

## Semen Analysis as A Tool to Assess Infertility Among Males

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### Abstract

Sperm analysis, also called sperm count, measures the amount and quality of sperm and semen. Sperm is a thick, white fluid that comes out of the vagina during orgasm. This release is called ejaculation. Sperm contains the sperm, the male cells carrying the genes. When a sperm unites with a woman's egg, it forms an embryo. Low sperm count or abnormal sperm formation or movement can make it difficult for a man to conceive a woman. Infertility is called infertility. Infertility can affect both men and women. About one-third of couples who are unable to have children, male fertility, an important factor needs to be considered. Sperm analysis can help determine the cause of male infertility.<sup>3</sup> The present study discussed about the sperm analysis and its use as per the standard guidelines to enable the researcher to carry out the research and get the quality outcome in terms of assessment of infertility among males.

**Keywords:** Azospermia, Sperm Analysis, Semen, Infertility.

### Introduction

Sperm, also known as semen, is a living fluid designed to produce sperm. It is released through the gonads (sex glands) and other genitals of the male or hermaphroditic organs and can fertilize the female ovum. In humans, sperm fluid contains several nutrients in addition to spermatozoa. These include proteolytic and other enzymes and fructose are the components of sperm fluid that promote spermatozoa survival, and provide a way to move

or "swim". Sperm is also produced from the seminal vesicle, which is located in the pelvis. The process that leads to sperm is called ejaculation. Sperm is a type of gene. In animals, sperm are collected for cryoconservation. Cryoconservation of animal genetic resources is a practice that requires the collection of genes in conservation efforts of a particular species.

Sperm testing is essential for a variety of reasons:

Testing for male reproductive function and the ability of the genital tract to provide appropriate reproductive treatment and to monitor the response to treatment;

Evaluation of fertility and choosing the appropriate treatment for the infertile couple. Measuring the effectiveness of male contraception (e.g. vas occlusion and interventions that include male hormonal contraception and other possible

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alternatives). Semen analysis may direct the clinician to determine how to proceed with the investigation and management of the infertile couple.<sup>2</sup>

### **Basic tests-Methods**

**Sperm count testing:** Sperm cleansing has been simplified, but 200 spermatozoa should be counted. The laboratory must not stop testing the amount of sperm at low concentrations (2 million / ml). The total number of sperm per ejaculate (ejaculation output) has a diagnostic value beyond the concentration of sperm, but in this case, the sperm volume should be accurately measured. A test phase of azoospermia is maintained. Procedures for centrifugation and staining of live sperm are also included for detecting sperm from unprepared samples for post-vasectomy sperm testing or when semen is significantly suppressing contraceptives.

**Sperm flow test.** Separation of sperm motility reverts to a continuous rapid, slow-moving, continuous motion and motion (grade a, b, c or d) because the presence (or absence) of rapidly developing sperm is clinically significant

### **Sperm test**

The procedure for testing for sperm formation using a systematic method is described and should be followed. In this issue, several micrographs of the best quality spermatozoa from unprocessed sperm samples considered normal, borderline, or abnormal are included, accompanied by explanations as to why each sperm is classified in the way it has been classified. It should help train specialists to explain the subtle features of spermatozoal morphology further.

### **Extended tests-Methods**

Unlike many other bodily secretions that have been tested for diagnostic or therapeutic purposes, ejaculate is a combination of secretions that are not present within the body before being expelled. In males, the ejaculate is produced from a concentrated suspension of spermatozoa. It is then stored in the paired epididymides, mixed with, and diluted by, primarily the prostatic fluid in the urethra, followed by the removal of seminal vesicles. Therefore, consecutive ejaculate components are not equally integrated.

The ejaculate has two large measurable properties.

The number of spermatozoa indicates sperm production by the testicles, the strength of the post-

testicular duct system, the efficiency of smooth muscle contraction in the epididymides and the vasa deferentia actively moving semen into the urethra, as well as the ability to ejaculate and ejaculate. Ejaculate filled with semen. The latter factors are influenced by sexual arousal - duration and quality - and are performed by emotional signals to smooth muscle cells (vasa deferentia, glands, urinary bladder sphincter) and smooth muscle cells that control blood flow and erectile dysfunction. The amount of fluid supplied by the various available glands reflects the secretory function of the glands and the smooth contraction of the muscles that secrete each gland. These activities are responsive to the emotional stimuli caused by arousal and as a preparation for arousal.<sup>1</sup>

The nature of spermatozoa (its strength, motility and morphology) and the formation of ejaculate fluid are also essential for sperm function. As a result of sexual intercourse, the first, affluent part of the prostatic sperm of the ejaculate may be connected to the cervical mucus that passes into the vagina without significant contact with the entire ejaculate. Conversely, in a laboratory setting, all ejaculate is collected in a single container, where sperm are trapped in a gel developed in proteins of seminal vesicular origin. In vitro, this gel is later converted to the action of prostatic protease, during which its osmolality increases. The total volume and content of spermatozoa for ejaculates varies depending on the conditions in which the ejaculate is produced. Ejaculates produced by masturbation and collected in containers in a room near the laboratory can result in lower yields than those found in non-sperm condoms used during sex at home. This difference may indicate a different level and duration of sexual arousal, as the time spent producing the sample by masturbation also affects ejaculate volume and content.<sup>3</sup>

Under the given conditions of collection, ejaculate factors depend on usually irreversible factors, such as sperm production, accessory discharge and recent infection (especially febrile), and other factors, such as urinary incontinence, should be recorded considered in interpreting results. The results of laboratory measurements of ejaculate symptoms will depend on the following. • That complete ejaculate is collected. During ejaculation, the first parts are excreted mainly by the prostatic fluid rich in semen, while the later parts are dominated by seminal vesicular fluid. Therefore, the loss of the first part (rich in sperm) of ejaculate has a more significant impact on the analysis results than the loss of the last part. Therefore, sperm concentration

is not a direct measure of testicular sperm release, as it is influenced by secretory function in other organs. The total amount of ejaculated spermatozoa (sperm concentration doubled by sperm volume) is, therefore, the best expression of sperm production capacity.

The time between ejaculated ejaculation and the most recent ejaculation (ejaculatory menopause "period of abstinence", sometimes called sexual abstinence). Spermatozoa collect in epididymides until they are complete, then excrete in the urethra and excrete in the urine; since epididymides are never wholly removed by a single discharge, some spermatozoa remain from the time of the previous discharge. This affects the age range and quality of spermatozoa in the ejaculate. Sperm strength and chromatin are not affected by the increased duration of sleep deprivation unless an epididymal function is impaired. In addition, extensive research to determine daily spermatozoa production has shown those 2-3 days of daily release is required to complete epididymal retention of spermatozoa. Therefore, a recommendation, based on clinical experience, asking men to collect ejaculate for testing after 2-7 days of abstinence can cause discrepancy and an unexplained boundary between expected and infertile outcomes. The extent of this influence is difficult to determine, and it is rarely considered.<sup>4</sup>

### ***Sperm Collection***

#### *Patient information*

The man should be given clear written and verbal instructions regarding collecting sperm samples. The doctor should provide the same information to the patient. The main recommendation is to collect ejaculate by masturbation. Coitus interrupts is not recommended and should only be used in exceptional cases due to the risk of incomplete collection and contamination of vaginal fluid and cells. Contraceptive condoms cannot be used due to sperm killing agents. Ordinary latex condoms should not be used for sperm collection because they contain substances that interfere with the movement of spermatozoa. Lubricants should be avoided, as they may contaminate the ejaculate and alter its properties. If necessary, certified non-spermatozoic certified lubricants should be used. The ejaculate needs to be fully collected, and the male should report any loss of any part of the sample. Ejaculate should be collected after at least two days and more than seven days of abstinence. Avoid sperm exposure to temperature fluctuations and control the time between collection and analysis, it is recommended that the sample be

collected in a private room near the laboratory. Ideally, the investigation should begin within 30 minutes after collection but at least within 60 minutes. Individual variations may be required, and each individual should be given appropriate advice about the possible risks and risks.<sup>5</sup>

Prior to ejaculating collection, the specimen container should be stored at a temperature of between 20 °C and 37 °C to avoid significant temperature changes that may affect sperm.

The sample container should be a clean, wide-brimmed plastic container from a collection that has been proven to be non-toxic to spermatozoa.<sup>12</sup>

The sample container, as well as the accompanying worksheets, should be labeled with identification consistent with the sampling acceptance procedures and continuous management, eliminating the risk of merging the samples with working papers. Legal requirements for container tag ownership may vary. It could be a man's name and ID number, date and time of collection, or unique identification numbers. The following information must be recorded on the acceptance sample and presented in the final report: the identity of the man (e.g. name, date of birth and personal code number) and his confirmation that the sample is his; pre-exit period; date and time of collection.<sup>13</sup>

The completeness of the sample and any difficulties in producing the sample (e.g. if the collection was not done in the laboratory); and turn off the volume.

#### ***Volume by weight***

Volume is best measured by measuring the sample in the container in which it is collected. It can best be done when a pre-heated container is received to incubate to drain the liquid.

Use a pre-measured container to collect the ejaculate, weight recorded on the container and lid. Empty sample containers usually have a different weight, so each container with a lid should be weighed in advance. The weight should be recorded on the container and its lid with a permanent marker pen before giving it to the patient. When labels are used - for example, tags - their weight should be included in the blank weight.<sup>6</sup>

Measure the vessel with ejaculate into it. 3. Remove the weight of the empty container. 4. Calculate the volume from the weighted sample, assuming the sperm concentration is 1 g / ml. (Sperm density reported to vary between 1.03 and 1.04 g / ml, 1.00 and 1.01 g / ml, and a dose of 1.01 g / ml).<sup>1,13</sup>

### *Macroscopic examination*

Macroscopic tests include several vital observations that may not be possible to diagnose with the exact number of numbers - and thus control the most common methods - but are still very important clinically. The average liquid ejaculate is a macroscopically pale, cream/grey colour. It may appear slightly opaque if sperm concentration is too low; colour may also vary - i.e. slightly yellow after a long period of abstinence, reddish-brown when red blood cells are present (haemospermia), or clear yellow in a patient with jaundice or taking specific vitamins or drugs. If the ejaculate appears vicious, completely clear and colourless, then the pre-ejaculate may come out only from Cowper glands, which are produced by males in various quantities during arousal; in this case, this should be discussed with the patient to determine if orgasm associated ejaculation occurs.<sup>6</sup>

### *Melting*

Immediately after ejaculation from the collection vessel, the ejaculate is usually a semi-solid coagulated mass or gel like clump. Usually, the ejaculate begins to melt (become thin) within a few minutes at a temperature equal to room, at which point a mixture of loose lumps will appear in the liquid. As liquefaction progresses, the ejaculate becomes homogeneous and watery but has a higher viscosity than water. In the final stages of melting, there are still small areas for thickening. A temperature of 37 °C will make it easier for the liquid. Also, a slow, rotating movement of the sample container will help the liquefaction dissolution complete. If a moving tray (orbital mixer) can be used during melting, the container must be rotated slightly for 15-30 seconds before starting a liquid macroscopic test.

Complete ejaculate liquefaction is usually achieved within 15-30 minutes at room temperature.

- If immersion is not completed within 30 minutes, this should be recorded and recorded in the final report. The ejaculate may be left at 37 °C for another 30 minutes.
- If immersion is not completed within 60 minutes, this should be included in the final report.
- Normal ejaculate can contain jelly-like granules (gelatinous bodies) that do not dissolve and do not appear to be clinically significant. The presence of mucous membranes, although it may interfere with ejaculate examination, should therefore be noted in the final report.

### *Ejaculate viscosity*

After liquefaction, the viscosity of the ejaculate

can be measured by gently squeezing it into a vast hole (approximately 1.5 mm wide) disposable plastic pipette (certified as non-toxic to the sperm and, if necessary, sterile), allowing sperm to drop. by gravity and to check the length of any string. Average fluid ejaculate falls like separate tiny droplets. If the viscosity is abnormal, the drop will form a thread more than 2 cm long.

### *Smell out*

There is a significant difference in the ability of different people to detect the typical odour of human ejaculate. Knowledge of the strong odour of urine or decay may be necessary for the clinic; it is, therefore, essential to note this in the report.<sup>7</sup>

### *Ejaculate pH*

The pH at the ejaculation rate depends on the relative contribution of acidic prostatic secretion and alkaline seminal vesicular secretion. In ejaculate, there is no effective control of the pH of the fluid. In vitro, there will be a continuous loss of CO<sub>2</sub>, resulting in a gradual increase in pH. The clinical interest in ejaculate pH is low. If pH is to be tested, it should be done simultaneously, preferably 30 minutes after collection, but in any case, within 1 hour of discharge. For standard samples, pH test strips of 6.0-10.0 should be used. 1. Mix the sperm sample well. 2. Distribute the sperm evenly across the pH line. 3. Wait for the colour of the affected area to match (<30 seconds). 4. Compare colour with measuring line to read pH.<sup>8</sup>

#### Preparation for a microscopic investigation

For reliable results of very little research, the tested aliquots must represent the entire ejaculate. The nature of the liquid ejaculate, which is more visible than water, makes taking a representative sample of sperm for analysis more difficult. If the sample is not well mixed, analyzing the two different aliquots is less likely to represent the entire ejaculate. It may show significant differences in sperm concentration, mobility, strength and morphology. Even if the liquid ejaculate is macroscopically homogeneous, small aliquots can have very different textures. Ejaculate mixing before removing the sperm aliquot from any test; mix the sample well in the first container, but not so hard that air bubbles form.<sup>9</sup>

Mixing can be achieved by placing the sample container on a moving tray during the liquid in an incubator at 37 °C. In the absence of an orbital mixer, basic mixing can be achieved in about 15-30 seconds of manual rotation.

### Damaged sample

Although liquefied macroscopically may look perfectly alike, there may be small but essential compounds with a different sperm structure and discharge. It is therefore essential: • To use at least 50  $\mu\text{l}$  repeated aliquots to purify the sperm concentration • To use at least 10  $\mu\text{l}$  repetitive aliquots to check sperm flow. Comparisons of those duplicate aliquots are required to reduce the risk of errors due to non-representative samples. Comparison methods are described under each testing strategy.<sup>10</sup>

To make a wet fix directly after the ejaculate is well mixed; remove the appropriate dose, not allowing time for the sperm to come out of the erection. Always re-mix the sperm sample before extracting duplicate aliquots. The movement test should be executed in two different ways, the newly prepared liquid (see the wet preparation instructions on page 65 for the background). 1. Place a 10  $\mu\text{l}$  aliquot mixed well on a clean microscope slide that is best heated to 37 ° C (e.g. in a sample incubator).

2. Place the 22 mm  $\times$  22 mm coverslip by carefully tossing it horizontally over the drop. The weight of the coverslip disperses the sample (so use the weight slip # 1½). 3. Check for recent wet repairs as soon as the content is no longer flooded. • If the flow does not stop within 1 minute after installing the coverslip, a new wet fix should be made.

Testing under a microscope A clear-contrast optics microscope is required for all tests of fresh sperm setting (Section 8.3 on page 221 on how to set up a microscope). 2.4.4.1 Low magnification. The first small test of ejaculate aliquot involves scanning correction for a total magnification of  $\times 100$  (i.e. a combination of a target lens of  $\times 10$  with  $\times 10$  ocular). It provides an overview of the sample, indicating whether the sperm is evenly distributed throughout the preparation, any visible mucous membranes, and sperm fusion or fusion. In the case of uneven distribution, the cause may be: insufficient mixing, high viscosity, insufficient fluid, sperm accumulation.<sup>11</sup>

High magnification Adjustments should then be tested at  $\times 200$  or  $\times 400$  total magnifications (i.e. a combination of  $\times 20$  or  $\times 40$  purposes with  $\times 10$  oculars).

It allows: Sperm flow testing; determining the purification required for accurate sperm count testing. Determining the presence of circular cells that require further testing; • determining the presence of cells outside of spermatozoa (e.g. epithelial cells) or "circular cells" (leukocytes and

immature virus cells).<sup>12</sup>

### Conclusion

The tests quality depends on the standard process which needs to be followed with minimizing errors. In case of sperm analysis the time management is crucial while performing a test for assessment of male infertility. The liquefaction after collection of the semen is important factor in addition to preservation of the sample. The process if followed meticulously then the battle is half win for the technologists performing the test.

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