Novel concepts in Male Infertility

Clinical and Laboratory Perspectives

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Background

Traditional seminal fluid analysis with the reviewed WHO 2021 criteria provide vital benchmarks, reflecting the 95th percentile from men who were able to impregnate their partners within a year of intimacy. However this falls short of addressing the qualitative aspects of the sperm DNA.

Parameters most frequently assessed in clinical settings, sperm count, motility and morphology, may not be the best predictors of a man's fertility potential as they do not account for the presence of immature sperm chromatin of fragmented DNA. Studies have shown that oxidative stress is a major cause of DNA fragmentation outside the testis. Patient specific characteristics and environmental factors are the most implicated. The use of sperm DNA fragmentation (SDF) could be a valuable tool in both diagnosis and management of male factor infertility.

Introduction

Infertility affects nearly 10 to 15 % of men in their prime reproductive age. Despite its multifactorial nature, male factor infertility is not yet fully understood and approximately half of the cases are deemed unexplained or idiopathic. Investigation of the conditions that compromise male fertility is usually undertaken by history, physical examination, and semen analysis. During this investigation, special attention should be given to issues that have major implication for diagnosis and management, including semen analysis, varicocele, and azoospermia.

It seems logical to adopt the latest WHO laboratory methods for the evaluation of human semen. A careful examination of its characteristics and limitations is advisable. Importantly, clinicians should not expect an analysis of the widely ranging parameters of the whole ejaculate to give robust discriminatory information of the male fertility potential.

Discussion

WHO lab methods and reference values

Routine semen analysis does not measure the fertilizing potential of spermatozoa and the complex changes that occur in the female reproductive tract before fertilization. Whether assisted reproduction technology is required depends not only on female fecundity but also on male factors.

Performing a routine semen analysis will provide leads as to whether the problem may be present in the male partner and an estimate of the severity of the problem. To find out the cause of the abnormality will require further testing that may include assessing the general health of the male partner (smoking, obesity, hypogonadism, and chronic diseases) and genetic testing.

Newer tests should predict the success of fertilization in vitro and the outcome of the progeny.

	WHO 2010	WHO 2021
Semen volume (mL)	1.5 (1.4 - 1.7)	1.4 (1.3 - 1.5)
Total sperm number (10^6 per ejaculate)	39 (33 - 46)	39 (35 - 44)
Total motility (%)	40 (38 - 42)	40 (40 - 43)
Progressive motility (%)	32 (31 - 34)	32 (29 - 31)
Non progressive motility (%)	1	1 (1 - 1)
Immotile sperm (%)	22	20 (19 - 20)
Vitality (%)	58 (55 - 63)	54 (50 - 56)
Normal forms (%)	4 (3 - 4)	4 (3.9 - 4)

Table 1: World Health Organization Laboratory Manual for the Examination and Processing of Human Semen.

Sperm DNA Fragmentation (SDF)

Sperm DNA fragmentation (SDF) refers to the presence of breaks in sperm DNA strands and higher SDF levels are linked to reduced fertility and adverse reproductive outcomes.

Despite being the parameters most frequently utilized in clinical settings, sperm count, motility, and morphology may not be the best predictors of a given man's fertility potential as they do not necessarily associate with the presence of immature sperm chromatin or fragmented DNA. Perhaps the major problem is that impaired sperm chromatin is found not only in men with abnormal semen parameters but also in those exhibiting semen analysis within normal ranges. SDF may originate from the testis and excurrent duct system. Abortive apoptosis and defective protamination theories are proposed to explain the generation of DNA fragmentation within the testis.

However, oxidative stress affecting sperm during transit through the epididymis and after ejaculation is the major cause of SDF outside the testis. Several etiological factors have been implicated in the impairment of sperm DNA integrity, including cigarette smoking, radiation, chemotherapy, leukocytospermia, varicocele, cancer, obesity, and advanced paternal age.
In approximately 15 % of the cases, results of the conventional semen analysis do not reveal these obvious abnormalities. However, it has been shown that spermatozoa of infertile men have lower DNA integrity than fertile men. This is important because genetic information passed on to the next generation depends on sperm DNA integrity. Since several etiological factors have been implicated in the impairment of sperm DNA content, assessment of sperm DNA fragmentation (SDF) may offer an opportunity to better understand and treat such sperm dysfunctions. Reduced fertility rates have been reported in cases of high SDF. Impaired embryo development compromised integrity of the embryonic genome, and increased rates of miscarriage have also been associated with high sperm DNA damage.

Sperm DNA fragmentation rates in ejaculated and testicular sperm

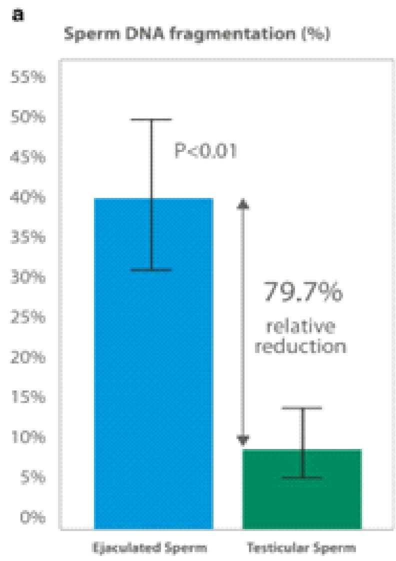


Figure 1: Comparison of sperm DNA fragmentation rates in ejaculated and testicular sperm of 81 infertile men undergoing ICSI. Use of testicular sperm for ICSI resulted in an absolute reduction of 32.6 % (relative reduction of 79.7 %) in SDF.

Collectively, increasing evidence suggests a negative impact of high SDF to natural conception and pregnancy outcomes in IVF and intracytoplasmic sperm injection (ICSI) cycles. In this context, the evaluation of SDF in addition to conventional semen analysis would be ideal as it evaluates an independent attribute of sperm quality.
The routine use of SDF testing in the clinical evaluation of male factor infertility is advocated. Cut-off levels of 30% are used to discriminate between specimens with normal and abnormal results. Importantly, patients are asked to abstain from ejaculation for a fixed period 1–2 days before semen collection due to the influence of longer abstinence periods on SDF results.

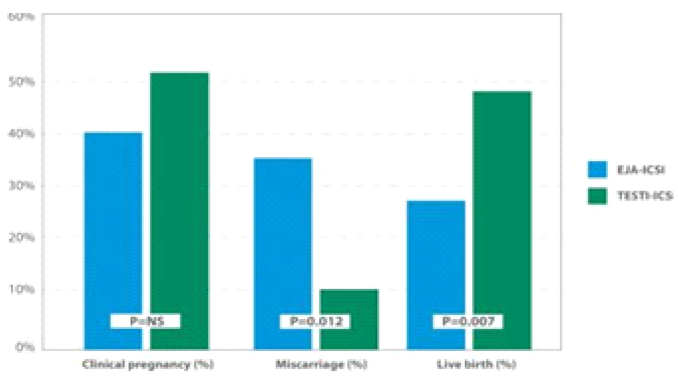
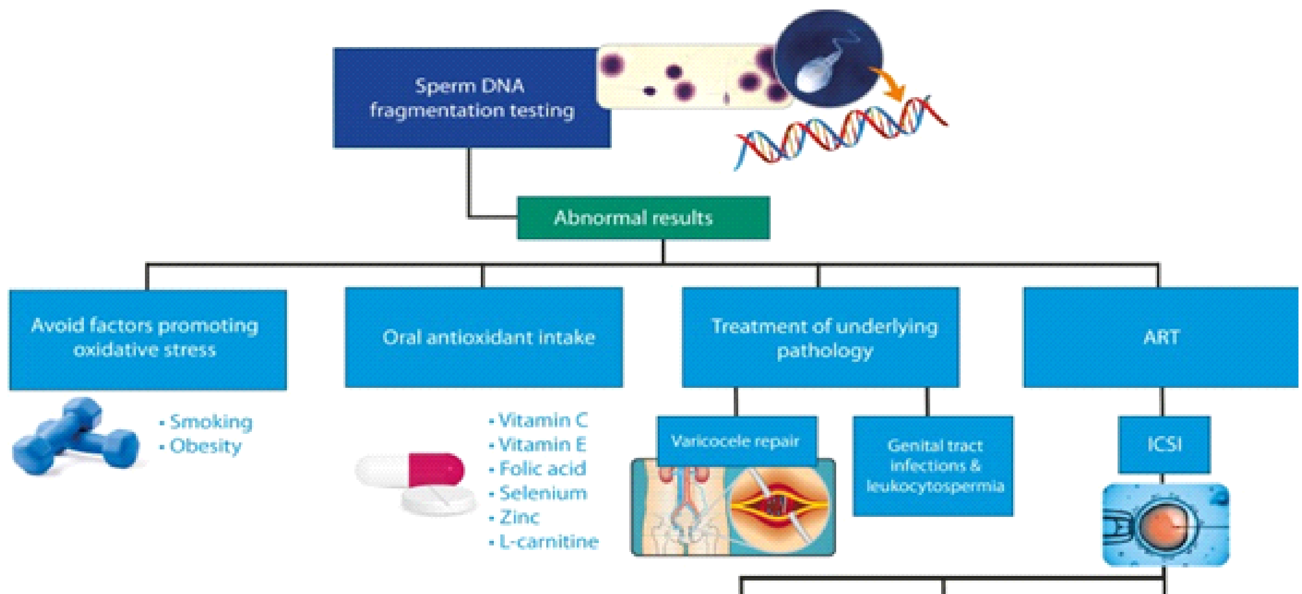


Figure 2: Clinical pregnancy, miscarriage, and live birth rates after sperm injections using either ejaculated sperm (EJA-ICSI; n = 91) or testicular sperm retrieved by TESE or TESA (TESTI-ICSI; n = 81) cohorts.

The role of SDF testing in effectively managing male infertility

Collectively, emerging evidence indicates that SDF tests provide valuable information to both diagnosis and management of male factor infertility. Testing may be recommended at initial workup to all men but not for those with azoospermia and severe oligozoospermia in whom the method may not be feasible. Abnormal testing results identify men in whom SDF is a contributory factor to infertility. SDF tests can be used to monitor results of interventions and to select the best ART strategy, as depicted in figure 3.

Novel concepts in the management of infertile men with non-obstructive azoospermia: Non-obstructive azoospermia is a serious type of male factor infertility, affecting approximately 1 % of all men and 10 to 15 % of infertile males. NOA is the absence of sperm in the ejaculate. Despite being untreatable and resulting from a spectrum of testicular disorders, approximately 50 % of men with NOA have sparse foci of sperm production within their dysfunctional testes, which can be extracted.



From a clinical perspective, we propose that the management of infertile men with NOA should consider the:

- Differential diagnosis of azoospermia.
- Selection of the eligible patients for sperm retrieval using molecular biology diagnosis. Identification of the affected men that might benefit of interventions prior to sperm retrieval.
- Application of the best method to surgically retrieve testicular spermatozoa; and
- Use of state-of-the-art IVF techniques.

Information obtained from the medical history, physical examination, and hormone analysis provides >90 % accuracy to determine the type of azoospermia (obstructive vs. nonobstructive). Etiology conditions associated with NOA include congenital and genetic abnormalities, endocrine disorders, exposure to gonadotoxins, postinfectious, varicocele, trauma, and idiopathic. Unlike NOA, obstructive azoospermia (OA) is attributed to mechanical blockage along the reproductive tract and is associated with favorable prognosis since spermatogenesis is intact.

Men with NOA usually have normal epididymis and palpable vasa. Small-sized testes (<15 mL) are otherwise encountered since about 85 % of testicular parenchyma is involved in spermatogenesis. However, testicular volume is often normal in men with spermatogenic maturation arrest, a condition characterized by a lack of mature spermatozoa despite the presence of normal numbers of germ cells. The serum levels of follicle-stimulating hormone (FSH) are usually elevated while low testosterone levels (<300 ng/dL) are found in approximately 50 % of the affected men. Abnormal testosterone levels reflect Leydig cell insufficiency, which is usually accompanied by elevated luteinizing hormone (LH) levels.

Ejaculates of men with NOA usually have normal volume and pH, which indicates patent ejaculatory ducts and functional seminal vesicles. It is important that such specimens are examined after high-speed (>1000g) centrifugation to exclude the presence of rare spermatozoa.
The finding of any spermatozoa may offer the chance of ART to be performed with ejaculated sperm, thus avoiding sperm retrieval (SR) methods. We perform centrifugation at 3000g for 15 min, which is followed by a careful examination of the pellet semen. Equally important is the examination of multiple specimens and on several occasions as azoospermia may be transient due to conditions such as fever and use of medication.

The gold-standard test for the confirmation of NOA is testicular histopathology analysis. Hypospermatogenesis, germ cell maturation arrest, germ cell aplasia (Sertoli cell-only syndrome, SCO), tubular sclerosis, or a combination of those, are the common phenotypes. Histopathology results may also predict sperm retrieval outcome.

However, testicular biopsies carried out with the sole purpose of histopathology evaluation could remove the rare foci of sperm production and thus jeopardize the chances of future retrieval attempts. Hence, we only perform diagnostic testicular biopsies when the type of azoospermia is equivocal. In such cases, a specimen is taken for wet examination in addition to conventional histopathology analysis. We routinely cryopreserve testicular spermatozoa when mature spermatozoa are found on wet specimens.

It has been estimated that 10 % of men with NOA harbour microdeletions within the long arm of the Y chromosome, which clusters the genes involved in spermatogenesis regulation. Genetic counselling is, therefore, essential to give information about the risks of conceiving a son with infertility and other genetic abnormalities.
To sum up, NOA candidates for SR and ICSI should be screened for Y chromosome microdeletions since diagnosing a deletion has prognostic value and influences the therapeutic options. Sperm retrieval is not recommended to men with complete deletions involving the AZFa and/or AZFb regions. Finally, genetic counselling should be offered to those patients with AZFc deletions because such deletions will be invariably transmitted from father to son.

Varicoceles and Male Infertility

Varicoceles are considered the leading cause of male infertility as they can impair spermatogenesis through several distinct pathophysiological mechanisms. Current evidence supports oxidative stress as the key element in the pathophysiology of varicocele-related infertility.

From the laboratory perspective, measurement of markers of oxidative stress, including SDF, can provide valuable information about the extent of oxidative stress and can guide therapeutic interventions. From the clinical perspective, repair of varicoceles has been advocated for alleviating oxidative stress-associated infertility, which may improve both natural fertility and assisted reproductive technology (ART) outcomes.

Varicoceles are found in approximately 5 % of men with NOA. While it is debatable whether varicoceles are merely coincidental or contributory to spermatogenesis disruption, surgical repair of clinical varicoceles has been carried out to improve sperm production in such men.

Three controlled studies which specifically evaluated sperm retrieval outcomes in men with varicoceles and NOA were examined. Sperm retrieval success was significantly higher in the group of men who had the varicocele treated than those with untreated varicoceles. Varicocele grade did not appear to have influenced the results. The appearance of sperm in postoperative ejaculates was associated with testicular histopathology findings.

Clinical Conclusions

The following summarizes the clinical and laboratory concepts in male factor infertility discussed in this review:

Semen analysis remains the cornerstone of infertility evaluation as it provides information on the status of the epididymis, seminiferous tubules, and accessory sex glands. The adoption of the latest WHO laboratory methods for the evaluation of human semen calls for a careful examination of its characteristics and limitations. Conventional semen analysis as routinely performed does not give robust discriminatory information about the male fertility potential.

Fair evidence indicates that sperm DNA fragmentation tests provide valuable information to both diagnosis and management of male factor infertility. Test results have both diagnostic and prognostic information complementary to, but distinct from conventional sperm parameters, and are useful to guide management and monitor intervention outcomes. Determining the values of SDF may be clinically informative for IUI or IVF and ICSI outcomes. Strategies to alleviate SDF include varicocele repair, oral antioxidant intake, short ejaculatory abstinence, laboratory sperm selection techniques, and use of testicular sperm for ICSI.

The importance of varicoceles to male factor infertility has been revisited. Oxidative stress is a central element in its pathophysiology and may lead to SDF. Repair of clinical varicoceles is effective to improve not only the conventional semen parameters but also sperm chromatin integrity. Furthermore, treatment of clinical varicoceles may improve both natural fertility and ART outcomes.

Non-obstructive azoospermia is the most severe presentation of male infertility. The optimal management of infertile men with NOA involves a series of steps that includes:

- Differential diagnosis of azoospermia;
- Selection of the eligible patients for sperm retrieval using molecular biology diagnosis;
- Identification of the affected men that might benefit of interventions prior to sperm retrieval;
- Application of the best method to surgically retrieve testicular spermatozoa; and
- (v) use of state-of-the-art IVF techniques.

Recommendations

Personalized treatment strategies based on novel insights.

Testicular histopathology analysis for men with non-obstructive azoospermia.

Genetic counselling should be offered to men with non-obstructive azoospermia.

Integration of advanced diagnostics like sperm DNA fragmentation tests in routine practice.

Medical therapy for management of infertile men with non-obstructive azoospermia.

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