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SYMPOSIUM OF SCIENTIFIC TRANINING OF THE POLISH SOCIETY OF ANDROLOGY – 18th DAY OF ANDROLOGY

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Report

The Conference of the Polish Society of Andrology – 18th Day of Andrology took place on September 30th – October 1st, 2016 in the Hotel Novotel Marina in Gdansk. The event was organized by the Conference Office from the Karol Marcinkowski Medical University in Poznan in cooperation with the Local Organizing Committee chaired by Dr. Mariusz Łukaszuk and the Scientific Committee chaired by Prof. Jolanta Słowikowska-Hilczer.

The Conference was preceded by a workshop entitled: "The criteria for the legitimacy of sexological intervention: between norm and pathology" conducted by Dr. Robert Kowalczyk, head of the Department of Sexology at the Faculty of Psychology and Human Sciences of the Andrzej Frycz Modrzewski Academy in Cracow.

The Conference was started by the greeting delivered by the Polish Society of Andrology President Prof. Jolanta Słowikowska-Hilczer. Prof. Ewa Rajpert-DeMeyts from the Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet) in Denmark was awarded a statuette and a diploma of the Polish Society of Andrology Honorary Member. Dr. Marta Olszewska from the Department of Reproductive Biology and Stem Cells, Institute of Human Genetics, Polish Academy of Sciences in Poznan, was awarded the Polish Society of Andrology Prize of Young Scientists named by Prof. Michał Bokiniec. Due to the fact that the winner could not attend the Conference, she presented her work through the Skype.

Dr. hab. Renata Walczak-Jędrzejowska announced the completion of work by Polish Society of Andrology and the National Chamber of Laboratory Diagnosticians on Polish Recommendations: "The basic semen analysis according to the standards of the World Health Organization in 2010". Prof. Jolanta Słowikowska-Hilczer presented medical doctors, who in 2016 received the title of clinical andrologist from the European Academy of Andrology (EAA). She announced also publication of the Polish version of the European Association of Urology (EAU) recommendations on male hypogonadism and male sexual dysfunction in the journal "Advances in Andrology *Online*". Prof. Piotr Jedrzejczak introduced the rules of the Polish Society of Andrology Certificate of clinical andrology. Prof. Malgorzata Piasecka discussed the current situation of the journal "Advances in Andrology *Online*".

Apart from the Polish lecturers the scientific part of the Conference was honored with the presence of foreign guests: Prof. Ewa Rajpert-DeMeyts from Denmark, Prof. Gerhard van der Horst and Prof. Stefan Du Plessiss from South Africa, Prof. Aleksander Giwercman from Sweden, Prof. Frederick Wu and Dr. Gulam Bahadur from the United Kingdom. The topics of the Conference included problems of male infertility, urological and endocrinological issues important in andrology, as well as achievements in the basic research on physiology and pathology of the male reproductive system. The results of current andrological research were presented in the form of short oral presentations.

The meeting ended with the invitation to the Polish Society of Andrology Conference – 19th Day of Andrology in 2017 in Krakow given by Dr. hab. Małgorzata Kotula-Balak and Dr. Leszek Bergier.

Abstracts of lectures

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REPRODUCTIVE PARAMETERS IN STZ--INDUCED DIABETIC MALE WISTAR RATS: BENEFICIAL ROLE OF AQUEOUS LEAVE EXTRACT OF *BASELLA ALBA*

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The aim of this study was to investigate the effects of streptozotocin-induced diabetes mellitus on Wistar rat sperm parameters and the possible role and mechanism of aqueous *Basella alba* leave extract on such effects. Forty mature male Wistar rats between 8–10 weeks of age were divided in four experimental groups (n = 10), namely; Healthy Control (Group A, oral normal saline 0.5 mL/100g daily), Diabetic Control (Group B, oral normal saline 0.5 mL/100 g daily), Healthy Treatment (Group C, Extract 200 mg/kg daily by gavage) and Diabetic Treatment (Group D, Extract 200 mg/kg daily by gavage). Diabetes was induced in groups B and D with a single intraperitoneal injection of streptozotocin (55 mg/kg). Fasting blood sugar and body weights were recorded during the 4 weeks treatment period. All animals were sacrificed at the end of treatment and blood samples, testes, and epididymis were collected and assayed. Weights (body, testes, epididymis) were measured, a semen analysis performed and serum gonadal hormone levels assessed. The results showed a significant ($p < 0.05$) decrease in body weight as well as testicular and epididymal weight in the untreated diabetic rats when compared to the healthy control. However, the relative organ weight of the epididymis (expressed as a % of bodyweight) was not affected by diabetes. There was a significant ($p < 0.0001$) increase in the relative testicular weights of both diabetic groups when compared to healthy controls. Likewise, sperm concentration, percentage viability and morphology, were all significantly ($p < 0.0001$) reduced in the untreated diabetic rats compared to the control rats. This improved significantly ($p < 0.05$) in the diabetic rats treated with the plant extract. The effect of diabetes and treatment with *Basella alba* on sperm motility was not statistically significant. However, the untreated diabetic group showed significant increases in serum testosterone ($p = 0.0002$) and luteinizing hormone ($p < 0.0001$) when compared to any of the other three groups. There was no significant effect on serum follicle stimulating hormone levels. We concluded from these findings that an aqueous extract of *Basella alba* played a major role in ameliorating some of the male reproductive complications caused by diabetes mellitus.

Gulam Bahadur

IMPROVING IUI PREGNANCY RATES WHILE OVERCOMING MALE FACTOR INFERTILITY

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Intrauterine insemination (IUI) has been a poorly practised and preferentially in favour of more expensive IVF (*in vitro* fertilization) treatment, which have greater associated risks and complexity. It is becoming more apparent that IUI if practised efficiently can provide equally good pregnancy rates, and benefit a global subfertile population who cannot access IVF facilities. The added costs necessary to achieve one additional healthy child in the IVF-SET (IVF-single embryo transfer) group compared with IUI-COH (IVF-controlled ovarian hyperstimulation) was €43,375 while the Cochrane reviews dismiss multiple birth rate concerns in the IUI procedure.

Often the limitation of IUI is defined by the availability of motile progressive sperm and the field has been severely restricted, thereby allowing the liberal use of ICSI procedures. In clinical settings one of the most patient requests is for improving the man's semen quality for which there is no established or tangibly proven procedure. There never has been consensus on optimal abstinence for subfertile men because the WHO 2010 reference range if 2–7 days is derived from fertile males in a number of countries over three continents. We introduced the concept of 'consecutive ejaculate' as a way of profiling subfertile men treatment and with a view to increasing the number of motile progressive sperm for insemination. We concluded, there was a significant improvement in the sperm motility, progression and morphology associations with no detriment to the sperm concentration in the consecutive ejaculate. Concentrations of rapid grade (Grade A) spermatozoa were significantly higher in the consecutive ejaculate. Favourable semen characteristics in consecutive ejaculates with short abstinence in subfertile men suggests this needs to be routinely investigated, as it could have a profound alteration in the management of the couple's subfertility investigation. While some clinics occasionally ask for a consecutive ejaculate it was surprising that semen parameters with very short abstinence were never published. In our work we introduced the idea of 'consecutive ejaculates' having an abstinence of under 30 minutes. Newer data elsewhere on sperm with shorter abstinence times suggest lowered DNA fragmentation rates along with other favourable molecular markers, although it would be early to speculate of the clinical benefits of a pregnancy outcome (*Bahadur et al.*: Reprod Biomed Online. 2016, 32, 323–328).

Following a number of changes such as IUI stimulation protocols away from clomid towards hMG (human menopausal gonadotropin), coupled with the use of `consecutive ejaculates' our clinics have made progressive improvements over the years to shift pregnancy rates

from around 7% per cycle to around 20% per cycle, with almost 25–33% of the cohort becoming pregnant. It appears the biggest determinant in the success of IUI is the clinical management of the patient. Generally clinics need a database to allow a real time monitoring of the progress of the IUI programme. We assert that the male patient be exhaustively assessed on a case by case basis to enable simpler low cost treatment option IUI treatment to be realistic and effective (*Bahadur et al.*: Hum Reprod. 2016, 31, 1141–1146).

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IMPACT OF SILVER NANOPARTICLES ON THE PARAMETERS OF ANTIOXIDANT DEFENSE IN THE GTESTIS OF MALE WISTAR RATS

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Metal nanoparticles, in particular silver nanoparticles (AgNPs) are among the most widely used nanomaterials. Silver nanoparticles because of its strong antibacterial effect are extensively used in personal care products, storing and processing of foods or in medical utilities. Due to the increasing prevalence of AgNPs in everyday life it is necessary to examine the effects of their impact on living organisms. The aim of the study was to determine the effect of a single administration of AgNPs on selected parameters of antioxidant defense in the testis of male Wistar rats.

The experiment was conducted on 84 adult male Wistar rats were divided into four groups: a control group and 3 experimental groups receiving intravenously AgNPs (20 nm of dimeter) at a dose of 5 mg/kg body weight (AgI group) or 10 mg/kg body weight (AgII group), or AgNPs (200 nm of diameter) at a dose of 5 mg/kg body weight (AgIII group). 24 hours, 7 days, and 28 days after the single injection, the animals were anesthetized and the testis were dissected. The antioxidant defense genes expression: superoxide dismutase (*Sod1*), glutathione peroxidase 1 and 4 (Gpx1, Gpx4), catalase (Cat), glutathione S-transferase pi 1 (Gstp1) and gene expression of heme oxygenase 1 (Ho1) in the testis were determined using polymerase chain reaction (real-time PCR) method.

The results indicated increase of gene expression (when comparing with control groups), including *Ho1* in AgI, AgII and AgIII groups ($p \leq 0{,}01$), *Cat* in AgIII group ($p \leq 0.05$) *Gpx4* in AgI and AgIII groups ($p \leq 0{,}01$), *Gstp1* in AgII and AgIII groups ($p \leq 0.05$) and *Sod1* in AgI and AgIII groups ($p \leq 0.05$).

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INFLUENCE OF BACTERIOSPERMIA AND LEUKOCYTOSPERMIA ON CONVENTIONAL AND NONCONVENTIONAL SEMEN PARAMETERS

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Urogenital tract infections are a growing problem in clinical andrology. Recent reports have demonstrated a wide range of microorganisms detected at the level of significant bacteriospermia (>10^4 CFU – colony-forming unit/mL) without clinical symptoms (*Hou et al.*: Fertil Steril. 2013, 100, 1261–1269). There are an increasing number of experimental studies which confirm the harmful effect of individual bacterial strains, both pathogenic and potentially pathogenic, on mature male gametes (*Fraczek* and *Kurpisz*: Folia Histochem Cytobiol. 2015, 53, 201–217). Invasion of bacteria into the male genital tract is associated with massive infiltration of activated leukocytes. However, clinical consequences of an increased number of seminal leukocytes in the presence or absence of an infectious agent (leukocytospermia coexisting with bacteriospermia and isolated leukocytospermia, respectively) still remain unexplained. The aim of the study was to analyse the influence of asymptomatic bacteriospermia and/or leukocytospermia on conventional (standard semen analysis) and nonconventional (subcellular changes of sperm) semen parameters in heathy males at the reproductive age.

Both bacteriospermia and leukocytospermia had a deleterious effect on standard semen parameters including sperm cells concentration, motility and morphology. The results of sperm membrane integrity, mitochondrial activity and DNA fragmentation have revealed that bacteria mainly participate in the intrinsic apoptotic sperm death mechanisms. This was evidenced by simultaneous increase in phosphatidylserine externalization, decrease in mitochondrial transmembrane potential, and increase in DNA fragmentation, in semen samples with bacteriospermia. Results

also showed a negative influence of leukocytes on sperm membrane lipid peroxidation and oxidoreductive capability of sperm mitochondria. In this context, oxidative stress may play a relevant role in diminished sperm conventional parameters during leukocytospermia.

In summary, the obtained results indicate the possibility of direct effect of both inflammatory mediators, bacteria and leukocytes, on subcellular parameters of male gamete, important for its biological function. The value of these observations may be important for the development of new algorithms for diagnosis and treatment of bacterial infections in the urogenital tract.

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YOUNG MALE CANCER SURVIVORS – CURED BUT NOT HEALTHY

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Thanks to the improvements of cancer therapy the survival rates for some of the most common malignant diseases of young age have increased significantly exceeding 95% for testicular germ cell cancer (TC) patients and 80% of childhood cancer (CC). However, recent data indicate that both TC and CC survivors (TCS, CCS) are at increased risk of long term morbidity and mortality. These adverse long term effects might be caused by a negative effect of cancer and its treatment on testicular function leading to hypogonadism (HG) and subsequently to serious diseases linked HG. The aim of our study was to: a) Estimate the prevalence of biochemical signs of HG among TCS and CCS; b) Identify disease – and treatment related factors predicting increased risk of HG; c) Elucidate whether there was an association between HG and signs of low bone mineral density, metabolic and cardiovascular disease.

Ninety-two TCS, 131 CCS (mean age 40 and 36 years, respectively; mean follow-up time 9.2 and 25 years, respectively) and a corresponding number of age-matched controls were included. Fasting morning blood samples were analyzed for total testosterone (TT) and luteinizing hormone (LH). The odds ratios (OR) for hypogonadism, defined as ongoing androgen replacement or TT <10 nmol/L and/or LH >10 IU/L, were calculated for TCS and CCS, and for subgroups defined by diagnosis and treatment. Bone mineral density was assessed by means of DEXA scan. Fasting plasma glucose and insulin were determined and in a selected subgroup of not anthracycline treated CCS and controls, two dimensional echocardiography was performed. HG was found in 25% of CCS and 36% of TCS, and was related to treatment modality. The risk of impaired insulin sensitivity and low bone mineral density were increased in patients with HG. Even those CCS who were not given anthracyclines, presented with signs of impaired cardiac function.

Follow-up of TCS and CCS should include assessment of endocrine and metabolic function as well as investigation of bone mineral density. Measures to treat and prevent long term morbidity may be warranted in significant proportion of these young men.

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TRACKING SPERM MOVEMENT IN FOUR DIMENSIONS AND MODELS FOR SPERM FUNCTIONALITY

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Basic semen analysis performed by means of either manual or Computer Aided Semen Analysis (CASA) is the first step in evaluating the quality of a semen sample but neither predict fertility outcome. The emphasis in male fertility research during the last decade has shifted to relate sperm function to both fertilization outcome as well as live birth outcome. The aim of our research was, firstly, to demonstrate the development and application of new quantitative, objective automated sperm functional tests such as sperm cervical mucous penetration (SCMP), hyperactivation (HA), vitality (Vit), hypo-osmotic swelling (HOS), and the acrosome reaction (AR) assay using CASA technology. Secondly, it is important to develop new approaches to contribute to our understanding of sperm function and its relationship to fertility. Laser based studies showed that sperm of most species swim in a helix. Our hypothesis is that reconstructed 3D/4D tracks from 2D tracks will provide new insights on sperm motility patterns and their relationship to fertility.

All the above sperm functional tests (SCMP, HA, VIT, HOS, AR) were tested/performed on human sperm using standard or modified protocols. SCA (Sperm Class Analyzer) CASA software was developed to accurately measure the different sperm parameters for each test objectively for potential fertility as obtained in the relevant literature (SCMP >5 mln sperm/ejaculate; HA > 20% of motile population; Vit and HOS >58% and for AR at least a difference of 15% between initial acrosome intact and acrosome reacted). Application of these newly developed CASA sperm functional tests showed good discrimination between good and poor sperm samples.

The most applicable kinematic parameters to formulate the 3D helix construction are amplitude of lateral head displacement (ALH, radius of helix), straight line velocity (VSL, distance from the first point to last point of track) and beat cross frequency (BCF, frequency of average path velocity/curvilinear velocity – VAP/VCL). This will describe the "distance between the 2D peaks" and as a first approximation we used VSL/(BCF/2).

The results obtained from this initial modelling showed that it is possible to construct 3D tracks from 2D CASA tracks. By simply changing any of the kinematic parameters ALH, BCF or VSL we can model spherical helices of almost any type. Furthermore, three new parameters derived from the helix model relate to length as a function of speed and curvature and torsion as components of the helix.

In conclusion, it is possible to measure several important sperm functional aspects objectively and routinely using CASA. In addition construction of 3D/4D sperm motility tracks may add important new perspectives to the meaning of sperm motility patterns. The important challenge in future is that we need to relate all these aspects to live birth outcome.

Piotr Jędrzejczak

MALE CONTRACEPTION – CURRENT PROBLEMS

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Unwanted pregnancy is a major global problem and with increasing world population there is a tremendous need for the development of effective male contraceptive method. Among all the approaches undertaken for the development of new male contraceptives, hormonal methods are potentially closest to a possible clinical application. The hormonal approach to male contraception is based on the suppression of gonadotropins leading to reversible inhibition of the spermatogenetic process. Supplementation with androgen is required to maintain physiological levels of testosterone and consequently androgen-dependent physiological functions.

Current strategy focuses on the administration of testosterone with a progestin. It was confirmed that sperm suppression achieved through such regimens may provide efficient contraceptive protection. One of the main obstacle for the development of hormonal male contraception is, that no major drug company has shown interest in leading this project to a market that does not seem profitable. In addition to approaches based on the hormonal compounds, there is a need to develop non-hormonal methods by identifying novel targets for contraceptive intervention. The goal may be either to inhibit spermatogenesis or to render sperm non-functional. The small molecule JQ1 (thieno-triazolo-1,4-diazepine) was designed as a prototype that effects at the level of spermatocyte to spermatid maturation. Vitamin A metabolism has long been considered as a target for male contraception. Retinoic acid receptor (RAR) antagonist (BMS-189453) causes a failure of spermatid alignment and sperm release. Adjudin is another potential compound for inhibiting sperm maturation at the testicular level. CatSper (sperm-specific Ca^{2+} channel) is essential for sperm capacitation. Studies for the development of pharmaceutical CatSper antagonist have been reported. Many observations would suggest that if new contraceptives for men were available, many couples worldwide would use them, so there is a need for future research.

Grzegorz Jakiel

THE ROLE OF ANDROLOGIST IN THE INFERTILITY CLINIC

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According to Polish Society of Andrology (PTA) definition, andrology is the area of the medical science responsible for the physiology and the pathology of male reproductive system in aspects of basic science, diagnostics and therapy. The andrologist is defined as a person conducting researches or simply having knowledge in the area of andrology.

Person working in the infertility clinics must be licensed in the one of two regulated by law professions: medical doctor or laboratory diagnostician. The law regulation is obligatory and completely independent from different certification: Polish one e.g, PTA certificate, as well as international e.g. European Academy of Andrology (EAA).

The laboratory diagnostician working in the infertility clinic should be able to perform the semen investigation according to Word Health Organization (WHO 2010) recommendations and concomitant specialty tests as hyaluronan binding assay (HBA), MAR test and DNA sperm fragmentation test. The sperm selection methods for assisted reproductive technologies (ART) as intracytoplasmic morphologically selected sperm injection (IMSI), the annexine selection and physiological intracytoplasmic sperm injection (PISCI) are also necessary competencies. The professional role of the laboratory diagnostician is complementary to the medical doctor responsibility but not exchangeable. The expertise in the laboratory practice is not essential in daily medical practice.

The medical doctor – andrologist is the member of the medical team of the infertility clinic and have the qualifications according to requirements for gynecology endocrinology and reproduction medicine. The qualifications should be enlarge by the specialty andrology knowledge.

Male factor is responsible for the half of the cases of the couple infertility in accordance to the actual data. The andrologist should be able to predict the male fertility based on diagnostic procedures and to manage the treatment in case of infertility. The first step is the analysis of the possibility for the casual treatment. The female fertility potential should be also consider. In case of the impossible causal treatment or the lack of success, the andrologist should propose ART method adapted to the needs of the treated couple. The proposed treatment plan

need to be based on the female fertility potential and answers of following questions:

1. Is the *in vitro* fertilization (IVF) necessary or maybe intrauterine insemination (IUI) is adequate for clinical status?
2. Is the micromanipulation necessary?
3. Is the sperm selection necessary?
4. Is the usage of the ejaculated sperms possible or microsurgical epididymal sperm aspiration (MESA) or testicular sperm aspiration (TESA) must be performed?
5. Is the pharmacological pretreatment necessary before sperm retrieval?
6. Is the medical status of patient indication for preimplantation genetic diagnosis (PGD)?

The presence of andrologist in the therapeutic team allows to personalize the treatment and to reach the more effective results. The optimal situation is the andrologist with the gynecological background. The specialty in gynecological endocrinology and andrology could be considered.

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PHTHALATE EXPOSURE AND MALE FERTILITY

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During the past decades a possible degradation in human semen quality has been debated intensively and has become an important public health issue. A controversial review article of 61 studies analyzing sperm concentrations in fertile men and in men of unknown fertility published between 1938 and 1990 by *Carlsen* and coworkers (*Carlsen et al.*: BMJ. 1992, 305, 609–613) showed a significant decrease in sperm concentrations and in semen volume. Although a tendency to decreasing semen quality over time has not been firmly established to date, such a possibility had raised new concerns about man made environmental endocrine disrupting factors such as phthalates, parabens, synthetic pyrethroids, bisphenol A which might affect human fertility. The aim of the study was to assess the association of phthalate metabolites levels in urine with semen parameters (sperm concentration, motility, morphology, CASA parameters – computer aided sperm analysis), sperm chromatin structure, sperm aneuploidy and reproductive hormones.

The study population consisted of 329 men who were attending an infertility clinic and had normal semen concentration (> 15 mln/mL) (WHO 2010, World Health Organization). Participants were interviewed and provided a semen sample. The phthalate metabolites were analyzed in the urine using a procedure based on the tandem mass spectrometry method (LC–MS/MS). The analyzed metabolites provided information about the exposure of men on the following phthalate: diethyl phthalate (DEP), monoethyl phthalate (MEP), di-(2-ethylhexyl) phthalate (DEHP), mono-(2-ethyl--5-hydroxyhexyl) phthalate (5-OH-MEHP), mono-(2--ethylhexenyl) phthalate (MEHP), diisobutyl phthalate (DBP), mono-n-butyl phthalate (MBP), benzyl butyl phthalate (BBzP), monobenzyl phthalate (MBzP), diisononyl phthalate (DINP), monoisononyl phthalate (MINP).

Urinary phthalate metabolites levels were significantly associated with a decrease in sperm motility (5-OH-MEHP, MEHP, MINP), CASA parameters (MBP), testosterone level (MEHP) and with an increase in sperm DNA damage (MBP) and sperm aneuploidy (MBzP, MBP, MEHP, MEP). In view of the importance of human reproductive health and the widespread usage of phthalates, it is important to further investigate these correlations.

Dariusz Kałka

MEDICAL POSITIVISM – SEX AND HEART IN A MAN´S LIFE

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Erectile dysfunction (ED) describes the prolonged inability to obtain and/or keep an erection sufficient enough to have satisfying sexual activity (the symptoms must last for minimum three months, unless ED is linked to trauma or a surgical procedure). It is estimated that 3 mln men in Poland are affected by ED and in the population of patients suffering from cardiovascular diseases (CVD) almost 80% are affected. Erectile dysfunction is directly associated with and CVD. Moreover, ED caused by dysfunction of the endothelium as well as atherosclerosis of penile arteries can be the first and only symptom of CVD with its severe complications. This situation is determined by the diameter of vessels – penile arteries are smaller and therefore blood flow impairment occurs eralier than in larger coronary arteries (*Montorsi et al.*: Eur. Eurol. 2003; 44: 360–364). Moreover, ED as well as CVD are characterized by the same modifiable risk factors such as: arterial hypertension, obesity, diabetes, hyperlipidemia, tobacco smoking and a sedentary lifestyle.

Sexual activity, especially in patients with CVD is often regarded as risky behaviour which could lead to life-threatening complications. However, opposed studies revealed evidence that in the majority of patients after myocardial infarction, sexual activity has a minimal risk of complications and can be performed without any limitations. The energetic input during this type of activity belongs to the group of medium input, which mobilize the cardiovascular system to moderate work, what makes that the risk of death during sexual activity is reasonably small (*Levine et al.*: Circulation 2012; 125 (8), 1058–1072).

According to the guidelines of the European Association of Urology from 2015, phosphodiesterase inhibitors type 5 (PDE5) together with vacuum erection devices and promising high hopes and treatment with low energy shock-wave therapy (LESWT), make up the first line treatment of ED. The administration of vasorelaxing agents directly into the corpus cavernosum belongs to the second-line treatment and third line treatment is composed of the implantation of a penile prostehsis (*Hatzimouratidis et al.*: 2015, http://uroweb.org/guideline/male-sexual-dysfunction/).

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COMPARISON OF MANUAL AND COMPUTER AIDED SPERM MORPHOLOGY ANALYSIS

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Sperm morphology assessment can be done in two ways: manual and Computer Aided Sperm Morphology Analysis (CASMA). During general semen analysis, both manual sperm morphology and its indices are calculated: 1) teratozoospermia index (TZI), which defines the coexistence of head, midpiece, tail defects and excess residual cytoplasm in abnormal spermatozoa (mean number of defects), 2) sperm deformity index (SDI), the number of the defects divided by the total number of normal and abnormal spermatozoa (mean number of defects) and 3) multiple anomalies index (MAI), the mean number of head, midpiece and tail defects per abnormal spermatozoon,

The aim of the research was to compare manual sperm morphology analysis versus CASMA and to evaluate sperm morphology indices as sperm quality predicting factors.

During the research 76 semen samples were examined according to WHO (World Health Organization) 2010. They were stained with the Papanicolau method (modified for staining spermatozoa) and examined using both manual and CASMA analysis (300 spermatozoa per patient). A detailed analysis of morphology and its indices was performed in 51 samples. Calculated indices were also compared with other semen parameters, such as vitality, progressive motility and total motility.

Bland and Altman analyses, as well as Passing and Bablok regressions confirmed that there is no significant differences between manual sperm morphology analysis and CASMA ($p = 0{,}48$). Highly significant correlation coefficients (r_s, rank Spearman correlation coefficient) between calculated indices versus the percentage normal sperm morphology were respectively established ($p < 0.0005$, $r_s = -0{,}47$ for TZI; $p < 0.0000$, $r_s = -0{,}76$ for SDI; $p < 0.0001$, $r_s = -0{,}53$ for MAI) in CASMA and manual analysis ($p < 0.008$, $r_s = -0{,}37$ for TZI; $p < 0.0001$, $r_s =$ ia0.52 for SDI; $p < 0.0002$, $r_s = -0.50$ for MAI).. Similarly significant, negative correlations were obtained between measurements and sperm motility.

The choice of diagnostic method (manual vs. CASMA) does not affect the outcome of sperm morphology evaluation. There is inversely proportional correlation between morphology sperm indices and percentages of sperm cells with normal morphology and motility. According to this, calculating the indices during general semen analysis is recommended.

Małgorzata Kotula-Balak, Laura Pardyak, Alicja Kaminska, Anna Hejmej, Barbara Bilińska

LEIDIG CELLS IN THE LIGHT OF RECENT RESEARCH

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Two populations of Leydig cells develop in the gonads of mammals: fetal Leydig cells population and adult Leydig cells population that exists during the male lifetime. Interestingly, in a boar the additional population of Leydig cells, perinatal one, has been described (*Geiger et al.*: Reprod Domest Anim. 1999, 17, 65–67). In that species the number of Leydig cells as well as the level of secreted estrogens are significantly higher in comparison to other mammals. The particular populations of Leydig cells have distinct morphology, biochemistry and functions. Estrogens secreted by Leydig cells have an effect on tissues by nuclear estrogen receptors (ER). Recent studies indicate the involvement of membrane estrogen receptor associated with G protein (GPER) in the signal transmission by estrogens. On the other hand, the involvement of estrogen-related receptors (ERR) in estrogen signaling in the male reproductive system and specific testicular cells is an unresolved issue. The role of estrogens in the Leydig cell function is also not fully understood. Research suggests that these hormones regulate such cellular processes as differentiation, proliferation, apoptosis and tumor transformation. The development of Leydigioma can be inhibited by administration of steroids, which inhibit proliferation and reduce aromatase activity in these cells (*Panza et al.*: Am J Pathol. 2016, 186, 1328–1339). In recent years, attempts are made to explain basic mechanisms of environmental estrogens (xenoestrogens) action and their participation in endogenous estrogen signaling. In Leydig cell, xenoestrogens can modify the activity of receptors binding estrogens through both genomic and non-genomic mechanisms by modulation of action of transcription factors and

proteins important for steroidogenesis. Also, the interaction of estrogenic compounds with receptors for other ligands such as aryl hydrocarbon receptor (AHR) is possible (*Zarzycka et al*.: Theriogenology. 2016, 86, 674–686). As a result, disturbances of hormonal balance required for the proper functioning of reproductive system are induced.

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THE IVF AND MALE FACTOR INFERTILITY

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As defined by the World Health Organization (WHO), infertility is defined as the absence of pregnancy after a year of sexual intercourse without the use of any contraceptive method. Epidemiological data estimate that may affect approximately 15% of couples. Among the causes of infertility significant role attributed to male factor. According to some data may constitute from 25% to 50% of the causes. The most important group of male infertility can be defined as: primary secondary ,obstructive and idiopathic. Male infertility is often a complex etiology and nearly half of the causes are undiagnosed despite performing, many hormonal, immunological, tests and sonography examinations . Sperm quality is still the primary method used to assess male fertility and one of the factors for predicting the chance of pregnancy. In 2010, the WHO defined new reference values for sperm parameters to discriminate between normal and abnormal semen samples. Abnormal semen are classified as oligozoospermia (O), asthenozoospermia (A), teratozoospermia (T), a combination of the these parameters and lack of spermatozoa in ejaculate – azoospermia. In the presence of male factor, described in 1992 intracytoplasmatic sperm injection (ICSI), has an advantage over the use of conventional /classical procedures of *in vitro* fertilization (IVF) Application of this method in cases of azoospermia, or cryptozoospermia, teratozoospermia allowed to obtain the maximum chance of fertilization. It is understood that ICSI is indicated when the total number of motile sperm is <1 mln/mL. Additionally ICSI is performed in cases where the spermatozoa are derived from the testis/epididymis in azoospermia. It should be emphasized, that the results of comparing the classical IVF with ICSI in the presence of isolated teratozoospermia (according Kruger's strict criteria: 4% normal sperm forms) are contradictory. In the last period, it is believed that a better predictor of getting clinical pregnancy is the total number of motile sperm count (TMSC). This parameter is also better predictor for ICSI outcomes than the parameters in accordance with the WHO classification.

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DO SEX ORGANS OF WOMEN AND MEN GROW OLD IN A SIMILAR WAY?

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Female sex organs grow old in physiological conditions in a different way than their male equivalents. In women physiological menopause, determined by hormonal decline, marks the beginning of progressive changes in the ovaries, fallopian tubes and uterus. The ovarian morphology and function alter in postmenopausal women. The ovaries lose their ability to perform the reproductive function. They lack ovarian follicles and the division into cortex and medulla. Instead, *corpora albicantia* and epithelial inclusion cysts can be observed. Morphological changes *i*n the fallopian tubes of postmenopausal women mainly affect epithelial cells, which are shorter, less ciliated, and atrophic. These changes may suggest that there are retrograde lesions in the fallopian tubes, and that this organ gradually loses its function. Morphological changes in the uterus of postmenopausal women, on the other hand, include atrophic lesions in the endometrium and myometrium. However, the proliferation index of the cells of the wall of the body of the uterus (Ki-67) remains unchanged, which allows concluding that endometrium is still ready to play its role in the reproductive process, e.g. after hormonal stimulation. Male menopause is a term introduced on the analogy of menopause in women. Still, it is criticized and rejected by scientific circles. In men, physiological aging of testes, epididymis and prostate progresses gradually. Although, testes tend to lose their hormonal function and spermatogenesis gets disturbed with time, men can retain fertility to the old age. In the testes of aging men, we observe morphological alterations in the seminiferous epithelial and Leydig's cells. In the epididymis there are also epithelial changes. Men over 45 years of age can suffer from benign prostatic hyperplasia, and are more likely to develop prostate cancer. We should not forget that physiological ageing of male and female sex organs may overlap with diseases resulting from genetic predisposition, specific lifestyle and environmental factors.

Yvonne Lundberg Giwercman

FSH-TREATMENT OF INFERTILE MEN

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The adult testis has two functions – producing testosterone and spermatogenesis. These processes are regulated by the pituitary hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Apart from its anabolic and extra gonadal actions, testosterone

is also stimulating spermatogenesis via its action on Sertoli cells. The cells are also the target of FSH, and thus both hormones regulate spermatogenesis in a similar indirect fashion (*Huhtaniemi*: Hormones, 2015, 14, 468–478). The isolated effect of FSH on human spermatogenesis is largely unknown. While it is well known that treatment with gonadotropins is effective in subjects with hypogonadotroph hypogonadism, restoring normal spermatogenesis, attempts to utilize FSH in men with poor spermatogenesis have shown contradicting results and since the classical biochemical picture in male infertility is high FSH, treatment with FSH may be regarded as of no use. However, recent placebo controlled studies have shown that supra physiological doses (300 IU every 2nd day for 4–5 month) are needed to significantly increase sperm counts instead of 75 or 150 IU for 3 months (*Paradisi et al.*: Andrologia 2014, 46, 1067–1072). The question is if all men benefit from FSH-treatment? Genetic studies have shown that in men hypophysectomized due to pituitary tumor, thus lacking FSH and LH, but with an FSH receptor (FSHR) activating mutation, spermatogenesis is normal (*Gromoll et al.*: J Clin Endocrinol Metab. 1996, 81, 1367–1370). This finding indicates that despite testosterone deficiency, as a consequence of absent LH, spermatogenesis can be ongoing. Nevertheless, also men with *inactivating* FSHR mutation can have relatively normal spermatogenesis (*Tapanainen et al.:* Nat Genet 1997;15:205–206). Thus, taken together, strong FSH stimulation seems to be able to compensate for the lack of testosterone and vice versa. Whether polymorphisms, which by definition are genetic variants found in at least 1% of the population can modify FSH action and treatment is an exciting topic and could possibly provide new insight into the role of FSH treatment of male infertility. Two frequently studied polymorphisms in the FSHR gene are A307T and N680S (*Simoni et al.*: J Clin Endocrinol Metab. 1999, 84, 751–755). In a case--control study from Italy, 70 men with oligozoospermia were treated with FSH for 3 months. Only those who were carriers of S680 had improved sperm parameters (*Selice et al.*: Int J Androl. 2011, 34, 306–312), indicating that to be effective, FSH therapy would need stratification according to genotype. Another study showed that men with a certain rare FSH beta subunit could benefit from FSH treatment (*Grigorova et al.*: J Clin Endocrinol Metab 2011;96:E1534–1541). In conclusion, weather FSH treatment of male infertility is efficient, and for whom, is still an open question and prospective studies warranted.

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DIFFERENTIATED ACTION OF ANDROGENS ON MEN'S HAIR

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Hair loss and their graying are associated with advanced age and are supposed to be source of decreased quality of live. Hair follicles are the unique structure that is subject to a continuous process of growth, involution and rest (*Rossi et al.*: Dermatol Ther. 2016, online; *Mirmirani et al.*: Mauritas. 2015, 80, 58–62). The processes of hair growth and their exchange are regulated by many hormones, including androgens, wherein their effects differ depending on the area of the body (*Inoue et al.*: Mol Cell Endocrinol. 2012, 362, 19–28, *Inui et al.*: Exp Dermatol. 2013; 22, 168–171). In men, androgens present in high concentrations, stimulate hair growth on the face, suprapubic area and chest. However, in the skin of the head, they can cause alopecia. A lower concentrations of androgens, in both sexes, stimulate hair follicles of axillary and lower pubic regions (*Elsner et al*: Br J Dermatol. 2012, 166, 2–5). Recent studies indicate that the hair follicles of the various areas of the human body react to androgens by the expression of the androgen receptor (AR) in the hair papilla cells (DPC, dermal papilla cells) (*Yip et al.*: Australas J Dermatol. 2011, 52, 81–88). AR expression and activity of 5α-reductase type II is much higher in the hair papilla cells of the beard area and regions that are subject to androgenic alopecia (*Slominski et al.*: J Steroid Biochem Mol Biol. 2013, 137, 107–123, *Westagate et al.*: Intern J Cosmet Sci. 2013, 35, 329–336). Androgenic alopecia (AGA) occurs in the majority of Caucasian men over 40 years of age. The essence of disease is the miniaturization of hair follicles under the influence of androgens, in people genetically predisposed. In the process of AGA in the hair follicle occur the change of interaction between the DPC and keratinocytes (*Lai et al.*: Arch Dermatol Res. 2012, 304, 499–510). High levels of androgens induce a loss of proliferative properties and apoptosis of DPC, increased expression of oxidative stress markers, like heat shock protein-27 (HSP-27), superoxide dismutase, catalase (*Bathta et al.*: J Invest Dermatol. 2008, 128, 1088–1094). The shorter anagen phase is observed as well as transforming the final hair follicles to the primary hair follicles and formation the shorter, thinner and discolored hair (*Makrantonaki et al.*: Curr Opin Endocrinol Diabetes Obes. 2009, 16, 240–245).

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AFLATOXINS IN THE SEMEN OF POLISH MEN

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The study veryfied the presence of aflatoxins in semen of Polish men. Of the five examined aflatoxins (AF), the presence of at least one was in 58 out of the 60 samples: AFB_1 – 80%, AFB_2 – 10%, AFG_1 – 72%, AFG_2 and AFM_1 – nearly 40% of samples. Concentrations of all examined aflatoxins in the semen of fertile men were lower than in semen of infertile men, but the difference was significant only for AFG_1 and AFG_2. This study also indicated that consumption of coffee, tobacco smoking and rural domiciled might increase in concentration of aflatoxins in human semen. For the first time, the concentrations of aflatoxins in semen of European men were determined. The obtained findings suggest that aflatoxins, even at low concentrations, may have a detrimental effect on male fertility.

Marcin Matuszewski, Wojciech Połom

MALE INFERTILITY – THE UOLOGIST PERSPECTIVE

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Infertility treatment should be undertaken within a multidisciplinary team. In Poland they are usually created by efforts of andrologists, gynecologists and endocrynologists. Urologist may play a vital additional role in such a team, especially as far as diagnostic and therapeutic surgical procedures are concern. What is more, due to a very deeply based opinion among patients as well as among family doctors about the crucial role of urology in the medical issues connected with male reproductive organ, doctors of this specialty are very often consulted and their opinions are very important in the preliminary stage of infertility consultation and treatment. It should be also taken into account that in Poland some political, religious and economic issues cause that some patients coming to see the urologist are not able to count on assisted fertilization techniques.

A lecture will include information about what should be analyzed by the urologist when consulting an infertile male patient. The following issues will be discussed on the basis of European Association of Urology (ECA) guidelines, 2016: 1) testicular dysgenesis syndrome with an importance of microcalcinosis of the testis, 2) varicocoele, 3) infections of the male accesory glands, 4) semen tract obstruction (CBAVD – congenital bilateral absence of the vas deferens, CUAVD – congenital unilateral absence of the vas deferens, Muller duct cyst, ejaculatory duct cyst), 5) TURED technique (transurethral resection of ejaculatory duct), 6) strategy for testicle cancer patients and 7) ejaculation disorders.

Marek Mędraś

ERGOGENIC AGENTS AND THE HYPOTHALAMO-PITUITARY-GONADAL AXIS

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Professional and recreational athletes often use substances that are to enhance sport performance. These are mainly hormones that either directly or indirectly affect the hypothalamo-pituitary-gonadal axis. Testosteron and its derivatives belong to the best known and the most available agents for doping purposes. The problem of the anabolic steroids-induced hypogonadism and the medical approach to such cases will be analysed in the lecture.

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INFLUENCE OF DIESEL ENGINE EXHAUST FROM COMBUSTION OF SECOND GENERATION BIODIESEL FUELS ON SELECTED PARAMETERS OF HORMONAL REGULATION OF REPRODUCTION IN ANIMAL MODEL

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Exhaust emissions resulting from the combustion of diesel fuels are an important source of air pollution, and diesel engines are the largest source of solid particles present in the atmosphere. Due to the necessity to reduce emissions from diesel engines the interest in the use of biofuels is increased. Therefore the aim of the study was to determine the effect of second-generation biofuels SHB20 (with a 20% addition of bio-components) on selected hormonal parameters involved in the regulation of reproductive function in animal model.

The experiment was conducted on 42 adult male Fischer 344 rats. The animals were divided into three groups: control and two experimental: SHB20+DPF (DPF, with diesel particle filter) and SHB20-DPF (no filter). Animals from experimental groups were inhaled air with the exhaust from the SHB20 fuel (1.5% v/v) in whole body inhalation chambers containing (or not) DPF. After 7 and 28 days of experiment half of the animals from each group were euthanized and blood and the testis were taken for further analysis. Plasma hormone levels of testosterone (T), dihydrotestosterone (DHT), and luteinizing hormone (LH) were determined using the enzyme-linked immunosorbent assay (ELISA). In

the testis 17β-estradiol (E2), T, DHT levels and protein levels of androgen receptor (AR), estrogen receptor type α and β (ERα, ERβ) and aromatase (Aro) were also analyzed.

A statistically significant increase in the serum T and DHT concentration as well as intratesticular DHT and protein level of Aro and ERα were observed 7 days after inhalation in SHB20-DPF group compared with the control group. There were no significant changes in the analyzed parameters after 28-day exposure.

The exposure of animals to diesel engine emission from second generation biodiesel fuel influences on the hormonal regulation of testicular function, which may be associated with the action of carbon nanoparticles and/or chemical compounds from the gaseous phase of diesel emission.
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Paweł Osemlak[1,2]

APPLICATION OF TESTICULAR PROSTHESES IN BOYS

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Lack of the testis may be caused in developmental age by anomaly in the form of agenesis, aplasia or atrophy, or the necessity of removing the testis because of torsion and necrosis, traumatic injury or tumor. Lack of the testis or testes in a boy is an indication for implantation of the prosthesis and the strict supervision of a pediatric urologist and endocrinologist. The problem of the use of implants in boys with a lack of testis is now rarely discussed in the literature (in contrast to adulthood). The aim of the studies was to answer to the question what are the long-term results of treatment and at what age operations should be performed.

The author analyzed material of the Clinical Department of Pediatric Surgery and Traumatology in Lublin, which consisted of boys who were implanted testicular prostheses from 2000 to 2010. The age at which the operation was performed, the reason for the absence of testis and cosmetic results (in the common judgment of the physician, the patient and the parents) were determined.

During 11 years in our Department 245 boys underwent implantation of testicular prosthesis. In more than 70% they were in infant and pre-school age. Indications for surgery resulted from lack of the testis caused by anomaly in 65% of cases, and from removing the testis because of torsion and necrosis in 20%. In the rest of boys orchidectomy had been previously performed because of injury or tumor. The prosthesis which had been implanted in early childhood was exchanged one time (for the final prosthesis) after completion of growth of the second testis, and in some cases 2 times – before and after puberty because of the large disparity in size between the testis and the prosthesis. Good outcome (prosthesis lies low in the scrotum, comparable in size with the testis) was achieved in 97% of cases. In the rest 3% of patients prosthesis was located at the top of the scrotum or was damage due to trauma.

The analysis gave conclusion that implantation of the prosthesis as early as possible (i.e. in lack of testis due to anomaly – during infant-preschool age, and due to orchidectomy because of torsion or tumor – in a few months) gives good long-term results because the implant works in a positive way, stimulating the scrotum to grow.

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METHODS FOR DETERMINATION OF SPERM DNA FRAGMENTATION

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Diagnosis of male infertility has traditionally relied upon microscopic assessment to determine human semen quality. However, none of these parameters addresses sperm DNA integrity and their clinical value in predicting fertility is questionable. Moreover, approximately 15% of patients with male factor infertility demonstrate normal spermiograms.

Sperm DNA integrity is required for accurate transmission of paternal genetic information and has been increasingly recognized as a promising biomarker of male infertility and a prognostic test for assisted reproductive techniques (ART) outcomes. There are many possible causes of sperm DNA damage including abortive apoptosis, oxidative stress associated with male genital tract infection, exposure to chemicals and defects of spermiogenesis. Physical factors such as exposure to electromagnetic radiation or scrotal heating can also induce DNA damage in spermatozoa.

Multiple methods used to measure DNA strand breaks have been developed and applied in clinical practice. The current range of sperm DNA tests measure different aspects of DNA damage and have different sensitivities. The most common DNA integrity tests are the sperm chromatin structure assay (SCSA), TUNEL method (the terminal deoxynucleotidyl transferase- mediated dUTP nick end labeling assay), the single cell gel electrophoresis assay (COMET), and Halo-test (SCD, the sperm chromatin dispersion test). Each of these tests provides a semi-quantitative estimate of the general state of DNA but does not provide an indication of specific DNA sequences that might be affected. These techniques provide limited information on the nature of the DNA

lesions detected, and none of them can fully depict the exact etiology and pathogenesis of impairment of sperm DNA integrity. Nonetheless, numerous studies utilizing the above techniques for assessing sperm DNA integrity support the existence of a significant association between sperm DNA damage and pregnancy outcomes. DNA damage in the male germline is associated with poor fertilization rates following *in vitro* fertilization, defective preimplantation embryonic development, and high rates of miscarriage and morbidity in the offspring, including childhood cancer.

Michał Rabijewski

RECENT GUIDELINES ON MALE HYPOGONADISM

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Hypogonadism in men is caused by the reduced secretion and action of testosterone, and is divided into primary (disorders of gonadal function), secondary (hypothalamo--pituitary axis disorders), and peripheral (androgen receptors polymorphism). in recent years it is also recognized late onset hypogonadism (LOH) observed in older men, becouse testosterone levels decrease slightly as a process of ageing: signs and symptoms caused by this decline can be considered a normal part of ageing. Testosterone plays a crucial role in the development and maintenance of male reproductive and sexual functions, body composition, bone health, and behaviour. Signs of testosterone deficiency depend on the age of patients, causes and its severity. In young male hypogonadism should be differentiated with delayed puberty while in older men the most characteristic symptoms of testosterone deficiency are: lack of sexual thoughts, no morning erections and erectile dysfunctions. Testosterone deficiency negatively affects the quality of life, sexuality and increases metabolic disorders (diabetes, obesity, atherosclerosis and osteoporosis). Hypogonadism is also associated with a 3-fold higher overall mortality and mortality subsidiary of cardiovascular diseases. In the treatment of hipogonadism we should take into account patients' age and his cause. In young men is a priority to achieve or maintain spermatogenesis while in older men we use testosterone replacement therapy, which is safe, well tolerated, reduces symptoms of hypogonadism, and improves quality of life. Recently published guidelines of the European Association of Urology (EAU) on the diagnosis and treatment of male hypogonadism, with the aim to provide practical recommendations on how to deal with primary hypogonadism and ageing-related decline in testosterone in male patients, as well as the treatment of testosterone deficiencies. These recommendations thoroughly discuss the indications for therapy, contraindications, side effects, and pay special attention to the potential impact of the therapy on the prostate and cardiovascular risk. This is particularly important in men with LOH because of the lack of specific symptoms, and not established lower reference limit of testosterone level.

Ewa Rajpert-De Meyts

IMPAIRED LEYDIG CELL FUNCTION IN TESTICULAR DYSGENESIS SYNDROME

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Leydig cells (LC) are the main source of androgen in the male throughout development and adult life. LC occur in the human testis as three distinct populations: fetal LC which are essential for masculinisation of the developing fetus, neonatal LC during mini-puberty, and adult LC, which are responsible for maintenance of the reproductive function in adulthood. Severe developmental disruption of androgen signalling (e.g. androgen receptor mutations) often leads to a disorder of sex development (DSD), while milder impairments of the LC function are suspected to play an important role in the pathogenesis of testicular dysgenesis syndrome (TDS). This syndrome comprises reproductive disorders that are linked to poor development of the testis, including testicular germ cell tumours (TGCT) of young adults (associated with germ cell neoplasia *in situ*/carcinoma *in situ*, cryptorchidism, mild hypospadias and impaired spermatogenesis, with small testis size and presence of dysgenic features (*Skakkebæk et al.*: Hum Reprod. 2001, 16, 972–978; *Hoei-Hansen et al.*: J Pathol. 2003, 200, 370–374). A very common feature in patients with TDS disorders is LC hyperplasia and presence of large LC clusters (micronodules), associated with increased luteinizing hormone/testosterone ratio, which may be exacerbated by hCG (human chorionic gonadotropin) secretion in a subset of TGCT (*Holm et al.*: J Pathol. 2003, 199, 378–386)). The presence of these nodules in testicular biopsies of patients with unilateral TGCT, in combination with their reproductive hormone profiles, can predict earlier appearance of hypogonadism in these men (*Tarsitano et al.*: new data). Micronodules are heterogeneous and contain LC at different maturation stages, distinguished by mutually exclusive expression of DLK1 (a marker of progenitor and immature LCs) or INSL3 (insulin-like peptide 3 – a marker of normal adult LC) (*Lottrup et al.*: Hum Reprod. 2014, 29, 1637–1650), and different content of Reinke crystals (*Sørensen et al.*, new data). Our analysis of the global gene expression in microdissected LC micronodules in comparison to fetal and adult LC populations revealed several novel LC maturation markers, and the profiles suggest that the changes in LC morphology and function observed in patients with TDS are linked to the increased adult LC renewal rather than the persistence of foetal cell population (*Lottrup et al.*, new data).

Stefan S. du Plessis

ROLE AND EFFECT OF REACTIVE OXYGEN SPECIES IN MALE REPRODUCTION

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Reactive oxygen species (ROS) play an important role in male fertility. Overproduction of ROS has been associated with a variety of male fertility complications, including leukocytospermia, varicocele and idiopathic infertility. The subsequent oxidative insult to spermatozoa can manifest as insufficient energy metabolism, lipid peroxidation and DNA damage, leading to loss of motility and viability. However, various studies have demonstrated that physiological amounts of ROS play important roles in the processes of spermatozoa maturation, capacitation, hyperactivation and acrosome reaction. It is therefore crucial to realize and define the delicate redox balance in order for a better understanding of both the positive and negative effects of ROS production on fertilizing ability. This presentation will discuss the role of ROS in male reproduction and explore the impact and contribution of various lifestyle factors. Finally, possible treatment options, the latest in ROS diagnostics and reference values will be alluded to.

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SHOULD MEN OVER 50 YEARS OF AGE TAKE MAGNESIUM AND ZINC SUPPLEMENTATION?

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Magnesium (Mg) is a cofactor for over 300 various enzymes in a human organism. It is mainly accumulated in bones, which is where 64% of the total Mg can be found. Mg deficiency favors the development of insulin resistance and type 2 diabetes, as well as lipid metabolism disorders, hypertension, and metabolic syndrome (*Swaninathan*: Clin Biochem Rev. 2003, 24,47–66; *Huang et al*.: Nutr J. 2012, 13, 41). Considering that age-related hypogonadism is more common in men with metabolic disorders, **hypomagnesemia seems to have the potential of contributing to** testosterone deficiency. In their study of elderly men, *Maggio et al*. (Int J Androl. 2011, 34, 594–600) demonstrated a positive correlation between the levels of Mg and anabolic hormones, including total testosterone (TT). *Cinar et al.* (Biol Trace Elem Res. 2011, 149, 18 –23) found evidence for a positive impact of Mg supplementation on TT and free testosterone (FT) levels. The increase in the level of testosterone was higher in those men who combined Mg supplementation with physical activity (*Maggio et al*.: Int J Endocrinol. 2014, 2014, 525249).

Zinc (Zn) is involved in steroidogenesis, creates favorable conditions for spermatozoa to mature and, as a 5α-reductase inhibitor, it regulates the concentration of dihydrotestosterone – a derivative of **testosterone** – whose surfeit plays a part in prostatic hyperplasia (*Fahim et al*.: Andrology. 1993, 25, 369–775; *Croxford et al*.: J Nutr. 2011, 141, 359–365). Available studies show that Zn deficiency is associated with a decrease in the testosterone level, fertility problems, and the decline in the number and vitality of spermatozoa (*Prasad*: Mol Med. 2008, 14, 353–357; *Yamaguchi et al*.: Proc Natl Sci USA. 2009, 30, 10859–10864). According to *Prasad* (2008), Zn supplementation in elderly men with a slight TT deficiency improves TT levels.

Our study of men between 50 and 75 years of age revealed a statistically significant relationship between Mg and TT levels, however, a similar connection between Mg and Zn was not observed. Mg supplementation seems justified in men over 50 years of age, especially those with metabolic disorders. More in-depth analysis is needed to find out whether supplementation with Zn is equally reasonable.

Waldemar Różański

OWN EXPERIENCE IN THE IMPLANTATION OF PENILE PROTHESIS

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Erectile dysfunction (ED) is defined as persistent inability to achieve and maintain a full erection of the penis allowing for satisfactory intercourse. Erectile dysfunction is the second, after the premature ejaculation, sexual ailment reported by men (*Lindan*: N Engl. J Med. 2007, 357, 762–774). According to *Porst* and *Sharlip* (Standard Practice in Sexual Medicine. 2006, 43–48) erectile dysfunction intensity varies depending on the age of the patient and is reported from 2% to 28.9% in the population of males between 30–39 year and from 41.9% to 83% in males between 70–80 year. The risk factors for ED include cardiovascular disease, sedentary lifestyle, obesity, smoking, hypercholesterolemia, metabolic disorders, diabetes, and iatrogenic damage after surgery on the pelvic organs (urological, surgical) (*Lee*: BJU Int. 2011, 107, 956–960).

First-line oral medications administered in erectile dysfunction in men are phosphodiesterase type 5 (PDE5) inhibitors: sildenafil (Viagra), vardenafil (Levitra), tadalafil (Cialis). Other modes of treatment that are commonly used include alprostadil (Caverject) injections into the corpora cavernosa of the penis and the use of vacuum to induce penile erection. In case of drug treatment insufficiency the treatment of choice is the implantation of corpora cavernosa prosthesis (*Bettolchi*: J Sex Med. 2010,

7, 304–309; *Minervini*: BJU Int. 2006, 97, 129–133). Complications that may occur after corpora cavernosa prosthesis implantation are following: infection and further need to replace the prosthesis, technical damage of the prosthesis, leakage of prosthesis, damage during aircraft flights at supersonic speed, getting bubbles to the prosthesis, corpora cavernosa erosion, urethral erosion (*Henry*: J Urol. 2005, 173, 89–92; *Carson*: Int J Impot Res. 2003, 15, 139–146).

Two standardized questionnaires are commonly used to assess the sexual quality of life in patients with erectile dysfunction: IIEF-5 (International Index of Erectile Function) and SHIM (International Index of Erectile Function). Comparison of the length of the penis in patients treated with injections (papaverine, phentolamine, prostaglandin E1) with the subjects who underwent corpora cavernosa prosthesis implantation was carried out after 6 weeks, 6 and 12 months after surgery. Shortening of the penis in patients with implanted corpora cavernosa prosthesis compared to those treated with injections has been observed. The length of the penis after injection was 13.2 +/- 0.4 cm. The length of the penis after prosthesis implantation was: after 6 weeks 12.4 +/- 0.3 cm; after 6 months 12.5 +/- 0.3 cm and after 12 months 12.5 +/- 0.4 cm. According to the SHIM questionnaire the quality of sexual life after implantation of the corpora cavernosa prosthesis is satisfactory, despite penis shortening. Patients with diabetes (n = 84) and after radical prostatectomy (n = 96) were compared. The findings of 12 month-observations were following: prosthesis infection took place in third patients, prosthesis erosion occurred in one patient, problems with the filling of the prosthesis (not observed during implantation) was noted in one patient. The average length of the penis of examined patients, obtained on the basis of questionnaires, were following: IIEF-5 – before surgery 8.2 +/- 4.0 cm, after treatment 20.6 +/- 2.7 cm; EDITS after treatment 72.2 +/- 20.7 cm (*Antonini et al.*: Int J Impot Res. 2016, 28, 2–8).

From 2013 to 2015 in the 2nd Department of Urology of Medical University in Lodz prostheses were implanted in three patients after radical prostatectomy. Evaluation of the sexual quality of life in patients with erectile dysfunction was performed on the basis of IIEF-5 and SHIM questionnaire. Responding to the IIEF questionnaire, all patients reported no or sporadic occurrence of erection and lack of sexual activity with a partner. After prosthesis implantation, only one patient reported that his relations are satisfactory in more than half times. The other two patients were fully satisfied with sexual intercourses with partners after prosthesis implantation. Responding to the SHIM questionnaire, two patients reported full satisfaction of intercourse to be completed. One patient stated that the intercourse after prosthesis implantation brings greater satisfaction his partner than himself.

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EFFECTS OF INCREASED MALE AGE ON SPERM CHROMATIN MATURITY

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Abnormal standard semen parameters as well as molecular changes of sperm chromatin can appear with increasing male age (> 35 y). However, the influence of paternal age on semen parameters are still unclear and controversial (*Sharma et al.*: Reprod Biol Endocrinol. 2015, 13, 35). The study was designed to comprehensive evaluate of sperm chromatin maturity (n = 1042) by means of chromomycin A3 (CMA3), aniline blue (AB), toluidine blue (TB) and TUNEL (terminal deodeoxynucleotidyl transferase-mediated dUTP nick end labeling) assay.

The men ≥ 40 and < 40 y did not differ significantly in sperm concentration (median: 40.30 vs. 35.35 mln/mL), total sperm count (median: 115.20 vs. 115.50 mln), its total motility (mediana: 57.50 vs. 59.00%), morphology (median: 4.00 vs. 4.00%), vitality (median: 69.00 vs. 68.00%), concentration of leucocytes (median: 0.00 vs. 0.00 mln/mL) and round cells (median: 0.10 vs. 0.16 mln/mL) (according to *WHO*, 2010). However, there were differences in volume of ejaculate (median: 3.00 vs. 3.50 mL), percentages of TUNEL-positive (median: 20.25 vs. 12.30%), AB-positive, (median: 15.00 vs. 13.00%) and TB-positive (median: 17.00 vs 14.00%) sperm cells. There was no difference in percentage of CMA3-positive sperm cells (median: 16.00 vs. 17.00%). Total sperm count, sperm morphology, motility and leucocyte concentration negatively correlated with the male age (respectively, $r_s = -0.092$, $r_s = -0.163$, $r_s = -0.106$, $r_s = -0.096$). Positive correlations were found between teratozoospermia index, AB-positive cells, TB-positive cells and TUNEL-positive cells (respectively, $r_s = 0{,}116$, $r_s = 0{,}088$, $r_s = 0{,}148$, $r_s = 0{,}229$). Although CMA3-postitive cells did not correlate with age.

Obtained data suggest no significant changes in standard semen characteristics of studied men ≥40 y, whereas molecular sperm chromatin abnormalities expressing as its abnormal structure and condensation can appear.

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OBESITY-RELATED TESTICULAR HISTOLOGICAL CHANGES EXPLAINED BY PROTEOMICS

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Obesity has become widespread (*Cabler et al.*: Asian J Androl. 2010, 12, 480–489) and has been linked to male infertility, where it results in poor sperm quality (*Du Plessis et al.*: Nat Rev Urol. 2010, 7, 153–161) and changes in the structure and function of reproductive organs. Mechanisms through which these changes occur are still open to further explanation. By using proteomics, this study aims to quantify differentially expressed proteins within the testes of obese male rats, making it possible for mechanisms of disease development to be further elucidated.

Forty male Wistar rats (200–220 g) were equally and randomly divided into two groups: control animals fed normal rat chow and a diet group fed a high caloric diet. After sixty weeks, animals were sacrificed by euthanasia. Testes were excised and prepared for histological and proteomic analysis. Proteomics was performed using an Orbitrap Mass Spectrometer (LC-MS/MS). Proteins with ≥ 2 unique peptides were quantified; a 1.5 fold change in expression and p value < 0.05 were considered significant.

In histological sections, seminiferous tubules of obese animals showed degeneration of the pseudostratified epithelial layer, evidenced by reductions in epithelial height (95.85 ±1.442 vs. 71.44 ±1.101; p < 0.0001) and increased luminal diameter (147.7 ±2.976 vs. 196.3 ±3.477, p < 0.0001). Proteomic data showed significant fold increases in oxidative stress related proteins, namely glutathione peroxidase 3 (2.2), and inflammation-related proteins including Fibronectin 1 (3.3). Metallothionein-3, a negative regulator of cell development, showed a 1.5 fold increase compared to control. Histone H1.1, expressed by pachytene spermatocytes, was overexpressed in obese animals.

Structural changes in the testes of obese male rats can be attributed oxidative stress, resulting in inflammatory processes that lead to damage of cellular structures. Cell development is dysregulated due to the overproduction of Metallothionein-3, prohibiting proper growth and survival. The overexpression of Histone H1.1 may point towards an arrest of some germ cells at the pachytene spermatocyte stage, as histological observations show less cells reaching spermatid stage.

Obesity causes structural changes within male testes, which proteomic data shows to be related to oxidative stress and inflammation, resulting in damage to organ structures and male germ cells.

Jolanta Słowikowska-Hilczer

RECOMMENDATIONS CONCERNING KLINEFELTER'S SYNDROME

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The most common (0.1–0.2% male newborns) genetic cause of infertility and hypergonadotropic hypogonadism in men is Klinefelter's syndrome. It develops as a result of numerical aberrations of chromosome X (usually 47, XXY). In childhood and early puberty function of the hypothalamic-pituitary-testes axis is usually correct. From the middle of puberty (GIII stage by Tanner's classification) clinical hypergonadotrophic hypogonadism develops due to the progressive degeneration of the structure and impaired testicular function (*Wikström and Dunkel*: Horm Res. 2008, 69, 317–326). The phenotype is diverse, ranging from almost normal to far below normal. Phenotype in neonates with Klinefelter's syndrome are usually normal male. Often the only clinical feature are small testes, which are usually identified only after puberty. Patients with this syndrome are mostly infertile, but in about half the cases, it is possible to find spermatozoa in the testes (*Rohayem et al.*: Andrology. 2015, 3, 5, 868–875). A higher risk includes also breast cancer, metabolic syndrome, cardiovascular disease, osteopenia / osteoporosis, autoimmune diseases. In addition there are in varying degrees cognitive, social, behavioral and learning difficulties. Early diagnosis is recommended to implement early therapy and prevention against associated diseases (*Aksglaede et al.*: Am J Med Genet C Semin Med Genet. 2013 163C, 1, 55–63; *Purwin and Słowikowska-Hilczer*: Advances in Andrology Online 2015 2, 2, 12–24; *Nieschlag et al.*: Andrology. 2016, 4, 3, 545–549).

Paweł Wiechno

LOW TESTOSTERONE LEVELS IN MEN WITH CANCER

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Cancer and its treatment can lead to testosterone deficiency with associated symptoms. Data from the literature and our own data on prevalence of testosterone deficiency in men treated for malignant disease and its health effects were analyzed.

The risk of testosterone deficiency in patients after the treatment of testicular cancer is between 5% and 25%, but more than 10. years after orchiectomy testosterone levels do not differ from those for the healthy population. Higher percentage of compensated hypogonadism is observed and that state is quite chronic (*Nord et al.*: Eur Urol. 2003, 44, 322–328). The clinical image includes, in addition to obvious reduction of the volume of hormonally active tissue, also damage of Leydig cells caused by cisplatin (*Berger et al.*: Br J Cancer. 1996, 73, 1108–1114). It is confirmed, that hypogonadism affects patient's well-being after unilateral orchiectomy. There are no studies on the results of testosterone supplementation in this group of men. Prostate cancer is an example of the androgen dependent tumour. Indication for surgical or pharmacological castration is metastatic prostate cancer (*Parker et al.*: Ann Oncol. 2015, 26, suppl.

5, 69–77). Moreover, adjuvant hormonotherapy combined with radical radiotherapy in men with high risk of progression is beneficial regarding the overall survival (*D'Amico et al.*: JAMA. 2008, 299, 289–295). However, such a treatment is associated with the risk of lipid disorders, diabetes, bone loss and cardiovascular events (*Mottet et al.*: Guidelines on Prostate Cancer. European Association of Urology, 2015). The percentage of men with testosterone deficiency in end-stage of cancer is estimated at 40–90%. Testosterone deficiency in these patients is a component of the cachexia (*Fuoco*: Ecancermedicalscience. 2015, 9, 561).

Testosterone deficiency and disorders of the pituitary--gonadal axis are common among men suffering from cancer. Androgen deficiency is associated with significant clinical effects impairing the men's quality of life.

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M-TESE – OUR EXPERIENCE IN THE YEARS 2010–2016

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In 1999 Schlegel was first described testicular biopsy with the use of the operating microscope (M-TESA, microsurgical testicular sperm extraction) in men with nonobstructive azoospermia (NOA), with negative biopsies in the past, with the intention of obtaining sperm to assisted reproduction IVF-ICSI (fertilization *in vitro*--intracytoplasmic sperm injection). The authors apply this technique successfully for the first time in Poland in October 2012. The objective of our studies was to evaluate the effectiveness of a biopsy of the M-TESE – sperm obtaining for IVF-ICSI.

Biopsies M-TESE was performed in nonhomogenic group of 108 men suffering from the NOA; age 17–44 (average 32 yr). M-TESE was the first biopsy for 14 men. Klinefelter syndrome 47 XXY, disorders of sex development 46, XY (DSD) and post-transurethral resection of ejaculatory ducts (post-TURED) were found in 11, 2 and 1 subject (respectively) out of these 14. M-TESE was the second biopsy in 89 men and was third biopsy in 5 subjects. In all patients, hormonal stimulation of spermatogenesis (6–12 weeks, androgen + antiestrogen with vasodilators, trace elements and vitamins) was carried out before the biopsy (*Adamopulous et al.*: Fertil Steril. 1995, 64, 818–824). The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone were monitored. All M-TESE was performed under general anesthesia, as a "one day surgery". Microscopes Seiler Evolution XR6, Carl Zeiss S7, Leica M860 2 x 2 (20–25x) were used. From both gonads sampled slices with 3 levels**.** Each slice was placed in liquid nitrogen (–196°C) to the future IVF with prior ad hoc assessment for the presence of sperm. Moreover, routine histological examination was performed.

The sperm cells were found in 28 out of 95 patients (29.5%) but were not observed in men with Klinefelter syndrome and with DSD. Achieved 16 pregnancies, 6 miscarriages were recorded and 6 children were born from 37 performed IVF procedures. Our results indicate, that testicular biopsy by using the operating microscope increases the chance of finding sperm in men with nonobstructive azoospermia and allows for enrolling the men in IVF-ICSI procedure.

Jan Karol Wolski

VITAMINS AND MICRONUTRIENT IN THE TREATMENT OF MALE INFERTILITY

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In the last 50 years is observed the increasing participation of the male factor in childlessness. It is estimated that up to 50% of the failures in reproduction lies with the man. Next to the urogenital malformations, varicocele, choked roads out semen, neurogenic ejaculation disorders most cases of male infertility are abnormal spermatogenesis and sperm. External factors, such as smoking, obesity, simple, environmental pollution, inflammation within the genitourinary system conducive to the occurrence of oxidative stress. This is a significant cause of the damaging sperm, estimated at 30–80%. The male generative cells are very sensitive to the effects of reactive oxygen species, which are associated with oxidative stress. Sperm cell membrane contains high amounts of unsaturated fatty acids, which are subject to oxidation, and in the cytoplasm is low levels of neutralizing enzymes.

Supply of oral vitamins and micronutrients (vitamins A, E, C, B, selenium, zinc, copper, chromium) may improve sperm quality by reducing oxidative stress associated with the level of reactive oxygen species. Many publications show a benefit from the use of this therapy. Most recent Cochrane analysis of 2014 (*Showell et al.*: Cochrane Database Syst Rev. 2014) states that there is evidence though relatively low quality in the case of four small randomized controlled trials. They suggest that antioxidant supplementation in men with reduced fertility beneficial effects on semen quality and can improve the number of live births. We need therefore further large, well designed, double randomized placebo control trials using commercial products specially designed for the treatment of male infertility.

Frederick C.W. Wu

FUNCTIONAL CHANGES IN THE HYPOTHALAMIC-PITUITARY-TESTICULAR (HPT) AXIS IN AGEING MEN – LONGITUDINAL DATA FROM THE EUROPEAN MALE AGEING STUDY (EMAS)

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Ageing is associated with multi-level alterations in the hypothalamic-pituitary-testicular (HPT) axis function affecting both the steroidogenic and gametogenic compartments. It is not clear how this aligns with the clinical scenario of symptomatic older men presenting with low or low normal testosterone. Whether the clinico--pathological constructs of hypogonadism, long established in young patients, can be translated to underpin the management of the burgeoning number of middle aged and older men being referred for possible androgen deficiency, is also uncertain.

I will present prospective follow-up data in the observational cohort of >3,000 men from the European Male Ageing Study (EMAS), which describe the natural history of two divergent tracks of HPT axis dysfunctional that underlie the age-related decline in testosterone (T), based on the physiological classification of hypogonadotrophic (secondary) or hypergondodotrophic (primary) 'hypogonadism'.

The main findings are: the vast majority of men in the general population do not become hypogonadal during ageing. Obesity is associated with the development of sexual symptoms with chronic but reversible hypothalamic/pituitary suppression (equivalent to secondary 'hypogonadism') independent of age, commonly affecting middle-aged rather than elderly men. In contrast, the less common equivalent of primary 'hypogonadism' found mainly in men over 70 year of age show a more severe phenotype with sexual and physical symptoms, insulin resistance and co-morbidity compatible with either androgen deficiency or general health deterioration. It is also relatively common to encounter elevated luteinizing hormone (LH) with normal testosterone in ageing men – this can be considered to be a state of compensatory 'eugonadism' since they do not have definite features of androgen deficiency, but amongst them will be a small minority who eventually transitions to primary 'hypogonadism'.

It is important to be reminded that epidemiological associations cannot differentiate between the co-linear symptoms of androgen deficiency and the non-specific features of ageing-related disability and chronic illness or infer causality in the presence of borderline low testosterone levels. Nevertheless, our data can improve our understanding of the aetiology and potential clinical significance of the age-related changes in the HPT axis thereby informing current clinical practice.