 5.6 mL. Time for appearance of sperm in semen was 16.1 ± 14.3 months. Maximum count of motile sperm was 10.9 ± 22.3 ×106/mL, although 5 CTx (24%) remained azoospermic. Forty four men (33.6 ± 5.8 y/o) received a total of 56 CTx desiring pregnacy of their wives. Baseline BTV was 18.4 ± 10.5 mL. Sperm in semen appeared at 7.4 ± 8.5 months. Maximum motile sperm count was 13.9 ± 26.2 ×106/mL, and 3 CTx (5%) persisted azoospermic. Pregnancy was obtained in 41 CTx (73%) and 33 couples (75%). Postpubertal HH and repeated CTx showed more successful spermatogenesis, while age >30 y/o at first CTx, cryptochidism and female factor were associated with worse spermatogenic and reproductive outcome.
Conclusion: Successful induction of spermatogenesis could be achieved in 90% of treatment courses, and pregnancies were produced in 75% of patients with HH wishing fertility. Factors influencing treatment outcome can be taken in account for the prognosis of individual patients.

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Poster cancelled

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Sclerosing sertoli cell tumor in infertile man - a case report

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Background: Sclerosing sertoli cell tumor (SSCT) is an extreme rare sex cord-stromal neoplasia of the testis, less than 1% of all gonadal cancers – until 2014 describes only 42 cases [Am J Surg Pathol, 2014].
Aim: Presentation the infertile man with the SSCT.
Methods: The 36-y.o. man; HCV 09.2012 (planned therapy interferon/Ribavirin), controlled hypertension; wife healthly; efforts to become pregnant >2 years. Patient was reported due to the sudden deterioration of the semen parameters: sperm concentration decreased from 20 to 2 million/mL within 3 months. In view of the 20-fold increased risk of testicular tumors in infertile men (Raman, 2005), an emergency diagnosis was done. Physical exam – gonads free of changes; ultrasound - polymorphic tumor 8 mm, the lower pole of the right testis; hormones, tumor markers, chest X-Ray – no changes. Right radical inguinal orchiectomy was performed, post-op no complications. An unusual morphological image, no diagnosis after a classic histologic colouring; immunohistochemical tests: Inhibin, Calretinin, SMA, Chromatografin, Synaptofizyn, CD56, CD10, PSA, CK AE1/3, MelanA were done.
Results: Pathologist report: the tumor of Sertoli cells, type SSCT; the cutting line of the seminal cord free of cancer;

CTscan chest and abdomen showed no pathology - pT1N0M0. This is benign lesion according to WHO classification, a routine control was planned.

Conclusion: Each infertile male must have made an assessment of the testis using ultrasound, in view of the 20-time increase in the likelihood of the occurrence of gonadal cancer.

If focal changes in the gonad was founded, regardless of the final histological diagnosis, radical inguinal orchidectomy is the recommended procedure.

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Evaluation of cytostatic therapy effects on the male gamete genome

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Background: The most frequent malignancies among men in reproductive age are testicular cancer, Hodgkin's and non-Hodgkin's lymphoma. The relatively low gonadotoxicity of therapies allows a rapid recovery of spermatogenesis and the future welfare of the offspring conceived by a father treated with cytotoxic therapy remains a major concern. The right timing for natural conception is still uncertain and based only on few studies.

Aim: To evaluate the effects of cytostatic therapies on the integrity/instability of the sperm genome.

Methods: 26 patients affected by testis cancer (n = 16) and lymphoma (n = 10) were analysed prior to and after cytotoxic therapy. Sperm DNA fragmentation analysis: based on terminal-uridine nick end assay (TUNEL) coupled to Flow Cytometry. Microsatellite instability (MSI) assessment: 7 selected loci (AR, ER, BAT25, BAT26, D2S123, D5S346, D17S250) of mono-, di- and tri-nucleotide tandem repeats were amplified by fluorescent PCR and analyzed on ABI Prism 310 sequence analyzer (GeneScan software). For comparison of the tested parameters, data on 90 fertile controls were used.

Results: A significantly higher DNA fragmentation was found after: (i) 2 years from the cytostatic therapy in the group of testis cancer (42.5 ± 22% vs. 34.04 ± 14.5%, p = 0.05; (ii) 1 year in the lymphoma group (53.1 ± 13.9% vs. 34.04± 14.5%, p = 0.001). No microsatellite instability was observed in any group.

Conclusion: Our pilot study shows that cytotoxic therapies do affect DNA integrity up to two years, whereas they do not induce microsatellite instability. Further enlargement of the study population and long term follow up is needed in order to get conclusive data.

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Testicular biopsies among azoospermic patients: prognostic factors of sperm recovery

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Background: The ability to achieve pregnancy with only a single testicular sperm has turned biopsy into a potentially therapeutic, as well as, diagnostic procedure. While testicular sperm recovery is successful in almost all cases of obstructive azoospermia, the recovery rate varies from 30 to 70% for non-obstructive azoospermia.

Aim: Present the results of testicular biopsy among obstructive and non-obstructive azoospermic patients in an infertility terciary referral centre analysing the predictive factors of sperm recovery.

Methods: A retrospective cohort study of 187 azoospermic patients submitted to testicular biopsy between February 2004 and March 2014. The following variables were analysed: patient data (age, testicular volume, FSH, LH, testosterone, prolactin, karyotype abnormalities,Y chromossome delections), technical variables(number of biopsies, laterality, type of technique for sperm extraction) and biopsy results(sperm retrieval and complications) and histological data.

Results: The mean age of patients was 36 years ± 5.26.The techniques for sperm retrieval were TESE, TESA,TESA+TESE combination and PESA in 46.5,12.9,33.3 and 2.2% of the cases. The median number of biopsies per patient was 4 [1–12] with 58.3% bilateral biopsies. The overall recovery rate was 54%. The mean value of FSH was 13.7 mUI/mL. Patients with an unsuccessful testicular biopsy had a higher mean FSH (19.3 vs. 8.3; $p < 0.001$) and LH (9.1 vs. 5.1 $p = 0.01$). Among non obstructive azoospermia we obtained the higher sperm recovery rate in patients with hypospermatogenesis (67.5%) and the lower recovery rate among patients with Sertoli cell-only syndrome (16.2%) ($p < 0.001$).No complications were observed.

Conclusion: Testicular biopsy is a safe and effective method for sperm retreival. In non obstructive azoospermia patients, the mean levels of LH and FSH were statistically higher in case of an unsuccessful testicular biopsy.

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Retrospective survey of 182 patients with Klinefelter syndrome in a specialized hospital

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Background: Klinefelter syndrome (KS) is the most common genetic cause of hypogonadism and male infertility. It is characterized by the presence of hyperploidy of X chromosomes in the karyotype.

Aim: To describe the main clinical and laboratory aspects of KS patients referred to our Centre during a period of 38 years.
Methods: Retrospective and descriptive analysis of clinical records from patients consulting at the Andrology Service and subsequently diagnosed with KS. Laboratory and histopathology data were reviewed in selected patients.
Results: One hundred eighty two patients were included in the study. Mean age was 33.7 y/o. Infertility was the main reason for consultation (74.7%) followed by hypogenitalism (8.8%) and erectile dysfunction (8.2%). Pure 47, XXY karyotype was found in 83.4% patients. Only 10.5% individuals showed the presence of sperm in semen analysis, with a mean concentration of 5.4 x106/mL and relative progressive motility of 27.2%. Testicular biopsy was performed in 26 men (14.2%), and positive sperm recovery was found in 9 (34.6%). The predominant histopathological pattern was tubular sclerohyalinosis (73.1%). Concentrations of testosterone, LH and FSH in azoospermic patients were 11.6 ± 7.1 nmol/L, 19.7 ± 9.7 and 29.4 ± 16.3 U/L respectively, not different than concentrations of those with positive sperm in semen or testis (9.7 ± 5.8 nmol/L, 17.7 ± 10.3 U/L and 30.1 ± 14.3 U/L of testosterone, LH and FSH).
Conclusion: The majority of patients with Klinefelter syndrome were diagnosed at consultation for infertility. Not all of them were azoospermic, but hormone levels were similar in both groups. Testes atrophy and sclerohyalinosis were invariable hystopatholocical findings.

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How relevant is mumps virus in the etiology of acute orchitis?

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Background: Acute orchitis represents a rare clinical entity. Data are mainly derived from patients suffering Mumps and in this context develop concomitant orchitis.
Aim: To investigate patients with acute isolated orchitis regarding the relevance of Mumps virus.
Methods: Between 2007 and 2013, 10 patients were diagnosed as having isolated orchitis. Inclusion criteria were (i) isolated testicular pain, (ii) testicular hyperemia in ultrasound and 3) elevated C-reactive protein (CRP). Exclusion criteria were (i) concomitant epididymitis and (ii) evidence of bacterial urinary tract infection (urine culture, PCR on sexually transmitted diseases, 16S rDNA analysis). Mumps analysis was performed by PCR in urine samples at first presentation (n = 10), as well as semen samples (n = 10) after relief of pain. In addition, serologic examinations were carried out at first presentation (n = 10).
Results: Median age of patients was 37 years (range: 25–47 years) with eight patients suffering unilateral and two patients bilateral orchitis. Three patients reported a respiratory tract infection and one parotitis within the previous two weeks. Two patients had fever >38 °C at first presentation. Median white blood cell count was 9.5 giga/L (range: 3.5–12.5 giga/L) and the median CRP value was 35 mg/L (range 5–52 mg/L). Mumps serology was consistent with previous infection/vaccination in nine patients, with only one patient having evidence for acute infection (increased IgM titers). Mumps virus was never recovered by PCR from urine and semen samples.
Conclusion: In most cases of acute isolated orchitis, Mumps virus does not seem to play a relevant etiologic role.

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Testicular microlithiasis – correlation with TDS (testicular dysgensis syndrome)

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Background: Results of clinical trials show unequivocal correlation between testicular microlithiasis and testicular germ cell tumours (TGCT) but the andrological aspects are less studied. In the last two decades publications introduced the testicular dysgenesis syndrome (TDS) which can explain the common origin of cryptorchidism, hypospadias, sub/infertility and TGCT.
Aim: The aim of the study is to determine the correlation between TM and classical sperm parameters, the presence of TGCT and TDS components.
Methods: Results of a cross-sectional controlled, ongoing evaluation are present to compare the oncology and fertility data of testicular microlithiasis positive (TM+, 79 patients) and negative (TM−, 20 patients) patients in infertile couples. Microscopic and computer assisted sperm analysis,scrotal colour Duplex ultrasound (SCDUS) was performed. The presence of TGCT and TDS components was also controlled.
Results: In the TM group the sperm concentration and progressive motility was significantly lower, (p = 0.033 and 0.037), incidence of oligozoospermia and azoospermia was significantly higher compared to the control group (p = 0.011 and p = 0.016). TGCT was diagnosed in seven patients which means higher incidence than in normal population (0.9% vs. 0.4%, respectively). In the TM group the average number of TDS components was significantly higher ($p < 0.001$).
Conclusion: TM shows statistically significant negative correlation with sperm concentration and progressive motility. A positive correlation of TM can be seen with TGCT. The presence of TDS components is significantly higher in the case of TM. These results suppose the theory that TM can be a component of TDS. Our data call the attention to the clinical impact of TM.

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FSH therapy in idiopathic oligo-asthenozoospermia

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Bacground: Follicle-stimulating hormone (FSH) therapy is a potential option for treatment of idiopathic male infertility. In fact, a recent Cochrane meta-analysis showed that FSH treatment of men affected by idiopathic oligozoospermia significantly improves pregnancy rate. Hyaluronic acid (HA) binding capacity of spermatozoa is considered a marker of functional competency, consequently the evaluation of the percentage of HA bound spermatozoa in the ejaculate may serve as a proxy of sperm fertilizing potential.
AIM: We studied the effect of FSH treatment on routine sperm parameters and on HA binding capacity of spermatozoa.
Materials and methods: 30 idiopathic oligo/asthenozoospermic men with FSH < 8 mU/mL. Sperm analysis and HB assay (performed by using coated slides with HA; Origio Inc.) before therapy and 1 and 3 months post-therapy (highly purified gonadotropin, 75UI 3 times/week).
Results: After 3 months we observed a significant increase of the total motile sperm count (TMC) (23 ± 29 vs. 36 ± 33 million spermatozoa/ejaculate $\pm$ SD); 56% of patients resulted 'responders' with an increase of total sperm number and TMC by more than 45% in respect to baseline. Similarly, the mean value of HA-bound spermatozoa showed a significant increase after one month (38 ± 23 vs. 47 ± 22) and 32% of patients resulted 'responder'.
Conclusion: Our study confirms that FSH therapy improves spermatogenesis, both quantitatively and qualitatively in about 50% of patients. For the first time, we report data showing a significant positive effect of FSH on a functional parameter, such as sperm HA binding. Pharmacogenetic studies are ongoing in order to gain more insights into the biological basis of FSH responsiveness.

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Outcome of electro ejaculation in 'difficult' cases

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Electro ejaculation (EEJ) is originally used for cases where the lower part of the spinal cord is damaged. It has been used in Hungary since 1998 in a total of 62 subjects. In this abstract three unusual cases are presented.
Case #1: 39 year old man, diagnosed with astrocytoma, and the surgical intervention was performed in Th11-12 aeria in 1989 which was totally removed in 1990 and subsequently treated by chemotherapy. No ejaculation. EEJ was performed in 2012 with very good quality anterograd ejaculate (total number: 150 M/ejaculate, motility: 30%, normal morphology: 4%) ICSI has been performed and pregnancy obtained after the first cycle. Healthy boy was born in term (2014).
Case #2: 32 year old man, diagnosed with Multiple Sclerosis and treated by Avonex and Tysabri for 5 years. Sperm cryconservation was not performed during treatment and the patient became unable to ejaculate after 5 years. EEJ was performed in 2011 with poor semen parameters (total number: 10 M/ejaculate, motility 10%, morphology: 3%) ICSI was performed in 2012 (28 year old wife) and twins pregnancy obtained and healthy twins were born in November 2012.
Case #3: 42 year old man, victim of car accident in 1987 (spinal cord damage, Th12), testicular biopsy was performed in 2008 and testicular sperm was used for ICSI (7 cycles) without obtaining pregnancy. In 2011 the couple (36 year old wife) performed an ICSI cycle with EEJ with sperm parameters (total number: 30 M/ejaculate, motility: 20% morphology: 5%). Pregnancy obtained and in the 34th week a boy was born with classification Appgar 9. On the 23rd of December in 2013 and he is healthy.
Indications for EEJ has been widening in the last few years. Data in the literature and in the present abstract show that EEJ is worth trying in every single case as it is shown in the above cases.

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Relationship between meiosis in testicular biopsy and DNA fragmentation, diploidy, and apoptosis on ejaculated sperm

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Background: Nowadays more sperm biomarkers are needed and being developed to give a more accurate diagnosis for individual male infertility cases.
Aim: To assess the relationship between results of meiosis found on testicular biopsy (TB) with sperm DNA fragmentation (% SDF), diploidy, and apoptosis found on ejaculate sperm.
Methods: 26 patients with both TB and ejaculate sperm sample were included. All had a history of implantation failure after two cycles of IVF and =6 embryos transferred in total. Meiosis results were altered in 13, while normal in the other 13. Each sample was analyzed for %SDF using the Sperm Chromatine Structure Assay (SCSA), diploidy test based on propidium iodide staining and apoptosis markers. All data were obtained by flow cytometry.
Results: When comparing altered meiosis group with normal group, no differences were found on the %SDF. None (0/13) of the normal meiosis, in comparison to 4/13 of the altered meiosis had a %SDF >30%. Average percentages of diploidy were 1.5% in normal TB and 2.4% in altered TB. 7.7% (1/13) of normal meiosis and 38.5% (5/13) of altered showed abnormal values of diploidy. Regarding apoptosis percentages, no differences were found between both groups. Nevertheless, only 33% (2/6) of patients with >40%

of apoptotic sperm had normal meiosis. Meanwhile, 55% (11/20) of patients with <40% showed normal meiosis.
Conclusion: Our results show a positive relationship between the results found on TB and those found on advanced sperm analysis. Due to the small number of cases, more patients are needed to confirm these preliminary data.

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Advanced semen quality analysis by flow cytometry

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Background: Even though every time better technologies are being developed to study functionality of the sperm cells, they aren't used routinely for the diagnosis of male factor.
Aim: The purpose of this study is to assess which additional information can be useful by systematically analyzing semen samples by flow cytometry.
Methods: This is a retrospective observational study with a total of 439 advanced semen analysis from different patients undergoing infertility treatment at the CIRH Clinic in Barcelona. All samples had both basic and advanced semen analysis, including DNA fragmentation test (SCSA), apoptosis, and diploidy. Data was obtained by flow cytometry. Results were sorted into 5 groups according to male factor's etiology: normozoospermic (35.3%), oligozoospermic (11.5%), asthenozoospermic (21%), teratospermic (27.8%), and oligoasthenozoospermic (4.4%).
Results: Values higher than 25% of DNA fragmentation, higher than 3.4% of diploid cells were considered abnormal. Oligoasthenozoospermic, followed by asthenozoospermic and oligozoospermic samples obtained the highest values of both DNA fragmentation and diploid percentages. Oligozoospermic and oligoasthenozoospermic groups obtained the highest values of cell apoptosis (>15%). Interestingly, 19.2% of the normozoospermic group showed altered sperm DNA fragmentation.
Conclusion: Our data show that even if basic semen analysis reports normozoospermic results, additional altered data can be found when advanced examination is made by flow cytometry. The incorporation of new diagnostic tests should improve fertility treatment outcomes, applying different techniques of sperm selection according to the etiology of each patient.

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Magnetic activated cell sorting (MACS) of non-apoptotic spermatozoa

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Background: MACS has been widely used for effective selection of the non-apoptotic spermatozoa among the semen samples, highly concentrating those spermatozoa that are non-apoptotic and with less DNA fragmentation percentages.
Aim: To assess if the quality of the semen samples improve by MACS of non-apoptotic spermatozoa.
Methods: A prospective study was made including 55 semen samples obtained between July 2013 and April 2014 from patients that attended CIRH Clinic in Barcelona. Each sample was capacitated by density gradients and sorted using MACS. Then, each sorted fraction was assessed for sperm DNA fragmentation (%SDF), apoptosis and vitality by flow cytometry. The results were analyzed using Spearman correlation test.
Results: The negative fraction obtained after capacitation and MACS sorting evidences a statistically significant difference (p-value = 0.000) in both sperm DNA fragmentation and live-apoptotic spermatozoa percentages in comparison with the natural semen and the non-sorted capacitated semen samples. Additionally, when comparing the negative fraction and the positive fraction after MACS sorting, higher statistically significant differences regarding the sperm DNA fragmentation and live-apoptotic sperm percentages are found.
Conclusion: Our data shows an important difference on the results found on semen obtained after MACS of non-apoptotic spermatozoa, making this technique a promising tool for sperm selection prior to an ICSI treatment. An increase in the number of patients is needed in order to confirm these preliminary data. Also, clinical outcome must be included in future studies.

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Testicular size at the onset of puberty assessed by orchidometer, ruler and ultrasound: finnish case-control study

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Background: We assessed testicular size using different tools (orchidometer, ruler and ultrasonography) in a longitudinal study of Finnish boys around the onset of puberty.
Aim: To evaluate whether early testicular damage (congenital cryptorchidism) causes changes in the timing of

puberty and to understand relationships between different testis measuring instruments during pubertal onset.
Methods: Healthy boys and boys with congenital cryptorchidism (n = 119) were followed from the age of 8.5 years biannually since 2005 (drop-outs: 15 boys).
Testis volume by orchidometer >3 mL and testis length by ruler >25 mm, respectively, were used as sign of onset of puberty. We calculated testicular volume by US using Lambert's formula (L × W × H × 0.71).
Results: Timing of pubertal onset did not differ between cases and controls: median age 11.5 (n = 44) vs. 11.5 (n = 55) by orchidometer, 11.5 (n = 44) vs. 11.5 (n = 55) by ruler.
Among all boys with 3 mL testis by orchidometer mean calculated US volume was 1.4 mL (95% CI 1.3–1.5 mL, n = 111). Mean US testicular volume in boys with 25 mm testicular length by ruler was 1.6 mL (95% CI 1.4–1.7 mL, n = 57). Thus, using Lambert's formula the limit of testis volume by ultrasound denoting the onset of puberty is around 1.5 mL.
Conclusion: There was no difference in the timing of onset of puberty between cryptorchid and healthy boys. Our study showed the high level of accuracy between orchidometer and ruler measurements. There was 86% agreement between definition of pubertal onset by orchidometer and by ruler, i.e. both methods were equally applicable for clinical evaluation.

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Male fertility in Chile: assessment of fertile potential in a high risk population for germinal dysfunction.

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Background: A decrease in fertility rates and semen quality have been reported in international literature. In Chile, reported marital infertility rates relatively low compared to industrialized countries 50 years ago, onmental pollution.
Aim: We investigated the potential of fertility in men at known high risk for germinal tubular dysfunction. In a previous own research in 102 patients with unilateral testicular cancer remarkable good spermatogenic patterns in the contralateral testis were found, highly favorable compared to earlier European reports (Berthelsen, 1983). To corroborate these findings, we made an extension of the study in a second prospective series of patients.
Methods: 63 patients presenting with unilateral testicular cancer at our institution, underwent to contralateral biopsy by the time of radical orchiectomy. The surgical specimens were studied at the Department of Pathology, University of Granada, Spain. Spermatogenic functionwas evaluated using the Johnsen score.
Results: 13% of patients showed histopathology consistent with irreversible infertility, 28% with signs of infertility and 59% normal spermatogenesis. This involve further damage of the germinal epithelium compared with our own previous series, but still better than those reported in European and American literature.
Conclusion: Male reproductive health has shown a progressive deterioration in Western countries. The results of this study reinforce our previous findings of a better childbearing potential in Chilean men compared to industrialized countries. However, remarkable differences between our own two series should alert us on possible effects of progressive enviromental pollution.

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Y chrosomosome microdeletions in Chilean infertile men: most comprehensive prevalence study in Latin America

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Background: Several studies have described the genetic disorders linked to impaired fertility in males with severe sperm alterations. Y chromosome microdeletions are considered important genetic causes of spermatogenesis failure in azoospermic and oligozoospermic men. Latinamerican studies addressing this issue are scarce, with small sample sizes
Aim: The aim of this study is to determine Y chromosome microdeletions prevalence in chilean infertile men
Methods: A group of 102 infertile men attending a fertility clinic, with azoospermia or severe oligozoospermia, were screened for microdeletions in the azoospermia factor (AZF) region of Y chromosome by multiplex polymerase chain reaction. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood samples. Each patient was analysed for the presence of sequence tagged sites in the AZFa, AZFb and AZFc regions
Results: 67 and 35 patients were found to have azoospermia and severe oligozoospermia, respectively. Microdeletions were found in 9.8% of patients. The most prevalent mutation was AZFc, affecting 3.9% of the sample. Followed by AZFbc with 2.9%, AZFa with 2.0% and AZFb with 1.0%. Only azoospermic men were found to present these genetic alterations
Conclusion: Prevalence of Y chromosome microdeletions in chilean infertile men is similar to the prevalence presented in international studies. As AZFa and AZFb mutations are associated with complete abscense of viable gamets, and AZFc has important consequences in the fertility potential of the offspring, these mutations have to be searched when facing an infertile patient with severe sperm alterations

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How does hyperestrogenism affect spermatogenesis and testicular functional integrity?

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Background: Male hyperestrogenism results from many factors such as hypogonadism, testicular insufficiency, or certain medications. The effects of long-term elevated levels of estrogen in men is poorly understood
Aim: To investigate the effects of high levels of estrogen on spermatogenesis and the functional integrity of the testis.
Methods: Samples of the testes were obtained post-orchiectomy from 10 transsexual men (aged 26–52) after surgery. Each patient had minimum 3 years estradiol treatment. For comparison, additional samples were obtained from microscopically unaltered testicular tissue surrounding tumors ($n = 7$). The tissues obtained were investigated by light microscopy (H&E, and Picrosirius staining), and histochemistry (Sudan Red, Periodic Acid Schiff Reaction, PAS).
Results: Spermatogenesis is strongly impaired resulting in mainly spermatogonia being present; only two patients showed spermatozoa and spermatids. Estrogen decreases the diameter of seminiferous tubules. There is fatty degeneration within the connective tissue surrounding the tubules and increased synthesis of collagen fibers in the extracellular matrix. Sertoli cell vacuolation is evident in the treated men. Distinct accumulations of glycoproteins are seen in the ground substance of the connective tissue surrounding the tubules, as well as in scattered Sertoli cells. Leydig cells are detected sporadically and in dedifferentiated form.
Conclusion: Increased estrogen leads to considerable impairment of spermatogenesis, and to changes in the composition and metabolism of the surrounding connective tissue. Sertoli cells and Leydig cells lose their functional integrity. Thus, successful therapy plans for fertility treatment in men with hyperestrogenism have to focus on improving metabolism of the connective tissue and supporting Sertoli cell and Leydig cell function.

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Effect in motility and DNA fragmentation in long term storage of cryopreserved semen

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Background: To evaluate sperm integrity in seminal samples that were cryopreserved for more than 10 years and because there are few reports that analyzed the effect of time in cryopreservation and seminal parameters, we performed a study to evaluate sperm motility and DNA fragmentation index (DFI) in the CEPAM Mexican Fertility Center
Aim: Assess motility and DNA fragmentation in thawed semen samples after 10 years of storage.
Methods: This is a longitudinal study in which were analyzed 19 semen samples after 10 years of cryopreservation for sperm motility and DFI. They were grouped in four groups: Group 1 (CX): Men in need of testicular surgery for a non oncologic reason. Group 2 (FIV): Normo-spermic men whose partner needed an IVF procedure and would not be able to assist to the clinic. Group 3 (OAT): Men with documented oligo-astheno-teratozoospermia and group 4 (QX): Men with cancer that were going to receive chemotherapy.
Results: After thawed all groups showed decrease in sperm motility (CX: 27.34%, FIV: 30.02%, OAT: 55.24%, QX: 10%). CX and FIV showed the least fragmentation index (3.5 ± 2.5 and 3.25 ± 3.01 respectively) compared with OAT and QX (9.8 ± 0.2 years 12.17 ± 3.9).
Conclusion: Although sperm motility decreased we recovered enough spermatozoa to performed ART techniques. Seminal samples cryopreserved for more than 10 years does not show a high fragmentation grade and thus may be used in ART techniques.

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Seminal, ultrasound and psychobiological parameters correlate with metabolic syndrome in male members of infertile couples

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Background: Metabolic syndrome (MetS) impact on male infertility has been poorly studied.
Aim: To systematically evaluate possible associations between MetS and clinical characteristics in males of infertile couples.
Methods: Out of 367 consecutive subjects, 351 men (36.0 ± 8.0 years) without genetic abnormalities were studied. MetS was defined according to IDF&AHA/NHLBI classification. All men underwent physical, hormonal, seminal and scrotal ultrasound evaluation. Erectile and ejaculatory functions were assessed by IIEF-15 erectile function domain (IIEF-15-EFD) and Premature Ejaculation Diagnostic Tool (PEDT), respectively; psychological symptoms by Middlesex Hospital Questionnaire.
Results: Out of 351 patients, 27 (7.7%) fulfilled MetS criteria. Among ultrasound features, in an age- adjusted logistic model, only testis inhomogeneity was significantly associated with increasing MetS factors (HR = 1.36 [1.09–1.70], $p < 0.01$). In an age-adjusted model, MetS was associated with a stepwise decline in total testosterone (TT) ($B = -1.25 \pm 0.33$, $p < 0.0001$), without a concomitant rise in gonadotropins. Among sperm parameters, in an age- and TT-adjusted multivariate model, only sperm morphology showed a significant negative association with increasing MetS components ($B = -1.418 \pm 0.42$;

$p = 0.001$). The risk of ED (IIEF-15-EFD score < 26) increased as a function of the number of MetS factors, even after adjusting for age and TT (HR = 1.45 [1.08–1.95], $p < 0.02$). No association between PEDT score and MetS was observed. Finally, after adjusting for age and TT, somatization and depressive symptoms were associated with increasing MetS components (B = 0.66 ± 0.03, $p < 0.05$; B = 0.69 ± 0.03, $p < 0.02$; respectively).

Conclusion: In males of infertile couples, MetS is associated with hypogonadism, poor sperm morphology, testis ultrasound inhomogeneity, ED, somatization and depression. Recognizing MetS could help patients to improve not only fertility but also sexual and overall health.

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Clinical implications of measuring prolactin levels in males of infertile couples

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Background: Although in females of infertile couples abnormal PRL has a definitive role in the medical flowchart, its role in males is less clear.

Aim: To systematically evaluate possible clinical and ultrasound correlates of PRL in males of infertile couples.

Methods: Out of 288 consecutive males of infertile couples, 269 (36.6 ± 4.4 years) without genetic abnormalities were studied. All men underwent physical, biochemical, seminal evaluation and scrotal and transrectal ultrasound before and after ejaculation. Ejaculatory and erectile functions were assessed by Premature Ejaculation Diagnostic Tool (PEDT) and IIEF-15, respectively.

Results: In a logistic ordinal model, adjusting for the aforementioned confounders and ejaculate volume, PRL was negatively associated with delaying ejaculation according to PEDT#1 score (Wald = 4.65, $p < 0.05$). No significant associations were found between PRL and other clinical parameters. Among semen parameters, only the positive association between PRL and ejaculate volume was significant, even adjusting for age, testosterone and TSH (adj. $r = 0.126$, $p < 0.05$). In an age- and ejaculate volume-adjusted, iterative binary logistic model, low PRL was associated with a five-fold risk of any failure in controlling ejaculation (HR = 5.15 [1.15–23], $p < 0.05$). Among scrotal and transrectal ultrasound features, we found a significant positive association between PRL and seminal vesicles (SV) volume. Associations with PRL were confirmed in nested 1:1 case-control analysis.

Conclusion: Low PRL is associated with a lessened ability to control ejaculation. For the first time, this study extends the concept of a trophic effect of PRL on male accessory glands from animals to humans. We report a positive association among PRL and ejaculate and SV volume, before and after ejaculation.

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Seminal, clinical and color-doppler ultrasound correlations of prostatitis-like symptoms in males of infertile couples

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Background: 'Prostatitis-like symptoms' (PLS) are a cluster of bothersome conditions defined as 'perineal and/or ejaculatory pain or discomfort and NIH-CPSI pain subdomain score = 4′ (Nickel's criteria). PLS may originate from the prostate or from other portions of the male genital tract. The NIH- CPSI is considered the gold-standard for assessing PLS severity.

Aim: This study was aimed at investigating possible associations among NIH-CPSI (total and subdomain) scores and PLS, with seminal, clinical and scrotal/transrectal color-Doppler ultrasound (CDU) parameters in a cohort of males of infertile couples.

Methods: PLS of 400 men (35.8 ± 7.2 years) with a suspected male factor were assessed by the NIH-CPSI. All patients underwent, during the same day, semen analysis, seminal plasma interleukin 8 (sIL-8, a marker of male genital tract inflammation), biochemical evaluation, urine/seminal cultures, scrotal/transrectal CDU.

Results: PLS was detected in 39 (9.8%) subjects. After adjusting for age, waist and total testosterone (TT), no association among NIH-CPSI scores or PLS and sperm parameters was observed. Yet we found a positive association with current positive urine and/or seminal cultures, sIL-8 levels and CDU features suggestive of inflammation of the epididymis, seminal vesicles, prostate, but not of the testis. The aforementioned significant associations of PLS were further confirmed by comparing PLS patients with age-, waist- and TT-matched PLS-free patients (1 : 3 ratio).

Conclusion: NIH-CPSI scores and PLS evaluated in males of infertile couples, are not related to sperm parameters, but mainly to clinical and CDU signs of infection/inflammation.

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Metabolic syndrome and prostate abnormalities in male subjects of infertile couples

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Background: No previous study evaluated systematically the relationship between metabolic syndrome (MetS) and prostate-related symptoms and signs in young, infertile, men.

Aim: To evaluate the MetS-prostate relationship.

Methods: We studied 171 (36.5 ± 8.3 years) males of infertile couples. MetS was defined based on NCEP- ATPIII classification. All men underwent hormonal (including total testosterone [TT] and insulin), seminal (including interleukin 8, sIL-8), scrotal and transrectal ultrasound

evaluations. Because we have previously assessed correlations between MetS and scrotal parameters in a larger cohort of infertile men, here we focused on transrectal features. Prostate-related symptoms were assessed using NIH-CPSI and IPSS questionnaires.
Results: Twenty-two subjects fulfilled MetS criteria. In an age-adjusted logistic ordinal model, insulin levels increased as a function of MetS components (Wald = 29.5, $p < 0.0001$) and showed an inverse correlation with TT (adj.$r = -0.359$, $p < 0.0001$). No association between MetS and NIH-CPSI or IPSS scores was observed. In an age-, TT-, insulin-adjusted logistic ordinal model, an increase in number of MetS components correlated negatively with normal sperm morphology (Wald = 5.59, $p < 0.02$) and positively with sIL-8 levels (Wald = 4.32, $p < 0.05$), a marker of prostate inflammation, with prostate total and transitional zone volume assessed using ultrasound (Wald = 17.6 and 12.5, both $p < 0.0001$), arterial peak systolic velocity (Wald = 9.57, $p = 0.002$), texture non-homogeneity (HR = 1.87 [1.05–3.33], $p < 0.05$), calcification size (Wald = 3.11, $p < 0.05$) but not with parameters of seminal vesicles.
Conclusion: In males of infertile couples, MetS is positively associated with prostate enlargement, biochemical (sIL8) and ultrasound-derived signs of prostate inflammation but not with prostate-related symptoms, which suggests MetS as a trigger for a subclinical, early-onset form of benign prostatic hyperplasia.

P112

Dysfunctional Leydig cell treated with low doses of recombinant human chorionic gonadotropin (HCGr)

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Background: Hypogonadism is a common cause of male infertility producing spermatogenesis damage; its origin may be multifactorial. Among the treatments are use antiandrogens, chorionic gonadotropin and testosterone.
Aim: Increase testosterone levels, with low doses of HCGr improving the spermatogenesis.
Methods: 28 patients with persistent hypogonadism, even controlling the endocrine/metabolic state, were selected for challenge with hCGr (250 μrs) and were treated with low doses of it (31.25 μrs) twice a week for 2–3 months.
Results: Mean age 37.14 ± 5.91 years; 15 patients with primary infertility (Evolution 7. 5 ± 2.7 years) and 13 patients with secondary infertility (evolution 4.6 ± 2.8 years). Total testosterone (Tt) (nmol/L) 10.72 basal ± 2. 73 and Free androgens index (FAI) (%) of 50.55 ± 20. 30; after the endocrine/metabolic control Tt 11. 73 ± 4.01, 52 FAI. 10 ± 18. 88 and post application hGCr 250 μrs. challenge Tt26.88 test links. 8, FAI 124. 10 ± 60.31%, and 2 months post treatment with 31.25 μrs Tt 15.74 ± 5. 93 and 71 FAI. 45 ± 27.33. The seminal parameter with greater modification was morphology: basal average 1.88 statistical mode 0, post treatment of 4.15, statistical mode 3. Three spontaneous pregnancies were reported.
Conclusion: The handling with hCGr at low doses in patients selected by dynamic testing improves clinical symptoms and spermatogenesis.

P113

Prevalence factors that alter the seminal parameters in male patients with recurrent pregnancy loss (RPL)

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Background: In the study of couples with RPL, we are dealing with the female factor because the binomial mother/son and do not take into consideration that male gamete contributes 50% of genomic material in embryonic development which also contributes to the placentation. So the sperm genetic and epigenetic can have important consequences as part of the etiology of the problem.
Aim: To determine prevalence of male factors that may be associated with RPL.
Methods: Retrospective study, of infertile patients evaluate for RPL at Andrology clinics of the INper, from January 2009 to January 2012. Data analysis made with frequencies and measures of central tendency (SPSS-18. Inc., Chicago, IL, USA).
Results: The prevalence of men send to study was 1.61% (17 patients). Age 35.35 ± 4.93 years. Testosterone level of 13.22 ± 5.74 nMol/L; seminal disorders: 52.9, 47.1% Teratozoospermia hypospermia. Risk factors: paternal age >40 years 23.5, 41.2%, glucose Varicocele >100 mg/dL. 64.7%, triglycerides >150 mg/dL, 76.5%, HDL <40 mg/dL, 82.4%, overweight and obesity 35.3% and metabolic syndrome with 64.7%.
Conclusion: We believe that the approach of the couple with RPL must be integral, studying both, this would improve the reproductive outcome and minimize risks, including lifestyle in women; and we propose the model of study by factors in the male similar to female study. Metabolic alterations were more prevalent, conditioning hypogonadism and its sperm alterations.

P114

Dynamic testing with recombinant human chorionic gonadotropin (HCGr) for challenge dysfunctional Leydig cell at male infertility with hypogonadism

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Background: Hypogonadism is caused by dysfunction or failure of Leydig cell; the differentiation between them gives us a therapeutic option.

Aim: To determine challenge with hCGr test (in a way similar to the urinary) determine the dysfunction or failure of Leydig, by the capability of response.
Methods: 47 patients with Leydig cell dysfunction (hypothalamus/pituitary positive response but refusal by Leydig cell /testicle) in clomiphene citrate (CC) test. Indicates challenging test with hCGr (250 mcgrs) measure basal and 72 h post-test blood samples of: Total testosterone (Tt), Estradiol (E2), Sex hormone- binding globulin (S.H.B.G.) and free androgens Index (FAI).
Results: Mean age 36. 12 ± 6. 29 years. Considered a positive response the increase of 50% of the basal values. Basal total testosterone (nMol/L) 11.81 ± 5. 06 and post-test 25. 60 ± 9. 86, Estradiol (pg/mL) basal 31. 15 ± 12. 18 and post-test 63.86 ± 28.01; Free androgens index (%) basal 56. 79 ± 22. 46 and post-test of 121. 80 ± 58. 41. S.H.B.G. does not change.
Conclusion: The challenge test reports the Leydig cell dysfunction, the grade of dysfunction, and identifies which have failure. The hCGr is easy to apply and minor local adverse effects.

P115

Therapy with clomiphene citrate (CC) in patients with multifactorial male infertility selected with CC challenge test

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Background: Testicular hormone deficiency promotes changes in spermatogenesis; clomiphene citrate (CC) treatment with 25 mg/day for endogenous stimulation of testosterone, has been used in non- obstructive azoospermia, varicocele, and hypogonadotropic hypogonadism.
Aim: Evaluate the improvement in seminal and hormonal parameters at patients treated with CC with multifactorial infertility and positive test challenge with CC.
Methods: 94 patients were selected (positive test) at 151 with CC test challenge; were treated with CC (25 mgrs per day), measuring basal/post treatment (two months): follicle-stimulating hormone (FSH), Luteinizing hormone (LH) Total testosterone (Tt), Estradiol (E2), Sex hormone-binding globulin (S.H.B.G.) and free androgens Index (FAI) and sperm capacitation test.
Results: Hormonal levels (basal/post treatment) average and standard deviation were: FSH 4.02 ± 2.4/4.44 ± 3.56 (mIU/mL), LH 2.71 ± 1.38/3.63 ± 2.28 (mIU/mL), Tt 13.05 ± 3.51/20.07 ± 7.61 (nmol/L), E2 34.05 ± 13.55/ 45.05 ± 18.57 (pg/ml), the seminal parameters (without treatment / treatment) average and standard deviation were: density pre 50.44 ± 41.7/51.9 ± 40.76 and 52.1 ± 39.76/66.96 ± 82.28 post capacitation test; motility pre 46.33 ± 20.80/45.63 ± 22.92 and 61.16 ± 27.21/ 73.54 ± 87.65 post capacitation test; morphology pre 2.51 ± 1.95/3 ± 2.31 and 3.62 ± 2.42/4.81 ± 3.07 post capacitation test, total number of mobile cells and total number of mobile cells with normal morphology (TNMCNM) pre 1.16/1.86, and 0.953/2.44 post capacitation test,total of mobile cells (TCM) pre 58.5/61.9 and 31.8/41.6 post capacitation test, 27 patients achieved clinical pregnancy (28.7%).
Conclusion: Improving testosterone levels, we see mainly increased the TCM and ICR that helped pregnancy; which suggest that the CC should be considered an economical management option with easy access for infertile patients.

P116

Risk of testicular carcinoma in situ in a cohort of 204 patients with non-obstructive azoospermia

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Background: Infertile men have a higher chance of malignant testicular germ cell tumors (TGCTs). The non-invasive stage of TGCT, also known as carcinoma in situ (CIS), can only be diagnosed by a testicular biopsy. Testicular CIS is found in 2.2% of the infertile male population.
Aim: We have not found reports on the prevalence of CIS in men undergoing TEsticular Sperm Extraction (TESE) for non-obstructive azoospermia (NOA). Our aim was to determine the chance of having CIS in this high risk population.
Methods: From 2007 to 2014 testicular histology was performed in all men that had TESE for NOA . In addition reproductive hormones, scrotal ultrasound, genetic analyses and risk factors for TGCT were determined.
Results: In 204 patients with NOA eight patients had CIS (4.0%). Four of these patients had a history of a TGCT. Of the remaining four patients with CIS (2.1%), one had bilateral CIS. Two of the four cases with CIS only had a history of cryptorchidism.
Conclusion: Testicular CIS is more prevalent in men with NOA than in the general male population. If a patient has no history of unilateral TGCT the chance of having CIS in the contralateral testis is equal to that of the male infertile population. In men with NOA and a history of TGCT the chance of having CIS in the contralateral testis is high (57%).

P117

Recurrent pregnancy loss and genomic instability of the male gamete

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Background: Recurrent pregnancy loss (RPL) is a multifactorial disease and in about 50% of cases the etiology is

not defined. Previous studies focused on the possible correlation with abnormal karyotype, sperm aneuploidy rate and complete AZF microdeletions on the Y chromosome.
Aim: We investigated on genetic factors in the male partner of RPL couples by considering: (i) complete AZF deletions; (ii) partial AZFc deletions; (iii) microsatellites instability (MSI).
Methods: For the first two objectives, we screened 140 RPL patients by ± PCR followed by AZFc gene dosage (Genescan software) and compared the data to 332 men with proven fertility. For the third objective, we analyzed 7 microsatellites (mono-, di- and trinucleotide loci) in DNA samples extracted from lymphocytes and spermatozoa (Genescan software) of 74 RPL patients and 90 fertile normozoospermic controls. A discrepancy of >40% between the markers in the two cell types defined an 'unstable' sample.
Results: No complete AZF deletions were found in RPL patients, whereas we observed a higher frequency of partial AZFc deletion subtypes in patients compared to controls (4/140, 2.85% vs. 1/332, 0.3%; $p = 0.013$; OR = 9.554, CI 95% = 1.077–84.715). The frequency of >40% MSI was higher in patients than controls, with a nearly significant p value (3/74; 4.1% vs. 0/90; 0.9% in controls, $p = 0.054$).
Conclusion: We found a significant correlation between partial AZFc deletion and RPL suggesting the involvement of this Y linked factor also in this pathological condition. The potential association between MSI and RPL needs further analysis on a larger study population.

P118

Factors affecting spermatogenesis upon gonadotropin replacement therapy: a meta-analytic study

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Background: Predictors of successful gonadotropin therapy in subjects with hypogonadotropic hypogonadism (HHG) and azoospermia are debated.
Aim: To systematically analyze the results of gonadotropin therapy in inducing spermatogenesis in HHG subjects and azoospermia.
Methods: An extensive Medline and Embase search was conducted for meta-analyzing data from all available studies on HHG subjects treated with hCG with or without FSH.
Results: 44 studies were retrieved, 43 considering the appearance of at least one sperm in semen and 26 considering sperm count. The combination of the study results showed a success rate of 77% (70–82) in achieving spermatogenesis, with a mean sperm count obtained of 9.57 (6.59–12.55) millions/ml. Results were significantly worse in studies involving only subjects with a pre-pubertal onset HHG, as compared with studies involving a mixed population of pre- and post-pubertal onset [70% (59–79) vs. 84% (76–89), $p = 0.024$ and 6.87(4.27-9.47) vs.12.45 (8.33–16.58) millions/mL, $p = 0.025$; for dichotomous and continuous data, respectively]. No difference in spermatogenesis achievement and sperm count was found for different FSH preparations. No significant correlation was found between prevalence of subjects with previous testosterone replacement therapy (TRT) and spermatogenesis achievement rate or sperm count. Finally, a higher success rate was found for subjects with lower levels of gonadotropins at the baseline and for those using both hCG and FSH.
Conclusion: Gonadotropin therapy, even with urinary derivatives, is a suitable option in inducing/restoring fertility in azoospermic HHG subjects. Gonadotropins appear to be more efficacious in subjects with a pure secondary nature (low gonadotropins) and a post-pubertal onset of the disorder, whereas previous TRT does not affect outcome.

P119

Coenzyme Q10 and aspartic acid exert protective effects on oxidative stress and DNA damage in idiopathic asthenozoospermic patients

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Background: Many reports show that male infertility is negatively influenced by reactive oxygen species and by other oxidant radicals.
Aim: The objective of this study was to assess the effects of Coenzyme Q10 (CoQ10) and Aspartic acid (D-Asp) on some parameters of sperm oxidative stress and DNA damage that were previously untested.
Methods: This was an observational longitudinal study, evaluating twenty patients affected by idiopathic asthenozoospermia. Subjects underwent the administration of oral dietary supplement, including CoQ10 and D-Asp. The following outcomes were evaluated: CoQ10 and D-Asp levels, superoxide dismutases (SOD) activity, nitric oxide (NO) and peroxynitrite levels and DNA damage (comet assay) in sperm.
Results: After treatment, NO and peroxynitrite levels diminished, whereas SOD activity increased significantly. Furthermore, tail intensity, a marker of DNA damage, decreased significantly after treatment. Correlation analysis showed a negative relationship between the increase of CoQ10 and the decrease of NO and tail intensity and a positive relationship between the increase of CoQ10 and the rise of SOD activity; no significant correlation was found between the increment of D-Asp and the changes of markers of oxidative stress and DNA damage. Increase of

SOD activity and decrease of NO levels were negatively and positively correlated with the diminishment of tail intensity, respectively.
Conclusion: According to our results, only CoQ10 seems to play a protective role against oxidative stress and DNA damage, thus contradicting some previous findings, which suggested such effects also for D- Asp.

P120

Detecting sperm DNA fragmentation to discriminate between fertile and infertile men

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Background: Sperm DNA fragmentation (sDF) is an anomaly of sperm genome consisting of single and double stranded DNA breaks. The ability of tests detecting sDF to predict the outcomes of reproduction is affected by many variables, including the sperm population where the damage is revealed. Using TUNEL/PI, which couples the detection of sDF to nuclear staining with propidium iodide (PI) our group unveiled two flow cytometric sperm populations that differ for PI staining (brighter and dimmer), for the amount of sDF and for viability. Dimmer sperm are dead and DNA fragmented whereas brighter sperm are both fragmented and non fragmented, both viable and non viable.
Aim: Based on these characteristics, we speculated that sDF of live brighter sperm is the DNA damage really impacting on reproduction.
Methods: To demonstrate this hypothesis, we set up a multicolor flow cytometric method able to detect sDF in viable besides in the total and brighter sperm population. Then we compared the levels of sDF in 33 fertile and 30 infertile men.
Results: We found that sDF was higher in infertile than in fertile subjects, both in total (44.1 $\pm$ 18.5 vs. 31.8 $\pm$ 12.6%, p <0.01), brighter (31.4 $\pm$ 15.8 vs. 22.4 $\pm$ 10%, $p < 0.01$) and live sperm (22.1 $\pm$ 18.2 vs. 13.1 $\pm$ 7.1 $p < 0.01$). However, the percentage increase in infertile vs. fertile subjects was much greater for viable sperm (68.7%) respect to PI brighter sperm (40%) and total population (38.7%).
Conclusion: In conclusion, the ability of sDF to discriminate between fertile and infertile men, increases by shifting from total to brighter to viable sperm.

P121

High prevalence of testosterone deficiency in subfertile men: adverse metabolic consequences

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Background: Subfertility may be associated with testosterone deficiency, which, if overlooked, may have serious adverse health effects.
Aim: The aim was to assess the frequency of hypogonadism and its metabolic consequences in subfertile men.
Methods: Men from infertile couples (n = 192, 18–50 years.) with sperm concentration <20 $\times$ 106/mL and age- matched men from the general population (n = 199) were enrolled. Serum/plasma levels of sex hormones, glucose, insulin and HbA1c were measured. Bone mineral density (BMD) was assessed by dual-energy X-ray absorptiometry at the lumbar spine (LS). Odds ratios (OR) for biochemical hypogonadism (BH), defined as total testosterone <8.0 nmol/L and/or LH >8.6 IU/L and/or ongoing androgen replacement therapy, were calculated. Differences in metabolic parameters between eugonadal and hypogonadal subfertile men were analysed. All calculations were adjusted for age, BMI and current smoking (BMD only).
Results: In subfertile men vs. controls, the OR of BH, was 10 (95% CI, 5.1, 22). BH subfertile men had higher fasting glucose (mean difference, 0.39 mmol/L; 95% CI, 0.07, 0.71; p = 0.016), higher fasting insulin (mean ratio, 1.41; 95% CI, 1.03, 1.92; p = 0.031), higher HOMA-index (mean ratio, 1.56; 95% CI, 1.13, 2.17; p = 0.007), higher HbA1c (mean diff, 2.2 mmol/mol; 95% CI, 0.28, 4.1; p = 0.025) and lower BMD-LS (mean diff, −0.05 g/cm^2; 95% CI, −0.10, −0.001; p = 0.045) compared to eugonadal subfertile men.
Conclusion: Subfertile men have increased risk of BH, which is associated with an adverse metabolic pattern and decreased BMD. Endocrine and metabolic follow up of these men after completion of fertility treatment is hence warranted.

P122

The human sperm proteome changes according to motility characteristics

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Background: Proteomics has contributed to the characterization of the human sperm protein composition (Amaral *et al.*, 2014, Hum Reprod Update 20 40–62). Recent improvements in mass spectrometry technology allow the application of high throughput approaches to quantify changes in the sperm proteome.

Aim: To determine whether there are differences in the sperm proteome of sperm cell subpopulations differing in motility (isolated from normozoospermic samples by classical swim-up procedure).

Methods: Normozoospermic semen samples ($n = 5$) were fractionated using the swim-up technique to obtain two different subpopulations (migrated and non-migrated sperm). Both subpopulations were then further purified individually by a Percoll gradient to remove any putative contaminating round cells. Proteins from migrating and non-migrating sperm fractions were extracted, differentially labelled with TMT-6plex isobaric tags, separated using SDS-PAGE and analysed by LC-MS/MS.

Results: A total of 887 proteins were identified. At least 93 proteins were differentially abundant in the two subpopulations. Mitochondrial proteins, those involved in metabolism and energy production (26%) and sperm flagella proteins (6%) were deregulated in less motile sperm. Bioinformatic analysis through David also detected a statistically significant enrichment (p -values < 0.05) of gene ontology terms in the lower motility sperm related to the mitochondrion and altered cellular pathways (Reactome) related to 'Integration of Energy Metabolism'.

Conclusion: High throughput differential proteomics has allowed indentifying differences in the relative abundance of proteins in different swim-up fractions providing new clues towards to understand the molecular basis of the human sperm motility. Supported by PI13/00699 and EU-FP7-PEOPLE-2011- ITN289880 to RO.

P123

Effects of percutaneous embolization of clinical varicocele on semen quality

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Background: Varicocele may impair spermatogenesis through several mechanisms and is found in about 35% of men who present for infertility evaluation. However the effect of varicocele repair in subfertile men is still controversial.

Aim: To evaluate changes in semen parameters after embolization of clinical varicocele in infertile men.

Methods: The authors prospectively evaluated a group of 157 infertile patients who underwent percutaneous embolization of clinical varicocele between January 2007 and January 2014 at Department of Human Reproduction of Centro Hospitalar e Universitário de Coimbra, Portugal. Pre and post embolization sperm concentration, A+B mobility and morphology were compared. These results were compared with a historic control of infertile patients who underwent surgical correction of clinical varicocele (high ligation – Palomo procedure). Statistical analysis was performed using SPSS V20.0.

Results: Mean sperm concentration improved from $12.0 \pm 16.4 \times 106$/mL pre-embolization to $22.1 \pm 25.4 \times 106$/mL post-embolization ($p < 0.001$). Conversely, A + B mobility evolved from $25.9 \pm 23.2\%$ to $33.9 \pm 23.6\%$ ($p = 0.001$) and normal morphology from $4.2 \pm 5.5\%$ to $4.9 \pm 5.2\%$ ($p = 0.176$). There was an increase in at least one of the semen parameters in 64.3% of patients, of which 22.9% improved in all three parameters. In historic controls there was also a statistically significant improvement in concentration and mobility, but not in morphology. We found no statistical differences in improvement of semen parameters in relation with the type of infertility or degree of varicocele.

Conclusion: Percutaneous embolization of clinical varicocele in men with fertility problems is clearly associated with a significant improvement of semen parameters, namely concentration and A + B mobility.

P124

CFTR genotype of genitourinary abnormalities in male patients

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Background: Mutations of CFTR gene have been firmly linked to congenital absence of vas deferens (CAVD) but this association remains controversial for other related abnormalities of the mesonephric tract.

Aim: To assess the frequency of CFTR mutations/variants in CAVD, distal malformations of genital tract (DMGT) and unilateral renal agenesis (RA), and to know the reproductive counseling of couples considering fertility.

Methods: Retrospective survey of clinical and laboratory information from 242 patients attending our hospital

(1990–2012). An extensive CFTR screening was performed in all patients, including mutations and variants described for the infertile phenotype. Female couples were screened for the most common CF mutations.

Results: Bilateral CAVD (CBAVD) was found in 122 patients, showing 73% of allele mutation (AM), while 43 men with unilateral CAVD (CUAVD) had 41.8% AM. DMGT were found in 10 patients, with 35% AM. The combination of CBAVD plus RA (n = 10) had 35% AM, and CUAVD plus RA (n = 30) had 21.6% AM. Finally, a group of 27 men with RA (with normal vasa) showed 14.8% AM. Among patients considering fertility (n = 39) 3 female partners were carriers (7.6%). Reproductive counselling indicated that the risk of severe CF in the offspring was low in these particular couples.

Conclusion: High prevalence of CFTR mutations/variants was confirmed in CAVD, but also in other congenital defects of distal genital tract. Contrary to previous reports, the presence of renal agenesis associated with either unilateral or bilateral CAVD showed high frequency of CFTR variants. Genetic screening of patient's female partners showed low reproductive risk for the offspring.

P125

Mast cells stabilizer desloratadine in the management of asthenozoospermia associated with elevated MAR-test

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Background: Desloratadine is a tricyclic antihistamine, which has a selective and peripheral H1- antagonist action, mast cell stabilizer.

Aim: Tthe aim of our study was to assess the potential role of Desloratadine (Eslotin) in the management of immunological infertility.

Methods: Inclution criteria to the study was asthenozoospremia (total sperm motility less 40%, progressive motility less 32%) and at the same time MAR test >10% of sperm agglutinated to beads. 45 couples with duration of infertility more than 2 years, with mean age of male 24.7 years, were randomized into two groups. Group I – 24 men with eslotin 5 mg daily administration and 21 men in control group II. In group I semen analysis and MAR-test level were re-assessed after 3 months of treatment, in control group same re-investigation was done again after 1 month.

Results: After Eslotin administration there was statistically significant improvement in spermatozoa motility. Total motility increased from 23% till 43% ($p < 0.05$), progressive motility from 15% till 28% ($p < 0.05$). There was no difference in motility in control group. At the same time in group I MAR –test level became significantly lower – mean level before treatment 22.84 and 9.78 after ($p < 0.05$). Again there was no changes in MAR – test level in control group.

Conclusion: Infertile men with elevated level of MAR-test and poor basic parameters of semen motility may be managed by Desloratadine (Eslotin). Sperm with antibodies on surface may play a major role in pathogenesis of idiopathic asthenozoospermia.

POSTER SESSION: MALE SEXUAL DYSFUNCTION

P126

Poster cancelled

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Erectil dysfunction management in a primary care center

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Background: Different studies have shown the relationship between erectile dysfunction and cardiovascular disease.
Aim: The aim of this study was to evaluate the cardiovascular profile and treatment of pacients diagnosed of erectile dysfunction (ED) in the Primary Care Center Gava-Begues.
Methods: We conducted a transversal study including 393 pacients who were diagnosed of ED. The main variables were: age, cardiovascular risk at the moment of diagnose and after, and the recommended treatment.
Results: Prevalence of ED: 1.85%. Average age: 57 ± 11. 84% were diagnosed during the last 5 years. The cardiovascular risk REGICOR: 7.2%. To 37.4% of cases had not been calculated. 9.9% had a testosteron deficit, up to 35.6% had not been calculated. Associated cardiovascular risk factors: 27% diabetes, 50.4% high blood pressure, 56% high cholesterol, 33.6% obesity and 37.9% smoking. Cardiovascular disease present at the moment of ED diagnose: 5.3% ischemic cardiopathy (CI), 3.1% cerebrovascular disease (CVD), 2% peripheral arteriopathy (PA); post-ED diagnose, 2.8% developed CI, 1.3% CVD and 2.8% PA. Recomanded treatments in frecuency order: tadalafil 20, sildenafil 100, sildenafil 50 and vardenafil 10.
Conclusion: In our area we have detected an infradiagnose of patients with ED and an incomplete evaluation of them at the moment of the diagnose. The cardiovascular risk factors prevalence is higher in these patients regarding the total population. The fact of not calculating the cardiovascular risk could be a consequence of not identifing ED soon enough to prevent cardiovascular disease. The drugs used and the dosis are optimal.

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Differential risk factors for metabolic syndrome in men consulting in urology offices according to the reason to seek care

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Background: Prevalence of metabolic syndrome (MetS) in known to be high among urological patients.
Aim: To identify risk factors for MetS, depending on the reason for consultation, among urological patients = 45.
Methods: Observational study performed in Spain. Age, waist circumference, body mass index (BMI), and SBP/DBP, total testosterone (TT) and MetS components were recorded. MetS diagnosis: NCEP- ATPIII criteria. Erectile dysfunction (ED) assessment: IIEF-EF. Bivariate and multivariate logistic regression analyses were performed to assess relationships
Results: 1.021 men aged 62.8 ± 8.2 years participated in the study. Sexual disorders accounted for 54.7% of visits, of which ED (primary/concomitant) 39.0%. Prostatic check-up accounted for 42.8%. MetS prevalence was 45.9%. Low T (TT <10.4 nmol/L) prevalence was 16.2%, and independent of age or reasons for consultation. ED prevalence was 86.1% in men with sexual complaints vs. 66.4% in others; $p < 0.001$; severe ED prevalence was similar. Seeking advice for ED (but no diagnosis/severity) and obesity/overweight significantly increased the odds for MetS in men with sexual complaints, while in other men were obesity and severe ED; low T and abdominal obesity increased the odds for MetS in both. On multivariate analysis, only low T and abdominal obesity remained as risk factors: OR 1.84 [95% CI 1.04–3.29] and 12.81 [95% CI 8.23–19.96] for men with sexual complaints, and 2.00 [95% CI 1.07–3.75] and 9.84 [95% CI 6.20–15.62] for others.
Conclusion: MetS prevalence among urological patients is high. Sexual complaints such as ED may indicate MetS but risk factors such as abdominal obesity and, particularly low T, should be considered in all men.

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Relationship between low testosterone levels and metabolic syndrome in men consulting in urology offices: is this an important reason for seeking care?

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Background: Metabolic syndrome (MetS) and testosterone deficiency (Low T) frequently coexist.
Aim: We assessed: (i) low T prevalence (Total T <10.4 nmol/L) and its relationship with MetS in urological patients = 45, and (ii) the differential risks for low T according to the reason to seek care (sexual complaints or others).
Methods: Observational study performed in Spain. Age, waist circumference (WC), body mass index (BMI), total T and MetS components (NCEP-ATPIII) were recorded. Erectile dysfunction (ED) assessment: IIEF-EF. Bivariate/multivariate logistic regression analyses were performed.
Results: 1021 men aged 62.8 ± 8.2 years participated in the study; 54.7% consulted for sexual complaints. Low T prevalence was 16.2%, and was independent of age, sexual disorders or IIEF-EF scores. MetS prevalence was 65.5% in men with low T vs. 34.6% in eugonadic men; $p < 0.001$). On multivariate analysis, obesity (BMI = 30 kg/m^2) was a risk factor for low T in men with sexual complaints (OR 4.57 [95% CI 1.99–10.49]), while abdominal obesity (WC >102) was in other men (OR 3.24 [95% CI 1.84–5.70]). SBP/DBP >130/85 and triglycerides >150 mg/mL were risk

factors for low T in the former, and fasting glucose >100 mg/mL in the latter (OR 2.09 [95% CI 1.05–4.13], 2.12 [95% CI 1.33–3.39], and 1.93 [95% CI 1.13–3.29], respectively).
Conclusion: Prevalence of MetS among urological patients is higher in men low T. Detection of men at risk for low T is crucial. Obesity, hypertension and hypertriglyceridemia in men consulting for sexual complaints, and high fasting glucose and abdominal obesity in men with other urological concerns, may be good indicators

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Hypogonadism, a clinical problem to resolve in patients with chronic pain and opioid treatment

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Background: Hypogonadism is considered prevalent among men that are chronically treated with opioids, and is one of the most frequent symptoms of Erectile Dysfunction. Treatment should include early detection along with multidisciplinary follow-up, and combined pharmacological treatment with hormone replacement therapy and phosphodiesterase 5 inhibitor.
Aim: Opioids impair gonadal function in men chronically treated with opioids, but their correlation with clinical and hormonal hypogonadism is not well established. Thus, we performed an observational, prospective study in order to correlate opioid treatment with hypogonadism.
Methods: A total of 77 patients with chronic pain and opioid treatment, presenting Erectile Dysfunction (ED) where evaluated during 6 months (clinically [International Erectile Function Index (IEFI), hormonally [FSH, LH, Total and free Testosterone] and psychologically [Anxiety and Depression test (HAD), Millon Personality Inventary].
Results: 34% of the patients with DE (average age 58, BMI 30 kg/cm^2) where treated with analgesics (21/61), being paracetamol the most prescribed (20%, 12/61). 34% where treated with second step opioids (21/61), being tramadol/paracetamol the most prescribed (25%, 15/61) and 57% with major opioids (35/61) being fentanile the most prescribed (26%, 16/61).
Conclusion: 44% of the patients with low levels of testosterone (2.05 ng/mL, VN = 3–10 ng/mL) and an average of 8 in the IEFI (moderate ED), 88 % received testosterone gel (22/25 cases), 32% of the patients were treated with vardenafil phosphodiesterase 5 inhibitor (8/25 cases) and 57% presented clinical recovery (8/14 cases) 3 months after initiating the treatment.

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Duration of erection: an objective measure of erectile function showing sensitivity to comorbidities

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Background: The presence of comorbidities: affect erectile function and dysfunction, including treatment response to PDE5 inhibitors.
Aim: To assess how the presence of comorbidities: hypertension, dyslipidaemia, diabetes mellitus, abdominal obesity and central obesity affect the efficacy of vardenafil orodispersible tablet (ODT) in terms of the objective endpoint duration of erection (DoE) vs. the subjective endpoint IIEF-EF.
Methods: Data were taken from a randomized, double-blind, placebo-controlled, multicenter study comparing the efficacy of 12-week treatment with vardenafil ODT 10 mg on-demand in 127 patients with ED. Primary efficacy endpoints: stopwatch-assessed DoE (min) both, at any attempt (DoE-AA) and when leading to successful intercourse (DoE-SEP3+), and IIEF-EF scores. Spearman's correlation tests and logistic regression analysis were performed to assess relationships.
Results: Age, comorbidities and efficacy outcomes were similar in both treatment groups. At baseline, only DoE-SEP3+ was significantly higher in men without DLP (8.8 $\pm$ 0.6 vs. 7.5 $\pm$ 0.4 when present; $p = 0.05$). DoE-AA and DoE-SEP3+ correlated with time since diagnosis of DM (Rho = -0.305; $p = 0.042$ and Rho = -0.398; $p = 0.040$, respectively). DM + O reduced DoE by 3.6 $\pm$ 1.6 min ($p = 0.032$) and DoE-SEP3+ by 2.9 $\pm$ 1.6 min ($p = 0.049$). No relationships were observed for IIEF-EF. After 12- week treatment, vardenafil ODT was significantly superior to placebo for all efficacy endpoints, regardless of the presence of any comorbidity. In multivariate analysis only DoE was affected by the presence of a comorbidity: HTN (3.7 $\pm$ 1.4 min less).
Conclusion: DoE (AA or SEP3+) seems to be sensitive to the presence of certain comorbidities, been able to provide objective information of their effect on sexual function besides of the effect of treatment.

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Early penile rehabilitation with once daily PDE 5 inhibitors in the initial management of peyronie's disease - our initial evaluation

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Background: The rationale for the use of PDE5i in Peyronie's disease comes from animal studies showing reduction in collagen/smooth muscle and collagen III/I ratios and increase the apoptotic index in the plaque. Long-term

continuous administration of PDE5i increase cGMP levels in target tissues.

Aim: Our preliminary study was designed to evaluate the role of long-term continuous PDE5 inhibitors as an adjunctive to conventional non-surgical management of peyronies disease.

Methods: 20 men with clinical symptoms suggestive of recent onset peyronies disease were evaluated for erectile dysfunction, pain, physical deformity etc. After detailed history, examination and counselling those patients who were in the initial stages of their disease were prescribed once daily dosage of PDE5i (Tadalafil 5 mg OD along with high dose Vitamin E for 3 months) for early penile rehabilitation with an aim to reduce the disease progression prior to deciding surgical intervention. They were followed up at 3 months and the outcome was assessed by successful attempts for sexual intercourse, pain relief and resolution of the plaque.

Results: Five patients (25%) had decrease in plaque size, 16 patients (80%) reported in resolution of pain and 18 patients (90%) reported improved satisfaction of their sexual performance

Conclusion: Our initial evaluation showed that with PDE5 inhibitors majority of the patients reported improved sexual performance and pain relief. Further prospective study is needed to establish the antifibrotic activity of PDE5i in peyronies disease.

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Performance of duplex ultrasound protocol with cavernous injection of alprostadil

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Background:

- Erectile dysfunction (ED) is the main complaint in male sexual medicine, with a high prevalence (52% in non-institutionalised men aged 40–70 years) and incidence.
- About 25–75% of men will develop ED after radical prostatectomy, an increasingly common technique in urology's daily practice.

Aim: We present the implementation of a diagnostic test for the ED.

Methods: Inclusion criteria: De novo ED; Absence of contraindication to phosphodiesterase inhibitors or alprostadil.

Exclusion criterion: Previous radical prostatectomy
Patients are quoted in ED specific consultation:

- Specific informed consent.
- History (includes IIEF) and physical examination.
- Analytical blood: Glucose and lipid profile; total testosterone.

Duplex-Ultrasonography: An urologist, a radiologist and a nurse are required.

- With flaccid penis Peak systolic velocity (PSV) and telediastolic velocity (TDV) in cavernosal arteries and dorsal artery are measured.
- The urologist inject 20 μg of alprostadil intracavernous and the team leave the patient alone.
- From 10 min response to the drug can be seen. One more time, in erection, the measures are repeated.

We use the European Assotiation of Urology parameters to asses normality:

- PSV of 35 cm/s or higher.
- TDV of 4 cm/s or lower.

Results: From March to April 2014, the first five patients have been studied.

- 3 have a normal study.
- 1 have a positive test.
- The other one had no response to the test.

Conclusion: Duplex ultrasound of penile arteries after intracavernous drug is the main vascular study in ED, as well as a safe and cheap diagnostic test.

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Cardiovascular risk associated to testosterone deficiency among men older than 40 years showing interest for a testosterone deficiency syndrome (SDT) awareness campaign: preliminary results

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Background: Testosterone deficiency is known to be largely underdiagnosed. Awareness campaigns may help to counteract this situation

Aim: To assess the prevalence of testosterone deficiency (total testosterone [TT] = 12 nmol/L) among men aged = 45 showing interest for a 'testosterone deficiency awareness campaign' and accepting to participate in the study.

Methods: The campaign was organized by the Spanish Society of Andrology and consisted of conferences for the general population and the delivery of written material to employees of participating companies. Age, body mass index (BMI) and comorbidities were recorded. Laboratory tests: TT, total-, LDL- and HDL-cholesterol, tryglicerides, and fasting glucose. Erectile dysfunction (ED) assessment: IIEF-5. Symptoms assessment: AMS scale. $p < 0.05$ for statistical significance.

Results: 1264 men aged 52.3 ± 7.7 years participated in the study. Prevalence of low T was 26.2% (n = 331). Men with low T had a greater prevalence of obesity (BMI = 30 kg/m^2; 30.0% vs. 13.5%), diabetes (10.6% vs. 4.9%), hypertension (30.2% vs. 21.4%), cardiac disease (8.8% vs. 4.4%) and depression/anxiety (13.6% vs. 8.2%).

Levels of fasting glucose and triglycerides were significantly higher; those of HDL-cholesterol were lower. Prevalence of ED was 53.8% and independent of low T. Prevalence of AMSS symptoms was 60.3%. Only prevalence of somatic symptoms was significantly higher in men with low T (90.6% vs. 85.6%).
Conclusion: Prevalence of low T was high among men interested in the awareness campaign and was associated with a higher cardiovascular risk. Awareness campaigns are efficacious. The high prevalence of ED indicates that sexual concerns are a drive to participate.

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Efficacy of combined therapy of intraurethral prostaglandin E1 and a PDE5 inhibitor for erectile dysfunction treatment

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Background: PDE5 inhibitors (PDE5i) increase cGMP availability, while prostaglandine E1 (PGE1) raises production of cAMP. Both mechanisms are responsible for the observed improvement in erectile dysfunction (ED) patients.
Previous studies showed that combining both might act complementarily to achieve an adequate response in patients with ED who failed to respond to either PDEi or intraurethral PGE1 treatment separately, and who are reluctant to more invasive therapies such as intracavernous injections or surgery.
Aim: Evaluate the efficacy of a combined therapy for ED.
Methods: During 15 months, 26 patients who did not respond to either PDE5i or intraurethral PGE1 as monotherapy were included. 58% resulted in ED after radical prostatectomy, while the rest showed other ED related factors such as DM, hypertension and smoking. Combined treatment with both medications was provided. The response was evaluated by comparing the international index of ED (IIFE-5) score at the beginning of treatment and after two and six months.
Results: From all 26 patients, 24 had either a moderate or a severe IIFE-5 initial score, with a mean IIEF- 5 score of 8.46. After 6 months of treatment, an average improvement of 7.46 points in the IIEF-5 score was observed, with a IIFE-5 mean score of 15.92.
46% of the patients had a favorable response (increase of 7 or more points in the EEIF-5 score) to the combined therapy.
Conclusion: Combined therapy with PDEi and intraurethral PGE1 can be an alternative for ED patients who are unwilling to more invasive therapies.
Further studies with broader samples can provide better understanding of this therapeutic option.

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Gynecomastia in subjects with sexual dysfunction

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Background: Gynecomastia can be a sign of hypogonadism. Low testosterone (T) is a common finding in patients with sexual dysfunction (SD).
Aim: To analyze possible relationships between gynecomastia and clinical and biochemical parameters in a large cohort of subjects with SD.
Methods: A series of 4023 men attending our Outpatient Clinic for SD was retrospectively studied.
Results: In subjects with Klinefelter's Syndrome (KS) the prevalence of gynecomastia was 30.8%; after excluding KS patients, the prevalence was 3.1%. After adjusting for lifestyle, gynecomastia was significantly associated with lower T levels. However, only 33.3% of subjects with gynecomastia were hypogonadal. Gynecomastia was associated with delayed puberty, history of testicular or hepatic diseases and cannabis abuse. Patients with gynecomastia more frequently reported sexual complaints, such as severe ED (odds ratio (OR) = 2.19 [1.26–3.86], $p = 0.006$), lower sexual desire and intercourse frequency (OR = 1.23 [1.06–1.58] and OR = 1.84 [1.22–2.78], respectively; both $p < 0.05$), orgasm difficulties (OR = 0.49 [0.28–0.83], $p = 0.008$), delayed ejaculation and lower ejaculate volume (OR = 1.89 [1.10–3.26] and OR = 1.51 [1.23–1.86], respectively; both $p < 0.05$). Gynecomastia was also positively associated with severe obesity, lower testis volume and luteinizing hormone (LH), and negatively with prostate specific antigen (PSA). The further adjustment for T did not affect these results, except for obesity. When considering gynecomastia severity, we found a stepwise,
T independent, decrease and increase of testis volume and LH, respectively. Gynecomastia was also associated with the use of several drugs in almost 40% of our patients.
Conclusion: Gynecomastia is a rare condition in subjects with SD, and could indicate a testosterone deficiency that deserves further investigation.

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Serum PSA as a predictor of testosterone deficiency

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Background: The relationship between serum prostate-specific antigen (PSA) and testosterone (T) levels is still controversial. According to the 'saturation hypothesis', a significant relationship is apparent only in the low T range.
Aim: To verify whether, in a large sample of male subjects seeking medical care for sexual dysfunction (SD), PSA might represent a reliable marker of T levels.
Methods: A consecutive series of 3,156 patients attending our unit for SD was studied. Only subjects without history of prostate disease and PSA levels <4 ng/mL (n = 2967) were analyzed.
Results: ROC curve analysis for predicting severe hypogonadism (T < 8 nmol/L) showed an accuracy of PSA = 0.612 ± 0.022 (p < 0.0001), with the best sensitivity and specificity at PSA < 0.65 ng/mL (65.2% and 55.5%, respectively). In the entire cohort, 254 subjects (8.6%) showed T < 8 nmol/L and, among them, more than half (4.8%) had PSA < 0.65 ng/mL. After adjusting for age, low PSA was associated with hypogonadism-related features and associated conditions, such as metabolic syndrome (HR = 1.506 [1.241–1.827]; p < 0.0001), type 2 diabetes (HR = 2.044 [1.675–2.494]; p < 0.0001), and cardiovascular diseases (HR = 1.275 [1.006–1.617]; p = 0.045). Furthermore, low PSA was associated with impaired sex- and sleep-related erections.These associations were retained after adjusting for T levels. Sensitivity and positive predictive values of low PSA increased, whereas specificity and negative predictive value decreased as a function of age.
Conclusion: PSA is a marker of T concentrations and it may represent a new tool in confirming hypogonadism. The determination of PSA levels might give insights not only on the circulating levels of total T but also on its active fractions.

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Beneficial effects of long-term testosterone replacement therapy (TRT) with testosterone undecanoate (TU) in hypogonadal men with cardiovascular diseases (CVD) in an observational registry study

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Background: Hypogonadism is associated with cardiometabolic risk. There are no long-term data of TRT in men with CVD.
Aim: To assess effects of long-term TRT in men with a history of CVD.
Methods: In a registry study from a single urologist's office, 300 men with testosterone = 12.1 nmol/L received TU injections for up to 6 years. In this subgroup analysis, 68 men with a previous diagnosis of coronary artery disease (CAD; n = 40) and/or a history of myocardial infarction (MI; n = 40) or stroke (n = 6) were analyzed.
Results: Mean age: 60.76 ± 4.94 years.
Mean weight (kg) decreased progressively from 115.07 ± 13.71 to 92.5 ± 9.64, waist circumference (cm) from 112.07 ± 7.97 to 99.89 ± 6.86, BMI from 37.27 ± 4.45 to 30.14 ± 3.21 (p <0.0001 for all). Mean weight loss was 17.05 ± 0.57%.
Mean fasting glucose decreased from 108.74 ± 17.08 to 96.0 ± 1.92 mg/dl, HbA1c from 7.81 ± 1.17 to 6.2 ± 0.62% (p <0.0001 for both).
Lipids (mg/dl): Total cholesterol (TC) decreased from 304.66 ± 34.09 to 189.32 ± 9.68, LDL from 184.28 ± 37.51 to 134 ± 27.91, triglycerides from 308.38 ± 56.3 to 187.71 ± 8.67 (p <0.0001 for all), and HDL increased slightly. The TC:HDL ratio declined from 5.16 ± 1.55 to 3.15 ± 0.87 (p <0.0001). Systolic BP decreased from 167.82 ± 11.01 to 142.36 ± 10.62, diastolic BP from 102.28 ± 8.23 to 81.25 ± 8.07 mmHg, pulse pressure from 65.54 ± 5.24 to 61.11 ± 4.66 (p <0.0001 for all). Liver enzymes (IU/L): AST decreased from 42.18 ± 14.11 to 22.14 ± 3.23, ALT from 42.62 ± 15.49 to 20.18 ± 2.72 (p <0.0001 for both).
C-reactive protein (CRP) declined from 4.08 ± 4.73 to 0.44 ± 0.6 mg/L. No cardiovascular events were reported during the observation time.
Conclusion: TRT in hypogonadal men with CVD resulted in sustained improvements of cardiometabolic risk factors and was well tolerated. There were no drop-outs during the observation time.

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Weight loss and waist size reduction in 362 hypogonadal men with obesity grades I to III under long-term treatment with testosterone undecanoate (TU): observational data from two registry studies

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Background: Obesity has a greater impact on the decline of testosterone than age.
Aim: To assess anthropometric changes in obese men under long-term testosterone treatment.
Methods: From two registries of 561 hypogonadal men, 362 men with obesity grade I-III were selected. All men received TU injections for up to 6 years. Measures were taken at each three-monthly visit.

Results: Grade I (n = 185, mean age: 58.4 ± 8.0: Weight (kg) decreased from 101.88 ± 6.2 to 89.34 ± 6.7. Change from baseline was −12.55 ± 0.44 kg, percent change from baseline −12.28 ± 0.44%. Waist circumference (WC; cm) decreased from 107.07 ± 7.57 to 97.09 ± 6.95. Change from baseline was −9.24 ± 0.3 cm. BMI (kg/m^2) decreased from 32.51 ± 1.39 to 28.63 ± 1.92, change from baseline: 3.99 ± 0.14 kg/m^2.
Grade II (n = 131, mean age: 60.6 ± 5.6 years): Weight (kg) decreased from 117.02 ± 6.99 to 96.78 ± 7.47. Change from baseline was −20.67 ± 0.51 kg, percent change from baseline −17.66 ± 0.43%. WC (cm) decreased from 114.23 ± 7.51 to 102.52 ± 6.5. Change from baseline was -12.29 ± 0.33 cm. BMI (kg/m^2) decreased from 37.39 ± 1.46 to 31.05 ± 2.02, change from baseline -6.58 ± 0.16 kg/m^2.
Grade III (n = 46, mean age: 60.3 ± 5.4 years): Weight (kg) decreased from 129.02 ± 5.67 to 103.33 ± 4.17. Change from baseline −27.15 ± 0.74 kg, percent change from baseline −20.83 ± 0.54%. WC (cm) decreased from 118.41 ± 5.69 to 106.48 ± 4.91. Change from baseline was −12.44 ± 0.36 cm. BMI (kg/m^2) decreased from 41.93 ± 1.5 to 33.62 ± 1.58, change from baseline −8.79 ± 0.23 kg/m^2.
Conclusion: Changes were more pronounced with increasing obesity. Changes were clinically meaningful and sustainable for the full observation period. TRT seems to be an effective approach to achieve sustained weight loss in obese hypogonadal men, thereby potentially reducing cardiometabolic risk.

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Improvement of metabolic syndrome (MetS) parameters in 362 obese hypogonadal men upon long-term treatment with testosterone undecanoate (TU) injections: observational data from two registry studies

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Background: Hypogonadism is associated with MetS.
Aim: To assess changes in MetS components in obese men under long-term testosterone treatment.
Methods: 362 men with obesity grade I-III from two registry studies. All received TU for up to 6 years.
Results: Grade I: Fasting glucose (FPG; mg/dL): 107.22 ± 30.2 to 97.87 ± 14.42 (p <0.0001) by −8.37 ± 1.83; HbA1c (%): 6.58 ± 1.24 to 5.6 ± 0.76 by −1.05 ± 0.06%. Total cholesterol (TC; mg/dL): 268.43 ± 44.24 to 191.47 ± 16.8; LDL (mg/dl): 158.75 ± 32.82 to 116.26 ± 34.65; triglycerides (TG; mg/dL): 257.49 ± 62.1 to 193.23 ± 29.01. HDL (mg/dL) increased from 46.53 ± 15.93 to 56.09 ± 15.71. TC:HDL ratio: 6.39 ± 2.41 to 3.64 ± 0.87 (p < 0.0001 for all). Systolic blood pressure (SBP; mmHg): 143.96 ± 15.09 to 130.11 ± 8.95; diastolic blood pressure (DBP): 85.54 ± 10.84 to 78.23 ± 5.82. C-reactive protein (CRP, mg/L): 2.11 ± 2.36 to 0.58 ± 0.46 (p <0.0001).
Grade II: FPG: 114.17 ± 27.04 to 99.3 ± 11.49 (p < 0.0001) by -14.83 ± 2.19 mg/dL; HbA1c: 7.63 ± 1.31 to 5.9 ± 0.73 −1.69 ± 0.07%. TC: 292.23 ± 41.07 to 196.78 ± 19.85; LDL: 174.5 ± 28.46 to 125.86 ± 35.8; TG: 292.12 ± 61.15 to 194.19 ± 20.66. HDL: 57.35 ± 19.17 to 67.41 ± 18.82. TC:HDL ratio: 5.86 ± 2.76 to 3.2 ± 1.12 (p < 0.0001 for all). SBP (mmHg): 159.15 ± 14.71 to 135.26 ± 10.97; DBP from 95.02 ± 11.86 to 79.66 ± 4.96. CRP: 3.34 ± 4.6 to 0.69 ± 0.97 (p < 0.0001).
Grade III: FPG: 115.48 ± 23.85 to 96.54 ± 2.9 (p < 0.0001) by -18.48 ± 2.96 mg/dL; HbA1c : 7.57 ± 1.38 to 6.08 ± 0.5 by −1.61 ± 0.13%. TC: 306.76 ± 43.03 to 192.23 ± 9.17; LDL: 190.57 ± 36.6 to 136.24 ± 28.07; TG: 326.87 ± 60.21 to 194.4 ± 12.59. HDL: 62.76 ± 18.7 to 72.55 ± 13.34. TC: HDL ratio: 5.47 ± 2.57 to 2.75 ± 0.59 (p < 0.0001 for all). SBP (mmHg): 161.04 ± 14.3 to 142.05 ± 9.57; DBP 97.07 ± 10.91 to 80.89 ± 6.76. CRP: 3.96 ± 4.31 to 0.57 ± 0.59 (p < 0.0001).
Conclusion: Changes were meaningful and sustained for the full observation time. TRT seems effective to improve MetS and cardiovascular risk profile in obese hypogonadal men.

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Poster cancelled

P142

Does the prevalence of erectile function of heterogeneous outpatients differ?

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Background: Patients with urological and cardiological diseases are exposed to erectile dysfunction.
Aim: Aim of the present study was to evaluate the erectile function of patients visiting urology, cardiology outpatient, general practitioner's (GP) office and to seek for any differences.
Methods: A form was given to outpatients and medical students served as controls. The validated international index of erectile function (IIEF-5) questionnaire was extended with 12 more questions regarding age, coffee, alcohol and drug consumption, smoking, cardiovascular disease, lower urinary tract symptoms (LUTS) and prostate cancer. Age-matched study groups were compared to each other.
Results: 1111 patients were enrolled in the study. 56.53% (n = 628) of all patients had ED, among them 17.19%

(n = 191) had mild to moderate, 8.64% (n = 96) moderate and 6.48% (n = 72) severe ED. Age, cardiovascular disease and prostate cancer had the most significant negative effect on erectile function ($p < 0.001$). There was no statistical significant difference between age-matched urology, cardiology and GP outpatients regarding IIEF scores based on confidence intervals. Alcohol consumption was significantly higher among medical students, cigarette abuse was less common regarding urology outpatients, cardiology outpatients were affected the most by cardiovascular disease and urology outpatients had more often LUTS or prostate cancer in comparison with other study groups.
Conclusion: The expected increase in prevalence of ED among urology and cardiology outpatients in comparison with GP patients was not proven by our study. This result suggests that due to the multifactorial background of ED GP patients have to be screened for ED as well in order to prevent severe cardiovascular complications.

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Does still is a place for paroxetine in the era of dapoxetine

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Background: PEDT questionnaire was translated and validated on Georgian language.
Aim: The aim of our study was management of patients with life long premature ejaculation (PE) by using PEDT (premature ejaculation diagnostic tool) on native language.
In Georgia dapoxetine is not available, so we want to show our experience with paroxetine.
Methods: 127 men with life long PE (age 18–42 years, mean age 23.8 years), were randomized into 3 groups. Group I – 47 men with Paroxetin 20 mg daily administration; group II – 49 men with Paroxetin 20 mg daily administration during 1 month, and after that on demand 2–3 h before intercourse; group III – 31 men with placebo daily. The primary endpoint was assessment of intravaginal ejaculatory latency time (IELT), secondary endpoints include evaluation of PEDT domains.
Results: At 3 months of drugs administration there were statistically significant increase in IELT in I and II groups compare to placebo ($p < 0.001$). IELT average increased by 7.8 fold in group I and by 6,8 in group II, at the same time IELT stay unchanged in placebo group. We found dramatically improvement in all domains of PEDT in both paroxetine groups compare to placebo. PEDT baseline average total score decreased from 17.6 till 5.4 and 6.7 in I and II groups respectively, and changed insignificantly in III group.
Conclusion: After validation of PEDT questionnaire on native language patients and specialists in Georgia will be able to use uniform tool in the assessment of results and future studies.

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Characteristics of compensated hypogonadism in patients with sexual dysfunction

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Background: It has been proposed that compensated hypogonadism represents a genuine clinical subgroup of individuals with late onset hypogonadism (LOH).
Aim: The aim of the present study is to investigate the association of compensated hypogonadism with clinical and psychological characteristics of male subjects complaining for sexual dysfunction (SD).
Methods: After excluding documented genetic causes of hypogonadism, an unselected consecutive series of 4173 patients consulting our Unit for SD was studied. Compensated hypogonadism was identified according to the European Male Aging Study criteria: total testosterone = 10.5 nmol/L and LH > 9.4 U/L.
Results: 170 (4.1%) subjects had compensated hypogonadism, whereas 827 (19.8%) had an overt hypogonadism. After the adjustment for confounding factors, non-specific sexual symptom was related to compensated hypogonadism. However, compensated hypogonadism individuals more often reported psychiatric symptoms, as detected by ?MHQ score, when compared to both eugonadal and overt hypogonadal subjects (adjusted OR = 1.018[1.005; 1.031]; 1.014 [1.001;1.028]; both $p < 0.005$). In addition, subjects with compensated or overt hypogonadism had an increased predicted CV risk (as assessed by Progetto Cuore risk algorithm) when compared to eugonadal individuals. Accordingly, major adverse cardiovascular events (MACE)-related mortality, but not MACE incidence, was significantly higher in subjects with both compensated and overt hypogonadism, when compared to eugonadal subjects.
Conclusion: Present data do not support the concept that compensated (subclinical) hypogonadism represents a new clinical entity. The possibility that subclinical hypogonadism could be a normal response of the hypothalamus-pituitary-testis axis to somatic illness should be considered. Further studies are urgently needed to clarify this latter point.

P145

Low testosterone syndrome protects subjects with high cardiovascular risk burden from major adverse cardiovascular events

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Background: The role of testosterone (T) in the cardiovascular (CV) health of men is controversial.

Aim: To analyze whether gonadal status might predict new CV event incidence according to a patient's previous history of CV events, in a cohort of subjects complaining of sexual dysfunction.

Methods: A consecutive series of 1687 patients was followed-up for a mean time of 4.3 ± 2.6 years for new occurrence of CV events, detecting 139 events.

Results: Hypogonadism (total T < 12 nmol/L) was not associated with an increased incidence of new CV events in the entire cohort. However, when considering patients with a previous history of CV events, hypogonadism was associated with a reduced risk of new CV events, even after adjusting for confounders (HR = 0.498 [0.240; 0.996]; $p = 0.049$), whereas no relationship was observed in subjects free of previous CV events. Similar results were observed when reduced testis volume (TV) was considered as a predictor of new CV events in subjects with previous CV events (HR = 0.486 [0.257; 0.920]; $p = 0.027$). In patients with a history of previous CV events, but not in those without previous CV events, having both low T and low TV was associated with a lower incidence of new CV events as compared with subjects with only one or none of these conditions, even after adjusting for confounders (HR = 0.514 [0.306; 0.864]; p for trend < 0.02). Notably, baseline CV risk was not different between hypogonadal and eugonadal subjects.

Conclusion: In conclusion, hypogonadism could be interpreted as a protective mechanism in unhealthy conditions, such as previous CV events, to avoid fatherhood and spare energy.

P146

Clinical correlates of enlarged prostate size in subjects with sexual dysfunction

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Background: Digito-rectal examination (DRE) of the prostate provides useful information on the state of prostate growth and on the presence of suspected peripheral nodules.

Aim: To describe the clinical and biochemical correlates of finding an enlarged prostate size at DRE in subjects with sexual dysfunction (SD).

Methods: A consecutive series of 2379 patients was retrospectively studied. The analysis was focused on a subset of subjects ($n = 1823$; mean age 54.7 ± 11.4) selected for being free from overt prostatic diseases. Several parameters were investigated.

Results: After adjusting for confounders, the presence of an enlarged prostate size at DRE was associated with a higher risk of metabolic syndrome (HR = 1.346 [1.129–1.759]; $p = 0.030$), type 2 diabetes mellitus (HR = 1.489 [1.120–1.980]; $p = 0.006$), increased LDL cholesterol (>100 mg/dL; HR = 1.354 [1.018–1.801]; $p = 0.037$) and increased mean blood pressure values (HR = 1.017 [1.007–1.027] for each mmHg increment; $p = 0.001$). Accordingly, enlarged prostate size was also associated with a higher risk of arteriogenic erectile dysfunction (ED), as well as with other andrological conditions, such as varicocele and premature ejaculation. PSA levels were significantly higher in subjects with enlarged prostate size when compared to the rest of the sample (HR = 3.318 [2.304; 4.799] for each log unit increment in PSA levels; $p < 0.0001$). Arteriogenic ED, according to different criteria, was also associated with increased PSA levels.

Conclusion: Our data support the need to examine prostate size either by clinical (DRE) or biochemical (PSA) inspection in subjects with SD, in order to have insights into the nature of the SD and the metabolic and cardiovascular background of the patient

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Frequency of sexual activity and cardiovascular risk in subjects with erectile dysfunction: cross sectional and longitudinal analyses

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Background: The relationship between sexual activity, and incident major adverse cardiovascular events (MACE) in subjects erectile dysfunction (ED) has been never investigated.

Aim: To investigate relationships between frequency of sexual attempts and incident MACE and to retrospectively explore its main determinants.

Methods: A consecutive series of 2187 subjects (mean age 49.9 ± 11.6 years old) attending the Outpatient Clinic for sexual dysfunction was retrospectively studied. A subset of the previous sample (N = 1687) was enrolled in a longitudinal study.

Results: In the whole sample, sexual attempts were an age- and testosterone-dependent phenomenon, while no association between frequency of sexual intercourse and ED or premature and delayed ejaculation, was observed. However, when the same analysis was performed according to age tertiles (I = 17–46, II = 47–59, III = 60–88 years old), ED was significantly associated with a higher risk of reduced sexual intercourse in younger (HR = 1.857 [1.066–3.234]; p = 0.029), but not in middle-aged or older individuals. The marital component, as assessed by SIEDY Scale 2, played a major role in regulating sexual frequency in all age bands. Depressive symptoms, represent another independent risk factor for reduced sexual activity (adj $r = -0.139$; $p < 0.0001$), in an age-dependent manner. When longitudinal data were analyzed, a higher frequency of sexual intercourse significantly reduced the risk of MACE even after adjusting for confounders.

Conclusion: Identifying among ED subjects those with lower frequency of sexual activity might provide an opportunity to modify their behavior and to discover subthreshold comorbidities, possibly preventing forthcoming CV events.

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Cardiovascular risk associated with testosterone boosting medications: a meta-analysis study

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Background: Recent reports significantly halted the enthusiasm regarding androgen boosting, suggesting that testosterone supplementation (TS) increases cardiovascular (CV) events.

Aim: To meta-analyze the effect of TS on male CV incidence.

Methods: An extensive Medline Embase and Cochrane search was performed. All randomized controlled trials (RCTs) comparing the effect of TS vs. placebo on different CV outcomes were included.

Results: Out of 2747 retrieved articles, 75 were included in the study. In addition, one completed but still unpublished study was also considered. Retrieved trials included 3040 TS treated and 2468 placebo treated men for a mean duration of 34 weeks. Our analyses, performed on the largest number of studies collected so far, indicate that TS is not related to any increase in CV risk even when composite (HR = 1.01 [0.58; 1.78]; p = 0.97]) or single adverse events were considered. In RCTs performed in subjects with metabolic derangements a protective effect of TS on CV risk was observed (HR = 0.19 [0.04; 0.85]; p = 0.03).

Conclusion: Present systematic analysis does not support a causal role between TS and adverse CV events. Our results are in agreement with a large body of literature from the last twenty years supporting TS of hypogonadal men as a valuable strategy in improving a patient's metabolic profile, reducing body fat and increasing lean muscle mass, which would ultimately reduce the risk of heart disease.

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Injectable testosterone undecanoate: a meta-analysis study

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Background: Injectable testosterone undecanoate (TU) is a long acting testosterone (T) (from 10 to 14 weeks) formulation available for the treatment of male hypogonadism since 2003 in several countries.

Aim: To assess the efficacy and safety of injectable TU by meta-analyzing available evidence.

Methods: An extensive Medline Embase and Cochrane search was performed. All uncontrolled and placebo-controlled randomized clinical trials (RCTs) evaluating the

effect of injectable TU on different outcomes were included

Results: Out of 98 retrieved articles, 33 were included in the study. Among those, 11 were placebo- controlled RCTs. Injectable TU was significantly associated with a reduction of fat mass and HbA1c in both controlled and uncontrolled trials, in particular when hypogonadal subjects were enrolled. Similar results were observed for the improvement of sexual function. In addition, TU ameliorated several other outcomes including blood pressure, lipid profile, waist circumference and BMI in uncontrolled studies but these data were not confirmed in placebo-controlled trials. The treatment was well tolerated and no risk of prostate cancer or cardiovascular disease observed.

Conclusion: Injectable TU is a safe and effective treatment for male hypogonadism. The possibility of a four time a year, therapeutic intervention frees the patient, at least partially, from having a chronic condition, maintaining a positive, active role in self-caring and helping hypogonadal men to keep his condition in long-way perspective.

P150

Clinical differences between treated and not treated hypogonadal men: results from the SIAMsO-NOI study (Società Italiana Di Andrologia E Medicina Della Sessualità-Osservatorio Nazionale Outcome Ipogonadismo)

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Background: Whether low testosterone-related symptoms improves upon treatment for hypogonadism (HG) is under debate.

Aim: To evaluate differences at the study entry between subjects who were already receiving treatment for HG and subjects who were not.

Methods: This is a baseline cross-sectional study on 420 HG subjects [median age 51 (39–63)] participating to SIAMsO-NOI, a multicenter observational longitudinal study enrolling HG patients (T < 12 nmol/L) in 15 Italian Endocrinology and/or Andrology centers. Decisions on treatment were made according to physician and patients choice, as in clinical practice. Each patient was interviewed with Structured Interview on Erectile Dysfunction and completed questionnaires on sexual, physical, psychological and urinary symptoms (IIEF-15, AMS and IPSS). Hormonal and biochemical parameters were also registered.

Results: Among the patients studied, 247 (58.8%) were treated for HG and 173 (41.2%) were not. After adjusting for age and center, subjects already treated for HG more often reported a history of pituitary disease (OR = 2.84 [1.54–5.26], $p = 0.001$) and delayed puberty (OR = 3.53 [1.67–7.47], $p = 0.001$), as compared with not treated. Treated patients had higher total and calculated free T and lower LH levels (Adj. $r = 0.38$, 0.36 and −0.17, all $p < 0.010$; respectively). Treated subjects less often reported severe erectile dysfunction, impaired spontaneous erections and low libido (OR = 0.46[0.27-0.80], OR = 0.45[0.30-0.68], OR = 0.43[0.28-0.65], all $p < 0.01$; respectively). Accordingly, they had higher IIEF-15 and lower AMS sexual scale scores (Adj. $r = 0.20$ and -0.28, both $p < 0.0001$; respectively).

Conclusion: HG subjects with a history of delayed puberty and pituitary diseases are more likely to be treated. Treated patients report less sexual concerns than not treated.

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Flaccid penile acceleration as a marker of cardiovascular risk in men without classical risk factors

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Background: Conventional cardiovascular (CV) risk factors identify only half of subjects with incident major adverse CV events (MACE). Hence new markers are needed. A role for dynamic peak systolic velocity (D-PSV) at penile color Doppler ultrasound (PCDU) has been suggested, but it is operator dependent and time consuming. Flaccid penile acceleration (FPA) is a PCDU parameter that reflects PSV, the systolic rise time (SRT), and end diastolic velocity (EDV), arithmetically defined as (PSV-EDV)/SRT.

Aim: The study aims to verify, in erectile dysfunction (ED) patients, whether FPA has a role in predicting MACE.

Methods: A selected series of 1,903 patients (aged 54.6 ± 11.7) with a suspected organic component for ED was retrospectively studied. A subset of this sample ($n = 622$) was enrolled in a longitudinal study. Several clinical, biochemical, and instrumental (PCDU) parameters were studied.

Results: Decreased FPA levels were associated with worse metabolic profile and sexual symptoms, lower total and calculated free testosterone. In the longitudinal study, unadjusted incidence of MACE was significantly associated with lower baseline FPA. When FPA was introduced in a multivariate model, along with D-PSV, lower FPA, but not D-PSV, was associated with incident MACE in lower-risk-i.e., younger (HR? = 0.48 [0.23–0.99]), non-hypertensive (HR = 0.59 [0.38–0.92]), non-obese (HR = 0.68 [0.49–0.96]), or non-diabetic (HR = 0.67 [0.49–0.96] subjects;all $p < 0.05$–but not in higher-risk ones. FPA demonstrated a threshold effect in predicting MACE at a value<1.17 m/s (2) which showed a threefold increase in incidence of MACE in apparently lower-risk individuals.

Conclusion: FPA is an easily obtained PCDU parameter and capable of identifying adverse metabolic and CV profiles, particularly in apparently lower-risk individuals with ED.

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Relationship of testis size and LH levels with incidence of major adverse cardiovascular events in older men with sexual dysfunction

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Background: Testis volume (TV) can predict reproductive fitness. However, the role of TV in overall and cardiovascular (CV) fitness has never been studied.
Aim: To analyze the clinical correlates of TV in patients with sexual dysfunction (SD) and to verify its value and that of its determinants (i.e.,LH levels) in predicting major adverse CV events (MACE).
Methods: A consecutive series of 2809 subjects without testiculopathy (age 51.2 ± 13.1) consulting for SD was retrospectively studied. A subset of this sample (n = 1395) was enrolled in a longitudinal study. Several clinical and biochemical parameters were investigated.
Results: After adjusting for confounders, TV was negatively associated with LH (Adj. $r = -0.234$; $p < 0.0001$) and FSH (Adj. $r = -0.326$; $p < 0.0001$). In addition overweight/obesity, smoking, and alcohol abuse increased as a function of TV (HR = 1.04 [1.02–1.06], $p < 0.0001$; 1.02 [1.01–1.04], $p = 0.012$; 1.06 [1.02–1.11], $p = 0.009$,respectively). Furthermore, mean blood pressure was positively related to increased TV (Adj. $r = 0.157$; $p < 0.0001$). The effect of these lifestyle factors on TV were only partially related to changes in gonadotropin levels. In the longitudinal analysis, after adjusting for confounders, TV was associated with a higher incidence of MACE (HR = 1.07 [1.01–1.12]; $p = 0.014$), and the stepwise introduction in the Cox model of lifestyle factors, mean blood pressure and BMI progressively smoothed out the association, which was no longer statistically significant in the fully adjusted model. Conversely, the association of higher LH levels with increased incidence of MACE was not attenuated by the progressive introduction of the aforementioned confounders in the model.
Conclusion: TV and LH are associated with an adverse CV risk profile that mediate the higher TV- associated incidence of MACE. High LH is an independent marker of CV risk.

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An integrated approach with vardenafil orodispersible (V) and cognitive-behavioral sex therapy (CBST) for the treatment of erectile dysfunction (ED)

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Background: Erectile Dysfunction (ED) is considered a multifactorial disease, where organic and psychological aspects are often interconnected.
Aim: To compare the efficacy of combined vardenafil (V) and Cognitive-Behavioral Sex Therapy (CBST) with vardenafil alone in improving sexual symptoms in both male and female partners.
Methods: 30 male patients with ED, and their partners, were randomly assigned to two different groups and treated for 10 weeks with V 10 mg orodispersible (Group A) or V 10 mg orodispersible +CBST (Group B). International Index of Erectile Dysfunction (IIEF-15), Female Sexual Function Index (FSFI) and Index of Sexual Satisfaction (ISS) were respectively administered to male, female and both partners at time(T) 0, 1 (+5 weeks of therapy) and 2 (+10 weeks of therapy).
Results: Groups A and B were similar for socio-demographic and clinical characteristics. T0 test scores did not significantly differ between the groups. In both Group A and B the IIEF-Erectile Function (EF) domain showed a significant improvement from T0 to T1 ($p = 0.008$ and $p = 0.001$ vs. T0, respectively) without any further change at T2 ($p = 0.68$ and $p = 0.61$ vs. T1 respectively). In Group A, FSFI and either male and female ISS did not show any significant change at T1 and T2 vs. T0. In Group B, a significant improvement at final time-point in FSFI and male and female ISS scores was reported ($p < 0.05$, T2 vs. T0 in all scores).
Conclusion: In our study, both V orodispersible alone and V orodispersible + CBST improve EF. However, only V+CBST ameliorates couple sexual satisfaction and female sexual function.

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Lack of sexual privacy affects psychological and marital domains of male sexual dysfunction

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Background: Sexual dysfunctions (SD) are dictated by predisposing, precipitating, maintaining, and contextual factors, the latter of which can help sexual problems to emerge. Even if the lack of sexual privacy is one of the most common contextual issues, it has not been extensively studied.
Aim: Investigation of sexual privacy in a large sample of men consulting for SD.
Methods: A consecutive series of 3736 men, attending the Outpatient Clinic for SD was retrospectively studied. Privacy during sexual intercourse was investigated with the following question 'During the last three months, have you had enough privacy during your sexual activity?', and rated 0 = yes, 1 = sometimes, 2 = rarely, 3 = never
Results: Among the 3736 patients studied, 83.9% reported enough privacy during sexual intercourse, while 8.6, 5.7 and 1.7% declared a decrease of sexual privacy of increasing severity. Lack of sexual privacy was associated with ejaculatory dysfunctions and with the inability to maintain an erection during intercourse. Subjects reporting lack of

sexual privacy had a higher risk of relational and intra-psychic impairments, as well as psychopathology at MHQ questionnaire, even after adjusting for confounders. Fatherhood was associated with sexual privacy issues only in the lowest quartiles. In subjects without children, the absence of cohabitation with the partner was associated with an increasing risk of not having enough privacy (HR = 1.837 [1.269–2.659], $p = 0.001$), data confirmed, after stratification for age, in the youngest subjects (I quartile HR = 2.159 [1.211–3.848], $p = 0.009$)

Conclusion: This study indicates that sexual privacy is often a poorly investigated item, which is important to evaluate in male SD.

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Estrogen mediates metabolic syndrome-induced erectile dysfunction: a study in the rabbit

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Background: ERa is critical in mediating the harmful effects of hyperestrogenism in fetal/neonatal life on the developing penis. In contrast, little is known on the impact of an excess of estrogens on penile function in adulthood.

Aim: The aim of this study was to investigate the effect of estrogens on metabolic syndrome (MetS)- associated erectile dysfunction (ED), in an animal model of MetS.

Methods: To understand the role of sex steroid milieu, we treated subgroups of MetS rabbits with either testosterone (T) or tamoxifen, a classical ERs antagonist. We evaluated acetylcholine (Ach)- penile responsiveness as well as the expression of genes related to penile smooth muscle relaxation and contractility.

Results: MetS was associated to elevated estradiol (E2) and low T levels. E2, not T, was independently and negatively associated with genes able to affect penile erection. Smooth muscle-related markers decreased as a function of E2 and were positively associated with all the variables investigated. Increasing concentrations of circulating E2 were negatively associated with Ach-induced relaxation. In HFD rabbits, in- vivo T dosing significantly improved MetS, and normalized circulating E2. Conversely, in-vivo tamoxifen dosing reduced visceral adiposity and partially restored T level. Ach-induced relaxation was severely impaired by HFD and significantly restored, up to the control level, by both tamoxifen and T. In rabbit smooth muscle cells culture 17 ß estradiol significantly reduced the expression of aSMA, SM22 and PDE5. Tamoxifen reverted completely these effects.

Conclusion: In conclusion, HFD-induced ED is more associated with a high estradiol, than to a low T, milieu.

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Nonalcoholic steatohepatitis as a novel player in metabolic syndrome- induced erectile dysfunction: an experimental study in the rabbit

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Background: A pathogenic link between erectile dysfunction (ED) and metabolic syndrome (MetS) is well established. Nonalcoholic steatohepatitis (NASH), the hepatic hallmark of MetS, is regarded as an active player in the pathogenesis of MetS-associated cardiovascular disease.

Aim: This study was aimed at evaluating the relationship between MetS-induced NASH and penile dysfunction.

Methods: We used a non-genomic, high- fat diet (HFD)-induced rabbit model of MetS and treated HFD rabbits with testosterone (T), with the farnesoid X receptor agonist obeticholic acid (OCA), or with the anti-TNFa mAb infliximab. Rabbits fed a regular diet were our controls.

Results: Liver histomorphological and gene expression analysis demonstrated NASH in HFD rabbits. Several genes related to inflammation (including TNFa), activation of stellate cells, fibrosis, lipid metabolism parameters were negatively associated to maximal acetylcholine (Ach)-induced–penile relaxation. When all these putative liver determinants of penile Ach responsiveness were tested as covariates in a multivariate model, only the association between hepatic TNFa expression and Ach response was confirmed. Accordingly, circulating levels of TNFa were increased in HFD rabbits. T and OCA in HFD rabbits both reduced TNFa liver expression and plasma levels, with a parallel increase of penile eNOS expression and Ach-responsiveness. Also neutralization of TNFa with infliximab treatment normalized HFD-induced hyporesponsiveness to Ach, as well as responsiveness to vardenafil, a PDE5 inhibitor.

Conclusion: MetS-induced NASH in HFD rabbits plays an active role in the pathogenesis of ED, through TNFa, as indicated by treatments reducing liver and circulating TNFa levels, or neutralizing TNFa action, which improve Ach-penile responsiveness in HFD rabbits.

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Metformin in vitro and in vivo increases adenosine signalling in rabbit corpora cavernosa

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Background: In subjects with erectile dysfunction responding poorly to sildenafil, metformin was reported to improve erections.
Aim: The aim of this study was to investigate metformin's mechanism of action on erectile function, focusing on adenosine (ADO) and nitric oxide (NO) signalling in an animal model of high fat diet (HFD)- induced metabolic syndrome.
Methods: In vitro contractility studies of penile strips. Penile expression of genes related to ADO or NO signaling was also evaluated.
Results: Expression of ADO receptor type 3 (A3R), ADO deaminase (ADA), AMP deaminase type 1 (AMPD1) and 2 (AMPD2) was decreased in HFD, as compared to RD. Accordingly, in HFD the ADO relaxant effect was potentiated as compared to RD. In vivo metformin treatment in both RD and HFD significantly increased the ADO relaxing effect, although to a different extent. In penile strips from HFD, in vivo metformin normalized A3R, ADA and AMPD1, further decreased AMPD2, increased dimethylarginine-dimethylamino-hydrolase and restored impaired Ach-induced relaxation. Ex vivo metformin time-and dose-dependently increased the relaxant effect of ADO in RD. The potentiating effect of metformin on ADO-induced relaxation was significantly reduced by pre-incubation with NOS inhibitor L-NAME. Interestingly, in vivo testosterone supplementation in HFD rabbits increased penile expression of eNOS, AMPD2 and restored metformin's potentiating effect on ADO induced relaxation, up to RD level.
Conclusion: Metformin in vivo and ex vivo increases ADO signalling in CC, most probably interfering with NO formation and ADO breakdown.

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Impaired masturbation induced erections: a new cardiovascular risk factor for male subjects with sexual dysfunction

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Background: Erectile dysfunction (ED) is considered an early surrogate marker of silent, or overt, cardiovascular diseases (CVD). Epidemiological studies evaluated only intercourse-related erections and not masturbation-induced erections as a possible predictor of major adverse cardiovascular events (MACE).
Aim: To evaluate the clinical correlates of impaired masturbation-induced erections and to verify their importance in predicting MACE.
Methods: A consecutive series of 4,031 male patients attending the Outpatient Clinic for sexual dysfunction for the first time was retrospectively studied. Among these subjects, only the 64% that reported autoeroticism during the last three months was considered in the study. In the longitudinal study, 862 subjects reporting autoeroticism were enrolled.
Results: Subjects with impaired masturbation-induced erections (46% of those reporting autoeroticism) had more often a positive personal or family history of CVD, a higher risk of reduced intercourse- and sleep-related erections, hypoactive sexual desire and impaired PGE1 test response. In the longitudinal study, unadjusted incidence of MACE was significantly associated with impaired masturbation-induced erections. When dividing the population according to the median age and diagnosis of diabetes, the association between impaired masturbation-induced erections and incidence of MACE was maintained only in the youngest (<55 year-old) and in non-diabetic subjects, even after adjusting for confounders (HR = 3.348[1.085–10.335], $p = 0.032$ and HR = 2.108 [1.002–4.433], $p = 0.049$; respectively).
Conclusion: This study indicates that, in subjects with male sexual dysfunction, evaluating an often neglected sexual parameter, such as masturbation-induced erections, can provide further insights on forthcoming MACE in particular in "low risk" subjects.

P159

Low adherence rates to the mediterranean diet are correlated with erectile dysfunction

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Background: Endothelial vascular damage is a key event in the pathophysiology of erectile dysfunction. Adherence to the Mediterranean diet, a nutritional life-style promoting fruits, vegetables and olive oil consumption, has been associated to improved endothelial vascular health and function.
Aim: Aim of this study was to investigate the relationship of adherence to the Mediterranean type of diet and the presence and severity of vasculogenic ED.
Methods: We studied 51 ED patients (aged 56 $\pm$ 11 years) and 26 non ED subjects (contol group) matched for age and cardiovascular risk factors. ED diagnosis and score were evaluated according to the International Index of Erectile Function questionnaire. Low IIEF score indicates

severe ED. Overall assessment of dietary habits was evaluated through the Med-Diet Score, (theoretical range 0–55), which assesses adherence to the Mediterranean dietary pattern. Higher values on the score indicate greater adherence to this pattern and consequently, healthier dietary habits.

Results: Compared to controls, ED patients had significantly lower Med-Diet Score (29 ± 5 vs. 34 ± 4 pmol/L, $p < 0.05$). In univariate analysis, Med-Diet Score was positively associated with erectile performance as expressed by SHIM-5 score ($r = 0.39$, $p < 0.01$). The association of Med Diet Score with SHIM-5 score remained significant in a linear regression model after controlling to age, cardiovascular risk factors and testosterone levels ($b = 0.27$, $p < 0.05$).

Conclusion: The poor long term adherence to the Mediterranean type of diet is related to an unfavorable effect on erectile function, irrespectively of other traditional risk factors. A healthier dietary life style may help preventing further vascular damage and assisting a better erectile performance.

P160

Plasma testosterone is associated with SCORE in erectile dysfunction patients

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Background: The systematic coronary risk evaluation (SCORE) predicts a patient's 10-year risk of developing cardiovascular disease. Many risk factors included in its calculation influence or are influenced by circulating testosterone. Low levels of testosterone are involved in the pathophysiology of erectile dysfunction (ED).

Aim: To investigate the possible association between testosterone and cardiovascular risk, as defined by SCORE, a database of ED patients was analyzed.

Methods: SCORE in relation to total testosterone (TT) were analyzed with proportional hazards models in 415 patients (mean age 56 years). Hypogonadism (HypG) was defined when TT levels were below 3.4 ng/mL. Exclusion criteria included pre-existing cardiovascular disease, stroke and diabetes.

Results: There was a positive association between TT and penile peak systolic velocity ($p < 0.01$) and negative associations between TT and body mass index ($p < 0.01$), systolic blood pressure ($p < 0.01$) and blood pressure medication use ($p < 0.01$). SCORE was negatively associated with TT ($p < 0.001$). Patients with high SCORE (>5%) were more likely to have laboratory HypG as compared to subjects with moderate (1–5%) and low SCORE (<1%).

Conclusion: Lower plasma TT may suggest the presence of cardiovascular risk factors and potentially increased risk for heart disease in ED patients.

POSTER SESSION: FEMALE SEXUAL DYSFUNCTION

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Poster cancelled

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Evaluation of female couple of patients with erectile dysfunction

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Background: In our experience the evaluation of partner of the patient with sexual dysfunction optimizes their approach.
Aim: Describe the sexual sphere of female partners of patients seen at Fundació Puigvert.
Methods: Female partners of patients with Erectile dysfunction (ED) who agreed to be visited (2006–2013) on female sexual dysfunction Unit (FSDU) Review medical records of patients and their partners.
Results: 327 records revised .76% has only ED.10% ED with Peyronie's disease. 7% ED with hypoactive sexual desire (HSD) and 7% ED with premature ejaculation. 37.6% patients with ED were treated with inhibitors 5 phosphodiesterase. 18% were carriers of penile prosthesis, 10% were using Intracavernosal inyection, using vacuum 8%; 8% were treated with psychological therapy and 6.8% with multimodal therapy (andrology treatment and psycotherapy). Women: 77% were menopausal. 20% received hormone replacement therapy. 25% had depression treated with antidepressants. 79% presented a sexual dysfunction: 44% HSD, dyspareunia 27%, 13% difficulty to achieve an orgasm and 1.8% have vaginismus. We made sexual counselling at the FSDU and add to them local vulvovaginal treatment. 46% of women showed improvement of HSD. 60% showed improvement to achieve an orgasm and 73% improve dispareunia.
Conclusion: 79% of female partners of men with DE have a sexual dysfunction, being the most comom HSD. Regardless of FSD, 50% women showed improvement of her dysfuction after treatment. Could be interesting to assess the partners of men with DE to evaluate her sexual sphere and to treat them if necessary.

P163

The global online sexuality survey: public perception of female genital cutting among internet users in the Middle East

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Background: Female genital cutting (FGC) is a ritual involving cutting part or all of the female external genitalia, performed primarily in Africa. Understanding the motivation behind FGC whether religious or otherwise is important for formulating the anti-FGC messages in prevention and awareness campaigns.
Aim: Investigation of opinion over FGC, the root motive/s behind it, in addition to the current prevalence of FGC among Internet users in the Middle East.
Methods: The Global Online Sexuality Survey (GOSS) was undertaken in the Middle East via paid advertising on Facebook®, comprising 146 questions.
Results: 31.6% of 992 participants experienced FGC at an average age of 9.6 ± 3.5 years, mostly in Egypt (50.2%). FGC was more prevalent among Muslims (36.9%) than Christians (18.8%), more in rural areas (78.7%) than urban (47.4%), and was performed primarily by doctors (54.7%) and nurses (9.5%). Whether or not it is necessary for female chastity, FGC was reported as highly necessary (22.5%), necessary (21.6%), more so among males, more among those with rural origin, with no difference as per educational level. Religious opinion among Muslims was: 55.4% anti-FGC and 44.6% pro-FGC. Only 3.7% saw it as a mandate of Islam.
Conclusion: An important motivation driving FGC seems to be males seeking female chastity rather than religion, especially with FGC not being an Islamic mandate. There is a shift towards doctors and nurses for performing FGC, which is both a threat and an opportunity if used to deliver the anti-FGC message.

P164

Sex-related variations of oxidative stress parameters in acquired growth hormone deficiency

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Background: Growth hormone deficiency (GHD) is associated with increased cardiovascular risk; few data are available on oxidative stress (OS) as mechanism underlying such phenomenon. However, little is known about the influence of steroid hormones in the regulation of antioxidant systems.
Aim: To investigate sex-related differences in OS parameters in GHD patients.
Methods: We have studied a group of 16 patients with GHD, diagnosed by GHRH + arginine dynamic test (5 females, aged 31–51, and 11 males, aged 36–57) evaluating plasma total antioxidant capacity (TAC) and the oxidized Coenzyme Q10 (CoQ10) as index of OS. TAC was evaluated using the system H202- metmyoglobin and the chromogen ABTS, whose radical cation is spectroscopically evidenciated. The latency time (LAG, sec) in the appearance of radical ABTS is proportional to the content of antioxidants in the sample. Coenzyme Q10 was measured by

electrochemical method, which allows to determine its reduced and oxidized form.
Results: When comparing results in males and females, we found a significant difference in both TAC (55 ± 3.1 in females and 88.3 ± 7.4 sec in males) and Oxidized CoQ10 (0.152 ± 0.02 vs. 0.07 ± 0.03 μg/mL, respectively), suggesting an higher OS in females.
Conclusion: These preliminary data suggest a possible influence of estrogens on indexes of OS and should be considered in the context of prooxidant effect of estrogens. Further study can clarify the clinical implications of such observation.

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Androgens positively regulate NO-mediated relaxant pathway in rat clitoris

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Background: Female sexual response is the result of a complex interplay between central and peripheral mechanisms Hormonal regulation of female sexual excitement is poorly understood.
Aim: To evaluate sex steroid regulation of the NO-dependent relaxant and RhoA/ROCK contractile pathways in clitoris.
Methods: Subgroups of ovariectomized rats were or left untreated or supplemented with estradiol, progesterone, testosterone (T) and T plus the aromatase inhibitor, letrozole. mRNA expression (qRTPCR) of genes of the relaxant NO-signaling, and genes of the contractile RhoA/ROCK pathway in clitoris.
Results: In- vivo treatment with T increased clitoris eNOS, nNOS, sGC1a3, sGC1b3, PDE5, PKG1 mRNAs, that were all further increased by cotreatment with letrozole. T also increased ROCK2mRNA. E2-supplementation increased RhoA and ROCK2 expression. All NO-signaling genes, and ROCK2 resulted positively associated with T plasma level, while E2 level was positively associated with RhoA, ROCK2 and sGC1a3. When T and E2 (ROCK2 determinants at univariate analysis) were introduced as covariates in a multivariate model, only the association between E2 and ROCK2 was confirmed. To further investigate the effect of T and E2, in isolated rat clitoris smooth muscle cells (clitSMC) we studied migration, as a read-out of RhoA/ROCK activity. E2 increased clitSMC migration, and the selective RhoA /ROCK inhibitors. Also T increased clitSMC migration. Letrozole pretreatment abrogated T- induced migration. The non aromatizable androgen, DHT, reduced clitSMC chemotaxis even below untreated cells.
Conclusion: Our data demonstrate that T improves the NO-mediated signaling, whilst E2 stimulates the contractile RhoA/ROCK signaling in clitoris.

POSTER SESSION: SURGICAL ANDROLOGY

P166

Assessment of lipid metabolism disorders in patients with prostatic hyperplasia qualified for surgical treatment

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Background: The relationship between lipid metabolism disorders and the pathogenesis of benign prostatic hyperplasia (BPH) has been substantiated in numerous studies. However, physiological mechanism of this association has not been fully understood, and available data concerning this issue are often ambiguous.
Aim: The aim of this study is to assess lipid metabolism disorders in patients with benign prostatic hyperplasia.
Methods: A study group included 150 men with a diagnosis of BPH, qualified for transurethral resection of the prostate (TURP) in the Clinic of Urology and Urological Oncology, the Pomeranian Medical University of Szczecin. A control group comprised of 131 patients recruited from primary health care institutions. These patients did not report any lower urinary tract symptoms (LUTS), did not take five-alpha-reductase inhibitors or alpha1-adrenergic receptor blockers, and were not under the care of an urologist. A general result obtained by these patients on the International Prostate Symptom Score (IPSS) was the lowest possible score, which suggested mild symptoms.
In both groups of patients, waist circumference measurement and body weight measurement were taken, and serum parameters of lipid metabolism were determined using standard diagnostic methods.
Results: Men with prostatic hyperplasia had significantly lower levels of cholesterol-HDL fraction and higher levels of total cholesterol. The study did not prove significant differences in the levels of triglycerides and cholesterol-LDL between the two groups of men.
Conclusion:

- Men with prostatic hyperplasia more often have lipid metabolism disorders, than those without hyperplasia of the prostate gland.
- Men with prostatic hyperplasia require more frequent screenings of lipid metabolism parameters.

P167

Laparoscopic hysterectomy in male

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Background: Persistent Müllerian duct syndrome is a rare disorder of sexual development characterized by the presence of Müllerian duct structures (uterus, fallopian tubes, proximal vagina) in males with normal genotype. Surgical intervention can be considered in order to reduce the risk of malignancy.
Aim: We report the case of a 19-year old male with unilateral cryptorchidism and presence Müllerian duct structures.
Methods: The patient was referred to our andrology outpatient due to anejaculation. He had correction of hypospadiasis and right inguinal testicular retention in his history. MRI scan revealed 6 cm long uterus, vagina and absence of the left testicle. Genetic analysis resulted in 46 XY male karyotype. Microsurgical sperm retrieval was unsuccessful, histology resulted in Johnsen Score 2. Interdisciplinary consultation indicated surgical removal of the Müllerian duct structures.
Results: We performed cystoscopy and hysteroscopy with 9.5 Ch pediatric cystoscope, since bladder catheter insertion entered first the vagina. Laparoscopic hysterectomy and removal of the vagina was performed via transperitoneal approach. The vagina entered the urethra via the small and atrophicprostate. The patient is still complaint less after uneventful postoperative period. Histology has proven the presence of the Müllerian duct structures. In addition detailed analysis of the left fallopian tube revealed the presence of an atrophic testicle and epididymis.
Conclusion: This is the first report of an atrophic testicle on an intrabdominal Müllerian duct structure. Laparoscopic approach provided good functional outcome with short postoperative stay in experienced laparoscopic center. Surgical removal of the uterus and left testicle can prevent development of malignancy.

P168

Erectile dysfunction after surgical treatment of ischaemic priapism

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Background: Surgical treatment of ischemic priapism allows restoring blood flow in the corpus cavernosum. An early surgical intervention may prevent the development of erectile dysfunction.
Aim: To characterize the impact of surgical treatment of ischemic priapism on erectile function.

Methods: Retrospective analysis of clinical data of 17 patients that underwent surgical treatment of ischemic priapism in an institution between 2007 and 2013.
Results: The median age of patients was 47 years (24–71). All patients presented with pain. Among predisposing factors, a psychotropic drug was identified in 10 cases and a blood dyscrasia was identified in one. No cause was identified in the other cases. The mean duration of priapism was 37.9 h (2.0–120.0 h). All patients underwent cavernous washing with a vasoactive agente previous to performing the cavernous-spongy shunt, which was distal in all cases. In 7 cases, the shunt wasn't enough for priapism resolution and reintervention was needed. When there was immediate intraoperative resolution, no fibrosis of the corpora cavernosa was identified, with these patients presenting a shorter priapism evolution compared with those without intraoperative resolution (13.3 h vs. 66.0 h). The group of patients who developed erectile dysfunction (ED) showed a longer time of evolution (66.0 h vs. 25.4 h). Additionally, none of the patients who didn't need reintervention developed ED. Within reoperated patients, only those with an early reintervention (<1 day) developed ED.
Conclusion: Intraoperative finding of fibrosis of the corpora cavernosa and surgical failure implying reintervention, especially if early, are important factors in the development of ED.

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Foreskin surgeries under Local Anesthesia (LA). A survey

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Background: Foreskin surgical procedures in adult male such as Circumcision, Prepuce-plasty, frenulo-plasty, dorsal slit etc are usually performed by both General Surgeons and Urologists electively. Eventhough sufficient anesthesia can be achieved by penile ring block or dorsal block, these procedures are often performed under general anesthesia.
Aim: Our aim was to evaluate the acceptance of performing these procedures in the Day Procedure Unit for elective procedures and in the Emergency department in acute presentations.
Methods: 100 patients who underwent foreskin surgeries under LA were evaluated with their experience. The patients were asked about their symptoms such as pain or discomfort during local infiltration of Lignocaine, discomfort during the actual procedure, post operative discomfort and general acceptance.
30 medical staff including junior, middle grade and consultants working in General Surgery, Urology and A&E participated in the survey. 10 junior doctors in Surgical training, 10 registrar/middle grade level doctors and 10 consultants participated in the survey. They were asked about their experience and their opinion about performing such procedures confidently under penile block without causing much patient discomfort.
Results: 50 patients underwent Circumcision, 30 patients underwent foreskin preserving operations such as frenuloplasty or prepuce-plasty and 20 patients underwent other procedures such as reduction of paraphimosis (10) and dorsal slit (10) under LA.
Conclusion: Penile block is a safe and effective way of providing sufficient anesthesia for foreskin surgeries. The technique is easy to learn. It reduces post operative pain as well and the risk and cost of General Anaesthesia.

P170

Is it feasible to perform vasovasostomies in a secondary-level hospital?

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Background: Vasovasostomy (VV) is the gold standard technique for vasectomy reversal. According to published series, patency and pregnancy rates are in average 90 and 50%, respectively.
Aim: The aim of this study was to analyse our VV series and compare them with the published literature.
Methods: A total of 20 VV procedures were performed between January 2001 and November 2013. SPSS v.17.0 was used for statistical analysis.
Results: Mean age of the operated patients was 43 years (29–62 years); mean obstructive interval was 9.1 years (1–30 years). The most common reason for VV was having a new partner (19/20); one case was due to post-vasectomy pain syndrome. VV was performed in 20 patients (two of them were lost during follow up). The employed technique in all patients was one-layer microsurgical VV. Complications were observed in 2 cases (1 orchitis and 1 scrotal haematoma). Patency was achieved in 17 cases (94%) and successful pregnancy in 5 cases (27.7%). When analysing the results in detail, only 12 patients tried to achieve conception, with a pregnancy rate of 42%; only two of the seven patients that did not achieve conception, asked for assisted reproduction.
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.

P171

Penile augmentation using the groin flap

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Background: Penile girth augmentation can be achieved by various techniques, among which are liposuction-injection, synthetic grafts and autologous grafts. Flaps are considered superior to grafts considering their uninterrupted blood supply.
Aim: The current work describes long term experience with penile girth augmentation using the superficial circumflex iliac artery and vein (SCIAV) flap.

Methods: SCIAV flap was used for penile girth augmentation in 40 candidates who followed up for a minimum of 18 months. The flap was mobilized from the groin region. The penis was pulled out of a peno-pubic incision. The flap was tunnelled under the pubic region to emerge at the base of the penis and was sutured to the subcoronal area and on either sides of the spongiosum. Another session was required for either de-bulking of the over-sized flap (four over-weight candidates), flap pedicle ($n = 6$) or for donor site scar revision ($n = 11$).
Results: Excluding dropouts ($n = 8$) and participants who had encountered de-bulking of the flap body ($n = 4$), forty participants had a preoperative average flaccid girth (AFG) of 9.3 ± 1.1 cm. Immediately postoperative AFG was 14.9 ± 1.1 cm ($p < 0.001$). Postoperative AFG at the final follow up visit (a minimum of 18 months) was 14.5 ± 1.1 cm (55.6% gain compared to base-line, $p < 0.001$).
Conclusion: SCIAV flap is a reliable option for long lasting and sizable penile girth augmentation. One-stage augmentation is more suited for non-obese candidates. A second session may be indicated in overweight candidates or for scar revision.

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Penile deviation re-visited: shortening-free correction of curvature

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Background: Correction of penile curvature by corporal rotation enabled correction of 90° ventral curvature with neither shortening nor erectile dysfunction. However, some limitations were described, and only one case was reported upon.
Aim: This work describes three year experience with corporeal rotation; modifications addressing and eliminating its drawbacks and limitations, as well as long term follow up of 22 patients.
Methods: Modified corporeal rotation was performed in 22 patients with various degrees of curvature. Degree of deviation, erect penile length, symmetry, and erectile function were evaluated and compared pre and post operatively.
Results: Full correction of curvature was achieved in 20 out of 22 patients, with no shortening, asymmetry or erectile dysfunction. Residual curvature in 2 patients was no more than 10 degrees.
Conclusion: Corporal rotation can restore straightness to the penis with no loss in phallic length, asymmetry, or erectile dysfunction. While a variety of surgical techniques are feasible for correction of milder degrees of curvature, we believe that severe degrees should be spared the shortening and corrected by corporeal rotation.

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Glans augmentation by grafting: Shaeer's technique

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Background: Augmentation of the glans penis may be indicated for cosmetic reasons, lack of glans tumescence following implantation of a penile prosthesis, or asymmetry following girth augmentation of the shaft. Many augmentation techniques have been offered to increase the length and girth of penile shaft, but not the glans penis, with the exception of Hyaluronic Acid gel injection that is known to decrease sensitivity of the glans and is restricted for cases with premature ejaculation.
Aim: This work is the first report on glans augmentation by grafting.
Methods: Ten males requesting augmentation of the glans were selected for the study after failing counseling, with normal erectile function and ejaculatory control. Two ventral incisions were cut along the ventral aspects of the coronal sulcus, one on either side of the frenulum. Lateral glans flaps were dissected on either side. The urethra was circumvented, creating a plane all around it. A dermal fat graft was inserted into the space created. The flaps were closed by simple absorbable sutures.
Results: Maximum circumference of the glans increased by 16.6% declining to 14.2% by the last follow up visit (10–12 months), a 2.3% decline. Self-reported impression of the augmented volume was high, and well maintained over the follow up period. Glans sensation, engorgement, erectile function and
ejaculatory control were preserved.
Conclusion: This pilot study on glans augmentation by grafting reports promising results with retention of the added volume at one year follow up, preservation sensitivity and engorgement, and no adverse effects on erectile function or ejaculatory control.

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Same-session dorsal vein ligation and testing by intracavernous injection prior to penile prosthesis implantation (DVL-ICI-PPI)

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Background: Complications of penile prosthesis implantation (PPI) are rare, nevertheless can be grave. In cases with veno-occlusive dysfunction (VOD), alternative surgical techniques such as dorsal vein ligation (DVL) are controversial. Some patients may opt for trial at DVL to avoid the possible complications of PPI. However, this may be associated with disappointment if DVL fails and another procedure is required.
Aim: To evaluate the results of dorsal vein ligation (DVL), same-session testing by intracavernous injection (ICI) of Prostaglandin E1 (PGE1), and immediate implantation of a penile prosthesis (PPI) in case of poor response to DVL.
Methods: 26 patients with refractory VOD were operated upon. Through a peno-pubic incision, DVL was performed, followed by ICI of 20 μg PGE1 in two divided doses, 10 μg each, 15 min apart.

Group-1 exhibited full rigidity in response to the first dose. Group -2 exhibited full rigidity in response to the second dose. PPI was not performed for either. Group-3 exhibited suboptimal response to both doses and PPI was performed through the same incision. Patients were followed up from 24 to 48 months using IIEF-5 scoring.
Results: For Group-1 (n = 8), 6 experienced normal erectile function following DVL throughout the whole follow-up period of 48 months (23.1% of all patients), and 2 patients relapsed. Group-2 (n = 6) (23.1%), reported normal erectile function for an average of 6 months, then relapsed. Group-3 (n = 12) had a PP implanted in the same setting.
Conclusion: Combined DVL-ICI-PPI can spare around 23.1% of young patients with VOD from PPI, at no additional risk.

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Morphometric study of spermatogenesis in the rat after vasectomy via inguinal canal

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Background: Utilizing a rabbit model, our previous study found firm evidence indicating that vasectomy-induced spermatogenic damage was pressure-mediated: the damage occurred when the occluded reproductive tract was unable to accommodate additional sperm produced by the testis (Andrologia 2011, 43: 129–38). More studies utilizing more commonly used rats showed, however, controversial or confusing results on whether and why the damage occurred, probably due partly to postoperative formation of sperm granuloma.
Aim: This study was undertaken with care to clarify the mechanism of the damage, if there would be.
Methods: 12 mature male Sprague-Dawley rats were subjected to unilateral vasectomy via the inguinal canal; 37 days postoperation, the testes, epididymides and granulomas (at the vasectomy site) were removed to obtain methacrylate resin-embedded sections and morphometric studies carried out with light microscopy.
Results: Marked damage to spermatogenesis in the testis on the vasectomized side, with many round spermatids seen in the ipsilateral epididymis, was demonstrated in five rats; in two of these rats, apparent granulomas were not formed while in the other three the granulomas formed were smaller than those formed in the other seven rats without spermatogenic damage. In addition, the mean thickness of the rete testis increased significantly after vasectomy.
Conclusion: It seems therefore that spermatogenic damage induced by vasectomy in the rat model was pressure-mediated as well.

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The use of biomaterial gore acuseal for grafting in the surgical treatment of Peyronie's disease

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Background: Plaque incision/excision and grafting remains the gold standard for correcting penile deformity in men with stable Peyronie's Disease (PD) if the following conditions: (i) adequate hardness, (ii) curvature more 60°, (iii) hour-glass.
Aim: We used biomaterial Gore Acuseal and evaluated short-term results of our treatment.
Methods: 22 patients were operated with biomaterial Gore Acuseal for grafting. There were men of 43.4 ± 10.8 years old with penis's dorsal curvature. We had the examination of our patients: (i) measuring the length of the erected penis and its photographing; (ii) questioning by IIEF and EHS. In the postoperative period we also tested patients with measuring of the penis during erection and special questionnaire to find out whether the patient regrets about the surgery and to find out all positive and negative aspects connected with the operation. Postoperatively all patients received sildenafil, vitamin E and traction therapy.
Results: All patients had no regrets about surgical treatment. We had no cases of infection or rejection of the biomaterial. In 3 (13.7%) cases there was residual curvature, which disappeared almost completely in 6 months after the surgery. 6 (27.3%) patients observed the increase of the penis in 3 months and 9 (41%) patients in 6 months after surgery. 11 (50%) patients had the glans penis sensitivity disorder during sex in 3 months after surgery, but in 6 months only 1 (4.5%) patient had sensitivity disorder.
Conclusion: We obtained positive results of the use of Gore Acuseal, so it gives us a possibility to use it for grafting in the surgical treatment of PD in prospect.
(Authors don't have financial conflict of interest or other relationship with a commercial Organization).

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Delay of surgical treatment of penile fracture results in poor functional outcome: results from a large retrospective multicentric European Study

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Background: Penile fracture is a rare event but, represents a urological emergency.
Aim: To review preoperative diagnostic evaluation, surgical treatment and related outcomes of penile fracture,

and to investigate the effect of delay of surgery on outcomes.
Methods: A retrospective analysis of the data obtained from 137 patients presenting a diagnosis of penile fracture from seven different European Academic centers was carried out. Parameters assessed were age, imaging modalities used, timing of surgical intervention, length of tunica albuginea defect, surgical technique, postoperative erectile function outcomes measured by IIEF-5 and presence of penile curvature.
Results: The mean age of the patients was 38.96 ± 13.55. All patients underwent routine clinical examination out of which 82 patients (59.85%) underwent penile Doppler ultrasound and 5 patients (3.64%) were evaluated with an MRI. Mean time between ER admission and surgical intervention was 780.6 ± 31.13 min. All of the patients were treated surgically. The mean length of tunica albuginea defect measured during surgery was 15.32 ± 8.30 mm. Mean IIEF-5 score was 15.09 ± 7.8 and 16.85 ± 8.96 after one and three months, respectively. If the surgical intervention was performed later than 8.23 h after admission, postoperative erectile function was significantly worse ($p = 0.0051$ at 1 month; $p = 0.0057$ at three months).
Conclusion: Penile fracture is a rare event. Our multicenter study shows that delaying surgical intervention for more than 8.23 hours from presentation in ER results in significantly decreased IIEF-5 scores in the follow up period, indicating that surgical treatment delay results in worse erectile recovery.

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Surgical keypoints in the simultaneous implant of an inflatable penile prosthesis and a male suburethral sling

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Background: Erectile dysfunction and urinary incontinence in men are pathologies that can occur at the same time. The most frequent cause is the iatrogenic injury that may come from either radical surgery of the prostate or bladder cancer. In both, theses sequels represent a poor postoperative functional outcome that considerably affects the patient′s quality of life. The penile prosthesis implant and the placement of either a suburethral sling or an artificial urinary sphincter are the definitive treatment for patients who do not respond to conventional treatment rehabilitation measures.
Aim: To describe step by step the surgical procedure (surgical video).
Methods: We present the case of one patient with severe erectile dysfunction, with no response to oral or intracavernous treatment, and moderate urinary incontinence. Eighteen months before he underwent a laparoscopic radical prostatectomy. We performed a simultaneous implant of a transobturator suburethral sling and an inflatable penile prosthesis. The most relevant anatomic and functional surgical aspects are analyzed in this report.
Results: The simultaneous implantation of an inflatable penile prosthesis and suburethral sling is a feasible therapeutic option for carefully selected patients with erectile dysfunction and mild to moderated urinary incontinence. This technique saves time and the total cost is lower than the implantation of both devices separately.
Conclusion: We believe that the simultaneous procedure is effective and safe. There is not a more risk of infection or complexity, although the risk of conflict between the different elements must be always minimized.

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Corporal rotation for surgical correction of congenital ventral penile curvature: modifications of the original technique

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Background: The classical way of correcting congenital penile curvature is based on two types of surgical techniques. One shortening the long side and the other elongating the short side of the tunica albuginea of the corpora cavernosa. Both obtain a high percentage of penile straightening. However, shortening involves some risk of loss of the length of the penis, and elongation greater risk of erectile dysfuntion and recurrence. In this context, a new surgical approach is proposed, 'the corporal rotation'.
Aim: To describe step by step the surgical procedure (surgical video).
Methods: We report one case of a twenty six years old male with congenital ventral penile curvature of fifty degrees. Following, the detailed procedure of the corporal rotation technique is exposed, as a modification of the original technique proposed by Dr. Shaeer .
Results: The advantages of this technique are; absence of shortening, nor palpable grooves or ridges and preservation of the erectile function. However, there are two limitations; first the inability of correction in lateral curves, and second the lack of experience in cases of ventral curve caused by La Peyronie's disease.
Conclusion: Despite of the lack of large published series, we think the corporal rotation technique must be considered when making a choice in severe cases of congenital ventral penile curvature.

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Venous ligation: a novel strategy for glans enhancement in penile prosthesis implantation

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Background: Penile implants remain the definitive solution for patients with refractory impotence but with common undesirable side effects, such as penile size reduction and cold glans syndrome.
Aim: We report results of a surgical method designed to ameliorate these issues.
Methods: From 2003 to 2013, 35 consecutive patients received one-piece penile implants. Of these, 15 men (treatment group) were also treated with ligation of the retrocoronal venous plexus, deep dorsal vein and cavernosal veins of the penis. The remaining 20 men (control group) were treated with a penile implant alone.
Results: Follow-up ranged from 1.1 to 10.0 years (mean 6.7 ± 1.5). Preoperative glanular dimensions did not differ significantly between the two groups. However there were significant respective differences at day one and one year postoperatively were found in glanular circumference (128.8 ± 6.8 mm vs. 115.3 ± 7.2 mm, 130.6 ± 7.2 mm vs. 100.5 ± 7.3 mm; both $p < 0.05$) and radius (38.8 ± 2.7 mm vs. 37.1 ± 2.8 mm, 41.5 ± 2.6 mm vs. 33.8 ± 2.9 mm; latter $p < 0.01$). Cold glans syndrome occurred in nine of the twenty (45%) in the control group compared to none in the treatment group. Overall satisfaction rate also differed between the treatment and control group (91.7% vs. 53.3%, $p < 0.05$).
Conclusion: Based on our results, selective venous ligation appears to enhance the penile glans both in size and temperature in implant patients.

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The penile venous occlusion mechanism: evidence derived from the electrocautery effect to the sinusoids on defrosted human cadavers

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Background: The venous occlusive mechanism is an important factor for maintaining penile rigidity during erections. This relationship between the veins and the tunica albuginea (TA) is not clear.
Aim: We aim to conduct an electrocautery study as an indirect means of describing venous leakage.
Methods: In 2010, seven adult male cadavers with intact penises were defrosted. A median dorsal longitudinal incision was made from the retrocoronal sulcus to the pubic region for vascular access. Two #19 scalp needles were placed with 4-0 silk sutures at the 3 and 9 o'clock positions. One needle was connected to an infusion pump used to inject 10% colloid into the corpora cavernosa, whereas the other was used to monitor the intracavernosal pressure (ICP). A 5 cm segment of the deep dorsal vein (DDV) was resected, followed by a 2.5 × 0.5 cm^2 block of dorsal corpus cavernosum proximal to the retro-coronal sulcus including an emissary vein. A watertight milieu was re-established by 6–0 nylon closure of the corporotomy. A similar tissue block was obtained after electrocautery at 45–60 watts, was applied to the emissary branches of the proximal DDV stump while the ICP was kept at 0, 50, 90, 130 and 150 mmHg to each cadaver in series. Two further cadavers were treated in a similar fashion at 90 and 130 mmHg of ICP. Tissue specimens were sent for special stains and analysis.
Results: The electrocautery effect penetrated categorically into the sinusoids when ICP <70 mmHg.
Conclusion: The emissary veins it seems closes at an ICP >130 mmHg if current penetration is used as a definition.

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Veno-occlusive erectile dysfunction in young patients resulting from the 'Jelqing maneuver': results of penile venous stripping surgery

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Background: Erectile dysfunction when it occurs in younger men is often deemed psychogenic. Organic causes such as veno-occlusive dysfunction should be explored.
Aim: We report a retrospective study on veno-occlusive erectile dysfunction in men who practice 'Jelqing' – a natural penile enlargement technique.
Methods: From October 2000 to September 2011, 37 men aged between 19 and 30 years with erectile dysfunction and a history in the use of the Jelqing maneuver were confirmed to have veno-occlusive dysfunction with cavernosography. 29 patients underwent penile vein stripping surgery and 8 men were treated with non-surgical means. All patients were followed with the abridged five-item version of the index of erectile function (IIEF-5) and cavernosography.
Results: In the surgical group, the pre-operative IIEF-5 score of 11.2 ± 2.3 ($n = 29$) was increased to 21.8 ± 1.9 ($p < 0.001$). In the non-surgical group however, the mean baseline IIEF-5 score of 11.5 ± 2.3 ($n = 8$) differed to 12.1 ± 2.6 ($p > 0.05$) after medical treatment only. Although there was no significant difference between the two groups' preoperative IIEF-5 score, there was a statistically significant difference after venous surgery was performed. The follow-up period ranged from 6 months to 11 years, with an average of 5.5 ± 1.7 years. Eventually both groups had six men each (26.9 and 75.0%) whom

required additional oral phosphodiesterase-5 inhibitor treatment. In the non-surgical group a man suffered from depression.
Conclusion: In this study, we may conclude that 'venous' aetiology should be an important factor rather than just the psychogenic origin in young males who suffer from erectile dysfunction who practice the Jelqing maneuver.

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Penile implants: new pump system: 100 cases, our experience

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Background: Penile prosthesis implants is the last option patients face when facing E.D. treatments, the increasing innovations in the devices have made them a more popular and demanded way of treating those patients where oral medication or inyected medication has no effects. The assessment of those innovations is important to evaluate benefits for patients and their partners.
Aim: The aim is to present our experience with Coloplast Titan™ Inflatable Penile Prosthesis (IPP) with One-Touch Release (OTR) pump. We will asses patient satisfaction and partner's satisfaction if there happens to be one. As well as present our experience with our first 100 cases between 3 Specialized Centers and 2 main surgeons. Hospital del Henares – Coslada (Madrid), Centro de Urología Andrología y Salud Sexual – Palma de Mallorca e Instituto de Medicina Sexual – Madrid.
Methods: Retrospective review was used to assess the Coloplast Titan™ OTR implant procedures/cases in our departments (from October 2009 to December 2012).
Results: Mean patient age (N = 100) was 54.2 years old (range: 20–77) with a mean length of follow-up of 21 months (range: 6–44).
Conclusion: The Coloplast Titan™ OTR IPP was easy to implant, inflate, and deflate with high levels of EF improvement, patient and partner satisfaction. A small number of postoperative teaching sessions were required for the patient to properly operate the device.
Keywords: erectile dysfunction; inflatable penile prosthesis; one-touch release pump (OTR).

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Testicular torsion - 25 years experience of a single urology department

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Background: Testicular torsion represents one of the few urological emergencies. The late referral to a urology department can lead to orchiectomy.
Aim: To evaluate the clinical history, demographic data and surgical exploration findings in all patients who underwent surgery exploration due to suspicion of testicular torsion.
Methods: Descriptive-retrospective study including all cases with a pre-operative diagnosis of testicular torsion, from 1989 to 2013, at our Department.
Results: A total of 194 patients, aged 11–45 years, underwent surgical exploration due to suspicion of testicular torsion. The age group 11–15 years was the most affected (n = 99; 51%). Testicular torsion was observed on the right-side in 52.1% (n = 101) and in 93 cases on left testicle (47.9%). Our testicular salvage rate was 79.9% (n = 155) and 39 patients underwent orchiectomy (20.1%). We observed a 100% testicular salvage rate in patients with symptoms duration under 6 h. On the other hand, most of the patients with more than 12 h of symptoms underwent orchiectomy (77.8%). The average time between first symptoms and surgical exploration was 10.1 h in patients in whom testicular salvage was possible. In those who underwent orchiectomy this time was 75.2 h. We did not find significative difference in testicular incidence between coldest and warmest months (p = 0.110).
Conclusion: The shorter the time to surgical exploration, the higher the chances to salvage the testicle. A 100% testicular salvage rate was obtained in patients with symptoms duration under 6 h.

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Primary penile prostheses implants: analysis of results at an andrology training centre

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Background: A great deal exists about infection and satisfaction rates of penile implants (PPI) at 'excellence' centres. Results are attributed to improvement of devices and surgical technique. There is little information about results obtained at teaching Centres.
Aim: To analyze the rate of infection and satisfaction of 'patients' and 'partners' with (PPI) and ascertain whether it is related to surgeon's experience.
Methods: PPI patients performed between January 2008 and December 2012 were reviewed after 12 months follow up postoperatively. Surgeons classified as 'experts' or 'trainees'. Infection was considered on the basis of clinical and/or positive culture's secretion. Patients and partners satisfaction scale: dissatisfied, satisfied, very satisfied. Fisher's test and Z test for proportions were applied to compare results. Between centres comparison is also provided.

Results: 255 patients were selected and 176 (mean age 60 ± 10) completed the study. 76% implanted by experts and 24% by trainees.

	176 patients		
	Surgeon experience		
	Experts	Trainees	*p*-value
Patient satisfaction	133	43	
Dissatisfied	17 (13%)	4 (9%)	0.78
Satisfied	4 (3%)	0 (0%)	–
Very satisfied	112 (84%)	39 (91%)	0.28
Partner satisfaction	107	34	
Dissatisfied	15 (14%)	4 (12%)	1
Satisfied	3 (3%)	0 (0%)	-
Very satisfied	89 (83%)	30 (88%)	0.48
Infection rate	3 (133) (1.5%)	4 (43) (6.6%)	0.06

Centres comparison of trainees

	PPI	Inflatable-PPI	PPI/year	Revision/ explant
Fundació Puigvert	60	54 (90%)	12	4/60 (6.6%)
University School	79	69 (87.3%)	24	7/79 (8–9%)

Conclusion: Satisfaction and infection rates are comparable between trainees and experts. Trainee's results are better to other centre probably due to an accurate teaching programme.

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