EDUCATIONAL COMMENTARY – SEMEN ANALYSIS AND FERTILITY

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**Florida licensees, please note: This exercise will appear in CE Broker under the specialty of Andrology.

Learning Objectives

On completion of this exercise, the participant should be able to

- discuss the test parameters that are part of the semen analysis;
- discuss sperm quantity necessary for fertilization; and
- discuss processes that influence sperm effectiveness.

Semen analysis is a highly variable, non-precise laboratory test. Although specific quantifiable measures are used for the analysis, the analysis itself is subjective and based on the knowledge and experience of the scientist as well as the integrity and quality of the specimen.

Semen analysis is usually performed to assess the outcome of vasectomy or for fertility issues. Many factors affect sperm function. This exercise will touch on some of the issues that affect the sperm’s ability to fertilize the egg.

Semen analysis involves four test parameters: ejaculate volume, number of sperm in the specimen, morphology and quality of the observed sperm, and sperm motility. Several conditions must be met to perform a good analysis of the semen.

The latest reference values are based on the 2010 World Health Organization (WHO) publication, *World Health Organization Reference Values for Human Semen Characteristics*, with strict criteria of 4,500 men from 14 countries. Of these men, the WHO chose data from those who were able to father children during the previous 12 months. The WHO statistically analyzed the range for the lowest number of sperm for conception. They were able to use the data from 1,953 of the men in the survey for the reference values shown in Table 1.
Table 1. One-sided lower reference limits for fertile men, generated from 1,953 men whose partners had a time to pregnancy (TTP) of ≤12 months.1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower Reference Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate (semen) volume, mL</td>
<td>1.5 mL (95% confidence interval, 1.4-1.7)</td>
</tr>
<tr>
<td>Sperm concentration (density), ×10^6/mL</td>
<td>15 (95% confidence interval, 12-16)</td>
</tr>
<tr>
<td>Total sperm per ejaculate, ×10^6</td>
<td>39 (95% confidence interval, 33-46)</td>
</tr>
<tr>
<td>Total motile sperm observed, %</td>
<td>40 (95% confidence interval, 38-42)</td>
</tr>
</tbody>
</table>

Volume

The specimen must be freshly collected, and analysis started within a short period of time. Specimens are usually obtained by masturbation after 2 to 7 days of abstinence. When first collected, the ejaculate is normally thick. After 5 to 10 minutes, it liquefies; at the point of liquefaction, the analysis begins. The ejaculate volume must be determined to quantify the concentration of sperm per milliliter. Ejaculate volume varies greatly among individuals. Some factors that influence the volume include duration of abstinence, time between ejaculation when there is “occasional/as usual” intercourse, and even the time of day.

Counts/Concentration

The concentration of sperm is affected by the ejaculate volume and the sperm count. The WHO study has determined the threshold sperm concentration for conception is 15×10^6/mL. The threshold total sperm per ejaculate is approximately 39×10^6. Sperm enumeration is accomplished by manual as well as instrumental methods. Please refer to the API 2010 educational commentary, “Current Methods to Determine Sperm Counts,” for additional information.2

The most common manual method is counting using the Neubauer hemocytometer for plating the semen. There are nine large squares, with the center square having finer grid lines. The volume of each square is 0.1 mL. The routine is to use the smaller four corner squares and the center square of the large (0.1 mL) center square. Formulas for calculation are as follows:

\[
\text{Area} = \text{Length} \times \text{Width} \\
\text{Volume} = \text{Length} \times \text{Width} \times \text{Height} \\
\text{Area of the smallest square: } \text{Length} \ (0.2 \text{ mm}) \times \text{Width} \ (0.2 \text{ mm}) = 0.04 \text{ mm}^2
\]

Using the five small squares counted, the total area would be 0.04 mm^2 × 5, or 0.2 mm^2. The height of the square is 0.1 mm, giving a volume of 0.02 mL. Therefore, the sperm/mL calculation would be as follows:
Sperm/mL = \frac{\text{No. of sperm counted} \times \text{dilution factor}}{0.02 \text{ mL}}

Owing to the nature of the semen specimen, the standard procedure from the WHO is to do at least a 200-cell (sperm) count in each chamber.¹ Two chambers are used and they must agree within 10%. If they do not agree, the count must be repeated with a freshly recharged hemocytometer.

Motility

The motility of the sperm is taken into account for natural intercourse and intrauterine insemination. Although it takes only one sperm to fertilize an egg, there are millions of sperm trying to do the same thing. For improved probability of fertilization, the quantity of sperm needed is unknown. The sperm must be able to swim the distance through the fallopian tubes for fertilization. Motility is estimated by observing the sample. A count is used for the estimate; however, it is not necessary to be exact. Only 400 sperm are used in the hemocytometer count to provide the estimate of motility. If 40% are motile in a specimen of 39 × 10⁶ sperm, there are still a large number in the ejaculate.

Sperm motility is graded. One common system uses five grades of sperm motility as shown in Table 2.

Table 2. A common system for grading sperm motility.³

<table>
<thead>
<tr>
<th>Grade</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No motility</td>
</tr>
<tr>
<td>1</td>
<td>Absent or minimal forward movement</td>
</tr>
<tr>
<td>2</td>
<td>Poor to fair movement with forward progression</td>
</tr>
<tr>
<td>3</td>
<td>Good activity, observed tail movement</td>
</tr>
<tr>
<td>4</td>
<td>Fully active with tail movement and rapid rate of speed</td>
</tr>
</tbody>
</table>

The grade is an average of motility for the whole specimen. Some laboratories report the percentage of observed sperm in each grade, which provides more detailed information. For example, the grades may be as follows:

0% Grade 0: Nonmotile
18% Grade 1: Minimal forward motility
12% Grade 2: Fair forward movement
25% Grade 3: Good activity with observed tail movement
45% Grade 4: Fully active with rapid movement
With the percentage of sperm in each grade and the sperm count, the quantity of sperm for each grade can be calculated. For example, with a sperm count of $50 \times 10^6$/mL, the above grades would have quantities as shown in Table 3 below.

Table 3. Example calculation of sperm quantity per grade.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Observed Motile, %</th>
<th>Quantity, $\times 10^6$/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>22.5</td>
</tr>
</tbody>
</table>

The grade and the quantity give the clinician important information about the sperm functionality. Another way to provide information on the viable sperm is to use the total motility count, the number of motile and morphologically normal sperm per milliliter. This takes into account the variable volume of the semen.

**Morphology**

The morphology of the sperm plays a part in fertilization. There are basically two parts of the sperm. The head contains the genetic material and the tail provides motility. The head shape and size affect the ability of the sperm to fertilize the egg. The head size determines what can be carried in the head. Too small (pinhead sperm) may not carry enough, or any, genetic material. Large or irregular sperm may not be able to penetrate the egg to deliver the DNA. Also, if the head of the sperm is large, the tail may not be strong enough to move it, making it unable to swim. Another abnormality are sperm with two heads and one tail. These double-headed sperm are rarely seen.

Important characteristics of the tail include its length, shape, and thickness. The tail is thickest where it connects to the head and tapers throughout its length. Some tails may not be able to stay physically connected or are easily broken off the sperm head while the tail is motile and “whipping around.” Thickness at the attachment to the head varies. The thickness affects the rate of movement by the tail. A normal tail tapers, allowing the tail to move and to “whip” around. The tail is the sperm’s mechanism for movement and its ability to swim up the fallopian tubes. The average tail length is 45 µm. The tail must be a sufficient length for proper movement. Too short a tail, and the sperm will not move far or fast enough: other, more normal, sperm, will be able to function better, and be able to fertilize the egg first.

There are, on occasion, sperm that have two tails and having the two tails impairs the sperm motility.
These are the four major factors for the semen analysis. Even if the sperm is mostly normal, there are other factors that make the job of the sperm more difficult. The subjective way morphology is described and reported influences the interpretation of sperm functionality. A study in 1986 by Thomas Kruger, MD, determined the minimum number of sperm needed to reach the cervix and the egg for fertilization to occur following natural intercourse. Known as the *Kruger Strict Criteria*, it determined that 4% normal sperm in the ejaculate are needed to reach the cervix. Based on this study, the WHO Guidelines were revised to include the Kruger strict criteria, and it was established that 4% of the total ejaculate must be normal sperm for fertilization to occur naturally.

Sperm are produced by the testicles and are suspended with very little fluid. The rest of the seminal fluid is produced by the prostate gland. Should the fluid be decreased, the concentration of sperm would be greater. For example, if $60 \times 10^6$ sperm are produced and there is 2 mL fluid volume, there would be $30 \times 10^6 / \text{mL}$ sperm in the ejaculate. If the prostate produces 1 mL of seminal fluid, the concentration would be $60 \times 10^6 \text{ sperm/mL}$.

Using the WHO strict criteria, the minimum amount of sperm needed for fertilization to occur via natural intercourse works out as follows:

- Minimum count: $15 \times 10^6 / \text{mL}$
- Percentage motile: 40%
- Required normal: 4%

$$15 \times 10^6 / \text{mL} \times 0.4 \times 0.04 = 240,000 \text{ normal motile sperm/mL}$$

At a minimum, the probability for fertilization requires 240,000 normal sperm per milliliter. Normal sperm are determined using the four parameters discussed previously in this commentary.

**Conclusion**

This commentary highlights the parameters that make up a semen analysis for fertilization via natural intercourse. In addition to the semen analysis parameters, there are other factors that affect sperm functionality. Conditions to consider include antisperm antibodies produced by the female body that may cause the sperm to clump together or interfere with the sperm-egg attachment. There are also the semen liquefaction (does not liquefy enough) and viscosity (too thick for the sperm to swim through or out of the ejaculate). The speed or velocity of the motile sperm affects its ability to reach the egg. The WHO standards are for natural intercourse. In vitro fertilization has other parameters that are studied. The reader is encouraged to seek additional information.
EDUCATIONAL COMMENTARY – SEMEN ANALYSIS AND FERTILITY (cont.)

References


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