Chapter

The Sperm: Parameters and Evaluation

Tanya Milachich and Desislava Dyulgerova-Nikolova

Abstract

Sperm abnormalities are a major factor of human infertility. Since 1987, there are several references in different editions of World Health Organization (WHO) manual defining optimal sperm parameters. Over the years, many reproductive specialists have been constantly debating, suggesting and remodeling the frame values in those guidelines. Semen parameters have a leading role both in natural conception and assisted reproduction technologies (ART) outcomes. Deviations expressed in lower sperm count, impaired motility, abnormal morphology, and high percentage of sperm DNA fragmentation are linked to reduced chances to achieve pregnancy. In cases with low sperm count, severe oligoasthenozoospermia (OA) or azoospermia, karyotyping or evaluation with sperm aneuploidy test (SAT) could be an option and genetic counseling will be necessary if there is an obvious deviation or aberration (e.g., translocation, aneuploidy, etc.). Taking care of lifestyle factors as body mass index (BMI), diets, alcohol intake, smoking, using some additional nutrition and vitamin supplements might affect sperm parameters and contribute to the chances of a couple to conceive.

Keywords: sperm count, OAT, azoospermia, ART, karyotyping, sperm aneuploidy, DNA fragmentation, genetic testing, lifestyle

1. Introduction

The link between semen quality and fertility has been studied in humans since 1930 [1]. Semen analysis, as a standard laboratory test, gives basic information on spermatogenesis, secretory activity of the gonads and patency of the male genital tract [2]. The results obtained during the semen sample analysis could point out absence of spermatozoa, severe or mild deviation in sperm parameters or normal values for semen volume, sperm count and concentration, motility and morphology of the spermatozoa. Over the years many reproductive specialists have been constantly debating, suggesting and remodeling the frame values of the semen in reference to male fertility. Since 1987, there are several updates in different editions of World Health Organization (WHO) manuals defining the optimal sperm parameters with reference to pregnancy outcomes. The last (fifth) edition of the manual, published in 2010, defines serious decrease in cutoff values for sperm parameters related to chances of achieving pregnancy and thus its significance was widely discussed [3, 4]. One of the strong limitations of semen analysis and the defined fertility potential references in the last WHO edition is the lack of correlation with the female age, as only 30% of infertility in couples is due to male factors alone [5, 6]. The sixth edition of WHO is in discussion as some of the directions of changes would be: semen analysis

references including the Asian population for reference establishment; additional separate chapters for sperm morphology and computer assisted sperm analysis (CASA); importance of microbiological assessment. Some of the inapplicable tests at the modern andrology lab tests, e.g., postcoital test, capillary tube, Hamster test, counting in glass chambers will be excluded from the manual. New techniques such as sperm DNA fragmentation tests, aneuploidy screening, acrosome reaction assay, motile sperm organelle morphology examination (MSOME), Calcium ionophore activation, Catsper channels activity examination, influence of epigenetics and miRNA will be described [7].

2. Semen sample parameters

Semen sample parameters could be influenced by various factors such as sexual abstinence periods [8, 9], gonadal activity [10], abnormal hormonal levels [11], testicle size [12], body mass index (BMI) [13–15], urogenital infections and antibiotics or anabolic substances intake [16–18], individual diet regiment [19–21], working environment and lifestyle [22–24]. Sperm parameters have a leading role both in natural conception and assisted reproduction technologies (ART) outcomes. In order to establish male fertility potential, at least two to three sperm samples in a 3-month period should be analyzed [25]. Attention to intraindividual variability in parameters has to be considered. Reports in various studies show fluctuation in sperm volume and count, concentration, motility and morphology in one individual [26–29]. There are limitations to semen analysis depending on the patient specificity and the use of good laboratory practice protocols. Only this analysis by itself has a contradictory clinical value and might not be a stand-alone predictor for male fertility [30, 31]. In conditions such as azoospermia, globozoospermia or necrozoospermia, exceptions are made and male infertility could be stated [32].

When there are no sperm cells detected through microscope observations (azoospermia), the condition needs further investigation. Performing at least two separate semen analysis is needed. Centrifugation of the whole ejaculated volume is necessary in order to detect specific conditions [33]. When several or sporadic sperm cells are routed out in the sediment of the centrifuged sample the definition would be cryptozoospermia [34].

2.1 Azoospermia

Approximately 10–15% of all infertile men are diagnosed with azoospermia. When according to laboratory test a patient is diagnosed with azoospermia, further hormonal and genetic tests along with andrology, urology, genetic consultation and ultrasound scan are needed [35].

Obstructive azoospermia (OA): could be due to obstruction in the epididymis, vas deferens or the ejaculatory duct [36]; it could also be the consequence of infections, inflammation, scrotal trauma, rare genetic conditions (cystic fibrosis), vasectomy or injury of vas deferens or previous surgery [37]. Depending on the specific case, microsurgery is an option for restoring the passage of the sperm cells. Different techniques for sperm retrieval: percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) could be applied in order to obtain reproductive cells for further use in in vitro fertilization (IVF) or ICSI treatment [38].

Nonobstructive azoospermia (NOA): could be the consequence of hormonal imbalance [39, 40], Y-chromosome deletion or altered karyotype [41, 42], long period of toxins exposure [43], chemotherapy or radiation treatment [44], certain

medications intake or varicocele [45]. Resurrecting the spermatogenesis process could be achieved depending on the factors inducing azoospermia. Another option is performing TESE and ICSI procedure when sperm cells are retrieved after the extraction. In order to suggest and apply the proper treatment for the patient with NOA, adequate genetic consulting and testing should be present [46, 47].

Oligozoospermia: the condition is characterized by reduced sperm density as sperm concentration below the fifth centile in fertile men was recently reduced from 20 to 15 million/ml [48]. In 75% of the cases with oligozoospermia the cause of infertility is considered idiopathic [49]. In men with severe oligozoospermia, concentration of less than 5×10^6 sperm/ml, possibility of residual spermatogenic function decline has been reported [50]. Fertility preservation via sperm freezing is an option. Another, yet controversial, issue reflecting in low sperm count is obesity. WHO consultation in 1997 [51] recognized obesity as a global epidemic affecting society in the developed countries [52, 53]. Studies point out correlation of obesity and overweight to increased risk of azoospermia and oligozoospermia [54] and adherence to healthy and diverse diet could improve male fertility [55].

2.2 Sperm morphology

Sperm cell morphology is strongly correlated to male reproduction. Abnormalities might affect sperm motility, sperm fertilize ability and conception. Some conditions such as globozoospermia or stunted tail sperm defects could lead to inability to father biological children as a consequence of natural conception [56–58].

Recently, the intact human flagellum has been studied using cryo-electron microscopy and tomography [59]. A novel structure—tail axoneme intra-lumenal spiral (TAILS)—was reviled and described [60]. This new discovery suggests the need of further exploration and observation of sperm structures—not only in order to connect them to sperm function but also to clarify their significance. As previous studies reported, abnormal tail structure is correlated to sperm motility disorders, as nonspecific flagellar anomalies (NSFAs) are found to be the most frequent flagellar pathology in severe asthenozoospermia, and thus reduces the chance for natural conception [61]. According to the new data revealing TAILS, the explanation to some cases considered as unexplained infertility might be reviled.

Link between sperm morphology and numerical or structural chromosome abnormalities are suggested and investigated [62–64]. In fertile men, who have different translocations the frequencies of sperm chromosomal abnormalities were high (33–92%) in comparison to those with normal karyotype [65].

Post-radiotherapy treatments also show in altered number of structural and numerical chromosome aneuploidies (from 6 to 67% respectively [65]. Studies on infertile men with teratozoospermia (<14% normal forms), globozoospermia and macrocephalic, multinucleated or multiflagellate spermatozoa show an increased incidence of sperm aneuploidy up to 50% [65, 66]. Sperm with normal chromosome constitutions can be exhibited in men with normal or abnormal sperm parameters [67, 68].

2.3 Sperm sample evaluation and references

Investigating male fertility potential initially is based on routine semen analysis. Establishment of certain values for semen in order to predict chances of conception generates the need of references for male fertility. Requirement for semen analysis and semen parameters have been set as recommended in successive editions of WHO in 1980, 1987, 1992, 1999 and 2010 [1]. The following table [69] represents changes for cut off values for semen parameters according consecutive WHO manuals:

Semen characteristics	WHO (1980)	WHO (1987)	WHO (1992)	WHO (1999)	WHO (2010)[a]
Volume (mL)	ND	≥2	≥2	≥2	1.5
Sperm count (10^6/mL)	20–200	≥20	≥20	≥20	15
Total sperm count (10^6)	ND	≥40	≥40	≥40	39
Total motility (% motile)	≥60	≥50	≥50	≥50	40
Progressive motility[b]	≥2[c]	≥25%	≥25% (grade a)	≥25% (grade a)	32% (a + b)
Vitality (% alive)	ND	≥50	≥75	≥75	58
Morphology (% normal forms)	80.5	≥50	≥30[d]	(14)[e]	4[f]
Leukocyte count (10^6/mL)	<4.7	<1.0	<1.0	<1.0	<1.0

[a] Lower reference limit obtained from the lower fifth centile value

[b] Grade a, rapid progressive motility (>25 μm/s); grade b, slow/sluggish progressive motility (5–25 μm/s); Normal, 50% motility (grades a + b) or 25% progressive motility (grade a) within 60 min of ejaculation

[c] Forward progression (scale 0–3)

[d] Arbitrary value

[e] Value not defined but strict criterion is suggested

[f] Strict (Tygerberg) criterion; *ND* not defined

Sperm sample evaluation in a modern andrology lab might be measured by the means of CASA. The use of computer aid does not exclude additional evaluation by the human eye [70, 71]. For sperm morphology evaluation, WHO [72] recommends criteria by strict morphology [73].

3. DNA fragmentation

Recently, DNA fragmentation tests have been widely incorporated in laboratory practice. DNA integrity and sperm hereditary information are essential to the offspring as male gametes has major contribution to the fertilization processes, embryo quality and embryo development even in early gestational stages [74–76]. Sperm contains almost 3000 different kinds of mRNA coded for proteins that are active in the early embryo development period. There are also some others still unknown and with no equivalent in the oocyte [77, 78].

3.1 Morphology evaluation and sperm selection in real time

Since the introduction of ICSI as routine procedure, the significance of standard semen analysis was neglected, as sperm concentration and motility have no longer such importance, since a single sperm cell has to be injected. When standard ICSI procedure under a Hoffman modulation contrast microscope, or Nomarski optics at magnification ×400 is performed visualization and assessment of sperm head (size and shape) mid-piece and tail are possible, but detailed ultrastructural morphology examination is limited [79, 80]. When conventional ICSI is performed, it would be difficult to evaluate and select morphologically normal sperm based on its detailed structural portrait: vacuolization, membrane invaginations, mid-piece thickness or deformity, etc. It is controversial whether high vacuolization in the sperm head is associated with higher DNA fragmentation and aneuploidy rate [81, 82] that may have adverse effect on embryo quality and postimplantation development and higher frequency of pregnancy loss at early gestational stages. Still, for some couples detailed sperm examination prior ICSI is preferable [83, 84].

Intracytoplasmic morphologically selected sperm injection (IMSI) is the cornerstone to sperm morphology evaluation. Based on the examination of motile sperm organelle morphology (MSOME) IMSI is the only real-time, unstained method used for selection of motile and morphologically normal spermatozoa for intracytoplasmic injection. IMSI was first introduced by Baratoov et al. [85]. MSOME selection

is made under inverted light emitting microscope with Differential interference contrast or Nomarski differential interference contrast optics and digital camera at high magnification ranging from ×6600 to ×13,000. Using MSOME criteria, the motile sperm fraction and each cell malformation is evaluated according to the morphological status of six organelles comprising the acrosome, post-acrosomal lamina, neck, mitochondria, tail and nucleus. Only 33% of spermatozoa from the examined samples appeared morphologically normal according to these criteria [86].

Defects defined for each area are:
acrosomal area—lack, partial or vesiculated;
post-acrosomal lamina—lack or vesiculated;
neck—abaxial, cytoplasmic droplet.
mitochondria—lack, partial, disorganization;
tail—lack, coiled, broken, multi, short;
nucleus—small or large oval, narrow, wide or short, regional disorder, vacuoles occupying more than 4% of the nuclear area [87, 88]. However, evaluation of motile spermatozoa might differently be determined by various scientists [89].

What seems to be the most important in the observation of motile spermatozoa under high magnification in real time is evaluating the presence of vacuoles in the head of the sperm cell—number, size and location. The precise origin of the vacuoles is still unknown, but different hypothesis suggest they derived from early stages of spermatogenesis during sperm maturation and their number increase on account of vacuole area [90]. Other studies suggest that vacuoles formation in spermatozoa starts in incubation and capacitation period after ejaculation [91]. Nevertheless, high vacuolization or the presence of large vacuoles in the sperm head might be associated with increased DNA fragmentation rates and increased level of chromatin immaturity and could influence fertilization and pregnancy rates [92–94].

Sperm morphology evaluation could be based on the Cassuto and Barak Score as a precise rate system for sperm selection [94]. For the establishment of the score six parameters of the spermatozoon were taken into account: head, acrosome, vacuole, basis, insertion, and cytoplasmic droplet ("HAVBIC"). Head, vacuole, and basis were considered as major criteria for abnormalities, and acrosome, insertion, and cytoplasmic droplet are minor criteria for sperm evaluation. The following equation was developed:

$$\text{Score of spermatozoa} = (2 \times \text{Head}) + (3 \times \text{Vacuole}) + (1 \times \text{Base}) \quad (1)$$

Based on the formula, sperm cells score could vary between 0 and up to 6, and in relation to the quality three groups were differentiated:

Class 1—High-quality spermatozoa (score 4–6);
Class 2—Medium-quality spermatozoa (score 1–3); and
Class 3—Low-quality spermatozoa (score 0) [81].

Since its introduction and based on the first articles demonstrating increase in the pregnancy rates using IMSI compared to ICSI [85, 87] the method became widely incorporated in laboratory practice despite it is a time-consuming technique.

Evaluation of motile spermatozoa under high magnification is suitable for patients with high levels of DNA fragmentation sperm aneuploidy, severe oligo- or oligoasthenozoospermia and/or teratozoospermia, recurrent implantation failures or history of repeated early miscarriages, advanced female age and advanced male age [80]. Subsequent studies provided further analysis and information for the importance of the new method evaluating sperm morphology for obtaining better results in patients with male factor infertility.

There are still controversial study conclusions for the impact of IMSI procedure on *in vitro* cycle outcomes. However, what seems to benefit its practice is the better understanding of sperm morphology and function [95].

3.2 Genetics and sperm count

Y-chromosome deletion is associated with azoospermia, oligozoospermia (low sperm count) or abnormal sperm morphology and motility [96, 97]. When AZFa and AZFb deletions are detected, testicular sperm retrieval would be ineffective [98], but it is successful option for most males with AZFc deletions [99–101]. There is a case [102] reporting natural conception and Y-microdeletion passing. When diagnosed, and considering that Y chromosome infertility is inherited in a Y-linked manner, the patients should discuss and consult the specific genetic condition with genetics specialists as this could lead to infertility in the next generations [103].

Another gene that could be investigated in order to obtain the option for TESE is the Testis expressed gene 11 (TEX 11). Studies show that *TEX11* (X-linked meiosis-specific gene) is mutated in azoospermic men [104]. Sperm retrieval is not applicable for those patients [105, 106].

Congenital bilateral absence of vas deferens (CBAVD) and the lack of sperm cells in the ejaculate are superable using microsurgical TESE or PESA followed by ICSI [107]. As CBAVD has been associated with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [108, 109] investigating the condition and genetic testing and consultation prior the procedures should be provided as there is a risk for the couple to have a child with cystic fibrosis [110].

In conclusion, genetic counseling as well as prenatal genetic diagnosis (PGD) or preimplantation genetic screening (PGS) should be offered as part of the fertility treatment [111, 112].

4. Cross points between cancer treatment and male fertility

Cancer healing by chemo- or radiation- therapy may disturb hormone production, ejaculation and spermatogenesis for long period of time or even permanently. These aggressive treatments could lead also to higher DNA sperm fragmentation [113, 114]. For cancer patients with assigned therapy, freezing one or several semen samples prior the start of any medicaments and manipulations (including operation, X-ray for additional diagnostics) is an option for further fertility preservation. Although cryopreservation of sperm does not guarantee preserving fertility or achieving pregnancy, it is substantial to consult and encourage the patient/couple to do it. Still, in patients with terminal loss of spermatogenesis due to cancer treatment, frozen samples are the only chance of hope to father a biological child. When a female partner is involved, counseling should consider the fact that female age is a leading factor in conception and postponing the ART or natural conception, could seriously decrease the chances of having a baby [115–119].

4.1 Using donor sperm in ART

Despite the advances of modern science and reproductive medicine, for some men, the only chance to father a child is through donated sperm [120]. In cases when after thorough examination male sterility was diagnosed [121]; severe hereditary conditions are established, or a couple with male factor infertility had numerous in vitro cycles [122] with no positive results, using donated sperm is an option. Employing donor spermatozoa in the fertility treatment could influence the

couple's psychological state, the relationship between the partners and their relatives [123, 124]. In order to perceive infertility and take informed choice for further fertility treatment psychological support could be of help [125].

5. Reactive oxygen species (ROS)

Superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hypochlorite (OHCl), and hydroxyl radical (OH) are highly reactive oxygen species (ROS) and their production occurs during normal metabolism of the cell. In semen ROS are produced mostly by the leukocytes and immature spermatozoa and are related to acrosome reaction, capacitation, mitochondrial stability, and fusion with oocyte. Imbalance between the formation of ROS and the inability of the antioxidants to neutralize the excessive production of ROS is defined as oxidative stress (OS). As seminal plasma contains antioxidants and has natural antioxidant capacity, it sustains the free radicals balance in the sperm; overproduction of ROS and OS results in lipid peroxidation, protein changes, DNA damage and sperm death, and this may affect male fertility [126, 127].

High concentrations of ROS as potential cause of male infertility have been studied since 1943 [128]. Potential internal yield of excess ROS could be consequence to damaged or abnormal spermatozoa, varicocele, cryptorchism, testicular torsion, infection, inflammation and aging. Some external factors such as exposure to toxins (toluene, methoxyethanol, sulfur dioxide), metals (cadmium) chemotherapy and ionizing radiation (cancer treatment) may also influence ROS levels and form OS.

Studies demonstrate association between elevated ROS levels and abnormal sperm concentration, motility, morphology, higher DNA damage and apoptosis. Comparison between infertile men and donors showed that excess ROS values had a sensitivity of 68.8% and specificity of 93.8% in correlation with poor semen parameters and could result in infertility [129, 130].

It is important to understand the physiological role of ROS as they are relevant to sperm capacitation, hyperactivation and sperm-egg fusion formation. ROS are involved in intracellular cyclic adenosine monophosphate (cAMP) increase followed by protein Kinase A activation and elevation of tyrosine phosphorylation. These changes lead to sperm capacitation and hyperactivation, sperm membrane becomes unstable and initiates acrosome reaction (releasing enzymes contained in the acrosome—nonzymogen acrosin, proacrosin, inhibitor-bound acrosin, hyaluronidase, acid phosphatase, beta-glucuronidase, beta-glucosidase, beta-N-acetylglucosaminidase, beta-galactosidase and beta-N-acetylgalactosaminidase) which allows the binding of sperm cell to oocytes zona pellucida (ZP) [131].

Imbalanced ROS levels could compromise semen quality and functions and keeping them in normal concentration is considered essential to fertility. Oxidative stress and nutritional status are of importance to every person as antioxidant deficiency and malnutrition may alter the health in general. ROS are also related to various respiratory and cardiovascular diseases, neurodegenerative, digestive disorders and even cancer. The clinical importance of OS in relation to fertility is thoroughly studied. The clinical awareness of nutritional balance in disease occurrence, progression and outcome is still limited, but the need of balanced diet nutrients and antioxidants is urged and necessary [132, 133].

6. Processing sperm samples *in vitro*

The ability of a men to become biological father is not only a consequence of normal sperm count but is also linked to the normal function of the male reproductive

tract and sperm activity. Failure in sperm production or low sperm count and motility, poor morphology, disturbance in sperm movement and progressive passage through the cervical mucus, uterus, ampulla of the oviducts, capacitation and acrosome reaction, binding zona peluccida, etc. can result in male infertility.

To overcome male infertility in ART different protocols for sperm processing have been developed. There are still many debates on the exact influence of specific techniques used for sperm processing and their benefit to achieve pregnancy. Selecting a proper technique must be strongly individual according the couple's infertility history and ART treatment plan along with semen quality. Isolating an optimal fraction (higher count with progressive movement, morphologically normal rates) of spermatozoa gives the opportunity for selection and usage of the spermatozoa with a better fertilizability and higher chances to contribute for a viable fetus, for intrauterine insemination (IUI), IVF or ICSI.

Two of the most explored methods for sperm processing in ART—density gradient centrifugation (DGS) and swim-up (SU)—are investigated in details. Compared to fresh sample, the processed one has lower DNA fragmentation rates [134, 135] and lower concentration of ROS regardless which method was used [136, 137].

As there are studies exploring telomere length in reproductive cells (oocytes and spermatozoa) and their connection to infertility, shorter telomeres in spermatozoa might be assumed as a factor causing idiopathic infertility [138]. Truncated telomeres and altered DNA integrity in sperm could negatively influence fertilization, pronuclei formation, embryo morphology and quality and thus could compromise blastocyst formation and implantation. Spermatozoa obtained by either DGC or SU have longer telomeres compared sperm cells in the raw semen [139].

Some substances such as pentoxifylline (methylxanthine derivate primarily used in intermittent claudication and other vascular disorders treatment) might enhance the motility and quantity of motile sperm after processing. By using pentoxifylline primarily on samples with poor quality increased sperm viability in infertile men with oligoasthenozoospermia, was observed. Samples obtained by PESA or TESE could also be improved by implementing this xanthine derivative in cultural media and thus improve sperm motility [140].

Sperm preparation methods along with technical advantages of MSOME allow the selection of sperm cells, with best predictive values, for ART treatments. There are some limitations related to each method used and that is now an open field to research and establish new noninvasive protocols for sperm selection in the routine practice.

7. Conclusion

Spermatogenesis is a complexed process of division and formation of male reproductive cells. It is highly sensitive to various internal (hormonal regulation, transmitters, growth factors) and external (nutritive substances, therapeutics, drugs, hormones and their metabolites, different toxic substances or X-radiation, increased temperature) factors [141]. Given that the time frame for formation of every new generation of spermatozoa takes approximately 3 months, it should be considered that unfavorable effects purge would be the consequence to time consuming treatment or lifestyle changes.

Modern day society—environment, lifestyle and diet are suspected to be harmful to different processes in the organism such as spermatogenesis and could negatively affect the quality and quantity of life through human lifespan including the ability to reproduce. Considering that sex formation takes place during early fetal development attention to mother's nocuous habits, lifestyle, and environmental specifics should be advert. Events during pregnancy could also influence male

fertility later in life [142]. In some specific cases, when there was a long exposure to high dosage of toxins, chemotherapy or radiotherapy, spermatogenesis regeneration would most probably take years or may never be restored. Healthy life style along with regular medical check and tests could indicate on time and even prevent urological or fertility problems.

Acknowledgements

The authors wish to thank their IVF unit colleagues at Ob/Gyn Hospital "Dr. Shterev" for most helpful discussions while summarizing this chapter.

Conflict of interest

The authors report no financial or commercial conflicts of interest.

Appendix: this appendix presents the definitions of terms/notations used through the report

AMA	advanced maternal age
ART	assisted reproduction technology
BMI	body mass index
CASA	computer assisted sperm analysis
cAMP	cyclic adenosine monophosphate
CBAVD	congenital bilateral absence of vas deferens
CFTR	cystic fibrosis transmembrane conductance regulator gene
DGC	density gradient centrifugation
ICSI	intracytoplasmic sperm injection
IMSI	intracytoplasmic morphologically selected sperm injection
IUI	intrauterine insemination
IVF	in vitro fertilization
MESA	microsurgical epididymal sperm aspiration
MSOME	motile sperm organelle morphology examination
NOA	nonobstructive oligoasthenozoospermia
OA	oligoasthenozoospermia
OS	oxidative stress
PESA	percutaneous epididymal sperm aspiration
PGD	preimplantation genetic diagnosis
PGS	preimplantation genetic screening extraction
ROS	reactive oxygen species
SAT	sperm aneuploidy test
SU	swim up
TAILS	tail axoneme intra-lumenal spital
TESE	testicular sperm
TEX 11	testis expressed gene
WHO	World Health Organization
ZP	zona pellucida
H_2O_2	hydrogen peroxide
O^{2-}	superoxide anion
OHCl	hypochlorite
OH	hydroxyl radical

Author details

Tanya Milachich* and Desislava Dyulgerova-Nikolova
SAGBAL "Dr. Shterev", Sofia, Bulgaria

*Address all correspondence to: tanya_ivf@yahoo.com

References

[1] Andrade-Rocha FT. On the origins of the semen analysis: A close relationship with the history of the reproductive medicine. Journal of Human Reproduction. 2017;**10**(4):242-255. DOI: 10.4103/jhrs.JHRS_97_17

[2] Vasan SS. Semen analysis and sperm function tests: How much to test? Indian Journal of Urology. 2011;**27**(1):41-48. DOI: 10.4103/0970-1591.78424

[3] Murray KS, Andrew J, McGeady J, Reed ML, Kuang WW, Nangia AK. The effect of the new 2010 World Health Organization criteria forsemen analyses on male infertility. Fertility and Sterility. 2012;**98**(6):1428-1431. DOI: 10.1016/j.fertnstert.2012.07.1130. [Epub: 24 August 2012]

[4] Alshahrani S, Aldossari K, Al-Zahrani J, Gabr AH, Henkel R, Ahmad G. Interpretation of semen analysis using WHO 1999 and WHO 2010 reference values: Abnormal becoming normal. First International Journal of Andrology Andrologia. 2018;**50**(2):e12838. DOI: 10.1111/and.12838

[5] Patel A, Leong JY, Ramasamy R. Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review. Arab Journal of Urology. 2018;**16**:96-102. DOI: 10.1016/j.aju.2017.10.005. eCollection 2018 Mar

[6] Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES Jr, Agarwal A. Critical appraisal of World Health Organization's New reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. Urology. 2012;**79**:16-22. DOI: 10.1016/j.urology.2011.08.003. [Epub: 08 November 2011]

[7] Baldi E, Festin MP. WHO manual: Status report and who perspectives. Abstracts of the 10th Congress of the European Academy of Andrology. Andrology. 2018;**6**(Suppl 2):23

[8] Sorena K, Craig JR, Zhang C. Impacts of abstinence time on semen parameters in a large population-based cohort of subfertile men. Urology. 2017;**108**:90-95

[9] De Jonge C, LaFromboise M, Bosmans E, et al. Influence of the abstinence period on human sperm quality. Fertility and Sterility. 2004;**82**(1):57-65

[10] Mahdi MA, Shwish DL, Almhmdi NA. Evaluation of the impact of the level of (Testosterone, Luteinizing Hormone, Follicle-Stimulating Hormone and Prolactin) on some semen parameters in infertile males. Tikrit Journal of Pure Science. 2018;**23**(7). ISSN: 18131662

[11] Miller MR, Mannowetz N, Iavarone AT, et al. Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. Science. 2016;**352**(6285):555-559. DOI: 10.1126/science.aad6887

[12] PapeMøller A. Ejaculate quality, testes size and sperm competition in primates. Journal of Human Evolution. 1988;**17**(5):479-488

[13] Veleva L, Milachich T, Bochev I. Relationship of somatic indicators with motility and velocity parameters of sperm movement. Reproductive Health. 2015;(22):9-13

[14] Sermondade N, Faure C, Fezeu L, et al. BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. Human Reproduction Update. 2013;**19**(3):221-231. DOI: 10.1093/humupd/dms050

[15] Andersen JM, Herning H, Aschim EL, Hjelmesæth J, Mala T,

Hanevik HI, et al. Body mass index is associated with impaired semen characteristics and reduced levels of anti-Müllerian hormone across a wide weight range. PLoS One. 2015;**10**(6):e0130210. DOI: 10.1371/journal.pone.0130210

[16] Rusz A, Pilatz A, Wagenlehner F, et al. Influence of urogenital infections and inflammation on semen quality and male fertility. The World Journal of Urology. 2012;**30**(1):23-30. DOI: 10.1007/s00345-011-0726-8. [Epub: 12 July 2011]

[17] Keck C, Gerber-Schäfer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: Impact on male fertility and treatment options. Human Reproduction Update. 1998;**4**(6):891-903. DOI: 10.1093/humupd/4.6.891

[18] Ochsendorf FR. Sexually transmitted infections: Impact on male fertility. Andrologia. 2008;**40**(2):72-75. DOI: 10.1111/j.1439-0272.2007.00825.x

[19] Salas-Huetos A. Adherence to the Mediterranean diet is positively associated with sperm motility: A cross-sectional analysis. Scientific Reports. 2019;**9**. DOI: 10.1038/s41598-019-39826-7. Article number: 3389

[20] Nassan FL et al. Diet and men's fertility: Does diet affect sperm quality? Fertility and Sterility. 2018;**110**(4):570-577. DOI: 10.1016/j.fertnstert.2018.05.025

[21] Luque EM, Tissera A, Gaggino MP, Molina RI, Mangeaud A, Vincenti LM, et al. Body mass index and human sperm quality: Neither one extreme nor the other. Reproduction, Fertility and Development. 2015;**29**:731-739. DOI: 10.1071/RD15351

[22] Sharma R, Harlev A, Agarwal A, Sandro C, Esteves SC. Cigarette smoking and semen quality: A new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. European Urology. 2016;**70**(4):635-645. DOI: 10.1016/j.eururo.2016.04.010. [Epub: 21 April 2016]

[23] Gaskins AJ, Mendiola J, Afeiche M, et al. Physical activity and television watching in relation to semen quality in young men. British Journal of Sports Medicine. 2015;**49**:265-270. DOI: 10.1136/bjsports-2012-091644. [Epub: 04 February 2013]

[24] Lähdetie J. Occupation- and exposure-related studies on human sperm. Journal of Occupational and Environmental Medicine. 1995;**37**(8):922-930. DOI: 10.1016/j.fertnstert.2005.06.048

[25] Keel AB. Within- and between-subject variation in semenparameters in infertile men and normal semen donors. Fertility and Sterility. 2006;**85**(1):128-134

[26] World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 3rd ed. Cambridge: Cambridge University Press; 1992

[27] Ossenbuhn S. Exogenous influences on human fertility: Fluctuations in sperm parameters and results of in-vitro fertilization coincide with conceptions in the normal population. Human Reproduction. 1998;**13**(8):2165-2171. DOI: 10.1093/humrep/13.8.2165

[28] Ombelet W, Maes M, Vandeput H, et al. Chronobiological fluctuations in semen parameters with a constant abstinence period. Archives of Andrology. 1996;**37**(2):91-96. DOI: 10.3109/01485019608988508

[29] De Giorgi A, Volpi R, Tiseo R, et al. Seasonal variation of human semen parameters: A retrospective study in Italy. Chronobiology International. 2015;**32**(5):711-716. DOI: 10.3109/07420528.2015.1024315. [Epub: 04 May 2015]

[30] Moazzam A, Choudhary MN, Muhammad I, et al. From basic to contemporary semen analysis: limitations and variability. The Journal of Animal & Plant Sciences. 2015;**25**(2):328-336. ISSN: 1018-7081

[31] Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertility and Sterility. 2014;**102**(6):1502-1507. DOI: 10.1016/j.fertnstert.2014.10.021

[32] Sikka SC, Hellstrom WJG. Current updates on laboratory techniques for the diagnosis of male reproductive failure. Asian Journal of Andrology. 2016;**18**:392-401. DOI: 10.4103/1008-682X.179161

[33] World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010

[34] Karabulut S, Keskin İ, Kutlu P, Delikara N, Atvar Ö, Öztürk MI. Male infertility, azoozpermia and cryptozoospermia incidence among three infertility clinics in Turkey. Turkish Journal of Urology. 2018;**44**(2):109-113. DOI: 10.5152/tud.2018.59196

[35] Practice committee of the American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology. Evaluation of the azoospermic male: A committee opinion. Fertility and Sterility. 2018;**109**(5):0015-0282. DOI: 10.1016/j.fertnstert.2018.01.043

[36] Cornud F, Belin X, Delafontaine D, et al. Imaging of obstructive azoospermia. European Radiology. 1997;**7**:1079. DOI: 10.1007/s003300050258

[37] Schlegel NP. Causes of azoospermia and their management. Reproduction, Fertility and Development;**16**(5):561-572. DOI: 10.1071/RD03087

[38] Baker K, Sabanegh E Jr. Obstructive azoospermia: reconstructive techniques and results. Clinics (Sao Paulo). 2013;**68**(Suppl 1):61-73. DOI: 10.6061/clinics/2013(sup01)07

[39] Kumar R. Medical management of non-obstructive azoospermia. Clinics (Sao Paulo). 2013;**68**(Suppl 1):75-79. DOI: 10.6061/clinics/2013(Sup01)08

[40] Emokpae MA, Uadia PO, Mohammed AZ, Omale-Itodo A. Hormonal abnormalities in azoospermic men in Kano, Northern Nigeria. The Indian Journal of Medical Research. 2006;**124**:299-304

[41] Nagvenkar P, Desai K, Hinduja I, Kusum Zaveri K. Chromosomal studies in infertile men with oligozoospermia & non-obstructive azoospermia. The Indian Journal of Medical Research. 2005;**122**:34-4234

[42] Xie C, Chen X, Liu Y, Wu Z, Ping P. Multicenter study of genetic abnormalities associated with severe oligospermia and non-obstructive azoospermia. Journal of International Medical Research. 2018;**46**(1):107-114. DOI: 10.1177/0300060517718771

[43] Benoff S, Jacob A, Hurley IR. Male infertility and environmental exposure to lead and cadmium. Human Reproduction Update. 2000;**6**(2):107-121. DOI: 10.1093/humupd/6.2.107

[44] Boekelheide K. Mechanisms of toxic damage to spermatogenesis. JNCI Monographs. 2005;**2005**(34):6-8. DOI: 10.1093/jncimonographs/lgi006

[45] Weedin JW, Khera M, Lipshultz LI. Varicocele repair in patients with nonobstructive azoospermia: A meta-analysis. The Journal of Urology. 2010;**183**(6):2309-2315. DOI: 10.1016/j.juro.2010.02.012. [Epub: 18 April 2010]

[46] Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. Clinics (São Paulo, Brazil). 2013;**68**(Suppl 1):39-60. DOI: 10.6061/clinics/2013(sup01)06

[47] Wosnitzer MS. Genetic evaluation of male infertility. Translational Andrology and Urology. 2014;**3**(1):17-26. DOI: 10.3978/j.issn.2223-4683.2014.02.04

[48] World Health Organization. World Health Organization Laboratory Manual for the Evaluation and Processing of Human Semen. 5th ed. Cambridge, UK: Cambridge University Press; 2010

[49] Punab M, Poolamets O, Paju P, et al. Causes of male infertility: A 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Human Reproduction. 2017;**32**(1):18-31. DOI: 10.1093/humrep/dew284

[50] Song S-H, Bak CW, et al. Natural course of severe oligozoospermia in infertile male: Influence on future fertility potential. Journal of Andrology. 2010;**31**(6). DOI: 10.2164/jandrol.110.010199. [Epub: 29 July 2010]

[51] World Health Organization. Obesity: Preventing and Managing the Global Epidemic, Report of a WHO Consultation (WHO Technical Report Series 894). Geneva, Switzerland: World Health Organization; 2000

[52] Caballero B. The global epidemic of obesity: An overview. Epidemiologic Reviews. 2007;**29**(1):1-5. DOI: 10.1093/epirev/mxm012

[53] Craig JR et al. Obesity, male infertility, and the sperm epigenome. Fertility and Sterility. 2017;**107**(4):848-859. DOI: 10.1016/j.fertnstert.2017.02.115

[54] Sermondade N, Faure C, Fezeu L, et al. Obesity and increased risk for oligozoospermia and azoospermia. Archives of Internal Medicine. 2012;**172**(5):440-442. DOI: 10.1001/archinternmed.2011.1382

[55] Salas-Huetos A, Bulló M, Salas-Salvadó J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: A systematic review of observational studies. Human Reproduction Update. 2017;**23**(4):371-389. DOI: 10.1093/humupd/dmx006

[56] Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. Human Reproduction Update. 2007;**13**(1):63-75. DOI: 10.1093/humupd/dml047. [Epub: 28 September 2006]

[57] Vicari E, Perdichizzi A, De Palma A, Burrello N, D'Agata R, Calogero AE. Globozoospermia is associated with chromatin structure abnormalities: Case report. Human Reproduction. 2002;**17**(8):2128-2133. DOI: 10.1093/humrep/17.8.2128

[58] Baccetti B et al. Notulae seminologicae. 2. The 'short tail' and 'stump' defect in human spermatozoa. Andrologia: First International Journal of Andrology. 1993;**25**(6):331-335. DOI: 10.1111/j.1439-0272.1993.tb02736.x

[59] Koning RI, Koster AJ. Cryo-electron tomography in biology and medicine. Annals of Anatomy—Anatomischer Anzeiger. 2009;**191**(5):427-445. DOI: 10.1016/j.aanat.2009.04.003

[60] Zabeo D, Heumann JM, Schwartz CL, Suzuki-Shinjo A, Morgan G, Widlund Per O, et al. A lumenal interrupted helix in human sperm tail microtubules. Scientific Reports. 2018;**8**:2727. DOI: 10.1038/s41598-018-21165-8

[61] Chemes HE, Sedo CA. Tales of the tail and sperm head aches: Changing concepts on the prognostic significance of sperm pathologies affecting the

head, neck and tail. Asian Journal of Andrology. 2012;**14**(1):14-23. DOI: 10.1038/aja.2011.168

[62] Wyrobek AJ, Bruce WR. The induction of sperm-shape abnormalities in mice and humans. In Chemical Mutagens: Principles and Methods for Their Detection Vol. 5. Hollaender A, de Serres FJ. New York, Plenum; (1978): 257-285.

[63] Martin RH, Rademaker A. The relationship between sperm chromosomal abnormalities and sperm morphology in humans. Mutation Research. 1988;**207**(3-4):159-164

[64] Ray P, Toure A, Metzler-Guillemain C, et al. Genetic abnormalities leading to qualitative defects of sperm morphology or function. Clinical Genetics. 2017;**91**(2):217-232. DOI: 10.1111/cge.12905, Special Issue: A Special Issue on Genetics and Human Reproduction. [Epub: 09 December 2016]

[65] Sun F, Ko E, Martin RH. Is there a relationship between sperm chromosome abnormalities and sperm morphology? Reproductive Biology and Endocrinology. 2006;**4**(1):1. DOI: 10.1186/1477-7827-4-1

[66] Devillard F, Metzler-Guillemain C, Pelletier R, DeRobertis C, Bergues U, Hennebicq S, et al. Polyploidy in large-headed sperm: FISH study of three cases. Human Reproduction. 2002;**17**(5):1292-1298. DOI: 10.1093/humrep/17.5.1292

[67] Boushaba S, Belaaloui G. Sperm DNA fragmentation and standard semen parameters in Algerian infertile male partners. The World Journal of Men's Health. 2015;**33**(1):1-7. DOI: 10.5534/wjmh.2015.33.1.1

[68] Belloc S, Benkhalifa M, Cohen-Bacrie M, Dalleac A, Chahine H, Amar E, et al. Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation. Journal of Assisted Reproduction and Genetics. 2014;**31**(5):527-532. DOI: 10.1007/s10815-014-0194-3

[69] Menkveld R, Wong WY, Lombard CJ, Wetzels AMM, Thomas CMG, Merkus H, et al. Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: An effort towards standardization of in-vivo thresholds. Human Reproduction. 2001;**16**(6):1165-1171. DOI: 10.1093/humrep/16.6.1165

[70] Engel KM, Grunewald S, Schiller J. Automated semen analysis by SQA Vision® versus the manual approach—A prospective double-blind study. Andrologia. 2019;**51**(1):e131490. DOI: 10.1111/and.13149

[71] Dearing C, Jayasena C, Lindsay K. Can the Sperm Class Analyser (SCA) CASA-Mot system for human sperm motility analysis reduce imprecision and operator subjectivity and improve semen analysis? Human Fertility. Published online May 6, 2019:1-11. DOI: 10.1080/14647273.2019.1610581

[72] World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge: Cambridge University Press; 1999

[73] World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed. Geneva: World Health Organization; 2010

[74] Kim SM, Kim SK, Jee BC, Kim SH. Effect of sperm DNA fragmentation on embryo quality in normal responder women in in vitro fertilization and intracytoplasmic sperm injection. Yonsei Medical Journal. 2019;**60**(5):461-466. DOI: 10.3349/ymj.2019.60.5.461

[75] Sedó CA, Bilinski M, Lorenzi D, et al. Effect of sperm DNA fragmentation on embryo development: Clinical and biological aspects. JBRA Assisted Reproduction;**21**(4):343-350. DOI: 10.5935/1518-0557.20170061

[76] Esquerré-Lamare C, Walschaerts M, Chansel Debordeaux L, et al. Sperm aneuploidy and DNA fragmentation in unexplained recurrent pregnancy loss: A multicenter case-control study. Basic and Clinical Andrology. 2018;**28**:4. DOI: 10.1186/s12610-018-0070-6. [Epub: 02 April 2018]

[77] Krawetz SA. Paternal contribution: New insights and future challenges. Nature Reviews. Genetics. 2005;**6**(8):633-642. DOI: 10.1038/nrg1654

[78] Bukowska D, Kempisty B, Piotrowska H, Sosinska P, Wozna M, Ciesiolka S, et al. The structure and role of mammalian sperm RNA: A review. Veterinární Medicína. 2013;**58**(2):57-64. DOI: 10.17221/6696-VETMED

[79] Berkovitz A, Eltes F, Lederman H, Peer S, Ellenbogen A, Feldberg B, et al. How to improve IVF–ICSI outcome by sperm selection. Reproductive Biomedicine Online. 2006;**12**(5):634-638. DOI: 10.1016/s1472-6483(10)61191-1

[80] Yona B, Ellenbogen A. Interacytoplasmic morphologically selected sperm injection: A tool for selecting the best sperm in real time. 2018. DOI: 10.5772/intechopen.73388 In book: Spermatozoa - Facts and Perspectives. License CC BY 3.0

[81] Gosálvez J et al. Single sperm selection and DNA fragmentation analysis: The case of MSOME/IMSI. Natural Science. 2013;**5**(7A):7-14. DOI: 10.4236/ns.2013.57A002

[82] Ebner T, Shebl O, Oppelt P, Mayer RB. Some reflections on intracytoplasmic morphologically selected sperm injection. International Journal of Fertility & Sterility. 2014;**8**(2):105-112

[83] Delaroche L, Yazbeck C, Gout C, Kahn V, Oger P, Rougier N. Intracytoplasmic morphologically selected sperm injection (IMSI) after repeated IVF or ICSI failures: A prospective comparative study. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2013;**167**(1):76-80. DOI: 10.1016/j.ejogrb.2012.11.011. [Epub: 29 December 2012]

[84] Boitrelle F, Guthauser B, Alter L, Bailly M, Bergere M, Wainer R, et al. High-magnification selection of spermatozoa prior to oocyte injection: Confirmed and potential indications. Reproductive Biomedicine Online. 2014;**28**(1):6-13. DOI: 10.1016/j.rbmo.2013.09.019. [Epub: 28 September 2013]

[85] Bartoov B, Berkovitz A, Eltes F. Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection. The New England Journal of Medicine. 2001;**345**(14):1067-1068. DOI: 10.1056/NEJM200110043451416

[86] Lo Monte G, Germond M, Marci R. Focus on intracytoplasmic morphologically selected sperm injection (IMSI): A mini-review. Asian Journal of Andrology. 2013. DOI: 10.1038/aja.2013.54

[87] Bartoov B, Berkovitz A, Eltes F, Kogosowski A, Menezo Y, Barak Y. Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. Journal of Andrology. 2002;**23**(1):1-8. DOI: 10.1002/j.1939-4640.2002.tb02595.x

[88] Antinori M, Vanderzwalmen P, Barak Y. IMSI as a valuable tool for sperm selection during ART. In: Racowsky C, Schlegel P, Fauser B,

Carrell D, editors. Biennial Review of Infertility. Vol. 2. Boston, MA: Springer; 2011. pp. 363-276. DOI: 10.1007/978-1-4419-8456-2_19

[89] Tanaka A, Nagayoshi M, Tanaka I, Kusunoki H. Human sperm head vacuoles are physiological structures formed during the sperm development and maturation process. Fertility and Sterility. 2012;**98**:315-320. DOI: 10.1016/j.fertnstert.2012.04.034. [Epub: 22 May 2012]

[90] Leahy T, Gadella BM. Sperm surface changes and physiological consequences induced by sperm handling and storage. Reproduction. 2011;**142**:759-778. DOI: 10.1530/REP-11-0310. [Epub: 29 September 2011]

[91] Boitrelle F, Ferfouri F, Petit JM. Large human sperm vacuoles observed in motile spermatozoa under high magnification: Nuclear thumbprints linked to failure of chromatin condensation. Human Reproduction. 2011;**26**(7):1650-1658. DOI: 10.1093/humrep/der129. [Epub: 02 May 2011]

[92] Sellami A, Chakroun N, Zarrouk SB, Sellami H, Kebaili S, Rebai T, et al. Assessment of chromatin maturity in human spermatozoa: Useful aniline blue assay for routine diagnosis of male infertility. Advances in Urology. 2013;**2013**:578631. DOI: 10.1155/2013/578631

[93] Vanderzwalmen P, Hiemer A, Rubner P, et al. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reproductive Biomedicine Online. 2008;**17**(5):617-627. DOI: 10.1016/s1472-6483(10)60308-2

[94] Cassuto NG et al. A new real-time morphology classification for human spermatozoa: A link for fertilization and improved embryo quality. Fertility and Sterility;**92**(5):1616-1625. DOI: 10.1016/j.fertnstert.2008.08.088. [Epub: 05 November 2008]

[95] Tosti E, Ménézo Y. IMSI, useful, useless or harmful? Fertilization: In vitro. 2012;**2**:e115. DOI: 10.4172/2165-7491.1000e115

[96] Akınsal EC, Baydilli N, Dündar M, Ekmekçioğlu O. The frequencies of Y chromosome microdeletions in infertile males. Turkish Journal of Urology. 2018;**44**(5):389-392. DOI: 10.5152/tud.2018.73669

[97] Elfateh F, Rulin D, Xin Y, Linlin L, Haibo Z, Liu RZ. Prevalence and patterns of Y chromosome microdeletion in infertile men with azoospermia and oligzoospermia in Northeast China. Iranian Journal of Reproductive Medicine. 2014;**12**(6):383-388

[98] Liu T, Song YX, Jiang YM. Early detection of Y chromosome microdeletions in infertile men is helpful to guide clinical reproductive treatments in southwest of China. Medicine (Baltimore). 2019;**98**(5):e14350. DOI: 10.1097/MD.0000000000014350

[99] Liu X.G, Hu H.Y, Guo Y.H, Sun Y.P. Correlation between Y chromosome microdeletion and male infertility. Genetics and Molecular Research 15 (2): 2016 DOI 10.4238/gmr.15028426

[100] Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Human Reproduction. 2003;**18**(8):1660-1665. DOI: 10.1093/humrep/deg348

[101] Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD, et al. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: Preliminary report of

a prognostic genetic test. Human Reproduction. 1998;**13**(10):2812-2815. DOI: 10.1093/humrep/13.10.2812

[102] Kuhnert B, Gromoll J, Kostova E, Tschanter P, Luetjens CM, Simoni M, et al. Case report: Natural transmission of an AZFc Y-chromosomal microdeletion from father to his sons. Human Reproduction. 2004;**19**(4): 886-888. DOI: 10.1093/humrep/deh186

[103] Dohle GR, Halley DJJ, Van Hemel JO, van den Ouwel AMW, Pieters MHEC, Weber RFA, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Human Reproduction. 2002;**17**(1):13-16. DOI: 10.1093/humrep/17.1.13

[104] Yatsenko AN, Georgiadis AP, Röpke A, et al. X-linked TEX11 mutations, meiotic arrest, and Azoospermia in infertile men. The New England Journal of Medicine. 2015;**372**:2097-2107. DOI: 10.1056/NEJMoa1406192

[105] Tang L, Zeng W, Clark RK, Dobrinski I. Characterization of the porcine testis-expressed gene 11 (Tex11). Spermatogenesis. 2011;**1**(2):147-151. DOI: 10.4161/spmg.1.2.16680

[106] Yang F, Silber S, Leu AN, Oates RD, Marszalek JD, Skaletsky H, et al. TEX 11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse. EMBO Molecular Medicine. 2015;**7**(9). DOI: 10.15252/emmm.201404967

[107] Samli H, Samli MM, Yilmaz E, Imirzalioglu N. Clinical, andrological and genetic characteristics of patients with congenital bilateral absence of vas deferens (CBAVD). Archives of Andrology. 2006;**52**(6):471-477. DOI: 10.1080/01485010600691993

[108] Anguiano A, Oates RD, Amos JA, et al. Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. Journal of the American Medical Association. 1992;**267**:1794-1797

[109] Kanavakis E et al. Cystic fibrosis mutation screening in CBAVD patients and men with obstructive azoospermia or severe oligozoospermia. Molecular Human Reproduction. 1998;**4**(4): 333-337. DOI: 10.1093/molehr/4.4.333

[110] Pauer HU, Hinney B, Michelmann HW, et al. Relevance of genetic counselling in couples prior to intracytoplasmic sperm injection. Human Reproduction. 1997, 1997;**12**(9):1909-1912. DOI: 10.1093/humrep/12.9.1909

[111] Harton GL, Magli MC, Lundin K, Montag M, Lemmen J, Harper JC. ESHRE PGD consortium/embryology special interest group—Best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Human Reproduction. 2011;**26**(1):41-46. DOI: 10.1093/humrep/deq265

[112] Harper J, Geraedts J, Borry P, et al. Current issues in medically assisted reproduction and genetics in Europe: Research, clinical practice, ethics, legal issues and policy. Human Reproduction. 2014;**29**(8):1603-1609. DOI: 10.1093/humrep/deu130. [Epub: 08 July 2014]

[113] Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR. Sperm DNA integrity in cancer patients before and after cytotoxic treatment. Human Reproduction. 2010;**25**(8):1877-1883. DOI: 10.1093/humrep/deq104

[114] Paoli D, Pallotti F, Andrea Lenzi A, Lombardo F. Fatherhood and sperm DNA damage in testicular cancer patients. Frontiers in Endocrinology. 2018;**9**:506. DOI: 10.3389/fendo.2018.00506

[115] Leridon H. Can assisted reproduction technology compensate for the natural decline in fertility with age? A model assessment. Human Reproduction. 2004;**19**(7):1548-1553. DOI: 10.1093/humrep/deh304

[116] Sauer VM. Reproduction at an advanced maternal age and maternal health. Fertility and Sterility. 2015;**103**(5):1136-1143. DOI: 10.1016/j.fertnstert.2015.03.004

[117] Ferrazzi E, Brembilla G, Cipriani S. Maternal age and body mass index at term: Risk factors for requiring an induced labour for a late-term pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2019;**233**:151-157. DOI: 10.1016/j.ejogrb.2018.12.018. [Epub: 20 Dec 2018]

[118] Wie HJ, Pak SE, Kim RY. Gestational age-specific risk of stillbirth during term pregnancy according to maternal age. Archives of Gynecology and Obstetrics. 2019;**299**(3):681-688. DOI: 10.1007/s00404-018-5022-z. [Epub: 21 December 2018]

[119] Pinheiro RL, Areia AL, Pinto AM, Donato H. Advanced maternal age: Adverse outcomes of pregnancy, a meta-analysis. Acta Medica Portuguesa. 2019;**32**(3). DOI: 10.20344/amp.11057. [Epub: 29 Mar 2019]

[120] Gudeloglu A, Parekattil SJ. Update in the evaluation of the azoospermic male. Clinics (Sao Paulo). 2013;**68**(Suppl 1):27-34. DOI: 10.6061/clinics/2013(sup01)04

[121] Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: A committee opinion. Fertility and Sterility. 2015;**103**(3):e18-e25. DOI: 10.1016/j.fertnstert.2014.12.103. [Epub: 15 Janaury 2015]

[122] Gorrill MJ, Burry KA, Patton PE. Pregnancy outcomes using donor sperm insemination after failed in vitro fertilization with intracytoplasmic sperm injection cycles in couples with complex infertility disorders. Fertility and Sterility. 2003;**80**(4):936-938. DOI: 10.1016/s0015-0282(03)01120-8

[123] Schover LR, Collins R, Richards S. Psychological aspects of donor insemination: Evaluation and follow-up of recipient couples. Fertility and Sterility. 1992;**57**(3):583-590. DOI: 10.1016/s0015-0282(16)54904-8

[124] Edelmann RJ, Humphrey M, Owens DJ. The meaning of parenthood and couples' reactions to male infertility. The British Journal of Medical Psychology. 1994;**67**(Pt 3):291-299. DOI: 10.1111/j.2044-8341.1994.tb01797.x

[125] Fisher JR, Hammarberg K. Psychological and social aspects of infertility in men: An overview of the evidence and implications for psychologically informed clinical care and future research. Asian Journal of Andrology. 2012;**14**(1):121-129. DOI: 10.1038/aja.2011.72

[126] Agarwal A, Ahmad G, Sharma R. Reference values of reactive oxygen species in seminal ejaculates using chemiluminescence assay. Journal of Assisted Reproduction and Genetics. 2015;**32**(12):1721-1729. DOI: 10.1007/s10815-015-0584-1. [Epub 02 October 2015]

[127] Wagner JH, Cheng W, Ko EY. Role of reactive oxygen species in male infertility: An updated review of literature. Arab Journal of Urology. 2018;**16**(1):35-43. DOI: 10.1016/j.aju.2017.11.001

[128] MacLeod J. The role of oxygen in the metabolism and motility of human spermatozoa. American Journal of Physiology. 1943;**138**:512-518. DOI: 10.1152/ajplegacy.1943.138.3.512

[129] Agarwal A, Sharma RK, Sharma R, Assidi M, Abuzenadah AM, Alshahrani S, et al. Characterizing semen parameters and their association with reactive oxygen species in infertile men. Reproductive Biology and Endocrinology. 2014;**12**:33. DOI: 10.1186/1477-7827-12-33

[130] Ko EY, Sabanegh ES Jr, Agarwal A. Male infertility testing: Reactive oxygen species and antioxidant capacity. Fertility and Sterility. 2014;**102**:1518-1527. DOI: 10.1016/j.fertnstert.2014.10.020

[131] Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. Etiologies of sperm oxidative stress. The International Journal of Reproductive BioMedicine (Yazd). 2016;**14**(4): 231-240

[132] Liu Z, Zh R, Zhang J, Chuang CC, Kandaswamy E, Zhou T, et al. Role of ROS and nutritional antioxidants in human diseases. Frontiers of Physiology. May 2018;**17**(9):477. DOI: 10.3389/fphys.2018.00477eCollection 2018

[133] Poljsak B. Strategies for reducing or preventing the generation of oxidative stress. Oxidative Medicine and Cellular Longevity. 2011. Article ID 194586, 15 p. DOI: 10.1155/2011/194586

[134] Rougier N. Changes in DNA fragmentation during sperm preparation for intracytoplasmic sperm injection over time. Fertility and Sterility. 2013;**100**(1):69-74. DOI: 10.1016/j.fertnstert.2013.03.005

[135] Quinn M, Jalalian L, Riberio S, et al. Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared to density gradient centrifugation with swim up in split semen samples. Human Reproduction. 2018;**33**(8):1388-1393. DOI: 10.1093/humrep/dey239

[136] Agarwal A, Ikemoto I, Loughlin KR. Levels of reactive oxygen species before and after sperm preparation: Comparison of swim-up and L4 filtration. Archives of Andrology. 1994;**32**(3):169-174. DOI: 10.3109/01485019408987783

[137] Li Z, Zhou Y, Liu R, et al. Effects of semen processing on the generation of reactive oxygen species and mitochondrial membrane potential of human spermatozoa. Andrologia: First International Journal of Andrology;**44**(3):157-163. DOI: 10.1111/j.1439-0272.2010.01123.x

[138] Thilagavathi J, Kumar M, Mishra SS, Venkatesh S, Kumar R, Dada R. Analysis of sperm telomere length in men with idiopathic infertility. Archives of Gynecology and Obstetrics. 2013;**287**:803-807. DOI: 10.1007/s00404-012-2632-8

[139] Zhao F, Yang Q, Shi S, Luo X, Sun Y. Semen preparation methods and sperm telomere length: density gradient centrifugation versus the swim up procedure. Scientific Reports. 2016;**6**:39051. DOI: 10.1038/srep39051. [Epub: 13 December 2016]

[140] Ghasemzadeh A, Karkon-Shayan F, Yousefzadeh S, Naghavi-Behzad M, Hamdi K. Study of pentoxifylline effects on motility and viability of spermatozoa from infertile asthenozoospermic males. Nigerian Medical Journal. 2016;**57**(6):324-328. DOI: 10.4103/0300-1652.193857

[141] Holstein AF, Schulze W, Davidoff M. Understanding spermatogenesis is a prerequisite for treatment. Reproductive Biology and Endocrinology. 2003;**1**:107. DOI: 10.1186/1477-7827-1-107

[142] Sharpe R. Environmental/lifestyle effects on spermatogenesis. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2010;**365**(1546):1697-1712. DOI: 10.1098/rstb.2009.0206