Indian Fertility Society

Volume 1 (Sep, 2024)

NEXUS

Semen Analysis:
Nuts and Bolts

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It is with pleasure and great privilege we introduce our first Nexus bulletin titled "Semen Analysis: Nuts and Bolts". The Nexus series are being updated to bridge the gap between ART Clinicians and Embryologists keeping in mind the latest recommendations ans guidelines. The series will cover topics pertaining to quality control, basic IVF techniques and laboratory protocols.

My heartfelt congratulations to Dr Rupali Goyal, and the entire editorial team and very best wishes for the future.

Dr Shweta Mittal
Secretary General - IFS

Indian Fertility Society feels proud and congratulates the Editorial team for relaunch of Nexus-E-bulletin. The bulletin aims to enlighten and broaden the spectra of knowledge in the field of Embryology and Andrology. This is an attempt to train and educate the budding embryologist and infertility specialists from basics, in step with the latest guidelines and recommendations. The content of the E-Bulletin will disseminate scientific knowledge, Ethical practices along with latest research and development across the globe.

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1 What are the equipments & disposables required in andrology lab for semen analysis?

Equipments required:

(a) Sperm counting chamber **(b)** laminar air flow **(c)** centrifugation machine **(d)** test tube warmer **(e)** binocular microscope **(f)** dry heat incubator.

a.

c.

b.

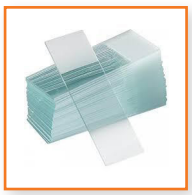
d.

Disposables required:

(a) Conical centrifuge tube **(b)** transfer pipettes **(c)** glass slides **(d)** glass cover slips **(e)** semen collection container (all disposable should be ART grade & Gamma sterilized).

a.

b.



c.

d.

e.

2 How to collect the semen sample? What are the requirements for semen collection room?

The sample should be collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis. The sample should be obtain by masturbation and ejaculated into a sterile clean wide-mouthed container.

The semen sample collection room should have the following facilities:

- Well-appointed room with privacy.
- Appropriate environment.
- Clean room.
- Must be a in-house facility.
- In a secluded area but close to laboratory.
- Well equipped, wash basin with soap, toilet not be used for any other purpose.
- Well written clear instructions for semen collection (optional). In English & local language.
- Sample collection window close to andrology laboratory.

3 What is the role of semen analysis in infertility treatment? What is the recommended abstinence interval for collecting semen samples?

Couples who are unable to conceive after 12 months of regular unprotected intercourse husband /male partner are recommended to undergo at least one semen analysis as per WHO guideline to rule out male factor infertility. The sample with abnormal semen parameters (low count and motility) in previous report should be given final opinion after doing repeat examination done after minimum of 1 month(ASRM 2021 guideline).There is no absolute requirement for doing repeat analysis in samples with normal semen parameters.

Abstinence period must be between 2 to 7 days. Shorter abstinence period (< 2 days) results in a lower sperm count whereas longer abstinence period (>7 days) leads to higher concentration of pus cells and debris than usual and also decreased motility can be observed due to more sperms stored in epididymis for a longer period

4 How to select the semen collection containers for semen analysis.

The semen collection container has to be tested for toxicity of the plastic and sterility before collecting patient sample in them. This is done by placing one half of the semen sample (high concentration, good motility) in a container that is known to be of non toxic material for semen sample (control). The other half of the sample is transferred in the new container being tested. The sperm motility is assessed at hourly intervals in replicate at room temperature or at 37°C for 4 hours. If there are no differences in motility between control and test then the test container can be considered non-toxic to spermatozoa and suitable for semen collection.

Semen collection container must be sealed, sterile, of non toxic material, wide mouth, flat bottom and free of any microorganisms. Container can be pre incubated at 37°C before sample collection in order to provide appropriate temperature conditions to semen.

Semen collection container must be labeled properly as follow (2 patient identifiers to be used to label these containers to avoid mixing of semen sample)

- Patient identification number
- Husband name / wife name or male patient name
- Age
- Date
- Procedure (HSA, IUI, IVF/ICSI, freezing)

Fig3. Semen collection container

5 What instructions must be given to patients for semen collection?

The patient should be given clear oral /written instruction for the collection as follow:

- Start collecting semen after passing urine.
- Hands must be washed properly with soap and water before giving sample.
- Container to opened only at the time of collection
- Collection method: masturbation
- To make sure that water or soap or any other thing other than semen sample must not enter container.
- Try to avoid sample spillage.
- In case of any spillage, must inform.

6 Who should perform semen analysis in the laboratory?

Semen analysis should be performed by embryologist / andrologist who is trained and experienced in processing samples as per methodology described in 6th edition of WHO manual.

7 How to handle the semen sample in the lab?

The collected semen sample should be allowed to liquefy, preferably in an incubator at 37 °C and, if possible, on an orbital mixing platform.

- The time between collection and the start of the ejaculate examination should be recorded at the start of macroscopic evaluation and presented in the final report. Preferably assessment should start within 30 minutes after collection and no more than 60 minutes after collection.
- Prolonged in vitro exposure to the liquefied ejaculate fluid will affect qualities such as motility and morphology.
- Ejaculates may contain dangerous infectious agents (e.g. human immunodeficiency virus (HIV), hepatitis viruses or herpes simplex virus) and should therefore be handled as a biohazard. Safety guidelines should be strictly followed; good laboratory practice is fundamental to laboratory safety.

8 What are all essential examinations carried out while performing semen analysis?

The essential macroscopic and microscopic examination that should be done in the liquefied sample after 15-30 minutes of sample collection

- Basic: sperm concentration, sperm motility, sperm morphology.
- Extended: assessment of leukocytes, immature germ cells, sperm antibodies; biochemical assays, sperm aneuploidy, sperm genetics, DNA fragmentation.
- Advance: assessment of reactive oxygen species and oxidative stress, membrane ion channels, acrosome reaction and sperm chromatn.

Macroscopic and microscopic semen examination

- Macroscopic examinations: volume, appearance, liquefaction, viscosity, odour, pH
- Microscopic examinations: concentration, motility, morphology, vitality, aggregation, agglutination.

9 Describe the time frame for performing tests for semen sample.

Ejaculated semen sample

↓ *Within first five minutes: measure volume*

Without any delay: place the semen sample preferably at 37°C in an incubator

↓ *Usually no more than 30-60 minutes*

- Liquefaction assessment and macroscopic appearance
- Sperm concentration and motility test by wet preparation
- Sperm morphology smears preparation
- Semen pH (if indicated)
- Sperm vitality (if low percentage of motile sperms)
- Assessing peroxidase- positive cells (if round cells are present)
- Biochemical markers (Centrifuge semen aliquots (if biochemical markers are to be assayed).
- Performing MAR test (Mixed Antiglobulin Reaction) (if required)
- Make smears for assessing sperm morphology, and fixing sears

↓ *Within 3 hours*

Microbiological/Cultural studies

↓ *Same day /subsequent day (if sample frozen)*

Sperm morphology (staining), biochemical analysis of accessory gland (optional)

10 How to calculate the semen volume?

The volume is best measured by weighing the sample in the container in which it has been collected. The volume of the ejaculate is mainly contributed by seminal vesicle and prostate gland with small amount of fluids from the bulbourethral glands and epididymides.

1. Use a pre-weighed container for collection of the ejaculate, with the weight noted on the container and lid.

*Empty specimen containers commonly have different weights, so each container with lid should be individually pre-weighed. The weight should be recorded on the container and its lid with a permanent marker pen before it is given to the patient. If labels are used – for example, for identity markers – their weight should be included in the empty weight. Sterile containers should not, and do not, require opening for this.

2. Weigh the vessel with the ejaculate in it.
3. Subtract the weight of the empty container.
4. Calculate the volume from the sample weight, assuming the density of semen to be 1 g/ml. (Semen density has been reported to vary between 1.03 and 1.04 g/ml , 1.00 and 1.01 g/ml , and an average of 1.01 g/ml .

11 What inference can be drawn from low sample volume?

The most of the semen ejaculate volume is contributed by secretion from seminal vesicles and prostate (>90%) gland. Ejaculate volume reflects the secretory functions of the auxiliary sex glands.

Semen sample with ejaculate volume ≤ 1.4 ml is termed hypospermia. In case of hypospermia, one must rule out any possibility of semen sample spillage and if yes then a repeat analysis is recommended. In cases of no spillage, there can be physiological reasons for some kind of abnormality or obstruction of ejaculatory ducts or either seminal vesicles.

12 What inference can be drawn from increased sample volume?

Semen sample with ejaculate volume >7 ml is termed as hyperspermia. The increased semen volume could be due to infection in seminal vesicles and prostate gland (prostatitis). The infection could result from presence of leukocytes and inflammatory substances in the accessory glands and male genital tract.

13 Define liquefaction and viscosity and what is their mechanism?

Liquefaction: Semen liquefaction is a proteolytic process where a gel-like ejaculated semen becomes watery due to the enzymatic activity of prostate-derived serine proteases in the female reproductive tract.

Viscosity: Viscosity is homogenous stickiness. Can be the result of an inflammatory condition, infection of the genital tract, genetic predisposition, and environmental factors. Studies have shown a relationship between high viscosity of seminal fluid and decreased motility, vitality, DNA integrity and fertilization potential of the sperm.

Fig4. Viscous sample: thread >2cm long

14 How would you handle delayed liquefaction of semen sample?

Semen samples sometime can have delayed liquefaction or may not liquefy after >30min of collection, making semen evaluation difficult. In these situations, additional pretreatments are proposed including mechanical mixing and enzymatic digestion to liquefy the sample.

This can be done by any of the method to promote liquefaction as summarized below:

- Semen sample can be allowed to liquefy for another 15-20 minutes in incubator.
- Semen sample can be placed in an orbital mixer as it facilitates liquefaction.
- Addition of physiological medium (e.g. Sperm Preparation Media, Dulbecco's phosphate-buffered saline) to an equal volume of semen sample followed by repeated pipetting.
- Mechanically in-homogeneity can be reduced by repeated (6–10 times) gentle passage through a blunt 18 gauge (internal diameter 0.84 mm) needle attached to a syringe.(However, this is not recommended, since the shearing forces are likely to damage spermatozoa and thereby negatively affect the DNA integrity).
- Enzymatic Digestion by Bromelain proteolytic enzyme (10IU/ml) prepared in Dulbecco's phosphate buffered saline) by mixing equal volume of semen sample in 1:2 ratio.

15 What are the indications for performing advanced sperm function test?

The patient with normal semen parameters may not be requiring any specialized tests. But in many cases of fertilization failures, recurrent pregnancy losses, sample with low sperm count, lower motiltiy and poor morphology may require a battery of sperm function tests to evaluate different sperms parameters. Sperm function tests provide useful insights to deduce the exact reason for fertilization failure and also guide infertility experts to adopt best possible individualized treatment based on sperm function assay results.

Various sperm function tests have been proposed and further endorsed by different researchers in addition to the routine evaluation of fertility. Some of the commonly advised sperm function tests are as follows:

- Hyalauronon binding assay (HBA)
- DNA fragmentation index (DFI)
- Vitality staining
- Seminal oxidative stress and reactive oxygen species test
- Semen Fructose detection
- Mixed antiglobulin reaction test (MAR) test

16 What is the role of microscopy in semen analysis?

Semen analysis is the first-tier screening test through routine microscopy and remains the cornerstone for the investigation of male infertility
A light microscope equipped with phase-contrast optics is necessary for all examinations of unstained preparations of fresh semen

Low magnification
An initial microscopic examination of the ejaculate aliquot involves scanning the preparation at a total magnification of ×100 [i.e. a combination of a×10 (yellow) objective lens with a×10 (ocular)]. This provides an overview of the sample, to reveal if spermatozoa are evenly distributed in the preparation, any visible mucus strands, and sperm aggregation or agglutination. In case of uneven distribution, the reason could be:

- Insufficient mixing
- High viscosity
- Insufficient liquefaction
- Sperm clumping.

Fig5a: Phase contrast binocular microscope

Fig5b: 10x, 20x, 40x, 100x objective lens

High magnification
The preparation should then be assessed at ×200 or × 400 total magnifications (i.e. a combination of a ×20 or a ×40 objective with a ×10 ocular). This permits:

- assessment of sperm motility;
- determination of the dilution required for accurate assessment of sperm number
- determination of the presence of round cells that requires further assessment
- determination of the presence of cells other than spermatozoa (e.g. epithelial cells) or "round cells" (leukocytes and immature germ cells).

Bright field for X100 (white) oil immersion objects (for morphology & vitality)
40X Negative -phase objective (optional for eosin vitality test).

17 How would you handle increased viscosity of semen sample?

Viscosity is assessed by using a plastic disposable pipette (verified as non-toxic to sperm and, if needed, sterile), allowing the semen to drop by gravity and observing the length of any thread.
Alternatively, the viscosity can be evaluated by introducing a glass rod into the sample and observing the length of the thread that forms upon withdrawal of the rod.
A normal liquefied ejaculate falls as small discrete drops.
If viscosity is abnormal the drop will form a thread will form > 2 cm long.
It can be reduced by doing additional pretreatments

- Addition of equal volume of physiological medium.
- Mechanical mixing by repeated gentle passage through broad gauge pipettes
- Enzymatic digestion by Bromelain.

High viscosity can interfere with semen analysis and determination of sperm motility, sperm concentration. It will also interfere in sperm preparation procedures, detection of antibody-coated spermatozoa and measurement of biochemical markers.

18 How to calculate sperm concentration utilizing various sperm counting chambers?

The recommended method of calculating sperm concentration is through use of available sperm counting chambers. These are used for increased level of accuracy, precision for counting spermatozoa. The sperm counting chambers for semen analysis are recommended in WHO Manual 6th edition and include glass cover slip chamber, haemocytometer, Cell-VU, and Makler® chamber as described below.

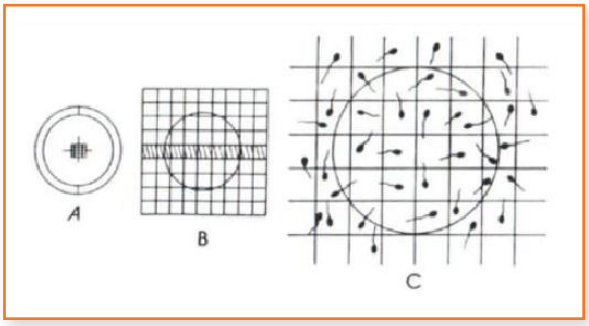


Fig6a. Sperms under microscope: Counting chamber grid

Fig6b. Sperms under microscope

Determining sperm concentration:

- Prepare the counting chamber.
- Load the counting chamber and leave it in a humid chamber to allow the spermatozoa to settle onto the bottom of the counting chamber.
- Assess the sperm numbers promptly after removal from the humid chamber (to avoid negative effects of evaporation from the counting chamber).

- Count the sperms in 3 rows or 3 columns and then take the average.
- Compare replicate counts to see if they are acceptably close. If so, proceed with calculations; if not, prepare new dilutions.
- Load the counting chamber with semen sample.
- Count the sperms in 3 rows or 3 columns and then take the average.
- Calculate the concentration in spermatozoa per ml.
- Calculate the number of spermatozoa per ejaculate.
- In the cases of oligospermic specimen, it is suggested to count sperm in the entire grid area. Five zeros are then added to the number counted and the result is the concentration in millions per mL.

- Makler® Chamber: The Makler® Chamber was designed specifically for the determination of sperm concentration and motility in undiluted semen. The grid area in the center of the coverslip is 1 mm × 1 mm and is divided into 100 smaller squares, each of which is 0.1 mm × 0.1 mm. A 5 µL volume of semen or sperm suspension is loaded into the chamber and the number of sperm counted as indicated above for the Cell-VU method. The reported depth of Makler® Chamber is 0.01 mm.

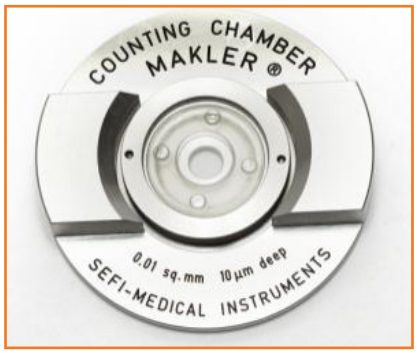


Fig7.Makler Chamber

- Laboratory Slide and Glass Cover Slip: The counting chamber using glass cover slip is the simplest method of calculating concentration of spermatozoa through wet preparations with chamber depth of approximately 20 µm deep. The volume of 10 µl of semen sample on a clean glass slide with a 22 mm × 22 mm coverslip (area 484 mm2) provides a chamber of depth of 20.7 µm. The depth of a preparation (D, µm) is obtained by dividing the volume of the sample (V,µl = mm3) by the area over which it is spread (A, mm2): D = V/A.

- Haemocytometer: The improved Neubauer haemocytometer has two separate counting chambers, each with 3 mm x 3 mm pattern of gridlines etched on the glass surface. It uses a special thick coverslip (thickness 0.44 mm), which lies over the grids supported by glass pillars 0.1 mm above the chamber floor. Each counting area is divided into nine 1 mm × 1 mm grids. The use of haemocytometer chamber with improved Neubauer ruling is recommended.

Fig9. Haemocytometer (Neubauer improved)

19 What is Agglutination and Aggregation? Elaborate their clinical relevance?

Agglutination: Agglutination specifically refers to adherence of motile spermatozoa sticking to each other through their head-to-head, tail-to-tail or in a mixed way. The motility is often vigorous, with a frantic shaking motion, but sometimes the spermatozoa are so agglutinated that their motion is limited.

Four categories for evaluating degree of agglutination:

- Isolated- <10 sperm/aggltuniate, may free sperm
- Moderate- 10 to 50 sperm/aggltuinate, free sperm
- Large- >50 sperm/agglutinate, some sperms still free.
- Gross- all sperm agglutinated and agglutinates interconnected

Abnormal agglutination is suggestive of immunological cause of infertility, further testing of antisperm antibodies required, however, sperm antibodies may be present without agglutination as well. It can severely impact sperm motility and concentration.

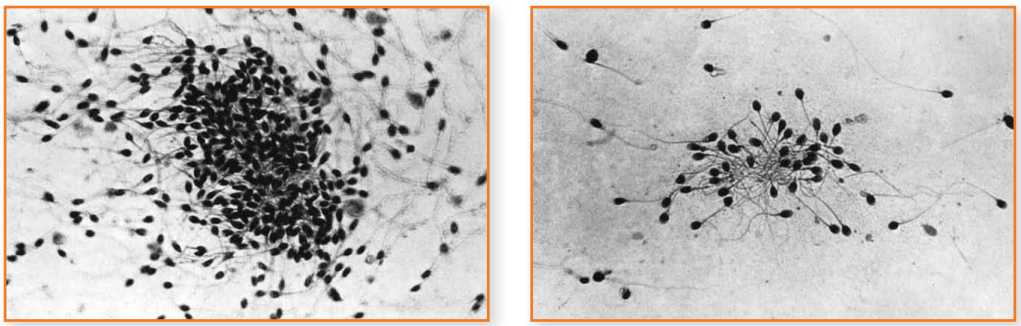


Fig10. Sperm agglutination

Aggregation: Aggregation is the adherence of immotile spermatozoa to each other or motile spermatozoa to mucus strands, gelatinous bodies, non-sperm cells (e.g. epithelial) or debris. Abnormal aggregation may interfere in semen analysis parameter.

One should always remember that motile spermatozoa stuck to the round cells or debris or immotile spermatozoa is aggregation and should not be scored as agglutination.

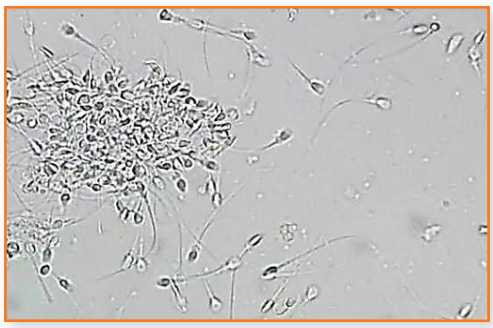


Fig11. Sperm aggregation

20 What is the importance of documentation in semen analysis?

Continuous monitoring and improvement through documentation techniques is an important part of Quality Assurance (QA) and should be elaborately described in laboratory Quality Manual. Documentation technique is an important exercise to detect and correct the problems and document to subsequent prevent the problems in future as part of corrective and preventive action (CAPA).

The QA programme should necessary include but not limited to following documents as a ready reference and assess to laboratory personnel's:Isolated- <10 sperm/aggltuniate, may free sperm

- Standard operating procedures (SOPs)
- Forms and Documents for temperature monitoring
- Instrument records of calibration and preventive maintenance of instruments
- Referral notes, Laboratory worksheet
- Report formats, Reference Ranges

Information leaflets to clients and referring clinicians.

21 What are the external and internal quality controls are as applied in semen analysis?

External quality control (maintains long term accuracy.):

- It's voluntary participation of laboratory performing semen analysis for getting samples as a part of EQC.
- Quality tests performed by an external body makes comparisons between different laboratories for several procedures.
- EQC is useful for detecting systematic variation and assessing accuracy of results.

Internal quality control (monitors the daily precision and accuracy of methodologies, personnel, and instruments):

- IQC materials for semen parameters (sperm concentration, morphology and motility) are included as a part of regular workload and monitor the semen analysis outcome.
- Internal quality control (IQC) monitors precision and indicates, through results outside the control limits, when the assay may be faulty, it also ensures that results are both accurate and precise.
- It's important to have successful internal quality control results of semen analysis before releasing patient results in clinical setting.

22 How is motility of sperms assessed and interpreted?

Motility is assessed by assessing approximately 200 spermatozoa. As per WHO Andrology Manual 6th edition, a four-category system for grading motility is recommended.

- **Rapidly Progressive:** Actively moving, either linearly or in a large circle with a speed of 25 µm/s or covering a distance of at least ½ tail length in a second.
- **Slowly Progressive:** Actively moving, either linearly or in a large circle with a speed of 5 to < 25 µm/s or covering distance of at least one head length to less than ½ tail length) in a second.
- **Non-Progressive:** All other patterns of active tail movements with an absence of progression – i.e. swimming in small circles, the flagellar force displacing the head less than 5 µm (one head length)
- **Immotile:** No active tail movements

Determining sperm motility:

- Calculate the proportions (%) of the four categories of motility.
- Report the average percentage for each motility grade to the nearest whole number.
- The sum of the four grades should be 100.

23 What is the importance of sperm DNA fragmentation test and what are the various techniques?

Sperm DNA integrity in important for normal function such as fertilization, implantation, pregnancy and fetal development. Sperm DNA damage can be defined as any chemical change in the normal structure of the DNA. Among these changes, sperm DNA fragmentation (sDF) is one of the most common disturbances affecting the genetic material in the form of single or double strand breaks. sDF may be triggered by different processes.

sDF may be triggered by different processes like:

- Defective packaging of the DNA during spermatogenesis.
- Processes of cell death and oxidative stress which may be associated with several pathological and environmental conditions.
- Lifestyle, aging, occupational and environmental factors are also found to be affecting sperm DNA integrity.
- Diseases such as varicoceles (disrupts spermatogenesis and results in high ROS production).
- Cancers such as as leukemia, Hodgkin's disease, and testicular cancers, either on their own or after receiving cytotoxic medication and/or radiation therapy.

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. sDF does not affect fertilization capacity but has been found to be affecting embryo development, implantation failure and clinical pregnancy rates.

The various methods have been developed to test sDF by making their way into the sperm chromatin to assess the presence of DNA fragmentation.

TUNNEL Assay (Terminal deoxynucleotidyl transferase dUTP nick-end labeling) , Sperm Chromatin Dispersion Test, Comet Assay, Acridine Orange Flow Cytometry

TUNNEL assay: In this assay, a template-independent DNA polymerase, the terminal deoxynucleotidyl transferase (TdT), adds the FITC-dUTP label to the nicks at the 3' hydroxyl end of the DNA. Thus, if there are more nicks in the DNA, more FITC-dUTP will be bound resulting in more cells fluorescing with a stronger fluorescent signal, which is then observed in a fluorescent microscope or measured by flow cytometry The terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase, adds the FITC-dUTP label to the nicks at the 3′ hydroxyl end of the DNA in the TUNEL experiment.
Therefore, more nicks in the DNA will result in more FITC-dUTP binding, which will cause more cells to glow with a greater signal that can be seen under a fluorescence microscope or quantified using flow cytometry.

Sperm chromatin dispersion test: Among all the test, this is the most commonly used test. It works on the principle that intact DNA loops expand following denaturation and extraction of nuclear proteins, whereas when DNA is fragmented, dispersion does not develop or is minimal. The formed halos can be classified as large, medium, small and no. Sperm with large and medium halos are considered to be non fragmented whereas the ones with small and no halo along with degraded sperms are considered as fragmented.

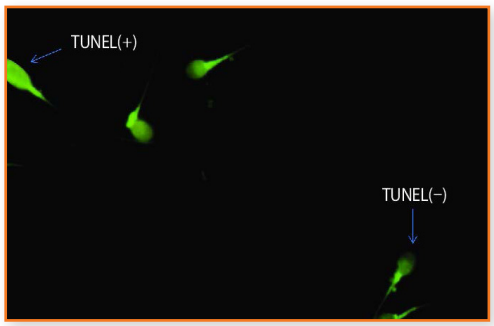


Fig12. TUNNEL Assay

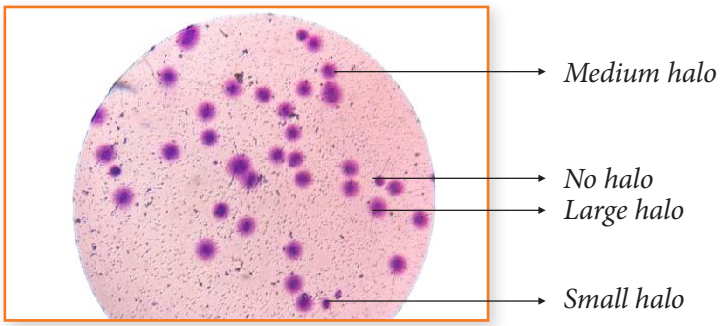


Fig13. Sperm halo formation (sDFI)

24 How do you asses semen sample with Retrograde Ejaculation?

In retrogragrade ejaculation semen passes into the bladder at ejaculation and results in aspermia with no apparent ejaculate. This can be confirmed by examining post-ejaculatory urine for the presence of spermatozoa. If pharmacological treatment is not possible or not successful, spermatozoa may be retrieved from the urine.

Without alkalinization treatment prior to sperm collection At the laboratory the man is asked to:

- Urinate without completely emptying the bladder;
- Produce an ejaculate by masturbation into a specimen container; and
- Urinate again into a second specimen vessel containing culture medium (to alkalinize the urine further).

Alkalinization treatment given prior to sperm collection

Alkalinization of the urine can be achieved by drinking water with sodium chloride and sodium bicarbonate 1–2 hours before attempt to collect an ejaculate as it will increase the chance that any spermatozoa passing into the urine retain its motility and vitality. Can be combined with alpha-1-receptor stimulator treatment.

At the laboratory the man should be asked to:

- Produce an ejaculate by masturbation into a specimen container; and
- Urinate after orgasm in a second container (volume at least 500 ml)
- Analysis of antegrade ejaculates and post-orgasmic urine

Both the ejaculate, if any, and urine samples should be analysed. Due to large volume of urine, it is often necessary to concentrate the specimen by centrifugation. (500g for 8 minutes) The retrograde specimen, once concentrated, and the antegrade specimen, if produced, can be most effectively processed using the density-gradient preparation method.

25 What is the correlation between RCF (relative centrifugal force)and RPM (revolution per minute)?

The force to which spermatozoa are subjected during centrifugation (relative centrifugal force, RCF) depends on the speed of rotation (N, revolutions per minute, r.p.m.) and the distance from the center of the rotor to the point at which the force is to be measured (usually the bottom of the centrifuge tube) (radius, R, cm).[Formula RCF (g): $1.118 \times 10\text{-}5 \times R \times N2$ (rpm) OR alternatively G-force = $0.000001118 \times R \times RPM^2$]

26 What is Azoospermia?

Azoospermia is defined as complete absence of spermatozoa in the ejaculate. The initial diagnosis of azoospermia is made when no spermatozoa cab be detected on high powered microscope examination of centrifuged seminal fluid on at least two occasion. The minimum initial evaluation of an azoospermic patient should be including a full medical history, physical examination, measurement of serum testosterone and FSH hormone levels.

Incidence of azoospermia in infertile men is approximately 10%, and it is usually non-obstructive. This is further differentiated as:

- Obstructive/Excretory Azoospermia (OA): Absence of spermatozoa and spermatogenetic cells in semen and post-ejaculate urine due to obstruction of the efferent ducts epididymis, vas deferens or ejaculatory duct) with reduced or normal semen volume and normal endocrinal parameters. In such cases obstruction may be treated or sperms can be obtained from epididymis or testes by surgical methods.
- Non Obstructive/Secretory Azoospermia (NOA): Absence of sperms in the ejaculate with normal semen volume with or without altered FSH, LH, Testosterone values. Therefore indicates absence of spermatogenesis.

Examine the centrifuged samples to detect spermatozoa:

When no spermatozoa are observed in wet preparation, the sample can be centrifuged to determine if any spermatozoa are present in the pellet. In presence of spermatozoa in either replicate report cryptozoospermia else report the sample as azoospermia (Centrifugation speed-3000g for 15 minutes).

27 What are the personal precautions to be taken while doing semen analysis?

Laboratory personnel must take following precautions while doing semen analysis

- No one should eat, drink, smoke, apply cosmetics or store food in the andrology laboratory.
- Wear disposable gloves while handling fresh or frozen semen or seminal plasma.
- Personnel should wash their hands regularly, especially before leaving the laboratory, after handling specimens and removing gowns and gloves.
- Measures should be taken to prevent, and where necessary contain, spillages of semen, blood or urine samples.
- Surgical masks should be worn by all staff performing procedures that could potentially create aerosols or droplets, e.g. vortexing and centrifuging of open containers.
- The last drops of semen specimens should not be forcibly expelled from pipettes, because this can cause droplets or aerosols to form.
- Must wear protective safety goggles, insulated gloves,

28 How to distinguish dead sperms from immotile sperms?

It's important to do hypo-osmotic swelling test (HOST) when there are all dead sperms in semen sample. The basic principle of HOS test is the ability of live spermatozoa to withstand moderate hypo-osmotic stress. Sperm membrane plays an important functional role during fertilization process as can be evaluated by the HOS test (HOST). Dead spermatozoa whose membranes are no longer intact do not swell in hypotonic media.

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. One can also use commercially available HEPES buffered reagent 'SpermMobil' to provide motility and differentiate dead sperms from immotile live sperms. All these sperms that demonstrate swelling on HOS and that gain motility are selected can be taken for Intra cytoplasmic Sperm Injection (ICSI).

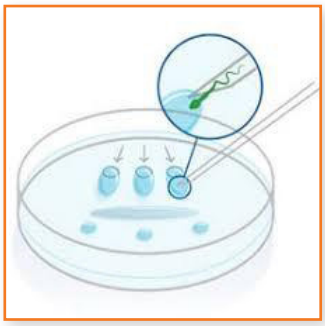
29 What is Physiological ICSI(PICSI) and Magnetic activating cell sorting technique (MACS)?

PICSI - physiologically selected intracytoplasmic sperm injection technique is based on the fact that the mature sperm head has a specific receptor that allows it to bind to hyaluronic acid (HA), the main component of the cumulus oophorous; this is in contrast to the immature spermatozoa. This technique is used to select sperm for ICSI, it involves placing the sperm in a dish with hyaluronic acid (HA,a natural compound found in the body. Selection is based on identifying sperm that can bind to hyaluronic acid. The bound sperm are considered mature and physiologically normal and are selected for fertilization. Spermatozoa that bind to HA have completed the spermatogenic process of remodeling the plasmatic membrane, cytoplasmic extrusion and nuclear maturity. Thus, they have a whole DNA and low frequency of aneuploidies and miscarriages.

PICSI is indicated for the patients with:

- Previous failure or low fertilization rate even after ICSI.
- Poor embryo quality or their failure to develop (not related to poor egg quality).
- Repeated miscarriages.
- High fragmentation of sperm chromatin.

The Magnetic activating cell sorting (MACS) technique, which is used to identify and positively eliminate apoptotic cell from ejaculate on the basis of the identification of externalized PS residues on apoptotic cell by annex in V-conjugated super paramagnetic microbeads. This technique reduces the proportion of sperm with fragmented DNA in the ejaculate before using it for ART procedures.
While studies have demonstrated that MACS/ PICSI reduce DNA fragmentation rates in the selected semen sample, their benefit on clinical or live birth needs to be established.



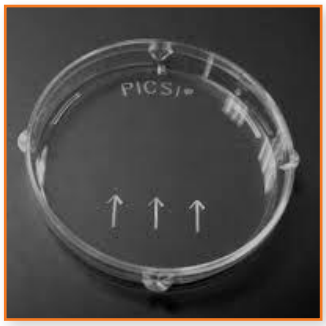


Fig15. PICSI dish

Fig14. MACS setup

30 What are the proposed decision limits as per WHO Andrology Manual 6th edition?

Decision limits 6th ed (5th percentiles and their 95% CIs) for semen characteristics

Parameters	Lower reference limit (range)	Nomenclature (for results < reference limit)
Semen volume (mL)	>1.4 (1.3-1.5)	Hypospermia
Total sperm number (106/ejaculate)	≥39 (35-40)	
Sperm concentration (106/mL)	≥16 (15-18)	Oligozoospermia
Total motility (PR + NP)%	≥42% (40-43)	Asthenospermia
Progressive motility (PR, %)	≥30 (29-31)	
Vitality (live spermatozoa, %)	≥54 (50-56)	
Sperm morphology (normal forms, %)	≥4 (3.9-4.0)	Teratozoospermia
Round cell concentration	1-2x106 / ml	Leukocytospermia/Pyospermia

CIs=confidence intervals; NP=non-progressive; PR=progressive

31 What is the purpose of sperm preparation?

Spermatozoa need to be separated from seminal plasma for a variety of purposes, such as diagnostic and research tests for functional competency, evaluation of effects of media composition, and sperm recovery for assisted reproductive technologies (ART).

An ideal sperm preparation technique should recover a highly functional sperm population that preserves DNA and does not induce dysfunction through the production of ROS by sperm and leukocytes.

The spermatozoa of all placental (eutherian) mammals, including humans, are in a protective, nonliable state at ejaculation and are incapable or poorly capable of fertilization even if they are placed in direct contact with an oocyte. They must undergo a subsequent period of final maturation (Capacitation) during which they acquire the capacity to interact with the oocyte–cumulus complex and achieve fertilization. Seminal plasma contains one or more factors (e.g. prostaglandins, zinc, leukocytes) that prevent spontaneous capacitation of spermatozoa upon ejaculation.

Prolonged exposure to seminal plasma has adverse effects on sperm function:

- Like the ability to penetrate cervical mucus
- Undergo the acrosome reaction in vitro, and the fertilization process in general

Consequently, in order for spermatozoa to have the capacity to fertilize an oocyte, they must be separated from the seminal plasma.

Sperm preparation techniques help to yield a final preparation that has high percentage of sperms that are motile, morphologically normal and free from debris, non-germ cell or dead sperms.

32 Mention the various semen preparation techniques.

Semen preparation techniques are used for assisted reproduction- IUI, IVF, ICSI. Some of the available sperm preparation techniques are described in the following s. For all of them, the culture medium suggested is a balanced salt solution supplemented with protein and containing a buffer appropriate for the environmental conditions in which the spermatozoa will be processed. If the incubator contains only atmospheric air and the temperature is 37 °C, the medium should be buffered. The final disposition of the processed spermatozoa will determine which buffered medium is appropriate.

Simple wash: Provides a high yield of spermatozoa if semen samples are of good quality, but it does not eliminate debris or leukocytes that are present in semen. Culture media is added to the semen sample and mixed well followed by centrifugation. Obtained pellet is resuspended in media.

Swim up: Motile sperms are allowed to swim up from seminal plasma through seminal plasma into culture media. The semen should preferably not be diluted and centrifuged prior to swim-up, because this can result in peroxidative damage to the sperm membranes. Therefore, direct swim up is recommended.

Density Gradient: This method uses centrifugation of semen over density gradients consisting of colloidal silica coated with saline, which separates cells by their density alone. A simple two-step discontinuous density-gradient preparation method is most widely applied. Results in a fraction of highly motile spermatozoa, free from debris, contaminating leukocytes, non-germ cells and degenerating germ cells.

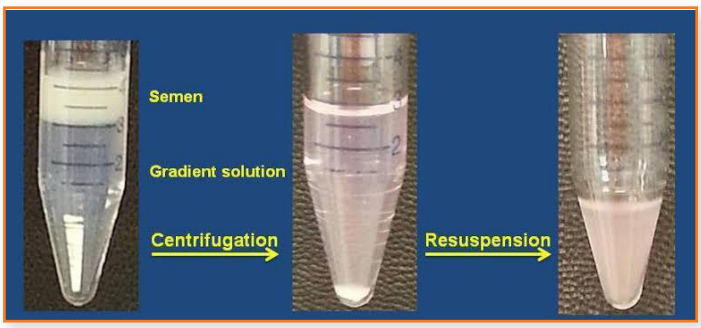


Fig15. Density Gradient

MACS: This technique separates apoptotic sperm cells from normal sperms. In MACS, damaged apoptotic sperm is labelled with magnetic nanoparticles, and then they are put through a column, where the apoptotic sperm is captured. Intact live sperm flow through the column and are collected for ART
procedure.

Fig16. MACS

Microfluidics: Microfluidics Sperm Sorting Technology help filter sperms that are more likely to carry DNA damage from normal sperms. This technique works on the principle that sperms with lower motility have higher DNA damage and motile sperms are less like to carry DNA damage.

Microfluidic sperm sorter separates morphologically normal sperms with high motility. Sperm selection in this method is done by two gravity driven laminar flows within a central microfluidic channels.

Fig17. Microfluidics devices

33 What is the purpose of sperm preparation?

Semen cryopreservation can be done for one's own future use (autologous ART) or donor banking (homologous ART).

Indications for semen cryopreservation:

- Fertility preservation in patients undergoing treatment with cytotoxic agents or radiotherapy.
- Fertility preservation in cases where the male partner undergoes vasectomy (in case if desires to procreate further).
- Fertility preservation in cases of active duty in dangerous occupations like military.
- In cases of sever OAT sample as backup frozen sample.
- In cases of special collections like spinal cord injury or retrograde ejaculation.
- For men with HIV controlled by antiretroviral therapy.
- Unavailability of male partner on the day of ART procedure.
- Donor semen samples.

Semen cryopreservation is done by the method of slow freezing in which cryoprotectant is added and mixed well with semen and then the temperature of the above mentioned mixture of semen and cryoprotectant is slowly brought down to a temperature of – 196 ° C. Semen cryopreservation can be done in raw as well as prepared semen samples.

References

1. A 1-22, 24-25, 27-28 & 30-33. "WHO manual for andrology 6th edition.pdf."
2. A 23. "WHO manual for andrology 6th edition.pdf.", Dutta, S., Henkel, R., & Agarwal, A. (2020). Comparative analysis of tests used to assess sperm chromatin integrity and DNA fragmentation. Andrologia, 53(2). https://doi.org/10.1111/and.13718
3. A 26. Physiological ICSI (PICSI) vs. Conventional ICSI in Couples with Male Factor: A Systematic Review, Georgina et al. doi: 10.5935/1518-0557.20180027; Pacheco, A. Blanco, F. Bronet, M. Cruz, J. García-Fernández, and J. A. García-Velasco, "Magnetic-Activated Cell Sorting (MACS): A Useful Sperm-Selection Technique in Cases of High Levels of Sperm DNA Fragmentation," J. Clin. Med., vol. 9, no. 12, p. 3976, Dec. 2020, doi: 10.3390/jcm9123976.
4. A 29. "The Evaluation of the Azoospermic Male: AUA Best Practice Statement."

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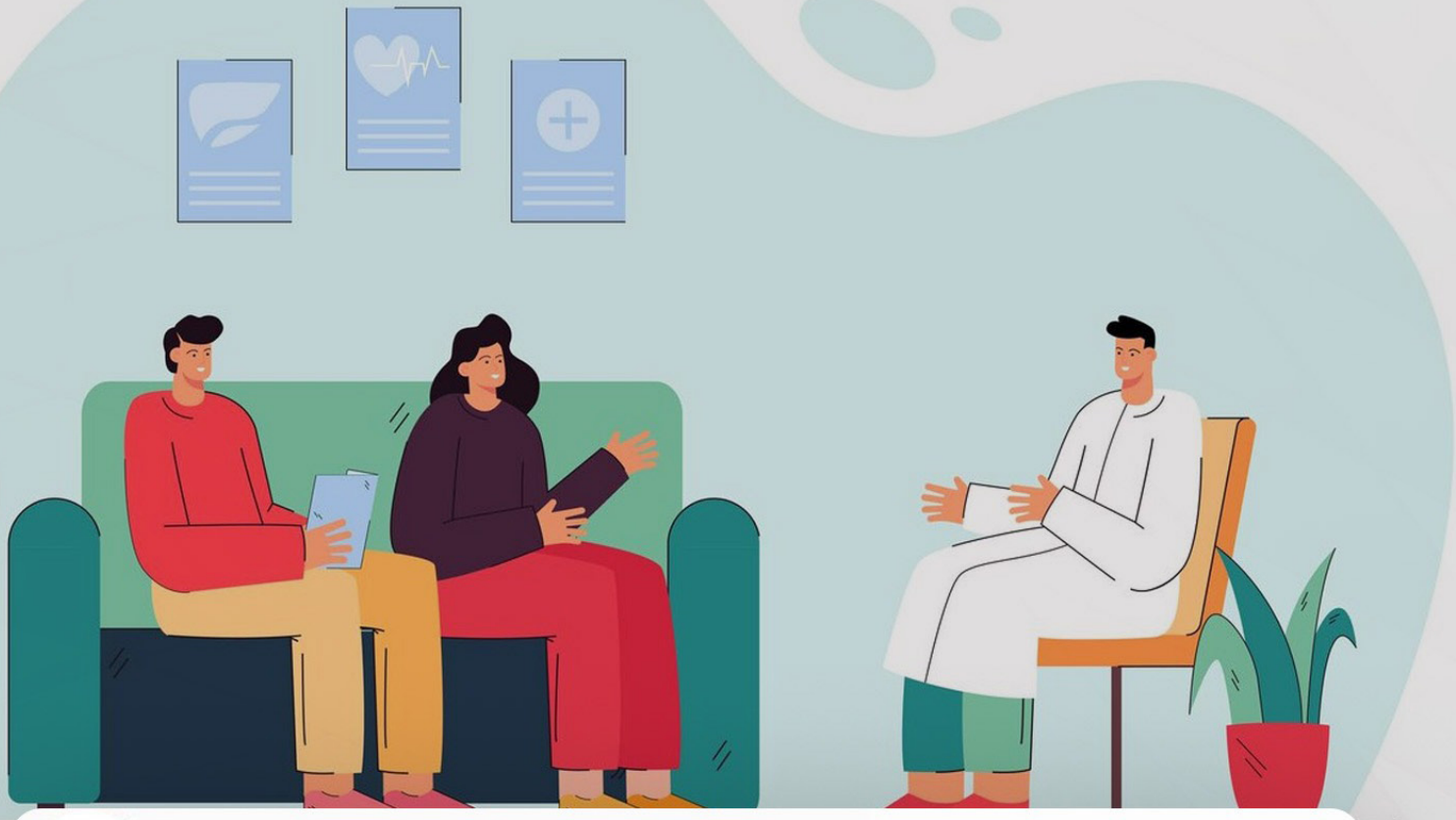

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