

## Lecture

### **Semen analysis**

**Semen analysis**: evaluates certain characteristics of a male's semen and the sperm contained therein. It is done to help evaluate male fertility, whether for those seeking pregnancy or the success of vasectomy. It is also commonly used for testing human donors for sperm donation.

A man will have a semen analysis done as part of routine pre-pregnancy testing. The semen and sperm test is not done unless specifically requested or there is a strong suspicion of pathology in one of these areas discovered during the medical history or during the physical examination.

### **Semen analysis Parameters**

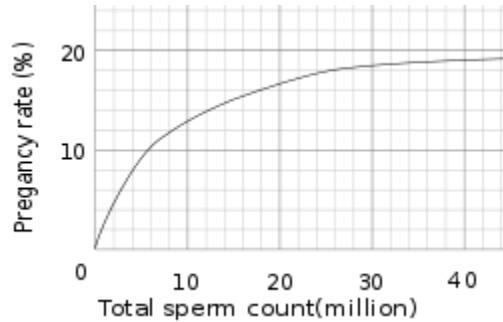
The Parameters measured in a semen analysis are:

**Sperm count, motility, morphology, volume, fructose level and pH.**

#### **❖ Sperm count**

Sperm count, or sperm concentration: measures the concentration of sperm in a man's ejaculate. **Over 15 million sperm per milliliter is considered normal,** according to the WHO in 2010. Older definitions state 20 million. A lower sperm count is considered Oligozoospermia. A vasectomy is considered successful if the sample is Azoospermia. Some define success with rare non-motile sperm are observed (fewer than 100,000 per milliliter). The average sperm count **today is between 20 and 40 million per milliliter in the Western world.**Fif(1)

Chips for home use are emerging that can give an accurate estimation of sperm count after three samples taken on different days. Such a chip may measure the concentration of sperm in a semen sample against a control liquid filled with polystyrene beads.



**Figure(1): The relation between Total sperm count and pregnancy rate**

### ❖ Motility

The WHO has a parameter of **vitality**, of 60% live spermatozoa and this must be measured within 60 minutes of collection. A man can have a total number of sperm far over the limit of 20 million sperm cells per milliliter, but still have bad quality because too few of them are motile. A man can have a sperm count far less than 20 million sperm cells per milliliter and still have good motility, if more than 60% of those observed sperm cells show good forward movement.

A more specified measure is **motility grade**, where the motility of sperm are divided into four different grades

**Grade a:** Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility **IV**.

**Grade b:** (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility **III**.

**Grade c:** These have non-progressive motility because they do not move forward despite the fact that they move their tails. Sometimes also denoted motility **II**.

**Grade d:** These are immotile and fail to move at all. Sometimes also denoted motility **I**.

### ❖ Morphology

Morphology is a predictor of success in fertilizing oocytes during fertilization. Regarding sperm morphology, the WHO criteria as described in 2010 state that a

sample is normal (samples from men whose partners had a pregnancy in the last 12 months) if 4% (or 5th centile) or more of the observed sperm have normal morphology. Fig(2)

Up to 10% of all spermatozoa have observable defects and as such are disadvantaged in terms of fertilizing an oocyte; also, sperm cells with tail-tip swelling patterns generally have lower frequency of aneuploidy



**Figure(2):**Normal and abnormal sperms morphology.

A **motile sperm organelle morphology examination (MSOME)** is a particular morphologic investigation wherein an inverted light microscope equipped with high-power optics and enhanced by digital imaging is used to achieve a magnification above x6000, which is much higher than the magnification used habitually by embryologists in spermatozoa selection for intracytoplasmic sperm injection (x200 to x400).

A potential finding on MSOME is the presence of sperm vacuoles, which are associated with sperm chromatin immaturity, particularly in the case of large vacuoles

### ❖ Volume

Normal semen volumes range between 1.0 mL and 6.5 ml. WHO regard 1.5 ml as the lower reference limit. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles.

In clinical practice, a volume of less than 2 mL in the setting of infertility and absent sperm should prompt an evaluation for obstructive azoospermia, be sure it has been at least 48 hours since the last ejaculation to time of sample collection.

### ❖ Fructose level

The normal level of fructose in the semen is 3 mg/mL. Absence of fructose may indicate a problem with the seminal vesicles

### ❖ pH

A semen normal pH range of 7.1- 8. WHO criteria specify normal as 7.2-7.8. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection . A pH value outside of the normal range is harmful to sperm.

### Liquefaction

The liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid. A liquefaction time within 60 minutes is regarded as within normal ranges

### MOT

MOT is a combination of sperm count and motility. It is a measure of how many million sperm cells per ml are highly motile, that is, approximately of grade a (>25 micrometer per 5 sek. at room temperature) and grade b (>25 micrometer per 25 sek. at room temperature).

### Total motile spermatozoa

Total motile spermatozoa (TMS) or total motile sperm count (TMSC) is a combination of sperm count, motility and volume, measuring how many million sperm cells in an entire ejaculate are motile.

Use of approximately 20 million sperm of motility **grade c or d** in intra-cervical insemination (ICI), and 5 million ones in intrauterine insemination (IUI) may be an approximate recommendation.

### **Others**

The sample may also be tested for white blood cells. A high level of white blood cells in semen is called leucospermia and may indicate an infection. Cutoffs may vary, but an example cutoff is over 1 million white blood cells per milliliter of semen

### **Semen abnormalities**

- Aspermia: absence of semen
- Azoospermia: absence of sperm
- Hypospermia: low semen volume
- Hyperspermia: high semen volume
- Oligozoospermia: Very low sperm count
- Asthenozoospermia: poor sperm motility
- Teratozoospermia: sperm carry more morphological defects than usual
- Necrozoospermia: all sperm in the ejaculate are dead
- Leucospermia: a high level of white blood cells in semen

### **Factors that influence results**

There are various methodological factors that may influence the results of semen analysis:

-Compared to samples obtained from masturbation, semen samples from collection condoms have higher total sperm counts, sperm motility, and percentage of sperm

with normal morphology .For this reason; they are believed to give more accurate results when used for semen analysis.

-If the results from a man's first sample are sub fertile, they must be verified with at least two more analyses. At least 2 to 4 weeks must be allowed between each analysis .Results for a single man may have a large amount of natural variation over time, meaning a single sample may not be representative of a man's average semen characteristics.

- In addition, the stress of producing an ejaculate sample for examination, often in an unfamiliar setting and without any lubrication (most lubricants are somewhat harmful to sperm), may explain why men's first samples often show poor results while later samples show normal results.

-A man may prefer to produce his sample at home rather than at the clinic. The site of semen collection does not affect the results of a semen analysis

### **Measurement methods**

-Volume can be determined by measuring the weight of the sample container, knowing the mass of the empty container.

-Sperm count and morphology can be calculated by microscopy. Sperm count can also be estimated by kits that measure the amount of a sperm-associated protein, and are suitable for home use

**-Computer Assisted Semen Analysis (CASA)** is automatic or semi-automatic semen analysis techniques. Most systems are based on image analysis, Computer-assisted techniques are most-often used for the assessment of sperm concentration and mobility characteristics, such as velocity and linear velocity. Nowadays, there are CASA systems, based on image analysis and using new techniques, with near perfect results, and doing full analysis in a few seconds. With some techniques, sperm concentration and motility measurements are at least as reliable as current manual methods

**-Raman spectroscopy** has made progress in its ability to perform characterization, identification and localization of sperm nuclear DNA damage.