Chapter

The Sperm: Parameters and Evaluation

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Abstract

Male factor infertility contribute approximately at 50% for the cause of infertility. The steady declination of semen quality in men for all over the world might be from various factors such as life style changes, environmental toxicity, dietary contribution and social problems. Assisted reproduction is the main treatment of choice for male infertility; However, in severe male factor infertility, the treatment outcomes could end up with recurrent implantation failure or recurrent pregnancy loss. Basic semen analysis still has limitation to explain the cause of failure for the part of male factors. The purposes of developing new sperm evaluation methods are to improve the diagnostic tools for identifying the sperm defects, appraise of fertility potential and provide suitable treatment for an infertile couple, explain the cause of treatment failure from male factor part and measure the efficacy of male contraception.

Keywords: male factor infertility, sperm DNA fragmentation, sperm oxidative stress, semen analysis, total antioxidant capacity, sperm motility, sperm morphology, sperm concentration

1. Introduction

According to the World Health Organization (WHO), infertility is a reproductive system disease that is defined as "a failure to conceive naturally after regular sexual intercourse without the use of contraception for at least a year" [1]. It is estimated that 8–12% of people who are of reproductive age encounter infertility problems [2]. The male factor is responsible for 50% of infertility cases [3].

Furthermore, the incidence of male factor infertility in men in the active reproductive age bracket has been reported to be as high as 15% [4]. The causes of male factor are multifactorial. The pathophysiology of the causes is not fully understood, and most of them are idiopathic [5]. Initial investigation of the male partner consists of a history, physical examination, and semen analysis; however, some may require specific hormonal investigations to determine the causes of male infertility [4].

Semen analysis is a basic method to investigate the cause of infertility in the male. Six versions of a semen assessment methods guideline have been created by WHO. The first manual was published in 1980 based on clinical experience and research. The following versions were published in 1987, 1992, 1999, 2010, and 2021. The reasons for revising the manual included improving the semen analysis methods and updating the semen parameters to be more compatible with normal male fertility [6–9].

Nevertheless, at least 15% of infertile men are found to have a normal semen analysis according to WHO criteria [10]. However, further abnormalities were detected by a sperm DNA integrity test [11–13]. In the latest edition of WHO's laboratory manual for human semen evaluation, revisions have been made to improve the accuracy of the test and eliminate the unnecessary steps of the evaluation [14].

2. Semen sample assessment

Semen analysis is the method of evaluating the ejaculate composed of sperm, which originates from the testes and seminal fluid secreted from the accessory glands. Components in seminal fluid facilitate the sperm's access to the female genital tract and its ability to fertilize the mature oocyte *in vivo* [15]. In the sixth edition of WHO's laboratory manual for human semen examination, the procedure is divided into three parts. The first part is a basic semen examination. The second is an extended analysis, which is specialized for specific clinical applications. The last part is an advanced procedure, which is not used in routine practice. It must be done in a special laboratory, and it is mainly used in research studies [16]. The basic assessment includes the measurement of ejaculate volume, macroscopic evaluation, and microscopic examination.

In the sixth WHO manual, revisions to the basic assessment are based on evidence-based practices that improve the process of assessment, reduce the workload in the laboratory, and promote inter-laboratory quality assurance. The WHO manual emphasizes the importance of precisely measuring the ejaculate volume, which reflects the true total sperm count and examining the extent of sperm motility, which is clinically related to the male fertility potential.

3. Basic examination of semen sample parameters

General semen quality has shown a steady decline across the world [17] due to various factors, such as lifestyle changes, environmental toxicity, dietary contribution, and social problems [18–20]. Today, there are multiple tools to evaluate the semen quality and find suitable treatments for each infertile couple. Each semen parameter reflects the individual cause that needs to be clarified.

3.1 Gross appearance

Normal ejaculate has a homogeneous grayish color. In the case of pale color or colorlessness, the patient should be asked if there is orgasm during seminal collection because the semen component might only be from the Cowper's glands, not the prostate gland, seminal vesicle, or seminiferous tubule. The former occurs from sexual arousal, while the latter occurs from an orgasm. The color could be a deep yellow in patients with jaundice or those taking vitamin supplements. Normally, the ejaculate is odorless and can be liquefied within 30 min at room temperature. A strong smell is not normal and should be noted in the report.

3.2 Volume

The ejaculate volume is clinically significant for the diagnosis of male infertility, as it reflects the total sperm count in the ejaculate. The sixth WHO manual focuses on

the accuracy of the volume measurement by instructing to weigh the ejaculate (pre-weighing the container and subtracting it from the total weight of the final specimen) and calculate back into the volume. The formula for calculating semen density is 1 g per 1 ml. Total sperm count is related to the sperm produced directly from the seminiferous tubules. This count is used as an indicator of spontaneous conception and treatment success in intrauterine insemination [21].

3.3 Microscopic examination

3.3.1 Sperm concentration

The clinical value of sperm concentration is less than the total sperm count per ejaculate because the sperm concentration depends on the amount of accessory gland secretion activity. Sperm concentration does not indicate the chance of success in intracytoplasmic sperm injection (ICSI) cycles [22].

3.3.2 Sperm motility

In the sixth WHO manual, the assessment of sperm motility has reverted to the four categorizations presented in the fourth WHO manual: rapidly progressive (≥25 μ m/s), slowly progressive (5 to <25 μ m/s), nonprogressive (<5 μ m/s) and immotile (no active tail movement) (they are classified as grade a, b, c, and d, respectively) movement. However, in the fifth edition, sperm motility was classified into just three categories: progressive motility, nonprogressive motility, and immotile.

The total number of progressively motile spermatozoa indicates the chance of success in intrauterine insemination [23], and the presence of rapidly progressive motile sperm is clinically significant [24]. Asthenozoospermia is a medical term defined as "lower sperm motility than the reference values." It could result from several factors.

3.3.2.1 Varicocele

Varicocele is a chronic disease involving the pampiniform plexus of veins. It creates the tortious vessels in the spermatic cord [25]. The mechanisms involved in sperm function in the case of varicocele have not been clearly explained. However, some mechanisms might be related to sperm motility. The most likely pathophysiologic cause of sperm impairment is increased scrotal temperature caused by tortious veins, which increase oxidative stress, and reflux of toxic substances from the kidneys and adrenal glands into the testes [26]. There is evidence that varicoceles impair sperm motility [27–29]. While a varicocelectomy can improve sperm motility, other sperm parameters are still controversial [30–33]. Adjuvant therapy using antioxidant supplements to improve sperm quality [34, 35] has had conflicting outcomes [36].

3.3.2.2 Sexual abstinence

The duration of abstinence impacts sperm quality, including sperm motility [37]. Consequently, the male partner should be advised to be abstinent for 2–7 days to maintain sperm analysis accuracy among patients and between laboratories [16]. The sperm kinematics was improved when the duration of abstinence was 2 hr. compared to 4–7 days, both in normal semen [38] and oligozoospermic semen [39].

However, contradictory outcomes were still reported, as some studies revealed that the duration of abstinence at 4–5 days had higher sperm motility than 2–3 days and 6–7 days of abstinence [40].

3.3.2.3 Lifestyle factors

A recent systematic review of the literature shows that smoking is a strong factor impacting sperm concentration and motility [41]. Even a moderate amount of smoking can impair progressive motility. The pathophysiology of tobacco that results in diminished sperm motility could be oxidative stress that leads to the axonemal and mitochondrial damage on the midpiece of the spermatozoa [42, 43]. Both smoking and alcohol consumption had a detrimental effect on sperm motility and other sperm parameters [44]. When considering alcohol consumption alone, it is shown to have some association with decreased sperm quality when the amount of consumption is significant and chronic [45]. In men with obesity, sperm quality is diminished in concentration and morphology but not sperm motility [46].

3.3.2.4 Genetic causes of male infertility

Some genetic defects, such as Kartagener's syndrome and primary ciliary dyskinesia, directly cause abnormal sperm motility due to their effects on the flagellar structure and function [46–48].

3.3.2.5 Mitochondrial DNA mutation

Mitochondria are the bioenergetic source for sperm activity. They are required for natural conception and *in vitro* fertilization. The mitochondrial DNA mutation could be one cause of male infertility [49–51]. Mitochondrial DNA is vulnerable to damage from reactive oxygen species due to the lack of histone protein. It is physically associated with the inner mitochondrial membrane, where free oxygen radicals are generated [50]. Recently, an association was discovered between the single nucleotide variants of the mitochondria cytochrome B gene (MT-CYB) and male infertility [52].

3.3.2.6 Anti-sperm antibody

The presence of an anti-sperm antibody in the ejaculate is correlated with semen quality—sperm count, motility, and morphology in terms of oligoasthenoteratozoospermia [53, 54]. However, the screening of an anti-sperm antibody test before ICSI is not meaningful because the process of ICSI already bypasses the natural ability of sperm to fertilize the oocyte [55].

3.3.2.7 Medication's effects on sperm motility

Several kinds of medications have deleterious effects on spermatogenesis. For example, chemotherapeutic drugs have a strong negative effect on sperm production [56–58]. However, the effect of chemotherapeutic drugs on sperm motility is still controversial. While psychotherapeutic drugs, such as imipramine hydrochloride, chlorpromazine, trifluoperazine are commonly used medications, there is strong evidence that they negatively affect sperm motility [59]. Additionally, acetaminophen, which is used as an antipyretic drug, and non-steroid anti-inflammatory drugs

(NSAIDs) affect sperm motility [60, 61]. Moreover, regular consumption of marijuana also results in decreased sperm motility [62].

3.3.2.8 Heat exposure

Normal spermatogenesis requires environmental temperatures to be 32 to 35°C, which is lower than human core temperature, around 2–5°C. Research in animal models has shown that that heat stress could impact sperm motility by downregulating mitochondria activity and decreasing ATP activity [63]. Furthermore, increasing whole body temperature could induce damage to the epididymal spermatozoa's membrane, resulting in apoptosis [64]. These findings support the conclusion that heat exposure can damage spermatozoa productivity and function.

3.3.2.9 Environmental factors

Due to the rapidly growing industrial and agricultural countries around the world, the environment is polluted with pesticides, herbicides, petrochemical agents, and volatile organic compounds. These are all endocrine-disrupting agents that can interfere with normal spermatogenesis and male endocrine function. Several published data support that pesticides [65], dioxins [66], phthalates [65], perfluorinated compounds [67], polychlorinated biphenyls [65], heavy metals [68], dichloro-diphenyl trichloroethane [69], and plasticizers [70] impact sperm motility.

3.3.3 Sperm morphology

Sperm morphology is an important indicator of male fertility. Teratozoospermia is the nomenclature in the fifth WHO edition that means "lower sperm morphology than the reference value."

In the sixth edition, there is no nomenclature such as teratozoospermia to clarify the semen quality (the sperm morphology is less than the reference value). However, the editors used the lower fifth percentile value of the sperm from men with a female counterpart who has had a spontaneous pregnancy within a year without contraception with a 95% confidence interval. In clinical practice, the clinician might need to use the reference value to discriminate between fertile and infertile men, as demonstrated in the fifth WHO edition that the normal morphology is less than 4% according to the strict Kruger's criteria [9]. The sperm morphology alone cannot be used to predict the success of intrauterine insemination [71]. Therefore, in teratozoospermia without other sperm abnormalities, the couple should not be excluded from the process of intrauterine insemination. In contrast, in a retrospective study in 22,000 assisted reproductive technologies cycles, there was a predictive value of sperm morphology with fertilization rate, clinical pregnancy rate, and live birth rate [72].

3.3.4 Sperm vitality

The sperm vitality test is not a routine step in the sperm assessment process. Vitality tests should be done in semen samples that have very low motile sperm. The purpose of sperm vitality is to distinguish the immotile living sperm from the immotile dead sperm (necrozoospermia). A high percentage of dead sperm in the ejaculate indicates pathology in the epididymis (testicular cause) [73, 74] or sperm damage from infection (extra-testicular cause) [75]. The vitality can be assessed by

an eosin-nigrosin (E-O) stain by evaluating sperm membrane integrity and permeability. The hypoosmotic swelling test is used to directly test the viability of the sperm without staining and evaluate the sperm membrane permeability [76].

4. Potential extended and advanced examination of semen sample parameters

4.1 Sperm DNA fragmentation

Conventional or basic semen analysis is used to identify male factor infertility. However, at least 15% of the infertile male partners have normal semen analysis based on conventional semen analysis [77, 78] However, basic semen analysis cannot detect some additional issues related to the fertilization rate, embryo development rate, and success rate in *in vitro* fertilization. Further investigation should be done on semen. Sperm DNA integrity is necessary for reproducing healthy offspring from one generation to the next generation. A DNA integrity test is a biomarker of intact chromatin and one of the independent tests available in male infertility besides basic semen analysis [79].

Reactive oxygen species (ROS) are free radicals of oxygen-producing hydroxyl radicals, superoxide anion, and hydrogen peroxide. During natural conception, low levels of reactive oxygen species are needed to maintain sperm capacitation, hyperactivation, acrosome reaction, and fertilization. DNA fragmentation occurs when there are more reactive oxygen species in the spermatozoa environment than the natural seminal antioxidant [80]. Some other external factors that impact the DNA fragmentation rate are as follows: obesity, psychogenic stress, smoking, alcoholic consumption, medication, and advanced paternal age [81].

DNA fragmentation is an important factor in detecting further male factor beyond basic semen analysis, which plays a role in IVF or ICSI failure. One publication demonstrated that the DNA fragmentation test is a useful tool for male factor evaluation [82]. High DNA fragmentation in the semen can interfere with the fertilization rate, embryo development rate, and implantation rate. Furthermore, it can increase the chance of spontaneous miscarriage [83–85]. The generation of sperm DNA fragmentation is initiated during maturation in the seminiferous tubule [86] and during sperm chromatin packaging in spermiogenesis [86–88].

In spermatozoa with low-level chromatin damage, fertilization capability remains intact due to the self-repairing action of the oocyte [89]. However, with higher chromatin damage, reproductive success depends on the extent of DNA damage and the repairing ability of the oocyte [90]. Young oocytes have a better repairing capacity than older oocytes [91]. In cases of severe sperm chromatin damage beyond repair, the embryo's development might fail to implant or be developmentally delayed [91, 92]. The sperm's DNA damage might not impact the fertilization rate, but the damage of the paternal part can have an effect later (late paternal effect), resulting in delayed embryo development during genomic activation—at the stage of development when there are 4–8 cells [93]—or later, at the time of implantation, leading to implantation failure or miscarriage.

Today's sperm DNA fragmentation tests have a variety of methods. The most commonly used tests in andrology laboratories are the sperm chromatin structural assay (SCSA), terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay, sperm chromatin dispersion (SCD) test, and the alkaline Comet test.

There are variations in clinical thresholds with cut-off level among these tests as well as the sites of the damaged DNA detected [94]. The best assay for DNA fragmentation has not been determined yet. In a systematic review and meta-analysis study, a threshold of 20% of fragmented DNA in the semen sample regarding the SCSA, TUNEL, and SCD tests can be used to differentiate between infertile and fertile men, with a sensitivity of 79% and specificity of 86% [95]. A threshold level of 20–30% of SCSA and SCD tests correlate with the duration of infertility, decreasing the chance of success in intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injecton (ICSI) and increasing the risk of miscarriage [96, 97]. The fragmented DNA is similar to the tip of the iceberg. The hidden part of the fragmented DNA cannot be detected; however, they are prone to be damaged during the process of *in vitro* manipulation of the sperm during assisted reproductive technology.

A DNA fragmentation test behaves the same as semen analysis in that it cannot discriminate between infertile from fertile men, nor can it be used to detect the success in assisted reproductive technology (ART) cycles. The test is not independent in that it still relies on the partner's factors—for example, oocyte quality and age. Basic semen analysis and sperm DNA fragmentation tests complement each other in the diagnosis of male infertility; however, they play a different part in the aspect of male fertility care.

In the clinical setting, sperm DNA fragmentation plays a role in fulfilling the diagnosis of male infertility, especially in the male partner who has specific conditions that require further analysis beyond the basic semen analysis.

4.1.1 Varicocele

Spermatozoa from a male partner with varicocele have a high potential to be affected by osmotic stress due to high temperature in the testicular environment. These factors result in sperm DNA fragmentation in at least 50% of cases [98]. A systematic review and meta-analysis demonstrated an improved DNA fragmentation rate after varicocelectomy and achievement of pregnancy in comparison to no surgery [99]. After varicocelectomy, a DNA fragmentation test can be a valuable prognostic tool to guide a suitable infertility treatment for a couple. A lower DNA fragmentation than the threshold can indicate a better outcome for natural conception, IVF, and ICSI. The type of treatment depends on the female factor. In the case of persistently high DNA fragmentation, the appropriate treatment can be assisted reproductive technology (ART), either with or without specific sperm selection for better sperm quality [100].

Sperm DNA fragmentation and male infertility are identified in subclinical varicocele. However, the controversial issue related to varicocele is that apparent vein dilation is not found upon examination; it is detected by doppler ultrasound. There was no significant difference in sperm DNA fragmentation rates between fertile and infertile men with subclinical varicocele [101]. A systematic review and meta-analysis study provided evidence that sperm DNA fragmentation rate is comparable between clinical and subclinical varicocele. However, varicocelectomy can only improve the fragmentation rate significantly in clinical varicocele [102].

4.1.2 Idiopathic infertility and unexplained infertility

Unexplained infertility is responsible for 15–30% of infertile patients. It means they have been investigated using a conventional diagnostic approach for the cause of

infertility, but no clear cause of infertility was found [103] However, about 40–50% of unexplained or idiopathic infertile couples have elevated sperm DNA fragmentation [104]. Likewise, men who have been diagnosed with idiopathic infertility are more likely to have abnormal semen parameters based on basic semen analysis without any obvious abnormality [105]. This information implies that an extended or advanced investigation might be necessary to uncover the causes of infertility in this group [105].

Sperm DNA damage might be one of the main causes of male infertility, especially in cases where infertility is idiopathic or unexplained. The added benefit of the sperm DNA fragmentation evaluation, apart from basic semen analysis, is that it might improve the chance of pregnancy, both natural conception and assisted reproduction that uses adjunctive treatment to improve sperm DNA integrity.

4.1.3 Recurrent pregnancy loss

The European Society of Human Reproduction and Embryology (ESHRE) has defined the terms of recurrent pregnancy loss as "at least two spontaneous miscarriages starting from natural conception until 24 weeks of gestation" [106]. The sperm DNA fragmentation rate is significantly higher in men with female partners who have had recurrent pregnancy loss than in a fertile female control group having at least one ongoing pregnancy or live birth [107, 108]. The mechanism of sperm fragmented DNA that initiates recurrent pregnancy loss has not been determined yet. However, one hypothesis is that the oocyte repair mechanism might be the main culprit, leading to poor blastocyst development, recurrent implantation failure, and pregnancy loss [108].

4.1.4 Intrauterine insemination

There is evidence that men with a sperm DNA fragmentation rate diagnosed above 27% by SCSA have higher early pregnancy loss and a lower pregnancy rate than the general infertile population with a lower sperm DNA fragmentation [109, 110]. According to this information, the sperm DNA fragmentation test has an additional benefit of guiding the clinician to choose the treatment of choice for each infertile couple. In case of the sperm DNA fragmentation being higher than the threshold, the couples should have complementary treatment before IUI to ameliorate the sperm DNA fragmentation rate. However, in couples with advanced female age, assisted reproduction should be considered early on due to the oocyte's diminished repair ability and the risk of chromosome abnormality, which are the main causes of treatment failure [94].

4.1.5 Assisted reproduction (in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI)

High sperm DNA fragmentation impacts the likelihood of successful pregnancy in a natural cycle, intrauterine insemination, and advanced treatments such as IVF and ICSI. There are several studies demonstrating that elevated sperm DNA fragmentation adversely impacts IVF and ICSI. Mainly, it increases spontaneous abortion and recurrent implantation failure. Additionally, it decreases the live birth rate [111–113]. Evidence suggests that the impact of sperm DNA fragmentation might be higher in IVF than in ICSI [114]. It was postulated that the sperm needs to be incubated with

the oocyte *in vitro* longer in IVF than in ICSI. The duration of exposure of sperm to the external environment during IVF might generate more DNA damage than ICSI, which requires only a short time after ejaculation until successful fertilization [115]. Additionally, the sperm is a significant source of reactive oxygen species. IVF requires the direct contact of at least one hundred thousand sperms and an oocyte for natural fertilization. The exposure of the oocyte to oxidative stress from sperm could adversely impact the embryo's development [116, 117].

Likewise, high sperm DNA fragmentation has an adverse effect that results in these couples having more spontaneous pregnancy losses than couples with low sperm DNA fragmentation [118]. The authors concluded that using a sperm selection method during assisted reproduction to choose sperm with low fragmented DNA could improve the pregnancy rate and decrease the miscarriage rate [118].

The pathophysiologic cause of an increasing rate of spontaneous abortion in high sperm DNA fragmentation in couples undergoing IVF or ICSI is still inconclusive. One proposed mechanism is that the genetic and epigenetic effects of sperm DNA damage could cause mutation or dysregulation of DNA methylation processes that are crucial for embryo development [119–121]. The other proposed mechanism is the oocyte's ability to repair the sperm DNA defect. In oocyte donation cycles, it was found that a good quality oocyte can counteract the defected sperm DNA. However, oocytes from women of advanced maternal age have diminished repair functions compared to young women [122]. Single-stranded DNA breakage is more likely to be repaired than double-stranded DNA breakage [123, 124]. Therefore, the final impact of sperm DNA fragmentation on the pregnancy outcome still relies on the balance of the oocyte repairing system and the extent of sperm DNA damage [90].

Persistent high sperm DNA fragmentation is one of the leading causes of IVF and ICSI failure. Detection of sperm DNA damage is crucial for treatment success. In cases where no causative factor of elevated sperm fragmented DNA is identified, testicular spermatozoa are the suggested method to improve treatment success in ICSI [125–132]. ICSI uses testicular spermatozoa instead of ejaculated spermatozoa, which could be related to the lower sperm DNA damage in the testicular sperm compared to ejaculated sperm that must transit from the epididymis to the male reproductive tract [132–136].

4.1.6 Other risk factors

Some lifestyle factors can increase sperm DNA fragmentation. Some examples are smoking, drinking, cannabis consumption, exposure to air pollutants, pesticides, polyaromatic hydrocarbons, and fertilizers. Among these factors, smoking has the most impact on sperm DNA fragmentation [137, 138]. Cannabis consumption also impairs sperm DNA integrity [139]. Advanced paternal age above 40 years also reduces the sperm DNA quality [140–142].

Obesity is another common problem that leads to poor sperm quality due to peripheral aromatization from testosterone to estradiol in the subcutaneous fat. The increasing testicular temperature from subpubic fat and the high estradiol levels in obese men might cause hypogonadism and sperm DNA damage [143].

4.1.7 Sperm cryopreservation

Sperm cryopreservation is a method to preserve male fertility for future use. This method allows men to preserve their semen. Candidates for sperm cryopreservation

include men who have cancer and require chemotherapy or radiation treatments or healthy men who need to preserve semen for future purposes before a vasectomy or after assisted reproduction. This technique is required for sperm donation. However, the process of sperm freezing and thawing can be harmful to sperm quality. It impacts the sperm's motility, viability, and normal morphology. Additionally, it increases osmotic stress on the spermatozoa and leads to sperm DNA damage [144, 145].

The vitrification technique has recently been developed for sperm cryopreservation. It has the potential to reduce sperm DNA damage compared to conventional slow freezing [146, 147]. However, more research is needed.

Sperm DNA fragmentation tests should be done before and after sperm cryopreservation to help improve the method to reduce the impact of cryopreservation on sperm parameters. Moreover, the tests provide the optimal treatment based on sperm quality. The addition of an antioxidant to the sperm freezing media is one technique to protect the sperm from the harsh conditions of the freezing and thawing process by reducing the osmotic stress-induced DNA damage [148].

4.2 Reactive oxygen species measurement

One of the causes of male infertility and sperm DNA damage is osmotic stress to the spermatozoa. Reactive oxygen species are derived from the metabolism of oxygen. They play a vital role in cellular signaling pathways, sperm maturation processes, and capacitation [149]. Excessive ROS can have a significant detrimental effect on sperm fertility potential [150]. All along the journey initiated from the seminiferous tubule and through the epididymis, *vas deferens,* and finally to the outlet of the male reproductive tract, spermatozoa are potentially assaulted from oxidative stress, which diminishes fertilizing capability and leads to recurrent implantation failure and pregnancy loss. External osmotic stress interferes with the sperm membrane, while internal osmotic stress acts on lipid peroxidation mechanisms, resulting in sperm DNA damage.

Possible mechanisms of oxidative damage to the spermatozoa are sperm membrane and DNA damage, which decrease sperm motility and fertilization ability. These types of damage can also cause poor embryo development, recurrent implantation failure, and early pregnancy loss [84, 151]. Sperm damage by ROS reduces sperm motility, as demonstrated in both conventional and computer-assisted semen analyses [152, 153].

Human semen consists of various kinds of cells, including mature and immature spermatozoa, epithelial cells, and leukocytes. The main sources of ROS in semen are leukocytes (i.e., extrinsic source) and spermatozoa (i.e., intrinsic source). In addition, environmental factors, such as heavy metals, smoking, varicocele, obesity, chronic illness, and genitourinary tract infection are potential sources of ROS, affecting sperm DNA and resulting in DNA fragmentation and damage [154].

Oxidative stress has been demonstrated to be a main factor responsible for male infertility via sperm dysfunction. Oxidative stress on spermatozoa mainly derives from the excessive ROS and inadequate antioxidants to counteract them. Excess ROS can damage sperm by several mechanisms via the oxidative pathway. Approximately 30–40% of infertile men have oxidative stress that causes male infertility [155].

Spermatozoa are unique biological cells in the human body that have limited self-repair capability due to the lack of a cytoplasmic repairing mechanism. This is the most important reason that spermatozoa are vulnerable to internal and external ROS damage [156]. In addition, the membrane of spermatozoa is composed of polyunsaturated fatty acid that is susceptible to oxygen-induced damage and, hence, lipid

peroxidation. One consequence of lipid peroxidation is damage to the axoneme and midpiece of spermatozoa, resulting in diminished sperm motility [157, 158]. High levels of ROS are detected in at least 25–40% of infertile men [159, 160] Levels of ROS above the semen's antioxidants result in oxidative stress to the sperm [160]. Currently, ROS measurement by chemiluminescence is the most well-described, advanced method of ROS detection in the seminal fluid [161–163].

4.3 Total antioxidant capacity measurement

Total antioxidant capacity (TAC) is a diagnostic test to measure enzymatic and non-enzymatic kinds of antioxidants in the seminal fluid during a male infertility work up. The value reflects the redox potential an antioxidant has to osmotic stress. There is substantial evidence that the utility of the TAC measurement lies in its ability to detect a lower TAC in infertile men than in a fertile control [164]. The imbalance between osmotic stress and TAC lead to male infertility. TAC is also used to detect who should have antioxidant supplementation before ICSI, especially in case of previously failed ICSI [165]. The role of an antioxidant supplement in male infertility has been reviewed with the potential to lead to a successful pregnancy [166].

4.4 Assessment of the presence of leukocytospermia and hematospermia

Normal leukocyte production occurs mainly in the epididymis, where they take responsibility for the immunosurveillance of abnormal sperm via phagocytosis. Leukocytes are composed of granulocytes at 50–60%, macrophages at 20–30%, and T-lymphocytes at 2–5% [167]. Leukocytospermia is an excessive amount of leukocytes—more than the threshold, according to the fifth WHO manual—which can impact the sperm quality, as leukocytes are the main source of ROS [154].

The best laboratory guideline for leukocyte assessment in semen is immunohistochemical staining against the various kinds of leukocytes; however, the method is complicated, time-consuming, and not well standardized [168]. The European Association of Urology recommends antibiotic treatment; however, evidence for improving pregnancy outcomes was not demonstrated [169]. Likewise, there is no clear evidence that either antioxidant or antibiotic treatment improves treatment success in infertile men with leukocytospermia [170].

Hematospermia is a term referring to the presence of gross and microscopic examination in the ejaculate. The pathophysiologic causes can be disorders in the ejaculatory ducts, accessory glands, and urethra. Alternatively, it can have iatrogenic causes. The extensive investigations in the case of hematospermia have been well-documented [171, 172]. There is some evidence of the relationship between hematospermia and male infertility [173, 174]. Red blood cells might be the main source of toxic substances leading to a decline in sperm quality. Hemolysis especially occurs during sperm cryopreservation and thawing [175].

5. Other extended and advanced examination of semen sample parameters

5.1 Sperm aneuploidy test

The sperm aneuploidy test is a direct evaluation of sperm chromosome complements that has been used in a couple with recurrent pregnancy loss. The test

evaluates both structural and numerical chromosome abnormality in spermatozoa. A systematic review and meta-analysis demonstrated the increasing incidence of aneuploidy in spermatozoa in cases of recurrent pregnancy loss [176]. The real benefit and implementation of sperm aneuploidy in a routine laboratory for male infertility has yet to be determined, as molecular analysis in miscarriage has revealed that most chromosome aneuploidy occurs during female meiosis, resulting in meiotic non-disjunction [177]. In addition, the final evaluation of chromosome abnormality on the blastocyst can provide both the meiotic and mitotic origin—from either oocyte or spermatozoa—with no need to investigate the oocyte or sperm before fertilization.

5.2 Cytokine assessment in the semen

Infection and inflammation of the male genitourinary tract play an important role in male infertility due to inflammatory mediators and ROS causing damage to the spermatozoa. Any suspicious male genitourinary tract infection should be thoroughly investigated; otherwise, irreversible sperm damage might occur. The biological markers of infection and inflammation in the ejaculate are leukocyte numbers>1 million/ml, granulocyte elastase >280 ng/ml, and proinflammatory cytokines (e.g., interleukin (IL)-6 > 30 pg./ml, IL-8 > 7000 pg./ml) [178]. Cytokine detection might play a potential role and be a sensitive marker of male genitourinary tract infection and inflammation, especially in asymptomatic and silent cases.

5.3 Immature germ cell assessment

Immature germ cells can be differentiated from leukocytes by Papanicolaou staining. There is no longer a cut-off value of immature germ cells provided in the fifth and sixth WHO manuals, as there is not a sufficient number of studies to confirm the clinical importance of the value. However, elevation of immature germ cell to more than 15% of total sperm in the ejaculate might be significant and indicative of sperm chromatin immaturity [179]. Investigation into the pathophysiologic cause of high shedding of immature germ cells in the ejaculate and the consequences that ensue are not warranted [179].

6. Conclusion

Currently, semen analysis has become a standard tool for evaluating male infertility and guiding clinicians to provide appropriate treatments for couples. The sperm parameters in the ejaculate are the biological markers of male fertility. A basic semen analysis provides the initial information related to identifying whether a man is fertile or infertile; however, there is no absolute cut-off value for the inability to achieve conception.

In the modern era of assisted reproduction and molecular genetics, new diagnostic techniques reveal the deeply detailed causes of male infertility to improve the treatment outcome. In the modern world, there is more likely to be an association between men's general health and the environment regarding sperm parameters than previously. Having knowledge of the consequences of these factors on sperm parameters can possibly lead to the development of pharmaceutical components or supplements that improve male fertility.

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