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ANDROLOGY

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Abstracts of the 9th Congress of the European Academy of Andrology

21 – 23 September 2016

Rotterdam, Netherlands

The merged journal of the American Society of Andrology and the European Academy of Andrology, now including the former *International Journal of Andrology and Journal of Andrology*

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American Society of Andrology

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ANDROLOGY

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WELCOME

Welcome message from the EAA President

Csilla Krausz
President of the EAA

On behalf of the European Academy of Andrology (EAA), it is my great pleasure to greeting you to the 9th European Congress of Andrology (ECA 2016) in Rotterdam. Dissemination of knowledge in the field of andrology represents one of the major missions of the EAA. Accordingly, a European Andrology Congress is organized every second year, providing an ideal platform for interaction between andrologists. The interest towards ECA 2016 appears similar to the previous meeting in Barcelona, which was attended by basic and clinical andrologists from over 40 countries. The excellent scientific program provides a forum for specialists to discuss the latest developments in male reproduction, onco-andrology, male and female sexual dysfunction, sexually transmitted diseases and some aspects of andrological surgery. I would like to thank the Program Organizing Committee, chaired by Prof Niels Skakkebaek, for their excellent work. Many thanks also to the participants who sent their abstracts. There will be plenty of opportunities to interact between clinicians and basic scientists during the guided poster sessions. The EAA has provided numerous Travel grants for young affiliated members and in addition poster prizes will be awarded to the best poster presenters.
As in the previous two editions, joint workshops with the European School of Urology (ESU) and the European Society of Andrological Urology (ESAU) have been organized, underpinning our latest efforts aimed at the establishment of a Multidisciplinary Joint Committee in andrology. We also continue with the traditional support of the symposium dedicated to the International Network for Young Researchers in Male Fertility.
It is clear that many unsolved questions in andrology should be addressed by large, multicenter studies requiring EU support. Hopefully such an important European event will also help bringing the attention of funding organisations to male reproductive and sexual health and will also represent a starting point for future joint projects.
I am confident that the meeting will be of major scientific interest and on behalf of the EAA I thank the Local Organizing Committee for all their efforts to organize the meeting in such a superb venue.
Have a great meeting!

Welcome by the Local organizer of ECA2016-

Dr. Gert Dohle, MD, PhD,
Clinical Andrologist EAA
Chairman of the Local
Organizing Committee

Dear friends-Andrologists,
The department of Andrology of Erasmus MC in Rotterdam, the Netherlands is hosting the ninth European Congress of Andrology from 21-23 September 2016. This special issue of Andrology highlights the scientific program of ECA2016. We are pleased that so many specialists in Andrology have registered for this meeting. The scientific level of the invited lectures and of the submitted abstract promise to make this meeting an outstanding congress. We are grateful to the program organizing committee for designing such an excellent program and to the support of the executive board of the European Academy of Andrology.

The congress bureau and myself have been working very hard to organize all logistics and the social program, dedicated to Antoni van Leeuwenhoek and the city of Delft, where he was the first to discover spermatozoa. We very much look forward to meeting you for this congress and are confident that it will be a most memorable event for all participants.
Sincerely,

Program organizing committee
Niels Erik Skakkebaek (Chairman, DK)
Jolanta Slowikowska – Hilczer (POL)
Emmanuele Jannini (IT)
Frederic Chalmel (FR)
Zsolt Kopa (HU)
Frank Tüttelmann (DE)
Herman Tournaye (BE)
Joao Ramalho-Santos (PT)
Linda Vignozzi (IT)

Local organizing committee
Gert Dohle (Chairman, NL)
Leendert Looijenga (NL)
Willy Baarends (NL)
Marij Dinkelman-Smit (NL)
Willem Boellaard (NL)
Luca Incrocci (NL)
Joop Laven (NL)

Congress organization
Marije Stofregen, Senior Conference manager– Congresbureau Erasmus MC

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GENERAL INFORMATION

Congress venue: De Doelen International Congress Centre is located nearby Rotterdam Central Railway Station in the city center of Rotterdam, surrounded by many hotels, shops and restaurants.

De Doelen International Congress Centre
Willem Burger Complex
Kruisplein 40
3012 CC Rotterdam
The Netherlands
Tel: +31 (0) 10 217 17 00

Smoking policy: De Doelen International Congress Centre is a non-smoking facility. Adjacent to the Willem Burger Foyer is a terrace where smoking is permitted.

Information and Registration desk: Located near the entrance on the ground floor of the Willem Burger Quarter (21 Sept and 22 Sept until 11.30 hrs) and in the Willem Burger Foyer on the 3rd floor (22 Sept from 12.00 hrs and 23 Sept).

Opening hours Registration desk: Wednesday 21 September: 08.00–09.30 hrs and 14.00–21.00
Thursday 22 September: 07.30–19.45 hrs
Friday 23 September: 08.00–14.00 hrs

Congress staff:

Het Congresbureau

If you have any questions please direct to the Congress staff: Marije Stofregen and Jiske George-Sluimer from Erasmus MC-Congress office. To be contacted at the Information and Registration desk.

Congress badge and Entrance policy: All people with a congress badge may enter the building and visit the scientific programme, attend the lunches and breaks. You have to wear your badge (visible at all times) during the whole congress period. People without a badge will be referred to the registration desk.

Speaker Service Centre (SSC): Invited faculty, satellite speakers and oral presenters can upload their presentation in the Speaker Service center. Please note to upload your presentation at least one hour before the start of your session. If you have an early morning session, upload your presentation the day before. Two laptops will be available to review your slides. A technician will be present for assistance.The SSC is located in the Hudig room (3rd floor).

Opening hours SSC:
Wednesday 21 September: 08.00–17.00 hrs
Thursday 22 September: 07.30–17.45
Friday 23 September: 08.00–13.00

Internet facilities (Wifi): In the Willem Burger Foyer (3rd floor), wireless internet is provided. Login details will be provide at the registration desk. Please note that the wireless internet is available to check your e-mail messages etc. The system is not suitable for heavy downloads.

Breaks and Lunches: All coffee/teabreaks and lunches will be served in the Willem Burger Foyer and will be served from buffets. Please note that you are able to pack your own lunch if you wish to attend the Satellite Symposium on 22 September in the Van Weelde room (4th floor).

List of attendance and Certificate of attendance: Please sign the list of attendance at the registration desk every day. This list is for accreditation purposes.

Participants will receive a digital certificate of attendance upon request after the congress. Requests can be sent to j.sluimer.1@erasmusmc.nl

Exhibitors: Exhibitors are situated in the Willem Burger Foyer (3rd floor). The exhibition will be open from Wednesday 14.00 hrs through Friday 13.00 hrs (September 21–23). Please visit the Exhibitors during the coffee breaks and lunches.

Rotterdam App: The Rotterdam App is a free mobile application that highlights the very best that Rotterdam has to offer, from attractions and museums to hotels, restaurants, clubs and shops. This mobile city guide features 500+ locations, an offline city map, walking tours and a dynamic events calendar that lets you discover what to see & do. To download the Rotterdam App, go to m.rotterdam.info/download on your mobile phone or download it via the App Store or Google Play.
More information: https://rotterdam.info/

Public transport: For overcoming longer distances within Rotterdam, you can travel by underground (metro), tram or bus. Rotterdam has several metro-lines. The metro is the fastest way to cross town. The Congress venue is within walking distance from most hotels.

Tickets:
If you travel by bus, underground (metro) and tram you must buy an OV chip card, available at the vending machines at the railway or metro stations. You can pay by bankcard, credit card or cash. There are three options in chip cards:

1 Disposable one trip card
2 Disposable (multi)day card
3 Anonymous chip card with credit which can be uploaded.

A credit chip card can be uploaded in the vending machines located at each metro station and per trip an amount is automatically deduced from card after entering and departing the sensor gates at the stations.

With all chip cards you must check-in and check-out with your chip card at the sensor gates at the stations.
Useful websites:
Trains (e.g. to go to the airport): www.ns.nl
Metro, bus or tram: www.ret.nl
OV chip card: www.ov-chipkaart.nl

Social event: Dinner and van Leeuwenhoek Lecture in Delft
Date: Thursday 22 September 2016
Time: 19.30–23.30 hrs
Price: € 50 per person

***Program*:**

19.30 hrs	Departure buses from congress venue
20.00 hrs	Arrival at the Museum Prinsenhof in Delft http://prinsenhof-delft.nl/
	Drinks, short lecture and buffet dinner
23.00 hrs	Departure buses to congress venue Rotterdam
23.30 hrs	Arrival at congress venue

Please note tickets must be purchased in advance via the registration website and seats are limited.

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PROGRAM

9th EUROPEAN CONGRESS OF ANDROLOGY – ECA2016 SCIENTIFIC PROGRAM

Wednesday 21/9/16

Post-graduate course Willem Burger Hall

09.00–10.00	**Diagnosis of male infertility PG1**	Chairs: M. Dinkelman-Smit (NL), A. Mahmoud (BE)
	F. Tüttelmann (DE): Genetic diagnostics of male infertility J. Ramalho-Santos (PT): Probes to monitor sperm function F. Chalmel (FR): Reproductive genomics and proteomics	
10.00–11.00	**Special problems in male infertility PG2**	Chairs: W. Boellaard (NL), T. Diemer (DE)
	A. Pilatz (DE): Urogenital infection and semen quality Z. Kopa (HU): Management of lesions of the testis H. Tournaye (BE): Klinefelter syndrome and fertility	
11.30–13.00	**Hypogonadism and male sexual dysfunction PG3**	Chairs: O. Apolikhin (RU), G. T'Sjoen (BE)
	J. Slowikowska-Hilczer (POL): Delayed puberty in boys S. Kliesch (DE): Hypogonadotrophic hypogonadism in male infertility L. Vignozzi (IT): Metabolic syndrome, systemic inflammation and erectile dysfunction	
13.15–14.45	Editorial board meeting of "Andrology"	
15.00–15.30	**Opening Ceremony Willem Burger Hall**	Chairs: G. Dohle (NL), C. Krausz (IT)
15.30–16.30	**Golden communications Willem Burger Hall**	Chairs: H.M. Behre (DE), G. Dohle (NL)
	LP Priskorn (DK): Is sedentary lifestyle associated with testicular function? A cross-sectional study of 1,210 men OR01 H.G.K.A. Angerer (DE): The German Male Sex-Study (GMS-Study): Differences in Sexual Behaviour and Number of lifetime Sexual Partners depending on Sexual Orientation Identity OR02 I. Tröndle (DE): Irradiation of juvenile primate testicular xenografts affects the somatic environment OR03 E. Lecluze (FR): Dynamics of the transcriptional landscape during human fetal gonad development OR04	
16.30–18.00	**State of the art lectures: (SA1) Willem Burger Hall**	Chairs: E. Rajpert-De Meyts (DK), N.E. Skakkebaek (DK)
	C. Krausz (IT): Novel Horizons in Andrology: a genetic perspective G. Buck Lewis (USA): Environmental Exposures and Male Fecundity and Related Impairments	
18.00–19.30	**Poster session 1a: Male infertility diagnosis MID 01- 28 Schadee Room**	Chairs: R. Mieusset (FR), M.Dinkelman-Smit (NL)
	Poster session 1b: Genetics, environment and spermatogenesis MIG 01-10, MIE 01-04, SP 01-08 Van Beuningen Room	Chairs: W. Baarends (NL), R. Oliva (ES)

Thursday 22/9/16

08.30–09.00	**ASA-exchange lecture Willem Burger Hall**	Chair: E. Nieschlag (DE)
	B. Robaire (CAN): Advanced Paternal Age and Reproductive Outcome	
09.00–11.00	**Evaluation of the infertile man: a discussion**	Chairs: A. Giwercman (SE), H. Tournaye (BE)
	S. Schlatt (DE), F.Wu (UK), N. Jørgensen (DK), F. Lotti (IT), S. Repping (NL)	
09.00–11.00	**ESAU-EAA session Van Weelde Room**	
09.00–10.00	**Surgery of male Infertility ESAU 1**	Chairs: T. Diemer (DE), N. Sofikitis (GR)
	M. Dinkelman-Smit (NL): Varicocele and non-obstructive azoospermia F. Fusco (IT): Obstructive azoospermia: Restoring patency and colleting sperm S. Minhas (UK): Failed TESE: what do you do next? Z. Kopa (HU): Testis sparing surgery- indications, technique and outcomes	
10.00–11.00	**Peyronie's disease ESAU 2**	Chairs: G. Dohle (NL), E.J.H. Meuleman (NL)
	E. Meuleman (NL): Technique selection for the individual Peyronies disease patient C. Bettocchi (IT): Surgical treatment of Peyronies disease patient with ED A. Kadioglu (TR): Management of complex deformities and Peyronies disease recurrence E. Ruis-Castane (ES): Peyronie's disease, lengthening techniques and graft selection	
11.30–12.30	**Andrological emergencies ESAU 3**	Chairs: S. Kliesch (DE), Z. Kopa (HU)
	A. Muneer (UK): Priapism – The EAU guidelines. P. Verze (IT): The acute scrotum and testicular trauma F. Lotti (IT): The role of Imaging in Andrological Emergencies	
11.30–12.30	**Hypothalamus-pituitary-testicular axis Willem Burger Hall**	Chairs: D.G. Goulis (GR), A. Isidori (IT)
	T. Kuiri-Hänninen (FI): Regulation of hypothalamus-pituitary-testicular axis in preterm and term boys	

(continued)

	A. Andersson (DK): pituitary gonadal axis and testosterone levels in health and disease K. Teerds (NL): Morphological and functional development of human Leydig cells	
12.30–13.00	**State of the art lecture Willem Burger Hall** S. Nef (CH): Dynamic Transcriptional Profile of Sertoli Cells During the Progression of Spermatogenesis	Chair: S. Schlatt (DE)
14.00–15.30	**Sperm function and selected orals Willem Burger Hall** S. Publicover (UK): Modulation of sperm behaviour by external factors – underlying mechanisms M. Eisenbach (IL): Fate of sperms in the female tract T. Strunker (DE): Compounds that might disrupt sperm functions **Selected oral presentations:** A. Amaral (DE): Sperm bioenergetics in mouse t-haplotype transmission ratio distortion OR05 E. Casamonti (IT): Short-term FSH therapy and sperm cellular maturity: a prospective study in idiopathic infertile men OR06	Chairs: S. Francavilla (IT), J. Ramalho-Santos (PT)
13.00–15.00	**Long-term treatment of hypogonadism – just a risky lifestyle intervention? Van Weelde Room** Industry Sponsered Symposium by Bayer A. Morgentaler (USA): Testosterone and the prostate gland: Risk-benefit of testosterone therapy in hypogonadal men L. Vignozzi (IT): Testosterone, inflammation and auto-immune diseases – what is old and what is new? K. Channer (UK): Testosterone and cardiovascular risk – what is it all about? F. Saad (DE): Testosterone, diabetes and weight management – what is the evidence?	Chair: S.Arver (SE)
15.00–16.00	**Ageing male Van Weelde Room** G. Rastrelli (IT): Development of and Recovery from Secondary Hypogonadism in Ageing Men: Prospective Results from the EMAS A. Isidori (IT): Phosphodiesterase type 5 (PDE5) inhibitors and the cardiovascular system H. Jones (UK): Diabetes, obesity and testosterone	Chairs: G. Forti (IT), G. Dohle (NL)
16.00–17.30	**Spermatogenesis Willem Burger Hall** W. Baarends (NL): Break it, to make it through male meiosis F. Tüttelmann (DE): genetics of spermatogenic arrest N. Kotaja (FI): Post-transcriptional gene regulation in spermatogenesis, the role of the chromatoid body K. Kula (PL): Hormonal regulation of spermatogenesis initiation	Chairs: F. Chalmel (FR), D. Jezek (HR)
16.30–17.30	**Sexual Medicine Van Weelde Room** P. Jern (FI): Evidence for a genetic etiology to ejaculatory dysfunction A. Barbonetti (IT): Sexual function in men with spinal cord injury R. Melcangi (IT): Post-finasteride persistant side effects on neuroactive steroids	Chairs: M. Maggi (IT), O. Rajmil (ES)
17.30–18.00	**State of the art lecture Willem Burger Hall** J. Toppari (Fi): Late effects in cryptorchidism	Chair: G. Dohle (NL)
18.00–19.30	**Poster session 2a: Male infertility and oncofertility MIT 01-23, OF 01-04, TC 01 Schadee Room** **Poster session 2b: Male Sexology SD 01-30 Van Beuningen Room** **Poster session 2c: Hypogonadism and Endocrinology HH 01-15, RE 01-13 Foyer**	Chairs: G. Balercia (IT), S. Kliesch (DE) Chairs: K. Shaeer (EG), D. W. Boellaard (NE) Chairs: G. Haidl (DE), D. Vanderschueren (BE)
18.30–19.30	EAA General Assembly	
Friday 23/9/16		
08.30–09.30	**Hypogonadotrophic hypogonadism Willen Burger Hall** M. Laan (ES): Pharmacogenetic potential of the polymorphisms in gonadotropin genes N. Pitteloud (CH): Gonadotrophin replacement for induction of fertility in hypogonadal men A. Juul (DK): FSH and FSH-R polymorphisms in relation to male puberty	Chairs: N. Jørgensen (DK), H. Behre (DE)
09.30–11.00	**Young researchers in Andrology (**INYRMF) Willem Burger Hall L. Turner (USA): Genomic Networks of Hybrid Sterility Discussed poster session of young researchers	Chairs: A. Amaral (DE), F. Tüttelmann (DE)
09.30–11.00	**Klinefelter syndrome Van Weelde Room** **Posters MID02, RE03, MID11, MIG03, OF02** G. Castellini (IT): Behavioural aspects of Klinefelter syndrome C. Gravholt (DK): Non-reproductive symptoms in Klinefelter syndrome H. Tournaye (BE): Sperm harvesting in boys and men with Klinefelter syndrome	Chairs: G. Corona (IT), A. Juul (DK)
11.30–12.30	**Oncology and germ cell cancer Van Weelde Room** A. Znaor (FR): Trends in testicular cancer L. Looijenga (NL): Development of germ cell tumours, new aspects MS. Dolci (IT); Differentiating or tumorigenic signals in spermatogonia	Chairs: A. Ferlin (IT), N.E. Skakkebaek (DK)

(continued)

11.30–12.30	**Selected oral presentations Willem Burger Hall**	Chairs: A. Meinhardt (DE), H.C. Schuppe (DE)
	Andreas Meinhardt (DE): Bacterial infection causes fibrotic remodelling and obstruction of the epididymis OR07 S Marchiani (IT): Search for new predictive parameters of Assisted Reproduction through analysis of male gamete OR08 T. Almont (FR): Testicular endocrine profiles in young boys operated for cryptorchidism OR09 S. d'Andrea (IT): Serum from patients with erectile dysfunction and vascular risk factors triggered oxidative stress-dependent mitochondrial apoptotic pathway in ex-vivo expanded circulating angiogenic cells of healthy men OR10 M. Whitfield (FR): The LXR-null mice: a model for dyslipidemia-induced male infertility and capacitation impairment OR11	
12.30–12.40	**Best paper in *"Andrology"* Willem Burger Hall**	Chairs: E. Rajpert-De Meyts (DK), Douglas T. Carrell (USA)
	J. Rohayem (DE): What predicts the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome?	
12.40–13.00	**Closing ceremony and awards Willem Burger Hall**	Chairs: G. Dohle (NL), C. Krausz (IT)

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PG – POST-GRADUATE COURSE IN CLINICAL ANDROLOGY

PG1 – Diagnostics of Male Infertility

Genetics of spermatogenic arrest

F. TÜTTELMANN
Institute of Human Genetics, University of Münster, Germany

Infertility affects 10–15% of couples and the causes of couple infertility are equally attributed to male and female (co-)factors. Male infertility is a genetically and clinically highly heterogeneous disease with a multitude of up to 1500 genes supposedly involved in spermatogenesis. Thus, unravelling the underlying causes and the pathophysiology is challenging and candidate gene approaches, e.g. picking one or several genes known to cause infertility in mice, did not identify novel genetic causes of infertility in men. Several distinct testicular phenotypes can be described comprising Sertoli-Cell-Only syndrome (with a complete lack of germ cells) and meiotic or maturation arrest at different stages. In the latter, genes involved in meiosis are the prime candidates for genetic causes for non-obstructive azoospermia (NOA).

The sex chromosomes are enriched for genes required for fertility and the Y-chromosomal AZF-microdeletions have been known for a long time to cause azoo- or severe oligozoospermia. However, X-chromosomal microdeletions and mutations in X-linked genes have only been analysed recently by utilising novel methods of genome-wide analyses. These have greatly expanded the toolbox for genetic studies and broadened the scope beyond single gene analyses. The power of such approaches is demonstrated by genome-wide array-Comparative Genomic Hybridisation (array-CGH) in groups of clinically well-characterised oligo- and azoospermic men. We were the first to report an excess of Copy Number Variations (CNVs) in infertile males especially on the sex-chromosomes. X-linked CNVs have also been proposed as recurrent cause for male infertility by others – comparable to the Y-chromosomal AZF-deletions.

Very recently, by using high-resolution array-CGH, we identified exon-deletions and nucleotide mutations in *TEX11* as the first common X-linked cause for meiotic arrest in about 15% of men with this phenotype. This breakthrough relied on phenotyping by testicular histology allowing specific selection of study subjects. Hemizygous mutations in *TEX11* were confirmed as an important cause for meiotic arrest already in another study. A few other genes implicated in meiosis from mouse and other studies, e.g. SYCP3, have also been found to cause meiotic arrest in men. However, the analysis of the currently known candidate genes would still only elucidate the genetic cause in about 20% of men with meiotic arrest. Thus, more concise efforts are needed to increase this diagnostic yield. Taken together, comprehensive screening for small deletions and sequencing of large numbers of genes in men with highly defined testicular phenotypes like meiotic arrest will likely result in fast identification of novel genetic causes for male infertility.

Probes to monitor sperm function

J. RAMALHO-SANTOS
Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Mammalian sperm in general, and human sperm in particular, is a challenging cell to work with. As it is a non-mitotic terminally differentiated cell with a small portion of cytoplasm and no transcriptional/translational activity any environmental or functional issues that require a cellular response must be met with existing resources, or by contact with reproductive fluids (epididymal secretions, seminal plasma, female tract). This also implies that many techniques normally used to dissect the molecular basis of function commonly applied to other cell types cannot be applied to sperm. Recent advances on human sperm function include several large scale proteomic analysis and the first studies on metabolomics. The ultimate goal would be to provide biomarkers that can predict sperm function in different contexts related to Assisted Reproduction technologies, or following treatment of male patients, given the notorious unreliability of classical spermiogram parameters in predicting reproductive success. However, these developments are still only in the conceptual stage. On a practical day to day basis several probes can be used in order to monitor distinct aspects of human sperm function, using both conventional and fluorescence microscopy, flow cytometry, and electrophoresis. Among aspects to be monitored one may include acrosomal status (using PSA-FITC), DNA status (using assays such as TUNEL, Dif-Quik, SCSA, COMET), mitochondrial function (using JC-1 or Mitotrackers), reactive oxygen species (ROS) production at distinct levels (MitoSOXRed, RedoxSensor Red, Cell ROX, Mito PY1, DHE), reactive nitrogen species (DAF2-DA), or calcium levels/flux (Fluo3, Oregon Green), among other properties. Of course the assays to be performed and the equipment to be used can be tailored to different research/clinical interests/capabilities. During this presentation practical aspects linked to several types of assays will be discussed, including appropriate controls that must be performed, what the assays can and cannot say, the advantages and disadvantages of different techniques (for example, fluorescence microscopy versus flow cytometry) and cost-benefit aspects for both research and diagnostic purposes.

Linking omics data to deciphering the testicular expression program

F. CHALMEL
Inserm U1085-Irset, Rennes, France

Spermatogenesis is a complex and tightly regulated process leading to the continuous production of male gametes, the spermatozoa. This developmental process requires the sequential and coordinated expression of thousands of genes, including many that are expressed in a testis-specific manner. The molecular networks underlying normal and pathological spermatogenesis have been widely investigated in recent decades, and many high-throughput expression studies have studied genes and proteins involved in male fertility. In this presentation, I will focus on studies that have attempted to correlate transcription

and translation during spermatogenesis by comparing the testicular transcriptome and proteome. I will also discuss the recent development and use of new transcriptomic approaches that provide a better proxy for the proteome, from both qualitative and quantitative perspectives. Finally, I will provide illustrations of how testis-derived transcriptomic and proteomic data can be integrated to address new questions and how the 'proteomics informed by transcriptomics' technique, by combining RNA-seq and MS-based proteomics, can contribute significantly to the discovery of new protein-coding genes or new protein isoforms expressed during spermatogenesis.

PG2 – Special Problems in Male Infertility

Urogenital infection and semen quality

A. PILATZ
Department of Urology, Pediatric Urology and Andrology, Justus Liebig University Giessen, Gießen, Germany

Infections in the urogenital tract are accepted causes for male infertility. Epidemiologic data indicate 6–10% of all males undergoing andrological work-up for infertility having an infectious etiology. Ascending urogenital tract infections include both enteric bacteria as well as sexually transmitted infections (STIs). In addition, systemic viral infections have to be considered. Several impairment mechanisms are known: (1) direct damage of spermatogenesis, (2) disturbance of sperm maturation, (3) accessory gland dysfunction, (4) obstruction within the urogenital tract, and (5) direct negative impact on sperm. This review gives a comprehensive overview on the most important urogenital tract infections (prostatitis, epididymitis, orchitis, male accessory gland infection – MAGI) and the impact on fertility. In addition, in males suffering infertility evidence is presented regarding an infectious etiology.

Management of non-palpable lesions of the testis

Z. KOPA
Department of Andrology and Urology, Semmelweis University Budapest, Hungary

Male infertility management involves ultrasound imaging which allows early discovery of non-palpable, small testicular masses. Most of these alterations are benign lesions so radical orchiectomy would mean an overtreatment. Testicular sparing surgery (TSS) can be recommended in selected cases using strict indication criteria with necessary precautions. The diagnostic procedure starts with the evaluation of known risk factors of testicular cancer, follows with physical examination and ultrasound imaging which has a high sensitivity detecting intratesticular masses but low specifity. Tumour serum markers and CT scan should be normal for patient selection. Fertility evaluation (semen analysis, endocrine assessement and cryopreservation) is an essential part of the management. TSS requires general anesthesia. Inguinal approach should be used and every patient should be informed and consented for the possibility of total testicular removal. The testicular mass should be correctly localized with US. Open testicular sparing surgery was introduced in 1986 and the technique was developed in 2001. Hopps and Goldstein introduced magnifying system for TSS in 2002. Microsurgery presents the best results with less complication rate. 6-25 × magnification can be used to help identification of testis and tumor blood supply. Tumour enucleation is performed taking multiple biopies from the tumour bed checking CIS in the surrounding tissue. Frozen section pathology examination (FSE) is a critical tool to differentiate between benign and malignant lesions and to identify the tumour margin and should always be adapted. The incidence of benign definitive histology is approximately 80%, overall complication rate is low (<6%). In this presentation we will introduce our results with microsurgical TSS coming to the conclusion that TSS is a viable treatment option for nonpalpable testicular masses less than 2 cm with normal markers and CT scan or in the case of bilateral tumors or solitary testicular masses. Frozen-section examination is a critical tool and has a high accuracy. TSS results less fertility alterations, less hormonal deficit and potential lower impact on sexual and psychosocial aspects. Intermediate to long-term follow-up results have not revealed any significant risk of local and/or distant recurrences after TSS. According to the EAU guidelines, an organ-sparing surgery (OSS) can be attempted in special cases and should be performed at experienced centers.

Sperm harvesting in boys and men with Klinefelter syndrome

H. TOURNAYE
Centre for Reproductive Medicine and EAA training centre for Andrology, Brussels Free University, Brussels, Belgium

At the onset of puberty, patients with Klinefelter syndrome face testicular stem cell loss. This loss is the result of both slowing-down the stem-cell self-renewal and intensified apoptosis. In the adult Klinefelter patient, testicular tubules show a Sertoli-cell-only pattern with sclerosis and fibrosis, however, occasional tubules showing active spermatogenesis may be observed in about half of them. In case, spermatozoa can be surgically harvested for ICSI. There exists some controversy on factors that may interfere with the success to recover spermatozoa from the testis. But also the eventual success rate of the combined strategy, i.e. TESE followed by ICSI is a matter of debate: while in many case-series chromosomally normal offspring has been reported, at present it remains unclear how successful this strategy can be overall since these reports tend to overestimate the actual outcome in a biased way. There is also great controversy in regards to whether pubertal adolescent Klinefelter boys should have TESE offered to preserve future fertility. The controversy is even greater for fertility preservation in pre-pubertal Klinefelter boys. At present, there is no data demonstrating any benefit of early fertility preservation in adolescents compared to the adult patients. No data at all exist on the benefit of testicular tissue banking at pre-pubertal age. Testicular tissue freezing in prepubertal 47, XXY boys requires further validation in a research framework. Not only do we need to scrutinize the potential benefits vs. drawbacks, but because of progressing fibrosis of the testes, the need for in-vitro maturation strategies should be investigated too.

PG3 – Hypogonadism and Male Sexual Dysfunction

Delayed puberty in boys

J. SLOWIKOWSKA-HILCZER
Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Lodz, Poland

Delayed puberty in boys is diagnosed if it has not ensued by age 14, an age that is 2–2.5 standard deviations above the mean for healthy children. Main categories of delayed puberty are as follows: (1) constitutional delay of growth and puberty, (2) functional hypogonadotropic hypogonadism caused by systemic illness or malnutrition, (3) hypogonadotropic hypogonadism caused by genetic or acquired defects in the hypothalamic-pituitary region and (4) hypergonadotropic hypogonadism secondary to primary gonadal failure. Medical history should include any history of systemic illness, eating disorders, excessive exercise, social and psychological problems. Boys with pubertal delay may have accompanying emotional and physical immaturity relative to their peers, which can be a source of anxiety. Physical examination should focus on height, arm span, weight, visual fields and secondary sex characteristics, including hair growth, testicular volume and phallic size. Testicular size >4 ml usually indicates that the child has entered puberty. The main diagnostic challenge is to distinguish boys with constitutional delay, who will start puberty 1–2 years later in comparison with most of their peers, from those with an underlying pathologic process. Constitutional delay should be suspected when there is a family history and when there are delayed bone age and short stature. A diagnosis of constitutional delay is made by exclusion that requires ongoing evaluation until the onset of puberty (activation of hypothalamo-pituitary-testicular axis) and the growth spurt. GnRH test, hCG test and imaging of hypothalamo-pituitary region and gonads may indicate the location of disorder.Waiting and reassurance without hormonal treatment is appropriate for many individuals with presumed constitutional delay of puberty. However, the impact of delayed growth and pubertal progression on a child's social relationships and school performance should be weighed. Late-maturing boys and their parents are often impatient and do not want to wait for the natural pubertal growth spurt. Therefore a brief course of testosterone to "jump-start" puberty is often offered. Therapy can begin with 25–50 mg testosterone enanthate or testosterone cypionate every 2 weeks, or by using a 2.5 mg testosterone patch or 25 mg testosterone gel. Testosterone treatment should be interrupted after 6 months to determine if endogenous LH and FSH secretion have ensued. Other causes of delayed puberty should be considered when there are associated clinical features or when boys do not enter puberty spontaneously after a year of observation or treatment. When the problem is either hypogonadotropic hypogonadism or damage to the testicles, testosterone is the treatment of choice, but the dose will need to be increased over time and it will need to be continued into the adulthood. Pubertal delay due to gonadotropin deficiency can be treated with gonadotropin substitution to achieve growth of testes, advance of spermatogenesis and normal testosterone level.

Testosterone, inflammation and auto-immune diseases – what is old and what is new?

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Several preclinical studies have demonstrated that androgens act as endogenous inhibitors of immune responses in several autoimmune processes, including those involved in non-alcoholic steatohepatitis (NASH), benign prostatic hyperplasia, chronic autoimmune thyroiditis, and autoimmune orchitis.

Recently, activation of androgen receptor (AR) by androgens has been demonstrated to reduce proliferation of CD4+ T clones and to markedly suppress the inflammatory response of human non-professional antigen presenting cells culture – such as those present within the prostate-, to inflammatory stimuli (such as TNFα- and the LPS) or to co-incubation with activated CD4+T lymphocytes, therefore suggesting that androgens could play a broad anti-inflammatory role in T cells. An abnormal immune response plays an important role in the development and perpetuation of the inflammatory cascade in a variety of chronic inflammatory diseases, including inflammatory bowel disease (IBD). Under most conditions, an immune competence of the gut would be beneficial to the host. However, in some situations a perturbation of the fine balance in immune regulation with a switch towards an effector Th1/Th17 phenotype might lead to the development of chronic intestinal inflammation, as observed in the pathogenesis of Crohn's disease. An overt, or even a subclinical, bacterial or viral infection is thought to cause intestinal inflammation (first hit) that could be auto-sustained or exacerbated by the presence of an altered and abnormal Th1 and Th17 immune response. Tumor necrosis factor (TNF)-α plays an important role in the pathogenesis of both Crohn and NASH, which indeed can be frequently seen in the same patient.

Normalizing serum testosterone in hypogonadal men with Crohn's disease has been described to have a positive effect on the clinical course, also evidenced by biochemical parameters. Among women, prediagnostic circulating testosterone is associated with a lower risk of Crohn's disease.

In a recent randomized, placebo-controlled trial in hypogonadal men with metabolic syndrome (MetS), testosterone replacement therapy (TRT) significantly reduced plasma inflammatory markers, including hsCRP and TNF-α. Similar results were obtained in observational studies.

In an open-label phase II trial including ten men with relapsing-remitting multiple sclerosis who received testosterone treatment for 12 months, a significant gray matter increase in the right frontal cortex was observed.

Moreover, in a single-centre, prospective registry study including of more than 340 hypogonadal men (total testosterone ≤12.1 nM), fifteen men with psoriasis were studied. Testosterone administration, improved the skin disease, which was paralleled with a significant improvement of Scores on the Psoriasis Area and Severity Index and Physician Global Assessment for Psoriasis.

Finally, there is increasing evidence from both clinical and experimental studies that testosterone has marked anti-inflammatory and immunomodulatory effects

SA1 – State of the Art Lectures

Novel Horizons in Andrology: A Genetic Perspective

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Male reproductive and sexual health are the two major topics of Andrology with intimate interconnections to other medical specializations such as endocrinology, urology, psychiatry/psychology, gynaecology, cardiology, dermatology and microbiology. Basic science investigations in this field are mainly focusing on sperm biology, stem cell biology, testis and prostate cancers and genetics/epigenetics of male infertility. Thanks to the diffusion of high throughput genome-wide and whole genome technologies, genetics is among the most dynamically progressing areas of medicine. The understanding of the genetic/epigenetic background of spermatogenesis and of the hormonal regulation of testis function are essential for the development of novel diagnostic and therapeutic tools (for instance personalized hormonal therapy based on genetic variants). Moreover, inherited genetic factors are likely to play a relevant role in translating the effect of environmental factors at the cellular level with possible implications for prevention. A genetic factor can be identified in about 20% of patients affected by severe oligozoospermia/azoospermia and this percentage is expected to further increase thanks to novel exome and genome studies. In the era of in vitro fertilization it is of outmost importance to identify the underlying genetic cause of impaired sperm production since there exists the risk of transmitting genetic disorders to the future offspring. In addition, recent genome-wide studies and our own studies on the X chromosome clearly show that a proportion of infertile men are carriers of a "deletion burden" with potential effect not only on spermatogenesis but also on general health. Whole Exome studies turned out to be successful for the identification of mutations in familial idiopathic azoospermia and for the identification of novel candidate genes of hypogonadotrophic hypogonadism. The identification of genetic factors in sporadic idiopathic oligo/azoospermia appears to be more complex and based on the currently available data from resequencing studies and from the first exome studies different "genetic" scenarios can be envisaged.

Environmental exposures and male fecundity and related impairments

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Growing evidence suggests that male fecundity and related impairments such as diminished semen quality or conception delay among couples trying for pregnancy may be impacted by various environmental factors, including contemporary lifestyles and endocrine disrupting chemicals (EDCs). Using data from the Longitudinal Investigation of Fertility and the Environment Study, this talk will focus on specific environmental exposures including lifestyle that were found to be associated with diminished semen quality and a longer time-to-pregnancy among couples trying for pregnancy. The talk will conclude with an illustration of novel environmental wide association study (GWAS) techniques, which are data-driven analytic methods for analyzing mixtures of environmental exposures, to assess patterns of semen quality endpoints and lifestyle exposures that may be predictive of conception delay or impaired male fecundity. The talk will be presented within a framework underscoring the importance of male fecundity across the lifespan, including its implications for health and disease.

AL – ASA Exchange Lecture

Advanced paternal age and reproductive outcome

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It is well-recognized that increased maternal age is associated with decreased fertility and a higher incidence of abnormalities and diseases in progeny; however, we are only now beginning to grasp the impact of paternal age on fertility and progeny outcome. There is a trend for men to start having children at an increasingly older age. Several epidemiological studies have established clear links between increasing paternal age and reduced fertility, as well as an increased incidence of a number of conditions in their children, such as autism, ADHD, diabetes, cardiovascular anomalies, and schizophrenia. Using animal studies, we have provided clear evidence that increasing paternal age affects progeny outcome, that sperm quality decreases as males age, and that oxidative stress may be an important factor in mediating these effects. We hypothesized that increasing age alters the expression of genes relating to oxidative stress and DNA damage/repair in germ cells. To test this hypothesis, we undertook a series of studies using isolated rat spermatogenic cells as well as mice over-expressing catalase (CAT), an enzyme that plays a key role in removing reactive oxygen species (ROS).

Pachytene spermatocytes and round spermatids were isolated from young and aged rats. Microarray analysis revealed changes in the expression of DNA damage/repair and oxidative stress genes in pachytene spermatocytes but not in round spermatids. Further analysis of pachytene spermatocytes demonstrated that transcripts and proteins involved in the base excision repair and nucleotide excision repair pathways were specifically altered during aging. Furthermore, in aged males there was an increase in 8-oxo-2′-deoxyguanosine (8-oxodG) immunoreactivity in the testes and in the number of spermatozoa positive for 8-oxodG. These studies established that aging is associated with differential regulation of DNA repair pathways; specifically, downregulation of the BER pathway leading to oxidative-stress related deficient repair of 8-oxo-dG lesions in germ cells.

To examine the effects of oxidative stress in aging on cellular responses in pachytene spermatocytes and round spermatids, we developed a culture protocol for maintaining

these cells for a protracted time period. Culturing these isolated germ cells from young and aged rats revealed that germ cells from aged males display an earlier decline in viability, elevated levels of ROS, and increased spermatocyte DNA damage. Oxidative insult induced by exposure to a pro-oxidant revealed that several transcripts for antioxidant enzymes, Sod1, Cat, and Prdxs, were up-regulated in response to ROS in germ cells from young males but not from aged ones. In contrast, the expression of DNA damage repair genes Rad50 and Atm were increased in the germ cells from aged animals. Thus, as germ cells proceed through spermatogenesis, there is an age-dependent adaptive response to altered oxidative stress.

The damage to spermatogenic cells from aged rats suggested that spermatogonial stem cells (SSC) may be affected. Using GFP-expressing germ cells from rats and transplantation studies, we discovered that aged rats had <50% of the colony numbers and extrapolated SSC numbers per donor testis compared to young rats, and that the colonies were shorter, suggesting a poorer quality. Microarray analysis of transcripts from a SSC-enriched spermatogonial population (CD9+ cells) revealed that CD9+ cells from aged rats had 60 transcripts that were upregulated and over 500 that were downregulated relative to young rats. An altered expression was found for transcripts involved in mitosis and in DNA damage response. These molecular alterations in the SSC-rich population of CD9+ aged cells imply that stem/progenitor spermatogonia are contributors to the germ cell origin of reproductive aging.

We used mice overexpressing CAT (MCAT) to determine whether CAT overexpression alleviates the redox dysfunction observed with aging. We found that MCAT mice did not exhibit the age-dependent loss of spermatozoa, nor did they show the aging associated loss in testicular germ and Sertoli cells seen in wild type (WT) mice. Low overall ROS and reduced peroxynitrite levels were detected in spermatocytes from aged MCAT mice following exposure to the pro-oxidant tert-butyl hydroperoxide. Germ cells from young MCAT mice showed elevated levels of DNA-damage repair markers, but this response was lost with aging. We also found that the 8-oxodG lesions found in sperm with aging were reduced in aged MCAT mice; furthermore, these mice showed no decrease in the age-dependent number of pups per litter. Thus aged MCAT mice generate sperm at the same rate as young mice; these sperm are protected from oxidative stress associated damage.

Together these findings clearly indicate that the decline in sperm quality is linked to oxidative damage and that appropriate anti-ROS treatment reduces damage to sperm chromatin. These studies were supported by CIHR.

ESAU 1 – Surgery of Male Infertility

The role of varicocele repair in men with non-obstructive azoospermia

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Background: After years of debate, the benefit of surgical repair of clinical varicoceles in terms of improvement of sperm parameters and increased spontaneous pregnancy rates in infertile men with oligospermia is now well documented [1].

Although the exact pathophysiology of testicular failure caused by varicocele remains unclear, it has been shown that clinical hallmarks of impaired spermatogenesis significantly improve as a result of varicocele repair in selected patients. Specifically, the significant postoperative decrease in sperm DNA fragmentation following varicocele repair in a majority of patients, indicates that varicocelectomy may reverse the detrimental effect of a varicocele on the spermatogenesis [2]. Non-obstructive azoospermia (NOA) is the most extreme feature in the clinical spectrum of testicular failure in male infertility. The incidence of NOA in men attending infertility clinics varies among institutions; concomitant varicoceles are estimated to be present in 5–15% of men with NOA.

Testicular sperm extraction (TESE) in combination with intracytoplasmatic sperm injection (ICSI) is a widely available treatment modality for NOA. Because specialized laboratories successfully perform TESE and ICSI even when only very few testicular sperm are harvested from testicular biopsies, a gradual shift can be observed in the use of very low numbers of viable sperm in ejaculated semen samples (cryptozoospermia) for ICSI. In their quest to offer patients with cryptozoospermia ICSI treatment and to optimize sperm retrieval rates in patients with NOA, urologists have a renewed interest in varicocele repair as a means to induce or maximize spermatogenesis in severe male infertility. Unfortunately, history seems to repeats itself, and again the benefit of varicocelectomy, this time indicated for men with NOA and cryptozoospermia, is controversial.

Aim and Methods: This overview aims to discuss the best available evidence and opposed views on the recovery of motile sperm in ejaculates of previously azoospermic men after varicocelectomy and the improvement of testicular sperm retrieval rates in patients with NOA following varicocele repair.

Results: Six non-randomized studies that included between 15 and 30 patients each, found that varicocelectomy induces spermatogenesis in men with NOA to the extent that low numbers of sperm reappear in the ejaculate in 20–55% of cases [3–8]. It should, however, be taken into account that not all studies included NOA patients with azoospermia in at least two centrifuged semen sample pellets. This inclusion criterion is important because cryptozoospermia can alternate azoospermia in repeat semen samples in the absence of intervention in up to 20% of patients.

An alternative application of varicocelectomy in patients with NOA is to improve the outcome of TESE. The best available evidence for this approach is presented in 4 observational studies that compared sperm retrieval rates in 529 untreated and treated patients with a concomitant NOA and a clinical varicocele [9–12]. A significantly increased sperm retrieval rate in favor of varicocele therapy was observed in 3 out of 4 studies. Two out of 3 studies that compared TESE-ICSI outcome in treated and untreated patients concluded that varicocelectomy significantly increased clinical pregnancy rate.

Conclusion: In summary, there seems to be a benefit of varicocele repair for patients with NOA. Varicocelectomy may result in the appearance of spermatozoa in the

ejaculate. Also, varicocele repair prior to TESE may enhance sperm retrieval. However, randomized controlled, adequate powered, multicenter trials with rigorous methodology are warranted to establish evidence based treatment algorithms to select responders and identify clinically relevant outcome parameters like testicular sperm retrieval rate and ICSI outcome.

Obstructive Azoospermia: Restoring patency and colleting sperm

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Obstructive azoospermia (OA) is the absence of spermatozoa and spermatogenetic cells in semen and post-ejaculate urine due to obstruction. It occurs in 15-20% of men with azoospermia and may result from testicular (15%), epididymal (30-67%), vasal (2-6%), or ejaculatory duct (1-3%) pathology. Severe genitourinary infections, iatrogenic injury during scrotal or inguinal surgical procedures and congenital anomalies are common causes of obstructive azoospermia. Vasectomy is the most common cause of vasal obstruction. Functional obstruction of the distal seminal ducts might also occur because of local neuropathy. Men with OA may father children either by surgical correction of the obstruction, which may produce pregnancy by intercourse and obviate the need for assisted reproductive technology; or by retrieval of sperm from the male reproductive system for in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI). Unfortunately, only some conditions are amenable to curative surgery. Surgical correction may be accomplished by microsurgical reconstruction of the vas and/or epididymis or, in cases of ejaculatory duct obstruction, by transurethral resection of the ejaculatory ducts (TURED).

Vasoepididymostomy is performed for congenital, infectious, postvasectomy or idiopathic epididymal obstruction. Following this type of microsurgery, 20-40% of couples achieve pregnancy through intercourse. Proximal vas obstruction after vasectomy requires microsurgical vasectomy reversal. Vasectomy reversal allows the return of sperm to the ejaculate in 70-95% of patients, and pregnancies are obtained without the need for assisted reproduction in 30-75% of couples. Large bilateral vas deferens defects, resulting from involuntary excision of the vasa deferens during hernia surgery in early childhood or previous orchidopexy are usually impossible to correct. In these cases testicular sperm extraction (TESE), Microsurgical epididymal sperm aspiration (MESA) or proximal vas deferens sperm aspiration can be used for cryopreservation for future ICSI. MESA is indicated in men with congenital bilateral absence of the vas deferens (CBAVD) .

The treatment of ejaculatory duct obstruction depends on its aetiology. TURED can be used in large postinflammatory obstruction and when one or both ejaculatory ducts empty into an intraprostatic midline cyst. Transurethral resection of the ejaculatory duct results in the appearance of sperm in the ejaculate in about one-half to three-fourths of cases. The pregnancy rate achieved by this surgery is about 25%. In cases of obstruction due to a midline intraprostatic cyst, incision or unroofing of the cyst is required. The alternatives to TURED are MESA, TESE, proximal vas deferens sperm aspiration, seminal vesicle ultrasonically guided aspiration, and direct cyst aspiration. Sperm retrieval and cryopreservation may be performed at the time of microsurgical reconstruction in order to avoid a second procedure in the event that the microsurgical reconstruction does not reverse a patient's azoospermia. In cases of intratesticular obstruction, only TESE allows sperm retrieval and is therefore recommended.

Testis sparing surgery- indications, technique and outcomes

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Testicular cancer is relatively rare but the most frequent malignancy in younger males and shows a doubled incidence in the last 40 years. The cure rate is excellent over 95%, especially in the early stage (near to 100%). Diagnosing testicular masses the classical treatment is radical orchiectomy but recently there is a trend to perform organ sparing surgical approach under special conditions when radical orchiectomy would mean an overtreatment.

The main goal of the testis sparing surgery is to avoid fertility, testosterone production and cosmetic function. Our recent guidelines do not indicate organ sparing surgery (OSS) in the case of non-tumoural contralateral testis. Partial orchiectomy can be attempted only in special cases with all the necessary precautions.

When suspected and pathologically confirmed of benign tumour or in the case of synchronous bilateral testicular tumours or metachronous contralateral tumours OSS can be indicated using very strict indication criteria. In a tumour in a solitary testis organ sparing approach can be attempted when the tumor volume is less than 30% of the testicular volume and the residual testicular tissue can have an avoided blood supply.

The organ preserving approach is not established for primary Germ Cell Tumour management but recently a trend can be observed to avoid the testicular tissue. OSS may have imperative indications (bilateral tumour or tumour in a solitary testis or when castration would render hormonal replacement). Preoperative hypogonadism, a compromised contralateral testis or microlithiasis mean relative indication criteria. Cases of non-palpable testicular masses, small tumours, polar or peripherial laesions with normal contralateral testis are elective indications.

The diagnostic procedure should focus to the risk factors, physical examination and the highly sensitive but less specific US imaging, tumour markers, CT scan and fertility evaluation. Psycho-emotional status is also an important part of patient selection.

Open organ sparing testicular surgery was introduced in 1986 and the technique was developed in 2001. Using a magnification system was introduced in 2002. Recently microsurgery can present the best results helping the identification of testis and tumour blood supply.

The procedure requires general anesthesia and uses inguinal approach. Frozen section pathological examination (FSE) is an essential requirement for diagnosis of benign

from malignant tumours and evaluation of margin. The overall complication rate is less than 6%.
The old dogma of performing an immediate orchiectomy for any testicular mass is refuted by the clinical experience. In patients in reproductive age before any treatment fertility assessment and semen cryopreservation should be offered.
Literature lacks studies with a high level of evidence on comparing OSS with radical surgery. For selected patients with testicular masses without compromising oncological and functional outcomes OSS might be considered: in organ confined tumor less than 20 mm., multiple biopsies of the tumor bed, close follow-up and high patient compliance.OSS can result in less fertility alteration and less hormonal deficit with lower impact on sexual and psychosocial aspects but indications are still controversial, specifically for patients with normal contralateral testis.

ESAU 2 – Peyronie's Disease

Technique selection for the individual Peyronie's disease patient

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To consider surgical correction, Peyronie's disease (PD) should be in a stable phase. Indications for surgery include presence of PD for 12 months with stability of disease for at least 3 months, the presence of a deformity compromising intercourse, and satisfactory quality of erections. Pain should be resolved. Surgery is not performed in cases of active or progressive disease, minimal degrees of curvature, or for "preventive" plaque resection. Surgical options include plication or wedge resection (shortening of the convex side), grafting (lengthening of the concave side), and implantation of a penile prosthesis. Preoperative counseling is paramount to address patient expectations about postoperative curve correction, penile length, and erectile function. Penile shortening often accompanies PD, and is dependent on direction and degree of curvature; stretched penile length should be documented and patient made aware that a grafting procedure is usually limited to adding 1–2 cm. On the other hand, the patient should also understand that in some cases, penile length may actually be lost using a grafting technique because of subsequent fibrosis. Technically, simpler plication surgeries, without tunical incision or excision, may result in a straight erection and essentially no risk of de novo ED secondary to surgery. However, these techniques do result in penile length loss. In addition to ED, patients must be aware of the risks of temporary or permanent penile hypo- or anesthesia, future plaque formation, new or recurrent deformity, and risks specific to the surgical approach utilized.
Plication or Wedge Resection
For a penis of adequate length and intact erectile function, a straightening procedure using nonincisional (pure plication) or wedge resection techniques is appropriate. The most commonly utilized procedures include:

- Nesbit procedure and its various modifications: one or more elliptical portions of the tunica albuginea at the most prominent portion of the curvature are excised and plicated
- Yachia procedure: longitudinal incisions in the tunica albuginea are closed horizontally in a Heineke–Mikulicz manner
- Pure plication techniques such as Lue's 16 or 24-dot minimal tension approach

All plication procedures result in penile length loss. Commonly reported complications include glans numbness (secondary to manipulation of the dorsal neurovascular bundle), penile narrowing/indentation, suture granuloma, palpable suture "knots" or "knot" pain, herniation, penile hematoma, phimosis, residual, recurrent or de novo curvature, and ED.
Grafting Procedures
For the short penis or complex deformity such as an hourglass or severe indentation and good erectile function, incision and application of a graft may be considered. Patients, especially those with borderline ED, must be aware of the increased risk of impotence secondary to this approach, and should be counseled regarding the option of a penile prosthesis. Grafting materials include autologous tissue (saphenous vein, dermis, and tunica vaginalis), allograft or xenograft materials (cadaveric pericardium and porcine small intestinal submucosa), or synthetic grafts.

Management of complex deformities and Peyronie's disease recurrence

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Background: Peyronie's disease (PD) is a relatively common ailment effecting 3–9% of males older than 40 years old. Although emerging minimal invasive treatments such as Clostridium Collagenase and Interferon are promising for the future, surgery is still the definitive golden standard for stable PD (>12–18 months episode, no pain for at least 6 months) surgery with its high success rate of curvature improvement. Complex deformities are hard to deal with for both the surgeon and patient because of their high complication rates, challenging surgical technique and so deserves a special attention.
Aim: The primary objective of this presentation is to focus on the complex deformities of Peyronie's disease and give practical management tips on difficult or recurrent cases.
Methods: The presentation was derived from evidence base literature on PD with insights from surgeon's personal experiences. The term complex deformity was defined as: Curvatures >60 °, prominent hourglass/notching deformities, severe penile length loss. Erectile dysfunction will be investigated as a complication of surgery. Recurrence of PD will be accepted as residual or relapsing curvature >15–20 degrees.
Results: Plaque incision and grafting is accepted as the primary surgical treatment for complex deformities without accompanying erectile dysfunction.. Grafts are applied to the defect caused by the incision of prominent point of

the convex penis side in which the plaque resides and thus lengthening of the penis is achieved. Plaque incision is preferred to excision because of the potential cavernosal dysfunction with the disruption of veno-occlusive mechanism. Among plaque incision techniques, no common approach was agreed on and surgeon may choose between H and Egydio according to his experience. Two different approaches for neurovascular bundle dissection, medial and lateral, are viable but there are no studies comparing the two techniques. The definition of the ideal graft material is available, pliable, inexpensive, resistant to infection and able to preserve erectile capacity. The graft type is also determined by surgeon's experience, patient preference and the type of deformity. Among graft materials, saphenous vein (autologous material), bovine or cadaveric pericardium, 4-layer SIS may be preferred. Novel grafts such as collagen fleece are under investigation and may be considered in near future. For hourglass deformities both longitudinal and transverse incisions should be made to have length and girth increase. Severe curvature degree (>60), age (>55), preoperative cavernosal dysfunction and Egydio's technique were associated with postoperative sexual dysfunction in one study. Lateral curvature and comorbidities were blamed as a reason for recurrence in difference studies but the results are contradictory.

Conclusion: Since the ideal surgical technique and graft type are not attained until now, PD surgery for complex deformities may be accepted as under development. Therefore a great variety of techniques and applications are reported throughout the world. Every study in this field makes the final destination one step closer.

Peyronie's disease, lengthening techniques and graft selection

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Background: Loss of penile length is a common complaint of men with Peyronie's disease (PD), both before and after corrective surgery. There are several techniques for maintaining or lengthening the penis in men with this disorder.

Aim: Describe the different types of tunical lengthening strategies for PD patients, with emphasis in graft material selection and novel surgical techniques description.

Results: Tunical lengthening procedures are used in patients with good erectile function and curvature >60°–70°, shaft narrowing, hinging and extensive plaque calcification. Several surgical strategies have been described in this setting, including partial incision/excision and grafting (PIG), the Egydio technique-PIG and PIG with corporal sparring. Satisfactory straightening is accomplished in 74–100% of patients, with postoperative erectile dysfunction reported in 5–53% of the cases; the postoperative rehabilitation period is critical to reduce the risk of postoperative erectile dysfunction and length loss. Graft selection is of outmost importance. Multiple grafts have been used historically, including fat, dermis, tunica vaginalis, duramater, temporalis fascia, saphenous vein, crura and buccal mucosa. Synthetic polyethylene terephthalate (Dacron) and polytetrafluoroethylene (Teflon) grafts have been used historically and are not recommended now. Allografts and xenografts have emerged in recent years, including processed pericardium from a bovine or human source, porcine intestinal submucosa and porcine skin. Processed human/bovine pericardium and small intestine submucosa grafts are the most common grafts currently used in clinical practice. Several tissue engineered graft materials are being investigated, with quite interesting results being expected in the next years. Currently, our group is involved in a study testing a ready-to-use fibrin sealant patch (TachoSil®) as grafting material after plaque incision. Innovating lengthening techniques have being described for PD patients in which penile prosthesis are being placed. Subcoronal prosthesis placement, prosthesis placement with corporal sparing, circumferential incision and grafting; together with the sliding technique have been presented in recent years. Finally, while performing PD corrective surgery some surgical alternatives have been used in an adjuvant manner to increase subjective and objective penile length. These include suspensory ligament release, suprapubic lipectomy and ventral phalloplasty.

Conclusion: The surgical approach in PD cases is very much dependent in multiple variables such as curvature grade, preoperative erectile function, prosthesis and grafts availability, surgical experience and personal choice. It is mandatory to involved the patient in the therapeutic process, explaining the benefits and risk of each technique.

ESAU 3 – Andrological Emergencies

Priapism- the EAU guidelines

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Priapism is defined as a prolonged penile erection lasting for more than 4 h in the absence of sexual stimulation and remains despite orgasm. It is commonly classified into three main subtypes non ischaemic (high flow), ischaemic (low flow) and stuttering (recurrent) subtypes. In ischaemic priapism (IP) has by far been the most extensive subtype undergoing investigation. The underlying pathophysiology is still not completely understood although the initiating events are likely to be multifactorial involving central neuronal pathways, alterations in the corpus cavernosum microenvironment, modulation of the smooth muscle contractile machinery and aberrant neurotransmitter regulation in the corpus cavernosum leading to dysregulation of the smooth muscle.Obstruction of the penile venous outflow, which leads to stasis of blood within the corpus cavernosum akin to a compartment syndrome results in the development of hypoxia, acidosis and glucopenia. It is the development of this ischaemic microenvironment that results in time dependent changes. At 24 to 48 h there is widespread endothelial destruction and exposure of the basement membrane. In addition to this the smooth muscle cells undergo transformation as well as necrosis. Persistent blood stasis and ischaemia lasting for longer than two days is associated with infiltration of the trabecular tissue with inflammatory cells and the smooth muscle cells undergoing necrosis or phenotypic change into fibroblast like cells.Although medical intervention for cases of shorter duration are effective using corporal blood

aspiration and sympathomimetic agents, refractory cases prove problematic. Through a combination of both in vivo and in vitro models and clinical case series, the last decade has seen a progressive change towards accepting that after a prolonged period of ischaemic priapism, there is a high probability of erectile dysfunction. With non-ischaemic priapism the absence of ischaemia within the corpus cavernosum has led to conservative management of patients before intervention using super selective embolization. However, it appears that some patients still develop distal corpus cavernosum smooth muscle dysfunction and fibrosis despite the absence of ischaemia. As to when we should embolise these cases is still uncertain but identifying these cases radiologically has become easier with the use of high resolution MRI scans. The lecture will aim to cover the role of shunt surgery for ischaemic priapism and the progress from basic science models to acute penile prosthesis insertion.

The Acute Scrotum and Testicular Trauma

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Although acute scrotal pain comprises fewer than 1% of overall emergency department visits, this presentation may provoke great anxiety for the patient or caretaker given its highly sensitive nature. An acute scrotum is defined as an acute painful swelling of the scrotum or its contents, accompanied by local signs or general symptoms. Although the list of diagnostic possibilities for a patient with an undifferentiated acute scrotum is extensive, early identification and skillful management of testicular torsion is critical, as it may threaten testicular viability and future fertility if not managed expediently and appropriately. Blunt trauma to the scrotum can cause testicular dislocation, testicular haematocoele, testicular rupture and/or scrotal haematoma. The cremasteric reflex and testicular sonography are frequently used, yet imperfect, diagnostic tools in assessing for testicular torsion. Traumatic dislocation of the testicle occurs rarely. Conservative management is recommended in haematoceles smaller than three times the size of the contralateral testis. In large haematoceles, non-operative management often fails, and delayed surgery (> three days) is often required. Testicular rupture is found in approximately 50% of cases of direct blunt scrotal trauma. It may occur under intense, traumatic compression of the testis against the inferior pubic ramus or symphysis, resulting in a rupture of the tunica albuginea of the testis. Penetrating injuries to the scrotum require surgical exploration with conservative debridement of non-viable tissue.

The Role of Imaging in Andrological Emergencies

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Color-Doppler ultrasound (CDUS) is the first imaging method for all andrological emergencies. It has a high accuracy in diagnosing acute andrological disorders, and it is extremely useful for following patients during therapy or after surgery, when required. Andrological emergencies include acute scrotal, penile and prostate-vesicular disorders. Acute scrotal disorders include testicular torsion, rupture, malignancy, orchitis and epididymitis. CDUS plays a critical role in their detection, leading to urgent surgical intervention or specific therapy to preserve testis function and general health. The main CDUS feature to be evaluated in most of these disorders is vascularization, differentiating testicular torsion (absent), infarction (absent or peripheral), epididymo-orchitis or some malignant conditions (i.e. leukemia, lymphoma) (enhanced), when considered along with other clinical features. Testicular torsion is characterized by pain and absent testicular vascularization at CDUS, although arterial flow can be maintained in partial torsion, and hyperemia mimicking orchitis can be seen after spontaneous detorsion. In the setting of trauma, the testis may develop hematoma, contusion, or rupture, the latter characterized by discontinuity of the tunica albuginea and possible protrusion of the seminiferous tubules. Testicular malignancy usually presents as vague scrotal discomfort, lump or painless swelling of the testis, but 10-20% of subjects complaint of dull or heavy pain, and CDUS detects small or large nodules, with or without internal vascularization, calcifications or cysts. Orchitis and epididymitis usually present with a painful hemiscrotum and testis or epididymis enlargement, respectively. CDUS plays a key role in identifying enhanced vascularization, diffuse enlargement, inhomogeneous, mainly hypoechoic echo-texture and reactive hydrocele. Finally, CDUS has good specificity in the differential diagnosis of spermatic cord and Morgagni's hydatid torsion, an acute scrotal affliction frequent in childhood. Acute penile disorders include priapism, penile Mondor's disease and fracture. Priapism is a condition characterized by a persistent painful erection not related to sexual desire. Priapism is classified as "low flow" or "ischemic" (more frequent), characterized by a disorder of venous blood outflow, and "high-flow", when unregulated cavernous arterial inflow is present. "Low flow" priapism may be secondary to vasoactive erectile agents, haematologic dyscrasias, neurogenic disorders and neoplasms, and is characterized by a painful full erection, while "high flow" priapism may be secondary to pelvic or penile trauma or surgery, and is characterized by an incomplete erection. CDUS demonstrates a high resistance waveform pattern in the former case, and a low resistance pattern in the latter case. Penile Mondor's disease is the thrombosis of the dorsal penile vein, appearing suddenly as a hard rope on the penile dorsal surface, usually associated with pain. CDUS detects absent flow in the superficial dorsal vein, allowing the beginning of a specific therapy. Penile fracture is the rupture of the penile tunica albuginea, typically resulting from blunt trauma or intercourse. Diagnosis is clinical, and CDUS provides a fast, noninvasive alternative to the more often used MRI. Among the prostate and seminal vesicles disorders, acute prostate-vesiculitis and prostatic abscess represent medical emergencies. CDUS can detect specific echo-patterns and can be used in the latter as a guide for percutaneous transperineal drainage.

SF- Sperm Function

Modulation of sperm behaviour by external factors – underlying mechanisms

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During human fertilisation the sperm must travel thousands of times its own length through fluids and viscous mucus, interact with the uterine and oviductal linings, find the oocyte, penetrate the surrounding cumulus mass then bind to and 'drill' through the zona that surrounds the oocyte. These tasks vary greatly in their mechanical and physiological requirements so diverse motility patterns (behaviours) are used, controlled by cues from the female reproductive tract. Freshly ejaculated sperm in seminal plasma swim with a low-amplitude, symmetrical flagellar beat that generates rapid, progressive movement in low-viscosity fluid. This pattern of movement, termed activated motility, which facilitates enable penetration into and through viscous environments such as cervical mucus. However, within the female tract sperm may display much more vigorous, whiplash-like motility. This change in behaviour, termed hyperactivation, is required for successful ascent to and progression within the oviduct where sperm bind to the oviduct wall and require hyperactivation to escape.

The central role of Ca^{2+} signaling in controlling mammalian sperm motility is well established. Increased amplitude and asymmetry of the flagellar beat and adoption of hyperactivated motility are associated with and dependent on increased $[Ca^{2+}]_i$. Recent progress in application of physiological techniques to sperm has greatly increased our knowledge of how sperm behaviour is controlled. Here we consider the central role of progesterone in regulating activity of human sperm through $[Ca^{2+}]_i$ and the significance of the different components of the sperm's Ca^{2+} signalling machinery (particularly the sperm specific Ca^{2+} channel CatSper and the intracellular Ca^{2+}-storage organelles) in determining the behaviour employed. Finally the incidence and potential effects of impaired Ca^{2+} signalling on human fertility (natural fertilisation and fertilisation by IVF) and the possibility of pharmacological treatment of such problems are considered.

Guidance of sperm in the female tract

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Mammalian spermatozoa must be guided for reaching the egg at the fertilization site [1]. Three potential sperm guidance mechanisms have been demonstrated to occur: chemotaxis, thermotaxis and rheotaxis. It is believed that, in the oviduct, spermatozoa are first guided to the fertilization site by the long-range mechanisms, thermotaxis and rheotaxis, and from there they are guided to the egg by two short-range processes of chemotaxis. One of the resulting puzzles is how, during thermotaxis, the spermatozoa can sense and respond to temperature changes as small as <0.0006°C. The identity of the sensing system that confers this exceptional sensitivity on spermatozoa is not known. Here we show that the temperature-sensing system of mammalian spermatozoa involves opsins, known to be G-protein-coupled receptors that act as photosensors in vision. We demonstrate by molecular, immunological, and functional approaches that opsins are present in human and mouse spermatozoa at specific sites, which depend on the species and the opsin type, and that they are involved in sperm thermotaxis via two signalling pathways—the phospholipase C and the cyclic-nucleotide pathways [2]. Our results suggest that, depending on the context and the tissue, mammalian opsins act not only as photosensors but also as thermosensors. As such, this may explain the puzzling presence of opsins in other non-photosensitive organs such as kidneys and lungs.

1 Eisenbach M & Giojalas LC (2006) Sperm guidance in mammals – an unpaved road to the egg. *Nature Rev. Mol. Cell Biol.* 7, 276–285.
2 Pérez-Cerezales S, Boryshpolets S, Afanzar O, Brandis A, Nevo R, Kiss V & Eisenbach M (2015) Involvement of opsins in mammalian sperm thermotaxis. *Sci Rep* 5, 16146.

Compounds that might disrupt sperm function

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Synthetic endocrine disrupting chemicals (EDCs) interfere with the endocrine system; EDCs that are omnipresent in food, air, textiles, pharmaceuticals, household products, and personal-care products mimic the action of natural hormones and affect their production or metabolism. EDCs have been implicated in adverse trends in human reproduction, including widespread infertility and increasing demand for assisted reproduction. However, it has been difficult to prove or refute the hypothesis that EDCs account for the decreasing reproductive success in the western world. We studied by Ca^{2+} fluorimetry, patch-clamp recordings, and motility analysis the action of 100 ubiquitous EDCs on human sperm. We show that structurally diverse EDCs, at physiologically relevant concentrations, directly activate the sperm-specific CatSper Ca^{2+} channel and, thereby, evoke an increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$), a motility response, and acrosomal exocytosis. EDCs compete with progesterone and prostaglandins for CatSper activation and desensitize sperm for these physiological ligands. Finally, in complex low-dose mixtures reflecting physiological exposure levels, EDCs act synergistically to elevate $[Ca^{2+}]_i$ in sperm. Our findings substantiate common concerns regarding the negative impact of EDCs on male reproductive health and should be considered for future regulations towards a more restrictive use of EDCs.

AM – Ageing Men

Development of and Recovery from Secondary Hypogonadism in Ageing Men: Prospective Results from the EMAS

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Cross-sectional studies show that obesity, independently of age, is associated with low T. The underlying mechanisms are still largely unknown and are likely to involve adipokines, pro-inflammatory cytokines and central insulin resistance. Obesity related decline in T is often modest and lower than that observed in the classical pathological male hypogonadism due to hypothalamic, pituitary or testicular diseases. Consistently, its clinical consequences are not well defined. According to the guidelines on clinical management of late onset hypogonadism (LOH), the conditions is defined for low T levels, accompanied by cognate symptoms of androgen deficiency. However, the thresholds for low T and the nature of symptoms are not agreed. There is overlap between the symptoms that are due to obesity, ageing and hypogonadism and it is difficult to discern which are due to low T. In the European Male Ageing Study (EMAS), a population based study involving more than 3,000 men form eight European centres, obesity at baseline and weight gain during follow-up predict with the occurrence of secondary hypogonadism (sHG). In turn, among a series of symptoms suggestive of hypogonadism, the occurrence of sHG was associated with sexual, but not physical and psychological ones. In the EMAS population, a sHG is a condition that can frequently remit. The recovery from sHG is predicted by normal weight and weight loss, thus mirroring the risk factors for occurrence of sHG. In contrast to evidence from randomized placebo-controlled clinical trials that shows that T replacement therapy is able to improve sexual function, recovery from sHG was not associated with improvement of sexual symptoms. The reasons for this apparent inconsistence are uncertain. It could be that the spontaneous increase in T may not represent a sufficiently sustained improvement to transmute into subjective symptomatic recovery or the increase may not be sufficient to drive improvements or resolution of sexual symptoms. Given the high prevalence of sexual symptoms irrespective of the concentration of circulating T, a further explanation could be that important non hormonal factors contribute to the persistence of symptoms, even after restoration of normal T levels.

Diabetes, obesity and testosterone

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The prevalence of type 2 diabetes (T2D) is increasing dramatically within countries of the western world and is a major health and economic burden. The main driver for this increase in prevalence is obesity. There is also a high prevalence of symptomatic testosterone deficiency in T2D in which the degree of central obesity negatively correlates with serum testosterone levels. Evidence suggests that there is a bidirectional mechanism involved in that low testosterone promotes fat deposition and the obesity itself leads to lower testosterone. This can be explained by the Hypogonadal-Obesity-Adipocytokine Hypothesis which implicates adipocytokines in the inhibition of the hypothalamic-pituitary testicular axis and increased adipose aromatase activity in reducing circulating testosterone. The prevalence of hypogonadism (symptomatic testosterone deficiency) in T2D is approximately 40%, with 17% having testosterone levels lower than the normal range.

Weight loss in diet controlled studies and as a result of bariatric surgery can lead to an increase in total testosterone provided there is a reduction by at least 10% body weight. Diet and exercise should always be part of any treatment plan in men with hypogonadism and obesity. The majority of TRT studies that have assessed body composition by bioimpedance and/or DEXA have reported an increase in lean mass and a reduction in fat mass. A number of trials found a decrease in waist circumference. The reduction in measures of obesity may continue to improve over several years.

Low testosterone states, in particular obesity, metabolic syndrome and T2D are associated with reduced insulin sensitivity. Insulin resistance is a major intermediary cardiovascular risk factor which contributes to hyperglycaemia, dyslipidaemia, hypertension and endothelial dysfunction. Interventional studies have demonstrated that testosterone replacement therapy (TRT) improves insulin resistance as assessed by HOMA-ir and hyperinsulinaemic-euglycaemic clamp studies. Whether or not TRT improves glycaemic control is not known for certain but there has been no definitive trial in men withT2D who have poor glycaemic control. TRT does however lower total and LDL-cholesterol, lipoprotein (a) and also HDL-cholesterol. No benefit or adverse effect on blood pressure has been identified. Overall the evidence suggests that TRT in hypogonadal men with T2D may have a greater beneficial effect on cardiovascular risk factors when compared to non-diabetic men.

Laboratory research has shown that testosterone has effects on increasing glucose uptake and utilization by liver and muscle cells by promoting glycolytic rate. Testosterone may also have a protective effect on the overspill of fatty acid uptake into non-adipose tissues and also visceral fat.

An epidemiological study has found that testosterone deficiency in diabetes is associated with a greater than two-fold increased mortality. A retrospective analysis in this study reported that those men receiving TRT had an improved survival to that of a T2D man with normal testosterone status. In a meta-analysis of men receiving TRT has shown in those subjects with metabolic conditions there was a reduction in cardiovascular events.

SM – Sexual Medicine

Evidence for a genetic etiology to ejaculatory dysfunction

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A heritable component in the etiology of premature ejaculation (PE) has been suggested for more than seven decades. In the last decade, empirical evidence for a heritable component in PE etiology of around 30% has been demonstrated, and subsequent studies have focused on identifying genes that contribute to PE etiology. However, all molecular genetic studies conducted on PE to date have employed methodological designs that are likely to produce spurious associations. Furthermore, sample sizes in all studies conducted to date have likely been too small to reliably detect the small effect sizes that are expected for genetic markers associated with complex phenotypes, such as PE. As a result, no molecular genetic studies of PE have been successfully replicated. To solve the problems outlined above, two undertakings need to be addressed: Firstly, the current clinical definition of PE (i.e., the diagnostic criteria) may not reflect the underlying biological reality, and as such, consensus needs to be reached with regards to the definition of the problem. Secondly, robust genetic associations are unlikely to be detected without vast resources and international collaboration in the form of consortia.

Sexual function in men with spinal cord injury

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Spinal cord injury (SCI) mainly occurs in sexually active young men, experiencing a broad spectrum of andrologic disorders, including erectile dysfunction (ED), ejaculation dysfunction, poor semen quality and androgen deficiency.

Primary sexual dysfunction, such as ED and ejaculation dysfunction, results from neurological lesions directly affecting the neural pathways subserving sexual function. Accordingly, men with complete high level SCI, albeit tetraplegic with a poor degree of functional independence (FI), usually preserve the functionality of their parasympathetic sacral centre controlling reflex erections, which are suitable for sexual intercourse. On the contrary, paraplegic men, who have a low degree of motor disability, exhibit a higher prevalence of ED and a poorer responsiveness to PDE-5 inhibitors. Therefore, although sexual dysfunction and the loss of FI represent two aspects of disability contributing to psychological issues, they usually do not occur to the same extent in each individual patient, and we reported that ED represents the major determinant of the psychological distress in men with chronic SCI, independently of the degree of FI impairment and bowel/bladder dysfunction (Barbonetti et al., J Sex Med, 2012;9:830–6).

Actually, men who undergo a traumatic SCI are rather complex patients who have to deal with various kinds of disability-related problems, which can lead to secondary sexual dysfunction: they include immobility, obesity, metabolic syndrome and muscle wasting, osteoporosis, poor bladder and bowel control, autonomic dysreflexia, neuropathic pain, spasticity, pressure ulcers and androgen deficiency. Although high rates of biochemical androgen deficiency have been reported in men with SCI, it is not established whether or to what extent it can result in clinical hypogonadism. Actually, the diagnosis of clinical hypogonadism poses specific challenges in spinal cord-injured men, because in the presence of SCI, not only sexual symptoms, but also most of the other putative clinical features of hypogonadism (e.g. changes in body composition, osteoporosis, anaemia, mood disorders), could overlap with direct or indirect consequences of neurological damage and disability. In this scenario, we recently demonstrated that poor leisure time physical activity, high body mass index and low sexual desire represent independent predictors of low testosterone in men with chronic SCI (Barbonetti et al., Andrology, 2014;2:721–8). The very high prevalence of androgen deficiency makes men with SCI an intriguing clinical model to explore the relationships of low testosterone with emerging aspects of hypogonadism, such as hypovitaminosis D (Barbonetti A. et al., J Spinal Cord Med. 2016;39:246–52) and non-alcoholic fatty liver disease (Barbonetti A. et al., Spinal Cord Med, 2016 Feb 25:1–7), independently from confounding factors shared by all of these conditions and peculiar to this population. In conclusion, in men with chronic SCI, sexual dysfunctions represent a key determinant of psychological distress contributing to general poor health status which can be reflected by biochemical androgens deficiency and its correlates.

Post-Finasteride persistent side effects on neuroactive steroids

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Finasteride is a reversible inhibitor of the enzyme 5alpha-reductase (5alpha-R) used for the treatment of human benign prostatic hyperplasia and androgenic alopecia. The 5alpha-R converts testosterone (T) and progesterone (PROG) into their 5alpha-reduced metabolites, dihydrotestosterone (DHT) and dihydroprogesterone (DHP), respectively. These neuroactive steroids, as well as their further metabolites, are important mediators for many physiological processes in the nervous system, affecting mood, behavior, reproduction, and cognition (Melcangi et al., Cell Mol Life Sci 65:777-797, 2008; Giatti et al., J Steroid Biochem Mol Biol 153:127-134, 2015). However, despite of the wide therapeutic use of finasteride, the effects of this inhibitor in the nervous system have been poorly explored. This aspect could be important, particularly because observations performed in a subset of man taking finasteride for androgenic alopecia show sexual dysfunction as well as anxious/depressive symptomatology. Very important, these side-effects were also reported in a subset of patients after discontinuation of the therapy (Traish et al., Rev Endocr Metab Disord 16:177-198, 2015). Interestingly, we demonstrated that post-finasteride patients showed altered neuroactive steroid levels in plasma and cerebrospinal fluid (CSF) in comparison to healthy individuals (Melcangi et al., J Sex Med 10: 2598-

2603, 2013; Caruso et al., J Steroid Biochem Mol Biol 146: 74-79, 2015). Thus, data obtained by liquid chromatography-tandem mass spectrometry show a general decrease of neuroactive steroid levels, and particularly of 5alpha-reduced metabolites of PROG and T. Data obtained in male rats after subchronic treatment with finasteride (i.e., 3 mg/kg/day for 20 days) indicate that after one month of withdrawal neuroactive steroids are also affected in brain structures (Giatti et al., Neuroendocrinology 2015, DOI: 10.1159/000442982). For instance, the levels of PROG and its metabolites (i.e., DHP, isopregnanolone and allopregnanolone), as well as of deydroepiandrosterone and 17-beta-estradiol were significantly decreased in the cerebral cortex. In the cerebellum, the levels of pregnenolone (PREG), DHP and T were increased while the levels of DHT and 5alpha-androstane-3beta, 17beta-diol were decreased. In the hippocampus, the levels of PREG and PROG were decreased while the levels of DHP were increased. In addition, changes in the expression of their receptors have been also reported. For instance, an upregulation of androgen receptor and estrogen receptor alpha and a downregulation of estrogen receptor beta were observed in the cerebral cortex. In addition, a decrease of alpha 4 and beta3 subunits of GABA-A receptor (i.e., the receptor able to bind allopregnanolone) has been observed in the cerebellum. Altogether these findings suggest that the block of the enzyme 5alpha-reductase by finasteride treatment may have broad consequences for the nervous system (We thank the Post-Finasteride Foundation for the financial support).

SA2 – State of the Art Lecture

Dynamic transcriptional profile of Sertoli cells during testis determination and the progression of spermatogenesis

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Sertoli cells (SCs) play a critical role in the formation of the testis, whereas during adulthood they are entirely committed to sustaining spermatogenesis. In males, Sertoli cells are the first somatic cells of the genital ridge to differentiate during sex determination. They orchestrate testis development by forming testis cords and by inducing fetal Leydig cell differentiation. In the adult, SCs, the only somatic cells within seminiferous tubules, associate intimately with developing germ cells. They not only provide physical and nutritional support but also secrete factors essential to the complex developmental processes of germ cell proliferation and differentiation. Due to the multiple evolving functions of SCs, the SC transcriptome must therefore adapt rapidly during early testis determination and later during the different stages of spermatogenesis. To address this question we combined total and single cell RNA seq. technology and report a comprehensive genome-wide expression profiles of SCs isolated at different stages during early mouse testis development and during the first wave of mouse spermatogenesis. Our results highlight the plastic transcriptional landscape of SCs during sex determination and the progression of spermatogenesis and provide valuable resources to better understand SC evolving functions and its related disorders, such as disorders of sexual development (DSD) and male infertility.

ANDROLOGY

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GC – GOLDEN COMMUNICATIONS
OR – ORAL PRESENTATIONS

OR01

Is sedentary lifestyle associated with testicular function? A cross-sectional study of 1210 men

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Background: A large proportion of young men from various European countries have impaired semen quality. The causes are debated but may include lifestyle factors e.g. smoking, alcohol, obesity and diet. Recently, an inverse association between television watching and semen quality was reported, an association modified by physical activity. This finding is interesting as sedentary behaviour has become an increasing part of modern life, including transportation, work and leisure time. Besides working and sleeping, watching television and other 'screen time' is the most common activity in many Western countries and contributes many hours of sedentary behaviour.

Aim: To examine the hypothesis that sedentary behaviour has adverse effects on the testicular function.

Methods: Based on cross-sectional data on 1210 healthy young Danish men we investigated whether sedentary lifestyle was associated with testicular function (semen quality and reproductive hormones) independent of physical activity. The men were invited to participate in the study when they attended a compulsory medical examination to determine their fitness for military service between 2008 and 2012. Information on sedentary behavior (television watching and computer time) and physical activity was obtained by questionnaire. The men had a physical examination, delivered a semen sample and had a blood sample drawn.

Results: Time spent watching television, but not time sitting in front of a computer, was associated with lower sperm counts. Men watching television more than 5 h/day had an adjusted sperm concentration of 37 million/mL (95% confidence interval (CI): 30, 44) vs. 52 million/mL (95% CI: 46, 62) among men not watching television and a total sperm count of 104 million (95% CI: 84, 126) vs. 158 million (95% CI: 130, 189). Furthermore, an increase in follicle-stimulating hormone and decreases in testosterone and the testosterone/luteinizing hormone ratio were detected in men watching many hours of television. Self-rated physical fitness, but not time spent on physical activity, was positively associated with sperm counts.

Conclusion: In conclusion, we detected some evidence that sedentary behaviour, particularly many hours of television watching, was associated with poorer testicular function, even among men who were physically active. The findings should be of public concern as many hours spent on television watching are widespread among young men.

OR02

The german male sex-study (GMS-study): differences in sexual behaviour and number of lifetime sexual partners depending on sexual orientation identity

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Background: Surveys investigating sexual behaviour in context of sexual orientation identity mainly focus on health risks and sexual transmitted diseases. Most of the time sexual orientation groups are examined separately and not simultaneously. As a result, little is known about sexuality in a community sample.

Aim: The purpose of this analysis was to assess the distribution of sexual orientation identity of men in Germany. Depending on sexual orientation identity different sexual behaviours, such as vaginal intercourse, oral sex, anal sex, solo masturbation and number of lifetime sexual partners were examined. Sexual orientation identity was categorized into heterosexual, gay or bisexual.

Methods: From May 2014 till November 2015 data was collected via a questionnaire within the PROBASE-trial (the German prostate cancer screening-trial). Only male participants, who were Caucasian and 45 years old at the time of answering the questionnaire were included in the sample (n = 9.603).

Results: 95.0% of men identified themselves as heterosexual, 3.9% as gay and 1.1% as bisexual. According to the number of lifetime sexual partners, the majority of heterosexual men have had up to 10 sexual partners (71.3%). A much higher amount of gay men reported more than 30 sexual partners than the two other groups (gay: 45.2% > bisexual: 25.7% > heterosexual: 5.9%; $p < 0.001$). Considering sexual activity within the past 3 months there was no significant difference between the three groups, but according to the type of sexual behaviour, significant differences could be noticed ($p < 0.001$). The most common sexual behaviour for heterosexual men was vaginal intercourse, followed by oral sex and anal sex (vaginal intercourse: 97.8% > oral sex: 58.3% > anal sex: 6.9%). Among gay men the most common sexual behaviour was oral sex, followed by anal sex and one in every 10 gay men reported on vaginal intercourse (oral sex: 90.0% > anal sex: 63.8% > vaginal intercourse: 9.8%). Amongst bisexual men almost equivalently high numbers have had vaginal intercourse and oral sex and about one third reported on anal sex within the past 3 months (vaginal intercourse: 77.3% > oral sex: 73.4% > anal sex: 34.7%). Additional to partnered sexual behaviours, solo masturbation was

analysed in general and depending on relationship status. Solo masturbation was very prevalent in all groups, but significantly more gay and bisexual men reported on solo masturbation within the past 3 months, than heterosexual men (gay: 93.9% > bisexual: 93.2% > heterosexual: 77.9%; $p < 0.001$). For homosexual and bisexual men relationship status barely influenced solo masturbation. In contrast to that for heterosexual men the difference in solo masturbation between partnered and single men was 14.8% (gay: 3.6%, bisexual: 6.8%).

Conclusion: There were substantial differences in sexual behaviour and experiences depending on men□s sexual orientation identity. Gay men have had the most sexual partners in life, whereby almost every second gay man reported more than 30 sexual partners. Oral sex was the most common sexual behaviour among gay men, whereas vaginal intercourse was the most common among heterosexual men. Masturbation was very prevalent throughout and partnership status showed to have an influence on it, especially for heterosexual men.

OR03

Irradiation of juvenile primate testicular xenografts affects the somatic environment

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Background & Aim: Improved cancer treatment regimens have promoted higher survival rates in prepubertal boys. However, these patients might face infertility in adulthood. Depending on the treatment dose, surviving spermatogonial stem cells (SSCs) are able to recolonize seminiferous tubules facilitating recovery of spermatogenesis and therefore fertility. SSCs reside along the basal membrane exposed to a niche composed of matrix and factors expressed by Sertoli cells, peritubular myoid cells (PTMCs) and interstitial cells affecting SSC self-renewal, differentiation and migration. While attention has been primarily devoted to the effects of irradiation on germ cells, little work considered irradiation effects on the somatic environment in primates. Here, we applied a xenografting approach facilitating the reduction of non-human primate animal numbers and the refinement of studies by performing the radiation exposure on dissected fragments in vitro. In combination with gene expression analysis and immunohistological stainings, irradiated xenografts obtained from prepubertal cynomolgus monkeys were investigated to evaluate the effect of irradiation on germ cells as well as the somatic cell population.

Methods: Two prepubertal macaques (*Macaca fascicularis*) aged 16 (MM687) and 22 (MM627) months were available from the inhouse breeding colony. Upon necropsy, four testes were dissected into 186 fragments which were equally distributed into three groups (untreated, 1 Gy and 4 Gy irradiated). Subsequently, fragments were grafted under the backskin of nude mice ($n = 36$). Fragments from each monkey were also processed as pregrafting controls. After 6.5 months, grafts were retrieved ($n = 98$) and examined using RT□ Profiler□ PCR arrays (34 marker genes) and histological analysis.

Results: Prior to grafting, testes of both monkeys consisted of seminiferous tubules with spermatogonia as the most advanced germ cell type. 6.5 months after grafting, spermatogenesis had started and 15.4% (MM687) and 1.8% (MM627) of seminiferous tubules contained spermatocytes as the most advanced germ cell types, respectively. In contrast, irradiation resulted in a dose-dependent decline of spermatogonia in the 1 and 4 Gy exposed fragments. Gene expression analysis revealed a dose-dependent decrease regarding germ cell marker genes *MAGEA4*, *BOLL*, *DDX4*, *GFRA1* and *FGFR1*. Interestingly, transcript levels of the Sertoli cell marker genes *AMH* as well as *GDNF* and *CXCL12*, which are described to regulate germ cell maintenance and migration, did not change following irradiation. In contrast, PTMCs were affected by irradiation as *ACTA2* and *CXCL11* expression levels as well as their protein expression pattern showed significant changes following treatment with 4 Gy compared with 1 Gy and untreated grafts, respectively.

Conclusion: This is the first study showing an effect of irradiation on primate testicular somatic cell populations combining prepubertal non-human primate xenografting with gene expression and morphological analysis. With this innovative approach, we were able to show that apart from the germ cell population, PTMCs were the main cell type affected by irradiation. Moreover, it remains to be elucidated how effects of irradiation in the somatic environment influences the SSC function and recolonization and hence male fertility.

OR04

Dynamics of the transcriptional landscape during human fetal gonad development.

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Fetal life is a crucial period for sexual reproduction when bipotential gonads differentiate into either a testis or an ovary. Gaining insights into the complex molecular events underlying this process is central to a better understanding of disorders of sexual development. The present work intends to improve the knowledge on molecular pathways at play during gonad development in humans using RNA-sequencing. This project particularly seeks to identify early transcriptional events that may play critical role in the regulatory network driving human sexual differentiation.

To address this issue, we defined the transcriptional landscape of fetal human gonads by sequencing total RNA extracted from testes and ovaries between 6 and 17 gestational weeks (GW). The resulting paired-end reads were mapped on the human genome (hg19) and then assembled into transcripts using the Tuxedo suite (Trapnell *et al*., 2012). We next defined a high-confidence set of transcripts showing differential expression across samples as described in (Chalmel et al., 2014). Clusters of co-expressed genes (*k-means* method) were subjected to

functional analysis (GO enrichment) performed in AMEN (Chalmel *et al*., 2008).
The analysis of this massive RNA-seq dataset has led to a high-confidence set of 27 721 assembled transcripts; among which 25 901 known and novel isoforms coding genes (mRNAs), 812 to long non-coding (lnc) RNAs and 133 to novel unannotated transcripts/genes (NUTs). Out of the 12 203 transcripts showing a differential expression during male and/or female gonad development, 7260 were sexually dimorphic and were classified into 23 expression patterns. Known gene markers and functional analysis confirmed that the resulting transcript clusters were significantly associated with biological processes relevant to fetal testis/ovary development. We next focused on 555 transcripts for which the sex-biased expression was evident even as early as 6GW and, among them, on 46 genes encoding transcription factors (e.g. SRY). Finally, we reconstructed a disease-regulatory network for human gonad development by integrating expression signals, regulome/interactome information, phenotypes and functional annotation data.
We next intend to focus on lncRNAs, and more precisely those localized in the antisense of sexually dimorphic genes. Indeed, such antisense lncRNAs might regulate the expression of the sense loci and thus contribute to the regulatory network at play during gonad development. We will also define the conserved expression program during gonadal development in mammals by integrating published datasets in rodents. Another important direction to this work would be to integrate genome-wide association studies to identify new candidate genes associated with disorders of sex development. Finally, this basic work paves the way for further molecular investigations on the impact of known and suspected reprotoxicants during the differentiation of human fetal gonads.

OP1 – SELECTED ORAL PRESENTATIONS 1

OR05

Sperm bioenergetics in mouse t-haplotype transmission ratio distortion

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The mouse t-haplotype is a selfish variant region on chromosome 17, spanning around 43 Mb, which is transmitted as a unit. Although homozygous (t/t) males are sterile, heterozygous (t/+) males transmit the t allele to up to 99% of their progeny. This transmission ratio distortion (TRD) phenomenon is postulated to be triggered by motility differences between t and + sperm in heterozygous males, with t-containing sperm being able to reach and fertilize oocytes faster. However, complete evidence that this is indeed the case is missing. Moreover, and although some of the molecular mechanisms controlling this postulated sperm competition in favor of t-carrying gametes have been deciphered, the complete picture of the sperm cellular features operating in TRD is not fully drawn.
The main goal of this project is to determine the physiological differences between t- and +-carrying sperm and to understand how do these are regulated and contribute to TRD.
To this end, we have compared sperm from mice from the three genotypes (+/+, t/+ and t/t) at different levels: (i) motility parameters (using computer assisted sperm motility analysis – CASA); (ii) quantitative proteomics (LC-MS/MS, by label free quantification (LFQ)); (iii) ATP levels (via a commercial kit based on the luciferin-luciferase reaction) and (iv) mitochondrial membrane potential (MMP; by means of JC-1 staining and flow cytometry).
CASA outcomes suggest that there are two subpopulations of motile sperm in t/+ mice, which might as well be the basis for TRD: one that moves more linearly and one whose motility has low progressivity. Proteome pathway enrichment analyses suggest that while in t/t samples there is a down-regulation of proteins involved in bioenergetics metabolism (when compared to +/+), sperm from t/+ mice have an up-regulation of several bioenergetic metabolic proteins, concomitant with a down-regulation of others. Sperm motility parameters and ATP levels are significantly lower in t/+ and t/t compared to +/+, while MMP is lower in t/t, but higher in t/+.
Our data indicate, for the first time, that metabolic differences may contribute to the distinct motility profiles of sub-populations of sperm derived from t/+ males, and thus to the TRD phenomenon.

OR06

Short-term FSH therapy and sperm cellular maturity: a prospective study in idiopathic infertile men

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Background: A number of previous studies reported that FSH administration for at least 3 months improves quantitative and functional sperm parameters in idiopathic infertility leading to an improvement of sperm fertilizing ability. Hyaluronic acid (HA) binding capacity of spermatozoa is a biomarker of functional competency. The percentage of HA bound spermatozoa in the ejaculate is a proxy of normal sperm maturation (both membrane and nuclear maturation) and fertilizing potential. No data is available on the effect of FSH therapy on *Hyaluronan Binding Assay* (HBA) values and on the potential effect of short term therapy, covering the last phase of spermatogenesis ('spermiogenesis').
Aim: The main objectives of the study were to evaluate the effects of highly purified FSH (hpFSH) treatment on sperm ability to bind hyaluronic acid (HBA) in relationship with: (i) 1 month therapy (spermiogenesis); (ii) 3 months therapy (the entire spermatogenesis). As secondary objective, we evaluated whether FSHβ (SNP -211G>T) and/or *FSHR* (SNP 2039A>G and SNP -29G>A) genotypes were able to predict response to treatment.
Materials and methods: 40 oligo- and/or asteno- and/or teratozoospermic male patients with idiopathic infertility, FSH <8 IU/L and HBA baseline values <60% were enrolled.

Patients were treated with hpFSH 75 IU/L s.c., every other day for a period of 3 months. HBA values were evaluated before (T0), after 1 (T1) and 3 months (T3) of therapy and 4–6 months after discontinuation of treatment ('washout'; second basal value). Molecular genetic analysis was performed by PCR/RFLP (*FSHβ*) and by PCR/HRMA (*FSHR*).

Results: A significant improvement in HBA value both after 1 (from 28 ± 13.4% to 38 ± 19.7%, $p < 0.001$), and 3 months (from 28 ± 13, 4% to 45 ± 23.4%; $p < 0.001$) of treatment was found. The average entity of the improvement was 35% and 61% at T1 and T3, respectively. 46% of patients (16/35) showed a significant increase of HBA value (more than twice the physiological variation observed in our cohort) at T1, and this percentage further increased at T3 (62%, 23/37). The HBA value returned to the pre-treatment value after 4-6 months from the end of the therapy. Concerning "clinical responders" (i.e. patients with HBA post-treatment values exceeding the threshold of normality, HBA>60%), 23% $^{8/35}$ of the patients after 1 month and 32% (12/37) after 3 months of therapy reached to normalization. Concerning the second objective: similar % of responders was found in distinct FSHβ and FSHR genotypes.

Conclusion: Our study showed that hpFSH improves sperm maturation expressed by the HBA value. The novelty of our study is that a significant positive effect on sperm maturity is already observed after 1 month in 46% of men opening novel therapeutic perspectives. The 'short cycle', by increasing the proportion of functionally competent cells, could be useful prior ART. The standard cycle, by further increasing the proportion of HBA responders, could lead to a higher chance for both natural or ART pregnancies. Our preliminary pharmacogenetics data does not support a clear-cut relationship between the FSHβ and *FSHR* genotypes and responsiveness to the treatment.

OP2 – SELECTED ORAL PRESENTATIONS 2

OR07

Bacterial infection causes fibrotic remodelling and obstruction of the epididymis

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Background: Despite antibiotic treatment, 40% of patients with epididymitis due to *Escherichia coli* serovars develop impaired fertility, which may result from epididymal obstruction.

Aim: Determine if *E. coli* infection of the epididymis causes fibrotic obstruction and identify the key mediators.

Methods: Patients with chronic epididymitis were examined. Epididymitis was induced by uropathogenic *E. coli* (UPEC) or commensal serovars in wildtype and *Myd88*$^{-/-}$ mice. Epididymal organ cultures were treated with activin A and bacteria. Epididymal histology was assessed by Azan staining and immunohistochemistry. Cytokines and fibrosis markers were analysed by qRT-PCR and hydroxyproline assay 7d post-infection or after activin A treatment in vitro. Activins A, B and follistatin were quantified by immunoassays. Statistical analysis was by one-way or two-way ANOVA.

Results: Patients with epididymitis showed severe fibrosis of the epididymal duct. In mice, UPEC infection caused fibrosis and obstruction in the cauda epididymis. Fibrotic marker (a-smooth muscle actin, fibronectin) and inflammatory cytokine (activin A, TNFa, IL1a, IL1□, IL6) mRNA levels and total collagen levels were elevated. The fibrotic response was inhibited by the absence of the pro-inflammatory adapter protein, MyD88. Activin A induced fibrosis in cultured epididymides, which was inhibited by the activin-binding protein, follistatin.

Conclusions: UPEC-induced epididymitis results in obstruction and fibrotic remodelling of the cauda epididymis. This damage appears to be largely attributable to the severity of the inflammatory response to the infection, rather than the infection itself. Activin A was identified as a potential causative agent in this transformation, which suggests the activin-binding protein follistatin as putative target to support antibiotic treatment and in blocking the activin A signalling axis and preserving fertility in these patients. As activin A was the only pro-fibrotic factor examined in this study, the pathology observed may also be influenced by other pro-fibrotic factors such as TGF-□ or CTGF.

OR08

Search for new predictive parameters of Assisted Reproduction through analysis of male gamete

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Background: Infertility is a worldwide health problem affecting about 15% of couples. A widely used treatment option for couple infertility is assisted reproduction techniques (ART). Despite recent improvements, ART success is still low. Currently no markers are available to predict ART outcomes. The identification of biomarkers of sperm quality might increase the percentages of successful ART treatments, avoiding psychological stress for couples and reducing costs. Aim and methods: to evaluate, in semen samples from male partners of couples undergoing ART at Demetra Center, the following parameters: (i) Sperm chromatin immaturity by using two techniques, Aniline Blue (AB, detecting histones retention) and Chromomycin A3 (CMA3, detecting protamine content); (ii) CatSper (sperm-specific calcium channel involved in several sperm functions) expression by immunofluorescence method coupled with flow cytometry, reported as mean fluorescence intensity (MFI). Chromatin immaturity and CatSper expression have been related to ART outcomes (Fertilization rate

(FR), Cleavage rate (CR), Embryo quality (EQ), Implantation rate (IR) and Pregnancy rate (PR)).
Results: (i) The two techniques AB and CMA3 are correlated ($r = 0.6$, $p < 0.0001$, $n = 123$). However, whereas the percentage of AB positive sperm is negatively associated with FR ($r = -0.2$, $p = 0.005$, $n = 137$), also after adjustment for female age and female factor ($\beta = -0.2$, $p = 0.02$), that of CMA3 was related only to EQ (24.0% [7.0–69.0, $n = 105$] in EQ < 0.5 vs. 11.5% [6.0–31.0, $n = 8$] in EQ = 0.5, $p = 0.006$). In couples with women = 35 years (median age of the group), the relationship between AB and FR is maintained ($r = -0.3$, $p = 0.02$, $n = 81$) and negative correlations between AB and IR ($r = -0.7$, $p = 0.004$, $n = 15$) and PR ($r = -0.7$, $p = 0.003$, $n = 14$) are revealed. Similarly, the relation between CMA3 positivity and low EQ is confirmed and negative associations between CMA3 and IR ($r = -0.6$, $p = 0.03$, $n = 15$) and PR ($r = -0.6$, $p = 0.04$, $n = 14$) are highlighted. (ii) CatSper MFI is significantly lower when EQ is worse (4.2 [2.0–14.3, $n = 100$] in EQ < 0.5 vs. 5.1 [3.0–11.1, $n = 15$] in EQ = 0.5, $p = 0.03$). Such data is confirmed also in the selected group of younger women (3.9 [2.0–13.1, $n = 52$] in EQ < 0.5 vs., 5.9 [4.0–11.1, $n = 8$] in EQ = 0.5, $p = 0.01$) where an association between CatSper MFI and achievement of pregnancy is revealed (MFI = 5.7 [3.4–11.1, $n = 17$] in pregnant vs. 3.8 [2.0–7.4, $n = 31$], in non-pregnant couples $p = 0.002$).
Conclusion: Our data indicate that the two methods for evaluation of chromatin immaturity are highly correlated but not equivalent. Indeed AB and CMA3 are differently related to ART outcomes and resulted to be predictive of fertilization ability, embryo quality, implantation and pregnancy. In the subgroup of younger women these associations become stricter and the role of male factor in the fertilization process and in the achievement of pregnancy is highlighted. These tests are easy and rapid to perform, are low cost and do not need sophisticated instruments. Therefore they could be rapidly introduced in routine diagnosis of male of infertile couples undergoing ART. This is the first study evaluating the association between sperm CatSper expression and ART outcomes. Our data suggest a role of the channel in the achievement of a good embryo quality and pregnancy.
INYRMF member.

OR09

Testicular endocrine profiles in young boys operated for cryptorchidism

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Background: Cryptorchidism is the most common male congenital malformation, with an increasing incidence within the last decades in industrialised countries. Cryptorchidism is also a major risk factor for infertility and for testis cancer. In addition to epidemiological studies assessing the environmental risk factors and professionals cryptorchidism, many studies have compared hormonal markers of the testicles (Testosterone, inhibin B and Anti-Mullerian Hormon or AMH) in cryptorchid and non-cryptorchid boys. Nevertheless, the results remain contradictory showing no difference in older studies, while more recent studies show a significant reduction of these hormones in children with cryptorchidism.
Aim: To compare reproductive hormone levels of Testosterone, Inhibin B and AMH in young boys with and without cryptorchidism.
Methods: We performed a hospital-based cross-sectional study. From surgery appointment records, we identified a case group of boys with unilateral or bilateral cryptorchidism, excluding any illness or other urogenital malformations, and a group undergoing dental care, minor osteoarticular (talipes, hallux valgus) or dermal surgery (naevi). Informed parental consent was obtained. Blood samples were taken during the surgical procedure for biological (cholesterol and triglycerides) and hormone assays (testosterone, inhibin B and AMH). Analyses were performed in the Biochemistry department of Toulouse University Hospital. Cholesterol and triglycerides concentrations were determined enzymatically with an Olympus analyzer (Beckman Coulter, Roissy, France). Testosterone was assessed through an RIA kit (Cis-Bio International, Gif-sur-Yvette, France). AMH was assayed by an enzyme-linked immunoassay (ELISA) kit provided by DSL-Beckman Coulter (Webster, TX, USA). Inhibin B was measured by ELISA with a kit supplied by OBI-DSL (Oxford, UK). Both AMH and Inhibin B were assayed in duplicate according to manufacturer's protocols.
Results: Two groups of 27 boys were included, different for age at surgery: 26.6 vs. 24.2 months ($p = 0.172$). All serum levels were lower in cryptorchid than in control boys. Testosterone levels, measured in 10 controls and 10 cases, were 18.10 ng/100 mL ± 4.07 and 11.60 ng/100 mL ± 1.26, respectively ($p < 0.001$). AMH levels were 86.63 ng/mL in cryptorchid boys and 134.56 ng/mL in controls ($p = 0.003$), while inhibin B levels were 96.89 and 133.44 pg/mL, respectively ($p = 0.007$). AMH and inhibin B were markedly lower in the bilateral cryptorchidism subgroup, being 50% lower than in the controls (both $p = 0.003$).
Conclusion: Cryptorchid patients had simultaneous significantly decreased serum testosterone, AMH and inhibin B levels, suggesting a functional defect of both Leydig and Sertoli cells. The Sertoli cell defect appeared more pronounced in bilateral cryptorchid boys and could potentially compromise their future fertility.

OR10

Serum from patients with erectile dysfunction and vascular risk factors triggered oxidative stress-dependent mitochondrial apoptotic pathway in ex-vivo expanded circulating angiogenic cells of healthy men

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Background: Erectile dysfunction (ED) in men with vascular risk factors (VRFs) is an early clinical manifestation of a

systemic vascular disease and is predictive for cardiovascular events. An endothelial damage/dysfunction and an impaired capacity for repairing the endothelial damage were proposed as a possible physiopathologic mechanism to contribute to the systemic vascular disease in men with ED and VRFs. Serum of men with ED and vascular risk VRFs inhibits mononuclear circulating cells (MNCs) to expand ex-vivo and differentiate circulating angiogenic cells (CACs), putatively involved in endothelial damage repair.

Aim: To explore the involvement of apoptosis in the inhibition of CACs differentiation from MNCs of healthy men, exerted by serum of men with ED and VRFs.

Methods: MNCs from healthy men were cultured in presence of serum from 10 healthy men [median age: 45 years (38.5–48.5 as 25th–75th quartiles)] or from 14 patients [median age: 58.0 years (52.5–62.0 as 25th–75th quartiles)]. CACs were identified by uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-labelled acetylated low-density lipoprotein (DiLDL) and concomitant Ulex europaeus agglutinin I (UEA-1) binding, assessed by fluorescence microscopy. Flow cytometry evaluation of mitochondrial membrane potential (??m), assessed with JC-1 dye, and of activated caspase-8, -9 and -3 in DiLDL-positive cells.

Results: The number of CACs (%) was significantly reduced by serum from patients compared to controls (66.8 ± 10.6% vs. 27.8 ± 4.7%, $p = 0.017$). This was associated to ΔΨm suppression, as indicated by the lower percentage of cells with red-orange JC-1 fluorescence (24.0 ± 17.0%) with respect to untreated cells (42.0 ± 14.0%, $p = 0.02$) and to cells exposed to serum from healthy controls (45.0 ± 19.0%, $p = 0.02$), and activation of caspase-9 (25.8 ± 8.2% vs. 13.5 ± 4.7%, $p = 0.008$) and caspase-3 (28.4 ± 8.1% vs. 1.0 ± 0.1%, $p < 0.001$), but not of caspase-8 (11.5 ± 3.3% vs 11.8 ± 3.0%, $p > 0.05$). This suggests an activation of the intrinsic (mitochondrial) pathway of apoptosis, while the death receptor activation of apoptosis was not involved. The exposure of MNCs to Trolox, a hydrophilic cell permeable vitamin E analog with high antioxidant capacity, prevented the activation induced by serum of patients with ED of caspase-9 (17.0 ± 6.7% vs. 25.8 ± 8.2%, $p = 0.04$) and caspase-3 (1.0 ± 0.2% vs. 28.4 ± 8.1%, $p = 0.003$).

Conclusion: An oxidative stress-dependent mitochondrial dysfunction was triggered in *ex-vivo* expanded CACs of healthy men by serum from men with VRFs and ED, the only clinical correlate for a diffuse vascular disease. The activation of apoptosis and inhibition of CACs differentiation might generate a defective mechanism of vascular repair.

OR11

The LXR-null mice: a model for dyslipidemia-induced male infertility and capacitation impairment

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Background: Lipid metabolism disorders (dyslipidemia) are known causes of male infertility, but the majority of studies on this topic have generally focused on the endocrine side of these disorders. The Liver-X-Receptors-null mice (*Lxr−/−*, cholesterol homeostasis regulators) are a model of dyslipidemia-induced post-testicular infertility. These male present a high plasma LDL (Low Density Lipoprotein) concentration, and develop infertility starting from the age 10 months, characterized by testicular and epididymal defects. Interestingly, an early sterility is triggered when young and normally fertile males (4 months old) are previously fed for four weeks with a high cholesterol diet (HCD). The diet-induced sterility is only associated with the epididymal phenotype.

Aim: The aim of this work was to understand the consequences of cholesterol homeostasis dysregulation observed in the epididymis on gamete maturation and capacitation. The impact of HCD on gamete lipid composition and function was studied.

Methods: Spermatozoa lipid composition was determined by liquid chromatography and mass spectrometry (cholesterol, phospholipids and oxysterols). Sperm ability to undergo the capacitation process was assessed by anti-phosphotyrosine western blot (a capacitation terminal marker). The kinetic evolutions of membrane fluidity and intracellular calcium concentration were studied by flow cytometry during in vitro capacitation, using merocyanine 540 and Fluo-4 AM probes, respectively.

Results: Our results showed that HCD increased the cholesterol / phospholipids ratio and disrupted the oxysterol composition of L*xr−/−* male gametes. Membrane cholesterol efflux was altered during capacitation, consequently impairing the normal increase in membrane fluidity. These alterations of the sperm membrane dynamics affected the in vitro capacitation process, mainly by limiting calcium influx and sperm-protein tyrosine phosphorylation.

Conclusion: Dyslipidemia negatively impacts sperm epididymal maturation, resulting in lipid composition and membrane dynamics alterations, ultimately leading to abnormal sperm capacitation. These results open new perspectives for the study and treatment of infertility in dyslipidemic men.

PS1A – POSTER SESSION NR.1

POSTER PRESENTATIONS

MID01

Impact of pyospermia on sperm dynamic motility parameters and DNA integrity

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Background : Pyospermia is a common finding in infertile men with controversial issues about its significance.
Objective: To evaluate effect of pyospermia on computerized semen (CASA) parameters, sperm DNA integrity and chromosomal aneuploidy in infertile men.
Subjects: The study included 50 infertile men with oligoasthenoteratozoospermia divided into 2 groups according to presence or absence of pyospermia.
Methods: The study included clinical evaluation, peroxidase stain, CASA, sperm DNA evaluation with acridine orange test and sperm FISH analysis of 18, x and Y chromosomes. Main outcome measure: Comparison between the infertile men with and without pyospermia CASA, sperm DNA fragmentation with acridine orange test and sperm FISH parameters. Also, to correlate between the number of pus cells and these parameters.
Results: Infertile men with pyospermia had significantly lower sperm progressive and total motility percentages. Also, motility parameters by CASA including curvilinear, straight line and average pathway velocities, straightness, and amplitude of lateral head displacement were significantly lower with pyospermia. Sperm DNA fragmentation index by AOT was significantly higher with pyospermia. Percentages of sperms with diasomy XY and 18 by FISH were higher with pyospermia. These changes in sperm motility parameters and DNA integrity correlated with the number of peroxidase positive leukocytes.
Conclusions: Pyospermia has a negative impact on sperm motility parameters and DNA integrity regardless infertility as a cofactor.

MID02

The influence of environment on the sperm quality: a comprehensive, retrospective, cohort study

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Background: Several studies proposed a relationship between environmental factors and semen quality. In particular, the negative effect of air pollution on spermatogenesis and gonadal function is currently suggested. However, no specific studies evaluated the environmental influence on semen quality in a specific geographical area and time frame.
Aim: The aim of this study was the assessment of the relationship of both air pollution and environmental parameters with quality-related sperm variables, during the coldest months of the year characterized by the most polluted air.
Methods: A retrospective, observational, cohort study was carried out in the province of Modena, located in the Emilia-Romagna region of Northern Italy. Semen analyses, environmental temperature, air humidity and air particulate matter (PM) measurements from the 1st of November, 2014 to the 19th of February, 2015 were acquired to the first database. A second, wider database was arranged, evaluating environmental exposure in the 3 months before semen collection (from August 1st 2014). All data included in the database were registered by geo-coding the residential address of the patients and the site of registration of environmental factors. The geo-codification of parameters was performed using Fusion Tables of Google available at https://www.google.com/fusiontables/data?dsrcid=implicit, considering the exact time of measurement.
Results: Average air temperature was inversely related to sperm concentration and to total sperm number ($p < 0.001$). Semen volume was inversely related only to the minimum ($p < 0.001$) and not to maximum recorded temperature ($p = 0.110$). Air humidity was not related to sperm quantity and quality. PM2.5 was directly related to total sperm number ($p < 0.001$). PM10 was directly related to both semen volume ($0 < 0.001$) and typical forms ($p < 0.001$), inversely related to atypical forms ($p < 0.001$), and related neither to sperm concentration ($p = 0.430$) nor to sperm motility. The extended analyses considering environmental parameters in the 3 months before semen collection, confirmed the relationship between air temperature and sperm quantity, whereas no influence was found between PM and sperm quality.
Conclusion: We found an influence of environmental temperature on semen quantity, without a clear effect of air pollution, as assessed through PM10 levels, on sperm parameters variations. Environmental temperature and humidity seem to not affect semen quality, although a wider bigdata approach could better explain this relationship.

MID03

Effect of Sperm DNA fragmentation on the clinical outcomes for couples with unexplained infertility undergoing in vitro fertilization

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Topic Male infertility-diagnosis
Keywords: DNA fragmentation, in vitro fertilization, male infertility
Background: Sperm analysis is the cornerstone in male factor study, however it is not enough to predict fertility, leading to the need of additional tests. One of these, is the sperm DNA fragmentation index (DFI) which evaluates sperm DNA integrity. A DFI > 15% has been asociated with worse prognosis for fertilization and pregnancy rates, blastocyst development and pregnancy loss. Some studies have

also shown that one third of couples classified as "unexplained infertility" have an abnormal sperm DNA integrity.
Aim: To evaluate the effects of sperm DNA fragmentation in pregnancy rates, blastocyst development, and pregnancy loss in normozoospermic males with unexplained infertility undergoing in vitro fertilization.
Methods: 117 couples were included. Univariate correlations between 3 different grades of sperm DNA fragmentation index measured by HALOsperm (Group 1: ≤ 15%, Group 2: 16-29%, and Group 3: ≥ 30%) and the occurrence of fertilization, pregnancy, blastocyst development and pregnancy loss were performed
Results: 29% of the normozoospermic males had > 16% of sperm DFI. There were significant correlations between pregnancy rate (r = -0.97) between groups 1 and 3; pregnancy loss rate (r = 0.92) and blastocyst development (r = -0.87)
Conclusion: Approximately one third of normozoospermic males with unexplained infertility had an abnormal sperm integrity test which correlates with worse fertility prognosis in terms of pregnancy rates, blastocyst development and pregnancy loss.

MID04

Bilateral testicular torsion with present circulation on one side

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Background: Testicular torsion occurs when the testicle rotates and consequently causing a strangulation of its blood supply leading to ischemia and cell death. About 2% of patients with symptoms of unilateral testicular torsion have both testes affected and are often misdiagnosed.
Aim: The aim of this case presentation is to emphasize the necessity of surgical exploration of both testicles in cases of unilateral testicular torsion.
Case description: The 17-year old male patient was referred to emergency ward due to pain in the right testicle with sudden onset in the morning around 9 a.m. Patient did not report fever, urethral discharge or previous trauma of the testicles. White blood cell count and other inflammatory markers (CRP, erythrocyte sedimentation rate) remained in the reference range. Ultrasound assessment performed by radiologist revealed decreased circulation in the right testicle along with enlargement of the right epididymis. The initial suggestion of the ER doctors was epididymitis, but patient was referred to urologist immediately. The patient arrived at the urology at 13:00 p.m. Status of the left testicle and epididymis was negative. The right scrotum was swollen, tender on touch, cremaster reflex was absent. Testicle was fixed in cranial position. Ultrasound evaluation revealed absent circulation in the right testicle, normal epididymises on both sides, and normal circulation in the left testicle. Immediate surgical exploration was indicated due to the absent circulation in the right testicle at 14:00 p.m. Right testicle was plum-blue. There was an approximately 1440 degree twist on the right funiculus (it was twisted 4 times). After detorquation the testicle was covered in warm towel till the exploration of the left testicle. However the color of the left testicle was normal, there was a 720 degree twist on the left funiculus (it was twisted 2 times) (Fig. 2). It was back twisted and fixed to the scrotum in a subvaginal pouch with 2 absorbable stitches. The right testicle was controlled, which has nice pink color. This testicle was fixed to the scrotum applying the same method described above. The scrotal pain was immediately ceased after the surgical exploration; the patient did not require postoperative analgesics. He was discharged from the hospital on the first postoperative day with present circulation in both testicles.
Conclusion: Although the overall prevalence is low, recent studies suggest bilateral testicular torsion is being reported at a higher frequency especially in perinatal period despite showing normal Doppler flow prior to the surgery. Bilateral scrotal exploration and orchidopexy of both testicles despite negative diagnostic findings greatly improves the overall outcome and significantly reduces the loss of testicles from prolonged ischemia. Furthermore both testicles must be fixed to the scrotum even in case of unilateral testicular torsion.

MID05

A prediction model for successful sperm retrieval in patients with non-obstructive azoospermia using serum FSH level, testicular volume and testicular histology

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Background: The chance of fathering a child for patients with non-obstructive azoospermia (NOA) depends from the likelihood of finding viable sperm in their testes. Several attempts have been made in order to find clinical factors that could predict the chance of successful sperm retrieval (SSR) in these patients, in order to assist surgeon for the appropriate counselling of these patients. Unfortunately, sperm retrieval is quite an unpredictable factor, given that FSH, testicular volume and other clinical characteristics have low sensibility and specificity when their predictive ability is individually evaluated. Yet, predictive models built putting together some preoperative clinical parameters have obtained slightly higher diagnostic accuracy (60.8%).
Aim: The present study sought to evaluate whether including testis histology in a predictive model evaluating also FSH level and testicular volume would increase its diagnostic accuracy.
Methods: 356 patients with NOA who underwent cTESE between June 2004 and July 2009 were retrospectively evaluated. FSH level and testis volume as assessed by scrotal ultrasound were available for all patients. Testicular histology was performed by a well-experienced pathologist on testicular samples obtained during surgical sperm retrieval. Binary logistic regression was used to evaluate the diagnostic accuracy of a predicting model built with serum FSH level, mean testicular volume and testis histology,

identifying SSR as binary dependent variable. The sample size requested for binary logistic regression, calculated according to the Peduzzi formula (N = 10 k/p), was 68 patients.

Results: The mean patients age was 36.8 years (18–63 years). Testicular sperm were retrieved in 158 out of 356 patients (44.3%). Histological diagnosis of Sertoly only syndrome (SCO) was obtained in 216 patients (60.6%), while 55 patients (15.4%) had maturation arrest (MA) and 85 (23.8%) had hypospermatogenesis (HYPO). The binary logistic regression model was statistically significant (c^2 = 96.792, $p < 0.0001$) and correctly classified 72.8% of cases (diagnostic accuracy) with 46.8% sensitivity (95% CI 38.86–54.931) and 93.4% specificity (95% CI 89.03–96.4), PPV 85.06%, NPV 68.7%, +Likelihood ratio (LR) 7.13 (95% CI 4.11–12.38), – LR 0.57 (95% CI 0.49–0.66). Only testicular histology was significant to the model, while FSH and testicular volume were not. SRR was significantly higher in patients with HYPO compared to patients with SCO or MA (88.2% vs. 30.5 and 30.9 respectively, $p < 0.0001$)

Conclusion: This study demonstrates that including testicular histology in a model for predicting sperm retrieval increases its diagnostic accuracy. According to our results, patients with histological diagnosis of HYPO would have good chances (88%) of having their sperm retrieved by another cTESE attempt, while patients with SCO or MA would have very low chances (around 30%) of SSR with this surgical technique. As a consequence, cTESE represents a cost-effective treatment for patients with HYPO, while patients with SCO or MA should be counseled to undergo microTESE in order to improve their chance of SRR. As testis histology is made available only to patients with previous testis surgery, the clinical application of this model would be limited to patients with previous sperm retrieval failure.

MID06

Evidence of a new pattern of ejaculation in men with spinal cord injury: ejaculation dyssynergia and implications for fertility

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Background: Ejaculation (E) may be significantly impaired in men with spinal cord injury (SCI). Antegrade ejaculation (AE) occurs in most cases (65%) but a number of patients experience retrograde ejaculation (RE). RE may be pure or combined with AE (16%). While the exact mechanism of RE is still unknown, there is some evidence that it may result from poor coordination between the external sphincter and the bladder neck during expulsion which can prevent AE or may reduce semen volume.

Aim: To search for semen in the prostatic urethra (PU), using a new technique which we developed, and to evaluate the characteristics of any semen found.

Methods: Among 104 patients with neurological disorders and ejaculatory dysfunction attended the Reproductive Medicine Centre (from November 2011 to December 2012), 33 who wished to father a child but had anejaculation during sexual intercourse or manual stimulation were prospectively enrolled.

All patients underwent the usual procedure to investigate ejaculation: catherization to completely empty all urine from the bladder, followed by the instillation of a pink buffering medium (40 mL of Ferticult□, Fertipro NV, Belgium) and then penile vibratory stimulations (PVS). If they failed to ejaculate, PVS was combined with oral midodrine (5 mg and increase in steps of 7.5 mg until the patient ejaculated, up to 30 mg).

Two-step catheterization was then performed: a catheter (Lofric Wellspect HealthCare, 12ch standard) was inserted through the urethral sphincter into the prostatic urethra to aspirate its content; then bladder catheterization was performed to collect the Ferticult□. The procedure was repeated in some patients after at least 1 week. The characteristics of the semen collected from each organ were analyzed and compared.

Results: Out of 33 patients, ejaculation (i.e. AE, RE, or PUE, either pure or combined) was achieved in 22 patients in whom 42 trials were obtained. Semen was found in the PU in 21 samples (50%) from 12 patients (11 with spinal cord injury, 1 with diabetes). The colour of all 21 prostatic urethra semen samples differed from the Ferticult□. Sperm motility was greater in 8 samples, sperm count higher in 10 and pH different in 10, compared with the bladder samples. The higher overall sperm quality allowed cryopreservation in 10 PU samples compared to only 5 bladder samples.

Four of the 5 patients with repeated trials had a reproducible pattern of PUE.

Conclusion: The presence of semen in the prostatic urethra most probably results from 'ejaculation dyssynergia', a lack of coordination between bladder neck and external sphincter. Semen from the prostatic urethra should be systematically sought to improve the outcome of assisted reproduction.

MID07

The spectrum of renal involvement in male patients with infertility related to excretory-system abnormalities: phenotypes, genotypes, and genetic counselling

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Background: While mutations of the *CFTR* gene account for a large percentage of patients with a uni/bilateral absence of vas deferens related infertility, most patients with the rare association of renal and genital-tract malformations do not harbor a mutation of *CFTR*.

Aim: To provide in-depth renal phenotyping of patients combining renal and genital tract malformations.

Methods: Retrospective (between 2000 and 2014), observational monocentric study of infertile male patients with both genital tract and renal malformations followed in the Male Sterility Centre and referred to the Renal Department

and to the Department of Medical Genetics. Data regarding semen parameters, imaging of the genital tract, kidneys and urinary tract, renal function, and *CFTR* and *HNF1B* genetic testing were collected.

Results: We identified 34 patients (median age 32 years [25–50]) with combined renal and genital tract disorders, including 28 (82%) without known renal disease before the assessment of the infertility. Four main renal phenotypes were observed: solitary kidney (n = 16, 47%); polycystic kidney disease suggestive of *PKD1* or *PKD2* mutations (n = 7, 20.6%); uni/bilateral hypoplastic kidneys (n = 7, 20.6%); and a complex renal phenotype (solitary cystic kidney or bilateral microcystic hyperechogenic kidneys) associated with a mutation of the *HNF1B* gene (n = 2, 5.9%).

A diagnosis of dominantly inherited renal disease was retained in 9/34 (26.5%) patients (ADPKD* [n = 7], *HNF1B*-related nephropathy [n = 2]). None of the patients had a biallelic *CFTR* mutation.

A uni/bilateral absence, hypotrophy or dilatation of the seminal vesicles was observed in 94% of patients; while 79% had a uni/bilateral absence (57.6%) or dilatation of the vas deferens (21.2%). Uni/bilateral absence of seminal vesicles and azoospermia were significantly associated with the presence of a solitary kidney, while dilatation of at least one seminal vesicle and necroasthenozoospermia were more suggestive of ADPKD. The finding of a complete dissociation between the testis and the epididymis was only observed in one of the two patients with a whole-gene deletion of *HNF1B*.

Conclusion: Male patients with a seminal-tract malformation (i.e. at least an absent, hypotrophic, or dilated seminal vesicle) should be referred to a nephrologist for routine renal assessment and to address the risk of inherited kidney disease before beginning a pregnancy either natural or with MART.

*ADPKD: autosomal dominant polycystic kidney disease.

MID08

A correlation between obesity and semen interleukin-6 with sperm concentration

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Background: Obesity has been defined as abnormal or excessive fat accumulation that may impair health while the Body mass index (BMI) is a simple index of the weight-to-height ratio that is commonly used in classifying overweight and obesity in adult populations.

Several studies showed the elevated IL-6 in obese subjects. These studies failed to explain the relationship between this peculiar molecule and its source from the adipose tissue.

Many semen parameters alteration can be ascribed to obesity like decreased sperm concentration, abnormal morphology, compromised chromatin integrity and abnormal motility. Many evidences showed an adverse effect of excess body fat on spermatogenesis.

The aim of the study: To evaluate the relationship between obesity and IL6 and their effects on semen parameters.

Methods: A total sample size composed of (87) Patients were grouped into three groups according to published BMI ranges as follows: normal weight, overweight and obese .A single semen sample was collected for each patient. Height in (m) and weight in (kg) were recorded on day of semen collection .BMI was calculated as BMI = kg/m^2. Sperm parameters evaluated according to World Health Organization guidelines. Quantitation of the IL6 in seminal plasma were accomplished using commercially available ELISA kit.

Results: In the current study, a positive correlation was found between body mass index and IL-6, on the contrary, a significant negative correlation was found among IL-6 level and progressive sperm motility and sperm concentration as well.

Conclusion: Positive correlation between body mass index and IL-6 level in seminal fluid.

Significant negative correlation was detected among IL-6 level and progressive sperm motility, non progressive sperm motility.

Significant decrease in sperm concentration in obese people.

The obesity reduces spermatogenesis by androgen deficiency result in low sperm concentration and low normal sperm motility.

MID09

No associations between sperm chromatin maturity and ICSI outcomes

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Background: The influence of standard semen parameters and sperm chromatin maturity on fertilization, embryo development and achieve pregnancy both in natural or assisted procreation is the subject of many reports presenting often conflicting opinions (Chan et al., 2015; Kazerooni et al., 2009; Ni et al., 2014; Rondanino et al., 2015; Talebi et al., 2012; Tamburrino et al., 2012; Wang et al., 2014). Therefore, our research was designed to find a relationships between these parameters and embryo development.

Subjects and Methods: The study was carried out on ejaculated spermatozoa of men (n = 209) from infertile couples treated with ICSI. The standard semen analysis was performed according to the WHO, 2010 recommendations. Assessment of sperm chromatin maturity was carried out using assay with chromomycin A3 (CMA3), aniline blue (AB) and toluidine blue (TB). Non-invasive, intravital assessment of embryo development in in vitro conditions was made (Gardner et al., 2000; Scott et al., 2000). The proportion of fertilized oocytes in the first day after conception (fertilization rate), the percentage of morphologically normal embryos assessed on 3rd day (top quality embryo rate on 3rd day) and 5th day (top quality embryo rate on

5th day) after fertilization as well as the percentage of pregnancies confirmed two weeks after embryo transfer were established.
Results: Comparison between a group of men from infertile couples treated by ICSI who achieved = 50% of normal embryo parameters and a group of men from infertile couples, who achieved >50% of these parameters showed no significant differences in standard semen parameters and sperm chromatin maturity. Similarly, a comparison between a group of men from infertile couples who did not achieve pregnancy and a group who achieved pregnancy showed no significant differences in the ICSI outcomes. The age of men, standard semen and sperm chromatin maturity results did not correlated with fertilization and embryo development. However, significantly older men originated from couples who did not achieve pregnancy (36.0 vs. 34.0 years of age). The age of men and the percentage of embryos with the highest morphological quality assessed on 5th day had a significant moderated (AUC = 0.604, cut off point = 38 years of age) or satisfactory (AUC = 0.702) diagnostic value for getting pregnant respectively.
Conclusion: Our results indicate that in the study group there is no associations between standard semen parameters, sperm chromatin maturity, fertilization, embryo development and achieve pregnancy in in vitro conditions. Can be assumed that the level of sperm chromatin damage of examined men enrolled to ICSI program was not clinically significant. However, it seems that men below 38 years of age and couples for achieving a morphologically normal embryos on 5th day may have a better chance of getting offspring.

MID10

The EAA/EMQN external quality control program critically improves the molecular diagnosis of Y chromosome microdeletions

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Background: Microdeletions in the long arm of the Y chromosome are one of the most frequent genetic causes of abnormal spermatogenesis. The molecular diagnosis of Y chromosome microdeletions is therefore a routine genetic test in the diagnostic workup of azoospermic and severely oligozoospermic (<5 million sperm/mL) patients. Owing to its diagnostic and prognostic value, it is crucial that Y chromosome microdeletion testing is performed according to the highest possible standards. For more than 15 years the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have been supporting the improvement of Y chromosome microdeletion testing through the publication of best practice guidelines and by offering an external quality control program.
Aim: To quantify the impact of external quality control in the molecular diagnosis of Y chromosome microdeletions.
Methods: Analysis over a 16-year period of the efficiency of the EAA/EMQN external quality control program in: (i) decreasing genotyping errors; and (ii) improving reporting practice. The EAA/EMQN external quality control program tests, on a yearly basis, the whole analytical process of a molecular genetics laboratory, its ability to interpret data in light of clinical information supplied with a referral, and the transmission of test results using clear and accurate reports. Lab performance is assessed through the analysis of 3 samples with mock clinical referral. The EAA/EMQN external quality control program accepts and evaluates lab reports in an extensive number of languages: English, German, Italian, Dutch, Portuguese, French and Spanish.
Results: The number of labs participating in the EAA/EMQN external quality control program has practically tripled since this service was first made available in 1999. This year, 137 labs across the globe signed up to be assessed. Importantly, the EAA/EMQN external quality control program has had a critical impact in reducing diagnostic errors (an incorrectly assigned genotype that would lead to misdiagnosis): at the start of this program the overall diagnostic error rate was almost 8%, after 6 years it was already reduced to half, and it is currently 0.74% of all reported cases. Furthermore, the program has also significantly improved the quality of reporting practice: the number of analyses with a full interpretation score has clearly increased since the start of the program and now corresponds to 68% of all analyses. Nevertheless, there is still room for improvement: several labs have yet to adopt the simple and cost-effective multiplex PCR set-up recommended by best practice guidelines, as well as to offer the now mandatory deletion extension analysis.
Conclusion: Participation in the EAA/EMQN external quality control program has lead to a critical improvement in the molecular diagnosis of Y chromosome microdeletions: not only diagnostic error rates have been dramatically reduced but also reporting practice is better and more consistent. Annual participation in the EAA/EMQN external quality control program is, therefore, strongly recommended for all labs performing molecular diagnosis of Y chromosome microdeletions.

MID11

2-Arachidonoylglycerol levels are increased in leukocytospermic ejaculates and correlate with semen concentration of macrophages and activated macrophages

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Background: Leukocytospermia represents the hallmark of the immune system response to inflammation and/or infection of the male genital tract, in the context of which macrophages are recruited and activated. As macrophages can actively generate endocannabinoids (eCBs) in response to inflammatory stimuli, we hypothesized that in the presence of leukocytospermia, macrophages might be an additional significant source of seminal eCBs, also derived from other cells and tissues of male urogenital tract, including spermatozoa.
Aim: In this study we evaluated whether seminal levels of the eCBs, *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), were higher in the presence of leukocytospermia compared with controls, and whether they were correlated with semen concentration of macrophages and activated macrophages.
Methods: The content of AEA and 2-AG was measured by high-performance liquid chromatography/mass spectrometry in seminal plasma of ejaculates from 18 leukocytospermic patients (>1 × 106 leukocytes/mL) and 21 normozoospermic controls, selected on the basis of no or ≤0.2 × 106 round cells/mL. In the same ejaculates, round cells were phenotypized by flow cytometry as leukocytes (CD45-positive cells), monocytes/macrophages (CD14-positive leukocytes) and antigen-presenting activated macrophages (HLA-DR-positive macrophages).
Results: Seminal plasma levels of 2-AG, but not of AEA, were significantly higher in ejaculates from leukocytospermic patients than in controls. In the whole study population, semen levels of 2-AG significantly correlated with the concentration of both macrophages and activated macrophages, but not with global leukocyte population. Noteworthy, a significant correlation between levels of 2-AG and activated macrophages was also observed when the analysis was restricted to leukocytospermic ejaculates ($r = 0.48$, $p = 0.04$).
At the multivariate linear regressions, 2-AG levels exhibited a significant independent association with both macrophages and activated macrophages after adjustment for semen volume and sperm concentration, as major confounding factors.
Conclusion: 2-AG seminal levels were higher in leukocytospermia and exhibited a significant independent association with macrophages and activated macrophages. As eCBs are involved in the control of several physiological processes, including reproduction, further studies are warranted to elucidate possible clinical reflections and applications in the diagnostic approach to leukocytospermia.

MID12

Sperm Chromatin Structure Assay in prediction of IVF/ICSI outcome

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Background: Nowadays, when the assisted reproductive technology has gained a major role in the treatment of infertility, the traditional semen test is not efficient in assessment of male fertility potential. A large number of new diagnostic methods focus on sperm chromatin integrity. Among them Sperm Chromatin Structure Assay (SCSA), expressed in DNA fragmentation index (DFI), is the most scrutinized technique and seems to be most promising from a clinical point of view.
Aim: To evaluate the association between sperm DFI and the outcome of standard in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) procedure.
Methods: Two studies were performed. They were based on a cohort of consecutive IVF/ICSI procedures. DFI values were categorized into intervals: DFI ≤ 10% (reference group), 10% < DFI ≤ 20%, 20% < DFI ≤ 30%, DFI > 30%. In the first study 1633 IVF/ICSI procedures were included. The following outcomes were analyzed in relation to the reference group: fertilization, good quality embryo, pregnancy, miscarriage, and live births. The second study involved 256 IVF and 383 ICSI treatments (totally 6117 oocytes). The endpoints were meantime of following early embryo morphokinetics recorded by time-lapse embryo monitoring system: formation of pronuclei, fading of pronuclei, early cleavage and starting blastulation. Data were analyzed in three ways: the interaction between DFI category and fertilization type; separately for IVF and ICSI and also ICSI vs. IVF.
Results: The first study illustrated that chance of live birth in IVF when DFI was above 20% was significantly lower than for those with lower DFI. Moreover, for the high DFI subgroup, live birth rates were significantly higher for ICSI as compared to IVF. The results corresponded with negative association between DFI and fertilization rate as well as the chance of obtaining at least one good quality embryo (GQE), in standard IVF but not in ICSI. Increased risk of miscarriage was seen in combined calculation for both IVF and ICSI when DFI exceeded 40%. The second study demonstrates that increased DFI makes some early embryo morphokinetics longer within IVF group and shorter or neutral in ICSI group which suggests that sperm DNA integrity plays an important role in early embryo development.
Conclusion: ICSI might be a preferred method of in vitro treatment in cases with high DFI. Sperm DNA damages

may lead to some changes of early embryo morphokinetics, which suggest that sperm chromatin integrity plays an important role not only in the fertilization act but also in early embryo development. SCSA and time-lapse technologies seem to be useful in future research, which examines this observation in more detail.

MID13

Descriptive statistics and 95% confidence intervals for three epididymal biomarkers assessed in the semen of 418 normozoospermic men screened in a French andrology centre

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Background: Epididymis shelters the post-testicular maturation of spermatozoa which is mandatory for an optimal fecundation process. Its secretory capacity, considered as a functional surrogate, can be assessed through the measurement of three biochemical constituents of the seminal plasma: free L-carnitine, alpha-glucosidase and glycerophosphocholine (GPC). These biomarkers can also be useful to identify acquired or congenital obstructive lesions of the male genital tract. However, their diagnostic validity remains questionable mainly because of the scarce availability of reference intervals.
Aim: To define and describe a two-sided distribution (5th and 95th centiles) for three epididymal biomarkers in the seminal plasma of a retrospective cohort of normozoospermic men.
Methods: We focused on semen analyses performed for infertile couples referred to our andrological centre (Toulouse, France) from January 2000 to September 2014. Thresholds that defined the normozoopermic status were the following: sperm count ³ 15.106/mL, semen volume ³ 1.5 mL, mobility ³ 30% and viability ³ 58%. Biochemical analyses were carried out according to inhouse procedures. A careful database search allowed identifying 418 normozoospermic men with complete medical records, semen analyses and seminal biochemical data set. Database building, variables description and statistical analyses were performed using R and SAS software.
Results: All the variables had a Gaussian distribution. Normozoospermic men had a mean (±SD) age of 34.3 (±5.8) years. The mean (±SD) of semen volume (mL), sperm count (106/mL), total sperm count (106/ejaculate), total progressive mobility (a+b at 1 hour, %) and viability (%) were respectively: 3.8 (±1.5), 70.5 (±55.2), 250 (±192), 43.8 (±8.2) and 75.7 (±7.9). The mean (±SD) of concentrations for free L-carnitine (μmol/L), alpha-glucosidase (IU/L) and GPC (mmol/L) were respectively: 391 (±170), 20.1 (±9.5) and 2.3 (±1.1). Analytical imprecisions of their biochemical assays were respectively of 7%, 11% and 13%. We then selected from the database a sub-group (G1) of 63 normozoospermic men with both absolutely normal andrological history and examination. The remaining patients (n = 355) constituted the G2 subgroup. The semen parameters of both sub-groups were no statistical different. The means (±SD) of total amount in the ejaculate for free L-carnitine (nmol), alphaglucosidase (mU), GPC (μmol) were respectively for G1: 1394 (582), 72 (37), 9,1 (5.4) and for G2: 1374 (582), 71 (34), 7.9 (3.9). There were no statistical differences between the two subgroups. Finally, we defined the 95% CI of the total amount in the ejaculate for the epididymal biomarkers using the entire cohort (n = 418). There were for free L-carnitine, 611 - 2460 nmol/ejaculate ; for alpha-glucosidase, 26 - 137 mIU/ejaculate and for GPC, 2.6 - 15.6 μmol/ejaculate.
Conclusion: We defined from a cohort of normozoospermic men the 95% CI for three epididymal biomarkers. This first step is useful to investigate their clinical validity, especially for etiological diagnosis for oligo- and azoospermic patients.

MID14

AZFb deletions compatible with sperm production?!

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Background: Deletions of the long arm of the Y chromosome are a well-known cause of male infertility. The detection of a Yq microdeletion is of diagnostic and prognostic use: only AZFc deletions are compatible with sperm production (in ~70% of patients). Since 2014 new EMQN guidelines have been endorsed for the detection and interpretation of Yq microdeletions. An important revision is the inclusion of the extension analysis, i.e. in case of the detection of a microdeletion, extra markers should be analysed.
Aim: In a routine setting, we test for the presence of Yq microdeletions according to the new guidelines. We have a special interest in the AZFb region.
Methods: The standard multiplex PCR reaction is performed in duplex, as instructed by Simoni et al. (2004) and Krausz et al. (2014). When a deletion is detected, we continue with the suggested extension analysis (Krausz et al., 2014). In case an AZFb deletion is detected, markers sY105, sY1224, sY1192 and sY153 (from proximal to distal) are tested. Markers sY1224 and sY1192 are expected to be absent in case of an AZFb deletion.
Results: We have detected two patients with residual sperm production, where the first Yq microdeletion test shows the absence of markers sY127 and sY134, located in the AZFb region. For the first patient, extension analysis showed that markers sY105 and sY1224 are absent, while markers sY1192 and sY153 are present. For the second patient, only sY1224 was shown to be absent.
Conclusion: We have detected two patients, were the 'old' Yq microdeletion test would classify the patients as having a 'complete AZFb' deletion. However, the extension analysis showed an atypical pattern. Presumably, a re-organisation of the AZFb region has occurred, resulting in a partial absence of genes in this region. Overall, these results show the importance of performing the extension analysis for patients with a Yq microdeletion, especially in case of an

AZFb deletion. For patients with an atypical AZFb deletion, testicular sperm extraction might still be an option.

MID15

Semen leukocytes and oxidative-dependent DNA damage of spermatozoa in male partners of subfertile couples with no symptoms of genital tract infection

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Background: Seminal leukocytes seem to mediate sperm damage, including DNA fragmentation, through secretion of reactive oxygen species (ROS). Sperm DNA fragmentation may also be the result of a persistence of DNA strand breaks and of abnormal chromatin condensation during spermiogenesis and it may be oxidatively induced This is substantially supported by the observation that sperm DNA fragmentation measured by the terminal deoxynucleotidyl transferase (TdT)-mediated fluorescein-dUTP nick end labelling (TUNEL) or sperm chromatin dispersion assays is highly associated with the expression of sperm 8-hydroxy-2-deoxyguanosine (8-OHdG). The latter is a bio product specific for an oxidant-induced DNA damage and can be measured on a single-cell basis in ejaculated spermatozoa therefore its determination may explore the relationship between semen infiltration of leukocytes and sperm DNA damage.
Aim: To clarify the association between the presence of specific leukocyte subset and the level of oxidative-related DNA damage of ejaculated spermatozoa and better define the association between oxidative-related DNA damage and quality of ejaculated spermatozoa in male partners of subfertile couples asymptomatic for a genital tract infection.
Methods: 111 ejaculates from men of subfertile couples attending the Andrology Centre at University of L'Aquila were first evaluated for the routine semen analysis. Seminal leukocytes subpopulations were detected using monoclonal antibodies anti-CD45, a pan-leukocyte marker, anti-CD14 for monocyte/macrophage lineage and anti HLA-DR for activated macrophages. 8-hydroxy-2-deoxyguanosine (8-OHdG) expression identified spermatozoa with DNA oxydative adducts while TUNEL assay detected spermatozoa with DNA fragmentation. Flow cytometry and immunocytochemistry was used for determinations.
Results: Seminal leukocyte subpopulations were strictly correlated ($p < 0.0001$), but no association was found between the concentration of leukocytes, sperm parameters, the percentage of TUNEL-positive and of 8-OHdG-positive spermatozoa. The lack of an association between leukocytes infiltration and 8-OHdG-positive spermatozoa, prompted to explore the relationship between the percentage of 8-OHdG-positive spermatozoa, sperm DNA fragmentation and sperm quality. The percentage of 8-OHdG-positive spermatozoa was positively correlated with the percentage of TUNEL-positive sperm ($r = 0.48$; $p < 0.0001$) and negatively correlated with sperm concentration ($r = -0.44$; $p < 0.0001$). Sperm concentration and the percentage of TUNEL-positive spermatozoa independently contributed ($r = -0.25$, $p = 0.008$; $r = 0.23$, $p = 0.05$ respectively) to the variation of percentage of 8-OHdG-positive spermatozoa after adjusting for age, abstinence time and smoking.
Conclusion: Oxidative dependent DNA damage in spermatozoa was associated to poor sperm quality but not to different leukocyte subpopulations in ejaculates of subfertile men asymptomatic for a genital tract infection.

MID16

Is thyroid hormone evaluation of clinical value in the work-up of males of infertile couples?

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Background: A few previous studies performed on a limited series of subjects reported a negative impact of hyper- and hypo-thyroidism on semen volume, sperm density, progressive motility and normal morphology. No previous study has systematically evaluated associations between thyroid hormones (TH) variation, semen parameters and ultrasound characteristics of the male genital tract.
Aim: To investigate the associations between TH levels, semen parameters and ultrasound characteristics of the male genital tract and to assess if TH evaluation is of clinical value in the work-up of males of infertile couples.
Methods: Cross-sectional study. Out of a consecutive series of 172 men with couple infertility, 163 (age 38.7 ± 7.6 years) free of genetic abnormalities were studied. All subjects underwent a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal colour-Doppler ultrasound (CDUS) and semen analysis (including seminal interleukin 8 levels, sIL-8) evaluation within the same day.
Results: Among the patients studied, 145 (88.9%) showed euthyroidism, 6 (3.7%) subclinical hyper- and 12 (7.4%) subclinical hypo-thyroidism. No subjects showed overt hyper- or hypo-thyroidism. At univariate analysis, no associations among TSH or TH levels and sperm parameters were observed. Conversely, we observed positive associations among fT3 and fT4 levels, ejaculate volume and seminal fructose levels. In a multivariate model, after adjusting for confounders such as age, body mass index, smoking habit, sexual abstinence, calculated free testosterone, prolactin and sIL-8 levels, only the associations found for fT3 levels were confirmed. When CDUS features were investigated, using the same multivariate model, we found positive associations between fT3 levels and seminal vesicles (SV) volume, both before and after ejaculation (adj.$r = 0.354$ and adj.$r = 0.318$, both $p < 0.0001$), as well as with SV emptying (SV volume; adj.$r = 0.346$, $p < 0.0001$) and echo-texture inhomogeneity. In addition, after adjusting for confounders, negative associations between fT4 levels and epididymal body and tail diameters were found. No significant associations between TSH or TH levels and CDUS features of other organs of the male genital tract, including testis and prostate, were found. Finally, when

the features of subjects with euthyroidism, subclinical hypo- and hyper-thyroidism were compared, no significant differences in seminal or hormonal parameters were found. Conversely, evaluating CDUS parameters, subjects with subclinical hyperthyroidism showed a higher difference between the SV longitudinal diameters measured before and after ejaculation as compared to that of subclinical hypothyroid men, even after adjusting for confounders ($p < 0.007$). All the other male genital tract CDUS characteristics did not differ among groups.

Conclusions: Although no associations between TH and sperm parameters were observed, present data support a positive effect of TH on SV size and a permissive role on the ejaculatory machinery, likely through an action on SV and epididymal contractility. This is the first study reporting such evidence. However, in contrast with the view that TH assessment is important for female fertility, our results do not support a systematic evaluation of thyroid function in males of infertile couples. How TH abnormalities impact male fertility needs to be addressed by further studies.

MID17

DNA fragmentation in two cytometric sperm populations: relationship with clinical and ultrasound characteristics of the male genital tract

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Background: Evaluating sperm DNA fragmentation (sDF) we previously reported the identification of two subpopulations, PI^{dimmer} and $PI^{brighter}$, with different biological characteristics and clinical relevance. In particular, PI^{dimmer} population is entirely formed by DNA unviable fragmented spermatozoa, and shows signs of apoptosis and negative correlations with semen quality. Conversely, $PI^{brighter}$ population consists of a variable percentage of sperm with DNA fragmentation, is formed by both viable and unviable sperm, shows signs of apoptosis and DNA oxidation, and is independent from semen quality. $PI^{brighter}$ sDF is the fraction that best discriminates fertile and infertile men independently from semen quality. The possible anatomic origin of these two subpopulations is presently unknown.

Aim: To investigate whether DNA fragmentation in two cytometric sperm populations (PI^{dimmer} and $PI^{brighter}$) is related to clinical and colour-Doppler ultrasound (CDUS) parameters of the male genital tract.

Methods: A consecutive series of 160 males of infertile couples without genetic abnormalities were evaluated for clinical, scrotal and transrectal CDUS characteristics, presence of prostatitis-like symptoms (with the National Institutes of Health-Chronic Prostatitis Symptom Index, NIH-CPSI) and sperm DNA fragmentation (sDF) in PI^{dimmer} and $PI^{brighter}$ populations (using TUNEL/PI method coupled with flow cytometry).

Results: Data were adjusted for age (Model 1) along with waistline, testosterone levels, smoking habit and sexual abstinence (Model 2). According to the statistical Model 2, PI^{dimmer} sDF was associated with testicular abnormalities, including lower clinical and ultrasound volume ($r = -0.205$ and $r = -0.204$, respectively; $p < 0.05$), higher FSH levels ($r = 0.338$, $p < 0.0001$) and occurrence of testicular inhomogeneity ($p < 0.05$) and hypoechogenicity ($p < 0.05$). $PI^{brighter}$ sDF was associated with prostate-related symptoms and abnormal signs, including higher NIH-CPSI total and subdomain scores, a higher prevalence of prostatitis-like symptoms and of CDUS alterations such as macro-calcifications, severe echo-texture inhomogeneity, hyperemia (all $p < 0.05$) and higher arterial peak systolic velocity ($r = 0.253$, $p < 0.05$).

Conclusions: Our results suggest that DNA fragmentation in PI^{dimmer} sperm, which is related to poor semen quality, mainly originates in the testicles, likely due to apoptosis. Conversely, DNA fragmentation in $PI^{brighter}$ sperm appears to mainly originate during or after transit through the prostate, increasing with the presence of an inflammatory status of the organ. These results could lead to new perspectives for the identification of therapeutic targets to reduce sDF.

MID18

Correlation between classical semen parameters, sperm nuclear condensation and DNA fragmentation index in infertile, oligozospermic males

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Background: Diagnosis and classification of male factor infertility depends in a large part on the results of conventional semen analysis, although it is generally accepted that quantitative assessment of semen has poor prognostic value in predicting outcomes of assisted reproductive cycles. Beside quantitative analysis there are many standardized methods to obtain qualitative data on sperm quality.

Aim: The aim of the research was to look for correlation between classical semen parameters and qualitative sperm characteristics.

Methods: Semen samples of 15 infertile, oligozoospermic men (OZ) and 15 age-matched fertile men were analyzed (mean age:33.1 years; 24–42 years). Classical semen analysis was performed. DNA fragmentation index was determined with fluorescent acridine-orange staining. Epigenetic maturation in the sperm includes the histone protamine exchange, which is the key step in chromatin condensation. The percentage of sperms with decondensed chromatin was determined with aniline-blue staining.

Result: The mean sperm concentration was 7.1×10^6/mL in the oligozoospermic group and 78.5×10^6/mL in the normozoospermic group. There was significant difference in the sperm progressive motility (16.8% vs. 39%), total motility (37.4% vs. 59.5%), morphology (12.7% vs. 3.7%) between the infertile, oligozoospermic and normozoospermic groups. The DNA fragmentation was significantly increased in the infertile, oligozoospermic group (26.4% vs. 14.5%). Chromatin condensation failure was present in the patient group in 45%, and in 17.9% in the control group ($p < 0.001$).

Correlation analysis revealed a connection between sperm concentration and DNA fragmentation ($r = -0.72$; $p < 0.05$) and also a stronger relationship between sperm concentration and chromatin decondensation ($r = -0.85$; $p < 0.05$).
Conclusion: Correlation between the quantitative and qualitative semen parameters can be explained by a common regulative mechanism, which plays role in different levels of the spermiogenesis. Higher DNA fragmentation index as well as increased chromatin condensation failure is known to reflect the fertility of the semen sample. By using both qualitative and quantitative methods in the analysis of sperm, essential diagnostics can be provided on the fertility of sperm; especially in cases of unexplained male infertility or recurrent pregnancy loss.

MID19

Extracellular miRNAs as biomarkers of male subfertility

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Background: Male subfertility contributes to about 50% of all infertility cases. Causes of male subfertility remain poorly understood and identification of subfertile men primarily relies on semen analysis. Serum biomarkers rely on reproductive hormone analysis and identify only a fraction of subfertile men with poor sensitivity and specificity. Serum nucleic acids have emerged as a new family of biomarkers for several conditions. Among these, miRNAs actively secreted from several tissues as paracrine or endocrine messengers are regarded as promising functionally significant biomarkers.
Aim: The aim of this study was to identify new serum miRNA-based biomarkers of male subfertility utilising a cohort of subfertile men and control population from Sk□ne region, Sweden, and to explore a possible association between their serum levels of hormonal and metabolic parameters.
Subjects and Methods: In total 117 men aged 23.3–54.7 years, 79 subfertile (median = 35.6, IQR = 15.2) and 38 age matched controls (median = 33.6, IQR = 15.2) were included in this study. The workup encompassed medical history and extensive clinical and laboratory evaluation of fertility-related parameters and indicators of metabolic syndrome. Subfertility was defined as sperm concentration below $20\square 10^6$/mL and more than 1 year of unsuccessful attempt of pregnancy. Sera were analyzed for presence of the following miRNAs: miR-155-5p, miR-122-5p, miR-200a-3p, and miR-200c-3p being previously associated with parameters of fertility as well as metabolic disorders. Extracellular circulating RNA was isolated from sera obtained from fasting blood samples with exogenous miRNA UniSp6 added as a normalization factor at the beginning of the extraction process. Total RNA was used to synthesize cDNA and selected miRNAs measured by qPCR using miRNA LNA PCR primers and external standards allowing for quantification. Differences in miRNA levels between subfertile men and controls were determined by the Mann–Whitney *U* test. Spearman's rank correlation analysis was applied to identify associations between the tested variables.
Results: Subfertile men had higher concentrations of miR-155-5p than controls (median (IQR): 0.118 (0.080) vs. 0.074 (0.075); $p = 0.003$) with sensitivity 76% and specificity 55% for the cut-off level at 0.0786 fM. For miR-200c-3p the difference was borderline statistically significant ($p = 0.054$). Median expression levels of other miRNAs were at comparable levels in both groups. Regarding metabolic parameters, statistically significant associations were found between miR-122-5p and glucose homeostasis and lipid metabolism (fasting insulin, HOMA-IR, HbA1c, leptin, adiponectin, triglycerides, cholesterol, HDL/LDL ratio), but no association with subfertility was identified.
Conclusions: Among the miRNAs analyzed, miR-155-5p and possibly also miR-200c, are promising serum-based biomarkers of male subfertility, suggesting its potential use in conjunction with reproductive hormone assessment. The remaining selected miRNAs might reflect coexisting medical conditions but not be associated with male subfertility per se.

MID20

Characterization of 85 cases of Azoospermia of the Andrology's Clinic, of the National Institute of Perinatology (INPer), Mexico: a prevalence study

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Introduction: Azoospermia (absence of sperm in the ejaculate) has been identified in approximately 1% of men of reproductive age, and 10–15% of infertile males. 60–70% are related to spermatogenesis failure and 30–35% due to obstructive problems. The causes can be congenital or acquired, and the reproductive prognosis is cause related. Other pathologies can be also present and should be treated before attempting to reproductive high complexity techniques.
Material and methods: Transversal, prospective, descriptive and retrolective study of 85 patients with azoospermia, which entered the clinic of Andrology of the INPer of January 2010 to April 2015. The patients were subdivided into: full group of azoospermia, and then in obstructive and non-obstructive Azoospermia in order to analyze the causes (acquired, congenital or idiopathic). The parameters assessed were: hormonal profiles (FSH, LH, testosterone, estradiol and prolactin), thyroid profile, testicular ultrasound, cytogenetic evaluation (chromosomal analysis, studies of microdeletions of chromosome Y), those with Congenital absence of the vas deferens requested genetic analysis of CFTR; patients suspected of obstructive azoospermia distal evaluated seminal fructose and prostate ultrasound and histopathological findings of patients with testicular biopsy histological.
Aim: To identify the characteristics of the population with azoospermia in our clinic, concomitant diseases and propound treatments that improve their reproductive prognosis.

Results: 85 of 1310 cases were included. Azoospermia prevalence was 6.48%, average age 34.12 years (range 22–59); 18 patients (21.17%) presented hypospermia. 36% of the patients had serum testosterone values between 10 and 14.9 nmol/L and 67.4% had FSH levels were lower than 6 mUI/mL, it was found that males in the group of non-obstructive azoospermia had a worst hormonal profile: 32% of males in this group had TSH values above 2.5 mUI/mL. Testicular ultrasound findings included: 23 with epididymal ectasia, 5 with vas deferens agenesis, 6 with cryptorchidism, 22 patients with varicocele (72% had left varicocele, 9% right varicocele and 11% bilateral varicocele) and 9 without pathology; 3 patients had an altered Karyotype and 3 with Y Microdeletions.
Conclusions: The prevalence of Azoospermia including genetic causes is lower than previously reported; the most common cause of obstructive azoospermia was an alteration of epididymis; the most frequent cause of non-obstructive azoospermia was tubular failure with hypogonadism.
Key words: Azoospermia, obstructive Azoospermia and non-obstructive Azoospermia.

MID21

Comparison between infertile males with monorquia and males with both testes

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Introduction: Unilateral surgical orchiectomy is performed mainly by cryptorchidism, testicular torsion, testicular trauma, surgical iatrogenic or oncological processes, or can be of congenital origin. Hormonal pattern in patients of reproductive age with monorquia is not well established, but it is assumed that depends on its origin.
Aim: To determine if infertile males with monorquia have an alteration in hormonal and seminal parameters in relation to infertile patients with two testicles and with normal testes and aggregate pathology.
Method: Retrospective, comparative, observational study. We included males with infertility and one testicle post orchiectomy (Group1), infertile males without aggregate pathology (Group 2), and infertile males with pathology (Group 3); The variables analyzed were: hormonal profile (LH, FSH, total testosterone, Estradiol, and prolactin), metabolic profile (glucose, lipids, etc), testicular ultrasound and semen analysis. Statistical analysis was performed with SPSS edition 21.
Results: The prevalence of monorquia was 0.8% (32/4151 patients). With an average age of 33.5 ± 5.8. In these patients, the statistical analysis of hormonal profiles showed elevation of LH and FSH. In group 1, had decrease in sperm concentration and motility compared with groups 2 and 3.No differences in levels of testosterone were found between groups.
Conclusions: Despite of etiology which originates the monorquia, there are hormonal similar patterns in all populations, showing increase of FSH and LH, without alterations in levels of testosterone, and the sperm proved to be the best indicator of testicular damage.

MID22

Prevalence of human papillomavirus in semen samples from Mexican patients with idiopathic asthenozoospermia

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Introduction: Asthenozoospermia has been reported in men with genital human papillomavirus (HPV) infection. However, the relation between the presence of human papillomavirus DNA in semen samples and asthenozoospermia is not clear.
Aim: To determine the prevalence of HPV-DNA sequences in semen samples with idiopathic asthenozoospermia
Method: 13 semen samples were collected with idiophatic astenozoospermia from patients enrolled for a infertility treatment with an age group between 28 and 43 years old. All cases were tested for HPV, in order to verify DNA suitability for polymerase chain reaction (PCR) the DNA obtained was amplified for the β-globin gene (PCO4/GH_2O) under conditions described by Resnick et al. Samples were latter submitted to HPV amplification with three sets of the following universal primers recognizing distinct size fragments of the L1 gene: L1C1/L1C2, MY09/MY11, and GP5/GP6. The criteria selection for asthenozoospermic were less than 30% of progressive motility. Data are presented as mean + SD. Differences between data were determined by two-tailed student's *t*-test after confirmation of normal distribution with the Kolmogorov-Smirnov test. *p*-values (two-sided) <0.05 were considered statistically significant.
Results: HPV DNA was found in 8/13 patients (61.5%), detecting types of high and low risk (58.68%, 28.82%), and one case with double infection (HPV44 and HPV58). There were statistical difference between progressive motility between samples HPV infected vs. HPV no infected. ($p = 0.05$).
Conclusions: Infertile males with idiopathic asthenozoospermia had a high prevalence of HPV infections which is an adverse factor affecting male fertility.

MID23

Sperm DNA fragmentation and sperm funtional maturity in men from infertile couples and men with testicular germ cell tumor

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Background: It has become apparent that routine sperm analysis is not sufficient for the determination of male

fertility. One of the reasons, which may restrict success rate of natural fertilization and artificial reproductive technologies, can be sperm DNA fragmentation and sperm functional immaturity.
Aim: The aim of the study was to show the sperm chromatin integrity and sperm functional maturity in men from infertile couples and men with testicular germ cell tumor (GCT).
Material and methods: Semen samples of 312 men, aged 25–58 years (median – 35), from infertile couples and 23 men, aged 24–48 years (median – 33), with GCT before oncological treatment were studied. The sperm chromatin dispersion test (Halosperm G2 Kit, Halotech, Spain) for the determination of sperm DNA fragmentation index (DFI) and assessment of the ability of sperms to bind with hyaluronic acid (HA) (Hyaluronan Binding Assay, Biocoat Inc., USA) for the determination of sperm functional maturity were performed.
Results: The results of both tests were normal in 24% of men from infertile couples and in only 4% of men with GCT. Both abnormal analyses were found in 23% of men from infertile couples and 48% of men with GCT. Most men from infertile couples (48%) and men with GCT (48%) revealed normal DFI (<30%) and decreased percentage of HA-bound sperms (<80%).
The significant negative correlation between DFI and the percentage of HA-bound sperms was found in the whole group ($r = -0.19$; $p < 0.001$), similarly in men from infertile couples ($r = -0.15$; $p < 0.01$) and men with GCT ($r = -0,48$; $p < 0.05$).
Conclusions: About 75% of men from infertile couples may have sperm DNA fragmentation and/or immature sperms (disturbed ability of sperms to bind with HA). The situation is worse in men with GCT, where 96% of cases may have these problems.
Increased DFI is correlated with decreased percentage of HA-bound sperms, indicating poor fertility potential of semen.
The assessment of sperm DFI and HA-binding should be performed in men from infertile couples, especially when the cause of infertility is not diagnosed by the basic semen analysis (idiopathic infertility).
These tests are important also in men with GCT because the results could predict the ability of sperms to fertilization.

MID24

Sperm DNA fragmentation and mitochondrial membrane potential are better for predicting natural pregnancy than semen analysis

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Background: Semen analysis is more useful in the diagnosis of extreme male factor infertility. An association between sperm DNA fragmentation, MMP and normal fertility potential has been reported previously. Sperm DNA damage has been associated with unsuccessful attempts to conceive. Data on a relationship between DNA sperm fragmentation, measured by TUNEL, and natural conception are scarce and inconsistent. MMP has been shown to be connected wit unsuccessful attempts to achieve assisted conception. An impact of MMP values has never been confirmed on the population of fertile men.
Aim: Standard semen analysis is not sufficient for natural pregnancy prediction i.e. in identifying those who are likely to conceive without medical assistance, and those who are not.
Methods: The infertile men were males from infertile couples coming for fertility evaluation with 12–18-month history of unsuccessful attempts to conceive, were observed for additional 6–12 months in terms of achieving a natural pregnancy. Control group were 51 men of currently pregnant women. Sperm DNA and MMP were measured by TUNEL and DiOC6(3) coupled with flow cytometry. The study was carried out at the outpatient infertility clinic, Andrology Unit of the University Medical Centre Ljubljana.
Results: Twenty-eight of 85 (33%) men from infertile couples conceived naturally after two year observational period. All 28 women delivered after 37 week of pregnancy. The median values of DNA fragmentation and MMP in infertile men were different to those in the fertile controls. Optimal threshold values of DNA fragmentation and MMP were 22.4% as determined by ROC analysis (AUC 0.69 [95% CI: 0.57–0.81]) and 62.5% (AUC 0.67 [95% CI: 0.55–0.79)]), respectively. The men in the infertile group with values of DNA fragmentation = 22.4% and with MMP values 62.5% had significantly higher odds for conception (OR 4.92 [95% CI: 1.86–13.02] and OR 4.61 [95% CI: 1.76–12.11], respectively). Both sperm function tests combined had significant odds for natural conception (OR 6.96 [95% CI: 2.48–19.58]) with probability of 0.64 (64%) for both normal values, and 0.19 (19%) for both abnormal values. Normal combined parameter also influenced time to natural pregnancy achievement.
Conclusion: Irrespective of the results of standard semen analysis these clearly defined infertile groups of men such as ours will benefit from combined sperm function tests in clinical decision making and hence might avoid unsuccessful attempts to conceive. Such decision making is time and cost effective and has positive effects.

MID25

Abstinence length and sperm parameters

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Subjects must remain abstinent between 2 and 7 days before collecting a semen sample for standard analysis according to the World Health Organization. The origin and foundation for this recommendation remains uncertain as several studies regarding this matter have shown contradictory results. This aim of this study was to compare a very short abstinence period to the prescribed abstinence time with regards to various sperm parameters in young healthy individuals.
Semen samples ($n = 100$) were collected from potentially fertile, healthy males (20–30 years). Donors abstained for

4 days prior to the first sample collection, while the second sample was collected from the same donor after 4 h of abstinence. Macroscopic (volume, pH), microscopic (vitality, motility, morphology) and advanced parameters (ROS) were measured and compared between groups. Data were analysed using a Student's paired *t*-tests (Graph Pad Prism□) and presented as Mean□SEM, while significance was set at $p < 0.05$.

As expected it was observed that short periods of abstinence significantly reduced semen volume and total sperm number. Furthermore, the samples collected after 4 hours of abstinence showed a significant increase in pH (7.69 ± 0.01 vs. 7.58 ± 0.016, $p = 0.0001$), total motility (62.29 ± 1.53 vs. 58.86 ± 1.51 $p = 0.0027$), progressive motility (49.58%±1.47 vs. 44.98%±1.37, $p = 0.0001$), as well as the following kinematic parameters: VCL (81.99 μm/s ± 1.68 vs. 76.24 μm/s ± 1.08, $p = 0.0001$), VSL (32.90 μm/s ± 0.61 vs. 29.81 μm/s ± 0.50, $p = 0.0001$) and LIN (40.89%±0.79 vs. 39.42%±0.59, $p = 0.0110$) compared to the sample collected after 4 days of abstinence. No significant differences were observed in morphology, ALH and O_2^-.

Short abstinence periods (4 h) were beneficial to sperm motility and kinematics and did not negatively affect any other important sperm parameters. At a clinical level these findings are important as it could be employed during fertility preservation and lead to improved ART outcomes.

MID26

Relationship between oxidative stress and sperm DNA fragmentation in male infertility

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Background: The pathological effects of free radicals in male reproductive tract are associated with DNA fragmentation, apoptosis, and lipid peroxidation. During evolution, organisms have developed antioxidant systems for protection – a complex of enzymatic and non-enzymatic activities, measured as total antioxidant capacity (TAC). DNA damage of the male germ cells is associated with poor performance in fertilization in vivo and in vitro, preimplantation embryonic defects and high incidence of abortion.

Aim: The purpose of this study was to evaluate the association between the levels of TAC and sperm DNA integrity (SDI) in seminal plasma of fertile and infertile men.

Methods: 48 men participated in the study, divided in two groups: Group 1 – fertile men ($n = 17$) and Group 2 – infertile men ($n = 31$). Sperm morphology was assessed by strict criteria of Kruger. Teratozoospermia index (TZI) was calculated. The assessment of total antioxidant capacity was performed according to the method of Koracevic. The integrity of sperm DNA was determined by flowcytometric Sperm DNA Integrity Test (SDIT).

Results: A negative correlation was established between: 1/ the levels of TAC and TZI of Group 1 (0.66 Alt0177 0.05 and 1.39 Alt0177 0.03) and Group 2 (0.33 Alt0177 0.03 and 1.45 Alt0177 0.02), p Alt0163 0.01; 2/ the levels of TAC and DFI of Group 1 (0.66 Alt0177 0.05 and 8.46 Alt0177 0.98) and Group 2 (0.33 Alt0177 0.03 and 14.48 Alt0177 1.57), p Alt0163 0.05. All men included in the study had normal sperm concentration and motility according to the WHO 5th reference values, in spite of that we established a significant difference in sperm morphology between Group 1 and Group 2 (7.1 Alt0177 0.76 and 4.0 Alt0177 0.38, respectively, p Alt0163 0.05).

Conclusion: Our results confirm the existence of association between oxidative stress and DNA fragmentation established by other authors. Elevated levels of TZI indicate an increased risk for impairment of spermatogenesis and concomitant presence of sperm dysfunction. Therefore a special attention is required in cases of normal sperm morphology (=4%), but high TZI.

MID27

Very high sperm DNA fragmentation index without increased reactive oxygen species. Link to hereditary cancer?

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Background: Sperm DNA Fragmentation Index (DFI) above 30%, as determined by Sperm Chromatin Structure Assay (SCSA) is considered as marker of infertility in vivo. It is anticipated that excessive levels of Reactive Oxygen Species (ROS) is the major cause of high DFI. However, errors in the DNA repair system may also contribute to increased sperm DNA fragmentation.

We here present a case of a 44 years old man with an extremely high and increasing DFI without high ROS levels.

Aim: To investigate if the extremely high DFI is a persisting or just temporary phenomenon and whether it is related to high ROS or length of abstinence time.

Method: Patient case: In 2012 the patient, born in 1972, and his female partner asked for medical aid for infertility. At the time his DFI, in two independent measures, was 37% (2 days of abstinence) and 46% (5 days of abstinence), respectively. Despite the high DFI, during the preparation for IVF, the couple achieved spontaneous pregnancy and got a live born child.

In 2014 the couple once again applied for help at fertility clinic because of secondary infertility. In 2015 a semen sample showed an extremely high DFI of 86% (3 days of abstinence). He was no smoker and had no fever or history of medication or any disease. However, his mother was diagnosed with breast cancer and his father and grandfather both suffered from prostate cancer.

Three additional semen samples were collected, all with different abstinence-time (1, 3 and 4 days) in order to find out whether high DFI was related to the length of the abstinence period, ROS levels were analysed by flow

cytometry, using MitoSOX Red (MSR) and dihydroethidium (DHE) as method.
Results: Date (days of abstinence)/DFI/mitochondrial ROS in viable sperms(MSR)/cytoplasmic ROS in viable sperms(DHE):

12 Oct 2015 (4 days)/39%/7.1%/7.1%
20 Jan 2016 (3 days)/86% /not analysed/not analysed
01 Apr 2016 (3 days)/89%/0.74%/0.26%
13 Apr 2016 (4 days)/98%/4.3%/2.6%
14 Apr 2016 (1 day)/98% /not analysed/not analysed

All reproductive hormone levels were within normal range, as was serum prostate specific antigen concentration (<3 ng/mL).
Conclusion: The extremely high DFI reported in this case was not related to ROS or abstinence-time. Both breast and prostate cancer may be related to impaired DNA repair and one can, therefore, speculate whether there is a pathogenic link between the patients' high DNA and the malignancy in his close relatives.

MID28

Sperm DNA integrity test – artificial reproductive procedures outcome and recurrent pregnancy losses

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Study question: To investigate influence of sperm DNA fragmentation index (DFI) on pregnancy outcome and pregnancy loss after ART procedure (autologus ICSI, donation eggs ICSI) and IUI.
Summary answer: The three group categories showed that samples with DFI > 27% were associated with increased risk of early pregnancy loss.
What is known already: Results from assessment of sperm DNA fragmentation [DFI] by analyzing sperm chromatin structure (DNA Integrity test), have impact on both natural pregnancy and that achieved through ART. Pilot studies have shown that high levels of DNA fragmentation [DFI > 27%] decrease fertility potential in patients undergoing ART procedure, even in men with completely normal standard sperm parameters Therefore, the lack of correlation between conventional parameters of sperm and DNA fragmentation determine DNA fragmentation as a potential source of male infertility in normozoospermic men. That is why further evaluation of sperm DNA fragmentation is required in the study of male infertility
Study design, size, duration: A prospective study.
Participants/materials, setting, methods: Patients: We investigated men from 531 couples undergoing autologous ICSI procedure [n = 416], from couples undergoing donation eggs procedure [n = 39] and IUI [n = 76]. Interventions: semen analysis, DNA integrity test, embryo scoring by Gardner and Schoolcraft grading system [1999]
Main results and the role of chance: The study shows no statistically significant differences between the group regarding pregnancy rate. [? 2 = 0.55. ? > 0.05, OR = 1.25]. However, with increased levels of DFI, the number of pregnancy losses became higher [including biochemical pregnancies and spontaneous abortions] at O r = 5.65. We examined the percentage of grade I blastocysts [by Gardner and Schoolcraft] before donation eggs embryo transfer and found a statistically significant correlation with both the DFI [2 = 7.80, $p < 0.05$] and sperm morphology [? 2 = 6.14; $p < 0.05$]. Analysis of the relationship between DFI and IUI output (clinical pregnancy, miscarriage) revealed significant correlations in both directions: between DFI and pregnancy rate after IUI (2 = 6.29, $p < 0.05$) and between the DFI and pregnancy development after IUI (2 = 6.87; $p < 0.05$).
Conclusion: Men with infertility should undergo DNA fragmentation assay in addition to the standard semen analysis. When DFI exceeds 27%, ICSI should be a method of choice, even in cases where the conventional parameters of semen analysis tests are normal.

PS1B1 – POSTER SESSION NR.1
POSTER PRESENTATIONS

MIG01

Case report: 32-year old male with azoospermia and partial AZFb Y microdeletion with positive spermatozoa findings

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Background: Infertility accounts about 10–15% of all couples. Half of them refer to men. Two major genetic infertility reasons are Klinefelter syndrome and microdeletions of the Y chromosome. Three regions are regularly checked in men with spermatozoa counting below 5 mill/mL (AZFa, AZFb and AZFc). Nowadays, even further microdeletions are investigated to help infertility treatment management.
Aim: Although patients with microdeletion of the Y chromosome in AZFa and AZFb region have virtually zero possibility of having children, we showed that azoospermic men with partial AZFb deletion of Y chromosome had positive spermatozoa findings and even successful IVF/ICSI procedure.
Methods: The patient was a 32-year old male with azoospermia. A detailed patient's history was taken and a genital examination was done. No infertility reason was observed during the examination. The patient's testicles demonstrated normal volume and consistency. Hormone, semen and genetic testing were done. FSH level was 5.7 IU/L, LH 7.7 IU/L and testosterone 20.46 nmol/L. During genetic analysis, a normal karyotype but a partial AZFb microdeletion of Y chromosome was diagnosed. Genetic counselling was also performed.
Results: After detailed analysis and genetic counselling, TESE procedure was performed. Histology findings revealed late spermatids that could be used in IVF/ICSI procedure. ET was successfully performed but after 6 weeks miscarriage occurred. Couple decided to make second TESE procedure and the same histology of the testicular parenchyma was observed. Second IVF/ICSI procedure is ongoing.

Conclusion: Even though patients with AZFb microdeletion of Y chromosome have small fathering chances, in some special cases and after proper genetic counselling it could be possible.

MIG02

X chromosome-linked CNVs and idiopathic male infertility in Chinese Han population

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Background: A recent study found that three X-lined copy number variation (CNV) deletions (CNV64, CNV67 and CNV69) are associated with idiopathic male infertility in Caucasians, especially the CNV67 deletion resembling the AZF deletions that merits further investigation among different populations.
Aim: To evaluate the deletion of these three CNVs in the idiopathic male infertility with different conditions, and fertile participants from different regions in Chinese Han population.
Methods: The subjects included 714 patients diagnosed as idiopathic infertility with different conditions (288 were with non-obstructive azoospermia, 210 with oligozoospermia and 216 with asthenospermia) and 836 fertile participants (vasectomized men). The fertile participants were from the representative regions including provinces of the north: Hebei and Shanxi, centre: Hubei and Jiangsu, and south: Guangdong. All patients were recruited from Hubei. Peripheral blood DNA was extracted from all subjects and then multiplex and minus polymerase chain reaction were performed to detect and confirm the three CNVs deletions.
Results: Similar rate of deletion of these three CNVs was observed between infertile men and fertile participants (Hubei), and among different conditions of infertility. Moreover, the CNVs map distribution geographically differed around China. Among the fertile group, the rate of CNV64 deletion in South (Guangdong population) was much lower, and CNV67 deletion was significantly higher than other regions. The three CNVs deletions in other regions excluding Guangdong were similar.
Conclusion: The CNV64 and CNV67 deletions in Chinese Han population are significantly different in geographical distribution. None of the three deletions may be associated with idiopathic male infertility in Chinese Han population. It seems that the association between deletions of these three CNVs and male infertility is related to ethnic difference, thus there is still need to screen the CNVs deletions in other ethnicities. Caution should be paid in clinical when prescribing CNVs deletions in male infertility as routine test screening.

MIG03

Association of MTHFR C677T polymorphism with male infertility in Pakistan

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Background: Male infertility, in 20–25% of the couples, is due to oligospermia, asthenospermia, teratozoospermia and azoospermia. Along with various other nutritional factors, folate deficiency also contributes to the male infertility. In this regard, less folate intake and mutation in enzymes for interconverting different impotent forms of folate into potent are responsible. One of these enzymes an important enzyme is methylenetetrahydrofolate reductase (*MTHFR*). The C677T (rs1801133) polymorphism in the *MTHFR* gene reduces the enzyme activity by 35 per cent resulting in impairment of nucleic acid metabolic pathways. However, the condition can be managed by folic acid supplementation for that determining the frequency of the *MTHFR* C677T mutation is pre-requisite. The results of many of the molecular epidemiological studies on the association of *MTHFR*677CT polymorphism and male infertility remain controversial due to small sample size, ambiguously defining infertility and confounding factors including ethnicity. In the present study, we determined an association between idiopathic sperm disorders and *MTHFR*677CT polymorphism.
Aim: The present study determined an association between idiopathic sperm disorders in a local Pakistani infertile male population and *MTHFR*677CT polymorphism.
Methods: A total 437 idiopathic infertile men including 57 azoospermic, 66 oligospermic, 44 asthenozoospermic, 29 teratozoospermic, 20 oligoasthenospermic and 221 infertile normospermic men were recruited, after ruling out non-genetic factors. Moreover, 218 normospermic fertile men had more than one child were taken as controls. The polymerase chain reaction (PCR)-restriction fragment length polymorphism technique was used to determine *MTHFR* C677T (rs1801133) polymorphism.
Results: A significant association of the minor *MTHFR* 677T allele with male infertility was observed ($p < 0.05$). In addition, men with *MTHFR* 677 CT and TT genotypes are at a greater risk (OR: 1.81, 95% CI: 1.17–2.80, $p = 0.008$ & OR: 9.24, 95% CI: 1.20–70.92, $p = 0.032$, respectively) of infertility. All the subgroups of male infertility (azoospermic, oligospermic, asthenospermic, OAT and normospermic infertile) had significantly ($p < 0.05$) higher frequencies of CT and TT genotype as compared to fertile men. The combined genotypes (CT + TT) were also found significantly (OR: 2.01, 95% CI: 1.31–3.08, $p < 0.001$) associated with male infertility.
Conclusion: The results suggest that the polymorphism might be a factor of male infertility in Pakistani population.

MIG04

Role of plasma membrane Ca^{2+} ATPase 4 gene in sperm motility and male infertility

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Background: Experiments on mice have demonstrated that spermatozoa of the animals with deleted PMCA4 gene lose their motility ability in the environment that requires the hyperactivation of sperms due to their defect in Ca^{2+} homoeostasis. There are no data available at the moment whether functional status of the PMCA4 (polymorphisms, mutations) in humans has any impact on sperm motility function and, thereby, on male fertility.

Aim: To study the role of the functional status of PMCA4 in male infertility.

Methods: Patient group consisted of 191 Estonian men from infertile couples, with low progressive sperm motility (A + B < 20%). Control groups consisted of 187 young men (age 18–25 years) from general population with normal semen quality, and 192 proven fertile men (4 and more children) with unknown semen quality.

The all coding exons and exon/intron boundaries of PMCA4 gene were analyzed by direct sequencing. Identified mutation was verified in control group by TagMan chemistry.

Results: A known polymorphism variant c.1981G>A (rs147729934) that causes the replacement of Glu to Lys has been detected in 11 patients in heterozygote state in exon 11, calling for the A allele frequency of 0.029. Among the young men from general population with normal semen quality, this polymorphism was detected only in two men out of 187. Hence, this PMCA4 variant was found statistically more frequent in the infertile men (OR = 5.6, $p = 0.02$). Analysis of the proven fertile men group is under the process, the data will be reported during the congress.

To assess whether the detected variant has been shown also in other populations, the available metadata was examined. It showed that rs147729934 A allele frequency is 0.004 among Europeans, according to 1000 Genome project, and 0.0019 worldwide, according to ExAc Aggregated Populations database. It makes the incidence of the mutation detected in this study among the 191 infertile male patients 15 times higher than in the other populations worldwide, and 7 times higher than in other European populations.

Since apart from our study this variant was found only in Finns and no other European populations, it can serve as a population specific marker. To expand our study, we additionally included samples from the ethnic groups of 200 Estonians, 190 Latvians, 185 Lithuanians, and 286 Russians, with unknown fertility (also women samples were included). The particular polymorphism in these groups was detected in 9 Estonian, 8 Latvian, 4 Lithuanian, and 11 Russian samples.

To assess the frequency of this variant in patients from population with another ethnicity, we also included 52 Slovenian men from infertile couples. This polymorphism was detected in two men from this group.

Conclusions: We have demonstrated that the polymorphism in PMCA4 gene is associated with low sperm motility in infertile men, and it could be part of the multifactorial mechanisms involved in disturbed sperm function. It seems to be a recent mutation that has originated in North-East European populations, spreading further to other populations as demonstrated by finding it in low frequency among Slovenian infertile men.

MIG05

Whole Exome Sequencing (WES) in Non-Obstructive Azoospermia

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Introduction: In a large proportion of Non-Obstructive Azoospermia (NOA) the etiology remains unknown and it is likely to be of genetic origin. Whole Exome Sequencing (WES) approach has proved to be a powerful tool for the detection of novel disease-causing genetic factors especially in consanguineous families. As far as the sporadic cases of male infertility are concerned, the X chromosome represents an ideal target for the discovery of novel genetic factors since it is enriched in genes with testis-specific expression and a compensatory allele is not available in case of pathogenic mutations.

Aims of the study: To identify the genetic cause(s) of NOA phenotype in patients (i) with Consanguineous Parents; (ii) with Unrelated Parents.

Materials and methods: WES analysis was performed (Illumina HiSeq2000 platform) in 9 patients affected by Sertoli Cell Only Syndrome (SCOS) or maturation arrest (at spermatogonia or spermatocyte stages). After standard filtering we have focused only on rare variants (MAF < 0.01). For patients with consanguineous parents we filtered rare homozygous variants (*recessive model approach*) whereas for patients with unrelated parents we extrapolated rare X-linked variants from the WES data. These selected variants were further prioritized depending on their functional effect (benign/damaging) according to 7 bioinformatic tools and according to their potential role during the early stages of spermatogenesis. Sanger sequencing was used for validation and screening of the selected variants in 100 Spanish fertile normozoospermic controls.

Results: We identified rare, highly damaging, homozygous mutations in *FANCA*, *MRO* and *ADAD2* genes for patients with consanguineous parents and hemyzygous, damaging X-linked mutations in *RBBP7*, *CT47B1* and *MAGT1* genes in patients with unrelated parents. The mutations were absent in 100 normozoospermic men. The most relevant finding concerns the patient who carried p.Arg880Gln in

FANCA gene (a functionally damaging mutation) since it is the first time that Fanconi Anemia (FA) is diagnosed following an exome analysis for SCOS (incidental finding) The patient did not show anemia and was not aware about having FA. Chromosome breakage test confirmed mosaic FA.

Conclusions: With the detection of 6 rare mutations in 5 novel candidate genes, our study represents an additional step towards elucidating the genetic basis of early spermatogenic failure. In addition, we made an important incidental finding of Fanconi Anemia (chromosome instability/cancer-prone condition), providing benefit to the patient's future health and allowing preventive measures. The reported FANCA mutation may add a novel piece of evidence for the previously reported higher morbidity/mortality rate in infertile men in respect to the fertile population.

MIG06

In silico analysis of Y chromosome AZF region gene deletions related with azoospermia or severe oligozoospermia

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Background: Infertility is a complex disorder with multiple genetic and environmental causes. Microdeletions on Y chromosome are well known genetic causes of infertility in azoospermic or severely oligozoospermic men. RNA transcript possessing miRNA response elements (MRE) may function as ceRNA and de-repress the activity of other RNAs with similar MRE by competing for the same miRNAs in the available miRNA pools.

Aim: To specify miRNAs targeting most of the genes deleted in each of AZF loci and upon the deletion of these specified genes to find out their corresponding ceRNAs.

Methods: miRNA targetting mRNAs of some genes (specific to azoospermia) deleted in AZF regions of Y chromosome are expected to look for new targets to bind. For in silico ceRNA analysis, AZFa, AZFb, AZFb-c and AZFc loci were investigated separately. Twelve miRNAs targeting most of the genes deleted in each of these loci were detected by using miRWalk database. Three loci targeted by all these 12 miRNAs and showing potential competing endogenious RNAs (ceRNA) activity were found by using ComiR database. All of separately detected ceRNAs for each AZF loci were put in GeneMANIA database for interaction prediction and the most possibly interacted genes with these ceRNAs were defined. Upon deletion of specified genes in AZF loci in azoospermia and oligozoospermia, miRNA targetting mRNAs of these genes look for new targets to bind. The expression of the most possibly genes targeted by these miRNAs and showing ceRNA activity are expected to decrease in silico manner.

Results: Our results have shown that *RPPA2*, *TMED3*, *STX7* and *ATRX*, *GOGLB1*, *KMT2C* genes are ceRNA for AZFa for AZFb, respectively. In addition, *CNB5*, *KCNC4*, *NDUFS1* and *TROBP*, *HEMK1* and *ORAI2* are potential ceRNA for AZFbc for AZFc, respectively.

Conclusion: Deletion of AZF loci may lead to change in post-transcriptional regulation of gene expression working through miRNA completion. Further experimental studies are required to conform these results.

MIG07

Association between FSHB and FSHR polymorphisms and testicular function in 2975 Danish men

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Background: Approximately 40% of young Danish men have sperm counts at a level that may reduce their fecundity. Adequate spermatogenesis depends on follicle-stimulating hormone (FSH). Genetic polymorphisms in the ligand; *FSHB* -211G/T, and the receptor: *FSHR* -29G/A and *FSHR* 2039A/G have been shown to be important for FSH signaling.

Aims: (i) To establish the allele and genotype frequencies of the *FSHB* and *FSHR* variants in the general Danish male population, (ii) to evaluate the association of the different genotypes with testicular function and, and lastly, (iii) to evaluate if distributions differ between men from the general population, fertile, and infertile me.

Methods: Semen parameters, hormone profiles, clinical parameters, and questionnaires from 1971 men (19 years) from the general Danish population, 571 fertile men (32 years), and 433 infertile men (34 years) were included in this study. Genotyping was done by competitive allele-specific quantitative PCR (KASP assays).

Results: The genotype frequencies in men from the general population were *for FSHB* -211G/T; GG: 68.7%, GT: 28.8%, TT: 2.5%, for *FSHR* -29G/A; GG: 53.8%, GA: 39.1%, AA: 7.1% and for *FSHR* 2039A/G; AA: 29.3%, AG: 48.8%, GG: 21.9%.

The *FSHB* polymorphism was not associated with semen quality in any of the three populations. However, carrying a *FSHB* T-allele was associated with lower serum levels of FSH and inhibin-B as well as smaller testis size and lower Testosterone/Luteinizing hormone-ratio. Carrying the A-allele of *FSHR* -29G/A was negatively associated with both sperm concentration and total sperm count in the general population, and in *FSHR* 2039A/G the G-allele was negatively associated to testicular size in both men from the general population and infertile men.

There were no significant differences in the distribution of the *FSHB* -211G/T genotypes between the three populations. The *FSHR* -29 G/A GG genotype was more frequent among the general population compared with both fertile men (48.6%, $p = 0.06$) and infertile men (48.1%, $p = 0.043$). The *FSHR* 2039A/G AA genotype was significantly less frequent among fertile men (22.3%, $p = 0.002$) compared to both infertile men (27.9%, $p = 0.03$) and men from the general population.

Conclusion: In this large study, the *FSHB* polymorphism was associated with lower levels of serum FSH, serum

inhibin-B and testicular size, but not semen quality. The *FSHR* -29G/A and *FSHR* 2039 A/G polymorphisms did not influence FSH levels, but were negatively associated with semen quality and testicular size. In conclusion, the three polymorphisms have a negative impact on testicular function but are not more frequently found in infertile Danish men.

MIG08

Zinner's syndrome in a patient with X-linked Kallmann syndrome: case report

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Background: Kallmann syndrome is a genetic disorder characterized by congenital hypogonadotropic hypogonadism and anosmia. Mutations in the X-linked KAL1 gene can be found in patients with a family history of Kallmann syndrome. Furthermore, about 30% of patients with mutations in the KAL1 gene have renal agenesis.
Case: We report a case of a 22-year-old male patient with Kallmann syndrome. He has anosmia and hypogonadotropic hypogonadism. Genetic analysis revealed a deletion of exons 2 to 14 in the X-linked KAL1 gene. Urogenital ultrasound showed testicular hypotrophy, a prominent tail of the left epididymis, but the left kidney and left renal artery were absent. Furthermore, a cystic structure was reported, located left from the prostate and next to the bladder wall. Additional MRI imaging suggested this was a seminal vesicle cyst and confirmed agenesis of the renal kidney, but also showed a hypertrophic left seminal vesicle, a tortuous dilated left ureter and a hypotrophic right seminal vesicle. These findings are suggestive of Zinner's syndrome, another rare cause of renal agenesis.
Discussion: Zinner's syndrome is a rare congenital disorder, in which an anomaly of the embryologic development of the Wolffian mesonephric duct occurs between the 4th and 13th week of gestation. This syndrome consists of a triad of unilateral renal agenesis, ipsilateral seminal vesicle cysts and ejaculatory duct obstruction. The etiology of Zinner's syndrome is not known. Although absence of the ipsilateral ductus deferens has been described in patients with KAL1 mutations and renal agenesis, seminal vesicle anomalies have not been reported previously. Furthermore, the underlying molecular mechanism causing urogenital anomalies during embryological development in patients with Kallmann syndrome or Zinner's syndrome may be linked.

MIG09

Sperm DNA fragmentation and BRCA1, BRCA2 gene promotor methylation in idiopathic infertility

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Background: Despite recent extensive efforts in understanding the molecular basis of male infertility, the underlying causes of 50% of all cases are still unknown. Epigenetic factors are important in the etiology of male infertility. Epigenetic alterations in DNA repair genes *BRCA1* and *BRAC2* are thought to play a role in sperm DNA fragmentation.
Aim: In this study, we aim to investigate the mechanism of sperm DNA fragmentation (SDF) in men diagnosed with idiopathic infertility.
Methods: 60 patients who were admitted to the Department of Urology at Ondokuz Mayis University Hospital were enrolled in this study. For this aim we were analysed DNA fragmentation using TUNEL assay. Semen samples were washed with PBS and centrifuged. The pelleted spermatozoa were fixed in 4% paraformaldehyde and allowed to settle on poly-L-lysine-coated slides overnight. Next day, cells were washed and permeabilized in 0.1% Triton-X-100 followed by incubation at 37C for 1 h with FITC-dUTP and TdT enzyme (TUNEL). After washing, cells were mounted with antifade solution including DAPI, and visualized under florescence microscope. Sperm DF (SDF) were calculated for four different groups (asthenozoospermic, oligozoospermic, oligoasthenozoospermic, oligoasthenoteratozoospermic) determined based on spermiogram analyses. Methylation spesific PCR (MSP) was used for DNA Methylation analysis. Semen samples were washed with Somatic Cell Lysis Buffer (SCLB). Genomic DNA was extracted after somatic cell lysis then treated with sodium bisulfide. Bisulfide modified DNA was amplified using methylation spesific and unmethylation specific primers for two genes. PCR products were analyzed by agarose gel electrophoresis.
Results: Mean of SDF was found 23.9% and 9.4% for patients and normospermic controls respectively. Significant differences were found in terms of andrological parameters in two groups with SDF above and below 20%. SDF was positively effected by sperm concentration (log transformed, ID as random factor and age as a covariate) using mixed model analysis ($F = 4.39$, $p = 0.041$). There was a negative relationship between SDF and both sperm motility and morphology (Linear stepwise regression, $r = 0.40$, $t = -3.20$, $p = 0.002$; $r = 0.47$, $t = -3.94$, $p < 0.001$, respectively). Altough there were interindividual differences of methylation profiles, we found that DNA methylation of CpG sites for *BRCA1* and *BRCA2* genes in all patients.Coclusion: Our results suggest that *BRCA1* and *BRCA2* promoter methylation could be observed functionally in the occurance of SDF and male infertility. Additionally, epigenetic relationship between SDF and *BRCA1* and *BRCA2* genes was reperted for the first time by this study

which will help understand the etiology of this condition. Preliminary results of which are presented herein, will proceed with various techniques, which will enable us to better establish a link between gene expression levels and DNA fragmentation.

MIG10

Discovery of a recessive mutation in the GnRHR associated to maternal hetero/isodisomy of chromosome 4

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Background: Congenital hypogonadotropic hypogonadism (CHH) can be caused by deficient production, secretion or action of gonadotropin-releasing hormone (GnRH). Isolated CHH typically manifests with delayed puberty and infertility. Homozygous or compound heterozygous inactivating mutations in the GnRH receptor gene are among the most frequent causes of normosmic CHH, accounting for about 10% of cases. The human *GNRHR* gene spans 18.7 kb of genomic sequence on the long arm of chromosome 4 (4q13.2) and consists of three exons encoding a seven-transmembrane domain G protein-coupled receptor (GPCR) expressed in pituitary gonadotropes.
Aim: Molecular characterization of a novel homozygous p.Gly99Glu mutation in patient A1385.
Materials and methods: We recruited 124 men affected by normosmic CHH for *GNRHR* mutation screening by Sanger sequencing. Specific molecular analysis in patient A1385 consisted in: (i) microsatellite STR analysis; (ii) gene dosage analysis (qPCR); (iii) SNP analysis.
Results: 14/124 (11%) patients resulted carriers of *GnRHR* mutation. In this cohort we came across to a peculiar case of normosmic delayed puberty due to a novel homozygous mutation (p.Gly99Glu) in exon 1 of the *GnRHR* gene. This missense mutation is predicted as disease causing by 3 in silico prediction tools (SIFT, Polyphen-2, Mutation Taster). The 20-year-old man presented eunuchoid body shape, absence of secondary terminal body hairs, Tanner Stage 1 and bilateral cryptorchidism at the first visit. Basal hormonal evaluation revealed low total testosterone (1.3 nmol/L), LH (<0.1 U/L) and FSH (<0.4 U/L) levels. Molecular analysis in the parents revealed the presence of the same mutation in heterozygosity in the mother, whereas the father was wild type. In order to define the mechanism by which the mutation has been acquired on both alleles, we performed a number of molecular genetic analyses: after confirming biological paternity, the analysis of 5 microsatellites on 4q indicated maternal heterodisomy (two distinct alleles derived from the mother) on this chromosome. Given that only one maternal allele carried the mutation we hypothesized three potential mechanisms for homozygosity: (i) deletion of the *GnRHR* gene (or part of it) on the wild type maternal allele; (ii) segmental maternal isodisomy; (iii) de novo mutation. Real-time PCR analyses (qPCR) showed that the patient has two copies of the mutant *GnRHR* gene. 3/7 SNPs analysed in the region containing the mutation (rs55844424, rs34530961, rs3822196) resulted informative and showed loss of heterozygosity (LOH), with an approximate extension of 20 Mb. This finding indicates maternal isodisomy (two identical copies of the maternal allele) restricted to a segment of the maternal allele containing the mutation (segmental maternal isodisomy).
Conclusion: We have demonstrated that the homozygous mutation in our patient originates from a maternal uniparental heterodisomy combined with a segmental isodisomy which contains the mutated gene. To our knowledge, it is the first case of nCHH due to this extremely rare chromosomal rearrangement.

PS1B2 – POSTER SESSION NR.1

POSTER PRESENTATIONS

MIE01

A functional approach: contrast enhanced ultrasound for visualizing altered testicular vascularization in 41,XXY* mice

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Background: Earlier findings in Klinefelter syndrome (KS) patients suspected Leydig cell disturbance to provoke serum testosterone deficiency. Addressing this issue in a KS mouse model (41,XX^{Y}*), we found LCs in contrast to be hyperplastic and hyper-reactive. Interestingly, also intratesticular testosterone (ITT) concentrations were comparable to controls. In addition, we could confirm this in a cohort of patients, therefore excluding insufficient ITT levels as the cause of hypogonadism. Recently, it was reported that arteries in KS patients are altered and impairing circulation.
Aim: We hypothesized changes in testicular vascularization might be involved in the endocrine phenotype and utilized our 41,XX^{Y}* mouse model to evaluate the testicular blood supply functionally.
Methods: We therefore performed a study in which an enhanced ultrasound based analysis of the testicular blood flow rate in 41,XX^{Y}* mice was conducted. Adult male 41, XX^{Y}* (n = 5) and littermate mice (n = 6) underwent ultrasound analyses with the Non-Targeted Contrast Agent Vevo MicroMarker. The agent containing gas filled microbubbles was administered intravenously for lower body perfusion. After initial perfusion, micro-bubbles were destroyed by high ultrasound pressure and the reperfusion period was documented and analysed. In parallel, electrocardiograms (ECGs) were taken. Afterwards mice were sacrificed and testes removed for histological analysis of the vascularization.
Results: Whilst ECGs did not reveal differences in heart function between the groups, the reperfusion time for testes was significantly increased in 41,XXY* mice (XXY* 28.8 ± 1.69s; XY* 19.9 ± 2.8s) Testes of 41,XXY* mice (XXY* 4.6 ± 0.10 mm²; XY* 11.1 ± 0.34 mm²) and the area

covered by blood vessels (XXY* 0.025 ± 0.003 mm²; XY* 0.040 ± 0.002 mm²) were significantly smaller as revealed by histology.

Conclusion: These functional data strengthen the assumption that the observation made previously and pointing to an affected vascular system in the disturbed testicular tissue of males with supernumerary X contributes to the endocrine phenotype seen in KS. Furthermore, a close relation between blood vessel formation and spermatogonial stem cell niches was reported and thus, the altered vascularization could also be involved in germ cell loss observed in KS. However, further studies have to be undertaken that confirm our observation also clinically.

MIE02

In vitro exposure of human spermatozoa to bisphenol A induces pro-oxidative/apoptotic mitochondrial dysfunction

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Background: Bisphenol A (BPA) is an industrial chemical widely used as key monomer of epoxy resins and polycarbonate plastics. It represents the first choice plasticizer due to its cross-linking properties, however, after polymerization, unbound monomers can leach into the environment, making possible their absorption by human body mainly through dietary ingestion. Once in the body, BPA can exert endocrine disrupting pro-estrogenic activities, thus negatively affecting spermatogenesis in animal models. Nevertheless, in vitro studies demonstrated that BPA can also directly promote biological damage by oxidative stress and apoptosis in several types of cells and mitochondrial dysfunction seems to play a major role in mediating these effects.

Aim: As, in spite of the increasing interest for the impact of BPA on male reproduction, adverse effects of the human spermatozoa exposure to BPA have not yet been investigated, in this study we explored whether the in vitro exposure to BPA could affect human sperm integrity through the induction of pro-oxidative/apoptotic mitochondrial dysfunction.

Methods: Motile sperm suspensions, obtained by swim up, were exposed to scalar concentrations of BPA from 10 to 800 μM. Sperm motility was evaluated by CASA. Flow cytometry was used to assess mitochondrial membrane potential (with JC-1), mitochondrial generation of superoxide anion (with MitoSOX red), caspase activation (with FITC-LEHD-FMK and FITC-DEVD-FMK, selective ligands of active caspase-9 and caspase-3, respectively) and DNA oxidative damage (with an anti-8OHdG monoclonal antibody).

Results: The sperm exposure to scalar BPA concentrations for 4 hours produced a significant decrease in the mitochondrial membrane potential, starting from 300 μM, as indicated by the lower percentage of sperm with red JC-1 fluorescence when exposed to BPA 300 μM (43.6 ± 5.4%), 400 μM (28.4 ± 3.5%) and 800 μM (3.2 ± 0.3%), with respect to untreated samples (70,1 ± 2,4%) and samples exposed to lower BPA concentrations (≥67.5%). It was associated with an increased mitochondrial generation of superoxide anion, activation of caspase-9 (the mitochondrial apoptotic pathway caspase) and caspase-3 (the downstream effector caspase), along with motility decrement. Vitality decline was observed at BPA ≥ 400 μM. Twenty hours exposure to 300 μM BPA, but not to lower concentrations, produced a significant loss in sperm vitality associated with a complete sperm immobilization. Finally, 300 μM BPA also produced a significant DNA oxidative damage, as revealed by a ~3.5-fold increase in the percentage of spermatozoa with 8OHdG adduct formation with respect to untreated control samples.

Conclusion: This study demonstrates that BPA, apart from the potential to affect spermatogenesis as an endocrine disruptor, also exhibits the potential to directly affect human sperm integrity through the induction of pro-oxidative and pro-apoptotic mitochondrial dysfunction. These in vitro findings should be considered in the assessment of the still uncertain BPA-related hazards for male fertility.

MIE03

Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples

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Background: Data on the effects of smoking on male fertility are conflicting. A correlation between smoking and reduced semen parameters has been reported, however, with a high heterogeneity among studies. An association between smoking behavior and higher testosterone levels in men has been described in several, but not all, the previous studies. No study has systematically evaluated the impact of smoking on the male genital tract ultrasound characteristics.

Aim: To evaluate the association between smoking behavior and seminal, hormonal and male genital tract ultrasound parameters in men with couple infertility.

Methods: Cross-sectional study. Out of a consecutive series of 426 men with couple infertility, 394 (age 36.0 ± 8.0 years) free of genetic abnormalities were studied. All subjects underwent a complete andrological and physical examination, biochemical and hormonal assessment, scrotal and transrectal color-Doppler ultrasound and semen analysis (including seminal interleukin 8 levels, sIL-8) within the same day.

Results: Among the patients evaluated, 229 were never smokers (NS), 56 past smokers (PS), and 109 current smokers (CS). When CS were compared to the rest of the sample (nonsmokers, NS + PS), in a multivariate model (analysis of covariance, ANCOVA) adjusted for age, lifestyle (including alcohol, cannabis and physical activity), BMI and sex hormone-binding globulin, significantly higher androgen (total testosterone, $p = 0.001$; calculated free testosterone, $p < 0.005$) and lower FSH ($p < 0.05$) levels were observed in CS. However, when total

testosterone was also included in the multivariate model as a further covariate, the difference in FSH levels was not confirmed. In a similar model, a lower ejaculate volume ($p < 0.01$) and a higher prevalence of normal sperm morphology ($p < 0.02$) were also detected in CS in comparison to the rest of the sample. However, when total testosterone was also included in the multivariate model as a further covariate, only the difference in ejaculate volume between CS and nonsmokers was confirmed (-0.61 ± 0.23 ml, $p < 0.01$). Finally, CS showed lower total seminal vesicles volume, before and after ejaculation, even after adjusting for confounders ($p = 0.02$ and $p < 0.01$, respectively). Similar results were observed when the reported number of cigarettes smoked or the number of pack-years was considered separately.

Conclusions: In males of infertile couples, CS, as compared to nonsmokers, show lower ejaculate and ultrasound-derived seminal vesicles volume despite higher testosterone levels. This is an apparent paradox in CS. However, our data suggest that smoking may negatively affect seminal vesicles volume in an independent manner, as the difference between CS and nonsmokers retained significance after adjusting for confounders including testosterone. This is the first study reporting such ultrasound evidence. How this new smoking-related alteration, along with low semen volume, impacts male fertility needs to be addressed by further studies.

MIE04

Perceived stress, but not hair cortisol levels, is associated with semen quality in 700 young Danish men

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Background: Stress is an important and frequent problem and 10–15% of young Danish men report a high stress level. At the same time approximately 40% of young Danish men have sperm counts at a level that may reduce fecundity. Previously, we have shown a negative association between high self-reported stress and semen quality in 1215 young Danish men (1).

Aim: To test if our previous results could be repeated in a new cohort of young men providing information on both stressful life events and perceived stress using additional validated stress scales (somatic stress scale and cognitive stress scale) and our own self-constructed scale, and perceived stress and semen quality was associated with cortisol concentration in hair.

Methods: Young men (median age 19 years) from the general Danish population were investigated in 2012–15. They completed a questionnaire on health and lifestyle, perceived stress (last four weeks) and stressful life events (last three months) prior to a physical examination and delivery of a semen sample. They also had a blood sample and a hair sample taken. Hair cortisol was assessed by LC-MS/MS.

Results: We detected a negative association between self-rated stress and semen quality as demonstrated in our previous work. All stress scales (somatic stress scale, cognitive stress scale, self-constructed stress scale) were associated with semen quality. We did not find an association between stressful life events and semen quality. Hair cortisol seems associated with semen parameters, however, in a U-shaped manner; men with both low and high hair cortisol levels had better semen quality than the intermediate group.

Conclusion: We confirmed our previous findings of a negative association between self-reported stress and semen quality using different reporting scales indicating that the association was not a chance finding but robust and reproducible. To evaluate stress in a more objective way we included hair cortisol as a biological stress marker. However, our findings are somehow contrary to what we expected. By now, we cannot explain this finding but are currently doing further more detailed analysis which hopefully may provide better insight into the possible biological mechanisms for this association.

Nordkap et al. Psychological stress and testicular function: a cross-sectional study of 1215 Danish men. Fertil Steril. 2016 Jan;105(1):174–87.

PS1B3 – POSTER SESSION NR.1

POSTER PRESENTATIONS

SP01

Immunization of male mice with B-cell epitopes of uPA inhibits fertility

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Background: Immunocontraception may provide a viable and valuable alternative for male contraception. We previously have demonstrated the antifertility effect of urokinase-type plasminogen activator (uPA) on male mice.

Aim: To evaluate the contraceptive ability of three B-cell epitopes in uPA which we denominated as GF34,K121, SP231.

Methods: The three predicted B-cell epitopes of uPA were synthesized to immunize male mice. BALB/c male mice were randomly divided into three experimental groups ($n = 12$ in each group) immunized with the three epitopes respectively, and two control groups (adjuvant control group and PBS control group, $n = 10$ in each group). Fertility, epididymal sperm function, and the presence and reactivity of antibodies in immunized males were investigated.

Results: Significant reduction of fertility was observed in GF34 and K121 groups in mating trial with no evident systemic illness or abnormal mating behavior. However, SP231 and control groups show similar normal results. Epididymal sperm of GF34 and K121 group males exhibited impaired progressive motility and ability to fertilize eggs in vitro. High titers of antibodies were induced in the sera in all three experimental groups.

Conclusion: Subcutaneous injection of uPA B-cell epitopes GF34 and K121 to male mice could effectively reduce their fertility, and these two epitopes could become

a new target for immunocontraception in male contraceptive development.

SP02

Evaluation of ezrin and fascin 1 in the PFOS treated Sertoli cell culture: an in vitro study

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Background: Junction restructuring of the blood-testis barrier (BTB) takes place at the Sertoli cell-cell interface called basal ectoplasmic specialization (ES) at to facilitate the transport of pre- leptotene spermatocytes across the barrier. Perfluorooctanesulfonate (PFOS), which is an environmental toxicant, have been implicated in male reproductive dysfunction. It is especially effective in perturbing the BTB function by disturbing F-actin organization in the Sertoli cells. Knockdowning of ezrin and of fascin 1 in vivo by RNAi ~60–70% induced defects in spermatid polarity, mediated by a mis-localization and/or down-regulation of actin-bundling proteins. And also was found to impede spermatid transport, causing defects in spermiation.
Aim: Ezrin and fascin 1 is involved in regulating actin microfilament organization at the ES in rat testes. Depends on the findings in knock downing of ezrin and of fascin 1 in vivo, we aim to show the defects or disruption of BTB structure and F-actin bundling after PFOS treatment in primary Sertoli cell culture.
Methods: After primary Sertoli cell isolation, dual-labeled immunofluorescence analysis to assess co-localization of fascin 1 with ezrin both in Sertoli cells and PFOS-treated (20 mM) Sertoli cells, was performed. Co-IP, using lysates of seminiferous tubules, was performed using actin and ezrin proteins to identify specific protein-protein interaction with fascin 1.
Results: Firstly, we showed that ezrin and fascin 1, which were components of the ES, were co-localized in the Sertoli cells and also they were interacted each other. Secondly, we indicated that they were dislocated in the PFOS-treated Sertoli cells in vitro. Because of PFOS (20 mM), the actin-based cytoskeleton was no longer capable of supporting the distribution and/or localization of actin-regulatory proteins at the cell-cell interface necessary to maintain localization of actin-regulatory at the BTB.
Conclusion: In summary, these findings suggest that ezrin and fascin 1 can work together to preserve BTB integrity by regulating F-actin organization in the PFOS-mediated Sertoli cell disruption.

SP03

Androglobin: a newly discovered globin preferentially expressed in testes

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Background: Androglobin (Adgb) is a recently discovered protein that is predominantly expressed in testis tissue of vertebrates. In silico expression analysis indicated a 4 fold higher expression level of Adgb in fertile men vs. infertile men. RNA expression analysis in mice showed a strong increase of Adgb at postnatal day 25 when postmeiotic spermatids are abundant, suggesting a potential role of Adgb during the late phases of spermatogenesis.
This newly discovered protein has a unique modular structure, possessing a N-terminal calpain-like domain, an internal circular permutated globin domain and an IQ-calmodulin binding motif. Alongside the well-established hemo- and myoglobin, and the more recently discovered neuro- and cytoglobin it represents already the fifth characterized human globin type.
Aim: Our aim is to define the molecular function of Adgb during spermatogenesis and to perform an in vitro biochemical characterization of the protein and its different domains.
Methods: We will perform a differential expression analysis of Adgb in semen samples and testis tissue of men with different types and grades of infertility using RT-qPCR and Western Blot. Furthermore, we will define the interaction partners of Adgb through a co-immunoprecipitation pull down experiment in mouse testis lysates. Finally, we will create a recombinant form of Adgb and perform a structural and functional characterization of the protein.
Results: The expression of Adgb in human spermatozoa and testis tissue was confirmed and a preliminary differential expression analysis in 4 human testis tissues revealed that Adgb is less expressed in biopsies of infertile men compared with a fertile biopsy.
The list of putative Adgb interaction partners generated by the co-immunoprecipitation pull down experiment contained many proteins that are linked with the chromatoid body (CB) of the male germ cell. CBs are characteristic spermatid organelles, which are suggested to function in RNA storage and small RNA processing but whose functions remain largely unknown.
As recombinant expression of Adgb was first in *E. coli* and *P. pastoris* remained successful due to folding problems, Adgb is currently recombinantly expressed using the baculovirus insect cell expression system, which is a higher eukaryotic expression system and can be easily scaled up.
Conclusion: Adgb plays a crucial role in spermatogenesis and is associated with late phases of spermatogenesis and with the chromatoid body of the male germ cell.

SP04

Olfactory receptors in semen and in the male tract: from proteome to proteins

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Topic Spermatogenesis
Keywords: chemiotaxis, olfactory receptor, sperm, sperm maturation

The estimated number of testicular ORs in mammals ranges between 20 and 66. Previous data reported the role of hOR17-4 and mOR23 in sperm-oocyte chemiotaxis, revealing that hOR17-4 has a chemiotactic function.
Proteomic analysis was performed to understand which are the ORs expressed in seminal plasma. Sperm and seminal plasma were collected in four fertile men. Seminal samples were analyzed by an Ultimate 3000Nano/Micro-HPLC apparatus coupled with an LTQ-Orbitrap XL hybrid mass spectrometer. Western blot analysis was performed to confirm proteomic data. The expression of ORs in sperm cells, testis and epidydimis was evaluated by confocal microscopy analysis. Testicular and epididymal tissues were collected from five patients (30-42 years) submitted to emasculation surgery. Moreover specimens from five testicular biopsies of patients (29-40 years) with spermatocyte maturative arrest were analyzed. One sample from testicular biopsy, finding a "Sertoli cell only syndrome", was collected from a 29 year-old patient as negative control.
In seminal plasma 13 different ORs were identified by proteomics and western blot confirmed these results. Confocal microscopy analysis revealed that OR4S1, OR4C13 and OR1I1 are expressed on the surface of sperm cells. In testicular tissue OR4S1 and OR4C13 are localized in the seminiferous epithelium with focal expression, while OR1I1 is expressed in the external cell compartment of the tubular walls. OR4S1, OR4C13 and OR1I1 had intense and diffuse staining in the epidydimis. OR4S1, OR1I1 and OR4C13 were absent in testicular tissue with spermatocytic arrest. Our results open new insights into OR involvment in sperm maturation and migration

SP05

DDX3Y, the major AZFa gene and its X homologue, DDX3X, control human male germ cell maturation before and after meiosis

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Background: The major AZFa gene on the human Y chromosome, *DDX3Y* and its X homologue, *DDX3X*, are functionally conserved from yeast to men and involved in the translational control of genes controlling the rate of cell cycles. In human, DDX3Y protein was found to be only expressed in male germ cells before meiosis (spermatogonia). Accordingly, AZFa deletions including *DDX3Y* cause a complete absence of male germ cells (SCO syndrome) but no somatic pathology. DDX3X protein is expressed only after meiosis in spermatids. This phase specific expression pattern is initiated by specific transcriptional control mechanisms for both genes functioning only in human male germ cells (Jaroszynski et al. 2011; Rauschendorf et al. 2011, 2014).
Aim: Our study is aimed to reveal putative functional links between expression of the germ cell specific *DDX3Y* and *DDX3X* transcript variants and germ cell specific signal pathways involved in spermatogonia differentiation control before meiosis (DDX3Y) and postmeiotic maturation of early spermatids after meiosis (DDX3X).
Methods: A large database of 150 infertile men with idiopathic azoospermia entering our clinical TESE was established. Their testicular pathologies were classified in the pathology department according to Sigg (1990) between grade 1 for hypospermatogenesis and grade 4 for SCO syndrome. Aliquots of their tissue samples were used for DNA, RNA and protein extraction and analyzed for expression of the *DDX3Y* and *DDX3X* germ cell specific transcript variants. Quantitative expression rates were compared with that of a set of genes known to be solely expressed during a specific germ cell phase.
Results: Analyses of the spermatogonia specific marker genes displayed a large and gene specific variation of their expression level when compared to that of the control sample (man with normal spermatogenesis). It points to a variable amount of spermatogonia in this patient group as expected, but also reveals a variable expression level of our marker genes in the spermatogonia still present. *GFRa1, UTF1* and *SLX4* displayed generally low expression rates in these germ cells, whereas *SALL4* is only reduced when *DDX3Y* expression is absent.
Rates of the germ cell specific *DDX3Y* transcript variants with 5'UTR exon T extension are heterogenous and not depending on germ cell number. It thus indicates a variable number of different spermatogonia types in the testis tubules of these patients.
Most variable were the germ cell specific *DDX3X* 3□UTR splicing variants first found when spermatids were present in the testis tubules. They seem to be involved in some postmeiotic germ cell specific transcription control mechanism essential for the production of mature sperms because divergence of their normal complexity pattern seems to be indicative for absence of mature sperms.
Conclusion: Diagnostic analysis of the *DDX3Y* and *DDX3X* germ cell specific transcript variants in patients with known arrest of their germ cell development before or after meiosis reveals more functional heterogeneities of human spermatogonia and spermatids not associated with their morphology but to the expression rates of other germ cell specific genes involved in their differentiation pathway towards spermatocytes, respectively, in further maturation events towards mature sperms.

SP06

Analysis of the epigenetic regulation of the Piwi-like 2 promoter in spermatogenesis

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Background: Piwi-like 2, a member of the Argonaute protein family, is exclusively expressed in pre-pachytene and pachytene stages of spermatogenesis. Piwi-like 2 acts in the germ cell development and the silencing of retrotransponsons to maintain genomic integrity and stem cell character. In the present study we investigated DNA methylation as potential mechanism for the regulation of human Piwi-like 2 expression in cell lines related to spermatozoa precursor cells.
Methods: For epigenetic regulation studies TCam2 and NT2D1 cell lines were used. We analyzed the expression of Piwi-like 2 by qRT-PCR and Western Blot after treatment with DNA methylation inhibitor 5-Aza-2′-deoxycytidine (5AzadC). Analysis of CpG methylation status of the Piwi-like 2 promoter was assessed by bisulfite sequencing. Piwi-like 2 promoter activity was demonstrated by luciferase reporter gene assay. The role of CpG methylation was studied by in vitro methylation of respective Piwi-like 2 promoter constructs.
Results: Piwi-like 2 mRNA (37fold; $p = 0.021$) and protein was upregulated in TCam2 cells after 5AzadC treatment. NT2D1 showed no change in Piwi-like 2 expression. Bioinformatics analysis identified 57 CpG dinucleotides in the promoter sequence from −300 to +600 bp. Bisulfite sequencing of the CpG site demonstrated a different basal methylation level of Piwi-like 2 in the cell lines (TCam2: 85%, NT2D1: 41%). Treatment of cells with 5-AzadC allows a partial demethylation of Piwil2 promoter in TCam2 (74%) and NT2D1 (30%). Transfection of cells with different Piwi-like 2 promoter constructs identified several regulatory regions by an increase of luciferase activity (Fragment 1: 35-fold ($p = 0.007$) increase in TCam2 and 10fold activation in NT2D1 ($p = 0.001$); Fragment 2: 3fold induction in TCam2 ($p = 0.049$) and a 6.5fold increase of luciferase activity in NT2D1 ($p = 0.034$). In vitro methylation of selected fragments suppressed Piwi-like 2 promoter activity.
Conclusion: We report an increase of Piwi-like 2 expression in hypermethylated cell line TCam2 by treatment with 5azadC. Piwi-like 2 promoter constructs of different length are able to drive luciferase expression in human cells to different extends. However, the activity was markedly reduced after in vitro methylation of these fragments. These data indicate that in humans DNA methylation is able to induce epigenetically silencing of Piwi-like 2 expression and provide first hints for epigenetic alterations during spermatogenesis.

SP07

Fertilizing capacity of spermatozoa generated in hamster testicular tissue transplanted in the anterior limbs of immune deficient rats: clinical implications

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Objectives: We evaluated the fertilizing capacity of spermatozoa generated in hamster testicular tissue (HTT) transplanted in the anterior limbs of immune deficient nude rats (ALsIRs).
Material and Methods: Fragments of hamster testicular tissue (HTT) recovered from mature animals ($n = 8$; 6-week-old) were transplanted microsurgically into the muscles of the ALsIRs ($n = 16$; 7-week-old). HTT from each one donor animal was transplanted to two recipient animals. Prior to transplantation, both hematoxyline eosin stain (HES) and transmission electron microscopy (TEM) in the transplanted HTT revealed full spermatogenesis. Ten weeks post-transplantation, all the recipient rats were killed and both TEM and HES demonstrated degeneration of the HTT in 3 recipients, arrest at the primary spermatocyte (PSs) stage in HTT in 6 recipients, arrest at the RS stage in HTT in 1 recipient, and complete spermatogenesis in few tubuli of the HTT in 6 recipients. At that time transplanted HTT was minced and evaluated for the presence of RSs or spermatozoa. Spermatozoa from 6 recipients were processed for ooplasmic injections into hamster oocytes using a Piezo-micromanipulator.
Results: Injections of hamster spermatozoa generated in HTT previously transplanted in ALsIRs resulted in fertilization rate of 46% and blastocyst development rate of 19%.
Conclusion: Transplantation of HTT into the ALsIRs results in the generation of donor spermatozoa within 37.5% of the recipient rats. Injections of hamster spermatozoa generated in ALsIRs (of nude rats) into hamster oocytes result in blastocysts. An attractive hypothesis is to autotransplant human testicular tissue to upper extremities in men who are going to receive scrotal or pelvic radiation. This approach may have an advantage compared with the testicular tissue freezing because (i) death of frozen diploid or haploid germ cells is avoided and additionally, (ii) the spermatogonia within the autotransplanted testicular tissue may undergo mitoses and increase their number post-transplantation.

SP08

The tyrosine kinase FER is responsible for the capacitation-associated increase in tyrosine phosphorylation

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Background: Sperm capacitation comprises all the physiological changes occurring to mammalian spermatozoa during the transit through the female reproductive tract and is necessary for successful fertilization both in vivo and in vitro. At the molecular level, the process is associated with a fast activation of cAMP-dependent phosphorylation by protein kinase A (PKA). The activation of PKA leads to a slow increase in the levels of tyrosine phosphorylation, another established hallmark of sperm capacitation. Several candidates to be responsible for the increase in tyrosine have been suggested. However, due to limitations in the use of knock-out approaches for sperm research, the identity of the kinase ultimately responsible for the increased levels of phosphorylation has not been demonstrated yet.
Aim: the aim of the present research was to investigate the identity of the tyrosine kinase responsible for the capacitation-induced increase of tyrosine phosphorylation.
Methods: In order to fulfill this purpose, both pharmacological inhibitors and genetically modified murine models were used. Levels of Tyrosine phosphorylation were tested by SDS-page, with antibodies directed against phosphorylated tyrosine residues. Effect of pharmacological inhibitors on recombinant kinases in vitro was tested by the use of radioactive assay.
Results: Mice lacking PYK2 tyrosine kinase display normal capacitation-associated increase in tyrosine phosphorylation. On the other hand, sperm from mice targeted with a mutation that abolish the activity of the FER kinase ($FER^{DR/DR}$), fail to undergo the increase in tyrosine phosphorylation associated with sperm capacitation. Our results indicate that FER tyrosine kinase is the tyrosine kinase responsible for the capacitation-associated increase in tyrosine phosphorylation. Interestingly, although sperm from $FER^{DR/DR}$ animals have significantly reduced capability to fertilize metaphase II-arrested eggs in vitro, these mice are fertile in vivo.
Conclusion: Our results suggest that FER is the kinase responsible for the regulation of the increase of tyrosine phosphorylation associated with sperm capacitation in murine.

PS2A1 – POSTER SESSION NR.2
POSTER PRESENTATIONS

OF01

Histological quantification of Leydig cell hyperplasia in testicular cancer patients: association with long-term Leydig cell dysfunction

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Background: A high prevalence of Leydig cell hyperplasia (LCH) has been observed in testicular biopsies from patients with testicular cancer. LCH has been associated with decreased testosterone/luteinizing hormone (T/LH) ratio as a sign of Leydig cell dysfunction. However, a clear histological definition of LCH is lacking.
Aims: The aims were (i) to establish a quantitative method to estimate the Leydig cell compartment in biopsies, and (ii) to investigate any associations between LCH in the contralateral biopsy of testicular cancer patients and long-term Leydig cell dysfunction.
Patients & Methods: The study population comprised 34 patients treated at Rigshospitalet (Copenhagen), selected from a long-term follow-up study of testicular cancer survivors (NCT02240966). All patients had been treated with unilateral orchiectomy and either (i) surveillance alone (ii) bleomycin, etoposide and cisplatin or (iii) radiotherapy. The contralateral biopsy taken at the time of diagnosis was evaluated for LCH. Patients with elevated hCG were excluded. Ten patients with obstructive azoospermia, normal testis histology, and serum T and LH levels within the normal range served as controls.
For each study subject, 4 of 10 archived hematoxylin/eosin-stained histological slides were scanned. Four sections from the contralateral biopsy or two sections from each of the two bilateral biopsies were evaluated in the patients and controls, respectively. For each slide an area with a minimum of 50 tubules was selected for analysis. Every Leydig cell group was delineated manually and the total Leydig cell area (LT) was calculated, using the open-source software ImageJ. To account for quality and heterogeneity of a histological section, the sum of tubules in the four sections was calculated. Four different methods/indexes were tested for quantification of the Leydig cell compartment: (i) LT/total selected area, (ii) LT/total area multiplied by number of tubules (LT/AxT), (iii) LT/total area divided by number of tubules and (iv) mean Leydig cell area. For each of the methods, the mean value was compared between patients and controls with independent samples *t*-test. LT/AxT index was transformed by natural logarithm to obtain a normal distribution. All statistical tests were computed in IBM SPSS Statistics, Version 22.0.
Results: In controls, LT/AxT was more homogeneously distributed than the other methods evaluated, so it was selected as the best fitting and used to quantify the Leydig cell compartment. The median LT/AxT was significantly

higher in patients with testicular cancer than in controls: 21.3 (range 4.9–72.7) vs. 12.7 (4.0–18.3), $p = 0.003$. The index logLT/AxT = 3 was selected as cut-off for LCH; and after a long-term follow-up (median 11.2 years), the patients with the logLT/AxT = 3 ($n = 17$) (termed □LCH') had significantly lower mean total testosterone (T 10.4 (4.0) nmol/L vs. 14.1 (4.0) nmol/L, $p = 0.01$) and lower T/LH (1.5 (1.0) vs. 3.1 (2.4), $p = 0.02$) than the patients with the logLT/AxT < 3 ($n = 17$).

Conclusion: A new quantitative method (LT/AxT index) was used to estimate Leydig cells in a testicular biopsy. The Leydig cell compartment was larger among testicular cancer patients compared to controls. A high LT/AxT value was associated with long-term risk of Leydig cell dysfunction.

(MGT and MB contributed equally).

OF02

A diagnostic germ cell score for evaluation of prepubertal and pubertal testicular biopsies stored for fertility preservation

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Background: Survival rates of cancer patients improved over the last decades. Therefore, quality of life of these patients after successful treatment has become an important concern. One side effect of treatment is temporary or permanent subfertility or even infertility. Cryopreservation of immature testicular tissues is therefore offered to prepubertal and pubertal boys at risk for germ cell loss at CeRA. In these tissues, germ cells have not initiated spermatogenic differentiation, but experimental protocols for the derivation of sperm from the spermatogonia that are present in these tissues are currently being developed. Critical factors for the success of these differentiation protocols are the absolute numbers of spermatogonia and the germ cell differentiation status.

Aim: The aim of the study was to evaluate if the combination of absolute number of spermatogonia and the differentiation status of the tissues are valuable parameters to determine a diagnostic germ cell score for each patient.

Materials & Methods: Testicular tissues from 39 patients were evaluated and divided into 3 groups. Group A: Patients suffering from diseases which do not directly affect the testes, for example Hodgkins lymphoma ($n = 6$; 6–14 years), group B: patients suffering from diseases that directly affect the testes, such as testicular tumors ($n = 14$; 2–17 years) and group C: Klinefelter patients ($n = 19$; 12–20 years). Immunohistochemical stainings (IHC) were performed for the germ cell markers UTF1 and MAGEA4. Two of these stained cross sections of each patient were evaluated to determine the absolute numbers of spermatogonia per mm^3 employing morphometric analyses. Also, the differentiation status of the testes was determined by UTF1 and MAGEA4 stainings.

Results: The calculated mean numbers of spermatogonia were 87041 (±20317), 33866 (±26843) and 4502 (±4253) per mm^3 in groups A, B and C, respectively. In group C, spermatogonia were only detected in 7 out of 19 patients. To determine a diagnostic germ cell score the 95th confidence interval of the absolute numbers of spermatogonia of group A was calculated (63684 spermatogonia per mm^3). Patients with spermatogonial numbers above this value were allocated a (2), below this number a (1) and patients without any spermatogonia a (0). Furthermore, the differentiation status of the tissues were evaluated by UTF1 stainings which stained mainly gonocytes and spermatogonia and MAGEA4 staining which labeled gonocytes, spermatogonia and spermatocytes. Four categories were applied: (a) gonocytes and spermatogonia, (b) spermatogonia, (c) spermatogonia and spermatocytes, (d) gonocytes, spermatogonia and spermatocytes. Each patient was assigned the letters a, b, c or d, respectively depending on the presence of the different germ cell types.

Conclusion: We conclude that a germ cell score considering the absolute numbers of spermatogonia and the differentiation status seems to be advisable, as these parameters will most likely affect the success of differentiation protocols that will be used in the future to derive sperm from these testicular tissues.

OF03

Identifying the need of discussing infertility concerns affecting testicular cancer patients; an evaluation (INDICATE study)

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Background: Men treated for testicular cancer (TC) might risk impaired fertility. Considering the overall 5-year survival rate of 95% and the onset during reproductive age, future fertility is of major concern to most TC patients. Patients with TC have a 15–30% higher risk of infertility than the normal population. In addition to psychological factors, this elevated risk could be the result of abnormal function of the remaining testis, azo□- or oligospermia due to treatment or retrograde ejaculation. After chemotherapy, azo□- or oligospermia occurs for 6–12 months, with 90% recovering within 2 years. Nerve-sparing *retroperitoneal lymph node dissection*, improved radiotherapy techniques and the use of other cytostatic agents have improved the fertility rate lately. Fertility problems may be prevented by semen cryopreservation before treatment and/or additional techniques of assisted reproduction. Patients should be informed about the risk of impaired fertility as a result of the (adjuvant) treatment and the possibility of cryopreservation.

Aim: This study was designed to evaluate the quality of discussions regarding fertility and cryopreservation after TC diagnosis according to survivors in retrospect. Furthermore, we determined how many patients performed cryopreservation and which percentage made use of or plans using the preserved semen. Also, the procedure and success of collecting semen for cryopreservation were addressed.

Methods: A cross-sectional survey was performed among TC patients diagnosed between 1995 and 2015, primarily

treated by or referred to a tertiary care hospital (n = 582). The questionnaire was designed by the authors. A patient panel of the Dutch Testicular Cancer Society piloted the questionnaire.

Results: Out of 574 survivors, 183 questionnaires were completed (response-rate 31.8%). Mean age of respondents was 44 years, mean age at time of diagnosis 34 years. Eighty four percent of the respondents was informed about possible impaired fertility in advance of treatment, of which 81% was satisfied about the provided information and counselling. Almost one out of five (18.1%) was not offered to discuss fertility concerns after treatment but would have liked to. Eighteen percent of the respondents reported grief due to impaired fertility, 9.4% stated to be less satisfied in life due to impaired fertility. The majority (54.2%) preferred the urologist for providing information about fertility. One third (31.3%) of all respondents had children after TC treatment, of which 8.8% had to make use of preserved semen and 5.3% with assisted reproduction (ICSI) due to poor semen quality. Semen cryopreservation was performed by 42.2%, of which 15.1% made use of the preserved semen, 6.9% still planned on using and 41.7% might make use of it if necessary. Collecting semen was troublesome but eventually successful according to 26.4%, 5.6% did not succeed in collecting semen for cryopreservation. Reasons for troublesome collection were high pressure due to disease, pain after surgery and uncomfortable setting.

Conclusion: Results of this survey indicate the importance of timely discussion of fertility issues with TC patients by healthcare professionals. Although discussed with most men, dissatisfaction, grief and reduced quality of life occur in some cases as a result of impaired fertility and a lack of attention for the subject.

OF04

Long term effects of cytotoxic therapy 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. While the relatively low gonado-toxicity of therapies allows a rapid recovery of spermatogenesis, the future welfare of the offspring conceived by a father treated with cytotoxic therapy remains a major concern. The right timing for natural conception after treatment is still uncertain and based only on few studies and with limited follow up (2 years).

Aims: To evaluate the integrity of the sperm genome (Sperm DNA Fragmentation, SDF) in patients affected by testis cancer (TC) and hematological malignancy (HM) up to 4 years post therapy.

Subjects and Methods: i) TC (n = 47) and HM (n = 18) patients after 2 years post-therapy; ii) TC (n = 36) and HM (n = 18) patients after 3 years, TC (n = 8) and HM (n = 2) after 4 years post-therapy. These patients were analyzed by terminal-uridine nick and labelling end assay (TUNEL). We evaluated %SDF as "total" and as "%SDF PIbr" which is significantly associated with sperm fertilizing potential. Data was compared to 108 healthy fertile men (CTRL) showing mean %SDF± SD=31.2 ± 11.7 (range: 11.8-60.0) and mean %SDF PIbr± SD= 19.1 ± 9.1 (range: 3.1-56.0).

Results: i) After 2 years in TC patients treated with ≥3 cycles (PEB or Carboplatine) both %SDF (47.2 ± 21.6) and %SDF PIbr (28.4 ± 13.7) were significantly higher in respect to controls (p < 0.001and p = 0.003, respectively); in HM patients treated with ≥5 cycles only %SDF PIbr showed significant difference vs controls: 30.0 ± 19.8 vs 19.1 ± 9.1 p = 0.001. In the entire cohort of the 2 years follow-up an SDF>60% was observed in: 23.4% of TC patients (11/47, two with 2 and four with 3 PEB cycles; one with 2 and one with 1 Carboplatine cycle and three with Radiotherapy); 11.1% of HM patients (2/18, both treated with 6 cycles of ABVD).

ii) After 3 years %SDF PIbr in TC and HM patients vs CTRL showed: 23.01 ± 11.3 and 24.17 ± 10.8 vs 19.1 ± 9.1, p = 0.038 and p = 0.040, respectively. After 4 years %SDF in TC vs CTRL was: 42.38 ± 17.7 vs 31.2 ± 11.2, p = 0.013 while in HM no significant increase has been observed. % SDF PIbr in TC and HM patients vs CTRL showed: 28.01 ± 12.99 and 26.94 ± 16.19 vs 19.1 ± 9.1, p = 0.011, n.s., respectively.

At 3 years an SDF value above 60% was observed in 6% of TC patients (2/36, one with CDDP+ETOP [3 cycles] and one with Carboplatine [1cycle+Radiotherapy] and 6% of HM patients (1/18, R-CHOP, 6 cycles). After 4 years, 25% TC patients (2/8, one with radiotherapy and one with Carboplatine [2 cycles]); whereas no HM patients showed >60% SDF.

Conclusion: Our study indicates a long-lasting effect of cytotoxic therapies on DNA integrity in a relatively large proportion of patients. A clearly pathological SDF value (above the maximum range reported in fertile controls) were observed in some patients even after 4 years post-therapy. DNA fragmentation analysis, especially %SDF PI^{br}, should be proposed as a pre-natural conception test both to monitor the long-term effect of cytotoxic therapies and to help in decision making on the timing of natural pregnancy.

PS2A2 – POSTER SESSION NR.2 POSTER PRESENTATIONS

MIT01

Regulation of C-type natriuretic peptide in sperm capacitation

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Background: C-type natriuretic peptide (CNP) is a newly discovered member of the natriuretic peptide family that mainly binds to its specific receptor NPR-B and causes biological effects. Recent studies have confirmed that CNP can affect sperm motility and the acrosome reaction, thus regulating the sperm capacitation.

Aim: To understand the regulation mechanism of CNP in human sperm capacitation.
Methods: We used an immunohistochemical technique to detect the expression and localization of CNP and its specific receptor NPR-B in the genital tract of female rats (e.g., cervix, uterus, oviduct). Then, enzyme linked immunosorbent assay was used to measure cyclic guanosine monophosphate(cGMP)content. Last, we detected Fluo3-AM Fluorescence probe-binding calcium ion in spermatozoa using flow cytometry and evaluated sperm protein tyrosine phosphorylation using western blots.
Results: Our results showed that CNP/NPR-B was expressed in the squamous epithelial cells of cervical mucosa, the endometrial columnar epithelial cells and ciliated cells of the mucosa epithelium of the oviduct; CNP significantly elevated cGMP content in sperm; and CNP and 8-Br-cGMP markedly improved sperm Ca^{2+} content, which was inhibited by KT5823. Nevertheless, the protein tyrosine phosphorylation levels of sperm did not significantly change.
Conclusion: Thus, it can be observed that CNP binds to its specific receptor NPR-B on sperm, elevating intracellular cGMP content, activating the downstream signaling molecule PKG, and increasing Ca^{2+} content to regulate sperm capacitation.

MIT02

The effects of antiestrogen administration on seminal parameters in men with idiopathic infertility: a systematic review and meta-analysis of randomized controlled trials

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Background: Antiestrogen has been extensively used as an empiric treatment for idiopathic male infertility for over 30 years. Most of early clinical trials yielded similar results that antiestrogen could improve seminal parameters and increase spontaneous pregnancy rates. However, recent studies have drawn variable conclusions about the effect of antiestrogen on seminal parameters.
Aim: To conduct a meta-analysis of randomized controlled trials (RCTs) evaluating the effects of antiestrogen on seminal parameters in men with idiopathic infertility.
Methods: We performed a comprehensive literature search in electronic database, including PubMed, Web of Science, Embase and CNKI, up to 2015. RCTs that compared the effect of antiestrogen administration (clomiphene citrate and tamoxifen) with placebo on idiopathic infertility male were considered for inclusion. Two of the authors independently evaluated the eligibility of all retrieved studies and extracted the relevant data from each study. Continuous data of seminal parameters were presented as mean differences and standard deviation and weighted mean differences (WMDs) with 95% confidence intervals (CIs) were calculated. Our meta-analysis was performed using RevMan5.3 software provided by the Cochrane Collaboration.
Results: Eleven RCTs were identified for inclusion involving 898 men presenting with idiopathic infertility. A meta-analysis was conducted following a quality evaluation. Compared with placebo, antiestrogen administration for idiopathic infertility patients could increase their sperm concentration (MD 8.46; 95% CI 6.56, 10.35; $p < 0.00001$) and sperm motility (MD 4.03; 95% CI 0.83, 7.24; $p = 0.01$) significantly. Meanwhile, antiestrogen administration reduced the percentage of sperm with abnormal morphology (MD −7.55; 95% CI −13.55, −1.55; $p = 0.01$). However, heterogeneity was present (I2 53–92%). Variations among included studies may increase the heterogeneity, such as countries and races of the patients, ages, methodology, drug doses, duration of treatment and follow-up. Conclusion: Evidence from available RCTs suggests that antiestrogen administration can improve semen parameters in cases of idiopathic infertility. Due to limited evidence, the additional high-quality RCTs with large sample size and longer follow-up duration are required to confirm the effect of antiestrogen on idiopathic male infertility.

MIT03

Prediction model for obtaining spermatozoa with TESE in men with non-obstructive azoospermia

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Study question: What factors pertain to the prediction of obtaining spermatozoa with testicular sperm extraction (TESE) in men with non-obstructive azoospermia (NOA) and how well does the resulting prognostic model perform in an external database?
Summary answer: Our prediction model including six variables was able to make a good distinction between men with a good chance and men with a poor chance of obtaining spermatozoa with TESE.
What is known already: The few models that have been developed to predict the chance of obtaining spermatozoa with TESE are all small and none of them have been validated externally.
Study design, size, duration: We performed a retrospective nationwide cohort study. Data of 1371 TESE procedures were collected between June 2007 and June 2015 in the fertility centres of the Radboud university medical center in Nijmegen and the Academic Medical Center in Amsterdam.
Participants/materials, setting, methods: All men with NOA undergoing their first TESE procedure as part of a fertility treatment were included. Primary endpoint was the presence of spermatozoa in the testicular biopsies. We constructed a model for the prediction of successful sperm retrieval, using univariable and multivariable binary logistic regression analysis and the dataset from one centre. This model was then validated using the dataset from the other centre. The area under receiver operating characteristic curve was calculated and model calibration was assessed.
Main results and the role of chance: There were 599 (43.7%) successful sperm retrievals after a first TESE

procedure. The prediction model, build after multivariable logistic regression analysis, demonstrated that higher male age, larger values for testosterone and lower values for FSH and LH were predictive for successful sperm retrieval. Diagnosis of idiopathic NOA and the presence of an *AZFc* deletion were predictive for unsuccessful sperm retrieval. The area under the curve was 0.69 (95% CI: 0.66–0.72). The difference between the mean observed chance and the mean predicted chance was less than 2.0% in all groups, indicating good calibration. In validation, the model had moderate discriminative capacity (area under the curve 0.65, 95% CI: 0.62–0.72) and moderate calibration, the predicted probability never differed by more than 9.2% of the mean observed probability.

Limitations, reasons for caution: The percentage of men with Klinefelter syndrome among men diagnosed with NOA is expected to be higher than in our study population, which is a potential selection bias.

Wider implications of the findings: This model can help in clinical decision making in men with NOA by reliably predicting the chance of obtaining spermatozoa with TESE.

MIT04

Effect of ejaculatory abstinence on sperm DNA integrity and longevity

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Background: The influence of ejaculatory abstinence on sperm DNA integrity may provide a valuable information on predicting successful clinical outcome.

Aim: To understand the influence of ejaculatory abstinence on sperm chromatin maturity, integrity, longevity and global methylation status.

Methods: This study included 76 ejaculates from 19 healthy volunteers who provided ejaculates after observing 1, 3, 5 and 7 days of abstinence. Sperm chromatin maturity and DNA integrity were assessed in the neat ejaculate whereas sperm motility, DNA integrity and longevity were assessed in the processed fraction of the fresh and frozen-thawed ejaculates to determine their association with the length of ejaculatory abstinence.

Results: Spermatozoa from 1 day ejaculatory abstinence displayed significantly higher level of sperm chromatin immaturity in comparison to 1 day ($p < 0.05$) and 5 day ejaculatory abstinence ($p < 0.01$). On the other hand, in vitro incubation of processed ejaculate from 1 day ejaculatory abstinence resulted in approximately 20 and 40 fold increase in the DNA fragmented spermatozoa at the end of 6 and 24 h respectively ($p < 0.01$–0.001).

Conclusion: Use of short-term EA for therapeutic fertilization would be a clinically valuable strategy to improve the DNA quality. However, use of such spermatozoa after prolonged incubation in vitro should be avoided as it can have negative impact on the reproductive outcome.

MIT05

Klinefelter syndrome and fertility: why early fertility preservation should not be offered to children with Klinefelter syndrome

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Background: KS is the most common chromosomal disorder in men leading to non-obstructive azoospermia and is caused by the presence of at least one additional X chromosome. The onset of puberty in adolescents with KS leads to progressive degeneration of the testicular environment. The impact of the subsequent tissue degeneration on fertility potential of patients with KS is unknown, but in previous literature it has been suggested to start fertility preservation in adolescents as early as possible. However, in adults with KS spermatozoa can be found by testicular sperm extraction (TESE) in about 50% despite severe testicular degeneration.

Aim: The aim of this review is to summarize and discuss current evidence for fertility preservation in children and adolescents and possible prognostic markers for fertility treatment in KS.

Methods: An extensive literature search has been conducted, searching Pubmed, Embase, Cinahl and Web of Science from origin till April 2016 for 'Klinefelter syndrome' and 'fertility' and various synonyms. Titles and abstracts have been scanned manually by the authors for eligibility.

Results: Various studies have shown that pre-pubertal children with KS already have a reduced number of germ cells despite a normal hormonal profile during childhood. The presence of spermatozoa in the ejaculate of adolescents with KS is extremely rare. Using (micro) TESE, the retrieval rates of spermatozoa for adolescents younger than 16 years old are much lower (0–20%) compared to adolescents and young adults between 16 and 30 years old (40–70%). Although Spermatogonia can be found by (m)TESE in about half of the peri-pubertal adolescents, there are currently no clinically functional techniques for their future use. Children and adolescents need to be informed that early fertility preservation before the age of 16 cannot guarantee fertility later in life and may even reduce the chances for offspring by removing functional immature germ cells which possibly had developed into spermatozoa after puberty. Furthermore, except for age of patients with KS there are no factors identified that can reliably be used as a predictive marker for fertility preservation.

Conclusion: Current evidence shows that fertility preservation should not be offered to adolescents with KS younger than 16 years old because of lower retrieval rates for spermatozoa by m(TESE) compared to adolescents and adults between 16 and 30 years old.

MIT06

Effect of Vitamin D on apoptosis and DNA integrity of human sperm

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Background: Vitamin D has important role for maintaining calcium, phosphorus homeostasis and promoting bone mineralization. Recently, localization of vitamin D receptor in the human sperm was detected. However, the action of this vitamin in human male reproduction has not yet been clarified.
Aim: In this study, we evaluated the effect of vitamin D on apoptosis, DNA integrity and other parameters of human sperm.
Method and materials: The study was carried out on semen of 7 fertile men who referred to IVF clinic of Emam hospital in Ahvaz Jundishapour University of Medical School. Samples were processed for swimming up. Supernatant was divided into two groups, one as control and another one had received 100 microliter of vitamin D as experimental group for 1 h.They were assessed for sperm motility by Makler chamber, DNA integrity with aniline and toluidine blue staining,and apoptosis of sperm with Annexin V assay.
Results: The results revealed that: (i) Apoptosis in sperm was significantly decrease (59.71% vs.17.09%, *p*value = 0.034) (ii) Total motile sperm was increased with vitamin D (31.91% vs. 24.3%) but there was no significant statistical difference (*p*value = 0.64) (iii) Fast motility was significant increase (10.1% vs. 1.87%, *p*value = 0.012) (iv) Positive aniline blue and toluidine blue were not decreases with vitamin D (35.59% vs. 40.092%, *p*value = 0.88 for aniline blue and 60.37% vs. 67.75%, *p*value = 0.73 for toluidine blue).
Conclusions: In this study, apoptosis and motility of sperm have improved after incubation with vitamin D so may be used it for therapeutic opportunities in sperm and male reproduction disorders.

MIT07

DBPC study in oligoasthenospermic men treated with metabolic and essential nutrients showed that progressive sperm motility was correlated to seminal carnitine levels

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Background: The main function of L-carnitine (L-C) and acetyl-L-carnitine (ALC) is to provide an energetic substrate for spermatozoa. Carnitine directly contributes to sperm motility and may be involved in the maturation of sperm. This is especially important since epididymal sperm use fatty acid oxidation as their main source of energy. L-C is highly concentrated in the epididymis and is necessary for transport of fatty acids into mitochondria. Studies have reported either no effect or that therapy with combined high dose L-C and ALC significantly increases sperm parameters (forward motility and concentration).
Aim: The study was randomized, double blind, placebo controlled (DBPC) and examined the effect of test formulation, Proxeed Plus, containing L-C 2 g and ALC 1 g, as well as antioxidants, vitamins and minerals, in men with oligoasthenozoospermia. The protocol was 2 months wash-out and 6 months treatment (T-2, T0, T+3, T+6), with test formulation (125 patients) or placebo (50 patients).
Methods: Men visiting the Andrology center, aged between 18 and 50 years and with history of difficulty in conceiving >12 months, were randomized to receive treatment or placebo in a double-blind protocol. Subjects with endocrine disorders, autoimmune disease, azoospermia, cystic fibrosis, testicular cancer etc., were excluded. Compliance was assessed at visits. Analysis of ejaculate was done according to WHO 5th guideline, and progressive sperm motility (A + B grade of rapid, progressive) was done manually.
Results: In the placebo group there was no significant improvement in the progressive sperm motility before, T0 = 20.14% (10.39 ± 28.11) and after the 6 months period, T6 = 23.44% (14.65 ± 38.00) with $p < 0.82$.
In the treated group there was statistically significant difference, $p = 0.004$ by McNemar-Boweker test, in the values of progressive sperm motility in the three different time periods: T0 = 22.50% (11.50 ± 38.00), T3 = 30.00% (12.00 ± 39.00) and T6 = 31.00% (20.00 ± 41.00). The seminal plasma carnitine at T0 was 700.50 μmol/L (625.50 ± 800.00) and at T6 = 751.50 μmol/L (671.10 ± 896.80), and this difference was significant ($p = 0.014$, by Wilcoxon signed-rank test). Further the Spearman's rank-order correlation test showed that the increase of seminal carnitine level influenced the progressive sperm motility ($r = 0.274$; $p = 0.023$), suggesting that if the seminal plasma carnitine level is higher, the progressive sperm motility is higher. Thus the correlation of seminal plasma carnitine and progressive sperm motility showed that in man, an increase of seminal carnitine of 7.7%, after six months therapy, would impact progressive sperm motility >10% with moderate accuracy (AUC = 726).
Conclusion: This randomized DBPC study showed significant improvement in percentage of progressive sperm motility after six months of therapy and also underlines the importance of duration of therapy (3 and 6 months) with antioxidant, metabolic and essential nutrients. Increased levels of carnitine, during and after the treatment, affected the sperm motility, showing that an increase of seminal carnitine positively impacted upon the patient progressive sperm motility.

MIT08

Left Varicocele in subfertile men: role of a continuous spermatic vein reflux before and after treatment to predict improved sperm parameters after varicocele correction

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Background: Varicocele repair is associated to improved sperm parameters in the male partner of subfertile couples. A continuous spermatic venous reflux (SVR) while standing is used, as an evidence of venous orthostatic hypertension present in men with varicocele but its variation after treatment to predict improved ejaculated sperm parameters is still undefined.
Aim: Outcome of this retrospective study was to define the association between changes of semen parameters after varicocele repair and the disappearance or the persistence of a continuous SVR after repair.
Methods: Subfertile patients with grade II or III left side varicocele were submitted to semen analysis, determination of blood hormone levels (FSH, LH, total Testosterone) and to scrotal color Doppler ultrasound (CDU) evaluation. Semen analysis and CDU were re-evaluated 6 months after varicocele repair through a retrograde internal spermatic vein embolization. The study included 60 men, who showed a disappearance of a continuous SVR (Group 1), and 40 men, which showed a persistence of a continuous SVR after varicocele repair (Group 2).
Results: No differences between the two groups were observed at baseline for age, testicular volume, the percentage of cases with left mean diameter of the venous vessels of the pampiniform plexus (MVD) >3 mm, the median value of a continuous SVR, which in all cases was >3.0 cm/s. Sperm parameters and hormone levels were also not different in the two groups. Venography of the internal left spermatic vein confirmed the presence of reflux and collateral venous branches in all cases. After correction both groups showed a significative improvement of sperm count, although this was much more significative in group 1. Total motile sperm count (TMC) after repair was negatively correlated with SVR after repair (r = −0.22; $p = 0.035$). Change of TMC (after repair minus baseline value) was negatively correlated with SVR after repair (r = -0.29; $p = 0.0050$). SVR after repair, adjusted for age, baseline FSH level, TMC and SVR, negatively predicted change of TMC (ß = −0.030; $p = 0.022$), suggesting that a lower SVR after varicocele repair was associated to a higher positive change of TMC. Adjusted baseline SVR positively predicted TMC change (ß = 0.179; $p = 0.0024$), suggesting that correction of higher values of baseline SVR resulted in more effective improvement of semen parameters after correction.
Conclusion: Subfertile men with clinical varicocele improved their sperm parameters after varicocele repair and this was more relevant in case of disappearance of a continuous SVR. Evaluation of a continuous left spermatic vein reflux is a relevant and objective method to assess a successful varicocele repair aimed to improve sperm parameters.

MIT09

Association between sperm DNA damage and human preimplantation embryo metabolism

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Background: Embryo metabolic response to sperm induced specific abnormalities could help in developing the metabolic markers to prevent the transfer of embryos carrying sperm mediated defects.
Aim: To understand the influence of sperm DNA damage on the ICSI derived human embryo metabolism.
Methods: In this prospective study, spent embryo culture media from 34 patients undergoing ICSI cycles were profiled using Nuclear magnetic resonance (NMR) spectroscopy. Processed ejaculates were tested for DNA damage using neutral comet assay. Relative intensities of the metabolites from 97 spent media samples were compared between 'male-factor' and control groups. Relative intensities in the subgroups derived from the extent of DNA damage from both the 'male factor' and control group were also compared.
Results: Sperm characteristics including DNA damage levels (Olive tail moment, OTM) were significantly different between 'male factor' and control groups ($p < 0.001$–0.0001). Of the metabolites analyzed, glutamine intensity was significantly lower in 'male factor' group ($p < 0.01$) whereas, glutamine, pyruvate and alanine intensities were significantly different between the embryos derived from sperm population having OTM <1.0 and >1.0 ($p = 0.03$, 0.003 & 0.005 respectively). Significant difference was also found in pyruvate to alanine ratio between the two OTM groups tested ($p < 0.0001$).
Conclusion: This study indicates that increased level of sperm DNA damage in the processed ejaculate affects embryo metabolism which could be related to embryonic genetic integrity.

MIT10

Reproductive status and outcome in patients with disorders of sex development

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It is generally believed that patients with disorders of sex development (DSD) have total lack or very low fertility and fecundity potential. However, in the face of modern medical technology opportunities these views change and fertility diagnosis takes a new meaning. dsd-LIFE is a comprehensive study investigating medical, surgical,

psychological and ethical issues to improve treatment and care of patients with the different diagnoses comprising DSD (www.dsd-life.eu). Among others, research questions were: To what extent is fertility compromised in different forms of DSD? How strong is the desire of DSD patients for fertility treatment? Was information on fertility problems and treatment possibilities given to DSD patients? Were the patients satisfied with how fertility problems were discussed? The multidisciplinary consortium consists of experienced European scientists in the areas of endocrinology, andrology, gynaecology, urology, surgery, psychology and ethics. The study is conducted in Germany, France, the Netherlands, Poland, Sweden and United Kingdom. Patients >16 years (in total 1161) with the following diagnoses participated: androgen insensitivity (complete and partial), gonadal dysgenesis (complete, partial, mixed), ovotestis, impaired androgen biosynthesis (5-alpha-reductase-2 deficiency, 17-beta-HSD-3 deficiency, LH-receptor defects), hypospadias, Klinefelter syndrome, Turner syndrome, XX male, congenital adrenal hyperplasia. The patients were given thorough information about the study and after informed consent the evaluation started. All patients underwent the same evaluation in each study centre. This included a medical questionnaire, which was answered by the physician seeing the patient. Data on surgery, hormone therapies and counseling were gathered from the patients' files after the patients' consent. A physical examination was conducted by a gynecologist/urologist. A standardized datasheet of clinical findings was completed for each patient. Hormonal and metabolic investigations were carried out, as well as ultrasonography of testes and ovaries and semen analysis for some patients. Psychosexual development and psychosocial adaption, health related quality of life and psychological well-being were evaluated by questionnaires answered by the patients online. The data were entered anonymized into the dsd-Life database. Here we present the preliminary results on the reproductive status and outcome, as well as satisfaction with care in patients with DSD from 6 European countries.

MIT11

Open Epididymal Sperm Aspiration (OESA): results of a twelve-year experience

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Background and aim: Obstructive azoospermia accounts for 40% percent of all azoospermia cases and 2% of all infertility cases in the Western world. Before the development of intracytoplasmic sperm injection, men suffering from obstructive azoospermia were unable to become genetically own fathers. Since then, several surgical procedures for the retrieval of spermatozoa exist. After various negative results with percutaneous epididymal sperm aspiration (PESA) in patients we on beforehand expected to have good results (e.g. proven fertile men after vasectomy or failed vasovasostomy), we decided to perform open epididymal sperm aspiration (OESA) at our facility. In this research paper, we report sperm retrieval rates and reproductive outcomes of OESA in our department.

Methods: We retrospectively reviewed the clinical records of 435 men undergoing OESA at a tertiary referral center between 2003 and 2015 and the clinical fertility records of their female partners. Characteristics obtained included age, endocrine profile, oocyte fertilization rate, no. of embryo transfers, no. of children born, gestational age, birthweight and method of birth. Patients were grouped according to obstruction cause; congenital bilateral absence of the vas deferens (CBAVD), previous vasectomy or bilateral epididymitis. The other/unknown group constituted of patients of whom the obstructive nature of the azoospermia was less clear, based on FSH levels and testis volume. We expected the far lowest success rate in this group. Sperm was retrieved by OESA. In short, the skin, tunica dartos and tunica vaginalis are incised in theater and the epididymis is mobilized. Then, using a 25 gauge butterfly needle, spermatozoa are aspirated. If we expected to use both epididymides, a scrotal midline incision was used. Some men underwent more than one OESA procedure. Retrieval rates, intracytoplasmic sperm injection results and neonatal outcomes were compared on univariate analysis.

Results: 459 OESA procedures were performed on 435 men. The cause of obstruction was CBAVD in 59 procedures, previous vasectomy in 184 cases, bilateral epididymitis in 20 cases, and other or unknown in 196 cases. Overall, sperm retrieval was successful in 244 procedures (53.2%). Sperm retrieval was lowest in the 'other or unknown diagnosis' group (23.3%). Men with a clear diagnosis of CBAVD, previous vasectomy or bilateral epididymitis had retrieval rates of 74.6%, 77.2% and 70.0%, respectively. FSH was significantly higher in the 'other or unknown diagnosis' group (9.49 IU/L $\pm$ 8.49 vs. overall average 7.30 IU/L $\pm$ 6.57) and mean combined testis volume was significantly lower (34.8 mL $\pm$ 12.12 vs. overall average 40.3 mL $\pm$ 14.34). On average, each woman had 5.0 ($\pm$4.36) embryos transferred. Of all embryo transfers, 70.8% resulted in live birth. A total of 170 children were born. On univariate analysis, no significant correlation between cause of obstruction and intracytoplasmic sperm injection outcome or neonatal outcome was proven.

Conclusion: OESA is a very reliable method for sperm retrieval in men suffering from obstructive azoospermia, provided the diagnosis is firmly established. Reproductive outcomes are not related to the cause of obstruction.

MIT12

Embolization of clinical varicocele: long term effects on semen quality, complication rates and satisfaction

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Background: Varicocele is among the most common causes of male infertility. The treatment of varicocele by

percutaneous embolization of the internal spermatic vein is a safe and effective minimally invasive procedure with very low morbidity, low complication rates and high long-term success rates. Embolization is an effective alternative to surgery and may be offered as primary therapy for varicocele treatment in subfertile patients.

Aim: To assess the long term improvement of semen parameters, complications and overall satisfaction of the patients after percutaneous embolization of varicocele.

Methods: Prospective evaluation of a population of 320 infertile men, followed in the Department of Reproductive Medicine of Coimbra Universitary Centre, who were submitted to percutaneous embolization of clinical varicocele between January of 2007 and March of 2016. Sperm concentration, mobility and morphology were evaluated before and at 3, 6 and 12 months after the embolization. The complications and overall patient satisfaction was also assessed. Statistical analysis was performed using IBM SPSS V.22.0.

Results: Sperm concentration improved from 11.1 ± 1.2x106/mL before the procedure to 19.9 ± 1.9x106/mL, 16.9 ± 3.1x106/mL and 23.0 ± 4.0x106/mL at 3, 6 and 12 months respectively ($p < 0.01$). Progressive mobility was initially 29.0 ± 1.8%. After the embolization progressive mobility improved to 33.8 ± 1.8% ($p = 0.041$), 35.4 ± 4.0% ($p > 0.05$) and 28.8 ± 3.6% ($p > 0.05$) at 3, 6 and 12 months. Normal morphology was 4.1 ± 0.4%, 4.6 ± 0.4%, 3.4 ± 0.6% and 3.5 ± 1.0%, before, at 3, 6 and 12 months after the procedure, respectively ($p > 0.05$).

Three months after the embolization there was an improvement in at least one of the semen parameters in 64% of patients, with 16% presenting an improvement in all of the considered semen parameters. We found no relation between the degree of improvement and the degree of the varicocele and whether it was a primary or secondary infertility. The overall complication rate was 5%, with post-operative infections and pain being the most frequent complaints (46% and 39% respectively). When questioned, 90% of the patients would perform the embolization again.

Conclusion: The percutaneous embolization of clinical varicocele in infertile men is clearly associated with an improvement of the sperm parameters, which is maintained in the long term, especially sperm concentration. Furthermore the complication rate is low, and most patients would repeat the procedure, if necessary.

MIT13

Spontaneous pregnancy and delivery rates after embolization of clinical varicocele in subfertil couples

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Background: Varicocele is a well-recognized cause of decreased testicular function and occurs in approximately 15–20% of all males and in 40% of infertile males, being the most common cause of male infertility. Although its correction can improve abnormal semen parameters, it is still debatable if it improves the odds of spontaneous pregnancy.

Aim: To evaluate the spontaneous pregnancy rate, the need to resort to medically assisted reproduction techniques and the birth rate in a population of infertile patients who were submitted to percutaneous embolization of clinical varicocele.

Methods: Prospective evaluation of a population of 320 infertile men, followed in the Department of Reproductive Medicine of Coimbra Universitary Centre, who were submitted to percutaneous embolization of clinical varicocele between January of 2007 and March of 2016. The need of assisted reproduction techniques, spontaneous pregnancy and birth rates were recorded and evaluated. Statistical analysis was performed using IBM SPSS V.22.0.

Results: After performance of the varicocele percutaneous embolization, and without accounting for the semen parameters, 42% of the couples resorted to assisted reproduction techniques. The overall pregnancy rate was 47% (52.4% were spontaneous and 47.6% occurred after assisted reproduction techniques). On average, the first pregnancy occurred 17.54 ± 14.4 months after the percutaneous procedure. 55.7% of the pregnancies occurred in the first 12 months after the treatment. The time between embolization and spontaneous pregnancy or pregnancy after assisted reproduction techniques is not significantly different ($p > 0.05$). Of these pregnancies, 83% resulted in delivery, mostly of a single newborn (81%). The patients that had higher mobility rates before the procedure had higher odds of pregnancy ($p = 0.031$). Couples with men with higher sperm concentration after the embolization had also higher pregnancy rates ($p = 0.012$).

Conclusions: The improvement of semen parameters after percutaneous embolization of clinical varicocele in a population of infertile men is correlated with higher odds of pregnancy after a short period of time (average <18 months). A significant portion of the couples were able to achieve a spontaneous pregnancy.

MIT14

Homozygote G-allels in FSHB promoter polymorphism 211G<T predicts successful testicular sperm extraction (TESE)

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A correlation between the FSH promoter polymorphism 211G>T (rs10835638) and male reproductive parameters is well documented. Significant correlations between the frequency of the T-allele and reduced serum FSH and testosterone levels, increased LH levels and reduced testicular volumes have been previously described, even in patients with Klinefelters syndrome (Busch et al. 2015).

Aim of this study was to analyze the relevance of the FSB promotor polymorphism in azoospermic patients in respect to TESE outcome.

We retrospectively analyzed 1025 patients who underwent TESE in our Center between 02/1997 until 10/2014 including obstructive and non-obstructive azoospermic patients. We determined preoperative hormones (LH, FSH, T), testicular volumes, histology and sperm retrieval rates and included these variables in binomial regression models.
Patients with GG-alleles had a significantly higher probability for successful sperm retrieval in the total cohort of patients (p-value: 0.035).
Further predictive factors have been identified (p-value: 0.001) (age of the patient, testicular volume, serum FSH, serum testosterone, and serum LH).
The previously described significant correlation between the number of the T-allels and reduced serum FSH could not be confirmed in azoospermic men.

MIT15

The wild African potato (*Hypoxis hemerocallidea*) supplementation on streptozotocin-induced diabetic Wistar rats ameliorates reproductive function

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Background: Diabetes mellitus (DM) has been reported as one of the greatest global public health threats and its prevalence is rapidly increasing. Hyperglycaemia has been linked to the overproduction of reactive oxygen species (ROS), also known as free radicals, consequently leading to oxidative stress (OS) that can impair the male reproductive function. DM can be treated pharmacologically with hypoglycaemic agents; however a reduced response as well as toxic side effects have been observed after prolonged use of these agents and have led to a search for chemicals of phytochemical origin. Several compounds have been extracted from *H. hemerocallidea* which may contribute to its therapeutic abilities.
Aim: The present study aims to observe whether the *H. hemerocallidea* can inhibit the destructive effects of oxidation and prevent damage to the reproductive system of diabetic male Wistar rats.
Methods: Sixty adult male Wistar rats (230–260 g) were randomly divided into 5 groups: normal, diabetic, diabetic + 800 mg/kg *Hypoxis*, diabetes+200 mg/kg *H. hemerocallidea (Hypoxis)* and non-diabetic + 800 mg/kg *Hypoxis*. Diabetes was induced with a single intraperitoneal injection of streptozotocin (STZ) and monitored during the study period.
Results: Blood glucose levels showed a significant increase ($p < 0.05$) 3 days after diabetes was induced using STZ. Blood samples, testes and epididymis were collected on the day of sacrifice. Testicular and epididymal lipid peroxidation (LPO) also showed a significant increase ($p < 0.05$) when diabetic control was compared to normal control. Body weights, epididymis and testicular weights, sperm motility and morphology, superoxide dismutase, catalase, total glutathione, total antioxidant capacity, testosterone and estradiol levels ($p < 0.05$) decreased in the diabetic Wistar rats. After 6 weeks administration of *H. hemerocallidea* there was a significant improvement in the above parameters in diabetic Wistar rats ($p < 0.05$). In addition, the non-diabetic + 800 mg/kg *Hypoxis* group showed improvement in some sperm motility parameters, epididymal GSHt, testosterone and estradiol levels when compared with the normal control group ($p < 0.05$).
Conclusion: These results indicated that *H. hemerocallidea* supplementation is an effective approach to ameliorate male infertility in diabetic rats.

MIT16

How successful is TESE-ICSI in non-mosaic 47,XXY syndrome? Cumulative delivery rates in an unselected consecutive patient cohort

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Background: Treating infertility in azoospermic 47,XXY men by TESE-ICSI (testicular sperm extraction and intracytoplasmic sperm injection) has become a routine procedure. In contrast to data on retrieval rates after TESE, reports on the reproductive outcome of TESE eventually followed by ICSI is scarce. Data available in the literature are limited to either sperm retrieval rates after TESE or outcome of ICSI once testicular sperm has been obtained, often in a selected subpopulation. Therefore, useful data for counselling azoospermic 47,XXY on their chances to become a biological father are still lacking.
Aim: This large retrospective consecutive cohort study aims to provide reliable data for counselling couples infertile because of azoospermia due to non-mosaic 47,XXY syndrome.
Methods: We identified all non-mosaic 47,XXY azoospermic patients having their first testicular biopsy in our centre between 1994 and 2013. Only patients having a female partner were included. Patients were followed longitudinally during TESE and consecutive ICSI cycles. The crude cumulative live birth delivery rate was calculated, as per ICSI cycle with testicular sperm (fresh and/or frozen) available for injection. Sperm retrieval rate and pregnancy rate were secondary outcome measures.
Results: Forty-eight (48) out of 138 azoospermic men with non-mosaic 47,XXY had sperm retrieved at their first TESE (34.8%). Of these 48 men, 4 did not start ICSI treatment. Five (5) couples dropped out during treatment because of sperm being unavailable after thawing on the moment of the first ICSI cycle and back-up fresh TESE was negative. In total, 39 couples had 62 ICSI cycles and 12 frozen embryo transfer cycles resulting in 20 pregnancies and 14 live birth deliveries (16 children). Cumulative pregnancy rate and delivery rate were 51.3% and 35.9% respectively. The live birth delivery rate for all patients included in this unselected azoospermic non-mosaic 47,XXY population was 10.1% per patient (14/138).
Conclusion: TESE-ICSI is a major breakthrough in the treatment of infertility in 47,XXY men with almost 4 out of 10 couples (35.9%) undergoing ICSI obtained a delivery when testicular sperm was available for injection. However, prior to undergoing TESE, patients should be counseled that eventually only 1 out of 10 azoospermic 47,XXY

men (10.1%) will father a genetically proper child when no selection criteria for intake are applied.

MIT17

Response to Clomiphene Citrate treatment in idiopathic oligozoospermia according to single nucleotide polymorphism of the FSH receptor

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Background and Aim: Clomiphene Citrate (CC) has been used to treat idiopathic oligozoospermia for the last four decades. However, until now it is not clear which patients will increase sperm concentration and which will remain unchanged. The objective of this study is to evaluate if the response to a daily treatment with CC to increase sperm concentration is related to Single Nucleotide Polymorphism (SNPs) of the FSH receptor (FSHr); specifically, to one of the most prevalent SNPs described for the FSHr, in exon 10; position 307 and position 680.

Methods: Thirty-six patients with idiopathic oligozoospermia (<15 mill/mL) in at least two semen analyses and normal FSH values were prospectively recruited. Patients with azoospermia and criptozoospermia were excluded. All patients received CC 50 mgs daily for 3 months. SNPs of the FSHr in position 307 (Thr/Thr – Ala/Thr – Ala/Ala) and 680 (Ser/Ser – Ser/Asn – Asn/Asn) were measured using Real Time PCR in DNA obtained from leukocytes isolated from peripheral blood. After three months all patients underwent two semen analyses.

Results: Globally after a 3-month treatment with CC, 55.6% of the patients increased Total Sperm Count (TSC). The mean increase was 83.6 million (range 2.1–381 millions); 22.2% percent of the patients decreased TSC (mean 7.5 million; range 3.0–9.5), and 22.2% of the patients had no significant changes (<2 million difference) in TSC. Regarding SNPs of the FSHr in position 307/680; in the sub-analysis of the nine possible combinations we found no statistically significant association demonstrating that a specific combination could predict an increase or decrease of TSC after CC treatment. However, in the group of patients who increased TSC (20/36), subjects with at least one Serine in position 680 had an statistically significantly higher increase compared to patients with no Serine in position 680 (112.7 ± 125.5 vs. 15.5 ± 8.6 million, $p < 0.05$, t test).

Conclusion: No specific SNPs combination of the FSHr in position 307/680 demonstrated to predict an increase or decrease in TSC after CC treatment. In the 55.6% of patients where an increase was observed, the presence of at least one serine in position 680 was associated with an statistically significantly higher increase compared to patients with no Serine in position 680. This finding has to be confirmed in further studies in order to establish a clinically significant association.

MIT18

The effect of Nigella sativa oil and Metformin on weight and male reproductive function in Wistar rats fed an obesogenic diet

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Background: *Nigella sativa* (Ns) is an annual plant native to the Middle East. Medicinally, oils extracted from the seeds show evidence indicating Ns may be as effective in obesity management in rats and humans, and may be as effective as metformin. Obesity is associated with poor reproductive parameters in males, and increased weight of reproductive organs.

Aim: The aim of the study was to investigate the impact of Ns, alongside metformin, on male reproductive organ weights and semen parameters compared to sham treatment in rats fed an obesogenic diet.

Methods: A total of 36 adult wild-type male Wistar rats ($n = 9$ per group) were fed a diet composed of 33% normal rat chow, 33% sucrose and 7% sugar dissolved in 27% water for 14 weeks. Intervention was force fed for the last 8 weeks of the 14 week experiments. This included a saline, metformin (75 mg/kg/day), low dose Ns (200 mg/kg/day) (NS200) and high dose Ns (400 mg/kg/day) (NS400) group. An additional 18 rats were either fed normal rat chow ($n = 9$) or the obesogenic diet with no intervention ($n = 9$) in order to control the impact of the diet on the parameters. Following euthanasia via cervical dislocation, total body weight, omentum weight, reproductive organ weights (prostate; testes; epididymis) and semen analysis (sperm concentration, motility, vitality and mitochondrial membrane potential (MMP)) was done.

Results: As expected, there were significant increases in all organ weights of the dietary induced obese rats (and saline group) compared to the normal rat chow group as expected. The obesogenic model also showed a significant decrease in sperm concentration, progressive motility, and an increase in static motility, compared to the normal chow group.

Experimental groups compared to the saline group showed that metformin, NS200 and NS400 significantly reduced total body weight, as well as prostate and epididymis weights. Although omentum weight was decreased in all experimental groups, only NS200 was significant. Testes weight was also decreased in all groups; however, this was significant for metformin and NS400 only. All treatments caused an increase is sperm concentration; however, this was significant only for metformin. After retrospective statistical sampling for the comparison between normal chow/metformin for the sperm concentration, the difference will be significant if 18 and 21 animals, respectively, would have been treated. Since this is a reasonable number, one can argue that metformin even increases the sperm count compared to the normal chow group. No significant changes for sperm motility (progressive; non-progressive; static) or vitality were observed in any group. Percentage of sperm with damaged MMP was significantly lowered in the metformin and NS400 groups, and non-significantly lowered in the NS200 group.

Conclusion: Ns is associated with weight loss and decreased omentum and reproductive organ weights,

comparative to metformin, in obese male rats, however, this had limited effect on sperm parameters in the experimental groups, with only sperm concentration notably increased and a positive impact on MMP. Metformin specifically may have an important role in treatment of male reproductive dysfunction associated with obesity.

MIT19

Electroejaculation performed in patients with spinal cord injuries: a single-center 21-year experience

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Background: During the last decades, electroejaculation (EEJ) has become an acceptable form of treatment for patients suffering from anejaculation as a result of a spinal cord injury (SCI). Although most EEJ procedures result in ejaculation, not all ejaculates contain (progressively motile) spermatozoa. When a semen sample yielded after a first EEJ does not contain progressively motile spermatozoa, a second EEJ can be considered. However, there is no consensus on how many attempts of EEJs should be done before proceeding to surgical sperm retrieval.
Aim: To evaluate treatment results of EEJ in 47 patients with SCI. To establish a possible additional value of repeated EEJ in patients when progressively motile spermatozoa could not be yielded during a first EEJ.
Methods: A retrospective chart analysis was performed of all SCI patients treated with EEJ at our center from January 1994 to January 2015. Data were collected on the patients' general medical history, hormonal profiles and ultrasonographic examination of scrotum and prostate. All patients underwent EEJ according to Seager. We evaluated sperm quality according to WHO-standards, pregnancy and delivery rates. Furthermore, we investigated the effect of repeated EEJ in patients in whom the first EEJ procedure had failed.
Results: A total of 230 EEJ procedures were performed in 47 patients. In 227/230 EEJs (98.7%) an ejaculate could be obtained. In 169/230 (73.5%) EEJ procedures it was possible to yield semen containing progressively motile spermatozoa. Median total motile sperm count was 4.6×10^6 progressively motile spermatozoa (IQR 1.9–19.7 $\times$ 10^6). In 18/47 (38%) of patients, no semen of sufficient quality could be yielded during the first EEJ; after performing one or more procedures, live spermatozoa were obtained in an additional seven patients. Procreation was attempted via IVF/ICSI in 17 patients; of these, 14/17 (82.4%) couples achieved pregnancy. In total, 15 healthy children were born.
Conclusion: In the majority of these patients (77%) treated with EEJ it was possible to obtain semen of sufficient quality to be used in artificial reproductive technologies. When the first EEJ procedure does not result in semen containing progressively motile spermatozoa, performing a second EEJ should be considered before proceeding to surgical sperm retrieval.

MIT20

M-TESE: testicular biopsy in men with nonobstructive azoospermia – our experience 2012–2016

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Background: Schlegel (1999) first reported testicular biopsy with the use of the operating microscope – M-TESE (Microsurgical Testicular Sperm Extraction). This technique allowed incorporated into the assisted reproduction IVF-ICSI (In Vitro Fertilization-Intracytoplasmic Sperm Injection) the next group of men with azoospermia, with previous negative biopsies in the past. The authors have entered this technique successfully for the first time in Poland in October 2012.
Aim: Analysis of the effectiveness of the M-TESE testicular biopsy – getting sperm to the IVF-ICSI procedure.
Methods: M-TESE biopsies were performed among the high selected, nonhomogeneous group of 108 men with nonobstructive azoospermia; age 17–44 (mean 32 years). For 14 men M-TESE was the first biopsy (11 – Klinefelter syndrome 47 XXY; 2 – DSD 46,XY; 1-post-TURED), for 89 – second, for 5-third. All patients before biopsy had hormonal stimulation of spermatogenesis (6–12 weeks): androgen + antiestrogen (Adamopoulos,1993) with the addition of vasodilator and trace elements with vitamins. The levels of LH, FSH and testosterone in the blood serum were controlled during therapy. All M-TESE procedures were performed under general anesthesia, as the day-case-surgery. The operating microscopes Seiler Evolution XR6 (94), Carl Zeiss S7 (6), Leica M860 2 $\times$ 2 (8) were used (magnification 20–25$\times$). From both gonads the testicular tissue were collected from 3 levels (Weidner, 2012). The current evaluation with following conservation at liquid nitrogen (−196 °C) for the future IVF were done immediately after surgery and the routine histological study (conservation in Bouin's fluid, Johnsen score) in each case was done.
Results: Sperm were found in 28/95 patients (29.5%), no sperm were noticed in subgroups: 47,XXY (11) and DSD 46,XY (2). 37 IVF-ICSI procedures have been done, 16 pregnancy and 6 miscarriage were noticed. Until then, 6 children were born.
Conclusion: M-TESE, testicular biopsy using the operating microscope, increases the chance to find sperm in men with nonobstructive azoospermia and finally allows incorporated them into the reproduction protocol – IVF-ICSI.

MIT21

Analysis of sperm parameters, pregnancy rate and complications after varicocelectomy subinguinal microsurgery in infertile men with varicocele visible or palpable

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Introduction: A varicocele is an abnormal enlargement of the pampiniform venous plexus. About 10–16% of male population is affected by this pathology. This frequency increases to 25–44% in primary infertile men and it can reach to 35–85% when the infertility is secondary. Surgical treatment is widely used but results remains controversial.
Objectives: To evaluate the effects of varicocelectomy analyzing the (i) semen quality before surgery and at 3 and 6 months after intervention (ii) relationship between age at the time of surgery, clinical grade of varicocele and semen parameters (iii) pregnancy rate after 1 year of varicocelectomy (iv) post-surgical complications.
Material and Methods: The study includes a cohort of 144 infertile patients with visible or palpable varicocele that underwent to subinguinal microsurgery varicocelectomy recruited between January 2010 and December 2014 at the Fundacio Puigvert. Couples of these men A stadndard seminograme was performed before surgery (T0) and at 3 (T1) and 6 (T2) months after intervention. Moreover seminograms were compared among patients depending on their age (=35 or >35 years old) and varicocele grade (I,II or III). Finally pregnancy rate was assessed after one year of surgery besides post-surgical complications.
Results: A general improvement of sperm parameters was observed among different spermiograms. Specially, a significant increased sperm concentration was identified being at $13{,}7x10^6$/ml at T0, 21.21×10^6/mL at T1 and 14.62×10^6/mL at T2 ($p < 0.05$). Regarding sperm motility a significant increase of progressive sperm motility was observed in the cohort, 24.2% at T0, 28.9% at T1 and 29.1% at T2 ($p < 0.01$). When the group of patients was divided in two depending on the age (=35 or >35 years old) and sperm parameters were analysed, a significant improvement of sperm parameters was observed in young patients ($p = 0.01$), however no significance was observed in the oldest patients. Concerning to the varicocele grade no conclusive results were obtained, no differences among varicocele grade and sperm parameters improvement were associated. With respect to pregnancy rate a 35.5% of couples become pregnant. Finally regarding to post-surgical complications they were extreamly rare; only a 5 of 144 operated patients suffer infections and 2 presented inguinoescrotal pain. None of the patients presented bruising or hydrocele after surgery.
Conclusions: The microsurgical varicocelectomy subinguinal improves sperm parameters (sperm concentration and motility) especially in those patients under 35 years independently of varicocele grade. The most interesting value concerns to pregnancy rate, in this study are reported that 35.5% of women (who have no fertility problems) get pregnant at the frist year after surgery. Moreover patients operated with microsurgery varicocelectomy at the Fundacio Puigvert presented very low incidence of the normal complications after this kind of intervention.

MIT22

Testicular sperm extraction in patients with testicular tumors and azoospermia or cryptozoospermia

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Background: Oncological therapies in patients affected by testicular neoplasias may adversely affect spermatogenesis and can lead to severe cryptozoospermia or even azoospermia. Even before therapy about 15% of such patients are azoospermic. In addition, putative effects of such therapies on Leydig Cell function have to be considered as primary hypogonadism may result. Regeneration of both testicular functions after oncological intervention has still to be elucidated. Cryopreservation of sperm in oncological patients is an option via testicular sperm extraction (mTESE), followed by assisted reproduction (e.g. ICSI).
Methods: A retrospective study design including a total of 116 patients with testicular neoplasia presenting with either azoospermia or cryptozoospermia and intented fertility preservation by mTESE prior to or after the end of oncological therapy (chemo- or radiotherapy). Age of patients at the time of diagnosis and at the time of mTESE, hormone profiles, testicular volumes, histology and outcome of mTESE were investigated in relation to oncological diagnosis and treatment.
Results: In 68 patients (58%) spermatozoa for cryopreservation were retrieved. The histological Bergmann-Kliesch score (BKS) was higher in patients with sperm following a U-shaped curve in relation to the interval between oncological therapy and mTESE ($p < 0.001$). BKS within the interval of 3 years after oncological therapy did not surpass 0.6, indicating severe damage of spermatogenesis. Non-linear binomial regression models revealed that mTESE should be either performed directly before begin of oncological therapy with a success rate of 56% or > 3 years after therapy with a success rate of 68% (p for overall model = 0.004). The type of tumor did not not predict mTESE results, but patients diagnosed with seminoma had a higher chance to develop Leydig Cell damage in comparison to patients diagnosed with non-seminoma (decreased total testosterone concentrations [$p = 0.009$] and increased LH levels [$p = 0.035$]).
Conclusions: Patients presenting with testicular tumors and severely impaired spermatogenesis maintain a chance to preserve fertility by cryopreservation of sperm retrieved by mTESE, depending on the time point of intervention. A likelihood of Leydig Cell impairment exists in relation to the tumor type and sex steroid profiles have to be monitored during follow-up.

MIT23

Sperm retrieval in subjects with Klinefelter syndrome: results from a meta-analysis study

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Background: Specific factors underlying successful surgical sperm retrieval rate (SRR) or pregnancy rate (PR) after testicular sperm extraction (TESE) in adult patients with Klinefelter syndrome (KS) are not completely clarified.
Aim: To meta-analyze currently available data regarding SRR in subject with KS.In addition, when available, PR and live birth rate (LBR) after intracytoplasmatic sperm injection (ICSI) technique have been also investigated.
Methods: All trials reporting SRR conventional-TESE (cTESE) or micro-TESE (mTESE) and its specific determinants without any arbitrary restriction were included. The identification of relevant studies was performed independently by three of the authors (F.L, A.P. and A.G), and conflicts were resolved by the fourth investigator (G.C.).
Results: Overall, 29 trials were included in the study enrolling 685 patients with a mean age of 30.2 ± 5.9 years. All studies, except 4 included non-mosaic KS. The majority of the studies (n = 19) applied mTESE, 8 cTESE and in one case testicular sperm aspiration (TESA) was used. Finally, one study used a mixed approach. Among the studies included, information on fertility outcome after ICSI were available for 15 trials. Overall, a SRR of 47[43–51] % was detected. Data were confirmed in sensitivity analysis when trials enrolling mosaic KS subjects were excluded from the analysis. In addition, similar results were observed when mTESE was compared to cTESE, (SRR 46[39;52] % vs. 50 [45;55] % for cTESE vs. micro-TESE, respectively; Q = 1.23, p = 0.27). Meta-regression analysis showed that none of the parameters tested, including age, testis volume as well as FSH, LH and testosterone (T) levels at enrolment, affects final SRR. Similarly, no difference was observed when a bilateral procedure was compared to a unilateral approach. However, a multiple biopsy approach was associated with a significant higher SRR when compared to a single biopsy method. Similar results were observed when only bilateral surgical approach was considered. No sufficient data were available to evaluate the effect of previous T treatment on SRR. Overall a total of 130 biochemical pregnancies after 269 ICSI cycles were observed (PR = 48 [40;56] %). Similar results were observed when LBR was analyzed. Similar to what observed for SRR no influence of KS age, mean testis volume, FSH and total T levels on both PR and LBR were observed. No sufficient data were available to test the effect of women age or other women fertility problems on PR and LBR. Finally, no difference in PR or LBR was observed when the use of fresh sperms was compared to the utilization of cryopreserved ones.
Conclusions: Present preliminary data suggest that performing TESE/micro-TESE in subjects with KS provide a SRR, PR or LBR of about 50% independent of any clinical or biochemical parameters tested.

PS2A3 – POSTER SESSION NR.2

POSTER PRESENTATION

TC01

Immune privilege and neoplasia in human testis: potential role and functional polarization of macrophages and dendritic cells*

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Introduction: Human testicular germ cell tumours, i.e. seminoma, and pre-invasive germ cell neoplasia in situ (GCNIS) are accompanied by infiltrating immune cells, previously identified as lymphocytes (T, B), macrophages and dendritic cells. Recent studies provide suggestive evidence that functional polarization and respective subtypes of macrophages (e.g. TAM, M1 and M2) play an important role in cancer development and surveillance. Also for dendritic cells, known as 'professional' antigen presenting cells, functionally different subsets (pDC, mDC) have been described. For both cell types and their subsets, however, putative immunopathological roles in testicular neoplasia remain to be elucidated. Therefore, we set out to identify and characterize subsets of macrophages as well as DC in seminoma and GCNIS in comparison to non-inflamed testis with intact spermatogenesis (Nsp).
Material and Methods: Bouin-fixed, paraffin-embedded tissue samples from human testis (seminoma n = 10; GCNIS n = 10; Nsp n = 5). The following markers were used for immunohistochemistry (IHC) and immunofluorescence (IF): macrophages M1: CD68; M2: CD163, CD206; dendritic cells mDC1: CD1c, CD11c; mDC2: CD11c, CD141; pDC: CD123, CD303, CD304 and S100 (DC). Preliminary measurements of transcripts encoding relevant cytokines were made using quantitative RT-PCR after RNA extraction from cryopreserved tissue samples (seminoma, GCNIS) and cDNA synthesis.
Results: In seminoma and GCNIS samples, IHC revealed positive staining with 7 DC-related markers tested, thus indicating presence of DC. Moreover, positive results were obtained for both M1- and M2-related markers. In ongoing experiments, IF double-staining is employed to further characterize extent and spatial distribution of infiltrating DC and macrophage subsets. In comparison, significantly lower numbers of macrophages and only isolated DC were observed in normal testis. Additional analysis of cytokine gene expression profiles using qRT-PCR reflects the interaction of different immune cells and functional polarization of macrophages and DC associated with testicular neoplasia.
Conclusion: Detailed functional characterization of infiltrating immune cells in testicular neoplasia, i.e. macrophages and DC, will help to understand the complex mechanisms of 'immune editing' during testis cancer development.

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PS2B1 – POSTER SESSION NR.2
POSTER PRESENTATIONS

RE01

Marital status and serum levels of PSA & testosterone in healthy men

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Background: The importance of marital status as a predictive factor of ill health has been subjected to extensive analysis in the general health literature.
Aim: The present study aims to investigate the impact of marital status on serum levels of testosterone and PSA based on data from 119 healthy men from the general population.
Methods: Testosterone and PSA levels were measured. Data was also gathered on age, BMI, waist circumference, hip circumference, waist/hip ratio, marital status, smoking, and alcohol consumption. Men were classified into two groups based on the marital status, married and unmarried men.
Results: The mean age of the subjects was 55 years (range 46-60 years). Married men had significantly lower testosterone levels as compared to unmarried men (14 nmol/L vs. 19 nmol/L, $p = 0.01$). The same trend was found regarding PSA levels although statistically not significant (1.3 ug/L vs. 1.6 ug/L, $p = 0.40$). In a multivariate regression analysis model adjusted for the age of subjects; BMI; waist circumference, hip circumference, waist/hip ratio, smoking; alcohol consumption, a significant negative associations between marital status and testosterone ($\beta = -05.30$; 95 % CI = −8.50, −2.15; $p = 0.01$), and PSA levels ($\beta = -0.50$; 95 % CI = −1.04, −.05; $p = 0.03$) were found.
Conclusions: Marriage was negatively associated with PSA levels. The negative impact of marital status on PSA levels seems to be attributed to the concomitant variation in testosterone levels observed in this group of men.

RE02

Ultrastructural alterations in junctional complexes at the blood-testis barrier in the seminiferous tubule of adult rats following flutamide treatment

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Background: In the testis, Sertoli cells serve as supporting cells and reside as a basal epithelial lining within the seminiferous epithelium. They create a specialized microenvironment to support the germ cell development especially through the formation of the blood-testis barrier (BTB). Testosterone has been shown as a direct regulator of the BTB function and dynamics. It is well known that cell-cell junctions that form this barrier are required for adequate functioning of the testis. We hypothesize that limited exposure to an anti-androgen flutamide may have a direct impact on the junctional complexes at the BTB, which may precede an impact on the tissue histology.
Aim: To get a deeper insight into the action of flutamide on the morphology of the BTB, the organization of the basal ectoplasmic specialization (ES) and intercellular junctions residing in this region was studied at the ultrastructural level.
Methods: Flutamide (50 mg/kg body weight) was administered to male rats daily from 82 to 88 postnatal day. Testes from 90-day-old control and flutamide-exposed rats were used for all analyses. Testis morphology was analyzed using light and electron microscopy (EM). The fixation procedure for EM was modified to enhance the contrast of plasma membranes, allowing observation of ES even at the level of light microscopy. Proliferation and apoptosis were examined by Western blot and TUNEL assay, respectively.
Results: As revealed by eosin-haematoxylin staining both Sertoli and germ cells displayed normal morphology without any loss of germ cells irrespective of the group of rats. In accord, no difference in the apoptosis and proliferation level was found between control and treated groups. Examination of semi-thin and ultra-thin sections revealed that cell surface occupied by the basal ES connecting neighboring Sertoli cells and the number of gap and tight junctions coexisting with the basal ES were apparently reduced in flutamide-treated rats. Moreover, the appearance of unconventional circular ES suggested enhanced internalization and degradation of these atypical junctional complexes. Equally important, in our fixation procedure potassium ferrocyanide contrasted only 'open' intercellular spaces, it did not penetrate between membranes sealed by tight junctions. Thus, in control rats the electron-dense tracer penetrated between non-specialized membranes and was stopped at the basal border of the ES, whereas in flutamide-treated rats, at least some junctional complexes (ES and tight junctions) were permeable and penetrated by the tracer.
Conclusion: Short-term treatment with flutamide applied to adult rats exerts its effect on the basal ES and coexisting junctional complexes without any apparent morphological alterations in the seminiferous epithelium. Altogether, these observations suggest that the cell-cell junctional complexes of adult rat testis are a primary target for flutamide action.
This work was supported by a grant HARMONIA3 from the National Science Centre.

RE03

Connexin 43 and zonula occludens-1 as primary targets for anti-androgen action in rat testis

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Background: Evidence is accumulating that intercellular communication via connexin 43 (Cx43)-based gap junctions is crucial for male fertility by its involvement in the control of Sertoli cell and germ cell proliferation, differentiation and apoptosis. Connexin 43 also participates in the local regulation of other proteins that constitute junctional complexes at the blood-testis barrier (BTB), such as zonula occludens-1 (ZO-1). Molecular interactions between Cx43 and ZO-1 take places also in non-physiological conditions with involvement of alternative signaling pathway. Recent findings indicate that ZO-1 protein, recruit other signaling proteins into Cx43-based gap junction. Taking into account our previous studies showing affected Cx43 expression in primary rat Sertoli cells treated in vitro with an anti-androgen flutamide, herein we focused on the *Cx43* and ZO-1 expression as a potential targets for anti-androgen in adult rat testis.

Aim: Present study was undertaken to explore the expression of Cx43 and ZO-1 mRNA and proteins in rat testes following short in vivo exposure to flutamide.

Methods: Flutamide (50 mg/kg body weight) was administered to male rats daily from 82 to 88 postnatal day. Testes from 90-day-old control and flutamide-exposed rats were used for all analyses. According to our preliminary studies, such regime of flutamide treatment allowed to reduce androgen signaling but without the loss of germ cells. Gene and protein expressions were analyzed by real-time RT-PCR and Western blotting, respectively, protein distribution was detected by immunohistochemistry.

Results: In the testes of flutamide-treated rats, the expression levels of Cx43 mRNA and protein significantly increased ($p < 0.05$) compared with the control. As revealed by immunohistochemistry in hypertrophic Leydig cells within the interstitium of flutamide-exposed males the Cx43 signal was significantly higher ($p < 0.01$) than that of the control what was confirmed by the optical density quantitative measurement. In contrast, flutamide led to the reduction of the Cx43 signal intensity ($p < 0.05$) and to the loss of linear staining pattern at the region of the BTB, while signal reminded unchanged at the apical compartment the seminiferous epithelium. These changes were accompanied by decreased ZO-1 expression ($p < 0.01$) and delocalization of ZO-1 protein from Sertoli cell surface to the cytoplasm.

Conclusions: Altered distribution of Cx43 and ZO-1 and reduced expression of both proteins at the BTB region suggest that coexisting junctional complexes and their proteins are primary targets for flutamide action. In addition, differential effects of the anti-androgen on Cx43 expression in the interstitial tissue and seminiferous epithelium indicate diverse mechanisms of flutamide action in different cellular targets within the testis.

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RE04

Effects of long-term testosterone undecanoate (TU) therapy in hypogonadal men with osteoporosis: real-life data from a registry study

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Objective: To assess long-term effects of testosterone therapy (TTh) with injectable TU in hypogonadal men with osteoporosis in a urological setting in comparison to an untreated hypogonadal control group.

Methods: Cumulative registry study in 656 men with total testosterone (T) levels below 12.1 nmol/L and symptoms of hypogonadism. 96 men with osteoporosis were analysed. 63 received parenteral TU 1000 mg/12 weeks following an initial 6-week interval. 33 men had opted against T therapy (TTh) and served as controls (CTRL). Median follow-up was 7 years in CTRL and 6 years in the T group. Measurements were taken at least twice a year, and 8-year data are presented. Mean changes over time between the groups were compared by a mixed effects model for repeated measures with a random effect for intercept and fixed effects for time, group and their interaction. Changes were adjusted for age, weight, waist circumference, blood pressure, and lipids to account for baseline differences between the two groups.

Results: Mean age: 57.9 ± 8.8 years. 30 patients in the T group had Klinefelter's syndrome.

T-scores improved from -3.36 ± 0.57 in the T group to −1.08 ± 0.61 after 8 years and worsened in CTRL from −3.01 ± 0.3 to -3.48 ± 0.18. The model-adjusted estimated difference between groups at 8 years was +1.79 ($p < 0.0001$ for all).

All but 6 patients in the T group achieved the category "osteopenia" (T-score of-1 to -2.5). The 6 patients who were still osteoporotic (T-score -2.5 or lower) had a short treatment duration of 18-36 months. In CTRL, all 33 patients remained in the osteoporosis category. 4 major fractures occurred in CTRL, none in the T group.

Waist circumference progressively decreased from 101.6 ± 5.9 to 96.3 ± 4 cm ($p < 0.0001$) in the T group and from 109.3 ± 6.6 to 107.5 ± 9.1 cm ($p < 0.01$) in CTRL. The model-adjusted estimated difference between groups at 8 years was -11.1 cm ($p < 0.0001$). Weight decreased from 98.3 ± 13.4 to 82.1 ± 9 kg ($p < 0.0001$) in the T group and from 96.3 ± 8.9 to 95.2 ± 14.6 kg (NS) in CTRL. Difference between groups: -22.7 kg ($p < 0.0001$).

Weight change from baseline was −15.9 ± 7.1% ($p < 0.0001$) in the T group and + 0.4 ± 2.6% (NS) in CTRL. Difference between groups: -20.9% ($p < 0.0001$).No patient dropped out. There was 1 death in the T group (traffic accident) and 1 in CTRL. In CTRL, there were 3 myocardial infarctions and 8 strokes.

Conclusions: Long-term TTh with TU in an unselected cohort of hypogonadal men with osteoporosis resulted in sustained improvements in T-scores and weight loss. In untreated controls, T-scores worsened and no weight loss occurred. Long-term TU was well tolerated. Because TU

injections were administered in the doctor's office, medication adherence was 100 per cent.

RE05

Clinical importance and (no) consequences of assessement of dehydroepiandrosterone sulfate (DHEAS) in Lithuania (2014)

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Background: Clinical indications of DHEAS assessement are not well defined. Usually patients with suspicion of adrenal disease are referred for investigation of DHEAS.
Aim: To evaluate clinical importance and consequences of assessement of DHEAS in Lithuania (2014) for men and woman diagnostics.
Methods: We thoroughly inspected 552 outpatient cases (185 men and 367 women) taken from the laboratory investigation list. DHEAS was reported by DHEAS ratio (DHEASR), consisting of ratio DHEAS in patient/ DHEAS highest value indicated in assay kit according to patients age.
Results: Most frequent age for referral for DHEAS determination in men was 0–24 years (77%). In contrast between women the reproductive age dominated- 15–44 years (71%). DHEASR was statistically lower in men as compared with women in ages of 0–14 (0.45 and 0.77 respectively, $p < 0{,}01$), 15–24 (0.64 and 0.91 respectively, $p < 0{,}001$), 65+ (0.22 and 0.67 respectively, $p < 0.0001$) and totally (0.63 and 0.88 respectively, 0.001). DHEASR did not differ significantly between men and women in age of 25–34, 35–44, 45–54, 55–64 and totally 25–64 years. Comparison of different clinical and laboratory parameters in groups of patients with 'normal' DHEASR -less than M + 1SD – 320 women and 157 men, and high DHEASR – more than M + 1SD – 47 women and 28 men – was performed. In both men and women body weight and body mass index were higher in high DHEASR groups. Only in men statistically significant increase of body weight during past 1 year was detected. In men with high DHEASR higher blood pressure was detected. In women increase of frequency of hirsutism ($p < 0.032$) and acnea ($p < 0.022$) were statistically higher in high DHEASR group. Testosterone concentration both in men and women were statistically higher in high DHEASR group.
Frequency of adrenal computer tomography or/and magnetic resonance imaging was the same in normal and high DHEASR groups, but was significantly more frequent in sonography of upper abdomen region of patients with high DHEASR.
Conclusion: Referral for DHEAS assessment in Lithuania (2014) in men was because of overweight, fast increase of weight during 1 year and detection of high blood pressure, predominately in younger men (0–24). Women were referred for determination DHEAS later, during reproductive age 15–44 years and having more pronounced hirsutism and acnea.
Results of DHEAS investigation had no impact on doctors decision to increase number of adrenal imaging investigations.

RE06

Anti-Müllerian hormone in adult males – relation to reproductive characteristics

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Background: Anti-Müllerian hormone (AMH) is a member of the transforming growth factor (TGF) beta superfamily and plays a central role in the regression of the Müllerian ducts during fetal life. The serum levels of AMH in adult males are very low compared with those found in infants. The hormone is produced by Sertoli cells, and the levels are higher in seminal plasma than in serum. The role of AMH in the adult male is poorly understood.
Aim: To investigate if the levels of AMH in seminal plasma and serum are associated with semen characteristics and reproductive hormones.
Methods: Men ≥18 years were recruited from the general population by advertising (n = 94), and from couples with female factor infertility attending a fertility clinic (n = 32). Semen analysis was performed according to WHO recommendations, and AMH analysis was performed with the improved Beckman Coulter method (2013).
Results: No correlation was found between AMH levels in seminal plasma and serum (Spearman's ρ=0.11, $p = 0.19$). Total AMH (pmol/ejaculate) in seminal plasma was positively associated with sperm concentration (B = 0.18, $p < 0.001$), total sperm count (B = 0.21, $p < 0.001$), and progressive sperm motility (B = 6.8, $p = 0.001$), when adjusted for possible confounding factors. No association was found between serum AMH and semen characteristics. Serum levels of inhibin B were positively correlated with total AMH in seminal plasma (B = 18.5, $p < 0.001$) and concentration of AMH in serum (B = 0.51, $p < 0.001$). A negative correlation with FSH was observed for AMH in serum, but not for seminal plasma AMH. Large inter-individual differences in seminal levels of AMH were observed.
Conclusion: We found a positive association between seminal plasma AMH and semen characteristics, which indicates a role for AMH in spermatogenesis.

RE07

Testicular function during puberty and young adulthood in patients with Klinefelter syndrome with and without sperm in seminal fluid

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Background: Patients with Klinefelter syndrome (KFS) experience progressive testicular degeneration resulting in

impaired endocrine function and azoospermia. What proportion of adolescents develop testosterone deficiency during puberty and how many show sperm in their semen is unclear to date.

Aim: We aimed to investigate testicular function during puberty and young adulthood in patients with KFS and to assess testosterone effects in target tissues.

Patients and Methods: Clinical data of non-mosaic KFS patients (47,XXY) aged 10–25 years without previous testosterone replacement were reviewed. In a subset of late pubertal adolescents, semen analyses were evaluated and testicular volumes, serum levels of LH, FSH, testosterone (T), the androgen receptor CAG repeat polymorphism (GAGn) length, semen volumes, haemoglobin levels (Hb) and final height data compared to those of age-matched controls with pubertal gynaecomastia.

Results: A total of 281 KFS patients and 233 control subjects were included. Spontaneous pubertal virilisation to Tanner stages IV-V occurred and serum T levels ≥10 nmol/l were reached in 62% of KFS patients and in 85% of controls at ages 15-25 (TKFS: 12.2 ± 5.4 vs. TC: 16.6 ± 7.2 nmol/l). LHKFS-levels were elevated >10 U/l in 84%, and normal in all controls (LHKFS: 18.6 ± 12.2 vs. LHC: 3.5 ± 1.6 U/l). In 9/130 (7%) KFS adolescents, sperm (oligozoospermia) were found in semen; all had T levels >7 nmol/l and 8/9 had LH-levels ≤18 U/l, while their mean hormone levels, mean number of CAG repeats and mean testicular volumes were not different from those of KFS adolescents with azoospermia. Controls had normal sperm concentrations in 73% (46/63). Semen volumesKFS were normal in 55% vs. 78% in controls, HbKFS was normal in 89% (HbC: 97%). Mean final heightKFS was 185 ± 8 cm vs. 181 ± 7 cm in controls.

Conclusions: Hypergonadotropic hypogonadism develops during early puberty in adolescents with KFS and remains compensated in over 60% during ages 15–25, with sufficient testosterone secretion for spontaneous accomplishment of pubertal development. Sperm in semen is rare and associated with T levels >7 nmol/L. Parameters reflecting androgen deficiency in target tissues may help to optimize timing of testosterone substitution, which should preferably not be initiated before fertility status has been clarified.

RE08

Differential response of anabolic hormones, parameters of oxidative stress and vascular reactivity in patients treated by insulin pump vs. multiple daily injection

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Background: It is known that in type 1 Diabetes Mellitus (T1DM) continuous subcutaneous insulin infusion (CSII) therapy improves metabolic control and reduces risk of hypoglycemia in comparison with multiple daily injection (MDI). Glycated Hb (HbA_{1c}), body mass index (BMI) and inflammatory parameters are usually considered as metabolic outcomes. However few data are available on pituitary and gonadal hormone responses, which are involved in metabolic processes, and on oxidative stress (OS) and endothelial function parameters.

Aim: To gain insight these relationships during CSII or MDI treatments, we have evaluated IGF-1, dehydroepiandrosterone sulphate (DHEAS), luteinizing hormone (LH), sex hormone binding globulin (SHBG), testosterone (T) levels, total antioxidant capacity (TAC) and flow-mediated dilation (FMD) in a cohort of T1DM patients, comparing these two different kinds of intensive insulin administration.

Methods: We enrolled 33 male patients, aged 20–60 years, 21 were treated by MDI (group 1) and 12 by CSII (group 2). The groups were similar for age, BMI, duration of DM. IGF-1 was assayed by ECLIA (Electro-chemiluminescent immunoassay); LH, SHBG, T and DHEAS by CMIA (Chemiluminescent Microparticle ImmunoAssay). FMD was measured by the method of Celermejer. Total antioxidant capacity (TAC) was evaluated with a colorimetric method, using the system metamyoglobin-H_2O_2 and the chromogen ABTS; the latency time (LAG, sec) in the appearance of ABTS radical species is proportional to antioxidant content of the system.

Results: Despite similar glycemic control (mean±SD % HbA1c : 7.7 ± 0.9 in patients of group 1; 7.5 ± 0.5 in patients of group 2), we found a differential pattern of the following hormones: in group 1, compared to group 2, a trend toward higher IGF-1 levels (124.7 ± 40.3 vs 106.8 ± 46.7 ng/ml) and a significant difference in DHEAS levels (2943.42 ± 1310.54 vs 2035.4 ± 573.22 ng/ml, $p < 0,05$); LH levels (2.7 ± 1.1 vs 4.2 ± 1.1 mUI/ml) and T levels (7.1 ± 2.7 vs 9.8 ± 2.6 ng/ml) were significantly lower in the same group. FMD values were significantly lower in patients of group 1 than group 2 (3.6 ± 2.5 vs 7.7 ± 1.7 %), but inversely correlated with LAG in patients treated by MDI ($r^2 = 0,3$ and $p < 0,05$), suggesting higher OS than in the group treated by CSII.

Conclusion: These preliminary data seem to indicate a differential hormone response in patients treated by CSII or MDI, despite similar glycemic control, with higher levels of pituitary-testicular axis hormones. Moreover, lower OS and better vascular response were observed in patients treated by CSII. The relationships between antioxidant modulation and hormone responses remain to be further elucidated, together with their prognostic and therapeutic implications

RE09

Mast cells in human foetal testis

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Background: In the available literature there are no data about mast cells in the human foetal testis. The hypothesis of this study has been as follows: the number of mast cells increases with gestational age of the foetus, coinciding with the increase of testicular volume, sex cords and interstitium.

Aim: The aim of the study was to visualize mast cells within the parenchyma of the foetal testis by histological staining methods and to investigate the development of these cells during the intrauterine development of the testis and its structures.

Methods: After the approval of the appropriate Ethical Committee, tissue samples were retrieved from historical paraffin blocks archive of Dept. Histology and Embryology. Foetal testes were obtained during the routine paedopathological autopsy of 39 spontaneously aborted/stillborn foetuses between 15 and 36 gestational weeks. Volume of each testis has been determined prior fixation and tissue processing. Mast cells were detected by immunohistochemistry and quantitatively analysed by stereological methods. The diameter of the sex cords was measured by ocular micrometer, whereas the volume of the interstitium and cords (per each testis) by stereology.
Results: During gestational weeks 15–29, mast cells were exclusively located within the tunica albuginea. After 30th gestational week, in addition to tunica albuginea, mast cells populated the loose connective tissue of the tunica vasculosa and the connective tissue of septa/interstitium within the parenchyma. The total number of mast cells increased over the investigated period. Quantitative stereological analysis showed that the development of mast cells coincided with the increase of the volume of the testis, sex cords and the interstitium. In contrast to these results, diameter of the sex cords remained unchanged during the investigated gestation period.
Conclusion: The development of mast cells in the foetal testis is probably regulated by paracrine factors secreted by cells of the interstitium and sex cords.

RE10

Delayed reversibility in RISUG mediated vas occlusion in rabbits

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Background: Intravasal injection of RISUG produces instant contraception. Safety and efficacy of RISUG have also been successfully demonstrated in human during Phase I and Phase II clinical trials and the multicentric Phase III clinical trial is in progress in India. However, in order to make the procedure more acceptable than that of the traditional vasectomy, reversal of vas occlusion, have been attempted in animal models.
Aim: The present study aimed to evaluate the effect and mechanism underlying reversal of RISUG induced vas occlusion with DMSO and sodium bicarbonate (NaHCO3) in adult male rabbits.
Methods: Animals were grouped into seven groups ($n = 5$), viz., sham operated control, vas occlusion with RISUG (5–7 μL) for 90 days and 360 days and reversal with DMSO (250–500 μL) and 5% $NaHCO_3$ (500–700 μL). Success of vas occlusion and reversal was established by periodical semen analysis, fertility tests and toxicological investigations.
Results: Fortnightly semen analysis revealed that sperm count steadily declined after vas occlusion and complete azoospermia was attained between 30 and 60 days of post injection. Spermatozoa reappeared between 60 and 75 days of reversal and normospermia was noticed between 135 and 150 days of post reversal. All spermatozoa were found to be non-motile and a decline in the percentage of viable sperms during 15–45 days of post-injection. Sperm abnormalities like head-tail separation, damaged acrosome, bent midpiece, coiled tail and bent tail were recorded in vas occluded animals (15–30 days of post-injection) and those subjected to reversal (60–120 days post-reversal). Presence of large number of macrophages were observed engulfing spermatozoa in the seminal plasma of the reversal groups. A slow but gradual recovery in sperm motility, viability and abnormality was observed which normalized during 105–135 days of reversal. Animals subjected to intravasal administration of RISUG were found to be sterile during the vas occlusion period. With the reappearance of spermatozoa following vas occlusion reversal, a gradual recovery in the fertility was noticed. Complete fertility was observed following 135–150 days of reversal when compared with sham operated control group. F1 progeny of reversed animals was found to be normal. Other parameters remained unaltered during all phases of the study.
Conclusion: The present study suggests instant sterility and safety following vas occlusion with RISUG. This study indicated a delayed reversibility compared with the previous study on rats. However, no significant difference was observed in the duration of reversibility using both approaches.

RE11

Morphological, immunohistochemical, hormonal and metabolic evaluation of men prostate with benign prostatic hyperplasia and testosterone deficiency syndrome

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Background: Benign prostatic hyperplasia (BPH) is a commonly occurring disease among elderly men. It is characterized by the proliferation of epithelial and stromal cells, followed by enlargement of the gland. Androgens are essential for maintaining normal morphology and the function of the prostate. Testosterone and the product of its enzymatic transformation, dihydrotestosterone (DHT), undeniably take part in the growth and proliferation of prostate cells. A slight decrease in blood testosterone level in men is a physiological state associated with the aging. However, the decline in the level of testosterone observed in BPH, accompanied by coexisting metabolic disorders is a controversial issue.
Aim: The aim of our study was to evaluate the occurrence of hormone and metabolic disorders, as well as the immunolocalization and immunoexpression of androgen receptors (AR) and estrogen-alpha receptors (ERa) in the prostates of men with benign prostatic hyperplasia (BPH) and coexisting testosterone deficiency syndrome (TDS).
Methods: The study involved 150 men, diagnosed with and receiving pharmacological treatment for BPH. Concentrations of glucose, total cholesterol (TCh), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and triglycerides (TG) were determined in blood serum. Serum concentrations of total testosterone (TT), free testosterone (FT), estradiol (E2), luteinizing hormone (LH), insulin (I), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), and insulin-like

growth factor 1 (IGF-1) were measured by ELISA. The number of AR-positive cells and ERa-positive cells were measured in prostate sections of men with BPH.
Results: Patients eligible for transurethral resection of the prostate and TDS were significantly more likely to have higher abdominal circumference and higher serum levels of insulin and IGF-1 as well as lower levels of FT and SHBG than control subjects with BPH and no TDS. Quantitative analysis revealed 35.8% AR-positive columnar epithelial cells and 24.3% AR-positive stromal cells in prostates of BPH patients with TDS and 30.5% and 23.0%, respectively, in BPH patients without TDS. However, the differences between the study and the control groups were statistically not significant. In prostates of BPH patients with TDS the immunoexpression of ERa was observed in 2.88% of the columnar epithelial cells and 0.39% of stromal cells. In BPH patients without TDS ERa-positive cells were only found in 0.04% of columnar epithelial cells and 0.62% of prostatic stromal cells.
Conclusion: Determining the relationship between BPH and TSD, changes the levels of other hormones, metabolic disorders could have important therapeutic implications for the treatment of diseases of aging in men. Considering the higher levels of insulin and IGF-1 and larger abdominal circumference of men with BPH and TDS, it can be supposed that visceral obesity and carbohydrate disorders may contribute to the reduction of serum testosterone concentration.

RE12

Histo-morphometric evaluation of testicular parameters in streptozotocin-nicotinamide-induced diabetic rats under antiretroviral therapy: any role for *Hypoxis hemerocallidea*?

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Background: Wide spectrum of metabolic alterations associated with highly active antiretroviral therapy (HAART) has been reported over decades with sparse information on testicular morphological aberrations.
Aim: We investigated the role of *Hypoxis hemerocallidea* (*HH*) on histomorphological & morphometric changes in the testes of streptozotocin-nicotinamide-induced diabetic rats under HAART.
Methods: Eighty adult male Sprague-Durley rats (188.98 ± 4.5 g) were divided into non-diabetic (A–D) groups of 6 animals each and diabetic (E, F. . .K) groups of 8 animals each. Diabetes was induced by a single intraperitoneal injection of nicotinamide (110 mg/kg bw) followed by streptozotocin (45 mg/kg bw) and the animals were then subjected to various treatment with HAART, *HH*, melatonin. Body and testicular weight, histological, histochemical, seminal fluid and morphometric analyses were carried out after autopsy.
Results: Body and testicular weights reduced significantly ($p < 0.05$) in diabetic rats in comparison with control. Histomorphological and morphometric studies revealed reduction in diameter of seminiferous tubules and germinal epithelium height, increase in basement membrane thickness with disruption of spermatogenesis process in diabetic rats.
Conclusion: The use of *HH* or melatonin does not improve testicular damage in diabetic male rats under antiretroviral therapy.

RE13

Sex hormone-binding globulin (SHBG) inhibits androgen bioactivity: in vitro and in vivo evidence

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Background: Sex hormone-binding globulin (SHBG) is a high-affinity binding protein for androgens and estrogens. According to the 'free hormone hypothesis', SHBG regulates the free sex steroid fraction and restricts androgen bioactivity. SHBG has also been independently associated with diabetes, osteoporosis etc., but whether this represents causality or residual confounding remains unconfirmed.
Methods and results: We studied mice transgenically overexpressing human SHBG under a 4.3 kb promoter (mice normally lack circulating SHBG). Using multiligand liquid chromatography tandem mass spectrometry (LC-MS/MS) we show that total concentrations of testosterone, DHT and other circulating androgens are ~100-fold increased, whereas urinary conjugation products were unaltered. Weights of androgen-sensitive organs (seminal vesicles and levator ani muscles) however are slightly (12–20%) but significantly ($p < 0.001$) reduced, indicating suppressed androgen bioactivity in vivo. Also in an in vitro luciferase androgen reporter bioassay, SHBG suppressed androgen bioactivity. Total estradiol was also increased in male mice but remained strikingly low, indicating that lack of circulating SHBG in mice is only slightly responsible for undetectable circulating estradiol levels in male mice. 3H-DHT and -T injections i.v. revealed that SHBG prolongs ligand half-life. In orchidectomized mice however, SHBG did not prolong the biological actions of androgens. Replacement experiments with anabolic testosterone doses showed that SHBG prevented against sex organ but not muscle hypertrophy, and restricts androgen entry into target tissues like bone. However, glucose sensitivity and bone mass were unaffected in SHBG-Tg mice.
Conclusions: Despite 100-fold higher total androgen levels in, bioactivity is reduced in SHBG-Tg mice. This genetic mouse model however revealed no independent influence of SHBG on bone or metabolic outcomes.
L Antonio and MR Laurent contributed equally to this work.

PS2B2 – POSTER SESSION NR.2

POSTER PRESENTATIONS

HH01

Metabolic and sexual effects of Testosterone replacement therapy plus Liraglutide in adult hypogonadal obese men with overt type 2 Diabetes mellitus

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Background: Recently, we have retrospectively studied a group of obese, diabetic (T2DM) men with overt hypogonadism (pre and postpubertal onset) who reached a better glycemic control (HbA1c = <58 nmol/mol) and a significant improvement of Erectile Dysfunction (ED) by means of a combined regimen of Testosterone supplementation (TS) plus Liraglutide (L), besides lifestyle changes and metformin (Met) therapy.
Aim: The purpose of this follow-up survey is to verify whether a stable good metabolic control obtained by the combined treatment with TS and L leads to a stable improvement of ED in these patients.
Material and Methods: Twenty eight obese (BMI = 32,4 ± 2,6) hypogonadal men (18 post pubertal and 10 prepubertal) affected by T2DM already under treatment with TS (Nebid 1000 fl 1 fl/12 weeks), Met 2000 mg/day and L (1,2 μg/day) for 21 ± 3 months were asked to continue this combined therapy for an additional 24 months. All patients were asked to fill in the International Index of Erectile Function (IIEF 15) questionnaire after 12 (T1) and 24 (T2) months of prolonged combined therapy to assess the possible relationship between maintenance of metabolic control and ED improvement. Serum HbA1c and Testosterone were measured in basal condition and at T1 and T2.
Results: At T2 22 subjects showed a good metabolic control (HbA1c = 55 ± 14 nmol/mol) whereas the last 6 subjects showed a poor metabolic control (HbA1c = 71 ± 15 nmol/mol).In all participants, there was a non-significant reduction of BMI (31,5 ± 2,0) and serum T was within the normal range for healthy adult men. IIEF score, however, was 17.0 ± 2,1 in men who had a good metabolic control, while it was 14,3 ± 1,3 (p = < 0.05) in those with poor metabolic control; results were similar at T1.
Conclusion: A durability good metabolic control can be reached with the combined therapy with TS and L, besides Met and lifestyle changes, in the vast majority of patients and could positively affect ED in obese diabetic men with overt hypogonadism. Randomized control studies are needed to confirm these data.

HH02

Long-term testosterone undecanoate (TU) therapy improves glycaemic control and weight in hypogonadal men with type 2 diabetes (T2DM): real-life data from a registry study

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Objective: To assess long-term effectiveness and safety of TU in a urological setting in comparison to an untreated hypogonadal control group.
Methods: Cumulative registry study in 656 men with total testosterone (T) levels below 12.1 nmol/L and symptoms of hypogonadism. 230 men with T2DM were analysed. 113 received parenteral TU 1000 mg/12 weeks following an initial 6-week interval. 117 men had opted against T therapy (TTh) and served as controls (CTRL). Median follow-up in both groups was 7 years. Measurements were taken at least twice a year, and 8-year data are presented. Mean changes over time between the groups were compared by a mixed effects model for repeated measures with a random effect for intercept and fixed effects for time, group and their interaction. Changes were adjusted for age, weight, waist circumference, blood pressure, and lipids to account for baseline differences between the two groups.
Results: Mean age: 63.4 ± 4.73 years.
Fasting glucose (mmol/L) decreased from 6.2 ± 0.8 to 5.2 ± 0.05 (p < 0.0001) in the T group and remained stable at 5.8 ± 0.3 in CTRL. Difference between groups: -0.9 (p < 0.0001). HbA1c decreased from 8.03 ± 0.83 to 5.77 ± 0.43% in the T group and increased from 7.44 ± 0.66 to 8.01 ± 0.78% in CTRL. Difference between groups: -2.52% (p < 0.0001 for all). At the last measurement, all but 9 patients in the T-group had achieved HbA1c target <6.5%. In CTRL, all but 9 patients' HbA1c had increased. The triglyceride:HDL ratio, a surrogate parameter for insulin resistance, decreased from 5.4 ± 2.4 to 2.3 ± 0.5 (p < 0.0001) in the T group and from 7.8 ± 4.4 to 7.0 ± 4.3 (p < 0.05) in CTRL. Difference between groups: -3.8 (p < 0.0001). The TyG index, another surrogate parameter for insulin resistance, decreased from 4.22 ± 0.1 to 3.95 ± 0.02 (p < 0.0001) in the T group and increased from 4.16 ± 0.08 to 4.19 ± 0.08 (NS) in CTRL. Difference between groups: -0.27 (p < 0.0001). Waist circumference (cm) progressively decreased from 110.7 ± 7.3 to 100.5 ± 5.4 in the T group and increased from 110.2 ± 7.2 to 110.7 ± 6.1 in CTRL. The model-adjusted estimated difference between groups at 8 years was -13.0 (p < 0.0001 for all). Weight (kg) decreased from 112.5 ± 13.2 to 91.8 ± 9 (p < 0.0001) in the T-group and from 97 ± 10.2 to 96.9 ± 9.0 (p < 0.05) in CTRL. Difference between groups: -21.9 (p < 0.0001). Weight change from baseline was -19 ± 5.6% (p < 0.0001) in the T group and + 1.1 ± 2.9% (p < 0.01) in CTRL. Difference between groups: -20.2% (p < 0.0001).BMI (kg/m2) decreased from 36.1 ± 4.2 to 29.7 ± 2.8 (p < 0.0001) in the T group and from 31 ± 3.5 to 30.9 ± 2.9 (NS) in CTRL. Difference between groups: -6.8 (p < 0.0001).
The waist:height ratio decreased from 0.63 ± 0.04 to 0.57 ± 0.03 in the T-group and increased from 0.62 ± 0.04 to 0.63 ± 0.04 in CTRL. Difference between groups:-0.07 (p < 0.0001 for all). No patient dropped out. There was 1

death in the T group. In CTRL, there were 14 myocardial infarctions, 16 strokes, and 9 deaths.

Conclusions: Long-term TTh with TU in an unselected cohort of hypogonadal men with T2DM resulted in improvements in anthropometric and metabolic parameters. Untreated controls gained weight and glycemic control worsened. Long-term TU was well tolerated and adherence excellent.

HH03

Incidence and severity of prostate cancer in 375 hypogonadal men treated with testosterone undecanoate injections for up to 10 years and 296 untreated hypogonadal controls

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Background: Testosterone therapy (TTh) in elderly men is still associated with concerns regarding prostate cancer (PCa). In this study, incidence of PCa in hypogonadal men on long-term treatment with testosterone undecanoate (TU) in comparison to an untreated hypogonadal control group (CTRL) was assessed.Methods:In an ongoing, cumulative registry study, 375 men (age range: 33–70) with total testosterone (T) = 12.1 nmol/L in the presence of symptoms received TU 1000 mg every 12 weeks following an initial interval of 6 weeks for up to 8 years. 296 hypogonadal men (age range: 57–74) decided against TTh, mainly due to financial reasons. Prostate volume (PV) and PSA were measured and digital rectal examination (DRE)/transrectal ultrasound (TRUS) performed before treatment initiation and then regularly every 3–6 months. Biopsies were performed when indicated according to EAU guidelines.

Results: In T-treated patients, 8 men (2.1%) were diagnosed with PCa. In the control group, 12 (4.1%) were diagnosed with PCa. The incidence per 10 000 years was 32 in the T group and 64 in CTRL. The mean baseline age of PCa patients was 65 years in both groups.

All patients underwent radical prostatectomy. In the T group, all patients had a Gleason score = 6 and a predominant Gleason score of 3 in all patients. Tumor grade was G2 in all 8 (100%), tumor stage T2a in 6 (75%) and T2b in 2 (25%) patients. Regional lymph nodes, distant metastases and surgical margin were negative (N0, M0, R0) in all 8 men.In CTRL, Gleason score was >6 in all 12 patients. Three men had a predominant Gleason score of 3, 8 had 4, and 1 had 5. Tumor grade was G2 in 5 (41.7%) and G3 in 7 (58.3%) patients, tumor stage T2b in 1 (8.3%), T2c in 1 (8.3%), T3b in 4 (33.3%) and T3c in 6 (50%) patients. 7 patients had positive lymph nodes and 7 patients had a positive surgical margin.

Conclusions: Long-term treatment with TU in hypogonadal men undergoing regular monitoring according to EAU guidelines does not increase the incidence of PCa in comparison to an untreated hypogonadal CTRL group. PCa was more severe in the CTRL group.

HH04

Secondary prevention of major adverse cardiovascular events (MACE) by long-term testosterone therapy hypogonadal men

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Objective: Controversies exist regarding potential cardiovascular risk associated with testosterone therapy (TTh). We investigated long-term TTh in hypogonadal men with preexisting cardiovascular events or diagnosis in a urological setting compared to an untreated hypogonadal control group.

Methods: Cumulative registry study in 656 men with total testosterone (T) levels below 12.1 nmol/L and symptoms of hypogonadism. 151 men with a CVD history were analysed. 68 received parenteral TU 1000 mg/12 weeks following an initial 6-week interval. 83 men had opted against T therapy (TTh) and served as controls (CTRL). Median follow-up was 6 years in CTRL and 9 years in the T-group. Measurements were taken at least twice a year, and 8-year data are presented. Mean changes over time between the groups were compared by a mixed effects model for repeated measures with a random effect for intercept and fixed effects for time, group and their interaction. Changes were adjusted for age, weight, waist circumference, blood pressure, and lipids to account for baseline differences between the two groups.

Results: Mean age: 63.4 ± 4.9 years. In the T-group, 40 men had had a myocardial infarction (MI), 6 stroke, and 40 had been diagnosed with coronary artery disease (CAD). In CTRL, 23 had had a MI, 24 stroke, and 65 CAD.

Waist circumference (cm) declined from 112.1 ± 8 to 99.9 ± 6.3 in the T-group and increased from 108.1 ± 9 to 109.4 ± 7.1 in CTRL. The model-adjusted estimated difference between groups at 8 years was -13.2 ($p < 0.0001$ for all). Fasting glucose (mmol/L) decreased from 6.0 ± 0.9 to 5.2 ± 0.1 ($p < 0.0001$) in the T-group and increased from 5.6 ± 0.4 to 5.7 ± 0.4 (NS) in CTRL. Difference between groups: -0.8 ($p < 0.0001$). HbA1c (%) decreased from 7.81 ± 1.16 to 5.76 ± 0.43 in the T-group and increased from 6.18 ± 1.24 to 7.15 ± 1.33 in CTRL. Difference between groups: -1.45 ($p < 0.0001$ for all). Lipids (mmol/L): HDL increased from 1.7 ± 0.5 to 2.1 ± 0.5 in the T-group and from 1.3 ± 0.5 to 1.4 ± 0.7 in CTRL. Difference between groups: 0.51 ($p < 0.0001$ for all). Triglycerides decreased from 3.5 ± 0.6 to 2.2 ± 0.1 ($p < 0.0001$) in the T-group and increased from 2.9 ± 0.5 to 3 ± 0.5 (NS) in CTRL. Difference between groups: -1.2 ($p < 0.0001$). Blood pressure (BP, mmHg): Systolic BP decreased from 167.8 ± 11.0 to 134.6 ± 7.5 ($p < 0.0001$) in the T-group and from 159 ± 9.3 to 156.6 ± 6.3 (NS) in CTRL, difference between groups: -36.9 ($p < 0.0001$). Diastolic BP decreased from 102.3 ± 8.2 to 76.4 ± 4.7 ($p < 0.0001$) in the T-group and increased from 89.1 ± 6.4 to 90.6 ± 5 (NS) in CTRL, difference between groups: -22.4 ($p < 0.0001$). Heart rate (bpm) decreased from 78.6 ± 3.6 to 72.9 ± 2.3 ($p < 0.0001$) in the T-group and from 76.3 ± 4.8 to 75.9 ± 3.6 (NS) in CTRL, difference between groups: -4.1 ($p < 0.005$). No patient dropped out. There were no MACE in the T-group. In CTRL, there were 12 MIs, 14 strokes, and 21 deaths.

Conclusions: In hypogonadal men with CVD history receiving long-term TTh, there was not a single MACE whereas there were 47 MACE in CTRL. Long-term TTh with TU may provide effective secondary prevention of cardiovascular events.

HH05

Long-term testosterone treatment prevents progression from prediabetes to diabetes type 2 in 109 hypogonadal men

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Background: While short-term studies using testosterone therapy (TTh) in hypogonadal men with type 2 diabetes mellitus (T2DM) have yielded inconsistent results, long-term treatment has shown beneficial effects of TTh. There is no information, however, whether TRT has benefits in hypogonadal men with prediabetes.
Methods: Men presenting to urological offices with various complaints were screened for the presence of hypogonadism and, if found hypogonadal, offered TTh. Those who had received at least 1 year of treatment with testosterone undecanoate 1000 mg injections (TU) were entered into two independent, prospective, observational, cumulative registry studies. 109 men with prediabetes, defined as a baseline HbA1c from 5.7% to 6.4%, were analysed. TU was administered in 3-month intervals following an initial 6-week interval for up to 8 years. At each or each other visit, anthropometric and metabolic parameters were measured. Patients whose TTh was temporarily interrupted were excluded from the analysis.
Results: Mean age was 57.37 ± 8.99 years. Mean weight decreased from 96.15 ± 13.05 to 84.14 ± 6.98 kg. Change from baseline was -14.58 ± 0.68 kg, percent change from baseline -14 ± 0.65%. Waist circumference decreased from 103.8 ± 6.88 to 94.32 ± 4.53 cm. Change from baseline was -9.62 ± 0.44 cm. BMI decreased from 30.55 ± 4.35 to 27.04 ± 2.55 kg/m2, change from baseline -4.66 ± 0.23 kg/m2. Waist-to-height ratio decreased from 0.58 ± 0.04 to 0.53 ± 0.03. All anthropometric measures were statistically significant vs. baseline ($p < 0.0001$) and improved progressively with statistical significance compared to the previous year for 6 to 7 years. Fasting glucose decreased from 5.43 ± 0.68 to 4.63 ± 0.67 mmol/L ($p < 0.0001$), change from baseline -0.94 ± 0.11 mmol/L reaching a plateau after 1 year. HbA1c decreased from 5.9 ± 0.21 to 5.38 ± 0.26% ($p < 0.0001$), change from baseline-0.59 ± 0.04% with statistical significance compared to the previous year for the first 3 years. The triglyceride to HDL ratio, a surrogate parameter of insulin resistance, declined from 5.62 ± 2.61 to 2.6 ± 0.74 ($p < 0.0001$). The product of fasting glucose and triglycerides (TyG Index), another surrogate for insulin resistance, improved from 4.04 ± 0.17 to 3.81 ± 0.14. No patient progressed from prediabetes to T2DM. All but 4 patients' last measured HbA1c was <5.7%. Lipid patterns, blood pressure, liver transaminases and C-reactive protein all improved significantly. 3 patients dropped out, 2 due to relocation to a different city, 1 was lost to follow-up. There were no major adverse cardiovascular events during the full observation time.

HH06

Does testosterone therapy protect against high-grade prostate cancer (PCa)? Incidence and severity of PCa in patients undergoing prostate biopsy in a urological office

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Background: There are still concerns regarding testosterone therapy (TTh) in middle-aged and elderly men and prostate cancer (PCa).Methods:Between 2008 and July 2013, 553 prostate biopsies were performed in our office. 22 patients refused biopsy. We investigated incidence and severity of PCa in three groups: hypogonadal ($T = 350$ ng/dL) men receiving TTh, hypogonadal untreated, and eugonadal men. All groups underwent similar screening intensity of at least once per year. Biopsies were performed when indicated according to EAU guidelines.
Results: In 42 hypogonadal men receiving TTh, 7 (16.7%) had a positive biopsy. Of these, 5 had a Gleason score = 6 (71.4%) and 2 a Gleason score >6 (28.6%). Predominant Gleason score was 3 in all 7 men (100%). Tumor grade was II in 6 (85.7%) and II-III in 1 (14.3%) men.
In 162 untreated hypogonadal men, 84 (51.9%) had a positive biopsy. Of these, 34 had a Gleason score = 6 (40.5%) and 50 a Gleason score >6 (59.5%). Predominant Gleason score was 3 in 65 (77.4%), 4 in 17 (20.2%) and 5 in 2 (2.4%) men. Tumor grade was II in 35 (41.7%), II-III in 10 (11.9%), III in 34 (40.5%) and IV in 5 (6.0%) men.
In 349 eugonadal men, 132 (37.8%) had a positive biopsy. Of these, 56 had a Gleason score = 6 (42.4%) and 76 a Gleason score >6 (57.6%). Predominant Gleason score was 3 in 109 (82.6%), 4 in 22 (16.7%) and 5 in 1 (0.8%) men. Tumor grade was II in 59 (44.7%), II–III in 6 (4.5%), III in 63 (47.7%) and IV in 4 (3.0%) men.
Conclusions: The incidence of positive prostate biopsies was lowest in hypogonadal men receiving TTh. The severity of PCa was significantly lower in hypogonadal patients receiving TTh. TTh may protect against high-grade PCa.

HH07

Can the lipid accumulation product index indicate sex hormone disorders in aging men?

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Background: The lipid accumulation product (LAP) is an index for the evaluation of lipid overaccumulation in adults with reference to an anthropometric factor – waist circumference (WC) and a biochemical factor – the serum levels of *triacylglycerols (TAG)*. LAP values are known to be strongly correlated with visceral fat. Visceral obesity, which is a major component of the metabolic syndrome (MetS), is associated with an increase in metabolic abnormalities, cardiovascular diseases, and late onset hypogonadism (LOH). Numerous studies and metaanalyses suggest the relationship between low testosterone and obesity.
Aim: To determine the relationship between the LAP value and the levels of total testosterone (TT), free testosterone (FT), estradiol (E2), *dehydroepiandrosterone sulfate (DHEA-S)*, and sex hormone binding globulin (SHBG) as well as a free androgen index (FAI) in men aged 50–75 years.
Material and Methods: The study involved 313 men, aged 50–75 years (mean age 61.3 years ± 6.3). The exclusion criteria were: oncological treatment, steroid (including testosterone) therapy, thyroid diseases, and receiving neuroleptic and antidepressant agents. Anthropometric measurements, among them waist measurement, were taken for the participants. The levels of *triacylglycerols* in blood serum were determined using a spectrophotometric method, and the levels of total testosterone, free testosterone, estradiol, *dehydroepiandrosterone sulfate*, and sex hormone binding globulin – using the ELISA method. The LAP index was calculated using the formula: LA $p = (WC - 65) \times TAG$ (mmol/L). The free androgen index (FAI) was calculated using the following formula: FAI = TT (nmol/L)/SHBG (nmol/L) × 100. Testosterone deficiency was diagnosed if TT <8 nmol/L or below 12 nmo/L if the patients had positive results for the Androgen Deficiency in Aging Male (ADAM) questionnaire. Statistical analysis of the results was performed. The level of significance was set at $p = 0.005$.
Result: Men with TT deficiency had statistically significantly higher LAP values ($p < 0.0001$). Correlation analysis demonstrated that LAP values significantly positively correlated with FAI ($p = 0.149$, $p = 0.009$) and the levels of DHEA-S ($p = 0.137$, $p = 0.016$), TT ($p = 0.321$, $p < 0.0001$) and SHGB ($p = -0.315$, $p < 0.0001$).
Linear regression analysis of the LAP values, conducted with reference to the levels of hormonal parameters and without reference to age, demonstrated that the LAP value depends on the level of SHGB ($B = -0.360$, $p = 0.001$) but does not depend on the levels of TT ($B = 2.547$, $p = 0.115$) and DHEAS ($B = 1.181$, $p = 0.679$).
Conclusion: The LAP index is a cheap and *easy-to-use* biomarker that can be applied to assess the risk of age-related TT deficiency disorders, but cannot substitute for hormone tests for menopausal men whose clinical symptoms may suggest a lower testosterone level.

HH08

Can the lipid accumulation product index indicate sex hormone disorders in aging men?

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Background: The lipid accumulation product (LAP) is an index for the evaluation of lipid overaccumulation in adults with reference to an anthropometric factor – waist circumference (WC) and a biochemical factor – the serum levels of triacylglycerols (TAG). LAP values are known to be strongly correlated with visceral fat. Visceral obesity, which is a major component of the metabolic syndrome (MetS), is associated with an increase in metabolic abnormalities, cardiovascular diseases, and late onset hypogonadism (LOH). Numerous studies and metaanalyses suggest the relationship between low testosterone and obesity.
Aim: To determine the relationship between the LAP value and the levels of total testosterone (TT), free testosterone (FT), estradiol (E2), dehydroepiandrosterone sulfate (DHEA-S), and sex hormone binding globulin (SHBG) as well as a free androgen index (FAI) in men aged 50–75 years. Material and Methods: The study involved 313 men, aged 50–75 years (mean age 61.3 years □ 6.3). The exclusion criteria were: oncological treatment, steroid (including testosterone) therapy, thyroid diseases, and receiving neuroleptic and antidepressant agents. Anthropometric measurements, among them waist measurement, were taken for the participants. The levels of triacylglycerols in blood serum were determined using a spectrophotometric method, and the levels of total testosterone, free testosterone, estradiol, dehydroepiandrosterone sulfate, and sex hormone binding globulin – using the ELISA method. The LAP index was calculated using the formula: LA $p = (WC - 65) \times TAG$ (mmol/L). The free androgen index (FAI) was calculated using the following formula: FAI = TT (nmol/L)/SHBG (nmol/L) × 100. Testosterone deficiency was diagnosed if TT <8 nmol/L or below 12 nmol/L if the patients had positive results for the Androgen Deficiency in Aging Male (ADAM) questionnaire. Statistical analysis of the results was performed. The level of significance was set at $p = 0.005$.Result: Men with TT deficiency had statistically significantly higher LAP values ($p < 0.0001$). Correlation analysis demonstrated that LAP values significantly positively correlated with FAI ($p = 0.149$, $p = 0.009$) and the levels of DHEA-S ($p = 0.137$, $p = 0.016$), TT ($p = 0.321$, $p < 0.0001$) and SHGB ($p = -0.315$, $p < 0.0001$).Linear regression analysis of the LAP values, conducted with reference to the levels of hormonal parameters and without reference to age, demonstrated that the LAP value depends on the level of SHGB ($B = -0.360$, $p = 0.001$) but does not depend on the levels of TT ($B = 2.547$, $p = 0.115$) and DHEAS ($B = 1.181$,

$p = 0.679$).Conclusion: The LAP index is a cheap and easy-to-use biomarker that can be applied to assess the risk of age-related TT deficiency disorders, but cannot substitute for hormone tests for menopausal men whose clinical symptoms may suggest a lower testosterone level.

HH09

Independent association between osteocalcin and testosterone levels in men with chronic spinal cord injury

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Background: Osteocalcin (OCN) is considered a marker of bone formation mainly produced by osteoblasts under vitamin D and insulin stimulation, but also released from the bone matrix during the resorption phase. In its under-carboxylated form, OCN represents a multifunctional hormone, promoting insulin synthesis in beta cells and insulin sensitivity in fat, liver and muscles. As OCN can also stimulate testosterone biosynthesis in Leydig cells, a role has been suggested for OCN network dysfunctions in the multidirectional mechanisms linking androgen deficiency (AD) with obesity and insulin resistance. Although observational studies have demonstrated that circulating OCN is positively correlated with testosterone levels, the impact of metabolic and lifestyle-related factors on this association remains elusive. In this light, men with chronic spinal cord injury (SCI) could represent a suitable clinical model of study, as they exhibit a high prevalence of AD, obesity and metabolic syndrome, along with hypovitaminosis D and a disrupted bone remodeling.
Aim: We explored whether an independent association of OCN with testosterone levels would be demonstrable in men with chronic SCI, after adjustments for metabolic and lifestyle-related confounding factors, highly prevalent in this population.
Methods: Fifty-five consecutive men (54.7□17.8 years) admitted to a rehabilitation program because of traumatic chronic SCI underwent clinical and biochemical evaluations, including measurements of total testosterone (TT), total OCN and 25(OH)D levels. Free testosterone (FT) levels were calculated by the Vermeulen formula. Insulin resistance was assessed by HOMA-index.
Results: A biochemical AD (TT<300 ng/dL) was observed in 15 patients (27.3%). They exhibited lower levels of OCN (13.5 ± 7.4 vs 21.3 ± 7.5 ng/mL, $p = 0.001$) and 25(OH)D (12.7 ± 6.2 vs 17.8 ± 7.9 ng/mL, $p = 0.02$), higher BMI (28.7 ± 4.8 vs 24.4 ± 3.4, $p = 0.002$), HOMA-index (3.1 ± 3.2 vs 1.5 ± 1.0, $p = 0.01$), triglycerides values (173.7 ± 88.1 vs 120.6 ± 74.0 mg/dL, $p = 0.03$) and were engaged in significantly poorer leisure time physical activity (LTPA, 288.0 ± 209.7 vs 708.2 ± 311.6 minutes/week, $p < 0.0001$) than men with TT≥300 ng/dL. TT was positively correlated with OCN (r = 0.4, $p = 0.002$), vitamin D and LTPA and negatively correlated with BMI and HOMA-index. Significant correlations of OCN were also found with FT (r = 0.34, $p = 0.01$), alkaline phosphatase (r = 0.37, $p = 0.004$), BMI (r = -0.31, $p = 0.02$) and HOMA-index (r = -0.31, $p = 0.02$). At the multiple linear regression analysis, lower OCN levels were associated with lower TT and FT levels after adjustment for age, level of the lesion, walking ability, years post-injury, BMI, HOMA-index and 25(OH)D levels; in the last model, fully adjusted also for LTPA, significant independent associations persisted between OCN and both TT (beta-coefficient: 0.45, CI:0.23-0.67, $p = 0.0007$) and FT (beta-coefficient: 0.33, CI:0.10-0.58, $p = 0.007$).
Conclusion: The evidence of an independent association between OCN and testosterone levels in men with chronic SCI, in spite of the many confounders peculiar to this population, reinforces the notion of a direct link between OCN and testosterone biosynthesis in Leydig cells.

HH10

Predictors and clinical consequences of starting androgen therapy in men with low testosterone: results from the SIAMO-NOI registry

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Background: Management of late onset hypogonadism (LOH) is not homogenous.
Aim: To observe the management of patients with low testosterone (T) in highly specialized Italian centres.
Methods: The SIAMO-NOI is an observational longitudinal disease registry for the evaluation of the clinical management of patients with low T levels (total T < 12 nmol/L, calculated free T < 225 pmol/L or already in treatment) in 15 Italian centers members of the Italian Society for Andrology and Sexual Medicine (SIAMS). Clinical and biochemical data were collected for four visits during 12 months of observation.
Results: 432 patients (mean age 50.9 ± 14.9 years) were enrolled. Of them, 247 men were receiving androgen therapy, whereas 145 were naive. After the first visit (V0), 80 men started androgen therapy, whereas 55 remained untreated during the entire observation. Younger age [odds ratio (OR) 0.57 (0.35–0.92)], total T < 8 nmol/L [OR 4.69 (1.59–13.81)], complaining at least one sexual symptom [OR 11.55 (2.01–66.35)] and reporting more severe

lower urinary tract symptoms [OR 1.27 (1.01–1.60)] predicted starting an androgen therapy. Sixty-four men started therapy immediately after V0 and maintained it until the observation end. When compared to V0, they reported an increase in all the domains of the International Index of Erectile Function-15 (IIEF-15), in the sexual and physical subdomains of the Aging Male Scale as well as in the International Prostate Symptom Score. Conversely, the untreated group reported a significant improvement, although lower than the treated group, only in the erectile function domain of the IIEF-15.
Conclusions: Management of LOH in SIAMS centres is in line with the international guidelines and the newest knowledge about the role of T on prostate health. Androgen therapy is associated with an improvement in all the aspects of sexual life and in the perception of physical strength.

HH11

How to define hypogonadism? Results from a population of men consulting for sexual dysfunction

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Background: The thresholds for testost erone (T) and the symptoms required for defining late onset hypogonadism (LOH) are under debate.
Aims: (i) To verify the association between total and calculated free T (cfT) and sexual symptoms; (ii) to identify thresholds for total and calculated free T to discriminate symptomatic from asymptomatic men.
Mehods: A consecutive series of 4890 men attending the outpatient clinic for sexual dysfunction was retrospectively studied. Biochemical parameters were collected. The relationships between symptoms and total or calculated free T were evaluated as LOESS curves.
Results: Severe impairment in morning erections, low libido and ED were reported by 14.6%, 2.7% and 60.2%, respectively. Simultaneous presence of severe ED and impaired morning erections or low desire was reported by 12.7% and 1.9%, respectively. Severely reduced desire and morning erections were complained of by 1.0%. The simultaneous presence of the three severe sexual symptoms was reported by 0.8%. Receiver operating characteristic (ROC) curve analysis showed that the highest accuracy for total T and cfT in detecting subjects with two symptoms was observed for reduced morning erections and desire (area under the ROC curve [AUC] = 0.670 ± 0.04 and 0.747 ± 0.04, for total T and cfT, respectively, both $p < 0.0001$). The addition of the third symptom, ED, further improved the accuracy (AUC = 0.681 ± 0.05 and 0.784 ± 0.04, for total T and cfT, respectively, both $p < 0.0001$). The assessment of the Youden index showed that the best thresholds for detecting men with androgen deficiency-related symptoms are 10.4 nmol/L for total T and ranges 225–260 pmol/L for cfT.
Conclusions: The simultaneous presence of reduced morning erections and desire is the cluster of symptoms that, along with total T < 10.4 nmol/L or cfT <225 pmol/L, defines LOH in a specific, evidence-based manner.

HH12

Interactions between depression and lower urinary tract symptoms: the role of adverse life events and inflammatory mechanisms. Results from the european male ageing study

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Background: Aging is associated with declines in multiple physiological systems, leading to an increased risk of both medical and psychological morbidity. Depression and lower urinary tract symptoms (LUTSs) have been found to co-occur among aging men.
Aim: The present study attempted to clarify the nature of this relationship, considering adverse life events as potential moderators and the inflammation as an underlying biological mechanism.
Methods: The relationship between depression and LUTS was evaluated using data from the European Male Ageing Study,the largest multicenter population-based study of aging in European men. The sample included 3369 men who wereassessed by means of several self-reported questionnaires, including the Beck Depression Inventory-II, the InternationalProstate Symptom Score, and the Adverse Life Events Scale. Participants were asked to provide information regarding generalhealth and life-style, and medical comorbidities. Biological measures including prostate-specific antigen, testosterone,and C-reactive protein were measured.Results: LUTS and depressive symptoms were correlated (R square = 0.32, β = 0.10, $p < 0.001$), even after adjusting for life-style, psychological, and medical variables. A history of adverse life events was associated with both higher LUTS and Beck Depression Inventory scores. Furthermore, adverse life events moderated the LUTS-depression association ($F = 22.62$, $b = 0.061$, $p < 0.001$), which increased as a function of the number of life events. C-reactive protein was found to mediatethe LUTS-depression association. This mediation effect was moderated by number of adverse life events.Conclusions: Participants with a history of adverse life events represent a vulnerable population in whom the associationbetween somatic and depressive symptoms is stronger. One of the biological mechanisms underlying this association couldbe an activation of the central inflammatory signaling pathways.

HH13

Testosterone supplementation and body composition: results from a meta-analysis study

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Background: To meta-analyze, in subjects with late onset hypogonadism, the effects of testosterone (T) supplementation (TS) on body composition and metabolic outcomes.
Methods: All observational studies and randomized controlled trials (RCTs) comparing the effect of TS on different endpoints were considered.
Results: Out of 824 retrieved articles, 91 were included in the study enrolling 6955 and 2066 patients in TS and control groups, respectively. In observational studies, but not in RCTs, TS was associated with a time-dependent reduction of body weight, and waist circumference (WC). The estimated weight loss and WC reduction at 24 months were −3.50[−5.21; −1.80] kg and −6.23[−7.94; −4.76] cm, respectively. TS was also associated with a significant reduction of fat and with an increase of lean mass as well as with a reduction of fasting glycaemia and insulin resistance in both observational studies and RCTs. When only RCTs enrolling hypogonadal (total T < 12 mol/L) subjects at baseline were considered, a reduction of total cholesterol as well as of triglyceride levels were observed in both observational studies and in RCTs. Conversely, an improvement in HDL cholesterol levels as well as in both systolic and diastolic blood pressure was observed only in observational studies, but not in RCTs.
Conclusions: Clinicians are strongly encouraged to check T in their obese subjects, because treating hypogonadism might help in decreasing fat mass, increasing muscle mass and, therefore, facilitating weight loss.

HH14

Treatment of hypogonadal men with clomiphene citrate: effects on semen quality, long-term results and predictors of successful biochemical response

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Background: Clomiphene citrate (CC) can be used to treat hypogonadal (subfertile) men. Several studies have reported that CC leads to increased serum gonadotropin and testosterone levels. However, studies lack long-term results and reports concerning the effect on semen quality are conflicting. Furthermore, information on possible predictors of treatment response is scarce.
Aim: To evaluate: (i) the effect of CC on semen quality, (ii) long-term treatment results of CC and (iii) possible predictors of successful biochemical response.
Methods: A retrospective chart analysis was performed of 105 men who were considered candidates for CC therapy from 1 June 2013 until 1 March 2016. 52 men with a baseline serum total $T = 12$ nmol/L who were treated with 25 mg every other day were included for analysis. Changes in mean serum hormonal levels and semen parameters were used as outcome measures. Successful biochemical response to CC was defined as an increase of serum total $T = 7$ nmol/L in combination with a serum total $T > 14$ nmol/L. Simple linear regression and the independent t-test were used to determine possible predictors of successful biochemical response to CC therapy.
Results: We observed a significant in serum SHBG (27.1 ± 12.3 nmol/L to 29.4 ± 11.5 nmol/L, $p = 0.02$), LH (4.4 ± 2.6 IU/L to 8.8 ± 5.0 IU/L, $p < 0.001$), FSH (11.6 ± 10.4 IU/L to 16.3 ± 11.4 IU/L), total T (8.5 ± 1.9 nmol/L to 18.5 ± 5.8 nmol/L) and free T (200.3 pmol/L ± 43.7 to 419.8 ± 125.0 pmol/L). Mean treatment duration was 53 days ± 39 days. Serum total T levels remained stable in 14 patients treated > 12 months. 36/52 (69.2%) patients successfully responded to CC. Mean baseline LH was significantly lower in the responders group: 3.9 IU/L ± 2.4 IU/L vs. 5.7 IU/L ± 2.4 IU/L in the non-responders ($p = 0.024$). The odds for a successful biochemical response to CC for patients with a baseline LH ≤ 5.0 IU/L compared to patients with a baseline LH > 5.0 IU/L was 4.24 (CI [95%]: 1.14 – 15.8, $p = 0.031$). Baseline LH (r = -0.72 [$p = 0.042$]), baseline FSH (r = -0.207 [$p = 0.018$]) and baseline total T (r = -1.17 [$p = 0.008$]) were negatively correlated with the increase in total T. Total testicular volume (mL) was positively correlated (r = 0.16, [$p = 0.029$]). In 3/15 (20.0%) patients who were initially azoospermic, progressively motile spermatozoa were present in the ejaculates during CC use. 3/7 (42.9%) patients who were initially oligoasthenozoospermic experienced a substantial increase in semen quality.
Conclusion: Treatment with CC results in a significant increase of serum LH, FSH, total T, free T and SHBG. The majority of patients (69.2%) has a successful biochemical response and a select number of patients experiences improvement of semen quality. Baseline LH is found to be the most important predictor of successful biochemical response.

HH15

Primary hypogonadism in the old male Wistar rats is associated with age related mitochondrial dysfunction

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Background: Male aging is accompanied by low level of circulating androgens as a consequence of primary or secondary hypogonadism. The primary hypogonadism reflects age dependent dysfunctionality of Leydig cells (Lc) followed by compromised LH-cAMP signaling and

downregulated protein machinery responsible for transport and transformation of cholesterol to testosterone. Since mitochondria play an essential role in initiation of testosterone biosynthesis, serve as power centers and represent a source of oxidative stress, a possible mitochondrial dysfunction could be connected with decreased activity of Leydig cells and lowered testosterone production during aging.

Aim: Here we chronologically analyzed age-related changes of mitochondrial function in rat Leydig cells and these changes were correlated with progressive decline in testosterone production.

Material: Experiments were performed on purified Leydig cells isolated from testes obtained from 3 to 24-month-old Wistar rats. Testosterone and LH were measured by RIA; relative gene expression were quantified with qRT-PCR; specific proteins were identified by Western blot analysis; mitochondrial membrane potential (??i) analyzed by Tetramethylrhodamine-dye; O_2 consumption was measured by Clark electrode; ATP level was measured by ATP bioluminescent kit. The results were statistically analyzed by Mann-Whitney's unpaired nonparametric two-tail test and by one-way ANOVA.

Results: Serum testosterone decreased significantly with age, starting from 12-mo and further progressed to 24-mo. Decline in serum testosterone was associated with: decreased expression of genes involved in initiations of testosterone synthesis (*Star* and *Cyp11a1*), increased mitochondrial membrane potential, lowered oxygen consumption and ATP production in isolated Leydig cells. qRT-PCR analysis showed changed expression of genes markers of mitochondrial function/homeostasis: decreased *Cox4i2* detected from 12–24-mo, and increased *Cytc* in 24-mo; transcription factors involved in regulation of respiratory chain elements expression, *Gabpa* and *Nrf1*, were elevated in 24-mo; *Sirt1* (activator of PGC1-mediated transcription) and *Pgc1a/b* were down-regulated in rats from 12 to 24-mo. The AMPK (energy sensor) was increased in 24-mo.

Conclusion: Obtained results showed disturbed mitochondrial function in Leydig cells during aging that could be connected with decreased testosterone production.

PS2C – POSTER SESSION NR.2

POSTER PRESENTATIONS

SD01

Evaluation of erectile function and hypothalamo-hypophysis-gonadal axis in patients undergoing methadone maintenance treatment

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Background: In implementing the National Programme for Prevention, Treatment and Rehabilitation of Drug Addiction in Bulgaria, a network of methadone maintenance treatment programs was created. The chronic use of opioids including methadone in men leads to symptoms such as delayed ejaculation, erectile dysfunction and significant reduction of sexual libido.

Aim: The aim of this study was to evaluate the effects of chronic use of methadone on erectile function and hypothalamo-hypophysis-gonadal axis status in men.

Methods: The study comprised of 75 male patients aged from 18 to 40 years, undergoing chronic methadone treatment in five Programs and 22 age-matched clinically healthy men. The administration of methadone was oral, every day; the average duration of exposure to methadone for the study group was 33.92 (Alt0177 24.2) months and the mean daily dose – 98.6 (Alt0177 40.6) mg. International survey IIEF (International Index of Erectile Function) in bulgarian language was used for assessment of erectile function. Hypothalamo-hypophysis-gonadal axis was evaluated by the measurement of serum levels of testosterone, LH, FSH and Inhibin B.

Results: We established significantly lower total scores on the scale for all components of the survey in the patients as compared to the healthy men ($p < 0.05$): EF (erectile function) (23.81 Alt0177 4.98 vs. 29.27 Alt0177 0.77); OF (orgasmic function) (8.04 Alt0177 2.1 vs. 9.14 Alt0177 1.36); SD (sexual desire) (7.16 Alt0177 1.62 vs. 9.05 Alt0177 0.95); IS (intercourse satisfaction) (10.09 Alt0177 3.10 vs. 13.05 Alt0177 1.62); OS (overall satisfaction) (7.18 Alt0177 1.94 vs. 9.23 Alt0177 0.87) The total mean score criterion EF (23.81) in the investigated group of patients indicated erectile dysfunction of slight degree. We also found data for impairment of the hypothalamo-hypophysis-gonadal axis($p < 0.05$): significantly lower levels of testosterone (3.91 Alt0177 1.81 vs. 5.15 Alt0177 1.58) ng/mL; higher levels of LH (5.16 Alt0177 2.96 vs. 3.68 Alt0177 1.46) IU/L and lower testosterone/LH ratio (0.91 Alt0177 0.46 vs. 1.53 Alt0177 0.84). Any significant differences in the levels of FSH, Inhibin B, FSH/Inibin B ratio and prolactin were not found.

Conclusions: The chronicexposure tomethadoneleads toimpairment of all components forassessmentof erectile functionincluded in the internationalsurveyIIEF.

Analysis of the results from our study definitely proved that the chronic use of methadone leads to erectile dysfunction.

The chronic exposure to methadone leads to impairment of gonadal function predominantly deteriorating the Leydig cells production.

SD02

Ureteral stone management in women and its effect on female sexual function

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Introduction: The aim of this study was to assess the effects of ureteroscopic stone management on perioperative female sexual function.

Materials and Methods: Thirty female patients with ureteral stones who were between the ages of 22 and 51 years

43.9 ± 7.5 (study group) and 30 healthy women aged between 23 and 58 years (38.7 ± 9.3 years) (control group) were enrolled in this study. Demographic characteristics, anaesthesia type, lower urinary tract symptoms andstone sizes were evaluated.Also, individual and total scores of the Turkish-validated version of the Female Sexual Function Index, Beck's depression scale and the Hamilton depression rating scale for evaluation of psychogenic status were compared between the two groups.
Results: Mean individual female sexual scores were statistically significant between the two groups with the exception of the preoperative values of the groups. The mean Beck scores and Hamilton anxiety scores of the groups were not significantly different between the two groups.
Conclusion: Sexual function can be affected by endourological procedures. In selected uncomplicated ureteral stone surgeries, not using JJ catheters or minimizing catheterization times may help improve sexual functions in women. Patients should be informed that they may experience temporary sexual dysfunction in the perioperative term.

SD03

The Global Online Sexuality Survey: ED in USA, 2016

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Background: The Global Online Sexuality Survey (GOSS) is an ongoing online epidemiologic study of male and female sexuality launched across the globe in different languages, aiming to provide knowledge about sexual issues in the general population. The first launch was in the Middle East in 2010 followed by USA in 2011.
Aim: This is the report on the prevalence of erectile dysfunction as well as trends in sexuality in USA; 2016.
Methods: GOSS was randomly offered to English-speaking male web surfers in the USA in 2016. A total of 100 questions were offered to the participants, including the abbreviated international index of erectile function, risk factors for ED, ejaculatory function, therapeutic trends, sexual preferences (including trends in marriage, polygamy, coital frequency, sexual positions and masturbation), and sexual orientation, among others.
Results: ED was encountered in 55.4% of participants from USA in 2016, compared to 37.7% in the 2011 survey. 86.6% were exclusively heterosexual compared to 81.9% in 2011. 52.3% were married, 75.8% reported a good or very good emotional relationship with partner, 20.1% had multiple partners simultaneously, 54% experienced one-night stands, with 33% never or rarely using condom on those occasions, 11.8% experienced a sexually transmitted infection with 34.4% never seeking treatment and for themselves and 31.7% never seeking treatment for their partners, among 100 parameters studied for the population surveyed.
Conclusion: In USA, 2016; ED prevalence is 55.4%, and may be on the rise compared to the 2010 report.

SD04

The German Male Sex-Study (GMS-Study): sexual satisfaction and importance of sexuality – association with self-concept of 45-year old men

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Background: Despite sexual health is known to have a positive impact on the psychological well-being almost no research exists on how sexual satisfaction and importance of sexuality are associated with the sexual self-concept of men.
Aim: Evaluation of sexual satisfaction and importance of sexuality and their relationship with 4 relevant sexual self-concept factors in 45-year-old men: Body image, sexual self-esteem, perceived social pressure and masculinity.
Methods: 45-year old Caucasian heterosexual men out of the ongoing PROBASE trial, who were recruited between March 2014 and March 2016, were included. Body image was evaluated with 3 questions of the Dresden body image questionnaire. Sexual self-esteem and perceived social pressure were examined by 3 respectively 4 new questions and masculinity with 3 questions of the Male Role Norms Scale. Sexual satisfaction and importance of sexuality each were assessed with 1 question. In both cases there were 5 response options which were classified as: Satisfied, more or less satisfied and unsatisfied respectively important, more or less important and unimportant. Factors of self-concept could take on values from 1 to 5. For body image and sexual self-esteem higher values indicate a positive body image and a high sexual self-esteem. Higher values also indicate higher perceived social pressure and a more modern understanding of masculinity. Mean value (mv) □ standard deviation, p value using F-test and effective size using Cohen's d were calculated.
Results: N = 11802 men were included. There was a tendency to a positive body image (mv 3.7 ± 0.6), a high sexual self-esteem (mv 3.8 ± 0.6), hardly noticed perceived social pressure (mv 1.7 ± 0.6) and a modern understanding of masculinity (mv 3.6 ± 0.7). Sexually satisfied men (49%) in comparison to unsatisfied men (19%) had significant higher scores regarding body image (mv 3.9 ± 0.6 vs. 3.5 ± 0.7; d = -0.54) and sexual self-esteem (mv 3.9 ± 0.5 vs. 3.7 ± 0.6; d = -0.42). They had lower scores in terms of perceived social pressure (mv 1.5 ± 0.5 vs. 1.9 ± 0.8; d = 0.63) and a more modern understanding of masculinity (mv 3.7 ± 0.7 vs. 3.5 ± 0.7 ;(all $p < 0.001$); d = -0.26). Men who gave importance to their sexuality (68%) in comparison to men who gave little importance to sexuality (4%) had significant higher scores regarding body image (mv 3.8 ± 0.6 vs. 3.4 ± 0.7; d = 0.80) and sexual self-esteem (mv 3.9 ± 0.5 vs. 3.4 ± 0.7; d = 1.05). They had lower scores in terms of perceived social pressure (mv 1.6 ± 0.6 vs. 1.8 ± 0.7; d = -0.30) and there was no

difference in understanding of masculinity (mv 3.6 ± 0.7 vs. 3.7 ± 0.7 ;(all $p < 0.001$); d = −0.15).

Conclusion: Sexual satisfaction and importance of sexuality among 45-year old men in Germany are associated with a positive body image, a high sexual self-esteem and a lower perceived social pressure. Weak association between sexual satisfaction and a more modern understanding of masculinity emerged, whereas there is no association between importance attached to sexuality and masculinity. The beneficial effects of sexual satisfaction and the perception of sexuality should be considered in sexuality education and in prevention programs.

SD05

The dual FXR/TGR5 agonist INT-767 counteracts nonalcoholic steatohepatitis and erectile dysfunction in a rabbit model of high fat diet-induced metabolic syndrome

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Background: It is well known from the literature the pathogenic relationship between erectile dysfunction (ED) and metabolic syndrome (MetS). Nonalcoholic steatohepatitis (NASH), the hepatic hallmark of MetS, is recently regarded as an active player in the pathogenesis of MetS-associated erectile dysfunction (ED). Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), highly expressed in the liver, are interesting pharmacological targets for the treatment of liver and metabolic diseases. We have recently demonstrated, in a high-fat diet (HFD)-induced rabbit model of MetS, that the selective FXR agonist, obeticholic acid (OCA), ameliorates not only metabolic profile (visceral adipose tissue dysfunction and insulin resistance), but also MetS-induced penile alterations, including hypo-responsiveness to acetylcholine (Ach).

Aim: The aim of this study is to investigate the effect of FXR/TGR5 dual agonists on liver and penile function in a rabbit model of HFD-induced MetS.

Methods: We used a non-genomic, HFD-induced, rabbit model of MetS, and treated subgroups of MetS rabbits with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30 mg/kg, orally, daily, 5 days a week for 12 weeks). Liver was studied by immunohistochemistry and RT-PCR. In vitro contractility studies were performed to evaluate the relaxant effect of Ach in corpora cavernosa strips.

Results: Treatment with increasing doses of the dual FXR/TGR5 agonist INT-767 in a rabbit model of HFD-induced MetS, characterized also by NASH, dose-dependently reduced several MetS-associated alterations, including hepatomegaly, insulin resistance, glucose and cholesterol levels, while significantly increasing HDL levels. Genes related to neutrophil apoptosis/apoptotic-neutrophil clearance (lactoferrin, eNOS, RAGE) and to extracellular matrix degradation (MMP2, TIMP2) were also increased by INT-767 treatment. INT-767 also reduced liver expression of IL-6, which preferentially skews the Th cell response towards a Th17-phenotype, while increasing Foxp3 expression, a Treg cell marker. Thus these data indicate that INT-767 can promote the neutrophil- and macrophage-driven resolution phase of inflammation and fibrosis regression. In addition, INT-767 increased genes related to hepatic fatty acid metabolism (PPARa, AR, CD36) and lipid droplet formation (SNAP23, VAMP4,syntaxin5, perilipin) therefore suggesting that INT-767 counteracts excess fatty acid mediated lipotoxicity in the liver. Genes related to insulin signaling (IRS1, SREBP1, G6Pase, and PEPCK) were also increased by INT-767. Moreover, immunohistochemical studies demonstrated that INT-767 treatment significantly reduced both HFD-induced liver inflammation and fibrosis. Finally, hypo-responsiveness to Ach MetS-induced was preserved by INT-767 treatment.

Conclusion: In conclusion, INT-767 treatment counteracts NASH and ED in a rabbit model of HFD-induced MetS, supporting a link between NASH and ED in MetS conditions.

SD06

Effects of physical activity or metformin on testosterone deficiency and erectile dysfunction associated to metabolic syndrome

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Background: Metabolic Syndrome (MetS) is a cluster of clinical conditions not only associated to an increased cardiovascular and metabolic risk, but also to hypogonadism and erectile dysfunction (ED). Lifestyle modification (including physical activity, PA) and metformin (MET) are well-known treatments for the condition. We previously established an animal model of MetS that recapitulates all the human phenotype, including andrologic derangements.

Aim: To elucidate in experimental MetS the effect of PA or MET on penile erection and on hormonal and metabolic parameters.

Methods: Control (regular diet, RD) and MetS rabbits were treated with MET (300 mg/kg the last 18 days) or exercise-trained to run on a 20% slope treadmill until exhaustion for 12 weeks. After this time, penile tissue was collected for in vitro contractility study or gene expression by qRT-PCR. Lactate levels were collected at the end of training resulting similar between groups, even though exercise tolerance was reduced by MetS with reduced running time and distance.

Results: MET induced a reduction in visceral adiposity ($p = 0.001$), blood pressure ($p < 0.0001$), triglycerides ($p = 0.049$), glucose level ($p = 0.024$) and tolerance ($p = 0.003$). None of the aforementioned parameters were significantly affected by PA. However both MET and PA increased HDL cholesterol ($p = 0.049$ and 0.013, respectively). MET increased testosterone (T, $p = 0.01$), whereas PA completely restored it ($p = 0.002$) up to the RD values. Ach-induced relaxation, hampered in MetS rabbits ($p < 0.0001$), was significantly ameliorated by MET

($p = 0.028$) and completely normalized by PA ($p = 0.001$). The effect of PA on Ach was confirmed at ANCOVA after adjusting for T. PA also decreased phenylephrine-induced contractility ($p = 0.002$) and normalized sodium nitroprusside (SNP)-induced relaxation ($p = 0.017$), that was enhanced in MetS rabbits ($p = 0.005$ vs. RD). Genes related to NO signaling, including guanylate cyclase subunits and PKG, were up-regulated by PA (all $p < 0.05$), but not by MET. Similar results were obtained for smooth muscle-related genes as smoothelin and aSMA (all $p < 0.02$). MET ($p = 0.001$), and to a lower extent PA ($p = 0.02$), increased DDAH1 the gene responsible for decreasing the formation of the endogenous NOS inhibitor ADMA. PDE5 expression was decreased in MetS rabbits ($p < 0.0001$) and completely restored by PA ($p < 0.0001$), but not MET, resulting even higher than in RD rabbits ($p = 0.006$). Differences were confirmed at ANCOVA after adjusting for T. Accordingly, sildenafil (100 nM)-induced increase in SNP relaxation was completely normalized by PA.

Conclusion: Physical activity more than pharmacological treatment (metformin) completely restored T levels and penile responsiveness to Ach and sildenafil in experimental MetS, even though it was less effective than metformin in reducing metabolic abnormalities. The effect of exercise training is most probably related to an improved NO signaling, including PDE5. Hence, physical activity can be considered a new strategy to treat hypogonadism and ED related to MetS.

SD07

Prevalence of premature ejaculation at urology outpatient clinic

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Introduction&objectives: Premature ejaculation (PE) is one of the most common sexual disorder among male patients. The prevalence is between 20% and 30% in every age group. The consequences can be anxiety, frustration and/or avoiding sexual intercourse. Our primary objective was to evaluate the prevalence of PE among patients visiting urology outpatient office.

Materials & methods: We applied the 'Premature Ejaculation Diagnostic Tool' (PEDT) questionnaire in our study. In addition we assessed the BMI, comorbidities of the patients and wether the patients had talked about their sexual dysfunction with their consultant. We analyzed the categorical variables with Khi square and Fisher exact test. The continuous variables were analyzed by multiparametric linear regression.

Results: 140 patients (between 15 and 79 years of age) filled the questionnaire correctly. The prevalence of premature ejaculation was 30% in the study population. The results in the age related groups were: 22% (15–39 years); 37% (40–64 years); 21% (65–79 years). The difference between the age related groups was not significant. Age, BMI and diabetes mellitus did not increase the risk of premature ejaculation. The rate of premature ejaculation was significantly higher ($p < 0{,}001$) among patients who had need to consult about the disease.

Conclusion: The prevalence of PE according to our study is similar to the results of different international publications. Furthermore there is a discrepancy between the treated and affected PE patients. Since premature ejaculation can decrease the patient's and also the partner's quality of life, thus screeening of the disease is recommended at least at urology outpatient offices, so treatment can be initiated in order to avoid social compications.

SD08

Post-Finasteride persistant side effects on neuroactive steroids

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Finasteride is a reversible inhibitor of the enzyme 5alpha-reductase (5alpha-R) used for the treatment of human benign prostatic hyperplasia and androgenic alopecia. The 5alpha-R converts testosterone (T) and progesterone (PROG) into their 5alpha-reduced metabolites, dihydrotestosterone (DHT) and dihydroprogesterone (DHP), respectively. These neuroactive steroids, as well as their further metabolites, are important mediators for many physiological processes in the nervous system, affecting mood, behavior, reproduction, and cognition (Melcangi et al., Cell Mol Life Sci 65:777–797, 2008; Giatti et al., J Steroid Biochem Mol Biol 153:127–134, 2015). However, despite of the wide therapeutic use of finasteride, the effects of this inhibitor in the nervous system have been poorly explored. This aspect could be important, particularly because observations performed in a subset of man taking finasteride for androgenic alopecia show sexual dysfunction as well as anxious/depressive symptomatology. Very important, these side-effects were also reported in a subset of patients after discontinuation of the therapy (Traish et al., Rev Endocr Metab Disord 16:177–198, 2015). Interestingly, we demonstrated that post-finasteride patients showed altered neuroactive steroid levels in plasma and cerebrospinal fluid (CSF) in comparison to healthy individuals (Melcangi et al., J Sex Med 10: 2598–2603, 2013; Caruso et al., J Steroid Biochem Mol Biol 146: 74–79, 2015). Thus, data obtained by liquid chromatography-tandem mass spectrometry show a general decrease of neuroactive steroid levels, and particularly of 5alpha-reduced metabolites of PROG and T. Data obtained in male rats after subchronic treatment with finasteride (i.e., 3 mg/kg/day for 20 days) indicate that after one month of withdrawal neuroactive steroids are also affected in brain structures (Giatti et al., Neuroendocrinology 2015, DOI: 10.1159/000442982). For instance, the levels of PROG and its metabolites (i.e., DHP, isopregnanolone and allopregnanolone), as well as of deydroepiandrosterone and 17-beta-estradiol were significantly decreased in the cerebral cortex. In the cerebellum, the levels of pregnenolone (PREG), DHP and T were increased while the levels of DHT and 5alpha-androstane-3beta, 17beta-diol were decreased. In the hippocampus, the levels of PREG and PROG were decreased while the levels of DHP were

increased. In addition, changes in the expression of their receptors have been also reported. For instance, an upregulation of androgen receptor and estrogen receptor alpha and a downregulation of estrogen receptor beta were observed in the cerebral cortex. In addition, a decrease of alpha 4 and beta3 subunits of GABA-A receptor (i.e., the receptor able to bind allopregnanolone) has been observed in the cerebellum.
Altogether these findings suggest that the block of the enzyme 5alpha-reductase by finasteride treatment may have broad consequences for the nervous system (We thank the Post-Finasteride Foundation for the financial support).

SD09

Vascular and chronological age in subjects with erectile dysfunction: cross-sectional and longitudinal results

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Background: Impaired penile color Doppler ultrasound (PCDU) predicts major adverse cardiovascular (CV) events (MACE), particularly in men at low-risk. However, PDCU is not recommended in routine clinical check-ups.
Aim: To evaluate whether difference between vascular age, as derived from the SCORE algorithm, and chronological age (dage) is a predictor of MACE in subjects consulting for erectile dysfunction (ED), independently from other CV risk factors, including PDCU parameters.
Methods: A consecutive series of 2853 male patients attending the Outpatient Clinic for ED for the first time was retrospectively studied. Among them, 85.4% (n = 2437) were free from previous MACE and were analyzed. In the longitudinal study, 739 subjects without previous MACE were enrolled. Several clinical, biochemical, and PDCU parameters were studied. Vascular age was derived from the SCORE algorithm and the difference between vascular and chronological age (dage) was considered.
Results: Higher dage is associated with several conventional (family history of CV diseases, lower education level, glycaemia, triglycerides and metabolic syndrome) and unconventional (severity of ED, frequency of sexual activity, alcohol abuse, fatherhood, extramarital affairs, compensated hypogonadism and low prolactin levels) risk factors. dage is inversely related to PDCU parameters, including flaccid and dynamic peak systolic velocity (PSV) and flaccid acceleration (β = −0.125, −0.113 and −0.134, respectively, all $p < 0.0001$). In the longitudinal study, dage was associated with incident MACE. When dividing the population according to the median age (56 years), the association between dage and MACE was maintained only in younger subjects, even after adjusting for confounders [HR = 1.09(1.03–1.16); $p = 0.003$], including PCDU parameters.
Conclusions: In subjects consulting for ED, dage is associated with an adverse cardio-metabolic profile and with incident MACE, in particular in low-risk men. The prediction of MACE by dage is independent from other risk factors including PDCU parameters, so that it can be used as a costless and safe surrogate marker of penile vascular damage.

SD10

Psychological, relational, and biological correlates of ego-dystonic masturbation in a clinical setting

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Background: Attitudes toward masturbation are extremely varied, and this practice is often perceived with a sense of guilt.
Aim: To evaluate the prevalence of ego-dystonic masturbation (EM), defined as masturbation activity followed by a sense of guilt, in a clinical setting of sexual medicine and the impact of EMon psychological and relational well-being.
Methods: A series of 4211 men attending an andrology and sexual medicine outpatient clinic was studied retrospectively. The presence and severity of EM were defined according to ANDROTEST items related to masturbation, determined by the mathematical product of the frequency of masturbation and the sense of guilt after masturbation. Clinical, biochemical, and psychological parameters were studied using the Structured Interview on Erectile Dysfunction, ANDROTEST, and modified Middlesex Hospital Questionnaire.
Results: Three hundred fifty-two subjects (8.4%) reported any sense of guilt after masturbation. Subjects with EM were younger than the remaining sample (mean age ± SD = 51.27 ± 13.43 vs 48.31 ± 12.04 years, $p < .0001$) and had more psychiatric comorbidities. EM severity was positively associated with higher freefloating (Wald = 35.94, $p < .001$) and depressive (Wald = 16.85, $p < .001$) symptoms, and subjects with a higher EM score reported less phobic anxiety (Wald ¼ 4.02, $p < .05$) and obsessive-compulsive symptoms (Wald = 7.6, $p < .01$). A higher EM score was associated with a higher alcohol intake. Subjects with EM more often reported the partner's lower frequency of climax and more problems achieving an erection during sexualintercourse. EM severity was positively associated with worse relational and intrapsychic domain scores.
Conclusions: Clinicians should consider that some subjects seeking treatment in a sexual medicine setting might report compulsive sexual behaviors. EM represents a clinically relevant cause of disability, given the high level of psychological distress reported by subjects with this condition, and the severe impact on quality of life in interpersonal relationships.

SD11

Evaluation of sexual habits of medical students

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Background: The sexual habits are changing of the present generation due to the severe behavior modifying effect of communication possibilities of the present days. Long term effects of these behavioral attitudes are unknown.
Aim: The primary objective of the present study was to assess the sexual habits of medical, dentistry and pharmacist students of the University of Debrecen (Hungary) with the aid of an anonymous questionnaire.
Methods: Age, gender, body mass index were assessed along with commination with sexual content, onset of sexual life, partner relationships, plans of childbearing, distress in regard with sexuality, frequency and type of sexual activity, contraception, satisfaction with sexual education at the university.
Results: Thousand students were invited, and finally 166 people intended to participate (55 males, 111 females. Average age was 23 years (range: 18–30 years). Mean BMI of females was 20, mean BMI of males was 23. 95% of the students had already sexual experience. The onset of the sexual life is 17 years (range 12–21 years). 81%; 36%; 3% of students discuss their sexual life with friends, parents and strangers, respectively. Average length of relationships is 14 and 21 months among males and females, respectively. Students have experience with petting, oral, vaginal and anal intercourse in 96%, 91%, 96% and 23%, respectively. Average number of sexual partners is 4 (1–15), and 47% of students had pickups previously (number of pickup partners is 3). 90% of students apply contraceptive method during sexual activity, most commonly condom or oral contraceptives. Only 11% of students had a request for sexual education at the university.
Conclusion: Results of the present study provide a realistic overview about the sexual habits of university students. Results are similar to previously published American studies, although present study provides additional information regarding sexual habits. Higher rate of anal intercourses and pickups carry the risk of long term infectious complications. Focused education should be considered.

SD12

Gender dysphoria: experience of genito-urinary and sexual reconstruction unit (urgus) of coimbra universitary centre

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Background: *Transsexualism* is defined by Word Health Organization as a 'desire to live and be accepted as a member of the opposite sex, usually accompanied by the wish to make one's body as congruent as possible with one's preferred sex through surgery and hormonal treatment. *Gender dysphoria* refers to the distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned gender, with an estimated prevalence of 0.005–0.014% of male patients and 0.002–0.003% for female patients at birth (DMS-V). The Genito-Urinary and Sexual Reconstruction Unit (URGUS) was created in 2011, and is the only national health system unit doing sex reassignment surgery. URGUS has a multidisciplinary team, including specialists in Psychiatry, Endocrinology, Plastic and Reconstructive Surgery, Urology and Gynecology.
Aim: Evaluate the sex reassignment surgeries and complication rates in URGUS.
Methods: The authors retrospectively evaluated a group of 91 patients observed by Psychiatry. 85 started hormonal treatment and 27 did sex reassignment surgeries. Sex reassignment surgeries, conducted between September 2011 and April 2016, and complication rates were assessed.
Results: Of the 27 patients, female to male (F–M) and male to female (M–F) surgeries were performed in 13 and 14 patients, respectively. In the F-M group (average age of 26.5 years) 9 bilateral mastectomies and 5 phalloplasties were performed; in the M-F group (average age of 32.2 years) 7 breast augmentations, 7 vagino/vulvoplasties and 1 facial feminization surgery were conducted. According to the Clavien-Dindo classification, the number of patients with grade 3 was 8 (29.6%). The most common surgical complication was the urethral fistula of the neophallus (n = 5) with the need of surgical revision in all cases. Four of these patients are able to urinate spontaneously by the neophallus and one is voiding by the urethral fistula. All of the patients with neophallus have a perfectly normal social life.
Conclusions: A multidisciplinary approach is essential for the treatment and to achieve patient satisfaction. Sex reassignment surgery is technically demanding, with the need for multiple procedures and usually re-interventions. Surgical treatment and achieved results are in agreement with international standard of care.

SD13

Penile low intensity shock wave therapy for PDE5i non-responders: A prospective, randomized, placebo-controlled study

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Background: Several animal and human studies have evaluated the role of low-intensity extracorporeal shock-wave therapy (LIST) in the management of multiple disorders such as chronic wounds, peripheral neuropathy and cardiac ischemic disease. LIST is thought to trigger a chain of events that releases angiogenic factors, recruits endothelial progenitor cells, induces neovascularization and enhances blood flow in treated areas. Recently, some studies with contradictory results have assessed the efficacy and safety of this therapy on patients suffering erectile dysfunction.

Aim: Investigate the effects of penile LIST on erectile function in patients suffering erectile dysfunction refractory to phosphodiesterase type 5 inhibitors (PDE5i).

Methods: Prospective, randomized, simple-blind, sham-controlled study. Fifty-eight patients with vasculogenic erectile dysfunction refractory to PDE5i were randomized into two groups. 30 were treated with electrohydraulic low intensity shock waves (1 session/week for 6 weeks; 1500 pulses of 0.10 mJ/mm^2 at 5 Hz) and 28 were treated with a sham probe. Eleven patients withdrew from the study and were lost to follow-up. All patients were evaluated at baseline and 1 month after the end of treatment using validated erectile dysfunction questionnaires. Demographic and clinical characteristics were recorded.

Results: 27 active-treated patients and 20 sham-treated patients completed the one-month follow-up. There was no significant difference between the two groups in baseline characteristics. Baseline five-item version of the International Index of Erectile Function (IIEF-5) mean scores, in the active and sham groups, were 10.0 ± 3.9 and 10.0 ± 4.5, respectively ($p = 0.863$). At baseline, 48.1% of patients in the active group and 50.0% of patients in the placebo group had a positive answer to the Sexual Encounter Profile (SEP) 2 question ($p = 1.000$); 11.1% of patients in the active group and 10.0% of patients in the placebo group had a positive answer to the SEP 3 question ($p = 1.000$). One month after treatment IIEF-5 scores mean changes from baseline, in the active and placebo group, were 1.6 ± 4.7 and 0.5 ± 4.4, respectively ($p = 0.478$). SEP 3 positive responders increased by 18.5% in the active group and by 0% in the placebo group ($p = 0.063$).

Conclusion: In this specific sample, electrohydraulic LIST produced non-significant changes in erectile function at one-month follow up, compared to sham treated patients. Type of energy, intensity, frequency of shockwaves and follow-up length, together with limited sample size, could be in part responsible for this finding. More studies with larger sample size and longer follow-up, comparing different lithotripters and shock wave protocols, are imperative to elucidate the real role of LIST in erectile dysfunction.

SD14

Which are the male factors associated with Female Sexual Dysfunction (FSD)?

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Background: It has been generally assumed that partner's erectile dysfunction, premature and delayed ejaculation play a significant role in determining Female sexual dysfunction (FSD).

Aim: This study aimed to evaluate the role of the male partner's sexual function, as perceived by women, in determining FSD.

Methods: A consecutive series of 156 heterosexual women consulting our clinic for FSD was retrospectively studied. All patients underwent a structured interview and completed the Female Sexual Function Index (FSFI).

Results: FSFI total score decreased as a function of partner's age, conflicts within the couple, relationship without cohabitation and the habit of engaging in intercourse to please the partner; FSFI total score increased as a function of frequency of intercourse, attempts to conceive and fertility-focused intercourse. FSFI total score showed a negative, stepwise correlation with partner's perceived hypoactive sexual desire ($r = -0.327$; $p < 0.0001$), whereas no significant correlation was found between FSFI and erectile dysfunction, premature and delayed ejaculation. In an age-adjusted model, partner's HSD was negatively related to FSFI total score (Wald = 9.196, $p = 0.002$), arousal (Wald = 7.893, $p = 0.005$), lubrication (Wald = 5.042, $p = 0.025$), orgasm (Wald = 9.293, $p = 0.002$), satisfaction (Wald = 12.764, $p < 0.0001$) and pain (Wald = 6.492, $p = 0.011$) domains. Partner's HSD (hypoactive sexual desire) was also significantly associated with somatized anxiety, low frequency of intercourse, low partner's care for the patient's sexual pleasure and with a higher frequency of masturbation, even after adjusting for age. In patients not reporting any reduction of libido, FSFI total score was significantly lower when their partner's libido was low ($p = 0.041$); the correlation disappeared if the patient also experienced HSD.

Conclusion: In conclusion, the presence of erectile dysfunction, premature and delayed ejaculation of the partner may not act as a primary contributing factor to FSD, as determined by FSFI scores; conversely, women's sexuality seems to be mostly impaired by the perceived reduction of their partner's sexual interest.

SD15

The interplay between premature ejaculation and erectile dysfunction: a systematic review and meta-analysis

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Background: The specific determinants and underlying factors linking erectile dysfunction (ED) and premature ejaculation (PE) have yet to be clearly identified.
Aim: To review and meta-analyze all available data regarding the link between ED and PE.
Methods: An extensive Medline Embase and Cochrane search was performed including the following words: 'premature ejaculation', 'erectile dysfunction'. All observational trials comparing the risk of ED in relation to PE were included. Data extraction was performed independently by two of the authors (G.R, G.C), and conflicts resolved by the third investigator (M.M).
Results: Out of 474 retrieved articles, 18 were included in the study for a total of 57 229 patients, of which 12 144 (21.2%) had PE. The presence of PE, however defined, was associated with a significant increase in ED risk (OR: 3.68 [2.61;5.18]; $p < 0.0001$). Meta-regression analysis showed that the risk of ED in PE subjects was higher in older individuals as well as in those with a lower level of education and in those who reported a stable relationshipless frequently. In addition, subjects with PE and ED more often reported anxiety and depressive symptoms and a lower prevalence of organic associated morbidities, including diabetes mellitus, hypertension and dyslipidemia. All the latter associations were confirmed even after adjustment for age. Finally the risk of PE-related ED increased with the increased proportion of acquired ejaculatory problems (adj $r = 0.414$; $p < 0.0001$ after the adjustment for age).
Conclusions: In conclusion, the present data showed that ED and PE are not distinctly separate entities but should be considered from a dimensional point of view. Understanding this dimensional perspective might help sexual health care professionals in providing the most appropriate therapeutic approach to realistically increase patient related outcomes in sexual medicine.

SD16

First generation PDE5i dropout: a comprehensive review and meta-analysis

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Backgroud: The discontinuation rate with phosphodiesterase type 5 inhibitors (PDE5i) remains very high.
Aim: To review and meta-analyze currently available data regarding dropout of the first-generation of PDE5i including sildenafil, vardenafil and tadalafil.
Methods: An extensive Medline Embase and Cochrane search was performed including the following words: 'PDE5i', 'discontinuation'. All observational studies reporting the dropout rate of PDE5i and its specific causes without any arbitrary restrictions were included.
Results: Out of 103 retrieved articles, 22 were included in the study. Retrieved trials included a total of 162 936 patients with a mean age of 58.8 $\pm$ 7.9 years. Prevalence of reported comorbid diabetes and hypertension were 27.7% and 36.9%, respectively. PDE5i were associated with a mean discontinuation rate of 4% per month (almost 50% after one year). This rate was higher in younger subjects and in those reporting a higher prevalence of associated morbidities. Six main reasons of PDE5i dropout were identified in the evaluated trials. Partner-related problems and lack of efficacy represented the most important reasons for PDE5i discontinuation, although no significant difference among factors was detected.
Conclusions: Despite their high efficacy and easy administration, the discontinuation rate and dissatisfaction with PDE5i are still very high. Our data showed that no single factor plays a major role in PDE5i dropout, suggesting that the discontinuation rate is usually due to a combination of both medical problems and psychosocial and relational factors.

SD17

Sexual dysfunction in subjects treated with inhibitors of 5a-reductase for benign prostatic hyperplasia: a comprehensive review and meta-analysis

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Background: Despite their efficacy in the treatment of benign prostatic hyperplasia (BPH) the popularity of

inhibitors of 5a-reductase (5ARIs) is limited by their association with adverse sexual side effects.
Aim: To review and meta-analyze currently available randomized clinical trials (RCTs), evaluating the rate of sexual side effects in men treated with 5ARIs.
Method: An extensive Medline Embase and Cochrane search was performed including the following words: 'finasteride', 'dutasteride', 'benign prostatic hyperplasia'. Only placebo controlled RCTs evaluating the effect of 5ARI in subjects with BPH were considered.
Results: Out of 380 retrieved articles, 15 were included in the study. RCTs enrolled 22761 in the active and 21637 patients in the placebo arms, respectively, with a mean follow-up of 112.9 weeks and mean age of 63.7 years. No difference was observed between trials using finasteride or dutasteride as active arm considering age, trial duration, prostate volume or IPSS score at enrolment. Overall, 5ARIs determined an increased risk of hypoactive sexual desire (HSD OR = 1.47[1.27;1.72]; $p < 0.0001$) and erectile dysfunction (ED; OR = 1.44[1.26;1.64]; $p < 0.0001$). No difference between finasteride and dutasteride on the risk of HSD and ED was observed. Meta-regression analysis showed that the risk of HSD and ED was higher in subjects with lower Qmax at enrolment and decreased as a function of trial follow up. Conversely, no effect of age, IPSS score or prostate volume at enrolment as well as Qmax at end-point was observed.
Conclusion: The use of 5ARI significantly increase the risk of ED and HSD in subjects with BPH. Patient should be adequately informed before 5ARIs prescription.

SD18

Sperm abnormalities are associated with sexual dysfunction according to their severity

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Background: Infertile men are at a higher risk for sexual dysfunction, psychopathological and general health disorders. However, it has never been systematically investigated if these problems are associated with sperm abnormality severity.
Aim: To investigate the relationship between sperm abnormality severity and sexual function in a cohort of males of infertile couples.
Methods: Cross-sectional analysis of a first-time evaluation of a cohort 448 men of infertile couples. In addition, 74 age-matched healthy, fertile men from a Florence spin-off of a European Academy of Andrology-sponsored ultrasound study on male fertility were also studied, for comparison. All subjects underwent a complete andrological and physical examination, hormonal and biochemical assessment, scrotal and flaccid penile colour-Doppler ultrasound and semen analysis. Validated tools, such as the International Index of Sexual Function-15 (IIEF-15), Premature Ejaculation Diagnostic Tool (PEDT), Middlesex Hospital Questionnaire (MHQ), National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI), International Prostate Symptom Score and Chronic Disease Score (CDS), were used to evaluate, respectively, sexual dysfunction, PE, psychopathological traits, prostatitis-like symptoms, lower urinary tract symptoms and general health status.
Results: Among men with couple infertility, 96 showed azoospermia (group #1), 245 at least one abnormality in sperm parameters (group #2) and 107 normozoospermia (group #3) according to World Health Organization. Fertile men were considered as a control group (group #4). After adjusting for age, we observed a higher prevalence of ED (IIEF-15-erectile function domain score <26) and PE (PEDT score >8) in males of infertile couples when compared with fertile men (18.3% vs. 0% and 12.9% vs. 4.1%). The prevalence of ED increases as a function of sperm abnormality severity ($p < 0.0001$), even after adjusting for confounders (age, CDS, MHQ and NIH-CPSI total score), despite similar hormonal, glyco-metabolic and penile vascular status. Compared to fertile men, all three groups of males with couple infertility showed a poorer erectile function, being the latter associated with an overall psychopathological burden (MHQ total score), particularly with somatized anxiety (MHQ-S). Azoospermic men showed the worst erectile function, as well as the most impaired general health. In this group, erectile function was negatively associated not only with psychopathological disturbances (MHQ total and MHQ-S scores; both $p < 0.0001$) but also with a less healthy phenotype (higher CDS; $p = 0.015$). In addition, azoospermic men reported a higher prevalence of PE, lower sexual desire and poorer orgasmic function when compared to fertile men (all $p < 0.05$), and all of these issues were related to psychopathological symptoms.
Conclusions: Our study shows that ED increases as a function of sperm abnormality, independently of conventional organic alterations and associated with mood disturbances. Azoospermic men reported the worst erectile function and general health status closely related to somatized anxiety. In addition, azoospermic subjects also had a higher prevalence of PE, lower sexual desire and poorer orgasmic function, all related to psychopathological symptoms. Investigation of sexual function, general health and psychological status of males of infertile couples, especially if azoospermic, is advisable, in order to improve not only reproductive fitness but also general health and sexual function.

SD19

Cardiometabolic risk and female sexuality: focus on clitoral vascular resistance

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Background: Clinical significance of genital vascular impairment in women and its consequences on sexual

functioning are not well defined. Clitoral colour Doppler ultrasound (CDU) with assessment of pulsatility index (PI), a variable reflecting downstream resistance to blood flow, has been proposed as non-invasive objective measurement of sexual functioning.

Aim: To investigate systematically the associations between clitoral PI and cardiometabolic risk factors, sexual function and self-perception of body image in women seeking medical care for sexual dysfunction.

Methods: Seventy-one adult heterosexual women in a stable heterosexual relationship attending our Sexual Medicine Outpatient Clinic at the University of Florence for sexual dysfunction were consecutively recruited. Patients underwent a physical, laboratory and clitoral CDU examination, and were asked to fulfill the Female Sexual Function Index (FSFI), the Middlesex Hospital Questionnaire (MHQ) and the Body Uneasiness Test (BUT).

Results: Clitoral PI was positively correlated with BMI (r = 0.441; $p < 0.0001$), waist circumference (r = 0.474; $p < 0.0001$), fasting glycaemia (r = 0.300; $p = 0.029$), insulin (r = 0.628; $p = 0.002$), HOMA index (r = 0.605; $p = 0.005$), triglycerides (r = 0.340, $p = 0.011$), total (r = 0.346, $p = 0.010$) and LDL cholesterol (r = 0.334, $p = 0.016$). All the relationships, with the exception of glycaemia, retained statistical significance after adjusting for age and years of menopause ($p < 0.0001$ for BMI, waist circumference, triglycerides and insulin; $p < 0.05$ for all the other associations). At ANCOVA, after adjusting for age and years of menopause, women with obesity or MetS showed significantly higher PI values (F = 17.79, $p = 0.001$; F = 7.37, $p = 0.019$, respectively). In particular, a stepwise increase of PI was found as a function of increasing MetS components (β=0.449, $p = 0.004$).

FSFI arousal and satisfaction scores decreased as a function of PI, even after adjusting for age and years since menopause (β= -0.325, $p = 0.012$; β= -0.292, $p = 0.024$ for arousal and satisfaction, respectively). Clitoral PI was also significantly associated with somatized anxiety symptoms (MHQ-S) even after adjusting for age, years of menopause and current use of psychiatric medication (β=0.351, $p = 0.011$). A positive, independent association was observed between PI and BUT Global severity index (GSI) (β=0.295, $p = 0.049$), Positive symptom distress index (PSDI) (β=0.316, $p = 0.042$) and BUT-B dislike of womb, genitals and breast (β=0.540, $p < 0.0001$; β=0.547, $p < 0.0001$; β=0.648, $p < 0.0001$, respectively). After introducing waist circumference as a further covariate, the associations between clitoral PI and BUT-PSDI, dislike of womb, genitals and breast retained statistical significance ($p = 0.042$ for PSDI and $p < 0.0001$ for the dislike of womb, genital and breast, respectively).

Conclusion: MetS, and in particular insulin resistance, is associated with a progressive increase of clitoral PI. Higher clitoral resistance is also positively related to reduced sexual arousal, increased somatized anxiety symptoms and body image concerns. Specifically designed clinical studies are needed to reveal whether treatment of metabolic abnormalities can induce an improvement in clitoral CDU indices and sexual outcome.

SD20

What can we learn from somatic symptoms in sexual medicine? Somatization in the determinism of male sexual dysfunction

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Background: An important feature of somatic symptom disorder is the subjective perception of the physical symptoms and its maladaptive interpretation. It is possible that they underlie at least a part of the symptoms in subjects complaining of sexual dysfunction.

Aim: To define the psychological, relational, and organic correlates of somatic symptoms in a large sample of patients complaining of sexual problems.

Methods: A consecutive series of 2833 men (mean age 50.2 ± 13.5 years) was retrospectively studied. Somatic symptoms were assessed using the subscale 'somatized anxiety symptoms' of the Middlesex Hospital Questionnaire (MHQ-S). Several clinical, biochemical, psychological, and relational parameters were studied. Patients were interviewed with the previously validated Structured Interview on Erectile Dysfunction (SIEDY), and ANDROTEST (a structured interview for the screening of hypogonadism in patients with sexual dysfunction).

Results: Among the 2833 patients studied, subjects scoring higher on somatic symptoms were older, more obese, reporting unhealthy lifestyle (current smoking, alcohol consumption) and a lower education. Moreover, they reported more often a general impairment of their sexuality, including erectile dysfunction, hypoactive sexual desire, reduced frequency of sexual intercourse and masturbation, and perceived reduced ejaculation. Interestly, we observed a significant association between MHQ-S scoringwith a reduced testosterone level and hypogonadism symptoms. Finally, we found a significant association between somatic symptoms and both SIEDY Scales 1 (organic domain of ED) and 3 (intrapsychic domain of ED).

Conclusions: According with the new DSM-5 position, the present study demonstrates that somatization is an expression of a complex disease encompassing both organic and psychological features. The consequences of this pattern have great clinical relevance in a sexual medicine setting, considering their severe impact on sexuality.

SD21

Erectile dysfunction is common among men with acromegaly and is associated with morbidities related to the disease

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Background: The prevalence of erectile dysfunction (ED) and its correlates in men with acromegaly has never been investigated.
Aim: To evaluate sexual function in men with acromegaly.
Methods: Multicenter-based, retrospective analysis of a non-selected series of 57 acromegalic subjects (mean age: 52.7 ± 14.2 years) was performed. Acromegalic subjects reporting ED (n = 24) were compared with matched ED-patients without acromegaly or pituitary disease (controls), selected from a cohort of more than 4000 subjects enrolled in the Flrence Sexual Medicine and Andrology Unit. Patients were interviewed using SIEDY structured interview, a 13-item tool for the assessment of ED-related morbidities. Several clinical and biochemical parameters were taken. Penile colour-Doppler ultrasound (PCDU) was performed in a subgroup of 37 acromegalic subjects.
Results: ED was reported by 42.1% of acromegalic subjects. After adjusting for age and testosterone, acromegalic subjects with ED had a higher prevalence of hypertension, and more often reported an impairment of sleep-related erections and a longer smoking habit. Accordingly, acromegaly-associated ED was characterized by a higher organic component and worse PCDU parameters. No relationship between ED and testosterone levels or other acromegaly-related parameters was found. However, acromegalic subjects with severe ED reported a longer disease duration. In a case-control analysis, comparing acromegalic subjects with ED-matched-controls free from acromegaly (1:5 ratio), acromegalic men had a worse ED problem and a higher organic component of ED, as derived from SIEDY score. In line with these data, acromegalic patients with ED had a higher prevalence of major adverse cardiovascular events (MACE) history at enrolment and lower PCDU parameters.
Conclusions: Subjects with complicated acromegaly are at an increased risk of developing ED, especially those with cardiovascular morbidities. Our data suggest including a sexual function evaluation in routine acromegaly follow-up.

SD22

High triglycerides predicts arteriogenic erectile dysfunction and major adverse cardiovascular events in subjects with sexual dysfunction

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Background: The atherogenic role of triglycerides (TG) remains controversial.
Aim: To analyse the contribution of TG in the pathogenesis of erectile dysfunction (ED) and to verify the value of elevated TG in predicting major adverse cardiovascular events (MACE).
Methods: An unselected series of 3990 attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample (n = 1687) was enrolled in a longitudinal study. Several clinical, biochemical and instrumental (penile color doppler ultrasound; PCDU) factors were evaluated.
Results: Among patients studied, after adjustment for confounders, higher TG levels were associated with arteriogenic ED and higher risk of clinical and biochemical hypogonadism. Conversely, no association between TG and other sexual dysfunctions was observed. When pathological PCDU parameters, including flaccid acceleration (<1.17 m/s^2) or dynamic peak systolic velocity (PSV <35 cm/s), were considered, the negative association between impaired penile flow and higher TG levels was confirmed, even when subjects taking lipid-lowering drugs or those with diabetes were excluded from the analysis (OR = 6.343[1.243;32.362], p = 0.026 and 3.576 [1.104;11.578]; p = 0.34 for impaired acceleration and PSV, respectively). Similarly, TG levels were associated with a higher of risk hypogonadism, independent of the criteria used for the definition, when the same adjusted model was applied (OR = 2.892[1.643;5.410], $p < 0.0001$ and 4.853[1.965;11.990]; p = 0.001 for total $T < 12$ and 8 nM, respectively). In the longitudinal study, after adjusting for confounders, elevated TG levels (IV quartile;: 162–1686 mg/dL) were independently associated with a higher incidence of MACE (HR = 2.469[1.019;5.981]; p = 0.045) when compared to the rest of the sample.
Conclusions: Our data indicate that TG play an independent role in the pathogenesis of arteriogenic ED in the ED cardiovascular (CV) risk stratification. Whether or not the use of TG lowering drugs might improve ED and its CV risk must be confirmed through specific trials.

SD23

Premature ejaculation and related distress: data from European Male Aging Study

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Background: Premature ejaculation (PE) is the most often reported sexual complaint in men under 60 years. However, there is currently a lack of comprehensive data regarding the prevalence and attitudes of PE among men from the general population, especially in Europe. Available information was mostly derived from specific, non representative, European countries through telephone or web interviews.
Aim: To investigate the prevalence of self-reported PE and its correlates in middle-aged and older men from different countries of the European Union.
Methods: This is across-sectional multicenter survey performed on a sample of 3,369 community dwelling men aged 40–79 years old (mean 60 ± 11 years). Subjects were randomly selected from eight European centres including [Florence (Italy), Leuven (Belgium), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Lodz (Poland), Szeged (Hungary), Tartu (Estonia)]. A self-administered sexual function questionnaire was completed by 3,112 men (93% response rate). PE was defined, using a specific item regarding unsatisfactory control of organs, due to too early climax. PE-related bother was also investigated.
Results: Among subjects with completed questionnaire data, 30.8% reported PE, of which only 7.3% claimed to be bothered. The prevalence of PE increased as a function of age, peaking in the 6th decade (32.7%) and decreasing thereafter. Similar results were observed for PE-related distress (8.8% in the 6th decade). Subjects with PE, and in particular those with a more severe PE-related distress, experienced a higher prevalence of erectile dysfunction, worse overall sexual functioning and couple fitness, reduced quality of life and higher depressive symptoms. Finally, PE was associated with higher prevalence of low urinary tract symptoms, as detected by the International Prostatic Symptoms Score (IPSS). All data were confirmed after adjusting for confounding factors including age, centre, education, smoking, mass-derived total testosterone and associated morbidities.
Conclusion: Present data confirm that PE is the most common male self-reported sexual complaint. However, PE-related concern is relatively uncommon in those who report PE. PE-concern, rather than PE alone, was associated with a more severe impairment of sexual function and with reduced couple fitness and quality of life. This suggests that PE, though common, is mostly a sub-clinical issue in male sexual experience. Further research to identify determinants of the severity and clinical consequences of PE is indicated.

SD24

Conflicts within the family and within the couple as contextual factors in the determinism of male sexual dysfunction

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Introduction: The deterioration of a couple's relationship has been previously associated with impairment in male sexual function. Besides a couple's dystonic relationship, other stressors can unfavorably influence dyadic intimacy. A largely neglected etiopathogenetic factor affecting couple sexuality is the frustration caused by conflicts within the family.
Aim: To evaluate the possible associations between male sexual dysfunction (SD) and conflictual relationships within the couple or the family.
Methods: A consecutive series of 3975 men, attending the Outpatient Clinic for SD for the first time, was retrospectively studied. Conflicts within the family and within the couple were assessed using two standard questions, 'Are there any conflicts at home', and 'Do you have a difficult relationship with your partner?', respectively, rating 0 = normal relationships, 1 = occasional quarrels, and 2 = frequent quarrels or always.
Main outcome measures: Several clinical, biochemical and psychological (Middlesex Hospital Questionnaire, MHQ) parameters were studied.
Results: Among the 3975 patients studied, we observed a high prevalence of conflicts within the family and within the couple (32% vs. 21.2%). When compared with the rest of the sample, subjects reporting both type of conflicts showed a higher prevalence of psychiatric comorbidities, hence all data were adjusted for this parameter and for age. Family and couple conflicts were significantly associated with free floating anxiety, depression symptoms and with a higher risk of subjective (self-reported) and objective (peak systolic velocity at the penile color Doppler ultrasound <35 mm/s^2) erectile dysfunction (ED) and hypoactive sexual desire (HSD). Female sexual function parameters, as reported by the patient, retained a significant association with both type of conflicts.
Conclusions: This study indicates that the presence of often unexplored issues, like conflicts within the family or within the couple, can represent an important contextual factor in the determinism of male SD.

SD25

Correlation between peak systolic velocity and diameter of cavernosal arteries in flaccid vs. dynamic state for the evaluation of erectile dysfunction

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Background and Aim: Previous studies have suggested that parameters measured in Flaccid State (FS) by Penile Duplex Doppler Ultrasound (PDDU) may predict results in Dynamic State (DS). The objective of this study is to evaluate if Peak Systolic Velocity (PSV) and diameter of cavernosal arteries in FS predict the response to intracavernosal injection of 20 mcg of e1 prostaglandin (PGe1).
Methods: Fifty patients who underwent PDDU for erectile dysfunction were prospectively enrolled. PSV and diameter of cavernosal arteries were measured in flaccid and dynamic state, and then correlated.
Results: PSV in FS demonstrated a significant correlation with PSV in DS ($p < 0.05$). Cavernosal artery diameter in FS showed no correlation with PSV after injection. Considering as normal a PSV = 35 cm/seg or =25 cm/seg in DS, three different cut points for PSV in FS were tested to evaluate sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) to detect an abnormal response to intracavernosal injection. None of the values obtained a combined sensitivity and specificity nor PPV and NPV >80%.
Conclusion: Even though, PSV in FS correlates with PSV in DS, predictive values are low, making it non-reliable to accurately foretell the response to intracavernosal injection of PGe1.

SD26

Differential effects of testosterone and estradiol on clitoris function: an experimental study in rats

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Background: Female sexual response is a complex phenomenon in which psychological, neurological, vascular mechanisms and hormonal factors interact. During the arousal phase they cooperate to increase genital blood flow, thus inducing engorgement of the clitoris and lubrication of the vagina. Regulation of vascular and non-vascular smooth muscle tone is the crucial event in the erectile process. Preclinical studies suggest that nitric oxide (NO) is the main vasodilator neurotransmitter modulating, through the second messenger cGMP, the clitoral flow vessels. Aim
The present study aims to investigate the effects of sexual steroid hormones on pro-erectile/relaxant (NO/cGMP mediated) and anti-erectile/contractile (RhoA/ROCK mediated) mechanisms in the clitoris using a validated animal model of female ovariectomized (OVX) Sprague-Dawley rats.
Methods: Subgroups of OVX were treated with either 17β-estradiol (E), progesterone, testosterone (T) or T and letrozole for six weeks. The experimental groups were compared with a control group of intact rats.
Results: Using real-time PCR, we observed that testosterone treatment upregulates the expression of NO-mediated pathway genes (eNOS, nNOS, GCSa3, GCSb3, PKG1, PDE5). Conversely, estrogen replacement upregulates the expression of the calcium-sensitizing RhoA/ROCK pathway genes. We also performed in vitro contractility studies on phenylephrine pre-contracted clitoris strips. OVX resulted associated with a reduced responsiveness to Y-27632, a ROCK inhibitor, which was fully restored by E supplementation. Finally, to further examine the effect of 17β-estradiol on the RhoA/ROCK pathway, we isolated smooth muscle cells from rat clitoris and evaluated their migration capacity.
Conclusion: Collectively, our data demonstrate that testosterone improves the vascular smooth muscle cells' relaxation through the NO/cGMP pathway, and that testosterone and 17β-estradiol are both necessary to maintain a functional contractile and relaxant machinery in the clitoris. This new concept might provide support for the concomitant use of estrogen and T during the treatment of sexual arousal disorders related to hormonal imbalance or insufficiency.

SD27

Satisfaction degree of surgical techniques for penile curvature correction. 6 years of our experience

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Introduction: The curvature of the penis by Peyronie's disease (PD) or Congenital Penile Curvature (CPC) is a common cause of consultation in Andrology and Urology services. These diseases are due to different etiologies and both share the curvature as clinical sign which limits the quality of sexual life mainly by the penile deformation and psychological effects derived from it. Surgery is considered the only effective treatment but it is entails some complication and side effects could appear.
Objectives: To evaluate patient's satisfaction about the results of surgical treatment of PD and CPC, depending on the surgical technique and know the changes that occurred in the quality of sexual life.
Material and Methods: Patients treated with corporoplasty in Andrology Service of the Puigvert Foundation between January/2007 to December/2012 (6 years) were included. All the sample answered a survey with quality life, quality sex life and satisfaction degree domains after surgical treatment with the aim to evaluate the quality of sex life after it; also we determine the side effects of surgery.

Results: The sample was 324 patients: 274 with PE and 50 with CPC. The 16 dots technique was the most surgical technique used (49%) followed by Yachia (25%), modified Nesbit (20%), Patch Surgisis (4%) and vaginal patch (2%). Overall 77% of patients were satisfied with the outcome of the corporoplasty, 80% considered the curvature has been corrected and 79% would recommend the treatment to other patients with the same health problem; there were no significant differences between the different techniques used (p-value not significant) and the evaluated domains. In relation to the changes that occurred in the sexual sphere after treatment, 65% reported improvement, 17% noticed no change and 18% said they had worsened and there was no significant difference in this respect between surgical techniques (p-value not significant). After surgery, 48 patients (14.8%) complained of penile shortening, 26 (8%) curvature recurrence and 14 (4.3%) pain during erections.
Conclusions: In our sample corporoplasty was accepted and highly valued as a treatment for patients with penile curvature with no significant differences in the degree of satisfaction among surgical techniques used. The surgery improved the quality of sexual life in more than two thirds of patients. The main complaint from the surgery was penile shortening. In our study no surgical technique showed superiority about the evaluated items.

SD28

The effect and mechanism of autologous adipose-derived stromal vascular fraction transplant in corpus cavernous of hypertensive erectile dysfunction rat

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Aim: To research the Effect and mechanism of stromal vascular fraction transplant in corpus cavernous of hypertensive erectile dysfunction rat, in order to provide theoretical basis for the treatment of hypertensive erectile dysfunction, open up new way for the treatment of hypertensive erectile dysfunction. Materials and Methods: Thirty weeks of male spontaneously hypertensive rats (SHR) (n = 40) and normotensive Wistar Kyoto rats (WKY, n = 20) were studied,Systolic blood pressure (SBP) was measured by noninvasive blood pressure instrument and erectile function was tested by injecting with apomorphine (APO), the rats were divided into 4 groups, namely A: control group (WKY, n = 20), hypertensive without erectile dysfunction group (SHR, n = 24), stromal vascular fraction transplant in hypertensive with erectile dysfunction group (SHR, n = 8) and PBS transplant in hypertensive with erectile dysfunction group (SHR, n = 8).Will autologous fat tissue of rats by collagenase enzyme elimination filter, centrifugal, SVF after removal of mature fat cells, will receive the SVF by corpus cavernosum injection transplantation in rat corpus cavernosum in group C (2×105 cells/20 μL) in rats. Three weeks later, respectively, the following tests: sponge body pressure (ICP), changes of the erectile function groups of rats.The expression of eNOS gene mRNA and protein was assessed by Western blotting and RT-PCR. Results: SHR group systolic blood pressure (197.47 + 6.82) mmHg, compared with WKY group (125.23–4.65) mmHg difference was statistically significant ($p < 0.01$).After 5v electrical stimulation, group D ICP/MAP value was significantly lower than that of group C (0.30 ± 0.04 V0.74, 0.05 mm) with statistical significance ($p < 0.05$).By Western blot and reverse transcription polymerase chain reaction (RT-PCR) shows that there are differences between the expression of eNOS protein and mRNA in each group, the group D was significantly lower than other three groups, with statistical difference ($p < 0.01$), the comparison between group A and group B and group C difference has no statistical significance ($p > 0.05$). Conclutions: SVF can improve erectile function in hypertensive rats.

SD29

Prevalence of endocrine and metabolic disorders in subjects with erectile dysfunction: a comparative study

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Background: Alterations of gonadal, thyroid, and pituitary hormones, along with metabolic disorders, might be involved in causing erectile dysfunction (ED).
Aim: The prevalence of endocrine abnormalities in two different cohorts from the general and the symptomatic populations of Florence was compared.
Methods: The first group is a general population sample derived from a Florentine spin-off of the European Male Aging Study (EMAS cohort; n = 202); the second group is a series of n = 3847 patients attending our clinic for ED (UNIFI cohort).
Results: Both primary and secondary hypogonadism were more often observed in the UNIFI than in the EMAS cohort (2.8% vs. 0%; $p < 0.05$ and 18.9% vs. 8%; $p < 0.001$, respectively). However, only the second association retained statistical significance after adjusting for age. Compensated hypogonadism was more common in the EMAS cohort (4.4% vs. 8.1%; $p < 0.05$). No statistically significant difference in the prevalence of overt thyroid disorders was observed. Conversely, subclinical hyperthyroidism was more prevalent in the EMAS cohort (2% vs. 4.1%, $p < 0.05$). No significant difference in the prevalence of hyperprolactinemia was detected, while the prevalence of hypoprolactinemia was significantly higher in the UNIFI than in the EMAS cohort (28.2% vs. 17.8%, $p = 0.001$), even after the adjustment for age, BMI, and testosterone ($p = 0.001$). Central obesity (waist = 102 cm), impaired fasting glucose (IFG), and type 2 diabetes mellitus (T2DM) were more often detected in UNIFI patients (31.7% vs. 22.8%, $p < 0.05$; 44.5% vs. 33.3%, $p < 0.05$; 20.1% vs. 1.0%, $p < 0.001$ in the UNIFI and EMAS cohort, respectively), even after adjusting for age. In contrast, the

prevalence of overweight and obesity did not differ between the two groups.
Conclusion: T2DM, IFG, central obesity, secondary hypogonadism, and hypoprolactinemia are more frequent in subjects consulting for ED than in the general population of the same geographic area. Our data suggest that these conditions could play a central role in determining consultation for ED.

SD30

'Forensic Andrology' in the context of Clinical Forensic Medicine

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Background: The examination of victims of sexual violence is part of the regular range of medico-legal activities. Besides capturing vagino-vulvar findings on victims, the inspection of the male genitalia, including collecting evidence, is an important aspect of the forensic investigation. It is not uncommon that during the criminal prosecution an alleged sexual offender states a medical condition leading to an impaired penile penetration capability. This claim must then be verified by a clinical urological examination.
Aim and Method: In this presentation, cases from the interface area between Andrology/Urology and Legal medicine in the context of sexual and violent offences are presented.
Results: In addition to the disclosure of an erectile dysfunction from varying causes, an IPP, consequences of a genital trauma or the alleged presence of a micropenis, a previous urological surgery or psychogenic illnesses are conditions that might be given as reasons for an inability to have performed an accused forced sexual intercourse. In this context we observe an increasing number of cases. Moreover, in some cases an expert opinion, whether a genital damage is permanent or temporary, is required.
Conclusion: In addition to the forensic expertise, the increased occurrence of highly specific andrologic questions during criminal prosecutions of alleged sexual offences, requires sometimes an additional urological assessment. A collaboration between these two medical fields can expand the range of Clinical Forensic Medicine and justify the need for a new specialized discipline – 'Forensic Andrology'.

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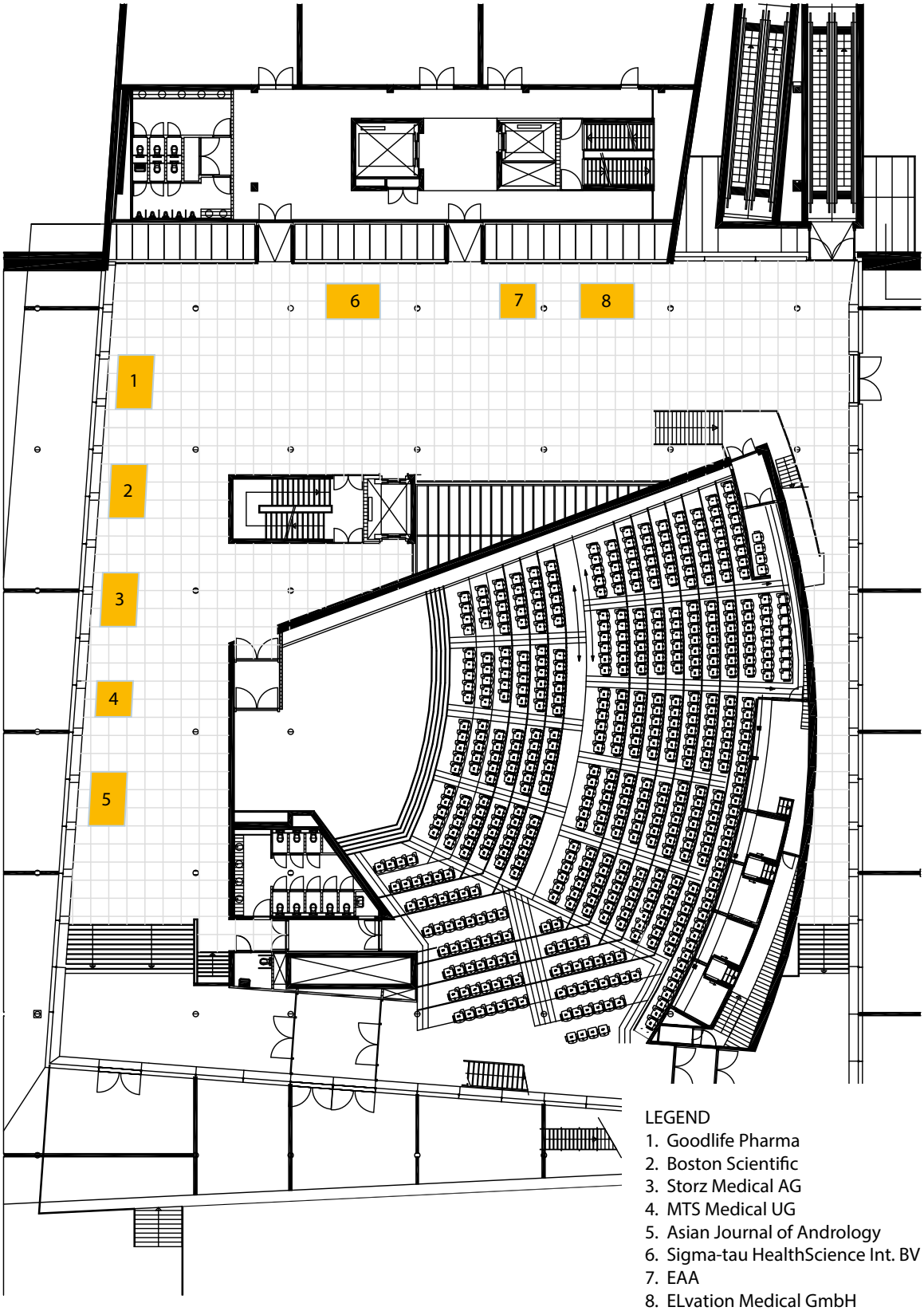
EXHIBITION PLAN



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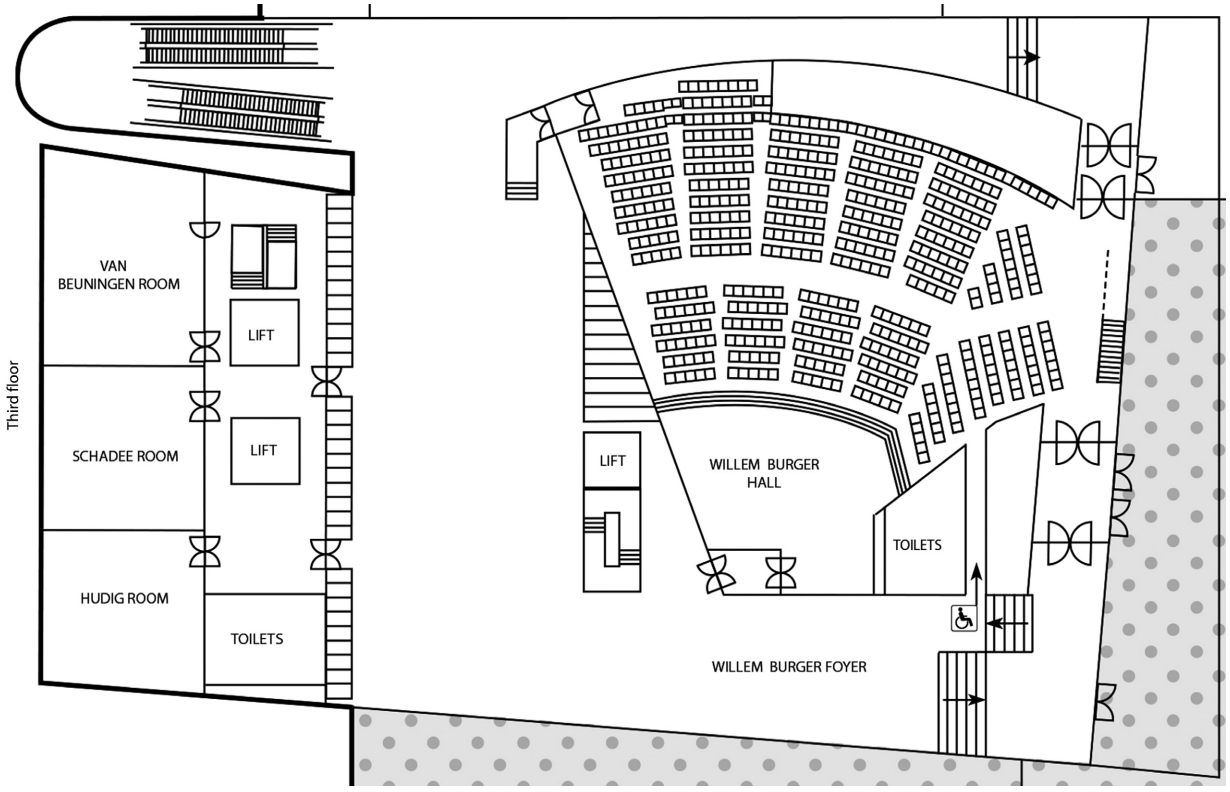

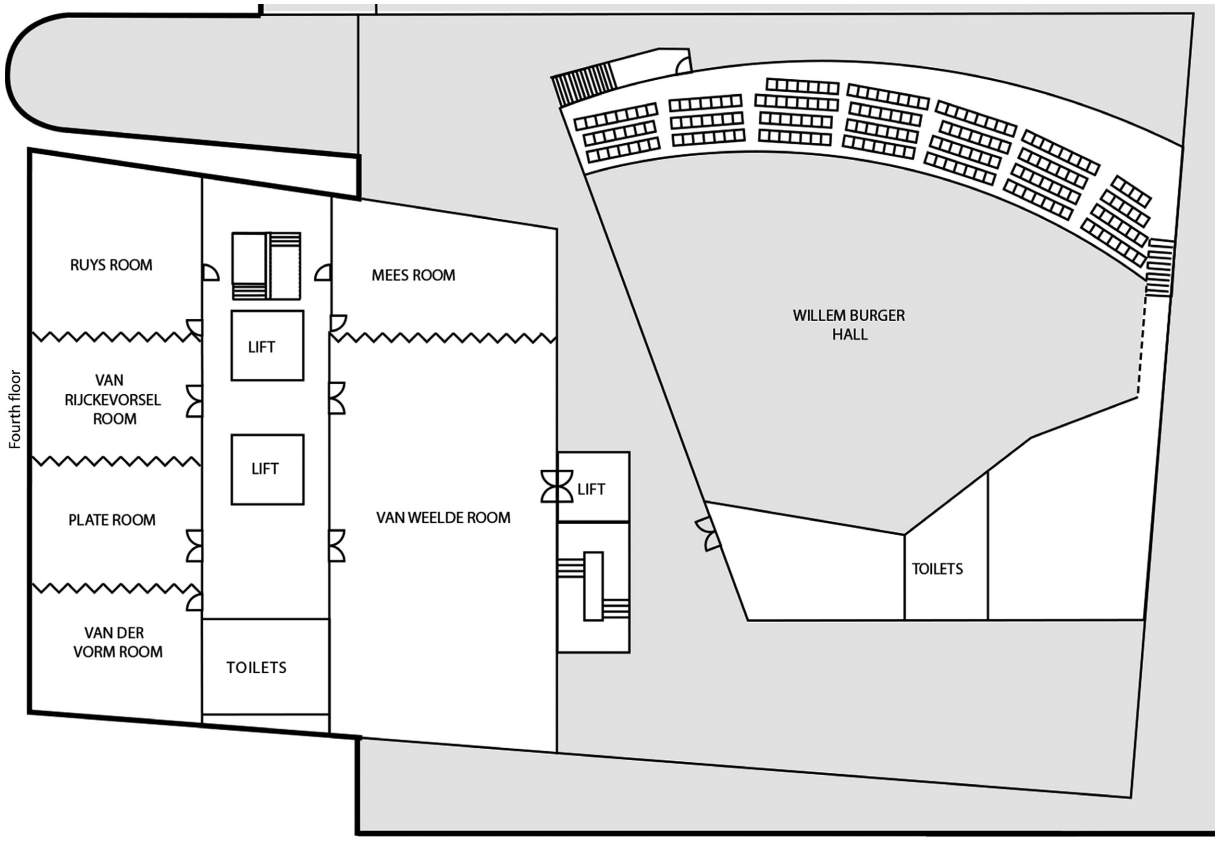

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Melcangi	R.C.	SD08	Post-Finasteride persistant side effects on neuroactive steroids.	32	PS2C
Micic	S.	MIT07	DBPC study in oligoasthenospermic men treated with metabolic and essential nutrients showed that progressive sperm motility was correlated to seminal carnitine levels.	28	PS2A2
Micillo	A.	MID15	Semen leukocytes and oxidative-dependent DNA damage of spermatozoa in male partners of subfertile couples with no symptoms of genital tract infection	7	PS1A
Mieusset	R.	MID06	Evidence of a new pattern of ejaculation in men with spinal cord injury: ejaculation dyssynergia and implications for fertility	7	PS1A
Mieusset	R.	MID07	The spectrum of renal involvement in male patients with infertility related to excretory-system abnormalities: phenotypes, genotypes, and genetic counselling	7	PS1A
Moreno Mendoza	D.	MIT21	Analysis of sperm parameters, pregnancy rate and complications after varicocelectomy subinguinal microsurgery in infertile men with varicocele visible or palpable	28	PS2A2
Mousa	W.	MIT22	Testicular sperm extraction in patients with testicular tumors and azoospermia or cryptozoospermia	28	PS2A2
Navarro-Costa	P.	MID10	The EAA/EMQN external quality control program critically improves the molecular diagnosis of Y chromosome microdeletions	7	PS1A
Oleszczuk	K.	MID12	Sperm Chromatin Structure Assay in prediction of IVF/ICSI outcome.	7	PS1A
Onyemaechi O Azu	OO	RE12	Histo-morphometric evaluation of testicular parameters in streptozotocin-nicotinamide-induced diabetic rats under antiretroviral therapy: Any role for Hypoxis hemerocallidea?	30	PS2B1
Petrov	S.V.P.	SD01	Evaluation of erectile function and hypothalamo-hypophysis-gonadal axis in patients undergoing methadone maintenance treatment	32	PS2C
Piasecka	M.P.	MID09	No associations between sperm chromatin maturity and ICSI outcomes	7	PS1A

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NAME	INITIALS	ABSTNR	TITLE	SESSION_ ID	SESSION_ CODE
Prahl	AP	MID27	Very high sperm DNA Fragmentation Index without increased Reactive Oxygen Species. Link to hereditary cancer?	7	PS1A
Priskorn	LP	OR01	Is sedentary lifestyle associated with testicular function? A cross-sectional study of 1,210 men	5	GC
Püschl	D.	TC01	Immune privilege and neoplasia in human testis: potential role and functional polarization of macrophages and dendritic cells*	29	PS2A3
Rastrelli	G.	HH10	Predictors and clinical consequences of starting androgen therapy in men with low testosterone: results from the SIAMO-NOI registry	31	PS2B2
Rastrelli	G.	HH11	How to define hypogonadism? Results from a population of men consulting for sexual dysfunction	31	PS2B2
Rastrelli	G.	SD09	Vascular and Chronological Age in Subjects with Erectile Dysfunction: Cross-sectional and Longitudinal Results	32	PS2C
Rilcheva	V.	MID28	Sperm DNA Integrity test - Artificial reproductive procedures outcome and recurrent pregnancy losses	7	PS1A
Rohayem	J.	RE07	Testicular function during puberty and young adulthood in patients with Klinefelter syndrome with and without sperm in seminal fluid	30	PS2B1
Rotter	I.	HH07	Can the lipid accumulation product index indicate sex hormone disorders in aging men?	31	PS2B2
Rotter	I.	HH08	Can the lipid accumulation product index indicate sex hormone disorders in aging men?	31	PS2B2
Ryl	A.	RE11	Morphological, immunohistochemical, hormonal and metabolic evaluation of men prostate with benign prostatic hyperplasia and testosterone deficiency syndrome	30	PS2B1
Saad	F.S.	HH02	Long-term testosterone undecanoate (TU) therapy improves glycaemic control and weight in hypogonadal men with type 2 diabetes (T2DM): real-life data from a registry study	31	PS2B2
Sánchez-Curbelo	J	SD27	Satisfaction degree of surgical techniques for penile curvature correction. 6 years of our experience	32	PS2C
Santi	D.	MID02	The influence of environment on the sperm quality: a comprehensive, retrospective, cohort study	7	PS1A
Sevgili	E.	MIG09	Sperm DNA fragmentation and BRCA1, BRCA2 gene promotor methylation in idiopathic infertility	8	PS1B1
Shaeer	K.	SD03	The Global Online Sexuality Survey: ED in USA, 2016	32	PS2C
Slowikowska-Hilczer	J.S.H.	MIT10	Reproductive status and outcome in patients with disorders of sex development	28	PS2A2
Soeterik	T.F.W.	MIT19	Electroejaculation performed in patients with spinal cord injuries: a single-center 21-year experience	28	PS2A2
Sokanovic	S.S.	HH15	Primary hypogonadism in the old male Wistar rats is associated with age related mitochondrial dysfunction	31	PS2B2
Stouffs	K.	MID14	AZFb deletions compatible with sperm production?!	7	PS1A
Taha	E.	MID01	Impact of pyospermia on sperm dynamic motility parameters and DNA integrity	7	PS1A
Taheri Moghadam	M.	MIT06	Effect of Vitamin D on apoptosis and DNA integrity of human sperm	28	PS2A2
Tarsitano	M.G.	OF01	Histological Quantification of Leydig Cell Hyperplasia in Testicular Cancer Patients: Association with long-term Leydig cell dysfunction	27	PS2A1
Thonneau	P.	OR09	Testicular endocrine profiles in young boys operated for cryptorchidism	39	OP2
Tröndle	I.	OR03	Irradiation of juvenile primate testicular xenografts affects the somatic environment	5	GC
Trzybulska	D.T.	MID19	Extracellular miRNAs as biomarkers of male subfertility	7	PS1A
Uppangala	S.	MIT09	Association between sperm DNA damage and human preimplantation embryo metabolism.	28	PS2A2
Valentinas Matulevicius	V.M.	RE05	Clinical importance and (no) consequences of assessement of dehydroepiandrosterone sulfate (DHEAS) in Lithuania (2014)	30	PS2B1
Vignozzi	L.	SD05	The dual FXR/TGR5 agonist INT-767 counteracts nonalcoholic steatohepatitis and erectile dysfunction in a rabbit model of high fat diet-induced metabolic syndrome	32	PS2C
Vignozzi	L.	SD06	Effects of physical activity or metformin on testosterone deficiency and erectile dysfunction associated to metabolic syndrome	32	PS2C
Vignozzi	L.	SD26	Differential effects of testosterone and estradiol on clitoris function: an experimental study in rats	32	PS2C
Vinay	J.	SD13	Penile low intensity shock wave therapy for PDE5i non-responders: A prospective, randomized, placebo-controlled study	32	PS2C
Vinci	S.	OF04	Long Term Effects of Cytotoxic Therapy on The Male Gamete Genome	27	PS2A1
Vloeberghs	V.	MIT16	How successful is TESE-ICSI in non-mosaic 47,XXY syndrome? Cumulative delivery rates in an unselected consecutive patient cohort.	28	PS2A2
Vogt	P.H.	SP05	DDX3Y, the major AZFa gene and its X homologue, DDX3X, control human male germ cell maturation before and after meiosis	10	PS1B3

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NAME	INITIALS	ABSTNR	TITLE	SESSION_ ID	SESSION_ CODE
Whitfield	M.W.	OR11	The LXR-null mice: a model for dyslipidemia-induced male infertility and capacitation impairment	39	OP2
Wolski	J.K.	MIT20	M-TESE: testicular biopsy in men with nonobstructive azoospermia – our experience 2012-2016	28	PS2A2
Xiong	C.	SP01	Immunization of male mice with B-cell epitopes of uPA inhibits fertility	10	PS1B3
Xiong	C.	MIG02	X Chromosome-linked CNVs and Idiopathic Male Infertility in Chinese Han Population	8	PS1B1
Yassin	A.	HH06	Does testosterone therapy protect against high-grade prostate cancer (PCa)? Incidence and severity of PCa in patients undergoing prostate biopsy in a urological office	31	PS2B2

ERRATA

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Abstract **MIG05**: **"Whole Exome Sequencing (WES) in Non-Obstructive Azoospermia"** listed the authors by their first name and did not include one of the two institutions. The correct list of authors and institutions:

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Abstract **TC-01** "**Immune privilege and neoplasia in human testis: potential role and functional polarization of macrophages and dendritic cells"** listed only the first author and one institution. The correct list of authors and institutions:

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Abstract **RE11**: **"Morphological, immunohistochemical, hormonal and metabolic evaluation of men prostate with benign prostatic hyperplasia and testosterone deficiency syndrome"** listed only the first author and incomplete affiliation. The correct list of authors and full affiliation:

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