# Sperm function test

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## ABSTRACT

With absolute normal semen analysis parameters it may not be necessary to shift to specialized tests early but in cases with borderline parameters or with history of fertilization failure in past it becomes necessary to do a battery of tests to evaluate different parameters of spermatozoa. Various sperm function tests are proposed and endorsed by different researchers in addition to the routine evaluation of fertility. These tests detect function of a certain part of spermatozoon and give insight on the events in fertilization of the oocyte. The sperms need to get nutrition from the seminal plasma in the form of fructose and citrate (this can be assessed by fructose qualitative and quantitative estimation, citrate estimation). They should be protected from the bad effects of pus cells and reactive oxygen species (ROS) (leukocyte detection test, ROS estimation). Their number should be in sufficient in terms of (count), structure normal to be able to fertilize eggs (semen morphology). Sperms should have intact and functioning membrane to survive harsh environment of vagina and uterine fluids (vitality and hypo-osmotic swelling test), should have good mitochondrial function to be able to provide energy (mitochondrial activity index test). They should also have satisfactory acrosome function to be able to burrow a hole in zona pellucida (acrosome intactness test, zona penetration test). Finally, they should have properly packed DNA in the nucleus to be able to transfer the male genes (nuclear chromatic decondensation test) to the oocyte during fertilization.

**KEY WORDS:** Fertilization, spermatozoa, sperm function test

## INTRODUCTION

Approximately, 10–15% couples of reproductive age group seek fertility assessment at various clinics worldwide. With an increasing population of working women and the postponement in the ages of marriage and initial childbearing, infertility services are being increasingly asked for. With the advent of assisted reproductive techniques (ART) and advances in embryology the evaluation of the male partner is often overlooked which is responsible for approximately half of the infertility cases. It is essential to identify the pathology and treat the male partner, which may allow couples to improve their fertility potential and conceive naturally too.

Only marginal improvement seen in implantation (IR) and pregnancy rates (PRs), despite major progress in stimulation protocols, drugs used for preventing premature luteinization, improved fertilization procedures with optimizing embryo culture conditions in the laboratory. We have concentrated only on oocyte quality, embryo quality, endometrial receptivity and uterine competence but not on the sperm.

Success of intracytoplasmic sperm injection (ICSI) had led to the cessation of research in sperm dysfunction and allowed the indiscriminate use of immature as well as defective sperms.

The new World Health Organization (WHO) guidelines on semen analysis are enthusing and makes one wonder whether we have over treated the male partners previously. The oligo-astheno-teratospermia syndrome is commonly encountered a problem in male infertility. This creates a challenging situation for the andrologists as this is a treatable condition if diagnosed at the correct time and treated accordingly. Understanding the human sperm and fertilization biochemistry has improved over the years. Objective data and information about important measures like kinematics of sperm capacitation, hyperactivation, the ability of sperm to bind zona-pellucida, undergo acrosome reaction,

and finally decondensation of the chromatin to form male pronucleus started coming in to improve our knowledge of events leading to fertilization and formation of embryo. Such recent information is helping us to diagnose new forms of male sub-fertility, predict the success of attempts at natural or assisted conception and to design *in vitro* sperm function tests. This is and also guiding us to design new treatments to overcome the diagnosed dysfunction.[1-4]

## Are sperm function test relevant in today's era?

Many feel and believe that sperm function testing is now irrelevant due to advances in, *in vitro* fertilization (IVF) technology, especially ICSI technique. ICSI has definitely given hope to couples who previously had virtually no chance of conception with their own gametes. Many couples without severe sperm defects can be treated by less invasive and expensive options provided we can provide better sperm function tests to ensure normal fertilization without the use of ICSI. Overrated and wrongly designed function tests in past also had their share of bad repute.[5,6] Only with an increased understanding of basic and applied biology of subfertility we can hope to address this burning issue.[7]

## What do we have in our armamentarium?

The semen analysis is one of the most basic laboratory tests for clinical assessment of the infertile couple. The parameters of semen evaluation provide information on sperm production by testes, patency and function of the male reproductive tract and activity of the accessory glands. The clinical usefulness of the semen evaluation is refining rapidly as more objective, standardized methodologies are being introduced.

This simple test provides essential information on the clinical and reproductive status of the individual. A number of clinical approaches have been used to identify the minimum standards for an ideal semen sample. Results are inconsistent in some of the criteria used for evaluations, and common shortcoming is the reliance on a single semen evaluation for prediction.

With absolute normal semen analysis parameters it may not be necessary to advise any specialized tests to the male but in many cases of borderline parameters it becomes obligatory to do a battery of sperm function tests to evaluate different parameters of sperms. Various sperm function tests have been proposed and further endorsed by different researchers in addition to the routine evaluation of fertility. These tests detect function of a certain part of spermatozoon and give insight on the events in fertilization of the oocyte. It is arduous to depend on a single group of tests for predicting fertility outcome as the fertility is dependent upon on sum total of all the functional parameters of the sperm and reliance on any one of them will be in appropriate in long run [Table 1].

## Sperm oocyte interaction and more

The steps leading to sperm maturation and fertilization occur during passage of spermatozoan in the female genital tract. To briefly enumerate, these are capacitation in the cervical mucus followed by acrosome reaction, sperm zona pellucida interaction, sperm-oocyte membrane fusion, decondensation of sperm nucleus and finally fusion of male and female pronucleus.[8,9] Each and every step is followed by the next one at a specific site and at critical times. Let us see how it happens in the body.

### *Capacitation*

Capacitation can be defined as time dependent, reversible changes sperm undergo especially in the female genital tract, which lead to the ability of sperm to acrosome react. The changes occur with loss of extrinsic proteins, including acrosome stabilizing factors along with the loss of membrane cholesterol. These intricate steps results in sperms having more fluid and pliable membranes. During capacitation sperm motility changes to the characteristic pattern, and induction of AR becomes possible. The rapid spiraling pattern of the sperm movement and subsequently hyperactivated sperm motility is commonly associated with sperm capacitation. Computer assisted semen analysis allows objective and repeatable quantification of these patterns.

### *Acrosome reaction*

A population of capacitated sperm can be stimulated to acrosome react and release acrosin in the culture medium.

**Table 1: Common sperm function test and their clinical significance**

| Pre requisities of an ideal sample | Assessed factor | Clinical test available |
|---|---|---|
| Adequate Nutrition from the seminal plasma | Fructose and citrate | Fructose qualitative and quantitative estimation, Citrate estimation |
| Protection from bad effects of leucocytes and routine apoptosis | Pus cells and reactive oxygen species | Leucocyte detection test, ROS estimation |
| Ideal morphological index | Structurally perfectly normal to be able to fertilize | Semen Morphology |
| Intact and functioning membrane | Survive harsh environment of vagina and uterine fluids | Vitality and HOS test |
| Optimal mitochondrial function | Provide energy to reach the site | Mitochondrial activity index test |
| Optimal acrosome function | Able to burrow hole in zona pellucida | Acrosome intactness test, zona penetration test |
| Ideally packed DNA in the nucleus | Transfer the male genes properly | Nuclear chromatic decondensation test |

DNA: Deoxyribonucleic acid, ROS: Reactive oxygen species, HOS: Hypo-osmotic swelling

Individual sperm that have acrosome reacted may be detected by variety of methods including labeling with fluorescent lectins, monoclonal antibodies to specific proteins, histochemical staining or by binding with antibody-bound beads. Flow cytometry also can be used to detect acrosome reacted sperm using specific fluorochrome staining.

*Sperm-zona pellucida interaction*

The zona plays a major role in controlling fertilization and it is the most potent and only physiological inducer of the acrosome reaction. To fertilize a human oocyte, sperm must recognize and tightly bind specific receptors on the zona, which are species specific. Zona binding and subsequent acrosome reaction induction is the precursor for final fertilization. Abnormal sperm zona pelucida interaction may be the major dysfunctional step in preventing fertilization. IVF treatment and observation of IVF oocytes can give insight into the zona binding of the sperm.

Two commonly used zona binding assays are hemizona assay and a competitive intact zona binding assay. Both use different methodologies, but they both use assessment of tight binding of sperm to zona as a primary endpoint. Significantly both assays have demonstrated to have a high predictive value for fertilization results under *in vitro* conditions. Both can detect cases likely to show failed or poor fertilization.[10,11]

*Sperm-oocyte interaction*

After achieving zona penetration, the acromose-reacted and acrosome-spent sperm must now bind and fuse with the plasma membrane of the oocyte. Specific receptors are involved in this process. The fusion process initiates the release of the oocyte cortical granules, which result in alteration of zona to prevent further sperm binding and penetration. Under the oocytes control the sperm is drawn into ooplasm where its nucleus decondenses and forms male pronucleus.

Tests for these steps (sperm penetration assay and IVF) are difficult to interpret as they involve 2 living cells, depend on the sequence of events and only final step can be observed. If sperm penetration test and fertilization is successful, then it is evident that at least one sperm performed all its required functions.[12,13]

### Ideal sperm function rest

Fertilization requires sperms to get nutrition from the seminal plasma in form of fructose and citrate. This provides energy to the spermatozoa. For this fructose qualitative and quantitative estimation test are available to us commercially. Further sperms should be protected from bad effects of pus cells and excessive reactive oxygen species (ROS). Leukocyte detection test and ROS estimation test may be offered to the males. Ejaculate should have sperms in sufficient numbers and should be morphologically normal. They should have intact and functioning plasma membrane to survive the harsh environment of the vagina and oxidative stress. These can be assessed by carrying out hypo-osmotic swelling (HOS) and vitality test. Further spermatozoon should have an adequate mitochondrial function to reach the eggs and fertilize them. This can be assessed by mitochondrial activity index test, which is presently not available to us. Sperms require adequate acrosome function to be able to penetrate zona pellucida and acrosome function test on lines of hyaluronic binding and gelatin assay may be offered when required. The nuclear DNA should be tightly packed in the nucleus for them to be able to transfer the male genes properly in the oocyte and form male pronucleus. Nuclear chromatic de-condensation and DNA fragmentation test may be carried out for such assessments [Table 1].

## COMMON TEST BEING CARRIED OUT IN DAY TO DAY PRACTICE

### Vitality

*Hypo-osmotic swelling test*

Water permeability is an important physiological property of all cell membranes and spermatozoa's are no exception. Membranes allow selective transport of fluids and molecules through it. Sperm membrane plays an important functional role during fertilization process as can be evaluated by the HOS test (HOST). A decent correlation ($r$ = 0·90) was witnessed between the percentage spermatozoa in a semen sample that were capable of undergoing swelling and the percentage of denuded hamster oocytes those penetrated by capacitated spermatozoa from the same semen sample.[14]

Hypo-osmotic swelling test is based on the ability of live spermatozoa to withstand moderate hypo-osmotic stress. With moderate hypo-osmotic stress membranes swell and reach steady state where fluid passing into the cells and that pumped out by intact functional membrane equal quantity. The cells will swell to varying degrees at this stage but will not burst open [Figure 1]. Dead spermatozoa whose membranes are no longer intact do not swell in hypotonic media. The clinical value of HOST is being constantly evaluated in the literature. HOS reacted sperm can be semi-quantitatively and subjectively graded as grade A to G based on the amount of swelling and curling of the tails. Percentage of each grade can be scored and reported as a percentage after counting 200 sperms. More than 60% HOS reacted sperms are considered as normal and abnormal if <50% show tail curling. Scores between 50% and 60% are considered intermediate. When correlated with DNA fragmentation status using HOS and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL), spermatozoan grades D, E, and F showed significantly less DNA fragmentation compared to grades A, B, and C within each of the total sperm population.

Correlation is evident in both types of samples those showing normal HOS and abnormal HOS scores. A study reported that low HOS values of neat semen samples were notably ($P < 0.001$) correlated with increased DNA damage identified by the DNA fragmentation index (DFI). DFI was assessed by sperm chromatin structure assay (SCSA) and TUNEL assay. The HOST value was highly predictive of an abnormal DFI value by receiver operating characteristic curve analysis ($P < 0.001$).[15,16] HOS can be generally used as an additional indicator of sperm vitality and can be used to diagnose spermatozoa with immotile cilia syndrome. Presently with the correlation with HOS grading and DNA fragmentation status HOS remains only reliable indicator for selection of spermatozoon for selection in ICSI.

### *Sperm vitality staining*

The sperm vitality is reflected in the proportion of spermatozoa that are "alive." It is measured by assessing the ability of sperm plasma membrane to exclude extra-cellular substances like dyes. Sperm vitality should be determined in semen samples with <50% motile spermatozoa. Vitality assessment also provides check on the accuracy of motility assessments; as the percentage of live spermatozoa should slightly exceed the total percentage of motile spermatozoa. Plain eosin staining can be used to assess vitality in wet smears. This provides a quick assessment at the same time of count and motility assessment. Spermatozoa that are white (unstained) are counted as live, and those showing any degree of pink or red are dead. Spermatozoa stained with this kit cannot be used for any further procedures. To differentiate live spermatozoa from dead spermatozoa for use in ICSI (low viability samples), the HOST should be used [Figure 2].

Eosin-nigrosin staining is also used for assessing vitality. The technique is based on the principle that dead cells will take up the eosin, and as a result stain pink. The nigrosin provides a dark background, which makes it easier to assess the slides [Figure 3]. The assessment can be carried out at any time and slides also can be preserved for future assessment and record.[17]

## Reactive oxygen species

Production of ROS is of concern because of potential pathological effects. Spermatozoa like any other cell constantly require $O_2$ for metabolism but the deleterious metabolites produced such as ROS can modify cell function and/or damage to endanger survival. Seminal plasma naturally contains different antioxidants' that help protect spermatozoa against such oxidants. Seminal plasma oxidative stress develops as a result of an imbalance between ROS generating and scavenging activities.[18]

Thirty-nine infertile men and 13 healthy fertile donors were studied by Aziz *et al*. and a significant negative correlation was detected between sperm ROS production and the proportion of sperm with normal morphology and borderline morphology. ROS production was positively correlated with the proportion of sperm with morphologically deformed sperms and leukocytes [Figure 4].[19]

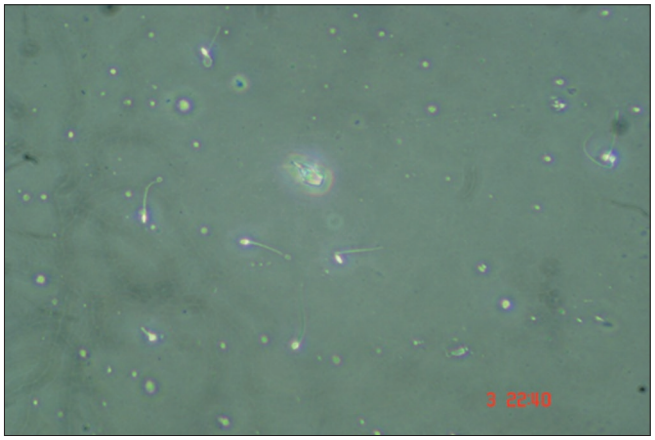

**Figure 1:** Healthy spermatozoon showing swelling and curling of the tail region

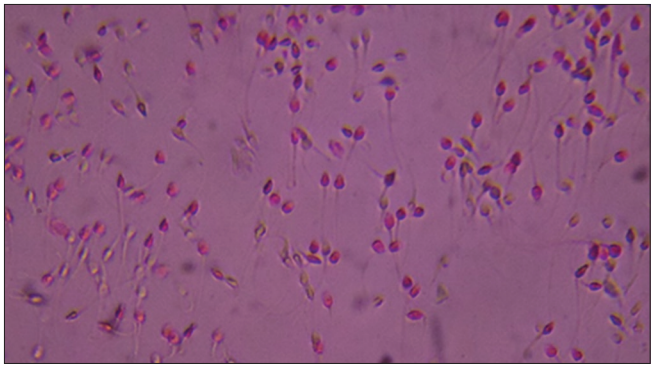

**Figure 2:** Eosin test: Pink stained sperms are non viable sperms as compared to white ones with intact memberanes

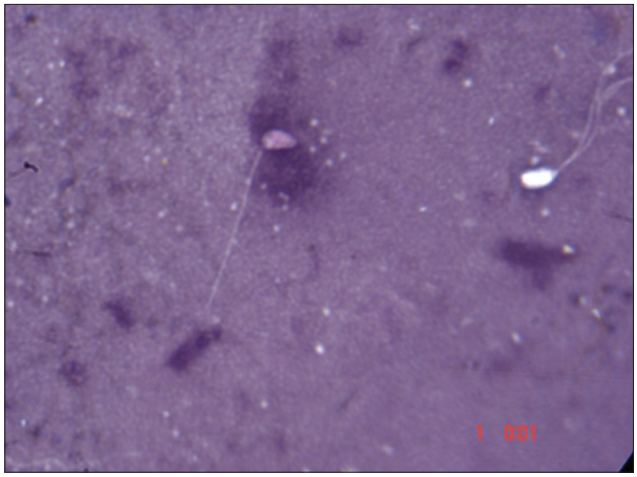

**Figure 3:** Eosin nogrosin staining: Pink stained sperms are non viable sperms. Nigrosin provides dark back ground

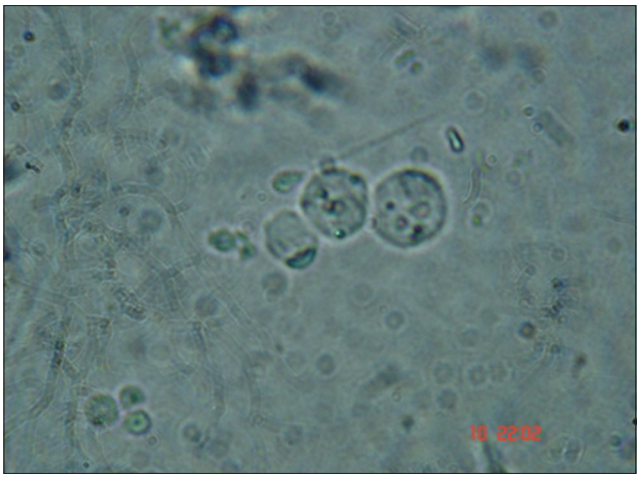

**Figure 4:** Poly morphonuclear leukocyte on a smear examination

Spermatozoa are particularly susceptible to ROS-induced damage compared to other cells as they have relatively large quantities of polyunsaturated fatty acids in the membrane, and their cytoplasm contains a low concentration of scavenging enzymes. ROS may be estimated in whole ejaculate using various chemiluminance methods or by semi-quantitative assays using oxidative indicators.[20,21]

### Mitochondrial activity index test

Sperms when released into seminiferous tubule lumen are immotile or at the most very feeble motile. This is due to immaturity of the plasma membrane of the testicular spermatozoa. This is true of testicular aspirated sperms also which do not show motility even after exposure to the culture medium. The energy required for this flagellar activity is derived from adenosine triphosphate (ATP) produced in the mitochondria concentrated in the midpiece of spermatozoa. The long passage of spermatozoa through female tract needs robust mitochondrial apparatus producing an optimal quantity of ATP.

The mitochondrial oxido-reductive enzyme apparatus can be tested using indicators like nitro blue tetrazolium, which product blue insoluble pigment in and around mid-piece. Evaluation can be carried out in smears showing stained mid-piece from good sperms to poor staining of nonmotile or poor mitochondrial activity sperms. Indirect semi-quantitative tests also are developed, which can give indirect evidence of mitochondrial activity depending on the quantity of color developed. The test was found to have a significant correlation with the sperm motility parameters. The $R^2$ value of the prediction equation was 88.74%.[22]

### DNA fragmentation tests

The integrity of paternal genome delivered by the spermatozoon is of paramount importance in the initiation of viable pregnancy. The fragmented DNA delivered by the sperm is incompatible with normal embryonic development.

DNA damage can occur due to apoptosis during spermatogenesis, DNA strand breaks during the remodeling of sperm chromatin during spermiogenesis, DNA fragmentation induced by ROS in the genital tract, or due to environmental toxins. Post testicular damage during passage through epididymis appears to play a major role in causing sperm DNA fragmentation.[23,24]

Sperm nucleus volume is 1/30$^{th}$ of the eukaryotic cell in spite of having just half the DNA quantity. This reduction of volume and orderly packaging of DNA requires very novel methods. DNA, which is packaged properly, can be studied by decondensation *in vitro*, induced by ethylenediaminetetraacetic acid (EDTA), glutathione (present in oocyte) or DTT. Sperms with damaged DNA or not properly packaged DNA do not show full DNA decondensation.[25,26]

DNA breaks detected by the test may be in an insignificant area like exons or introns of the chromosome with no consequence on pregnancy development. The proportion of sperms showing damage is important but few sperms, which show normal DNA may be sufficient for good pregnancy development. Predictive value is always uncertainty and cannot have 100% negative predictive value. Main indications for sperm DNA testing include idiopathic infertility, repeated pregnancy failure in IVF and recurrent miscarriages.

Indications for DNA fragmentation testing:

- Unexplained or persistent infertility
- Failure to conceive after 5–6 intrauterine insemination (IUI) cycles despite good count and motility
- Low fertilization rates or poor embryo quality in IVF cycles
- IR failure after IVF
- Recurrent miscarriage
- Prolonged stay in an environment that exposes to reproductive toxins
- Abnormal semen analysis
- Advancing male age (>45 years).

### *Nuclear chromatin de-condensation test*

The chromatin in spermatozoa is in a highly condensed state prior to fertilization. The appropriate nuclear chromatin de-condensation and subsequent male pronucleus formation is essential for fertilization and normal zygote development. Highly condensed nuclear chromatin in spermatozoa is because of the formation of S-S cross links

between its histone units. The cleavage of S-S link can be induced *in vitro* by sodium dodecyl sulfate and EDTA. Such induced decondensation is a predictor of the good fertilizing ability of spermatozoa. The result of the test is expressed as the percent swollen cells or NCD reacted cells to total sperms [Figure 5]. More than 70% sperms in normal samples show NCD positivity. Normospermic samples were evaluated for their decondensation capacity with regards to their fertilizing performance in IVF program. Fertilization favorably occurred when the decondensation % of the sperm nuclear chromatin was beyond 70%. The effective sperm count was significantly ($P < 0.05$) lesser in the unfertilized group.[27]

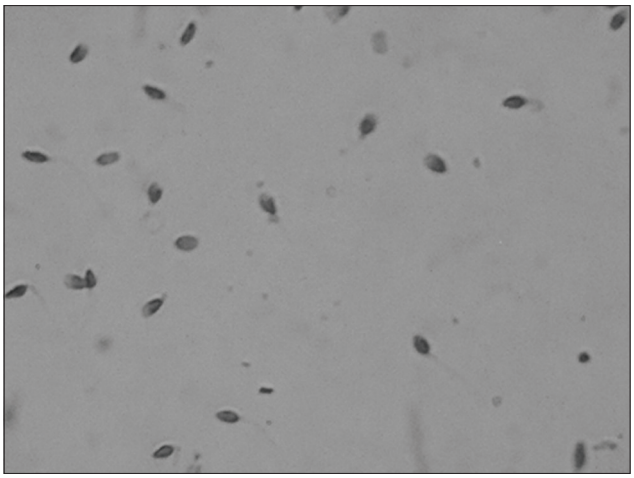

**Figure 5:** NCD Test; The cells showing swelling of nucleus with uniform chromatin are considered positive and the cells showing intact nucleus are considered negative for NCD

### *DNA fragmentation index*

In the recent years, there has been a developing global interest among the reproductive biologist that sperm nuclear integrity should be assessed using SCSA, sperm chromatin dispersion [Figure 6], TUNEL or the comet assay. DNA fragmentation was investigated by TUNEL assay in the spermatozoa by Henkel *et al*. The percentage of TUNEL-positive spermatozoa were negatively correlated with normal sperm morphology ($r = -0.4423$; $P = 0.0026$), which itself was positively correlated with motility ($r = 0.4818$; $P = 0.0008$) and progressive motility ($r = 0.4545$; $P = 0.0017$).[28] The causes for increased sperm DNA fragmentation are shown in the Figure 7.

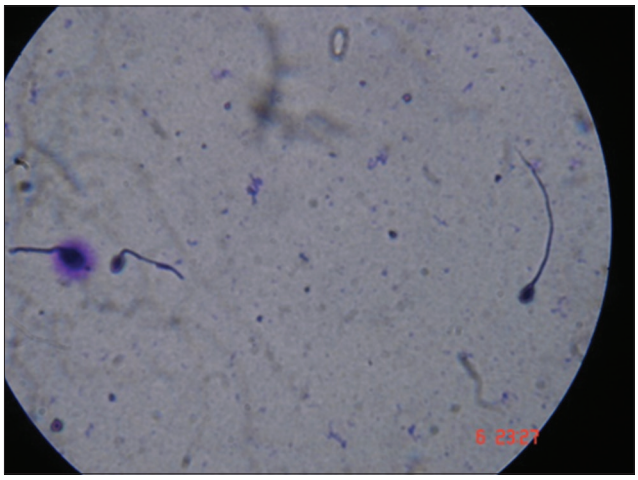

**Figure 6:** DNA fragmentation test. cells showing halo around the nucleus are good sperms

Increased intervention from IUI to IVF to ICSI, the lesser the impact of sperm DNA damage has on early fertility checkpoints. However, in IVF and ICSI pregnancy loss, DNA damage has a moderate positive effect.

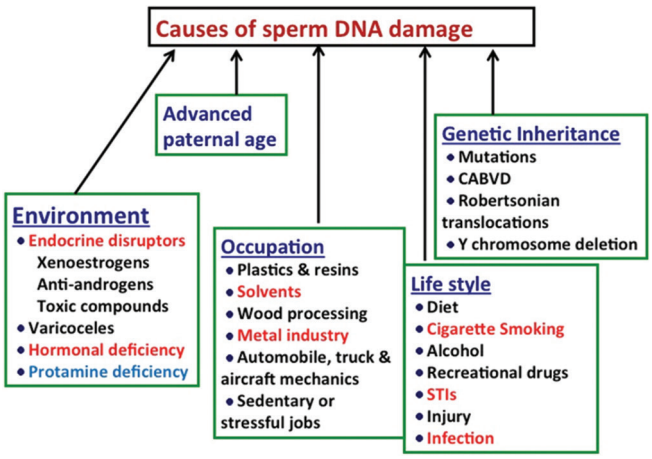


**Figure 7:** Causes of increased DNA fragmentation index

## Acrosomal assay test

The sperm acrosome is located on the top of the sperm head. During acrosome reaction, the outer acrosomal membrane and the plasma membrane fuse and vesiculate to discharge the acrosomal contents. One of the most important sperm membrane property related to maturation is ability to bind to hyaluronic acid. The study of hyaluronic acid bound sperm reveals that the sperms exhibit uniform morphology as well as nuclear maturation indicated by aniline blue staining. Hyaluronic acid receptors on sperm head are important for inducing specific capacitation motility pattern as well as for zona binding. *In vitro* binding test is a very good indicator of sperm membrane receptor and is performed by binding to hyaluronic acid immobilized on plastic of glass surfaces.[29]

### *Gelatin assay test*

Multiple enzymes are present in the sperm acrosome including several acid hydrolases commonly found in lysosomes. Acrosomal enzymes help the sperm to penetrate the cumulus mass and the zona pellucida. Gelatin

film lysis is one such method, which detects acrosome proteolytic activity in terms of gelatin digestion in the area surrounding the sperm head. The precoated gelatin film on the glass slide is lysed coming in contact with proteolytic enzymes leaching out of acrosome and allow light to pass through creating a halo around the sperm head which can be examined using a microscope. The sperm heads showing no halos are considered negative for acrosomal status and function.

### Biochemical test

*Fructose assessment*

Seminal plasma is a complex secretion with various substances some of which are specific to the accessory gland and contribute to a fraction of ejaculate. Important secretions are from seminal vesicles and prostate glands. Each secretion has a characteristic marker to detect presence, absence, dysfunction or infection of specific glands.

It is necessary to include more than one marker for each gland and to standardize time for collecting aliquot from well mixed complete ejaculate. Any abnormal or unusual value must be reconfirmed before drawing any conclusions or taking therapeutic decisions. Common secretions of the male genital tract are as mentioned below:

- Prostate gland: The secretions of acid phosphatase, citric acid, zinc and magnesium are specific markers of prostate gland
- Seminal vesicles: Fructose and prostaglandins
- Epididymis: L - carnitine, alpha-glucosidase, and glycerophsophocholine.

The Precise role of these specific substances contributing to sperm function is still obscure. Zinc has a probable role in protecting and stabilizing condensed sperm chromatin. Fructose is secreted by seminal vesicles and provides an energy source to spermatozoa for anaerobic metabolism. This is an important energy source for the sperm and exclusion of the seminal vesicular component from the ejaculate will result in almost completely immotile sperm.

It is a marker for seminal vesicle function, and there levels in semen are androgen dependent. Fructose levels should be determined in any patient with azoospermia and especially in those whose ejaculate volume is <1 ml, suggesting seminal obstruction or atresia or ejaculatory tract duct obstruction.

Disorders of the seminal vesicles and a subsequent reduction in the fructose concentration in semen will also result in a reduced motility of semen. Another situation where fructose estimates are helpful is in men with polyzoospermia and low motility. Occasionally in men with very high sperm concentrations (more than 350 million sperm/ml), the sperm are immotile due to a relative deficiency of fructose.

Concentration of fructose in semen ranges from 63 to 500 mg/dl (3.5–28 mmol/l). It must be remembered that as sample ages; the fructose level will fall due to the utilization of fructose by spermatozoa.

### Morphology

Variations of sperm morphology make an assessment of morphology difficult. By strict application of certain criteria of sperm morphology, relationship between the percentage of normal forms and various fertility pointers have been established [Figure 8].

The classification system evolved by Kruger *et al*. and subsequently accepted by WHO give very low threshold for accepting normal morphology and very few samples show more than 25% normal spermatozoa with most in the range of 4–30% in fertile population.[30] Patients with <4% normal forms had a low fertilization rate of 7.6%. Those with normal morphology between 4% and 14% had a considerably enhanced fertilization rate of 63.9% ($P < 0.0001$). Spermatozoa with >14% normal forms fertilized within the normal range for the laboratory.[31]

According to strict accepted criteria considering normal spermatozoan are:

- Normal sperm head is considered to be 3–5 μ in length and 2–3 μ in width with perfect oval shape
- Mid-piece is about 1 μ in diameter with straight and regular outline. It must be aligned to the longitudinal axis of the head and should be 7–8 μ in length
- The tail must be slender, uncoiled and at least 45 μ in length.

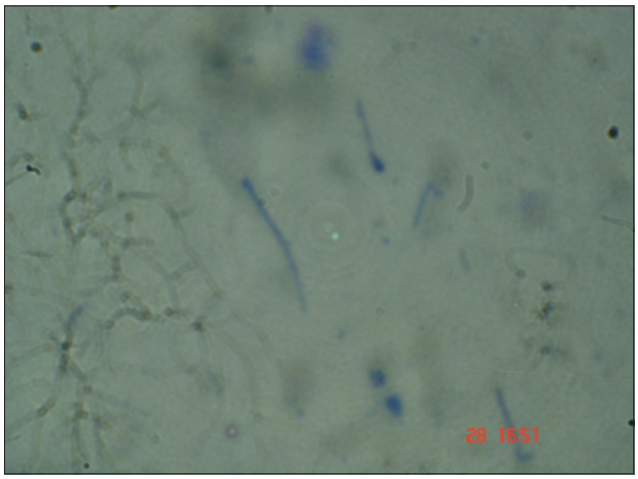

**Figure 8:** Semen morphology slide showing pin head sperms

Any sperm not meeting these criteria is considered abnormal in strict criteria.

The morphology is evaluated by using air dried smears prepared from whole semen on glass slides and stained using Diff-Quick or PAP method. Both give satisfactory results. Preferable 200 spermatozoan are counted for various abnormalities and only those showing all parts normal are considered normal.

*Teratozoospermia index*

Teratozoospermia index (TZI) is a good predictor of fertilization and PR. It is a multiple anomalies index where a total number of 100 sperms are counted. Record of number of normal and abnormal sperms is kept and then each abnormal sperm is seen for a maximal of 4 abnormalities.

Total number of all separate abnormalities is then divided by total number of abnormal spermatozoa counted, which gives the TZI. The normal TZI is <1.6. If TZI is >1.8 ICSI is done irrespective of the count and motility.

### Anti sperm antibodies

The first hurdle sperms encounter in the female genital tract is cervical mucus. Their penetration through cervical mucus and passage through it is essential to sieve out unwanted components in semen. Though cervical mucus is not hostile to sperms, it effectively blocks poorly motile and structurally abnormal sperms. The mucus during mid-cycle also can function as a store of spermatozoa, which are released to uterine cavity slowly. It protects the sperm from the harsh acidic pH of the vagina.[32]

Cervical mucus receptivity to sperms changes cyclically and it is at a peak at the time of ovulation, and thus it forms the basis for post-coital test. The cervical mucus is collected 2 h postcoitus, during ovulation period and examined for the presence of motile sperms. Presence of motile sperms in the cervical mucus denotes good mucus. Kremer's test apparatus is used for semi-quantitative evaluation of the cervical mucus penetration.

Antisperm antibodies can form in females due to repeated exposure to sperms, and these can be secreted in cervical mucus (IgA type) or can be circulating in the blood and other body fluids (IgG type). Though it cannot be determined to what extent the antibodies can affect fertility of sperms, various tests can be carried out to detect the antibodies:

- Immunobead test
- Mixed antiglobulin reaction (coomb's)
- Elisa estimation of antibodies.

Direct immunobead test and mixed antiglobulin reactions are most common tests performed, and the presence of more than 50% immunobead reacted or mixed antiglobulin reacted sperms is significant cause of subfertility. Bussaca *et al*. studied the prevalence of the immune response against spermatozoa in infertile couples using immunobead test. He deduced a significant difference ($P \leq 0.02$) in the conception rate between immune and nonimmune couples (19.3% vs. 42%). The pregnancy outcome of immune couples was favorable only in 50% of the cases.

## CONCLUSION

There is still a gap in understanding of the complex nature of molecular interactions between oocyte and sperm leading to fertilization. Exact chain of events needs further clarification, with identification of specific molecules and gamete function should become the basis of new diagnostic tests. Since there is more than one sperm function involved, no single test of a single function will have perfect sensitivity. Therefore, batteries of tests are required for diagnosis. A major goal of new tests should be the identification of molecular defects in sperm function, allowing rapid development of biochemical tests, with the goal to specifically target therapies toward male subfertility. Tests with high sensitivity, high predictive value, and low false positive rate are desirable. Valid and more predictive tests need to be developed with strict validation and specificity.

Points to be kept in mind when prescribing for sperm function test:

- High-quality clinical data missing on sperm function test
- For a test to be useful, it must have strong predictive value for pregnancy outcome and have little overlap between fertile and infertile samples
- Multi-centric trials essential to develop robust thresholds in order to integrate sperm function test especially DNA tests into routine clinical practice as they could predict ART clinical outcome
- They may be useful for identifying a male factor contributing to unexplained infertility or for selecting therapy
- There is a need for targeted sperm function testing, but to whom and which one(s) is unclear
- New tools which are simple, cheap, reliable, repeatable, effective combined with more robust assessments urgently required.

Sperm function test should not be routine investigations as they are complex, expensive, not rigorously tested, do not always provide clinically useful information and typically do not affect treatment.

## REFERENCES

1. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Sperm. 5th ed. Geneva: WHO; 2010.

2. WHO Laboratory Manual for Examination of Semen and Semen-Cervical Mucus Interaction. 4th ed. Cambridge University Press; 1999.
3. WHO Laboratory Manual for Examination of Semen and Semen-Cervical Mucus Interaction. 3rd ed. Cambridge University Press; 1992.
4. WHO Laboratory Manual for Examination of Semen and Semen-Cervical Mucus Interaction. 2nd ed. Cambridge University Press; 1987.
5. Consensus workshop on advanced diagnostic andrology techniques. ESHRE (European Society of Human Reproduction and Embryology) Andrology Special Interest Group. Hum Reprod 1996;11:1463-79.
6. De Jonge C. Attributes of fertile spermatozoa: An update. J Androl 1999;20:463-73.
7. Franken DR, Oehninger S. Semen analysis and sperm function testing. Asian J Androl 2012;14:6-13.
8. Melendrez CS, Meizel S, Berger T. Comparison of the ability of progesterone and heat solubilized porcine zona pellucida to initiate the porcine sperm acrosome reaction *in vitro*. Mol Reprod Dev 1994;39:433-8.
9. Roldan ER, Murase T, Shi QX. Exocytosis in spermatozoa in response to progesterone and zona pellucida. Science 1994;266:1578-81.
10. Yanagimachi R. Mechanisms of fertilization in mammals. In: Mastroianni L, Biggers JD, editors. Fertilization and Embryonic Development *In vitro*. New York: Plenum Press; 1981. p. 81-187.
11. Overstreet JW, Hembree WC. Penetration of the zona pellucida of nonliving human oocytes by human spermatozoa *in vitro*. Fertil Steril 1976;27:815-31.
12. Yanagimachi R. Zona-free hamster eggs and their use in assessing fertilizing capacity and examining chromosomes of human spermatozoa. Gamete Res 1984;10:187-232.
13. Aitken J. On the future of the hamster oocyte penetration assay. Fertil Steril 1994;62:17-9.
14. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil 1984;70:219-28.
15. Hossain AM, Rizk B, Barik S, Huff C, Thorneycroft IH. Time course of hypo-osmotic swellings of human spermatozoa: Evidence of ordered transition between swelling subtypes. Hum Reprod 1998;13:1578-83.
16. Stanger JD, Vo L, Yovich JL, Almahbobi G. Hypo-osmotic swelling test identifies individual spermatozoa with minimal DNA fragmentation. Reprod Biomed Online 2010;21:474-84.
17. Björndahl L, Söderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum Reprod 2003;18:813-6.
18. Henkel R, Kierspel E, Stalf T, Mehnert C, Menkveld R, Tinneberg HR, *et al.* Effect of reactive oxygen species produced by spermatozoa and leukocytes on sperm functions in non-leukocytospermic patients. Fertil Steril 2005;83:635-42.
19. Aziz N, Saleh RA, Sharma RK, Lewis-Jones I, Esfandiari N, Thomas AJ Jr, *et al.* Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. Fertil Steril 2004;81:349-54.
20. Sikka SC, Rajasekaran M, Hellstrom WJ. Role of oxidative stress and antioxidants in male infertility. J Androl 1995;16:464-8.
21. Wolff H. The biologic significance of white blood cells in semen. Fertil Steril 1995;63:1143-57.
22. Mukhopadhyay CS, Verma A, Joshi BK, Chakravarty AK. Association of sperm mitochondrial activity index with gross seminal parameters and fertility in crossbred bulls. Indian J Anim Res 2008;42:282-4.
23. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, *et al.* Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 2005;84:833-42.
24. Noblac A, Kocer A, Drevet JR. Recent knowledge concerning mammalian spern chromatin organization and its potential weakness when facing oxidative challenge. Basic Clin Androl 2014;24:6.
25. Tomlinson MJ, Moffatt O, Manicardi GC, Bizzaro D, Afnan M, Sakkas D. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: Implications for assisted conception. Hum Reprod 2001;16:2160-5.
26. Palermo GD, Neri QV, Cozzubbo T, Rosenwaks Z. Perspectives on the assessment of human sperm chromatin integrity. Fertil Steril 2014;102:1508-17.
27. Gopalkrishnan K, Hinduja IN, Kumar TC. *In vitro* decondensation of nuclear chromatin of human spermatozoa: Assessing fertilizing potential. Arch Androl 1991;27:43-50.
28. Henkel R, Kierspel E, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, *et al.* DNA fragmentation of spermatozoa and assisted reproduction technology. Reprod Biomed Online 2003;7:477-84.
29. Huszar G, Ozenci CC, Cayli S, Zavaczki Z, Hansch E, Vigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril 2003;79 Suppl 3:1616-24.
30. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, *et al.* Sperm morphologic features as a prognostic factor in *in vitro* fertilization. Fertil Steril 1986;46:1118-23.
31. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in *in vitro* fertilization. Fertil Steril 1988;49:112-7.
32. Busacca M, Fusi F, Brigante C, Doldi N, Smid M, Viganò P. Evaluation of antisperm antibodies in infertile couples with immunobead test: Prevalence and prognostic value. Acta Eur Fertil 1989;20:77-82.


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