IN THE NAME OF GOD

Clinical information of male infertility from Laboratories

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Introduction

Infertility affects nearly 10 to 15 % of men in their reproductive age .

Despite its multifactorial nature, male factor infertility is yet not 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.

S/A

Semen analyses serve as a diagnostic cornerstone in male fertility.

On the most basic level, it measures the function of the testicles for sperm production and accesses the fluid volume contributed by accessory glands .

WHO has standardized semen analysis in a protocol .

Volume	1.4 mL (1.3-1.5)
Viscosity	< 2 cm after liquefaction
Gross and microscopic appearance	Opaque, cream-colored, ≤ 1–3 WBC/high-power field No agglutination present
pH	7.2
Total sperm number	39 million sperm per ejaculate or more (35-40)
Total motility	42% (40-43)
Progressive motility	> 30% (29-31)
Nonprogressive motility	30% (29-31)
Morphology	> 4% normal forms using the Tyberberg method (3.9-4)
Fructose	> 13 micromoles per ejaculate (indicating at least 1 ejaculatory duct is patent)
Vitality	> 54% (50-56)
WBC count	< 1,000,000/mL

WBC = white blood cells

*Reference: World Health Organization (WHO): WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO

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Assessment of Motility

Sperm are now categorized as progressively motile, nonprogressively motile, and immotile.

Progressive motility constitutes spermatozoa moving actively,

linearly, or in large circles, while nonprogressive motility constitutes lesser motility with reduced progression or limited circles.

It is generally recognized that the percentage of progressively

motile sperm is associated with pregnancy rates, While overall motility remains a poor predictor of fertility.

Assessment of Concentration and Volume

Semen volume should be greater than 1.5 ml, with deficient volume resulting from incomplete sample collection, low testosterone, retrograde ejaculation, or ejaculatory duct obstruction .

Oligozoospermia refers to sperm concentration below the WHO reference value (15 million/ml).

Azoospermia refers to complete absence of sperm in the ejaculate.

Asthenozoospermia is the state of having total and/or forward progressive motility below WHO lower reference values (40 and 32 %, respectively.

Assessment of Morphology

Sperm morphology is an oval-shaped head with smooth contour, head length of 4–5 µm, and sperm width of 2.5–3 µm.

Acrosomal region, comprising 40–70 % of the area of the head. The midsection should be slender and in proportion to the head, while the tail should be uniform, uncoiled, and approximately 45 µm in length .

The WHO protocol, Particular areas of concern in abnormal spermatozoa include vacuolated heads (>20 % of head area occupied by unstained vacuolar areas), heads with reduced acrosomal area (<40 %), double heads, and other gross deformities.

Common defects of the midsection include bent neck, asymmetrical mounting of mid-piece and head, and gross irregularities

Abnormal tails are commonly short, multiple, bent, of irregular girth, or otherwise defective .

Assessment of Morphology

For years, sperm morphology has been a debated indicator of male fertility and success with assisted reproductive technologies (ARTs) While subfertile men have a lower percentage of normal forms when compared with men with proven fertility.

Does form impact function? While an assessment of sperm morphology is a component of the standard semen analysis, the clinical utility of this is debated.

The WHO currently recommends use of the strict morphology criteria to evaluate sperm morphology and has established 4% normal forms

as the lower limit of normal .

later data showed that successful oocyte fertilization and pregnancies were reported even in couples with normal sperm morphology of 0% .

Further complicating matters, a recent study found that many physicians believe that for men with normal morphology <4%, natural means will not be successful in achieving a pregnancy.

Natural Conception

likelihood of achieving pregnancy without the use of ART in men with severe teratozoospermia, 0% normal forms(Kovac)

Twenty-four men with 0% normal forms were compared to 27 randomly selected men with ≥4% normal forms over a 3-year period. While the natural conception rate was higher in men with ≥ 4% normal forms

compared to the severe teratozoospermia group (51.8% vs 25%, $p \leq 0.05$), men with 0% normal forms were still able to conceive naturally in 25% of cases.

In cases where men with 0% normal forms conceived naturally,100% of these men had another child via natural conception.The authors concluded that strict morphology should not be used to predict fertilization, pregnancy, or live birth potential,and in men with 0% normal forms, alternative reproductive modalities should be considered before immediate IVF.

Intrauterine Insemination

IUI success has been shown to be strongly dependent on the number of total motile sperm post-wash.

According to the WHO 5th edition, the minimum threshold for number of motile sperm post-wash necessary for possible IUI success is one million.

However, there is no consensus for the percentage of morphologically normal sperm needed for IUI success.

In conclusion, while earlier data were conflicting, the most recent data do not seem to show a strong correlation between sperm morphology and IUI success rates.

Intracytoplasmic Sperm Injection

The true impact of sperm morphology on embryo quality and development is unclear based on the very little evidence in this area.

Others in semen

Men with increased round cells on SA (>1million/mL) should be evaluated further to differentiate white blood cells (pyospermia) from germ cells. (Expert Opinion)

Patients with pyospermia should be evaluated for the presence of infection. (Clinical Principle)

Routine semen cultures have not been prospectively demonstrated to benefit infertile couples, so many male reproductive experts do not routinely screen for infection unless pyospermia is present.

Antisperm antibody (ASA) testing should not be done in the initial evaluation of male infertility.(Expert Opinion)

ASA testing should only be considered if it will affect management of the patient,for example, to suggest the presence of reproductivetract obstruction.

Continue

For couples with RPL, men should be evaluated with karyotype (Expert Opinion) and spermDNA fragmentation. (Moderate Recommendation; Evidence Level: Grade C

Limitations of Semen Analysis

1. Insufficient in determining the fertility
2. Poor indicator of sperm function
3. prognostic value is limited
4. Inherent biological heterogeneity and vary by country, region, and individual
5. These tests provide little to no insight into the location, cause, or time frame of the origins of abnormalities in ejaculated sperm.

Sperm DNA fragmentation testing and clinical implications for male factor infertility

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 .

ASRM:Sperm DNA fragmentation analysis is not recommended in the initial evaluation of the infertile couple. (Moderate Recommendation;

Evidence Level: Grade C)

(Since there are no prospective studies that have directly evaluated the impact of DNA fragmentation testing on the clinical management of infertile couples this assay should not be routinely performed in the initial evaluation of the infertile male.)

Sperm DNA fragmentation

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.

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(oxidative stress), cancer, obesity, and advanced paternal age.

Sperm DNA fragmentation

Sperm DNA fragmentation may adversely affect:

1. The outcome of ART treatments
2. Natural fertility
3. Increased miscarriage rate

ASRM

Clinical utility

ASRM has recently conceded that determining the values of SDF may be clinically informative for intrauterine insemination (IUI) or IVF and ICSI outcomes.

Repair of clinical varicocele has been shown to reduce SDF. This decrease is usually observed 3 months after varicocelectomy It is hypothesized that improvements in sperm function, including sperm chromatin integrity, would be a plausible explanation for the observed beneficial effect of prior varicocelectomy on ICSI outcomes.

Clinical implication of SDF assay

- **Varicocele**
- **Unexplained Male Infertility (UMI)**(Higher levels of SDF and its positive association with oxidative stress have been demonstrated in men with UMI with normal sperm parameters)
- **Intrauterine Insemination (IUI)**
- **In Vitro Fertilization (IVF)/ICSI**
- **Recurrent Pregnancy Loss (RPL) and Miscarriage** (defined as at least two prior pregnancy losses at less than 20 weeks of gestation)

Strategies to reduce SDF

- Antioxidant therapy
- Short ejaculatory abstinence
- Sperm processing and preparation
- Sperm selection strategies

Antioxidant therapy

- With increasing reports on the detrimental effects of oxidative stress on sperm DNA integrity, the **possible beneficial** indicated by various clinical studies.

- **Further studies are required for standardization of treatment regimen** as contradictory reports on the **negative impact** of antioxidant therapy on male infertility have been reported.

Short ejaculatory abstinence

- **Repetitive** ejaculation with **short abstinence** has been reported to reduce SDF significantly .

- This is due to significant reduction in the duration of **epididymal transit of spermatozoa and its exposure to ROS.**

Sperm processing and preparation

- **Several strategies have been reported to reduce the negative effects of sperm preparation on SDF, which includes:**

shorter incubation time,

storage at room temperature,

low speed centrifugation,

and addition of antioxidants/ cryoprotectants to culture media.

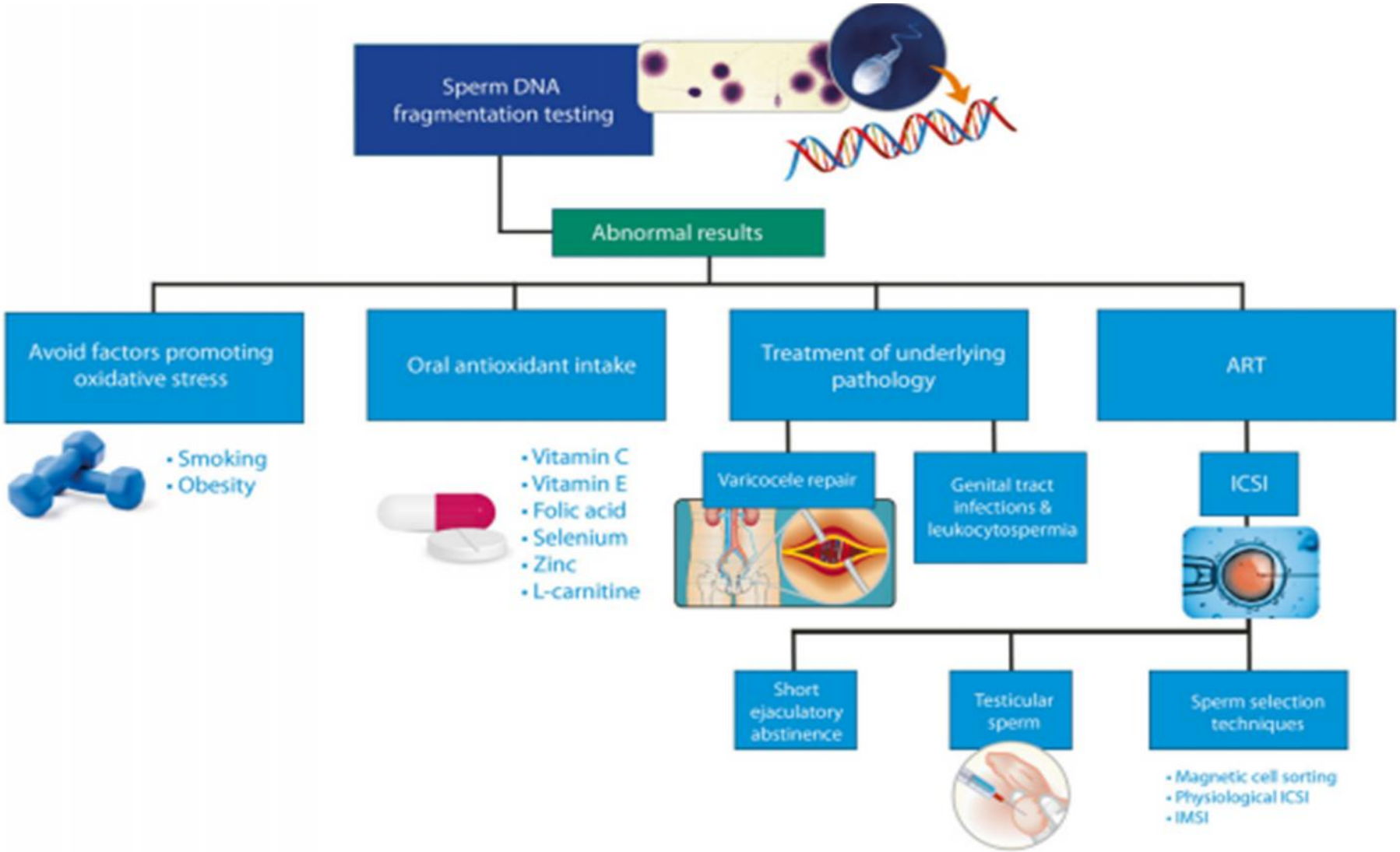
- Density gradient centrifugation (DGC) is a common sperm preparation protocol before ART and **has been reported to elevate SDF significantly** resulting in lower pregnancy rate.

- The negative influence of DGC on SDF was reported when higher centrifugation force and Percoll gradients were used . Conversely, reduction in SDF following DGC has also been reported.

Sperm selection strategies

- **Sperm selection strategies include techniques such as:**
- magnetic cell sorting (MACS),
- intracytoplasmic morphologically selected sperm injection (IMSI),
- physiological ICSI with hyaluronic acid binding assay (PICSI),
- testicular sperm extraction (TESE) and testicular sperm aspiration (TESA).

- A recent study conducted by Bradley et al. **showed highest improvement in live birth rates with TESE/TESA (49.8%)**, and modest improvement with IMSI(28.7%) and PICSI (38.3%) when compared with no intervention (24.2%).


Sperm DNA fragmentation testing
Abnormal results
Avoid factors promoting oxidative stress
• Smoking
• Obesity
Oral antioxidant intake
• Vitamin C
• Vitamin E
• Folic acid
• Selenium
• Zinc
• L-carnitine
Treatment of underlying pathology
Varicocele repair
Genital tract infections & leukocytospermia
ART
ICSI
Short ejaculatory abstinence
Testicular sperm
Sperm selection techniques
• Magnetic cell sorting
• Physiological ICSI
• IMSI

Endocrine evaluation

An endocrine evaluation of an infertile male should be performed to check:

(1) an abnormal testicular size and/or pathology

(2) abnormal semen parameters;

(3)impaired sexual function

(4) other clinical findings suggestive of a specific endocrinopathy.

Initial evaluation should include levels of serum testosterone (T), luteinizing hormone (LH), follicular stimulating hormone (FSH), inhibin and also prolactin (if erectile dysfunction is suspected).

Since serum gonadotropins and T are secreted in a pulsatile manner, time of collection is important to determine a patient's endocrine status.

If the T level is low, a repeat measurement of total and free/bioavailable T and serum LH is important.

A normal serum FSH level does not guarantee the presence of intact spermatogenesis. Abnormal serum FSH and inhibin are indicative of impaired spermatogenesis

Table 1: Reference values of male reproductive hormones

Hormone (units)	*Normal reference range*
Total testosterone (ng dl^{-1}) >20 years	240–950
Testosterone, bioavailable (ng dl^{-1})	
20–39 years	72–257
40–69 years	40–213
Testosterone, free (ng dl^{-1})	
20–39 years	1.4–20.3
40–69 years	0.6–16.8
Estradiol adult (pg ml^{-1})*	11.6–41.6
Follicle stimulating hormone adult (mIU ml^{-1})*	0.9–15
Luteinizing hormone adult (mIU ml^{-1})*	1.3–13
Inhibin B (pg ml^{-1})	47–308
Prolactin (ng ml^{-1})	2–15

*Some variation observed between various reference laboratories

Table 2: Male reproductive hormone evaluation profile as related to clinical condition

Clinical condition	*Follicle stimulating hormone*	*Luteinizing hormone*	*Testosterone*	*Prolactin*
Normal spermatogenesis	Normal	Normal	Normal	Normal
Abnormal spermatogenesis	High	Normal	Normal	Normal
Hypogonadotropic hypogonadism	Low	Low	Low	Normal
Hypergonadotropic hypogonadism/complete testicular failure	High	High	Low	Normal
Prolactin-secreting pituitary tumor	Normal/low	Normal/ low	Low	High

Table 3. *Hormonal assessment expected in azoospermic men with severely impaired spermatogenesis, obstruction, and hypogonadotropic hypogondadism*

	Severely impaired spermatogenesis	Obstructive azoospermia	Hypogonadotropic hypogonadism
LH	↑ or NI	NI	↓
FSH	↑	NI	↓
Testosterone	↓ or NI	NI	↓

Genetic Testing

1. Karyotype and Y-chromosome microdeletion analysis should be recommended for men with primary infertility and azoospermia or severe oligozoospermia (<5 million sperm/mL)
2. Clinicians should recommend Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutation carrier testing (including assessment of the 5T allele) in men with vasal agenesis or idiopathic obstructive azoospermia.(Expert Opinion)
3. For men who harbor a CFTR mutation, genetic evaluation of the female partner should be recommended. (Expert Opinion)

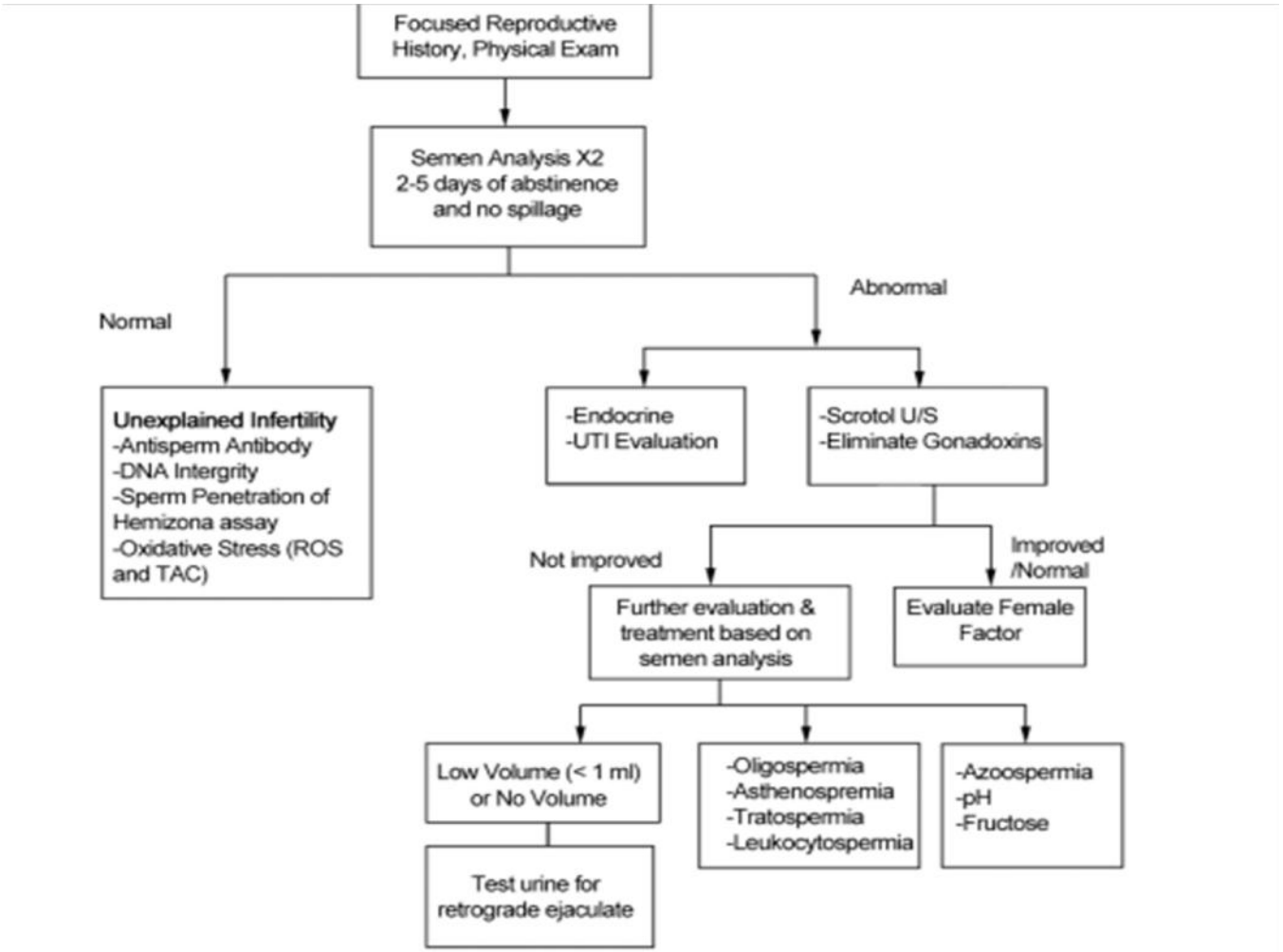


Figure 1: Flow chart for the evaluation of male infertility and reproductive. Failure flowchart demonstrating various methods available to measure seminal oxidative stress. GSH: reduced glutathione; GSSG: oxidized glutathione; ROS: reactive oxygen species; TAC: total antioxidant capacity.

THE END