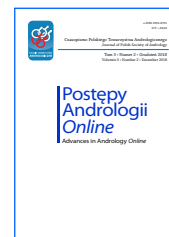




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SYMPOSIUM OF SCIENTIFIC TRAINING OF THE POLISH SOCIETY OF ANDROLOGY – 20th DAY OF ANDROLOGY AND 6th SCIENTIFIC AND TRAINING CONFERENCE ON INNOVATIVE TECHNOLOGIES IN MEDICINE – DAYS OF THREE CULTURES

Lublin, 26–27.10.2018; www.pta2018.pl

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Report

On October 26–27, 2018, in the Center for the Meeting of Cultures in Lublin, the jubilee Conference – 20th Andrological Day organized jointly by the Polish Andrological Society (PTA) and the International Scientific Society for the Support and Development of Medical Technology (ISSSDMT). The practical side of organizing this event was taken by the Cumulus Agency in cooperation with the Organizing Committee of the Symposium headed by MD PhD Szymon Bakalczuk and MD PhD Artur Wdowiak and the Scientific Committee chaired by prof. Jolanta Słowikowska-Hilczer.

The meeting was preceded on October 25 by a test exam in clinical andrology, organized for the second time by the PTA. 19 medical doctors participated in it, of which 17 obtained a positive result and received the PTA Certificate in clinical andrology.

The scientific part of the Conference began with a greeting given by the PTA President prof. Jolanta Słowikowska-Hilczer, President of the ISSSDMT MD PhD Artur Wdowiak and the Chairman of the Organizational Committee of MD PhD Szymon Bakalczuk.

Society Award named by Prof. Michał Bokinić for The Young Polish Scientist in Andrology for 2017 received PhD Aleksandra Rył from Pomeranian Medical University in Szczecin. The laureate presented the awarded work in a short presentation.

The first lecture was given by prof. Zbigniew Izdebski from the Department of Biomedical Fundamentals of Development and Sexology, University of Warsaw and the Department of Humanization of Medicine and Sexology, University of Zielona Góra on sexual health of men in Poland.

In the academics sessions lectures were conducted by foreign guests: prof. Gerhard Van der Horst and prof. Stefan Du Plessis from Republic of South Africa, prof. Birute Žilajtiene from Lithuania, PhD Hiva Alipour and PhD Fereshteh Dardmeh from Denmark and Dr. Thomas Tang from Ireland. In addition, lectures were given by many outstanding experts in the field from Poland. The subject concerned issues related to male fertility, the influence of endocrine disorders on the activity of the male reproductive system, the possibilities of urological treatments in erectile dysfunction and many others. Discussed were also diagnostic methods and therapeutic options in infertility. The representatives of basic sciences presented the results of the latest research related to the physiology and pathology of the male reproductive system. The session of short scientific presentations was also very popular. In the ISSSDMT session issues related to pregnancy problems after *in vitro* fertilization were discussed.

The meeting ended with thanks to the lecturers, participants, organizers and sponsors of the Conference and the invitation of the PTA President for the Polish Andrological Society Conference in 2019, which will take place in Łódź.

Abstracts of lectures

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IMPROVED CONVENTIONAL AND FUNCTIONAL SEMINAL PARAMETERS AFTER SHORT VERSUS LONG EJACULATION ABSTINENCE

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The ejaculatory abstinence (EA) period can greatly affect sperm quality. However, the optimal EA period before delivering samples for semen analysis or infertility treatment is still a topic of debate. Previous studies on the influence of different abstinence times on sperm quality in populations with sub-optimal sperm quality have provided controversial results. Therefore, this study assessed the effects of short (two hours) versus long (4–7 days) ejaculatory abstinence periods on sperm quality parameters including sperm concentration and counts, motility and detailed kinematic parameters, morphology and DNA fragmentation in normozoospermic men. Metabolomic profiles of the seminal plasma were also investigated to provide a possible insight into the mechanisms underlying the observed differences. The results demonstrated lower volume, sperm concentration and sperm number in total ejaculate, but significantly increased percentages of progressive and rapid-velocity sperm, with a lower percentage of DNA fragmented sperm in samples obtained after two hours versus 4–7 days of abstinence. Metabolomic profiling of the samples using nuclear magnetic resonance spectroscopy demonstrated higher absolute amounts of pyruvate and taurine per spermatozoa in ejaculates collected after the shorter abstinence time, which may be considered as an underlying mechanism behind the better motility observed in the second ejaculates. Despite the lower volume, concentration and total sperm number in ejaculates collected after short abstinence, the higher percentages of spermatozoa with better motility and lower DNA damage can, in theory, increase the possibility of selecting higher quality sperm for procedures not requiring many sperm (IVF – *in vitro* fertilization, ICSI – intracytoplasmic sperm injection). Procedures such as intrauterine inseminations which require a higher number of motile spermatozoa may also possibly benefit from using the second ejaculate, or pooling of two consecutive ejaculates to improve fertilization rates. The lower levels of DNA fragmentation in the second sample could also result in reduced risk of miscarriage after IVF/ICSI.

Leszek Bergier

REFERENCE VALUES FOR SERUM TOTAL TESTOSTERONE

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Total testosterone reference values depend strongly on the measurement method. The variety of methods used results in multiple reference ranges, commonly known as “normal values”.

Methods used for TT assessment: 1) reference method – mass spectrometry, preceded by extraction, gas or liquid chromatography, 2) direct immunochemical methods – used in most laboratories due to their simplicity and short TAT.

The quality of TT assessment is based on analytical parameters of the method used: specificity, sensitivity and precision. MS is more precise than direct methods, as defined by lower coefficient of variation (CV): 6% vs 15–20%, respectively. Immunochemical methods tend to show high CV especially for samples with low or high TT concentrations and may be subjected to interference of heterophilic antibodies. The choice of the method should be based on the profile of patients tended by the provider and multiple factors on the side of the laboratory: technology available, experience of the personnel and economical determinants.

No matter that each laboratory is encouraged to establish its own reference ranges, in practice it is not possible and hence lab reports display “normal values” provided by test reagents producer. TT concentrations are expressed in the following units: ng/mL, ng/dL and nmol/L. The most commonly seen reference values range is between 240 ng/dL and 1100 ng/dL.

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CORRELATION OF SELECTED MARKERS OF SPERM APOPTOSIS AND OXIDATIVE STRESS PARAMETERS WITH SEMEN QUALITY IN MEN EXPOSED TO GENITAL HEAT STRESS

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In order to verify the molecular pathomechanism by which scrotal hyperthermia can compromise the fertilizing potential of human germ cells, some selected markers of sperm apoptosis and necrosis (mitochondrial transmembrane potential, membrane integrity, phosphatidylserine translocation, lipid membrane asymmetry, DNA fragmentation) and oxidative stress parameters (total antioxidant capacity, activity of superoxide dismutase and catalase, malondialdehyde concentration, production of sperm mitochondrial superoxide anion) in semen of men exposed to thermogenic factors were determined. The research was conducted in a group of 84 men at reproductive age. The studied male cohort was classified into one of the four following study groups: 1) professional drivers (n = 23), 2) infertile men with cryptorchidism in childhood (n = 15), 3) infertile men with varicocele (n = 34), and 4) fertile individuals as control group (n = 12). A strong deterioration in routine sperm parameters was found in men exposed to both clinical and environmental thermogenic factors; and this effect was the strongest in the group with cryptorchidism. A significant increase in the population of necrotic sperm was observed in all the studied groups compared to the fertile control group. Analysis of subcellular sperm parameters has revealed an increase in the expression of all the classic markers of early and late apoptosis in the group of infertile men with cryptorchidism. In addition, in this group positive correlations of the percentage of sperm with sperm membrane asymmetry with progressive motility and viability were observed. In the group of occupational drivers, the activity of superoxide dismutase was positively correlated with progressive motility. As for the group of infertile men with varicocele, negative correlations of sperm with DNA fragmentation and progressive motility as well as total antioxidant capacity were observed. Both clinical and environmental genital heat stress leads to the strong deterioration of semen quality. The obtained results indicated the possibility of harmful influence of the thermogenic factor on the subcellular structures of male gametes as a result of the joint action of oxidative stress and apoptosis, and the pattern of these changes may be characteristic for particular pathological groups.

The study was financed by National Science Centre, Poland (Grant No 2015/19/B/NZ5/02241).

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CHRONIC PAIN, OBESITY AND MALE FERTILITY POTENTIAL – THE DISREGARDED TRIANGLE

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Obesity is known to increase the risk of developing musculoskeletal pain and negatively affecting the male hormonal balance and fertility potential. However, very limited is known about the coexisting condition of obesity, pain, and infertility, and further investigations are crucial to offer better patient treatment strategies. This study aimed at finding the possible effects of separate and co-existing conditions of obesity and chronic pain on male fertility potential, while assessing the ability of the *Lactobacillus rhamnosus* PBO1 probiotic supplementation as a novel strategy to reverse the negative effects of the mentioned complications. This project comprised of human (observational) and animal (interventional) phases. In the human phase, sperm concentration, motility and kinematic parameters, DNA fragmentation and morphology were assessed by computer aided semen analysis (CASA) and correlated to pressure pain thresholds (PPT) measured by a handheld algometer in pre-defined points in chronic pain patients and pain-free healthy matched controls. The animal phase was performed on normal weight and diet-induced obesity (DIO) male mice models, randomly divided into two equal sub-groups receiving a single daily dose (1×10^9 CFU) of *L. rhamnosus* (test group) or physiological saline (control group) and the same diet for 4 weeks. Sensitivity to mechanical stimulation was assessed by an electronic Von Frey device every two weeks. Serum total antioxidant capacity (TAC), reproductive hormone levels, and lipid profiles were assessed by enzyme linked immunosorbent assay (ELISA). Results of the human phase demonstrated that PPT values were generally lower in the overweight chronic pain patient-group compared to the respective control groups, however insignificant. Slim men with chronic musculoskeletal pain demonstrated a significantly lower percentage of progressively motile sperm and insignificantly lower concentration, lower normal morphology, and higher DNA fragmentation levels. The overweight chronic pain group had a tendency towards a lower concentration and percentage of progressively motile sperm and significantly lower kinematic parameters (VCL – curvilinear velocity, STR – straightness and WOB – wobble) compared to the overweight control groups. In the animal phase, the DIO group demonstrated a clear reduction in several kinematic parameters (VCL, VSL – straight-line velocity, average VAP – path velocity, STR and LIN – linearity) including the percentage of progressive motile sperm, which were reversed proportionally in the DIO probiotics supplemented group. Collectively, the results demonstrated the negative effect of chronic pain and obesity on the male fertility potential. Additionally, the oral supplementation of *L. rhamnosus* can be suggested as a potential innovative approach for the management of weight and nociception, while also positively affecting the male fertility potential, especially in cases of obesity.

Stefan S du Plessis

TO ABSTAIN OR NOT TO ABSTAIN, THAT IS THE QUESTION

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Disparities in ejaculatory abstinence durations as suggested by various regulatory bodies (e.g. ESHRE, NAFA, WHO) have resulted in a growing concern as to what the most suitable period of ejaculatory abstinence for collecting of an optimal semen sample ought to be (Ayad et al.: *Int J Fertil Steril*. 2018, 11, 238–246). Several studies have been undertaken to examine the relationship between the length of sexual abstinence and semen characteristics. Not all studies, however, have come to the same conclusions. The aim of this presentation is twofold: 1) provide a brief overview of the existing literature pertaining to the influence of ejaculatory abstinence on semen quality and 2) highlight findings from personal research projects related to short ejaculatory abstinence periods.

The weighted evidence from the literature suggests that the decline in semen volume and sperm concentration with shorter abstinence periods is accompanied by a substantial improvement in sperm quality (Ayad et al.: *Int J Fertil Steril*. 2018; 11, 238-246).

From the results of a number of our prospective studies it is evident that abstinence periods as short as a single day led to improved sperm viability and decreased DNA fragmentation, which was accompanied by a reduction in reactive oxygen species (Agarwal et al.: *Urology*. 2016, 94, 102–110; Mayorga-Torres et al.: *Reprod Biol Endocrinol*. 2015, 13, 47).

Comparing the effect of only 4 hours of ejaculatory abstinence to a period of 4 days furthermore showed a significant increase in total and progressive motility, as well as kinematic parameters (Ayad et al.: *Middle East Fertil Soc J*. 2018, 23, 37–43). These findings will be further explained at the hand of the differences observed in accessory sex gland secretions and changes in seminal plasma protein profiles.

In conclusion, considering all data, shortening of the abstinence period may be beneficial to sperm quality and it is therefore recommend that the current guidelines regarding the prescribed abstinence period should be revisited.

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RELATIONSHIPS AMONGST REACTIVE OXYGEN SPECIES, RESTRICTION ENZYMES AND DNA FRAGMENTATION IN SPERM

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Reactive oxygen species (ROS) are considered to be the main reason for DNA fragmentation in sperm. Recent research confirms the presence of restriction enzymes in seminal plasma. Therefore, the aim of our correlation study was to determine DNA fragmentation in human sperm in relation to ROS concentration and activity of restriction enzymes (DNase I, topoisomerase IIB). Analyses were carried out at initial time (control group), after 2 and 24 hours of incubation at 37°C. Ten semen samples were collected from healthy donors. Sperm DNA fragmentation was assessed using the Halosperm® kit (HalotechDNA, Spain). The activity of enzymes was measured by commercial ELISA kit (Thermo Fisher Scientific Inc.). The obtained results were statistically evaluated by one-way ANOVA with Dunnett's test and Pearson correlation in GraphPad Prism. After 2 hours, the ROS quantity increased significantly ($p < 0.05$) and with an even higher significance level ($p < 0.001$) following 24 hours. Similarly, sperm DNA fragmentation increased after 2 hours significantly ($p < 0.05$) and after 24 hours with a significance level of $p < 0.001$. The activity of both enzymes was nearly stable throughout the experiment. The correlation analysis showed a weak correlation ($r = 0.317$) between DNA fragmentation and ROS quantity after 2 hours. Surprisingly, the correlation between DNA fragmentation and enzymes was moderate ($r_{\text{DNase I}} = 0.691$; $r_{\text{Topoisomerase IIB}} = 0.668$). Inversely, the results observed after 24 hours between DNA fragmentation and ROS showed a strong correlation ($r = 0.715$). On the other hand, after 24 hours, the correlation between DNA fragmentation and enzymes was weak ($r_{\text{DNase I}} = 0.345^*$; $r_{\text{Topoisomerase IIB}} = 0.342$). Overall, the present study showed that both ROS and restriction enzymes, are responsible for sperm DNA fragmentation. In the first 2 hours, the restriction enzymes revealed to have the prevalent effect on DNA fragmentation. After 24 hours, oxidative stress prevailed in relation to DNA fragmentation. Our study suggests to remove and replace seminal plasma during cryopreservation process due to the harmful effect of restriction enzymes on sperm DNA integrity. This research was supported by the APVV-15-0544 and KEGA 009SPU-4/2018 grants as well as by the CeRA Team of Excellence.

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THE ROLE OF NOTCH SIGNALING PATHWAY IN MALE GONAD

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In the mammalian testis hormonal signaling and local cell-cell communication interact with each other, forming a complex network whose proper functioning is crucial for

testicular tissue homeostasis and spermatogenesis. Local cell–cell interactions include direct contact-dependent pathway, called Notch signaling pathway. Activation of this pathway is induced by binding of membrane Notch receptors with membrane ligands (Jagged, JAG and Delta-like, DLL) present on neighboring cells. It was shown that experimental disturbances of Notch signaling adversely affected spermatogenesis (Murta et al.: PLoS One 2014, 9: e113365; Garcia et al.: Development. 2014, 141, 4468–4478). In addition, altered activity of Notch signaling was associated with male fertility disorders in both animals and humans (Hayashi et al.: J Androl. 2001, 22, 999–1011; Sahin et al.: Fertil Steril. 2005, 83, 86–94).

During fetal development Notch pathway regulates proliferation and differentiation of Leydig cells and spermatogonial stem cells (Barsoum and Yao: J Androl. 2010, 3, 11–15; Garcia and Hofmann: Cell Cycle. 2013, 12, 2538–2545). In adult testis the role of this pathway is still not fully characterized. Recently, it was demonstrated that the production of glial-derived neurotrophic factor (GDNF), crucial for spermatogonial stem cells proliferation and self-renewal, is controlled by Notch signaling pathway in Sertoli cells (Garcia et al.: Stem Cells Dev. 2017, 26, 585–598).

The results of our recent study indicate that Notch pathway is involved in the control of androgen receptor expression in rodent Sertoli cells. Blockade of Notch pathway activity (using pharmaceutical inhibitor DAPT or silencing of *Rbpj* gene, that encodes protein necessary for Notch pathway activity) led to increased expression of nuclear androgen receptor (AR) and membrane androgen receptor (Zrt- and Irt-like protein 9, ZIP9) in mouse Sertoli cell line TM4. Changes in androgen receptors expression were followed by increased expression of androgen-dependent genes encoding blood-testis barrier proteins, claudins (*Cldn5* and *Cldn11*) and increased 3',5'-cyclic adenosine monophosphate (cAMP) level. To sum up, disturbed Notch signaling in seminiferous epithelium may affect androgen signaling and androgen-regulated functions of Sertoli cells.

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Stanisław Horák

THE QUALITY OF SPERMATOZOON AND QUALITY OF PREGNANCY

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The course and result of pregnancy depends on many factors, most important of which is the quality of gametes. The classic spermogram abnormalities such as sperm

concentration, motility and morphology are usually an obstacle to the very process of joining a sperm with an oocyte. This problem has been resolved, though not fully, for 27 years by technics of intracytoplasmic sperm injection. The further development of an embryo and a fetus depends on the genetic material supplied by spermatozoon, similarly as in the case of natural conception.

The impact of exo- and endogenous factors on the quality of the genetic material contained in the spermatozoon such as the age of male, his health, medication, habits and lifestyle on the course and result of pregnancy is presented. The most significant of them seems to be the age, because in elder males the intensification of DNA fragmentation and higher level of DNA adducts in spermatozoa is stated. Sperm DNA fragmentation is assessed using flow cytometry (TUNEL – terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling i SCSA –sperm chromatin structure assay) as well as light microscopy (comet assay and SCD – sperm chromatin dispersion test). The DNA adducts level in sperm is most often assayed by ³²P-postlabeling method.

Having a limited influence on the natural procreation, one should think about the choice of the most suitable spermatozoon in terms of the genetic material involved performing the assisted reproduction procedures. Some tests are performed using the correlation of DNA quality of the sperm with its characteristics which may be examined intravitaly. The most important of them are discussed, such as microscopically selected sperm intracytoplasmic injection (IMSI), sperm preselected using hyaluronic acid binding and its intracytoplasmic injection (PICSI), using sperm after hypoosmotic swelling (HOS) and magnetic-activated cell sorting (MACS).

Zbigniew Izdebski

SEXUAL HEALTH OF MEN IN POLAND

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The presentation will be based on the results of the author's research on "Sexuality of Poles 2017" carried out on a nationwide sample of n = 2,500 at the age of 18+ (men was 1196). The subject of the analysis were men who have begun regular sexual life. For 48% of respondents, sex played an important role in their lives, while for men aged 50+ the response rate was 27%.

More than a half (55%) of respondents are satisfied with their sexual life, however, over the years, the frequency of sexual contact decreases. In comparison with 1997, the percentage was 86%, while in 2017 – 76%. 48% of respondents indicated difficulties during sexual intercourse. For 21% of men, the cause of problems was fatigue and stress. The fear of unwanted pregnancy concerned

13% of respondents. The illness and a malaise was a cause for 9% of men, also 9% of respondents indicated an intoxication of the partner. However, 16% of respondents showed fear that will not be able to have sex.

The average age of the occurrence of difficulties in the sexual life of men was 46 years. In the group of men having problems 10% were looking for help. Most indications related to information searching via Internet (32%). 69% of the surveyed men declared a positive attitude towards drugs to improve sexual performance.

Over the years the fear of HIV (human immunodeficiency virus) infection has decreased clearly in Poland. Currently, this concerns 5% of respondents. At the same time, only 7% have ever made the HIV testing. Regarding the preventive medical examination of the prostate, more than a half (52%) of men aged 50+ do not carry out of these examinations.

Furthermore, there will be discussed the issues related to the forms of sexual activity and risky sexual behaviours of men.

Grzegorz Jakiel

OXIDATIVE STRESS AS THE NEW MARKER IN DIAGNOSIS OF MALE INFERTILITY

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Despite the new WHO semen norms, established in 2010 year and based on Cooper et al. (Int J Androl. 2002, 25, 306–311; Hum Reprod Update. 2010, 16, 231–245) study, classical routine semen analysis has limited predictive value for individual fertility. The publications of Ombelet et al. in the 1990s (Int J Androl. 1997, 20, 367–772; Hum Reprod. 1997, 12, 987–993) demonstrated the difference between the average parameters in the group of fertile and subfertile males, but the ranges in both groups are almost the same. This finding demonstrated in several paper and also in clinical practice is a reason for looking for new parameters which would better predict the fertility of individual. First step in this type of research is an answer to a question what is faulty in routine semen analysis. It is believed that routine semen analysis is poorly correlated with stability of the genome and condition of DNA structure. In consequence, some new laboratory tests were introduced to the daily practice. Most important of them is sperm DNA fragmentation test. Sperm DNA fragmentation can be resulted from apoptosis, high level of the caspases and a direct effect of reactive oxygen species. The new data published last couple of years established that almost 80% of human DNA sperm fragmentation is caused by reactive oxygen species (ROS). As it is already known, a small level of ROS is needed for appropriate sperm function but a high level gives the pathological changes and

affects sperm function as well as embryo development. The consequence is infertility and early pregnancy loss. The main reason of high ROS production seems to be inflammation and varicocele.

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SEXUAL AND RELATIONAL PROBLEMS OF UNCONSUMMATED REALATIONSHIPS

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Unconsummated marriage/relationship has been rarely described in medical literature. However, the problems regards about 4% of partners, and is observed more often in the Middle East, Sub-Saharan Africa, Turkey, Pakistan and India (Özdemir i wsp.: J Sex Marital Ther. 2008, 34, 268–279). Despite the will of both partners to have a sexual intercourse it is impossible for them to have vaginal sex. Female reason is mostly vaginismus (80%). Male reasons are: erectile disorders, premature ejaculation or delayed ejaculation. Fear is also a significant problem. (Zargooshi J. Unconsummated marriage: clarification of aetiology; treatment with intracorporeal injection. BJU Int. 2000, 86, 75–79). Despite the impossibility of penetration over the course of years the relation between the partners is satisfactory (measured by cooperation, understanding, compassion). Few publications can be found about men in unconsummated relationships (Gindin et al.: J Sex Marital Ther. 2002, 28, 85–99).

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SPERM MORPHOLOGY AND SPERM CHROMATIN STATUS IN INFERTILE MEN

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Because morphological sperm evaluation and assessment of their chromatin status seems to be an important predictor of reproductive success (Majzoub et al.: Arab J Urol. 2018, 16, 87–95), current research attempts to reveal the relationship between sperm structure and their chromatin maturity. Sperm morphology was analyzed on the basis of Papanicolau-stained smear (WHO, 2010), while structural abnormalities of male

reproductive cells and teratozoospermia index (TZI) were evaluated according to the developed morphogram. The male gametes were processed to define level of DNA fragmentation (SDF%) using sperm chromatin dispersion test (HaloSperm test).

Infertile men ($n = 481$) showed significantly lower morphology and more defects of sperm heads, midpieces, tails and more cells with residual cytoplasm, as well as a higher value TZI vs. healthy volunteers with normozoospermia ($n = 403$). SDF% was significantly higher in infertile men (median: 24% vs. 14%). The lower proportion of men (19.42%) with low SDF level ($\leq 15\%$ sperm cells with SDF) and higher incidence of men (36.36%) with high SDF level ($>30\%$) was found in infertile men vs. healthy volunteers (respectively: 58.52%, 4.44%). Moreover, infertile men had significantly lower odds ratios (ORs) for having low SDF level (OR: 0.17) and higher ORs for showing high SDF level (OR: 12.29). Lower proportion of men with low SDF level (16.44%) and higher incidence of men with high SDF level (38.81%) was found in men with teratozoospermia vs. healthy volunteers (respectively: 56.96%, 5.70%). Similarly, men with teratozoospermia had a lower ORs for having low SDF level (OR: 0.15), and higher ORs for showing high SDF level (OR: 10.50) compared to men with normal sperm morphology. Sperm nuclear DNA damage was significantly negatively correlated to sperm morphology but positively correlated to TZI, percentage of head, insert and tail defects, with the percentage of sperm with short and bent tail and with the percentage of sperm with residual cytoplasm.

Male infertility was shown to have a negative impact on sperm morphology and chromatin maturity. There is a relationship between sperm morphological defects and sperm nuclear DNA fragmentation, which suggests that reduced sperm morphology can be accompanied by chromatin damage. Moreover, research suggests that the chance of a high level of sperm chromatin damage increases 10-12 times in infertile men.

The study was supported by Pomeranian Medical University in Szczecin (project No. WNoZ-322-04/S/2016, FSN-322-5/2016).

Marek Janicki

HEALTH ENGINEERING IN ANDROLOGY AS A SUPPORT TREATMENT

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Infertility should be considered in the partner category. 60% of partner infertility is due to the male factor. Male factor – male infertility may have a reason: a) pre-testicular: chromosomal, hormonal, and sexual; b) testicular: congenital, infectious, vascular, antispermatogenic, immunological, idiopathic; c) extra-nuclear: obstructive,

hostility of the epididymis, infections of the additional glands, immunological.

Balneoclimatology and physical medicine is a branch of medical knowledge that uses natural methods to treat, prevent and rehabilitate chronic diseases. Balneological methods use for treatment mineral water, gases, peloids, muds, slits, climatic factors and physical factors. Other basic methods are also used, such as kinesitherapy, diet, physical therapy and pharmacotherapy. Balneological treatment is comprehensive. Climatology uses for treatment natural healing environment. Krenotherapy is treatment based on drinking mineral water. Physiotherapy is divided into kinesitherapy and physical therapy. Kinesitherapy is a movement treatment- gymnastics in the gym, outdoors or in the water. Physical therapy uses as treatment body massage, hot or cold temperature, electricity, magnetic field, lasers, acupuncture, electroacupuncture and hydrotherapy. Water cure is hydrotherapy (industrial water) and balneohydrotherapia (healing water is used). The treatment is used in Health Resorts.

Types of curative waters occurring in Poland: chloride-sodium (brine), bicarbonate (with CO₂), sulphide-sulphydric, radium, thermal (above 20°C – hot springs). Curative gases: carbon dioxide, hydrogen sulphide, radon, oxygen, ozone. Various types of baths are used – water treatments: kinesitherapy baths, whirlpool baths, showers, underwater massage, pearl baths, specific hydrotherapy. The thermal effect of water treatments is important. The water temperature of 33–35°C is felt as neutral; tepid – 20–27°C, lukewarm – 28–32°C, warm – 36–37°C, hot – 38–42°C. In Andrology, it is advisable to use cold and lukewarm water for therapeutic baths.

Peloidotherapy is the use of peat or mud in the form of a paste, baths, solutions, peat packs. Mud treatments in urology, gynecology, proctology have a form of tampons, hip-baths, partial polutice, mud pants. The therapeutic effect of the mud is anti-inflammatory, astringent, bacteriostatic and bactericidal, hyperemic and regenerating tissues, hormonal, thermal (minimal).

Piotr Jędrzejczak

CAN SPERM ANALYSIS OR ITS PREPARATION HELP COUPLES WITH RECURRENT MISCARRIAGE?

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Recurrent miscarriage is defined as the loss of two or more consecutive pregnancies before the 22nd week of pregnancy. It is assumed that approximately 5% of women at reproductive age experience this, and this percentage increases significantly after the age of 35. In view of the fact that more and more women postpone the decision to become pregnant later in life, it should be assumed that

this problem will be more common in our population. Recurrent miscarriages are extremely stressful for the couple. Often the reason remains unknown or difficult to discover, therefore, the issue of precise diagnostics and effective treatment of this disease is becoming important. So far, little space has been devoted to the analysis of the male partner, in addition to the karyotype. Recent reports provide some information on the evaluation of some semen parameters helpful in diagnosis. This is especially true for fragmentation of sperm DNA. The importance of a proper lifestyle of a partner is also raised to reduce the risk of recurrent miscarriages in female partners. During the presentation, these issues will be discussed, with particular emphasis on the importance of reproductive potential of male gametes in the possibility of reducing recurrent miscarriage.

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ANTISPERM ANTIBODIES AND TESTICULAR HYPERTHERMIA

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Extended testicular/scrotal hyperthermia may induce oxidative stress response and may trigger the immune-based reactions. The assessment of antisperm antibodies (AsA) is an essential tool for the diagnosis of male immunological infertility, however, AsA formation can be the end point of complex immunological reactions. Therefore, immune-based "male factor" infertility should be considered in a broad context. The aim of this study was to find a correlation between AsA and oxidative stress response in men exposed to elevated testicular/scrotal temperature.

The studied male cohort was classified into the following groups: infertile patients with varicocele (n = 53), infertile patients with cryptorchidism (n = 23), professional drivers (n = 22), men with idiopathic infertility (n = 20), males with immunological infertility (n = 5) and fertile individuals as control group (n = 15). The indirect MAR test and flow cytometry were used for the detection

of AsA in seminal plasma. Additionally, direct MAR test was used for the detection of AsA on the surface of live spermatozoa. The oxidative stress parameters such as total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) activities and malondialdehyde level (MDA) were measured in seminal plasma using spectrophotometry.

The low AsA incidence in MAR test as well as in flow cytometry have been found. The percentage of AsA in flow cytometry analysis was found to be increased in the group of professional drivers compared to fertile men, and these differences were statistically significant for IgA and IgG immunoglobulin classes. The TAC values were significantly lower in men with immunological infertility compared to other tested groups. As for CAT activity the statistically significant difference between professional drivers and fertile men has also been found. Numerous correlations between AsA incidence and oxidative stress parameters (CAT and MDA) have been found.

Based on the results obtained, it can be concluded that hyperthermia does not significantly increase the AsA incidence. However, in the group of males with past cryptorchidism (clinical hyperthermia), an increase in local AsA levels can be related to persistent oxidative stress in semen.

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Marek Krakós

METHODS OF EXTERNAL GENITALIA RECONSTRUCTION IN PATIENTS WITH DISORDERS OF SEX DEVELOPMENT

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Every newborn with abnormal external genitals requires an urgent diagnosis in order to determine whether this is a symptom of a state which is an immediate life threat, e.g.: congenital adrenal hyperplasia with salt loss. The next important aspect is determining the gender in which the child will be brought up and then planning therapeutic treatment.

After selecting the gender, the external genitourinary organs should be reconstructed to ensure the best possible psychosexual development of the patient. In the case of girls, all elements should be made: the outer and inner labia, the separate orifice of the urethra and vagina, and the clitoris should be reduced. The reconstruction of the male external organs most often consists in performing a procedure such as the proximal form of hypospadias. Afaia (the lack of penis) is still a big challenge because to this day we are not able to create a completely effective penis.

Beata Kurowicka

LIFE IN HEAT: DOES IT ALWAYS IMPAIR THE REPRODUCTIVE FUNCTION

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The organism's long-term exposure to high ambient temperature, as opposed to short-term high-intensity thermal stress, allows for the full development of the body's adaptation processes (Assayag et al.: Cell Stress Chaper. 2010, 15, 651–664). As such, you can expect better protection of reproductive function compared to the effects of heat stress applied to non-adopted gonads. However, few studies tackle the adaptation mechanisms of male testes under heat acclimation conditions. Evaluating the functions of the male reproductive system of rats heat-acclimated (34°C) after puberty and neonatal heat-acclimated rats (Kurowicka et al.: Cell Physiol Biochem. 2015, 35, 1729–1743; Kurowicka et al.: Reprod Biol. 2015, 15, 1–8; Kurowicka et al.: Pol J Vet Sci. 2016, 19, 379–386) we have found that a high ambient temperature during the neonatal period changes rats' pituitary (LH, FSH and prolactin) and thyroid secretory activities. Changes in the receptor expression for these hormones in the testicles and the expression and activity of the steroid hormones' synthesis pathway might partially compensate the hormonal changes, as we have found almost unchanged concentrations of sex steroids in peripheral blood. An adequate concentration of steroid hormones led to undisturbed spermatogenesis, and consequently maintained unchanged cauda epididymis sperm count. The only negative effect of the heat acclimation, during the development of the reproductive system of male rats was the enlargement of additional sexual glands and impaired curvilinear movement of sperm.

Long lasting exposure of adult male rats to high ambient temperature, although it did not substantially alter the concentration of pituitary gonadotrophic hormones and prolactin, as well as the plasma concentrations of sex steroids, it led to partial degeneration of the testicular seminal epithelium and the reduction of cauda epididymis sperm count.

These results indicate significant adaptability of male gonads in the early stages of development of the reproductive system, aided by the adaptation of metabolism and temperature regulatory system.

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FERTILITY IMPROVEMENT BY OPTIMIZATION OF SPERMATOGENESIS TEMPERATURE

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The fraction of couples with infertility has rapidly increased in developed countries. Male infertility affects up to 8% of men's population worldwide. Impaired production of sperm could be the result of long-lasting overheating of the testicles, caused by varicoele, wearing tight-fitting underwear and the lifestyle. The outer position of the scrotum is necessary to maintain the spermatogenesis temperature approx. 2°C lower, than the temperature of the interior of the body. Elevated temperature can cause apoptosis of spermatogonia and sperm maturation impairment. Many attempts have been made to decrease testicular temperature for fertility improvement so far. Promising early results of those studies did not cause break-through in treatment and many obstacles that were met to implement cooling to therapy. We present results of recent studies, as well as, assumptions that were made to construct and perform an effectiveness trial of a novel device. The prototype of the device will allow constant optimization of spermatogenesis temperature. It is based on innovative technical solutions, that were not available in the past, and could contribute to improvement of infertility treatment of specific groups of males with testicle overheating.

Katarzyna Marchlewska

THE BEST METHOD FOR ASSESSING SPERM FERTILIZING ABILITY

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According to the World Health Organization definition, infertility is diagnosed in a couple who did not get pregnant within 12 months of regular sexual intercourse without using contraception. In special cases, diagnosis begins earlier, before 12 months, for example if there are additional disorders that may lower the fertility of a woman or a man, or the age of a woman is above 30 years, and men over 35 years of age, due to limited reproductive period and a risk of increased incidence of fertility disorders.

Diagnostics of the causes of infertility should be carried out simultaneously in a woman and a man. In men, the first laboratory test is the basic examination of the semen. It has a screening significance and cannot be crucial for the diagnosis of infertility. Even the finding of azoospermia (lack of sperm in semen) or a high degree of oligozoospermia (concentration of sperm <5 million/mL) does not determine the permanent, incurable infertility of a man. In turn, the correct result of basic semen analysis cannot be the only basis to determine a male fertility potential. The result of sperm analysis only directs further diagnostic activities to explain the causes of infertility and possible treatment.

Fertility problems in men may be associated with impaired function and quality of sperm. For this reason,

after excluding the female factor of infertility, one can consider performing other tests in a man, among others: functional tests of sperm, such as the “swim up” test evaluating the sperm migration capacity, the hyaluronic acid test, sperm chromatin integrity test, semen oxidoreductive potential test, tests assessing the immunological causes i.e. presence of anti-sperm antibodies in semen, and microbiological, biochemical and molecular tests aimed at detecting the causes of infection, genetic tests that can explain the cause of infertility and help to decide about future therapeutic management.

Andrzej Milewicz

INSULIN RESISTANCE AND INFERTILE COUPLE

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Although both in obese women and men, disturbances of hypothalamus function, gonads or adipokines secretion are observed, these abnormalities are most common in insulin resistance cases. This impairment of tissue sensitivity to insulin is observed only in metabolic or abdominal obesity found in normal body mass patients. Insulin resistance is the key to the whole complex of hormonal or fertility disturbances in women with diagnosed polycystic ovaries. Insulin resistance coexisting with obesity is the reason for early miscarriages and small efficacy of reproduction supportive techniques. In extreme cases, bariatric procedures resulted in the increase of pregnancy numbers in obese women. In the case of male abdominal obesity, ejaculation volume was diminished and semen quality was restricted. Also, obesity has a negative impact on sex in both males and females. Metformin is a drug of choice in the case of insulin resistance treatment. It influences positively hormonal and metabolic disturbances as well as miscarriages in females and it also increases testosterone level and semen quality in males.

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LONG-TERM RESULTS OF SURGICAL TREATMENT IN BOYS WITH TESTICULAR TORSION – EVALUATION OF CHOSEN IMMUNOLOGICAL, HORMONAL PARAMETERS AND GROWTH FACTORS

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Testicular torsion is the second cause of “acute scrotum” in the developmental period after torsion of Morgagni appendix. It is a potential threat to physio-sexual development of boys. The aim of this study was to determine risk for boys’ fertility caused by specific antibodies induced by testicular torsion. To determine probable hormonal disturbances in boys operated on because of testicular torsion. Evaluation of selected growth factors in the aspect of testicular torsion.

In 2012–2015 we operated in the Department of Paediatric Surgery and Traumatology on 80 boys due to testicular torsion. The study group consisted of 28 boys subjected to a minimum of 1-year outpatient observation. Levels of antibodies against sperm and Leydig cells in serum were determined. Levels of selected hormones of the hypothalamic-pituitary-gonadal axis and cellular growth factors were determined. The control group consisted of 12 boys with normal sexual development, without any hormonal disorders who were hospitalized in the Paediatric Surgery Department. Patients were divided into 3 age groups: prepubertal group, early pubertal and pubertal.

There was no production of antibodies against sperm or Leydig cells in boys treated surgically due to torsion of the testis. The level of follicle-stimulating hormone is higher in the prepubertal group than in other age groups and in the control group. The level of progesterone is higher in the pubertal group than in the control group. The level of insulin-like growth factor (IGF-1) is higher in the prepubertal group than in the control group. The same relationship was found for protein 3 binding insulin-like growth factor (IGFBP-3).

The immunological risk associated with testicular torsion for patients in the developmental period has not been confirmed in our material. The consequences of testicular torsion force increased the secretory function of the pituitary gland, which ensures correct testicular steroidogenesis. The surgical treatment of boys with testicular torsion does not result in over-production of vascular growth factors in the long follow-up.

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PREGNANCY RESULTING FROM IVF – OBSTETRIC PERSPECTIVE

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The risk of adverse obstetric and neonatal outcomes in pregnancies resulting from *in vitro* fertilization (IVF) is higher compared to spontaneously conceived pregnancies (Talaulikar al.: Eur J Obstet Gynecol Reprod Biol. 2013,

170, 13–19). It might be the result of IVF method itself (hormonal stimulation of the ovulation, gamete manipulations, in vitro culture) (Mainigi et al.: *Biol Reprod.* 2014, 90, 1–9), but mostly it might be due to the maternal underlying factors (the advanced maternal age, chronic diseases, the cause of infertility) (Romundstad et al.: *Lancet.* 2008, 372, 737–743). The pregnancy after IVF more often results in the pathology of the first trimester: the spontaneous miscarriage, ectopic pregnancy. The risk of preeclampsia development, gestational diabetes mellitus, and placental abruption is also higher in pregnant patients after IVF: the advanced maternal age, chronic conditions (obesity, diabetes mellitus, hypertension) and abnormal placentation all are reported to be the risk factors (Talaulikar et al.: *Obstet Gynecol Surv.* 2012, 67, 566–583). The abnormal placentation (placenta praevia, accreta, increta) observed in IVF group is thought to be responsible for higher incidence of intrapartum hemorrhage (Romundstad et al.: *Hum Reprod.* 2006, 21, 2353–2358). Multiple pregnancies and their complications like preterm labor and low birth weight are substantially increased after IVF compared to natural conception (Kulkarni et al.: *N Engl J Med.* 2013, 369, 2218–2225) although singleton pregnancies after IVF also tend to deliver prematurely, with lower newborn birth weight (Pandey et al.: *Hum Reprod Update.* 2012, 18, 485–503). The data suggests better perinatal outcomes for twins after IVF than natural twins, while singleton pregnancies after IVF carry the increased risk of the adverse outcome if compared to spontaneous singleton pregnancies (Talaulikar et al.: *Eur J Obstet Gynecol Reprod Biol.* 2013, 170, 13–19). Thus the pregnancies resulting from IVF should be found as high risk pregnancies – the prophylaxis and early complications diagnosis are of great importance. Pregnant women after IVF should be strongly encouraged to follow the healthy diet with the appropriate amount of folic acid, vitamin D3 and to modify their lifestyle – avoid smoking and alcohol drinking. The preeclampsia prophylaxis with aspirin should be considered. Since the higher risk of congenital abnormalities and the low birth weight in IVF children are reported, the regular ultrasound assessment of fetal anatomy and growth is mandatory.

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INFLAMMATION IN THE DEVELOPMENT OF PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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Both the risk of going down with prostate cancer (PCa) and benign prostatic hyperplasia (BPH) rises with age. The incidence of PCa, which belongs to the leading causes of cancer-related death, is a global problem. BPH is also commonly diagnosed in aging men – as many as 50% of the male population aged 50 suffer from pathological BPH-related symptoms, and this percentage increases up to 80% of men aged 80 (De Nunzio et al.: *Eur Urol.* 2011, 60, 106–117).

BPH and PCa develop in different parts of the prostate gland. While BPH is mainly observed in the transitional and central zones, PCa affects the peripheral zone. Despite different molecular and pathogenetic mechanisms, these two diseases have one factor in common. This factor is inflammation (Krušlin et al.: *Front Oncol.* 2017, 7, 77; Gandaglia et al.: *Curr Opin Urol.* 2017, 27, 99–106).

Immunohistochemical reactions have confirmed the presence of immune system cells in the biopsies of prostates with benign hyperplasia. Inflammation is then characterized by the inflow of T CD3⁺ and CD4⁺ lymphocytes, B CD19⁺ and CD20⁺ lymphocytes, CD163⁺ and CD68⁺ macrophages, and mastocytes to the prostate gland (Norström et al., *Oncotarget.* 2016, 7, 23581–23593). Immunological cells promote the emergence of inflammatory environment, and the cytokines that are produced induce, among others, cell growth factors, thus leading to the development of BPH (Steiner et al., *Lab Invest.* 2003, 83, 1131–1146; Bardan et al., *Clin Biochem.* 2014, 47, 909–915; Jiang et al.: *Med Sci Monit.* 2015, 21, 2976–2985; Ou et al., *Oncotarget.* 2017, 8, 59156–59164). This is believed to be caused by microbiological infections, urinary retention, the influence of dietary components and hormones, and by an autoimmune reaction (Robert et al.: *Prostate.* 2011, 71, 1701–179).

Many studies link chronic inflammatory states with the course and pathogenesis of PCa, which has been confirmed by both molecular and histological research. Apart from immune system cells, an important part in PCa is also ascribed to cellular stress factors. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are regarded as epigenetic modifying factors. They can lead to the DNA damage, alterations in gene function, as well as disturbances of apoptosis, protein structure, and DNA repair. What is more, they can enhance the expression of cytokines and proliferation of damaged cells, which entails the progression of cancer (Pace et al.: *Int Braz J Urol.* 2011, 37, 617–622; Sfanos et al.: *Nat Rev Urol.* 2018, 15, 11–24). Currently, a lot of attention is paid to the role of microorganisms in the development of PCa (Porter et al.: *Prostate Cancer Prostatic Dis.* 2018, 21, 345–354). Also disturbances of the human microbiome, including the intestinal microflora, have been described as potentially involved in prostate gland tumorigenesis (Golombos et al.: *Urology.* 2018, 111, 122–128).

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SLEEP DISORDERS IN PATIENTS WITH ERECTILE DYSFUNCTION

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Obstructive sleep apnoea (OSA) is a breathing disorder characterized by episodes of hypoxia, disturbed sleep and daytime somnolence due to repetitive upper airway obstruction. OSA is proven to coexist with hypertension and significant cardiovascular diseases. It is suggested that OSA can initiate and accelerate cardiovascular disease. Erectile dysfunction (ED) and obstructive sleep apnoea share many risk factors, are both increasing in prevalence and may co-exist. 10-69% of men with OSA is estimated to have erectile dysfunction. (Campos-Juanatey et al.: *Asian J Androl.* 2017, 19, 303–310; Pastore et al.: *Int J Clin Pract.* 2014, 68, 995–1000; Husnu i wsp.: *Afr Health Sci.* 2015, 15, 171–179). The exact pathogenetic link between OSA and ED remains unclear, but is likely multifactorial. Both OSA and ED also represent opportunities to modify risk, prevent future cardiovascular disease and its complications. OSA may be under recognized in clinical practice.

The lecture will present single andrology center assessment of the prevalence of OSA in men presenting with ED, analyze pathophysiology, comorbidities and risk factors of these diseases. In our experience, men presenting to the andrology clinic with erectile dysfunction are at a significant risk of having undiagnosed sleep apnoea and subthreshold insomnia. This has serious adverse health consequences as well as being associated with potential dangers at work and travel. It is feasible to screen this population for sleep disturbance and this should be part of the ED assessment.

Piotr Paweł Świniarski

ERECTILE DYSFUNCTION – WHAT NEXT IF VIAGRA FAILS?

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Erectile dysfunction (ED) is a serious problem of 15% of men, but almost all men are at risk of ED and can experience temporary or situational ED. Difficulties in gaining erection have positive correlation with patient's age: the older the patient is, the higher risk of ED he has. A revolution in ED treatment started with a market introduction of a small, blue pill. The Viagra is well known

all over the world and every new drug for ED, besides a new name and a new active substance, is described by patients as a "next Viagra".

But what to do if a wonderful pill doesn't work? First check if the patient takes a correct dose, sufficiently earlier, in an intimate situation with his partner and how many times he attempts intercourse. It is also worth conducting an interview to exclude psychological background of ED, because psychological factors sometimes appear as the reason of ED, and almost always as result of sexual fails. Even if all recommendations are fulfilled and the patient still does not respond for the treatment, it is worth checking the rest of phosphodiesterase type 5 inhibitors (iPDE5). A lack of response to the highest dose of sildenafil, tadalafil, vardenafil and avanafil confirmed in at least a dozen attempts, qualify a patient for a more aggressive treatment. Prostaglandin E1 (alprostadil) is used as an intraurethral, local on penile skin or as-intracavernosal injections. As a vasoactive drug it relaxes the vessel's smooth muscles, enlarges arteries' lumen and increases blood flow into the penis.

Penile rehabilitation with low-intensity shockwave therapy (LiSWT) is promising, but still as an experimental method waits for final conclusions about its effectiveness. Vacuum erection device (VED) is very efficient in some patients, but it is necessary to have a broad spectrum of silicon rings to adjust a correct strength of squeeze. In extreme ED, if the patient does not respond to the above treatment or refuses it, semirigid or hydraulic penile implants are used. Implantation of penile prosthesis provide the patient with the certainty of a hard and long erection. Although the erection is mechanic, all feelings of pleasure and climax is undisturbed and natural, and if seminal ducts are uninterrupted (e.g. like after radical prostatectomy) the patient preserves ejaculation function.

Sylwia Szpak-Ulczoł

HYPERPROLACTINEMIA IN MEN - DIAGNOSIS AND TREATMENT

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Hyperprolactinaemia in men is defined by the presence of serum prolactin level more than $>15 \mu\text{g/L}$. Important causes of mild hyperprolactinaemia are physiological stress and exercise. Medication-induced hyperprolactinaemia is usually associated with prolactin levels from 25 to $100 \mu\text{g/L}$, but metoclopramide, risperidone, and phenothiazines can raise prolactin level to $>200 \mu\text{g/L}$. Prolactinoma is the common cause of hyperprolactinemia. Clinical presentation of prolactinomas is pressure over optic chiasm and limitation of visual field. Patients can

also present decreased libido (Dabbous and Atkin: Arab J Urol. 2017, 16, 44–52).

Hyperprolactinemia is the cause of infertility in 11% of oligospermic males. Prolactin excess inhibits the pulsatile secretion of the gonadotrophin releasing hormone, which causes decreased activity of follicle stimulating hormone and luteinizing hormone, and consequently testosterone. Hyperprolactinemia is the reason of spermatogenic arrest, impaired sperm motility and quality. It leads to secondary hypogonadism and infertility. Hyperprolactinemia also directly influences spermatogenesis and steroidogenesis by prolactin receptors present in Sertoli and Leydig cells in testes, and it leads to primary hypogonadism and infertility.

Hyperprolactinemia is a reversible cause of male infertility, and has very good results with dopamine agonist drugs (bromocriptine and cabergoline). In male patients present with decreased libido, ED, and hypogonadism, and oligospermia or azoospermia, and impaired sperm motility, or altered sperm quality, a routine evaluation of serum prolactin level should be assessed.

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Anovulation subfertility is one of the most common causes of conception delay and accounts for one-third of the referrals to infertility clinics. Ovulatory disorders are broadly classified by the WHO as: Group I has hypothalamic-pituitary failure (15%), Group II has hypothalamic-pituitary-ovarian axis dysfunction (80%), Group III has ovarian failure (5%).

Ovulation induction therapies are highly effective in achieving pregnancy (Group I and II) in excess of 70% when there are no other factors in a couple's conception delay. Nevertheless, age and life-style factors negatively influence the success of treatment. Polycystic ovary syndrome (Group II) is the predominant cause of anovulatory subfertility (80%).

This update is going to cover the recent recommendations from the international evidence-based guideline for the assessment and management for PCOS (*Human Reproduction July 18*) including the use of metformin, anti-oestrogen therapies, aromatase inhibitors, ovarian drilling as well as gonadotropins. Furthermore, recent discoveries on Kisspeptin and Neurokinin B neuro-endocrine pathways may create more options in ovulation induction treatments. Although any new therapy should need to be compared with more established ovulation induction agents such as Clomifene in terms of success and patient safety.

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ANALYSIS OF THE ACTIVITY OF GLUTATHIONE PEROXIDASE AND TBA SUBSTANCES REACTIVE IN THE SEMINAL PLASMA IN THE CONDITIONS OF NORMOZOOSPERM AND SEMEN PATHOLOGY

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Oxidative stress is one of the main factors that impair the morphology and function of human spermatozoa, i.e. its ability to move, or the process of fusion with the egg cell. It occurs when the production of reactive oxygen species (ROS) reaches a critical point. Imbalanced ROS and antioxidant prevents the proper defense of the body with the use of antioxidative mechanisms located in sperm and seminal plasma.

In this article, the activity of antioxidant of glutathione peroxidase (GSH-Px) and the activity of thiobarbituric acid reactive substance (TBARS) in semen plasma under conditions of normozoospermia and semen pathology were investigated. The study involved 69 men treated for male infertility for more than 1 year in the absence of the female infertility factor. For research purposes, the group was divided into the WHO(+) group with normozoospermia in which 26 men were included, and WHO(-) group with abnormal semen parameters to which 43 men were qualified.

Comparing the WHO(+) and WHO(-) groups, statistically significantly higher activity of glutathione peroxidase in semen plasma was found in men with abnormal semen parameters. Higher TBARS levels were also found in the studies, which nevertheless were not statistically significant. There was a statistically significantly positive correlation between plasma glutathione peroxidase (GSH-Px-Plz) activity and TBARS plasma level (TBARS-Plz) in the WHO(+) group and no positive correlation in WHO(-). The above data suggests a relation between an oxidoreductive balance disorder and some cases of male infertility.

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THE PRESENCE OF BACTERIAL SPECIES IN HUMAN SEMEN AND THEIR IMPACT ON SPERM QUALITY

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This study was focused to assess whether bacteria detected in ejaculates from healthy donors could contribute to a decreased sperm quality. Samples were collected from 30 clinically healthy donors, and the presence of any bacterial strain was assessed using matrix assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS). For the semen quality assessment, Computer-assisted sperm analysis was used to evaluate sperm motility, Annexin V-Propidium Iodide stain was applied to study membrane integrity, while reactive oxygen species (ROS) production was investigated using luminol-based luminometry. DNA integrity was evaluated with the chromatin dispersion test, and mitochondrial membrane potential was assessed using the JC-1 fluorescent dye. The samples were distributed into groups depending on the bacterial strains identified, which were subsequently subjected to One Way ANOVA and the Dunnett test using GraphPad Prism. MALDI-TOF MS detected 10 bacterial strains in 20 samples, among which *Staphylococcus* and *Enterococcus* were the prevalent genera. The correlation analysis revealed that *Staphylococcus hominis*, *Enterococcus faecalis* and *Staphylococcus aureus* were associated with a significantly decreased sperm motility ($p < 0.001$), membrane integrity ($p < 0.001$) as well as mitochondrial activity ($p < 0.05$), followed by the highest DNA fragmentation index and ROS production ($p < 0.001$). Inversely, the presence of *Corynebacterium singulare*, *Corynebacterium aurimucosum* and *Streptococcus agalactiae* had no impact on sperm motility or mitochondrial activity, although semen positive for these bacteria exhibited a significantly lower sperm membrane and DNA integrity ($p < 0.05$). The results from this preliminary study reveal that bacteria detectable in semen from healthy humans may exhibit the potential to decrease sperm quality. As such, more attention must be paid to eradicate the potential factors leading to bacteriospermia and its detrimental effects on semen quality.

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BIRDS AND BEES DO IT RIGHT: WHAT IS WRONG WITH HUMAN SPERM?

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This paper looks in a holistic way at different reproductive patterns, sperm design and motility patterns in diverse insect and bird species and compares them with human sperm. The emphasis is on sperm biology and

pattern recognition and what may be derived from it. Are birds and bees really more successful when compared to humans? Is there a genuine problem concerning a global decline in human sperm quality and male fertility? Is the problem largely restricted to First World countries when contrasted with for example African and Arab countries? Let us first look at some sperm characteristics in the three diverse Animalia groups. Why these three groups? Insects form 95% of the animal kingdom with the most amazing diverse reproductive patterns and predominantly high levels of sperm competition and high quality sperm. Birds represent all levels of sperm competition up to monogamous groups but generally with a conservative Bauplan. Humans appear to have been monogamous for the last 25 000 years on the basis of genetic studies and accordingly have a low level of sperm competition and relatively poor sperm characteristics, as in gorillas and in the monogamous naked mole rat (van der Horst and Maree: Mol Reprod. 2014, 81, 204–216).

Sperm structure in insects is very complex and bees and black soldier flies have mitochondrial derivatives and tails as long as 220 μm and in honey bees' sperm concentration is 7 to 30 $\times 10^6/\text{mL}$ with almost 100% motile sperm. In African penguins' sperm concentration reaches about 3.3 $\times 10^9/\text{mL}$ with approximately 70% motility. In humans the average for donor sperm seems to be about 45 $\times 10^6/\text{mL}$ with approximately 60% motile sperm. Is this simply a numbers game that makes one group more successful than the others or is it an adaptation to the need to survive within a specific pattern of reproduction?

The Polish scientist, Mandelbrot, formulated the mathematical basis surrounding pattern recognition. In this paper we will try and explain pattern recognition in terms of reproductive strategies as well as in patterns of sperm motion in the above three groups. We hope to provide some clues to the current so-called panic mania about declining fertility and impending zero sperm number in humans.

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SEMEN ANALYSIS IN INFERTILITY MANAGEMENT

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Semen analysis according to WHO 2010 criteria is the first step to assess male fertility. Spermogram is an inseparable part of the diagnostic minimum in infertility therapy. The assessment of sperm parameters is used to select the method of infertility treatment. Standard semen analysis, however, is not able to assess fully the sperm's ability to fertilize the oocyte. The development of new diagnostic techniques has now created the

opportunity to study other sperm properties. In addition to the WHO 2010 semen analysis, it is possible to test: oxidative stress, DNA fragmentation and binding to hyaluronic acid.

Previous scientific reports on other semen tests and their relationship to the chance of achieving pregnancy relate mainly to the procedure of in vitro fertilization, which makes it difficult to interpret them unequivocally in the case of natural conception. The presented research results are also not consistent.

Spermogram and other semen tests provide the basis for conducting a therapeutic process in partners with reproductive problems. The choice of infertility treatment should, however, take into account not only the issue of the male factor, but also the reproductive potential of the woman.

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PLACENTA INCRETA – AN INCREASING PROBLEM IN CONTEMPORARY OBSTETRICS

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Placenta increta still constitutes a high risk condition leading to massive perinatal haemorrhage and morbidity and mortality both in mothers and their newborns. Recently an increase in placenta increta incidence has been observed in up to approximately 1/500 deliveries. An increase in perinatal hysterectomy due to placenta increta has been noticed in up to about 78%. A decrease in perinatal hysterectomy due to uterine atony has been found in up to 12% (Xiao-Yu Pan et al.: Chinese Medical Journal. 2015, 128, 2189–2193). Main risk factors for placenta increta include past medical history of a Caesarean section, placenta previa, maternal age of above 35 years old and surgery involving uterine tearing.

Management of placenta increta still belongs to the greatest challenges of contemporary obstetrics. Prenatal diagnosis and arrangements made in advance within an interdisciplinary team play a key role in the management. Ultrasonography, particularly 3D power Doppler US with the highest predictive value and magnetic resonance (MRI) have achieved the greatest usefulness in making a diagnosis.

At present, the following three treatments are being discussed: excision of involved uterine tissue with the placenta and reconstruction of the uterus; excision of the uterus with the placenta as well as leaving the placenta in situ. Preoperative arterial balloon catheterisation and urethral sounding have contributed to significantly limited operative complications.

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PROTECTION OF MALE FERTILITY BEFORE THE CANCER THERAPY

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The negative impact of cancer on fertility inter-individual has long been known. The disease process can damage temporarily or permanently gonads, seminal tract, penis, central nervous system (pituitary gland). Treatment therapies – orchidectomy – deprive the patient of generative cells; penile surgery - is a disorder of efficient deposition of sperm in the vagina or impotence, bladder neck surgery – results in retrograde ejaculation. Chemotherapy and radiation therapy damages generative cells, often irreversibly. Although the problem of losing fertility is associated primarily with testicular cancer which represents only 1% of all malignant tumors, but due to the fact most frequent malignant tumor in the group 15–35 year olds, is the flagship example of the impact of disease and cancer therapy. However, any cancer disease has a negative impact on fertility. Opportunities for successful cryopreservation of male generative cells and using them for medically assisted reproduction are known from 1949. Guidelines of the European Association of Urology strongly recommended to inform each oncological patient – during the procreation years – about protection of fertility. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells precisely describes the conditions of security of fertility in patients with cancer. Procedure should be initiated before the start of anticancer therapy. Cryoprotection apply to semen, part of the testis obtained during the biopsy, or the whole gonad. Storage at the temperature of liquid nitrogen does not damage generative cells. Protection of male fertility before cancer therapy gives the chance for fatherhood after recovering in such cases and for returning to normal social life.

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THE ASSESSMENT AND INVESTIGATION OF THE INFERTILE COUPLE

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Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. After screening for infection and cervical screening male and female partners should be investigated in parallel. Evaluation in primary and secondary male infertility is the

same. Initial male partner evaluation is medical history and semen analysis. Complete evaluation for male infertility is performed by a specialist in male reproduction. The indications for complete male infertility evaluation are: abnormal reproductive history, abnormal semen parameters, couples with unexplained infertility, couples remaining infertile after successful treatment of identified female infertility factors. This evaluation consists of complete medical history, physical examination, serial semen analyses, endocrine evaluation, postejaculatory urinalysis, ultrasonography, special tests on semen and sperm, genetic screening.

The initial examination of infertile women consists of reproductive history and physical examination.

Ovulatory function tests as mid-luteal progesterone, transvaginal ultrasound and, in the cases of amenorrhea and oligomenorrhea – thyroid-stimulating hormone (TSH), prolactin, follicle-stimulating hormone (FSH), estradiol – are recommended for standard evaluation. Ovarian reserve tests – early follicular phase FSH and estradiol, antral follicle count, serum anti-Müllerian hormone AMH – are considered to be useful to predict efficacy of ovulation stimulation. Methods for investigation of tubal and uterine abnormalities – hysterosalpingography, hysterosalpingo-contrast-ultrasonography, laparoscopy, hysteroscopy – are selected depending on the suspected comorbidities.