Acta Scientific Reproductive Biology (ASRB)

Volume 1 Issue 1 September 2020

Research Article

Male Infertility According to the WHO, Semen Analysis 2010, DNA Exploitation, Genetic Tests and Functional Examination

Mustafa Zakaria[1]*, Noureddine Louanjli[2], Mohamed Ennaji[3], Malak Jamil[3], Amal Kabit[3], Achraf Zakaria[4] and Mohamed Zarqaoui[5]

[1]General Practitioner, MSc of Reproductive Biology, Clinical Pathology and Assisted Reproductive Technology, Consultant at IRIFIV Fertility Center, Casablanca, Morocco

[2]Reproductive Biology, Head of LABOMAC Laboratory, of clinical Analysis and Assisted Reproductive Technology IRIFIV Center, AFC Center, Casablanca, Morocco

[3]Senior Clinical Embryologist at IRIFIV Fertility Center Morocco

[4]Sperm Analysis ART Technical Biologist at Labomac | Medical Analysis Laboratory, Morocco

[5]Endoscopic Surgery Fertility Specialist, Gynecologist obstetrician, General Medical Coordinator of IRIFIV Fertility Center Casablanca, Morocco

***Corresponding Author:** Mustafa Zakaria, General Practitioner, MSc of Reproductive Biology, Clinical Pathology and Assisted Reproductive Technology, Consultant at IRIFIV Fertility Center, Casablanca, Morocco.

Received: July 27, 2020

Published: August 31, 2020

Abstract

Infertility is a reproductive system pathology defined by absence of pregnancy, without contraception, after at least 12 months of frequent sexual intercourse. Male infertility affects 10 to 15 per cent of men of reproductive age and impacts more than 50 per cent of cases of infertility, whether or not associated with a female cause. Thanks to the introduction of new concepts and medical progress at the diagnostic and the therapeutic levels, the management of male infertility constantly evolves. It is difficult to identify the causes of male infertility, since it is often complex and related. They may contribute to the different stages of sperm production and/or transport of spermatozoa, and may be acquired or congenital. The involvement of the environment in male infertility has been the subject of a lot of research for several years. When a man is treated for infertility, all factors which may affect fertility must be considered and a full assessment must be made. Exploration must begin with an interrogation and a clinical examination. The first thing to do is to suggest a semen analysis, as known as spermogram. More specialized sperm tests can help refine the diagnosis. Medical imagery, hormonal examination and genetic testing are needed in some situations. Etiological management of male infertility is sometimes possible. In the absence of a known cause, symptomatic treatments are available. Medically assisted procreation techniques are offered as a last resort.

After conducting several researches in MEDLINE (PubMed), UpToDate, this article will review male infertility and its causes, semen analysis, the etiology and mechanisms of sperm DNA damage, genetic defects, and improvement of male infertility factor within ICSI.

Keywords: Male Infertility; Sperm Testing and Semen Analysis; Sperm morphology; The Genetic Control of Male Infertility and Understanding the Y chromosome; Sperm DNA testing on male infertility. Chromosomal Abnormalities and DNA Damage; Etiologies Immune Infertility; Oxidative Stress; Genetic Defects; Improvement of Male Infertility Factor in ICSI

Abbreviations

SDF: Sperm DNA fragmentation; WHO: World Health Organization; STIS: Sexually Transmitted Infections; FIGO: International Foundation for Obstetrics and Gynecology; ICSI: Intracytoplasmic Sperm Injection; TESE: Testicular Sperm Extraction; IVF: *In Vitro* Fertilization; AO: Acridine Orange; AB: Aniline Blue; CMA#: Chromomycin A3; TB: Toluidine Blue; ART: Assisted Reproductive Technology; SSR: Surgical Sperm Retrieval; DNA: Deoxyribonucleic Acid; dUTP: Deoxy-Nucleotidyl Transferase-Mediated Deoxyuridine Triphosphate; ASA: Antisperm Antibody; RLU: Relative Light Units; POLG: Polymerase Gamma

Introduction

Semen analysis is the basis for assessing the male partner in a subfertility couple. Compared to several other tests used in the assessment of infertile couples, semen analysis has been standardized across the world. This has been made possible through the efforts of the World Health Organization (WHO) since the 1970s by creating, editing, updating and disseminating the Semen Analysis Manual [1]. The manual provides step-by - step methods for the routine semen analysis, instructions for internally and externally managed quality management of these measures and guidelines for more commonly used sperm function tests. The purpose of the manual is to upgrade the standards of semen analysis and to guarantee that the semen and sperm parameters assessed in one laboratory using this manual will be similar to those carried out in another laboratory using the same manual. Global and national societies of andrology, reproductive medicine, human reproduction and pathology have contributed by supplying hands-on training to maintain that technologists use these defined methods to assess semen and sperm quality. This allows comparative studies and the pooling of data from around the globe for epidemiology studies to determine semen content [2,3] and establish reference ranges for parameters of semen and sperm. Semen analysis should be conducted in laboratories with qualified technologists who have been trained for routine clinical semen evaluation in these standardized methods. Despite our ability to assess the quality of sperm through a semen analysis methodology harmonized throughout other laboratories, the use of such parameters cannot correctly and accurately predict the fertility of a person presenting to a clinician.

Male infertility

According to the WHO (World Health Organization), infertility is defined as the inability of a couple to procreate after two years of unprotected sex. According to a study published in 2007, the prevalence of infertility is however closer to 9%, which still affects nearly 70 million couples worldwide. Infertility is a major public health problem and represents an important medical and scientific issue.

In about 50% of cases, it involves either exclusively the man, or both members of the couple. The causes of male infertility are many and multifactorial. There are secretory causes, the most frequent, accompanied by a defect in spermatogenesis, and excretory causes preventing the excretion of spermatozoa. A complete clinical assessment can attribute approximately 30% of cases of azoospermia and oligozoospermia (total absence of sperm in the sperm or abnormally reduced amount) to chromosomal abnormalities or to gene mutations affecting genes involved in production or the function of germ cells. In addition, 30% of infertility remains unexplained and almost 40% has uncertain causes. Thus, male infertility of genetic origin could affect nearly 1 in 40 men. Among the genetic causes that are currently well established are chromosomal abnormalities, Y chromosome microdeletions and mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene.

Sperm testing and semen analysis

The factor for carrying out a test depends on whether the results during management will be of value. There are currently no generally recognized standard protocols for investigating subfertile couples, though recommendations have been established by the World Health Organization (WHO) [4] the Cambridge University Press in 1993, and the Royal College of Obstetricians and Gynecologists in London in 1998.

The causes and therapy of male infertility have been the focus of several years of debate. In the past four decades, several treatments have been commonly advocated for male infertility, like clomiphene citrate, testosterone, human menopausal gonadotropin, human chorionic gonadotropin, corticosteroids (for sperm antibodies), vitamins, and many more recently popularly advertised nutritional supplements, but with no recorded proof of efficacy [5]. Even the operation of varicocelectomy has come into severe doubt. It is apparent that most spermatogenic defects are in fact hereditary in nature and completely impervious to improvement with any therapy. In addition, the development of intracytoplasmic sperm injection (ICSI) as a useful therapy with all male infertility

cases has led to a huge reassessment and critical analysis of male infertility diagnostic and therapeutic approaches. Finally, even the concept of male infertility can be very vague, because we know that men can successfully impregnate a very young, fertile woman even with very small sperm counts.

Sperm morphology

The inability of the ordinary semen analysis to correctly estimate couple fertility, and a clear lack of a threshold value below which it can clearly establish that a man is infertile, have contributed to the implementation of several more advanced tests to measure sperm function. One of the simplest of these measurements is the "strict criteria" evaluation of sperm morphology [6]. For years, the WHO has described the lower limit of normal for sperm morphology in the semen analysis as 30% [7]. This parameter has not been very effective in predicting fertility (Figure 1 and 2) [8]. However, the simple categories of normal (oval-headed), amorphous (irregular-headed), tapered headed, and small-headed sperm have now been replaced by strict criteria**.** The "strict criteria" method of determining morphology precisely measures the length and width of the oval spermatozoa head to a more exacting degree, and a sperm head could only be called "normal" if it fits within this narrow range (2.5-3.5 µm wide and 5-6 µm long). The acrosome had to account for 40% or more of the sperm head, and other perhaps less important measurements of the mid-piece (1 µm wide and 7.5-9 µm long) and tail of the sperm (45-mm long and uncoiled) had to be "strictly" applied. With these strict criteria, it was suggested that the lower limit of normal was 14% rather than 30%. Those with less than 4% normal morphology by strict criteria had only a 7.6% fertilization rate with IVF, those with 4-14% normal forms by strict criteria had a 64% fertilization rate with IVF, and those with greater than 14% normal morphology by strict criteria had a 91% fertilization rate [9]**.**

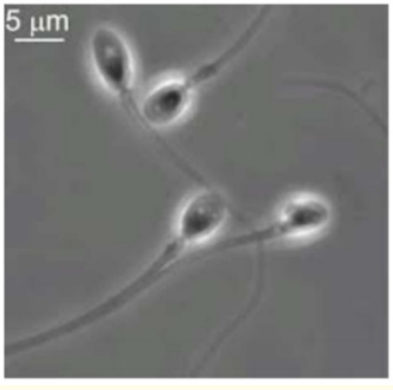

Figure 1: Under 800× magnification (IMSI), detailed sperm morphology reveals a perfectly oval shaped head and a "growing" acrosomal cap.

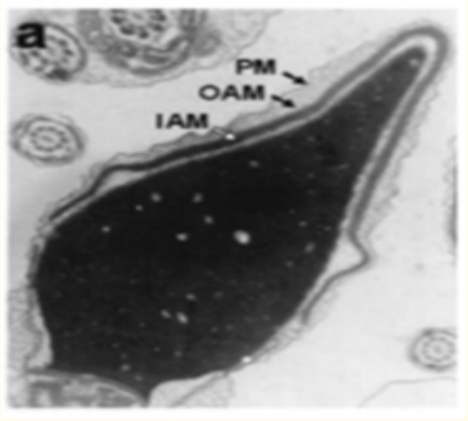

Figure 2: Under 5000 electron microscopy (E/M), a normal non-senescent sperm head is noted to have no "sperm DNA fragmentation".

The genetic control of male infertility and understanding the Y chromosome

The Y chromosome was a very rich way to consider the study of the genetic control of spermatogenesis. The Y chromosome consists of sixty multicopy genes made up of nine different gene families concentrated in multiple sequencing regions called amplicons arranged as palindromes. This covers a wide range of testis-specific spermatogenesis genes. This very confusing pattern is likely to delete itself by homologous recombination and may explain the presence in otherwise azoospermic people, of small amounts of sperm. It is also the beginning of the understanding of spermatogenesis' genetic regulation. We started to study the genetic causes of male infertility by initially mapping the Y chromosome in azoospermic men and fertile male control populations, eventually leading to the complete sequencing of the Y chromosome, almost in parallel with the production of intracytoplasmic sperm injection (ICSi) and testicular sperm extraction (TESE) for the purposes of azoospermia in 1993. In the end, this contributed to a clarification as to why the testis of azoospermic men, generally expected to produce no sperm, still contain small quantities of it [10-16]. Subsequently, much of the work on male infertility genetics focused on Y chromosome aberrations. This specific male chromosome, the Y chromosome, includes several genes implicated in spermatogenesis, arranged in an unusual pattern of nucleotide repeats and mirror image inversions, named amplicons and palindromes. Deletions affecting these regions of the Y are present in 15% of highly infertile men and have been reported to be passed to male children through ICSI, which is likely to trigger fertility complications in these children later in the future.

Sperm DNA testing on male infertility

Male factor accounts for almost 50 percent of all infertility causes. Nearly 30-40% of cases are called idiopathic, because there are no known causes that describe the findings of an irregular semen study. Semen analysis (concentration of sperm, motility and morphology) is still required for routine assessment of male infertility; however, these parameters have been found to be restricted as surrogate markers of male fertility. In fact, about 15 percent of infertile male patients have a regular semen analysis.

Impaired sperm DNA integrity affects the biological sperm structure, which may undoubtedly result in poor pregnancy results in couples with some rather unclear subfertility [miscarriage, recurrent *in vitro* fertilization (IVF) failure]. The biological composition of the sperm, however, cannot be determined with standard semen analysis. To this purpose, advanced sperm function experiments involving DNA sperm fragmentation (SDF) and reactive oxygen species were used. DNA damage in sperm may arise from the testis or/and during transit through the reproductive duct system (epididymis, etc.) During its journey through the epididymis, the spermatozoon acquires progressive motility and fertility. The epididymis epithelium 's normal secretion and absorption function delivers the right microenvironment for proper maturation of sperm. Oxidative stress, however, may have an effect on sperm chromatin during the process of transit through the epididymis. Paternal age, smoking, radiation, varicocele, obesity, cancers and leukocytospermia are the causative factors in SDF**.** The integrity of the sperm DNA is essential for normal embryogenesis. Several studies have strongly shown during the past decade that elevated rates of DNA sperm damage are correlated with poor outcomes with regard to natural conception. In addition, SDF has been shown to be considerably higher in infertile male patients compared to fertile counterparts. Many other tests for measuring the SDF rates have been developed. TUNEL, SCSA, Comet assay, and SCD test are used more frequently than acridine orange (AO), aniline blue (AB), chromomycin A3 (CMA3), and toluidine blue (TB).

Another controversial varicocelectomy indication relates to assisted reproductive technology (ART) for patients. Esteves., *et al*. recently published a systematic review and meta-analysis to establish the purpose of varicocelectomy in non-azoospermic infertile men with clinical varicocele on ART outcomes. The cited study pooled 4 retrospective studies that accounted for 870 cycles of intracytoplasmic sperm injection (ICSI) (438 with varicocelectomy, 432 without varicocelectomy).

In four studies, varicocelectomy patients had higher clinical pregnancies and higher live birth rates with ICSI than patients untreated. The outcome of this meta-analysis indicated that varicocele repair would improve ART results [17].

Male infertility and semen tests continued

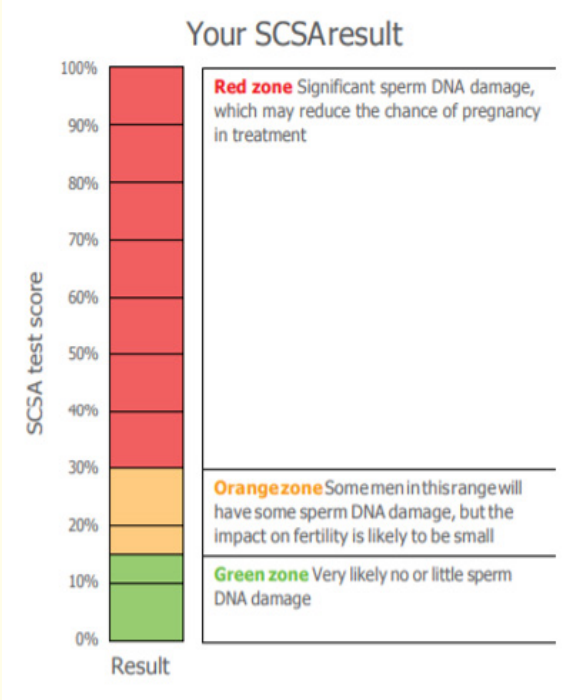

Figure a

However, a sperm DNA test doesn't provide a black or white response, as most fertility tests do. The higher the result of the test, the more damage to sperm DNA and the greater the probability that the damage may affect your chance of pregnancy. In the graph, the results are divided into green, orange and red zones to show the potential impact of your SCSA test results. Since IVF is a treatment, sometimes the result can be diagnostic. Low or no fertilization in IVF, for example, can sometimes indicate a functional sperm problem.

Treatments for male infertility

It is infrequent for the sperm problem to be due to an FSH hormone deficiency, but if so, treatment with drugs over several months usually induces enough sperm for pregnancy to occur naturally or for the sperm to be used in fertility treatment. Sometimes

an epididymis obstruction that causes no sperm in the ejaculate (azoospermia) can be resolved by micro surgery but now most people choose sperm retrieval and IVF/ICSI because it is more successful. If the obstruction is due to vasectomy, the best approach is often a micro-surgical vasectomy reversal but there are many factors to consider and it is best to discuss all the issues first with a fertility expert. Men with non-obstructive azoospermia often have areas within their tubules that produce enough sperm to be harvested for use in IVF with ICSI by Surgical Sperm Retrieval (SSR).

The importance and limitations of routine semen analysis in unexplained infertility

In addition to detailed medical history and thorough physical examination, routine semen analysis remains the basis of the male factor infertility assessment currently [18]. This approach is founded on the premise that it has been demonstrated that semen parameters such as sperm concentration, motility and morphology are strongly related to pregnancy. Moreover, being a cost-effective and non-invasive test has resulted in the common use of semen analysis in the initial evaluation of infertile men [19]. However, the criteria for normal semen parameters differ according to which edition of the WHO laboratory manual is used for human semen examination and processing [20].

In 2010, the World Health Organization (WHO) introduced new reference values for characteristics of human semen which are significantly lower than those previously reported. Nearly two thousand men from eight different countries whose partners had a time-to-pregnancy of less or equivalent to 12 months were selected as individuals to provide reference distributions for semen parameters. Despite the use of controlled studies involving couples with known pregnancy time to establish the new limits, reference studies were limited in terms of the population analyzed and the methods used for semen assessment. The use of the new WHO manual reference values into clinical practice will probably result in many of the infertile couples being re-classified. In particular, those couples earlier classified as having male-factor infertility with sperm parameters above the latest reference limits but below the old values are now diagnosed as having unclear infertility or female-factor infertility.

Chromosomal abnormalities and DNA damage

There are several methods to detect and evaluate sperm chromosomal and DNA abnormalities, such as Karyotype sperm and in situ hybridization fluorescence (FISH). FISH is not only a highly sensitive and specific method, it also allows the study of much larger sperm counts, thus increasing both the accuracy and efficiency of the process of detecting sperm chromosomal aneuploidy rates in infertile men. However, it should be noted that chromosomal sperm anomalies are exceedingly rare in UMI patients.

On the other hand, sperm DNA integrity assessment is of higher significance for approximately 10 per cent of men with normal semen analysis. These men may harbor fragmentation of single or double-stranded DNA. There are various tests that allow sperm DNA damage to be detected and assessed in spermatozoa, and that can be classified as either direct or indirect measurement of DNA damage. Comet assay, also known as electrophoresis of a single cell gel, is a sensitive technique which directly measures damage to DNA.

Another sensitive and specific method for measuring sperm DNA damage is the terminal deoxy-nucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) assay. TUNEL is capable of detecting both single and double strand breaks at the same time, unlike Comet [21]. It also reveals only the number of cells with DNA damage in a population, whereas Comet can quantify the degree of DNA damage in each cell [22].

Etiologies Immune infertility

Spermatogenesis doesn't take place until the blood-testis barrier keeps the onset of puberty and sperm separated from the immune system. If for any reason the blood test barrier is breached and sperm antigens come into contact with the immune system, they will be handled as foreign agents resulting in the formation of antisperm antibody (ASA) [23].

While previous trauma, infection and obstruction have been involved as valid etiologies for ASA formation, several cases of immune infertility have not had these events. Antisperm antibody formation has been reported in 42% of men with unexplained infertility, 10.7% of men undergoing infertility evaluations, 10% of men in couples undergoing IVF treatment but only in 2% of fertile men.

Immunoglobulin classes A (IgA) and G (IgG) are the practically important antibodies with respect to male infertility as IgM have high molecular weight and cannot perforate the blood testis barrier. Those antibodies attach to the sperm and reduce the ability to fertilize. Clark., *et al*. asserted a 27% fertilization rate when more

or equivalent to 80% of sperm contained sperm-bound IgA and IgG, while fertilization rate of 72% was seen when less or equivalent to 80% of sperm had sperm-bound ASA**.** It is not evident whether the location of the sperm-bound, whether sperm head or tail, ASA is significant, as there are conflicting reports assessing the value of localization and its relation to fertilization capacity [24].

Oxidative stress

Oxidative stress refers to elevated intracellular levels of reactive oxygen species (*ROS*), and it is a chemiluminescence assay that indirectly measures seminal levels of ROS. It registers the light intensity generated from the reaction of the luminol probe with the ROS in relative light units (RLU). Both intra and extracellular ROS are measured by chemiluminescence. Semen samples should contain sperm concentration 1 x 10^6 / mL or greater to ensure accurate readings and should be analyzed within the first hour of collection. It is also possible to use flow cytometry to measure intracellular sperm ROS; however, this is a much more expensive tool and therefore not as practical for widespread clinical use.

There are many treatment options for excess ROS. The patient should be given guidance and advices to avoid tobacco use immediately, as abstinence from tobacco use might help lower seminal ROS levels. Modifications in lifestyle such as weight loss for obese men, eating fruit and vegetables are also helpful. Recent reports further support the use of antioxidants for the treatment of male infertility related to oxidative stress. In order to avoid excessive development of ROS and subsequent destruction, antioxidants disrupt free radical chain reactions and create a non-harmful non-radical end product. In cases of male fertility, some clinical trials were able to show beneficial effects of antioxidant therapy in terms of improving semen parameters, pregnancy rates and sperm DNA fragmentation index (measure for DNA integrity defects)**.** Vitamin E, vitamin C, coenzyme Q-10, selenium, zinc, lycopene, and carnitine are all useful antioxidants. A recent Cochrane meta-analysis on the use of oral antioxidants in male infertility revealed that these agents improved significantly rates of pregnancy and live births, and reduced sperm DNA damage [25].

The evidence demonstrates that antioxidant supplementation in subfertility males may improve the results of live birth and pregnancy rate for subfertility couples undergoing fertility treatment. That being said, large clinical trials are still vital to define the superiority of one antioxidant over the other in different subpopulations of infertile males, as well as other important aspects such as dose and duration of therapy. Finally, Hamada., *et al*. reported that even low level leukocytospermia (important source of ROS) could be harmful and prescribing 200 mg of doxycycline twice daily for three weeks leads to significant improvements in pregnancy rates.

Genetic defects

Genetic sperm damage can occur at many levels, all of which have the potential to cause men to become infertile. Chromosomal sperm abnormalities are most commonly seen in men with reduced sperm count, meaning that they have oligozoospermia, decreased motility in case of asthenozoospermia, or a high percentage of morphologically abnormal sperm found in teratozoospermic men [26]. Several reports showed disomy rates for autosomes and sex chromosomes to be 0.11% and 0.44% for normozoospermic infertile men, and diploidy rates to be 0.3-1%. Among infertile males, the probability of sex chromosomal anomalies is 15 times greater than in the general population, whereas autosomal disorders arise at a level six times higher. Gene mutations and polymorphism have also been identified in infertile men with normal spermiograms. Examples of such gene abnormalities are the CatSper gene 1 mutation, later described under hyperactivation defects, and CAG repeat polymorphism in the gene coding for polymerase gamma (POLG). Polymerase gamma is the catalytic subunit of the enzyme mitochondrial DNA polymerase that is responsible for synthesis and repair of mitochondrial DNA. Mitochondrial DNA encodes many mitochondrial proteins which are important in energy and ROS production. PLOG gene polymorphism is found in infertile men with normal spermiogram. The sperm from these men have lesser oocyte penetration ability and fertilization rates [27].

Comprehensive assessment and improvement of male infertility factor in ICSI

Couples confronting unexplained infertility are characterized by being childless despite presence of normal semen parameters and normal female partner evaluation. Even when detailed history taking and physical examination are always essential to reveal erectile dysfunction or infrequent intercourse, more novel expensive tests are needed to scrutinize hidden sperm functional defects. ICSI will help to address the unclear male infertility issue and bypass all the normal obstacles a dysfunctional sperm has to overcome to trigger fertilization. Such therapy is, however, not without risks and complications. The successful pregnancy obtained by using a dysfunctional sperm brings a possibility of a risk of transmission of the same infertility traits to the male child.

Moreover, the paternal part of the embryonic genome is actively demonstrated at the four- to eight-cell stage in human embryos. Hence the later stages of embryonic development can be adversely affected by sperm DNA strand breaks that cannot be repaired by the oocyte DNA repair system. Aitken and Kraus have recognized that the damage to sperm DNA is premutagenic and can cause mutations after fertilization [28].

Mutations developed at the very early stage of embryonic development would be fixed in the germline and can result in induction of infertility, childhood cancer and higher risk of offspring imprinting diseases**.** Yet, however, short term follow-up studies of offspring born after conventional IVF have not been definitive regarding the risks of congenital malformations, imprinting diseases and health problems in general. Long term research on the risks and complications of ICSI on the produced offspring are crucially needed.

Discussions

The discovery of infertility genes once again requires a very strong collaboration between clinicians, cytogeneticists and molecular biologists. ICSI now allows egg fertilization by sperm pre-read directly in the testicle. Such a technique applied to a man with extreme oligospermia may allow him to have a child. But the cause of his anomaly may be genetic, for example an interstitial deletion of the Y chromosome. His son will inherit this defective chromosome, and will have the same phenotype. Other genetic defects could be passed on to subsequent generations. Knowledge of the genes responsible for infertility may first enable the mutation responsible for the abnormality to be clearly identified, to provide the couple and their families with genetic advice explaining the risks of recurrence of the disease, and to direct them to the most appropriate solution to their problem, from ICSI to donor insemination. Once the precise role of these genes is known, targeted treatments may be considered to correct the defective function. Although these types of therapies are still under study, investigating the presence and frequency of metabolic targets that help guide the therapeutic research plans specifically to correct these metabolic alterations. In fact, the detection of such anomalies in such studies, e.g. sperm DNA integrity defects, can inform the couples in pre-ICSI therapy about the advantages as well as possible ART procedure failures and complications.

Conclusion

The cause of male infertility is various, ranging from genetic mutations to lifestyle choices and to medical sickness. Despite improvements in male infertility awareness, idiopathic sperm abnormalities still account for around 30% of male infertility. The analysis of sperm parameters is of paramount importance in the initial investigation of the fertility of the male partner and in the interpretation of his reproductive capacity. The elements to take into consideration in the first line in the semen analysis, are first of all the sperm concentration, but also the volume of the ejaculate, the percentage of living healthy sperm in the semen (vitality), and the sperm motility. Abnormalities observed in the semen analysis should be considered as a symptom whose causes may be multiple. Also, augmented fragmentation of sperm DNA is considered to have undesirable effects on pregnancy rates. Lately, controversy has emerged about the utility of DNA fragmentation tests in anticipating ART results. Given the lack of standardization between many tests and the incapability in smaller studies to anticipate outcomes, prior guidelines had cautioned practitioners in testing for sperm DNA damage. That being said, Simon., *et al*. recently published a systematic review and meta-analysis asserting that DNA damage has a negative effect on clinical pregnancy rates following both *in vitro* fertilization and ICSI. More recommendations are now available based on existing evidence for these measures of DNA fragmentation. Due to the data on DNA fragmentation, several studies tried to find efficient and efficient means of sperm cell sorting for the identification of the undamaged sperm and cautiously use these for ART. The discovery of ICSI was decisive to the extent that, men, who were previously unable to obtain children, now have the opportunity to have a paternity. Indeed, medically assisted procreation, with the ICSI, has done tremendous good in the treatment of male infertility. However, father-son vertical transmission of genetic anomalies, such as microdeletion of chromosome Y, may well result in pathologies involving the vital prognosis, if they do not remain stable over the generations. Thus, the couple must be provided with complete information (genetic advice) as well as the potential risks enabling them to understand and assume their share of I.C.S.I. responsibility.

Bibliography

1. World Health Organization. World Health Organization Laboratory Manual for the Examination and Processing of Human Semen. 5. Geneva, Swtizerland: World Health Organization (2010).

2. Carlsen E., *et al*. "Evidence for decreasing quality of semen during past 50 years". *British Medical Journal* 305 (1992): 609-613.

3. Swan SH., *et al*. "Geographic differences in semen quality of fertile U.S. males". *Environmental Health Perspectives* 111 (2003): 414-420.

4. Baker HWG., *et al*. "Testicular vein ligation and fertility in men with varicoceles". *British Medical Journal* 291 (1985): 1678-1680.

5. Rodriguez-Ragui LJ., *et al*. "Relationship of varicocele to sperm output and fertility of male partners in infertile couples". *Journal of Urology* 120 (1978): 691-694.

6. Polge C. "Low-temperature storage of mammalian spermatozoa". *Proceedings of the Royal Society B: Biological Sciences* 147 (1957): 498-508.

7. Bunge RG and Sherman JK. "Fertilizing capacity of frozen human spermatozoa". *Nature* 172 (1953): 767-768.

8. Brinster RL. "Male germline stem cells: from mice to men". *Science* 316 (2007): 404-405.

9. Bahadur G., *et al*. "Testicular tissue cryopreservation in boys. Ethical and legal issues: case report". *Human Reproduction* 15 (2000): 1416-1420.

10. Keel BA and Webster BW. "CRC handbook of the laboratory diagnosis and treatment of infertility". CRC Press, Boca Raton, FL (1990).

11. Mortimer D. "Practical laboratory andrology". Oxford University Press, New York (1994)

12. Morshedi M., *et al*. "Cryopreserved/thawed semen for in vitro fertilization: results from fertile donors and infertile patients". *Fertility and Sterility* 54 (1990): 1093-1099.

13. Oshima H and Burger HG. "Current topics in andrology". *Japan Society of Andrology*, Tokyo (1993).

14. Hallak J., *et al*. "Cryopreservation of human spermatozoa: comparison of TESTyolk buffer and glycerol". *International Journal of Fertility* 45.1 (2000): 38-42.

15. Bhattacharya J., *et al*. "A comparative study on TEST-yolk buffer and human sperm preservation medium on post thaw characteristics of human sperm from prefreeze specimens". *Fertility and Sterility* 86 (2006): S200.

16. Hammadeh M., *et al*. "Comparison between human sperm preservation medium and TEST-yolk buffer on protecting chromatin and morphology integrity of human spermatozoa in fertile and subfertile men after freeze-thawing procedure". *Journal of Andrology* 22.6 (2001): 1012-1018.

17. Ioannou D., *et al*. "Impact of sperm DNA chromatin in the clinic". *Journal of Assisted Reproduction and Genetics* 33 (2016): 157-166.

18. Esteves SC., *et al*. "An update on the clinical assessment of the infertile male". Clinics (Sao Paulo). 66 (2011): 691-700.

19. Agarwal A., *et al*. "Assessing sperm function". *Urologic Clinics of North America* 35 (2008): 157-171.

20. Esteves SC., *et al*. "Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men". *Urology* 79 (2012): 16-22.

21. Fraser L. "Structural damage to nuclear DNA in mammalian spermatozoa: its evaluation techniques and relationship with male infertility". *Polish Journal of Veterinary Sciences* 7 (2004): 311-321.

22. Shamsi MB., *et al*. "Evaluation of nuclear DNA damage in human spermatozoa in men opting for assisted reproduction". *Indian Journal of Medical Research* 127 (2008): 115-123.

23. Mandelbaum SL., *et al*. "The impact of antisperm antibodies on human infertility". *Journal of Urology* 138 (1987): 1-8.

24. Chiu WW and Chamley LW. "Clinical associations and mechanisms of action of antisperm antibodies". *Fertility and Sterility* 82 (2004): 529-535.

25. Hamada A., *et al*. "Empirical treatment of low-level leukocytospermia with doxycycline in male infertility patients". *Urology* 78 (2011): 1320-1325.

26. Weber RF., *et al*. "Clinical laboratory evaluation of male subfertility". *Advances in Clinical Chemistry* 40 (2005): 317-364.

27. Jensen M., *et al*. "Frequent polymorphism of the mitochondrial DNA polymerase gamma gene (POLG) in patients with normal spermiograms and unexplained subfertility". *Human Reproduction* 19 (2004): 65-70.

28. Aitken RJ and Krausz C. "Oxidative stress, DNA damage and the Y chromosome". *Reproduction* 122 (2001): 497-506.