INTRODUCTION

Semen analysis is a laboratory test that is primarily used for evaluating fertility potential and for assessing success following a vasectomy procedure. The composition of semen, also known as seminal fluid, consists of spermatozoa (sperm cells) and secretions.

Semen analysis is a unique laboratory test in which multiple parameters are evaluated to determine the physical and chemical properties of a seminal fluid sample. Semen analysis consists of macroscopic and microscopic examinations, which provide information on the physical, functional, and biochemical properties of seminal fluid.

Within the male genital tract, various anatomical structures contribute to the formation of seminal fluid. These include the testes (testicles), epididymes, vas deferens, seminal vesicles, and prostate gland. The maturation of spermatozoa, known as spermatogenesis, is a rather intricate developmental process. In spermatogenesis, sperm cells undergo a series of changes, which result in the formation of mature motile spermatozoa. In addition, biochemical substances are secreted. These substances provide a nutrient environment for spermatozoa and as a means for transporting sperm cells.

In performing semen analysis, various factors can impact the validity of the test results and can occur during the pre-analytic and analytic phases. To prevent erroneous results, it is imperative to have at a minimum:

1. Properly obtained semen sample,
2. Use of standardized test procedures, and
3. Staff proficient in the interpretation of multiple semen analysis test parameters.

The main objective of this educational activity is to provide the reader with an overview of semen analysis. Information is given on recognizing factors that can adversely affect semen analysis test results and their impact on subsequent medical management of the patient. Discussion is focused on the manual methods of semen analysis that are used in most laboratories. The readers are encouraged to review the listed references for further information. This includes the most recent edition of the World Health Organization (WHO) manual (4th edition), which is used as the main source of reference values of semen variables.¹

ANATOMY AND PHYSIOLOGY

The development of mature sperm cells is a complex process involving multiple anatomic sites within the male genital tract. The illustration on the male reproductive system and spermatogenesis (Figure 1. in the Appendix) should be used as a supplement to the descriptive overview given below.

Sperm maturation occurs within the scrotum, primarily within the testes and epididymides. Since the scrotum is located outside the body, it is at a lower temperature. Decreased body temperature is essential to sperm maturation.
The male hormone testosterone is produced in the testes. Within the testes are seminiferous tubules, which are coiled and convoluted. This network of tubules can be identified on microscopic examination of the testes. It is within the seminiferous tubules that sperm cell maturation occurs. In the process of sperm development, undifferentiated germ cells, or spermatogonia, undergo changes in the testes. This involves a unique form of cell division known as meiosis. Spermatogonia undergo divisions resulting in primary and secondary spermatocytes, respectively. The secondary spermatocytes undergo further meiosis and evolve into spermatids. The spermatids undergo further development into immature immotile sperm. Within the testes are sertoli cells that provide support and nourishment to the developing sperm cells.

Following maturation in the testes, immature spermatozoa are carried within fluid to the epididymides. Each epididymis is also located within the scrotum. Within the epididymis, the spermatozoa undergo the final stages of maturation and become motile. Mature sperm cells remain in the epididymis until ejaculation occurs.

When spermatozoa leave the epididymis, these sperm cells are carried through the vas deferens, which connects to an ejaculatory duct. Each ejaculatory duct is in proximity to the seminal vesicles and transverses into the prostate gland. The seminal vesicles and prostate glands are accessory glands in the male reproductive tract. They produce biochemical substances that provide a nutrient milieu for sperm cells. Seminal vesicles produce most of the fluid component in semen. Fructose is produced in the seminal vesicles. The prostate gland secretes protein, zinc, and enzymes, including acid phosphatase. The fluids from the prostate gland and seminal vesicles are added to the ejaculatory duct.

The results of semen analysis can indicate an anatomic abnormality of the male genital tract. The absence of spermatozoa (azoospermia) or a marked decrease in the number of sperm cells (oligospermia) on semen analysis can signify an obstruction to the vas deferens or ejaculatory duct. These findings can prompt a more comprehensive urological evaluation for the patient.

**SEMINAL FLUID SPECIMEN AND SEMEN ANALYSIS**

In performing a semen analysis, it is crucial to maintain specimen integrity throughout all phases of testing. A suboptimal specimen can adversely affect the semen analysis results.

**Pre-Analytical Test Phase**

Patients should be instructed on specimen collection. The instructions should include an emphasis on prompt delivery of the semen sample to the laboratory, especially if collected off site and not subjecting the semen sample to extreme temperature fluctuations during transit to the laboratory. If the specimen is obtained in proximity to the laboratory, an appropriate and comfortable environment should be made available to the patient.
The following items should be included in patients’ instructions for collection of semen samples:

- The semen sample should be collected after a period of sexual abstinence of at least 48 hours, but not more than 7 days.
- The patient produces a semen sample by masturbation and ejaculation into a wide mouthed container. The laboratory should ensure that the containers used are not toxic to sperm prior to their usage.¹
- Lubricants and condoms should not be used in specimen procurement, since these can potentially affect the validity of the test results.
- It is important to obtain collection of a complete semen specimen. An incomplete specimen collection may not provide accurate results. Since sperm concentration is highest in the first portion of the ejaculate, an initial loss of specimen could result in a spurious decrease in sperm count.

**Analytical Test Phase**

Semen analysis testing in the laboratory should commence within one hour of specimen procurement. The sample should be well mixed.

The evaluated parameters of a semen analysis include macroscopic (visual) and microscopic examinations. The macroscopic evaluation includes: appearance, volume, liquefaction, viscosity, and pH. The microscopic examination includes sperm count, motility, and morphology. Other microscopic findings may be seen, such as sperm agglutination or the presence of other cells, (e.g. white blood cells). These findings and their potential clinical impact will be further discussed.

**Macroscopic Evaluation**

**Color/Appearance**

The appearance of normal semen is a homogeneous gray-opalescent or creamy color. If the color is red or brownish, this may indicate the presence of blood in seminal fluid. If a semen sample appears white and turbid, this can signify inflammation and possible infection.

**Volume**

Normal volume is 2 to 5 mL. If a patient has low volume especially on repeat semen samples, this finding may indicate an anatomic abnormality of the genital tract. An increased semen volume can be seen in retrograde ejaculation. In this condition, seminal fluid, which would normally be passed through the urethra, enters into the bladder. Diagnosis is made when sperm cells are noted on urinalysis.

**pH**

Normal semen reference pH range is 7.2 to 7.8.¹²

Variations in volume and low pH may be due to congenital abnormalities of the genital tract or to obstruction. A pH of 8.0 or greater can be associated with infection of the prostate, seminal vesicle, or epididymis.³
Liquefaction & Viscosity
When ejaculated, semen has a thick consistency that reverts to a liquid. Normal seminal fluid samples should liquify within 60 minutes at room temperature, although most samples will liquify in a shorter period of time, (i.e., 15 minutes or less).

In highly viscous semen, liquefaction may be incomplete. Failure of complete liquefaction can be evaluated by aspirating a portion of the semen sample into a pipette. As the sample is slowly expelled from the pipette, the semen should form small discrete droplets. Semen samples of high viscosity will not form droplets and will demonstrate an elongated thread-like consistency.

Increased semen viscosity and abnormal liquefaction can affect sperm count and morphology results.

Microscopic Examination
Sperm Count
This parameter refers to the number of sperm cells per milliliter (mL) or concentration. A sperm count or density greater than 20 million sperm / mL is considered within normal limits.¹

A counting chamber is used to assess sperm count. Different counting chambers exist, and procedures for counting spermatozoa vary among chambers. Procedural differences can include the number of fields or grids examined or the need to dilute a semen sample prior to determining the count.

If the number of sperm cells varies considerably from field to field, the specimen may not be homogeneous and should be thoroughly mixed. Other factors that can contribute to count variations are specimen dilution, microscopic viewing of grids versus fields, and for some chambers, mathematical calculations. Duplicate counts of 200 spermatozoa are advised to obviate counting error.¹

Aside from technical/procedural differences between counting chambers, intrinsic abnormalities in a given semen sample, namely increased viscosity, can affect sperm count. If no sperm cells are seen on initial examination, the semen specimen should be centrifuged and re-examined. If no spermatozoa are identified following these steps, then the absence of sperm cells should be reported.

Motility
This parameter determines the percentage of spermatozoa with progressive forward movement. At least five microscopic fields are examined to grade the movement of 200 spermatozoa as rapid progressive, slow progressive, non-progressive motility, or non-motility. There is subjective interpretation in grading sperm cell motility.

If a large percentage of non-motile spermatozoa are present, this may signify either a sperm cell flagellum defect or the presence of dead sperm. Distinguishing between live and dead spermatozoa can
be done through a supravital stain such as eosin Y. In principle, dead spermatozoa have damaged cell membranes, which consequently take up the stain; whereas live spermatozoa have intact cell membranes and do not take up the stain. The dead sperm cells stain red and live sperm cells are unstained.

Poor sperm motility can affect fertility potential. Decreased motility can impede spermatozoa from entering the cervix and moving into the fallopian tubes and ovum.

**Morphology**

A spermatozoan or sperm cell is comprised of a head, neck, acrosome, and tail. A sperm cell is generally considered normal if it has a smooth and oval head, an acrosome comprising 40-70% of the sperm head, symmetrical neck insertion into head, a midpiece with no large cytoplasmic droplets (greater than 30% of head size), and a uniform tail of regular width and length without coiling or bent configuration. In addition to these morphological criteria, there are measurement criteria pertaining to length and width of a sperm cell.¹ An ocular micrometer should be used in determining spermatozoan measurements.

Sperm cells are evaluated on stained slides using bright field microscopy. At least 200 consecutive spermatozoa are examined when possible. Abnormalities of a sperm cell may involve a single region (e.g., head, tail, etc.) or involve multiple regions (e.g., head and tail, head and neck, etc.), or involve abnormal length or width.

As the results of the proficiency tests indicate, participating laboratories use various classifications and stains in assessing sperm morphology. Stains include Papanicolaou, Spermac, Shorr, Wright Giemsa, and Diff-Quik. Within selected subgroups using similar classification and stain, variations in reported morphology results were noted. Adhering to standard staining protocols is important since technical deviations can affect the quality of the slide. Artifacts on the slide can interfere with accurate sperm cell interpretation. Other factors can impact sperm morphology evaluation. Sperm concentrations, particularly in the high and low ranges, can impact the quality of smear preparation. There may be a subjective interpretation of classifying sperm cells as normal or abnormal, especially if an abnormality is subtle.

The Kruger (Tygerberg) classification uses strict criteria in determining whether or not spermatozoa are normal. Based on the percentage of normal spermatozoa, there are three threshold groups:

- 0-4% normal forms
- 5-14% normal forms
- Greater than 14% normal forms

These groups are used clinically as a predictive value in determining in-vitro fertilization outcomes.
In the most recent WHO semen manual (4th edition), the reference value for normal sperm morphology has changed from that of previous editions (WHO 2\textsuperscript{nd} and 3\textsuperscript{rd} eds.). The reference value is based on strict criteria.

From a clinical perspective, morphological abnormalities provide information on sperm functionality. For example, if sperm cells have significant tail defects, the migratory activity of spermatozoa through cervical mucus can be decreased. An acrosome defect may impede a sperm cell from penetrating an oocyte (egg) membrane for fertilization.

**Post-Analytic Test Phase**

In semen analysis, there can be unexpected findings that may prompt further evaluation. When sperm cells are absent (azoospermia), a fructose test can be performed to determine if a congenital abnormality or obstruction is present. As previously mentioned, fructose is produced in the seminal vesicles and secreted. A fructose level can be low if there are conational anomalies or obstruction involving the seminal vesicles, vas deferens, or ejaculatory ducts.

When motile spermatozoa stick to each other, this is agglutination. Sperm cells can agglutinate in a head to head, or tail to tail, or head to tail configuration. If the sperm cells demonstrate a consistent pattern of agglutination on microscopic examination, this finding can indicate an immunological etiology, such as the presence of antisperm antibodies.

Round cells may be seen on microscopic review. These cells may represent immature sperm cells, white blood cell leukocytes, or epithelial cells undergoing degenerative changes. If bacteria and/or leukocytes are noted, seminal cultures should be considered for further evaluation.

**CLINICAL MANAGEMENT**

The findings of semen analysis can influence medical management of an infertile couple. In male patients with low sperm counts or abnormal sperm morphology, assisted reproductive techniques such as in-vitro fertilization or intracytoplasmic sperm injection may be used to enhance the likelihood of fertilization.

**SUMMARY**

In semen analysis, many factors during the pre-analytic and analytic test phases can impact the validity of the results. It is vital that laboratories maintain good standard consistent practices and technical competency.

Since multiple factors can affect results of semen analysis, repeat testing on a different sample should be made especially prior to costly and time consuming assisted reproductive technologies and procedures.
References

Figure 1.

The Male Reproductive System and Spermatogenesis

Within the wall of the seminiferous tubules, the spermatogenic cells undergo mitosis, meiosis, and spermatogenesis. The sertoli cells provide nourishment to these germ cells.

mitosis and differentiation
- primary spermatocyte
- secondary spermatocytes
- early spermatids
- late spermatids
- spermatids

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