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Report of the Centre of Reproductive Medicine and Andrology (CeRA) of the University Hospital of Münster and the University of Münster, Germany, as an EAA Training Centre, reporting period 2010-2013

Date of report:
19 February, 2014

Centre of Reproductive Medicine and Andrology and Institute of Reproductive and Regenerative Biology:
Director: Prof. Dr. rer. nat. Stefan Schlatt since July 1, 2008

Department of Clinical Andrology:
Chair: Prof. Dr. med. Sabine Kliesch since June 1, 2008 / Director of the EAA training Center

World Health Organization
WHO Collaborating Centre for Research in Male Reproduction

Training Centre
European Academy of Andrology
E·A·A

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In June 2010, the Centre of Reproductive Medicine and Andrology (CeRA) was positively re-evaluated as an EAA training center. The Centre consists of two main institutions, the Institute of Reproductive and Regenerative Biology (IRRB) and the Department of Clinical Andrology. The Department of Clinical Andrology is responsible for the patient care, the medical training of clinical andrologist, for the andrological teaching of medical students as well as clinical research. In addition to the andrological, endocrinological and urological work-up of andrological patients, the CeRA is also responsible for the IVF laboratory of the Fertility Centre of the University. The following report covers the time from June 2010 until now.

Since 1986 the Centre has been and continues to be a WHO Collaborating Centre for Research in Human Reproduction. It was recognized as an EAA Training Centre in 1994 which was successfully re-evaluated in 2001 and 2010. From 1999 onwards, the former IRM and the present CeRA received Quality Management Accreditation (ISO 9000-2) from the TÜV Rheinland for the fields of Andrology,

Endocrinology, Reproductive Medicine and Urology, recently renewed in January 2014.

Present staff

The staff of the Centre has undergone major changes in the last three years.

The vacant position for an Associate professorship was advertised in 2009/2010 to be filled with a candidate of a scientific background in the field of "Regenerative Biomedicine". After having several top candidates selected after public lectures at the Centre, the final agreement between the Medical Faculty and the candidates failed. A second round was started in 2013 with new candidates of the field. There will be public lectures given by five top scientiest on 24 February, 2014. The professorship belongs to Professor Schlatt's institution, and we are confident that the vacancy will be filled in the coming months.

Dr. Con Mallidis, who is an expert in andrology, took over the responsibility for the andrology lab and the research field of physiology of reproduction. He implemented new diagnostic tools such as DNA fragmentation analysis and RAMAN spectroscopy. He is closely interacting with the clinic.

Prof. rer. nat. Jörg Gromoll, Prof. Dr. med. Michael Zitzmann, Dr. rer. nat. Jochen Wistuba and Dr. med. Claudia Krallmann have permanent positions. Professor Gromoll and Dr Wistuba are part of the IRRB, Professor Zitzmann and Dr Krallmann belong to the clinical department.

7 research assistants, 9 medical doctors, 5 post-graduates, 2 master students, 13 technical assistants and 10 (patients´) secretarial staff as well as 2 animal caretakers comprise the rest of the Centre and are distributed between the two institutions as detailed in Table 1. Altogether, 15 positions are funded by the University, 21 are funded by the University Hospital, and the others are funded as specified in Table 1.

Professor Kliesch is Secretary of the Board of the German Society of Urology (DGU) since 2008 responsible for public relations of the Society, and she was again a member of the Board of the German Society of Andrology (DGA) from 2011 to 2013. In December 2013, the fifth interdisciplinary Congress on Reproductive Medicine and Andrology took place in Münster under her presidency. More than 660 scientists participated. Moreover, she is the president of the Quality control program of the German State Medical Board (Bundesärztekammer) for semen analysis and member of the German State Medical Board quality control program for laboratory diagnostics. The is elected member of the German Society of Cancer Diseases (DKG, Deutsche Krebsgesellschaft) and represents the Working Group for Urologic Cancer. She is the speaker of the interdisciplinary German Testicular Cancer Study Group of the DKG. She is member of the ESHRE Task Force on fertility preservation in men. Since 2010 she is member of the advisory board for men´s health of the German State Central for Health Explanation (Bundeszentrale für gesundheitliche Aufklärung, BZgA). She is the Director of the WHO Collaboration Center Münster and Director of the EAA Training Center Münster.

Professor Schlatt is Secretary of the International Society of Andrology (ISA) since April 2009 and a member of the Board of the German Society of Andrology (DGA) since 2010. He is speaker of the male subgroup of the ESHRE Task Force on fertility preservation and coordinator of the Special Interest group Andrology (SIGA) of ESHRE. Since 2012 he is chairman of the KFR (Clinical Research Group for Reproductive Medicine e.V.), supporting research acitivities of the CeRA.

Professor Gromoll is President of the German Society of Endocrinology (DGE). Professor Zitzmann is a member of the Board of the International Society for Men's Health and since 2014 Member of the Board of the German Society of Andrology.

Professor Nieschlag is still active and Chairman of the quality controll programm of the German Society of Andrology (QuaDeGA) since 2009. In 2013 more than 600 laboratories participated, 10% from abroad. Until 2012 he was chairman, since then he is secretary of the KFR

(Clinical Research Group for Reproductive Medicine e.V.). As a Member of the Scientific Advisory Council of the German Federal Medical Board (Bundesärztekammer) since 2004, he was recently especially involved in a Memorandum on Preimplantation Diagnostic (2012), in a position paper on The Future of University Medicine (2012) and Chairman of a working group on Guidelines for Diagnosis and Treatment of Children and Adolescents with DSD (disorders of sexual development (since 2013). He was member of the International Committe on Contraception Research (ICCR) of the Population Council / Rockefeller Foundation New York 1999 – 2012 and since then Emeritus Advisor. Since 2013 Professor Nieschlag is a HiCi Scientist (Highly Cited) at the Center of Excellence in Genomic Medicine Research of the King Abdulaziz University, Jeddah, Saudi Arabia since 2013. He acts as an advisor on Thrapeutic Use Exemptions for Athletes (since 2012) and on the development of Athlete Biological Passports (ABP) (since 2013) at the World Anti Doping Agency (WADA). He was President of the European Society of Endocrinology (ESE) 2007 – 2011 and received the Honorary membership of the German Society of Endocrinology (DGE) in 2011.

Several research prizes have been received by members of the staff over the past three years. Professor Nieschlag received the outstanding **Career Achievement Award** of the International Society of Men's Health 2011 and in 2012 the **Berthold Medal** of the German Society of Endocrinology. The collaborative working group of our center with focus on **the FSHB and FSHR polymorphism** has been awarded by the International Award for Publishing Excellence in the JCEM in 2012 and with the Young Andrologist Award 2013 (first authors Frank Tüttelmann, Institute of Human genetics and senior author Jörg Gromoll, CeRA).

Proff. Gromoll, Kliesch, Nieschlag, Schlatt and Zitzmann, as well as Dres. Mallidis, Nordhoff, Rohayem and Wistuba are members of Editorial Boards and function as Associate Editors for leading journals in the field of andrology, endocrinology, urology and reproductive medicine/biology.

Collaboration with other institutions

The nature and members of collaborations with several institutions of the University of Münster have been intensified and re-structured after the change in the leadership of the Centre 2008. The collaboration with the Women's Hospital and with the Institutes of Human Genetics, Microbiology, Radiology, Clinical Chemistry, Medical Psychology and the University Children's Hospital, have continued. New local collaborative research links have been established with the Clinic for Psychosomatic Diseases (Director: Prof. Dr. med. Gereon Heuft), the Eye Clinic (Prof. Dr. Thanos), various research groups at the Cell Biology department in the Centre for Molecular Biology in Inflammation (ZMBE, Prof. Dr. Raz, Dr. J. Schwamborn), the Centre for Nanotechnology (Prof. L. Chi) and the Clinical and Experimental Transplantation Hepatology (Dr. A. Zibert) in Münster. The collaboration with the Urology Department was intensified, as Professor Kliesch is a former co-worker of this department, and with her change to the Centre she took over all andrological aspects from the Department of Urology.

Due to Professor Schlatt's activities in stem cell research and his close contact with Prof. Dr. rer. nat. Hans Schöler, with whom he already collaborated during a postdoc period at the University of Pennsylvania in 2001, the connections with the Max Planck Institute for molecular Biomedicine were intensified over the last years. Prof. Dr. Schlatt was the local representative of the Westfälische-Wilhelms Universität for the Northrhein-Westfalian Network in Stem Cell Research until 2013 and acts as chairman of the Research Focus Area "Reproductive and Regenerative Medicine" at the Medical Faculty of the University. In 2010 a new collaboration was established through a grant from the stem cell network with Prof. Schorle from the department of Pathology in Bonn on germ cell transplantation experiments together with CeRA scientists using the well characterized human seminoma cell line TCAM-2. As part of our research and clinical activities in regard to testicular cancer, several more collaborations could be established with the German and the European Cancer Collaborative Study Groups by Professor Kliesch. The long lasting close collaboration with Professor Bergmann, Veterinary Anatomy and Prof. Meinhardt, Anatomy of the University of Gießen, has been intensified as well as the well established co-operation with the Department of Urology and Andrology of the University of Gießen (Director: Prof. Dr. med. W.

Weidner) and the Center for Reproductive Medicine and Andrology of the University of Halle (Director: Prof. Dr. med. H. M. Behre). Many international collaborations are well established. A constant flow of young scientists from Germany and abroad visits the CeRA for training. As an example since June 2010 more than 10 research and clinical fellows from Armenia, India, Italy, Portugal, Switzerland and the UK worked at CeRA, many of them being sponsored by fellowships from their home countries or stipends from the ERASMUS program, the Alexander von Humboldt Foundation, the German Aacademic Exchange Service (DAAD) and the German Association of Urology (East-West-Exchange Programme). The many existing international collaborations, new scientific links and joined projects were continued since July 2010. Among those are already financially supported collaborations with the Centre for Research in Reproductive Physiology at the University Pittsburgh, USA, the Department of Woman and Child Health at Karolinska University, Sweden, the MRC Human Reproductive Science Unit, UK, the Department of Zoology at the University of Coimbra, Portugal and the Division of Reproductive Endocrinology at the Department of Reproductive Medicine at the University of California San Diego, USA.

Management of andrological patients

The Department of Clinical Andrology is active in the diagnosis and treatment of infertile patients, hypogonadal males, patients with endocrine disorders and those with disorders of ejaculation and erection. Oncological patients are provided with care regarding their reproductive health concerns. In case of germ cell tumours, our Department is accredited as a second opinion center of the DKG. Most of the patients present with severe problems, as the Department of Clinical Andrology is acknowledged as a secondary and tertiary referral centre. The number of consultations and diagnostic and routine laboratory tests are listed in Table 2 and 3 and increased steadily since 2008.

Since October 2012 we have a dependance in the City of Münster (Von-Vincke-Straße 14). It is a Private Ambulance for Andrology and belongs to the Department of Clinical Andrology under the responsibility of Prof. Kliesch. The diagnostic and therapeutic work-flows are identical for the patients seen in the private ambulance. The ambulance has a

small andrological laboratory for the semen tests and analysis to be performed freshly. Serum samples, DNA analysis and seminal plasma samples are transferred to the Domagkstreet for central analysis. In December 2013, the private ambulance has installed a DXA scan for measurements of bone density and body composition. After beeing licensed first measurements have been performed in January 2014.

Fertility Clinic (ART)

- In collaboration with the Women's Hospital, childless couples are cared for in the fertility outpatient clinic. Our department concentrates on the male partner and the IVF laboratory. We conduct a joint programme for assisted reproductive techniques including intrauterine inseminations, in-vitro fertilisation and intracytoplasmic sperm injection. The Fertility Clinic was reorganised with the aim to increase patient numbers treated under the responsibility of PD Dr. Med. A. Schüring specialised for Gynecological Endocrinology and Reproductive Medicine. By now, a constant personal staff is present in the Fertilty Clinic with 2 gynecologists for councelling and treating the female patients. Weekly interdisciplinary conferences are part of the routine work up of the couple. An external review process was initiated in 2012 and 2013, both covering the gynecological clinical and the andrological laboratory part of the processes to optimize diagnostic and treatment of the infertile couple. As a result of the structural changes, the patient numbers treated by IVF and ICSI could be increased and resulted in more thann 300 cycles at the end of 2013. As a consequence, also infrastructural improvements result in 2014, with a second new intracytoplasmic sperm injection (ICSI) microscope. The Nicon Ti-5 microscope is equipped with two Eppendorf micromanipulators which operate electronically with a memory function and are thereby faster during an ICSI. This decreases the amount of time oocytes remain outside of the incubator. In addition, the new system features two new options, a laser and a polarisation filter (PolarAIDETM Octax) which detects the birefringence of dense objects in the oocyte. These attributes represent the latest developments in the field of clinical embryology.
  The new laser is useful in selecting sperm for ICSI particularly in azoospermic men, where sperm are retrieved by testicular sperm

extraction (TESE). As being an andrological center, severe andrological cases with only few and immotile sperm in semen or testicular samples are frequent. 22% of our patients receive ICSI treatment with testicular spermatozoa. By using the laser microscopy, the distinction of viability of spermatozoa is possible. As a consequence live sperm can be selected and used for injection. When the number of sperms is smaller than the number of oocytes, the polarisation feature helps select the best oocyte for these sperm. The utilisation of these two new features has enhanced our fertilisation rates for TESE-ICSI and by improving the selection of PN increased our pregnancy rates. In addition, we implemented new techniques in the IVF laboratory setting: vitrification of oocytes is offered since the end of 2013 and Ca-ionophor assisted ART was implemented in January 2014. Since 2013, PGD methods are trained and applicable in the frame of the strict German law regulations.

Male Infertility

Apart from the close interaction with the Fertility Clinic of our Department, we cooperate with a Private Practice for Reproductive Medicine in Münster (Dr. med. A. Mempel/Mrs. S. Stratmann). We see all males of the infertile couples treated there. Diagnostic procedures and therapeutic options for the male are performed in our Department. Weekly interdisciplinary conferences ensure the best treatment for the couple.

Endocrine disorders/hypogonadism

- The endocrinology outpatient clinic deals with diagnosis and treatment of primary and secondary hypogonadism, including Klinefelter syndrome, delayed pubertal development and the ageing male. Patients with gynecomastia are also diagnosed and treated. Male patients with late-onset hypogonadism are diagnosed and treated according to the actually available guidelines. All relevant clinical and hormonal tests are provided. All modern hormone replacement schemes (oral, transdermal and injectable testosterone preparations) are available as well as stimulatory treatment protocols in males with secondary hypogonadal patients and infertility.

Impotence and ejaculation disorders

- Patients with ejaculatory or erectile dysfunction are seen in the outpatient clinic in increasing numbers. This part of the clinic deals with erectile dysfunction, Peyronie's disease, penile curvatures and ejaculation disorders. Diagnostics include duplex sonography of the penile vessels and pharmacostimulatory tests. The treatment options available cover the whole spectrum of medical and pharmacological intracavernous treatment, reconstruction of the penile curvature and implantation of penile prostheses. Especially in patients with Peyronie´s disease surgery comprises plaque incision and grafting techniques as well as penile implants.

Oncological andrology:

- This part of our clinic provides the infrastructure and expertise for patients seeking cryopreservation of their semen (or spermatozoa of the testis if azoospermia is diagnosed) prior to undergoing oncological treatment. Most patients have testicular cancer, leukemia or lymphoma at the time of diagnosis. We see an increasing number of patients seeking help concerning persistent azoospermia after recovery from the oncological disease. We have also established a new collaboration with the Oncological Department of the Children's Hospital to provide options to pubertal boys with oncological diseases and their need for fertility preservation. Most patients are referred to us by the Department of Paediatrics, the Department of Oncology andthe Department of Urology. In 2012 we founded the Network "Androprotect" with the intention to establish a network of pediatric oncologist, urologists and andrologists that offers male patients with childhood cancer and male patients with prepubertal gonadal insufficency the option to preserve gonadal stem cells. Meanwhile, an European Task Force has been started to propagate fertility preservation options for prepubertal and adolescent boys. A first publication has been initiated, creating a consensus report that is presently under review. From Germany, Prof. Kliesch and Prof. Schlatt are involved in the Committee of the "ESHRE Task Force on Male Fertility Preservation".

Surgical andrology (urological andrology):

- Since June 2008, we have the ability to offer all andrological surgical procedures to patients in our Department.
- These treatment options include diagnostic testicular biopsies (to detect TIN), all microsurgical procedures such as microsurgical testicular and epididymal sperm extraction techniques, microsurgical varicocele ligation, microsurgical vasovasostomy and vasotubulostomy for refertilisation.
- Concerning the severest group of infertile males, the azoospermic patients, we extended our activities regarding TESE-ICSI and MESA-ICSI cycles in the last five years. The German IVF Registry shows a frequency of 3-5 % of these procedures in all ICSI cycles. In our Centre, the proportion of treatment with TESE or MESA sperm is 22% of patients. Since June 1, 2008, responsibility for these surgical procedures was transferred from Urology to our Department. The **microsurgical testicular sperm extraction** technique is implemented as a standard procedure for non-obstructive azoospermia since 2008.
- Patients with erectile disorders can be treated by the implantation of penile prostheses. In case of penile curvatures, mainly in combination with Peyronie´s disease, the surgical correction of penile curvature can be offered either with simple Nesbit procedures or with plaque incision and grafting procedures with or without combination with penile implants.
- Hypogonadal patients or cancer patients whose treatment results in anorchia, can be provided with testicular prostheses.
- Testicular cancer patients diagnosed at our department can receive surgical treatment in our Clinic. Further treatment involving chemotherapy or radiotherapy is undertaken in close collaboration with the Department of Urology and the Department of Radiotherapy.
- The surgical procedures are done by Prof. Kliesch and two to three trained urologists or trainees at our Department. Since 2008, patient numbers treated are steadily increasing (2008 : 70 pts., 2009: 160 pts. , 2013: 253 patients).

Diagnostic and laboratory tests:

- Ultrasonography of the scrotal content is performed routinely as a complement to the physical investigations. Transrectal

ultrasonography of the prostate and seminal vesicles is performed in all hypogonadal patients and in infertile men where indicated (e. g. obstruction). Ultrasonography and duplex sonography of the penile structures is routinely performed in patients with erectile dysfunction or ejaculation disorders. The tests include duplex sonography in pharmacologically stimulated erectile dysfunction patients. Ultrasonography of the retroperitoneum is offered as well as sonography of the thyroid gland and the peripheral arteries.

- Semen analysis is performed according to the WHO guidelines with an established internal quality control programme. In certain studies, semen analysis is supplemented by a computer assisted sperm motility analysis (CASA: Hamilton-Thorne). Beyond its daily diagnostic responsibilities, the andrology laboratory serves as a WHO reference laboratory providing information and guidance on techniques and instruments employed in the analysis of semen. As such, procedures are conducted as per the WHO guidelines and the veracity of the results assessed by internal and external quality control programmes. The laboratory participates in the British (UKNEQAS), the European (ESHRE) as well as the German (QuaDeGa) scheme, where it also serves as the instigating centre. The accuracy and precision of seminal plasma biochemistry assays are gauged by participation in the Karolinska Institute programme.
- Bacterial culture of semen is performed in collaboration with the Institute of Microbiology.
- For endocrinological and infertility diagnosis, in 2013 about 26,000 hormone determinations per year are performed for patient care as well as for research purposes. In 1996, a fully automatic hormone analyser was introduced which has permitted much higher asssay precision. All hormone assays are subject to quality control according to the stringent criteria of national and international regulating agencies. The development of new techniques for measuring well-known hormones as well as the introduction of methods for new hormones are one of the Centre's core activities. Inhibin B, AMH and DHT have now been added to the list of laboratory tests measured.
- Testicular histology: Testicular biopsies are examined for diagnostic and treatment purposes in cases of azoospermia, for

detection of testicular intraepithelial neoplasia and testicular cancer, and for sperm retrieval. Annually, more than 1,650 PAS and 850 PLAP stainings are performed in our histology lab by trained technicians. For semi-quantitative analysis, the score system according to Bergmann &Kliesch is used.

- Genetic diagnostics: Like in the last years we continued to screen infertile men with sperm counts >1 million for microdeletions in the AZF region. For this we are applying a multiplex PCR recommended by the EAA guidelines and we are successfully participating on an annual basis in the EMQN External Assessment Quality Schemes for AZF. In addition we are determining in patients with signs of androgen resistance the CAG repeat number in the androgen receptor, being known to be a modulator of androgen action. The unique setting at the CeRA not only allows to perform routine diagnostics (such as AZF deletions and CAG repeat analysis), but also paves the way for DNA based association studies, screens for mutation analysis of candidate genes as well as the development of new genetic markers for male infertility. Very recently we added epigenetics to our DNA diagnostics. We now are studying whether aberrant methylation could contribute to impaired spermatogenesis. We have set up a normal range of methylation profiles to study infertile patients in much more depth. Apart from this, FSH receptor and promotor polymorphism are within the focus of our very recent and successful work to study genetic causes of male infertility.
- Bone density and body composition measurements:
  Since January 2014 bone densitiy can be measured by DXA scan, indicated especially in mals hypogonadal patients which are at risk for osteopenia. In addition, in patients with metabolic syndrome, body composition can be measured as well. Especially for future studies in hypogonadal patients, this instrument will give access to important and new data.

Clinical database Androbase

Due to Professor Nieschlag's activities and the commitment of PD Dr. Frank Tüttelmann, former co-worker of the center and now scientist in the Institute of Human Genetics of the University, a well adopted database for andrological patients was established. Named

Androbase©, this software allows the digital documentation of all patient- and proband-related entries, all laboratory results and all clinical findings/diagnoses. Furthermore, this software also allows to do continuous development and to build up an enormously valuable database providing the basis for systematic analysis of andrological patients and andrological diseases. After an official evaluation of the University IT Department, Androbase has become the official and fully supported database for the CeRA and the Institute of Human Genetics (Genesys). Within the last 3 years all prerequisites were established to switch from patient records to a fully digital patient record system. When starting the Private Ambulance in the VVS, we started on a merely electronic patient data system with Androbase and have the aim, to fully implement it also for the Domagkstraße in 2014 to get rid of the written documentation system were possible.

Medical education and clinical training programme in andrology

MDs receive training in all clinical activities: fertility clinic, endocrinology, erectile dysfunction, oncological andrology including cryopreservation, surgical andrology and, in collaboration with the Women's Hospital, assisted reproduction. New MDs are first assigned to a senior assistant and are gradually permitted more responsibility in the clinical care of patients. All patients/couples are presented to the Clinical Director or the Senior Registrar and all cases are discussed in the weekly clinical rounds (3 hours every Wednesday). MDs perform and/or interpret diagnostic procedures themselves (i.e. endocrine function tests, semen analysis, imaging procedures) and pass through the clinical laboratories to train in semen analysis and hormone assays for several weeks. Those remaining longer than one year (on average, MDs stay for 3 to 5 years) receive training in ultrasonography, including transrectal and duplex sonography and histological evaluation of testis samples.

All MDs participate in weekly rounds with the gynaecologists. They are also involved in clinical trials and hence receive training in conducting trials subject to the strict rules of the European and USA regulatory agencies. MDs also engage in laboratory work which may be clinical and for basic research. In their individual research projects and by participating in the regular Progress Reports of all collaborators (every

Friday), they learn how to conduct research. Interaction of the Centre members involved in this interdisciplinary research occurs at weekly meetings where recent results and the direction of current projects are discussed. New research topics, techniques and highlights from meetings attended are discussed in the weekly Journal Club. To compile grants, individual counselling is offered and extended scientific meetings (XXL Meeting) on a monthly basis have been implemented in 2013. The weekly and monthly research meetings and the Journal Club are presented in English promoting the use of scientific language for all scientific co-workers.
From the German authorities, Professor Kliesch is licenced to conduct full training in andrology and the 12 month urology training. Professor Zitzmann is licenced for training in internal medicine for twelve months and endocrinology for another twelve months. Since 2010, another 4 andrologists (R. Bongers 2012, J. Rohayem 2012, F. Gottardo 2010, E. Vorona 2010) have passed the EAA exam and 3 the German exam at the State Medical Board (U. Eppelmann 2012, A. Tiemann 2013, J. Rohayem 2013). Moreover, three colleagues were trained in Urology for 12 months each in our Department.
Medical students regularly visit our Centre for shorter or longer teaching periods. Since 2009, the Centre has been engaged in the curricular teaching program of the Medical Faculty during the regular semester. For this purpose, we have compiled a case for an e-learning module concerning a male infertile patient. This e-learning program was first introduced in 2009 and has been well accepted and well evaluated by the medical students of the fifth clinical semester. Since 2010, another two electronic cases have been developed. We cover the field of male hypogonadism, erectile dysfunction and male infertility by these electronic patients. Moreover, the scientific co-workers of both our institutions take part in regular teaching lessons. Once per year a three-day Summer School in Reproductive Biology and Medicine is offered to advanced biology and medical students. This Summer Academy is restricted to a maximum of 40 participnats and will be presented for the 23rd time in 2014. Since 2010 it is included into the curriculum of the Medical Faculty.

The Centre maintains a very well equipped library maintaining all relevant Journals in the field of reproductive medicine and biology, reproductive endocrinology and andrology. Furthermore an impressive

list of monographs and text books is available. The library is equipped with electronic access to the Central and medical libraries and the Internet and also provides modern IT equipment for PPT-presentations. Since the library holds space for up to 50 people it presents a perfect and heavily frequented place for all scientific meetings and educational events with up to 50 participants. The library is also the forum for regular seminars on topics in reproductive medicine/biology. Seminars are offered either on Wednesday to our own staff but also to members of the medical and other faculties at the University Münster. Despite of Seminars by Invited Speakers the Centre organized a Seminar series entitled “Six to Six”. This series is specifically dedicated to translational topics and usually is presented by a basic reseacher and a clinician presenting a focussed topic from different point of views.

Apart from the medical teaching and the teaching of the residents in urology and andrology, the Centre offers regular training courses (8 to 10 per year) for semen analysis for medical doctors and technical assistants. Up to now, 87 courses have taken place with 1187 participants. During a one day-course, the participants are trained in the practical performance of semen analysis and the evaluation of its results and learn the essentials of the internal and external quality control.

Research activities

The CeRA is engaged in a wide-ranging research programme, resulting in an impressive list of publications. Some of the most relevant topics being studied are explained in the following chapter:

- **DFG Research Group of Germ Cell Potential (Coordinator: Prof. Dr. rer. nat. J. Gromoll)**

The Research Unit aims to explore and exploit the potential of germ cells. It combines stem cell research and reproductive medicine. Using modern cellular and molecular techniques (stem cell culture, 2D and 3D microscopy, methylation analysis, expression profiling and proteomics), it investigates the development and decline of the germ cell potential during in vivo and in vitro gametogenesis. A major focus is the genetic and epigenetic determinants of the germ cell potential.

The projects take advantage of the particular strengths of well-established animal models and clinical studies.
The Research Unit is composed of basic and clinical scientists, who work in gynecology, andrology, veterinary medicine, genetics and developmental biology. We expect that this multidisciplinary approach will not only advance our understanding of the germ cell, but also generate the basis for novel treatment protocols.

List of projects

Project 1: Segregation of genomic sequence variations and contribution to loss of diploid and haploid germ cells.
F. Tüttelmann. A. Röpke (Münster)
Project 2: Spermatogonial stem cells and their potential.
J. Nolte (Göttingen), U. Zechner (Mainz), W. Schulze (Hamburg), W. Engel (Göttingen)
Project 3: Features and developmental changes of spermatogonial stem cells and their niches in marmoset monkeys.(Callithrix jacchus).
St. Schlatt (Münster), J. Gromoll (Münster), R. Behr (Göttingen
Project 4 (new) : Exploring the contribution of human testicular peritubular cells on testicular stem cells and their niches: A "secretome" analysis and in vitro studies
A. Mayerhofer (München), G. Arnold (München), St. Schlatt (Münster)
Project 5 (new): Epimutations in the male germ cell and possible consequences for ART outcome.
J. Gromoll (Münster), Th. Haaf (Würzburg)
Project 6: In vitro derivation and maturation of oocytes from murine embryonic stem cells.
H. Schöler, K. Hübner (Münster)
Project 7: Molecular analysis of disorders of gonadal development
P. Wieacker (Münster), S. Ledig (Münster)
Project 8: Developmental potential, epigenetic status, proteome profile and health of mammalian oocytes and embryos from cryopreservation and improved multistep follicle culture.
U. Eichenlaub-Ritter (Bielefeld), G. Griesinger (Lübeck), T. Haaf (Mainz), G.J. Arnold (München)
Project 9: Epigenetic analysis of the bovine model in oocyte and embryo development.
H. Niemann (Mariensee), C. Wrenzycki (Hannover), T. Haaf (Mainz)
Project 10: Structural, molecular and functional analysis of early bovine embryogenesis: readouts for oocyte potential after in vitro vs. in vivo maturation.
F. Habermann, G. Arnold, E. Wolf (München)
Project 11: In vitro maturation (IVM) - clinical follow up of pregnancies achieved by in vitro maturation and consecutive IVF and analysis of IVM oocytes in the human and a mouse model.
Th. Strowitzki (Heidelberg), Th. Haaf (Würzburg) , U. Eichenlaub-Ritter (Bielefeld)
Project M: Management of the Research Unit.
J. Gromoll (Münster)

In vitro *spermatogenesis*

To transfer male germ line differentiation into a culture dish has been approached by reproductive scientist for almost a century (reviewed in Reuter et al 2012) but still remains experimental. The topic has faced increasing importance as the need for methods providing in vitro

matured spermatozoa is forced by an increasing number of in particular young patients suffering from malignant diseases which can be fortunately cured nowadays and become long term survivors. However the therapies used still provoke long-term infertility as a severe side effect of treatment and no sufficient method is available to preserve fertility in those boys which can not provide a semen sample for cryopreservation before surgery.

As conventional cell cultures do not provide the spatial arrangements which testicular cells encounter in their natural environment, we developed three dimensional cell culture approaches to reconstitute tubulogenesis and spermatogenic differentiation in matrices as well as artificial scaffolds using murine models; providing a microenvironment that resembles the three-dimensional (3D) *in situ* organization of the seminiferous epithelium better.

We demonstrated previously that male mouse germ cells in 3D matrices soft agar culture system; methylcellulose culture system) are able to form morphologically mature elongated spermatids. In a more recent approach granted by the medical faculty, we used collagenous sponges as artificial scaffold and were successful in colonization of these by isolated testicular cells which formed clusters resembling features of early tubulogenesis (Reuter et al, 2014, in press), but although undifferentiated spermatogonia could be maintained in this system for several week , no spermatogenic progress was observed yet, very likely due to different culture media needed from a yet unidentified stage onwards. Nevertheless these approaches will contribute important basic data along the route to develop strategies for fertility preservation by culture of the male germ line. Currently we transferred the method to primate testicular cells from marmosets and could confirm the observations made in rodents (unpublished data). These studies are now continued systematically in order to translate the methodology into human spermatogenesis.

Innovative studies exploring the effects of nanostructures of cell culture surfaces and the co-culture with pluripotent stem cells were applied to understand the process of testicular morphogenesis. A collaboration with physicists at the CENTECH nanotechnology center provided access to cutting edge technology. The reassembly of cord-like structures in vitro could facilitate the construction of cell culture

systems to promote spermatogenesis. We have recently published our data on cord formation using rat primary Sertoli cells (Pan et al., 2013). We are convinced that these data provide the foundation for novel concepts into creating testicular cultures to promote spermatogenic activation.

Stem cell characterisation and physiology

A combined approach facilitates the reliable detection of human spermatogonia *in vitro:* While some studies have reported that human spermatogonial stem cells (SSC) can be maintained *in vitro* and show characteristics of pluripotency, the germ cell origin and the differentiation potential of these cells was subsequently called into question. The aim of our study was therefore to establish an integral approach for the unequivocal detection of human germ cells and particularly of spermatogonia *in vitro*. We succeeded in conclusively showing that an integral approach comprising the 1. detailed characterization of patients and tissue samples prior to the selection of biopsies, 2. the use of unambiguous markers for the characterization of cultures and 3. the use of biopsies lacking the germ cell population as negative control is the prerequisite for the establishment of human SSC cultures. Thus, this study forms the basis for future long term cultures of human SSCs which may facilitate the development of novel therapeutic strategies for the treatment of male infertility.

The role of the CXCL12-CXCR4 system in the homing process of human adult testicular stem cells: It has been shown in several organs that an up-regulation of the chemokine Cxcl12 occurs following stem cell loss which facilitates the recruitment of $Cxcr4^{+}$ adult stem cells and thereby aids tissue regeneration. As it remains unknown so far whether the loss of spermatogonial stem cells results in an increased expression of Cxcl12, the aim of this study was to investigate the expression pattern of testicular somatic markers as well as of Cxcl12 and Cxcr4 following an induced germ cell loss in adult mouse testes. Interestingly, while expression levels of most somatic marker genes did not change significantly, expression levels of *Cxcl12* were significantly increased after treatment. Our data therefore demonstrates that germ cell loss is associated with an up-regulation of

Cxcl12 expression, indicating a role of the Cxcl12 /Cxcr4 interaction for the recovery of spermatogenesis.

Sperm physiology

The working group lead by Con Mallidis, PhD, has focused on investigating the causes, identifying the manifestations and developing methods of circumventing sperm nuclear DNA damage. Specifically we have studied different instigators of oxidative stress, be they physiological (i.e. diabetes, infection) or induced (i.e. UV irradiation, Fenton reaction), chronicling their effects and characterizing their actions. We have developed a novel means of assessing individual sperm allowing us to non-invasively and non-destructively appraise the chemical and molecular integrity of each cell. The technique, Raman microspectroscopy, also opens the possibility of developing a means of assessing, selecting and providing nDNA intact sperm for use in ART. In a parallel study, the clinical applicability of the technique has been assessed, characterizing tissue and cells from germ cell tumours in an effort to identify markers that will allow for more accurate and timely therapeutic options. In the passed three years, these studies have resulted in the publication of 10 original research articles, 3 reviews, 2 book chapters, 2 technical notes and garnered 4 favourable commentaries.

Assisted reproductive technology: Testing of IVF culture media and the development of microfluidics for embryo selection

Culture media and their influence on early embryonic life span: The DFG granted a bilateral application from the Netherlands and Germany regarding human culture media and their influence on early mouse embryonic development. The results have created international awareness that research on this topic is mostly lacking. We have designed studies exploring the effects of culture media using the mouse model. We found effects on pre- and post-implantation development, e.g. cell numbers in blastocysts from different culture media were affected as well as the type of fertilisation had effects. After implantation no major effects were detectable. This indicates that implantation is an important check point from which morphological

development seems to be normal, regardless of early influences of culture media. More studies are necessary and planned, especially epigenetic questions have to be analysed in the future.

ART unit: The use of ART has increased worldwide. But still the success rates remain low. Commonly the best embryo is chosen for embryo transfer. But in our opinion it is of more importance to start at the beginning of assisted reproduction procedures: the gametes. Therefore further research has to be done on the selection of the best sperm for the best oocyte. New techniques have been implemented in our IVF laboratory and several questions have been studied, e.g. the laser-selection of immotile spermatozoa for ICSI, TESE indication for retrieval of spermatozoa, polarisation microscopy for ICSI timing and maturation status of metaphase I oocytes.

Epi-genetic diagnosis and research on male infertility

Very recently we implemented the routine screening for the FSHB haploptype in all patients at the CeRA. It turned out that the FSHB TT haplotype is strongly associated with impaired spermatogenesis and reduced testicular volume. Together with the FSH receptor single nucleotide polymorphism we have now a genetic setup in which we could identify a subgroup of patients which would benefit from a FSH treatment, allowing for the first time a pharmacological approach to cure male infertility.

As a new avenue in the genetic diagnostic set up for male infertility, we introduced the first steps of routine screening programme in sperm of infertile men for aberrant DNA methylation. The first results, obtained from an imprinted gene (MEST) are promising and might lead to a new parameter in the andrology lab eventually allowing to prospectively predict fertility.

We have developed a sensitive method based on the laser-capture microdissection of routinely fixed testicular biopsies that enables the analysis of the DNA methylation levels of several genes in cell-specific samples. Using this strategy, we are studying the DNA methylation levels of several genes in individual cell types derived from testicular biopsies ranging from complete spermatogenesis to meiotic arrest. In addition, by using micromanipulation, we are able to obtain samples

containing a maximum of 10 spermatozoa. The measurement of DNA methylation levels in these small sperm samples is less affected by intra-individual variability and a large screening of epigenetic heterogeneity in sperm samples in currently ongoing.

Clinicl studies on male infertility, male hypogonadism and hormonal male contraception

Male Contraception has always been a key target in the research of our Centre. A hormonal approach to reach this goal has been targeted by our researchers in collaboration with other centres. In 2009, the world-wide largest efficacy trial on hormonal male contraception under the auspices of the WHO and CONRAD was initiated using testosterone undecanoate and norethisterone in injectable forms. Muenster participates as a leading centre with more than 50 couples and is the centre laboratory for this multi-national trial. Analysis of serum samples is still ongoing and will be finalised this year.

In 2009, we started with a controlled observational trial on the effects of testicular cancer and its treatment on the occurance of hypogonadism and the metabolic syndrome. This project is ready for publication by now.

In 2010, a clinical research award of the Medical Faculty was given to the researchers of the Centre in colloboration with the Human Genetics Department to investigate the epigenetic effects of the supernumerary X-Chromosome in Klinefelter men in relation to parental origin, gene activation, the CAG repeat polymorphism, cardiovascular risk and inflammation as well as fertility. The study is finished and the manuscript presently under review. The project was named the **Münster EXAKT project**: epigenetics and clinical applications in Klinefelter Syndrome. The study is finished and the manuscript presently under review.

In addition, the clinical results of microsurgical TESE procedures in Klinefelter patients since 2008 were analysed and parameters were identified to predict TESE outcome, especially in respect to age. Age and markers of Leydig cell function, but not of Sertoli cell function could be identified to predict the success of sperm retrieval in a cohort of 135 adolescents and adults with Klinefelter's syndrome.

In 2011, a clinical prospective multicentre trial started to investigate the induction of puberty and fertility with hCG/rFSH in boys with hypogonadotropic hypogonadism. The aim of the study is to elucidate the best induction treatment in respect to the psychological and physical health of boys and adolescents. The recruitment of the study is finished and treatment phase will be accomplished at the end of 2014.

In addition, with the help of a medical thesis the best differential diagnosis between constitutional delay of puberty and true hypogonadotropic hypogonadism was re-evaluated in a cohort of 50 young adolescents. Serum Inhibin B, less so INSL 3, and AMH levels were identified to be helpful in discriminating between constitutional delay of growth and puberty (CDGP) and hypogonadotropic hypogonadism (HH) in boys with delayed puberty.

In 2012/2013 a clinical prospective controlled international multicentre trial investigating the efficacy and tolerability of a new oral testosterone preparation was performed. Patient enrollment and treatment is finished and evaluation is ongoing.

In 2013, one of the andrological urological trainees evaluated the results of our microsurgical vasovasostomy programme in post-vasectomy patients. An overall patency rate of 85% with a clinical pregnancy rate of 49% could be documented in more than 200 patients with more than 400 microsurgical procedures.
Our Centre participated in worldwide register study on the treatment of male hypogonadism in 1,438 men. Several trials concerning testosterone and its action and modulation by CAG polymophisms were performed concerning spatial abilities and psychological issues and published.

A study in conjunction with the Radiology Department on the effects of sex steroids on functional brain imaging (fMRI) in healthy men and women was initiated in 2010 and is being performed and still ongoing.

In 2013, a clinical international multicenter trial (prospective, randomized, controlled study) for investigation the efficacy and

tolerability of a new transdermal axillary testosterone was started. Patient enrolment is ongoing.

Oncological Andrology

A retrospective cohort analysis of oncological patients cryopreserving their spermatozoa was initiated. The analysis of follow up investigations after 4-5 years after end of treatment revealed relevant results on recovery rate of spermatogenesis. The data were collected in the frame of a medical thesis which was finished in January 2014.

To clarify the significance of microlithiasis testis as an indicator for TIN in infertile patients, another medical thesis was initiated and is still under investigation.

To explore the possibilities of detecting carcinoma cells in semen samples of male infertile patients at risk for germ cell cancer, a pilot project was started to detect TIN and seminoma cells by RAMAN spectroscopy.

Another project deals with the differentiation of seminomas stage I and stage II to III by RAMAN spectroscopy and is still ongoing. Within a medical thesis, first results of differences of protein structure of seminoma cells with the potential to metastasize could be obtained.

We have recently begun to perform germ cell transplantation experiments using seminoma cell lines to explore the cancerogenic potential of these cells in the testicular microenvironment. These transplantation approaches of cells and tissues will continue at CeRA. We anticipate that these strategies will provide us with models to study human testis cancer development which are currently not available. These unique and promising animal models will therefore lead us to an improved knowledge on the development of testic cancer from precursor cells.

Animal models at the CeRA

Pathophysiology of sex-chromosome imbalance in the male: *The 41, XX^{Y*} mouse model*

A supernumerary X chromosome is the hallmark of the most frequent genetic cause of male infertility, the Klinefelter Syndrome. Klinefelter men exhibit a karyotype of 47,XXY linked to dramatic phenotypical changes compared to normal XY males affecting endocrinological regulation and reproductive function accompanied by cognitive and behavioral defects but also resulting in increased mortality and morbidity (reviewed in Wistuba 2010, Wistuba et al 2013) .
Although addressed in many studies since its discovery, the knowledge and understanding of molecular and genetic mechanisms underlying the syndrome are still limited, mainly due to the lack of experimental access that requires reliable animal models.

Almost a decade ago we therefore started to establish and characterize a Klinefelter mouse model at the institutes facilities and succeeded in breeding male mice with two X chromosomes (XX^{Y*}) from fathers carrying a structurally rearranged Y chromosome (Y*). Animals displaying the karyotype 41, XX^{Y*} were found to present the Y-chromosomal signal adjacent to one of the X chromosomes.
Our mice were found to closely resemble the features of the human KS as germ cell loss, Leydig cell hyperplasia, normal inactivation of the second X-chromosome, hypergonadotropic hypogonadism, and cognitive deficits (for review see Wistuba et al. 2013).

In a continuing follow up project granted by the DFG we made use of these model animals over the last years to analyze in detail the postnatal developmental course of the germ cell loss. We described that spermatogonial stem cell marker expression is fading very early in life, we observed changes in the Sertoli cell number and physiology and we could provide evidence that the testicular vascularization is disturbed and might be involved into both, the germ cell loss as well as the endocrine phenotype (Werler et al. 2014, in press, Tuettelmann et al. 2014 in press). Similarly, we could also establish a link between findings from the mouse model concerning genes escaping from X inactivation which we were able to determine to be expressed in a tissue and gene specific manner in the mice with findings from the Münster EXAKT project in which these escapee genes were shown to play also an important role in the human patients (Werler et al. 2011, Zitzmann et al., under revision).

We intend to continue this research by exploring germ cell loss, genetic imbalance and angiogenesis in our mouse model. We would like to put the focus on the most relevant period of disturbed embryonic testicular development in our XXY model mouse which is obviously *in utero*.

Stem cells in the Mexican axolotl (Ambystoma mexicanum)

Our Centre is maintaining an axolotl breeding colony in order to provide our studies with embryonic, larval and adult axolotls at any ontogenetic stage of development. The axolotl is a neotene salamander (Amphibia, Urodela) from central Mexico. One of the outstanding features of this species is the enormous regenerative potential allowing regrwoth of limbs and extremeties after injury. We have recently explored if the enormously efficient regeneration of tissues and organs is also present in inner organs. Interestingly removal of the testes did not lead to recovery and regrowth of the organ indicating that the high regenerative potential is not a general phenomenon of this species.

Pluripotent stem cells in the primate testis

Unipotent spermatogonia are derived from potentially pluripotent primordial germ cells. Primate testes contain two types of spermatogonia: a) reserve stem cells and b) self renewing progenitors. While rodents generate about 4000 sperm per initial stem cell division, in man an initial progenitor division creates only 16 sperm, leading to very different kinetics in spermatogenesis in rodents and primates. So far, no systematic studies have explored the ontogenetic differentiation of male germ line stem cells in primates and it is not known, whether truly pluripotent stem cells remain in the testes even in adulthood. The adult primate testis contains two types of undifferentiated spermatogonia: the Adark-spermatogonia, reserve stem cells, and the Apale-spermato-gonia, self renewing progenitor cells. Histological criteria have been used for decades to distinguish the spermatogonial subpopulations, but so far no conclusive data have been generated on the biological roles of each of these subpopulations, and definitive biomolecular markers for each subpopulation are not yet known. In this ongoing study we address whether pluripotent spermatogonial precursors in the Cynomolgus Monkey (Macaca fascicularis) are gradually eliminated from the developing testis by (potentially reversible) differentiation into unipotent spermatogonia. We also

examine, whether the distinct spermatogonial subpopulations in the adult testis of a non-human primate, the Rhesus Monkey (Macaca mulatta), express distinct biomolecular marker combinations, and we have recently extended our study, now also including adult human testicular tissue. We use a panel of markers which allows us to histologically detect spermatogonia at various stages of differentiation in the adult monkey's testis. Using the expression of Oct3/4, Ap-2γ, VASA, GFRα1, Nanog, PGP 9.5, Tra -1-81, and Tra-1-60 we identify sub-populations of spermatogonia, aiming to determine the total number of pluripotent cells in a non-human primate's testis.
These studies will provide insight into the mechanisms behind differentiation of pluripotent germ line cells into unipotent testicular stem cells in primates. Our data may enable us to select a target population in fetal, neonatal and adult primate testes for the isolation and derivation of pluripotent stem cells. Recently we have explored morphogenesis of seminiferous tubules in xenografts and collagenous matrices. We have shown that soluble factors and surface modalities which can be engineered by nanostructure technologies influence the aggregation and tubule formation of testicular somatic cells. It is our goal to prepare *in vitro* conditions for generation of sperm from stem cells *ex vivo*.

Research funding

The main sources of current funding are the German Research Council (DFG), Federal Ministry of Health (BMG), Federal Ministry of Education (BMBF), The Northrhine Westfalian Ministry for Innovation, Science and Technology, the Interdisciplinary Clinical Research Centre of the Medical Faculty (IZKF), Innovative Medical Research of the Medical Faculty (IMF), World Health Organisation (WHO), the Friedrich-Krupp von Bohlen und Halbach Stiftung, the Deutsche Krebshilfe, the DAAD and the ERASMUS program.
Industrial funds also provide support for other projects and for our own tax exempt foundation (Klinische Forschergruppe für Reproduktionsmedizin) which provides funds for travel of scientists, small research equipment, costs for probands and student salaries.

Publications

Since the last site visit in 2010, more than 176 peer-reviewed articles have been published and 51 book chapters have been written. 391

scientific talks have been given on national and international congresses. Two editions of an andrology book (in both German and English) in this time. All these can be found in the attached list.

Table 1 - Staff list and sources of funding

(blue – Dep. Clinical Andrology,; white - IRRB

Title	Christian name	Name	Project
Scientific and Medical staff			
Prof. Dr. rer. nat.	Stefan	Schlatt	WWU
Prof. Dr. med.	Sabine	Kliesch	UKM
Prof. Dr. rer. nat.	Jörg	Gromoll	WWU
Prof. Dr. med.	Michael	Zitzmann	UKM
M.D.	Natalya	Brauckmann	Research
Dr. med.	Karen	Czeloth	UKM
Dr. med.	Claudia	Krallmann	UKM
M.D.	Waseem	Moussa	UKM
Dr. med.	Julia	Rohayem	WWU / UKM
M.D.	Georgios	Spiliopoulos	UKM / Research grant
Dr. med.	Arne	Tiemann	UKM
Dipl. Biol.	Oliver	Damm	DFG
Dr. rer.nat.	Nina	Kossack	WWU
M. sc.	Daniel	Langenstroth	DFG
Dr. rer. nat.	Verena	Nordhoff	WWU
Dr.	Con	Mallidis	WWU
Dipl. Biol.	Oliver	Damm	DFG
Dr. rer. medic.	Klaus	Redmann	WWU
Dipl. Biol.	Karin	Reuter	IMF
Dr. rer. nat.	Sandra	Laurentino	DFG
Dr. rer. nat.	Joachim	Wistuba	WWU
Dipl. Biol.	Birgit	Westernstroer	IMF
Dipl. Biol.	Stefanie	Werler	DFG

Technical assistants

Titel	Vorname	Name	Project
	Joachim	Esselmann	WWU
	Sabine	Forsthoff	WWU
	Daniela	Hanke	UKM
	Barbara	Hellenkemper	WWU / QuaDeGA
	Jolanta	Körber	DFG/WWU
	Heidi	Kersebom	UKM
	Elke	Kößer	UKM
	Raphaele	Kürten	UKM
	Lisa	Lahrmann	WWU
	Sabine	Rehr	UKM
	Jutta	Salzig	UKM / BMBF
	Reinhild	Sandhowe	WWU
	Nicole	Terwort	WWU

Secretaries/Administration

Title	Christian name	Name	Project
	Anne	Olerink	WWU / DFG
	Ingrid	Rambow	UKM
	Ursula	Rüschhoff	WWU
	Barbara	Fischer-Rittmeyer	DGE

Doctor's receptionists

Titel	Vorname	Name	Project
	Birgit	Conrads	UKM
	Christina	Rein	UKM
	Jacqueline	Wilke	UKM
	Susanne	Orlowski	UKM
Trainee	Valerie	Wall	UKM
Trainee	Agnes	Badura	UKM

Animal caretakers

Title	Christian name	Name	Project
	Martin	Heuermann	WWU
	Günter	Stelke	WWU

Guests/Scholarship Holders

Title	Christian name	Name	Project
	Victoria	Sanchez	DAAD
	Kuong	Tran	CEDAD

Extraordinary Professorship

Prof. Dr. phil. Gerhard F. Weinbauer Covance Laboratories, Münster
Prof. Dr. med. Axel Kamischke Kinderwunschzentrum Münster

Sources of Salary:

WWU = Institute for Reproductive and Regenerative Biology, Westf. Wilhelms University
UKM = Department of Clinical Andrology, University Clinic

BMBF = Federal Ministry of Education, Science, Research and Technology
DAAD = German Academic Exchange Service
DFG = German Research Council
IMF = Innovative Medical Research Centre of the Medical Faculty Münster

IZKF = Interdisciplinary Clinical Research Centre of the Medical Faculty Münster

QuaDeGA= External Quality Control Assessment of the German Society of Andrology

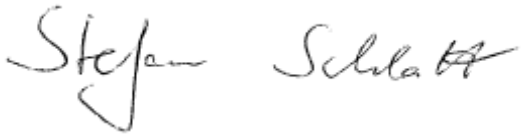
Table 2. Number of consultations over the last 5 years

	2009	2010	2011	2012	2013
New patients	926	851	837	932	989
Patients with previous consultations	2060	1948	2154	2355	2316
Total	**2986**	**2799**	**2991**	**3287**	**3305**
	2005	2006	2007	2008	2009
New patients	618	673	729	879	926
Patients with previous consultations	1081	1135	1262	1453	2060
Total	**1699**	**1808**	**1991**	**2332**	**2986**

Table 3. Number of semen and hormone analyses, histology, genetic and laboratory tests over the last 5 years

YEAR	2009	2010	2011	2012	2013
Semen analysis	2288	2959	3111	2853	2894
Antibody (MAR) test	1135	1344	1390	1518	1408
Eosine	1848	1947	1985	1989	1863
Culture	1050	1156	1425	1557	1636
Chlamydia	905	946	1118	1170	1122
Chlamydia PCR	15	9	14	4	7
Combur	13	8	5	7	14
Probe-Swim-Up	518	608	760	773	730
Sperm survival test	499	549	616	768	578
Insemination	125	144	114	91	97
IVF	48	29	34	41	26
ICSI	181	148	165	142	166
Computer analysis (CASA)	90	408	451	549	483
TESE	5	6	3	0	0
Microsurgical TESE	100	127	120	141	170
MESA	8	13	13	9	5
Cryopreservation procedures	196	262	343	253	268
Patients for cryopreservation	102	123	154	111	133
TESE long-time storage	63	61	68	78	104
Enzymatic preparation	780	1530	1404	1584	1929
Mechanic preparation	68	26	23	52	38
Semen analysis from urine	28	21	32	10	16
Quality control internal	40	40	38	44	48
Quality control external	39	29	40	28	20

Glucosidase	1531	2164	2187	2088	2106
Fructose	1534	2164	2186	2116	2107
Zinc	1535	2163	2175	2116	2102
Histology patients	161	197	214	194	221
Samples prepared for histology patients	659	853	876	786	873
PAS-staining	1173	1642	1762	1503	1671
PLAP-staining	887	1019	974	814	851
hLH FIA	2669	3497	3357	3510	3335
hFSH	2668	3495	3355	3511	3323
hPRL FIA	2633	3156	3267	3391	3243
T EIA	2727	3518	3369	3542	3408
E2 FIA	2628	3176	3272	3419	3319
SHBG	2634	3159	3278	3435	3330
PSA	2217	2806	2930	2676	2499
free PSA	80	139	150	125	161
free T	2633	3148	3128	3419	3315
Ultrasonography	1980	2646	2603	2640	2649
CAG CeRA	216	163	108	108	110
AZF CeRA	42	24	23	42	37
AZF IHG	220	186	182	214	200
CAG IHG	179	163	223	171	130
Inhibin B					116
AMH					60



Professor Sabine Kliesch, MD

Professor Stefan Schlatt, PhD