In the name of GOD
Semen Analysis

the cornerstone of the laboratory evaluation of the infertile man

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Semen Analysis

Primary source of information on:
– Sperm production
– Hormone integrity
– Reproductive tract patency

An accurately performed semen analysis remains an important tool for the evaluation of the infertile man.
Semen analysis is not a measure of fertility. An abnormal semen analysis simply suggests the likelihood of decreased fertility.

Except in cases of azoospermia, the semen analysis does not allow for the definitive separation of patients into sterile and fertile groups.

As semen parameters decrease in quality, the statistical chance of conception decreases but does not reach zero.
The Human Semen

- During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the paired epididymides, mixed with, and diluted by, fluid secretions from the accessory sex organs. It is emitted in several boluses.
The percentage contribution of each of the secretions that make up the seminal fluid

<table>
<thead>
<tr>
<th>Source of secretions</th>
<th>Ejaculate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes and excurrent ducts</td>
<td>5</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>46-80</td>
</tr>
<tr>
<td>Prostate</td>
<td>13-33</td>
</tr>
<tr>
<td>Bulbourethral and urethral glands</td>
<td>2-5</td>
</tr>
</tbody>
</table>
The Human Semen

Semen has two major quantifiable attributes:

I. The total number of spermatozoa. This reflects sperm production by the testes and the patency of the post-testicular duct system.

II. The total fluid volume contributed by the various accessory glands. This reflects the secretory activity of the glands.

The nature of the spermatozoa (their vitality, motility and morphology) and the composition of seminal fluid are also important for sperm function.
Semen Quality

- Sperm production by testes
- Accessory organ secretions
- Recent (febrile) illnesses
- Abstinence time
Results of laboratory measurements:

- Complete sample collection
- The activity of accessory glands
- The time since the last sexual activity
- The penultimate abstinence period
- The size of testes
Fig. 2.1 Variation in total number of spermatozoa and sperm concentration over a one-and-a-half-year period

Data courtesy of Schering Plough and Bayer Schering Pharma AG.
Table A1.1 Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower reference limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>1.5 (1.4–1.7)</td>
</tr>
<tr>
<td>Total sperm number ($10^6$ per ejaculate)</td>
<td>39 (33–46)</td>
</tr>
<tr>
<td>Sperm concentration ($10^6$ per ml)</td>
<td>15 (12–16)</td>
</tr>
<tr>
<td>Total motility (PR+NP, %)</td>
<td>40 (38–42)</td>
</tr>
<tr>
<td>Progressive motility (PR, %)</td>
<td>32 (31–34)</td>
</tr>
<tr>
<td>Vitality (live spermatozoa, %)</td>
<td>58 (55–63)</td>
</tr>
<tr>
<td>Sperm morphology (normal forms, %)</td>
<td>4 (3.0–4.0)</td>
</tr>
</tbody>
</table>

Other consensus threshold values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>$\geq 7.2$</td>
</tr>
<tr>
<td>Peroxidase-positive leukocytes ($10^6$ per ml)</td>
<td>$&lt; 1.0$</td>
</tr>
<tr>
<td>MAR test (motile spermatozoa with bound particles, %)</td>
<td>$&lt; 50$</td>
</tr>
<tr>
<td>Immunobead test (motile spermatozoa with bound beads, %)</td>
<td>$&lt; 50$</td>
</tr>
<tr>
<td>Seminal zinc ($\mu$mol/ejaculate)</td>
<td>$\geq 2.4$</td>
</tr>
<tr>
<td>Seminal fructose ($\mu$mol/ejaculate)</td>
<td>$\geq 13$</td>
</tr>
<tr>
<td>Seminal neutral glucosidase (mU/ejaculate)</td>
<td>$\geq 20$</td>
</tr>
<tr>
<td>Nomenclature</td>
<td>Description</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>aspermia</td>
<td>no semen (no or retrograde ejaculation)</td>
</tr>
<tr>
<td>asthenozoospermia</td>
<td>percentage of progressively motile (PR) spermatozoa below the lower reference limit</td>
</tr>
<tr>
<td>asthenoteratozoospermia</td>
<td>percentages of both progressively motile (PR) and morphologically normal spermatozoa below the lower reference limits</td>
</tr>
<tr>
<td>azoospermia</td>
<td>no spermatozoa in the ejaculate (given as the limit of quantification for the assessment method employed)</td>
</tr>
<tr>
<td>cryptozoospermia</td>
<td>spermatozoa absent from fresh preparations but observed in a centrifuged pellet</td>
</tr>
<tr>
<td>haemospermia (haematospermia)</td>
<td>presence of erythrocytes in the ejaculate</td>
</tr>
<tr>
<td>leukospermia (leukocytopspermia, pyospermia)</td>
<td>presence of leukocytes in the ejaculate above the threshold value</td>
</tr>
<tr>
<td>necrozoospermia</td>
<td>low percentage of live, and high percentage of immotile, spermatozoa in the ejaculate</td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>normozoospermia</td>
<td>total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits</td>
</tr>
<tr>
<td>oligoasthenozoospermia</td>
<td>total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of progressively motile (PR) spermatozoa, below the lower reference limits</td>
</tr>
<tr>
<td>oligoasthenoteratozoospermia</td>
<td>total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits</td>
</tr>
<tr>
<td>oligoteratozoospermia</td>
<td>total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of morphologically normal spermatozoa, below the lower reference limits</td>
</tr>
<tr>
<td>oligozoospermia</td>
<td>total number (or concentration, depending on outcome reported)* of spermatozoa below the lower reference limit</td>
</tr>
<tr>
<td>teratozoospermia</td>
<td>percentage of morphologically normal spermatozoa below the lower reference limit</td>
</tr>
</tbody>
</table>
Examination of Human Semen

• STANDARD METHODS: Which are robust routine procedures for determining semen quality

 OPTIONAL TESTS: Which may be used in certain situations or by choice of the laboratory

 RESEARCH TESTS: Which are not currently regarded as routine
STANDARD PROCEDURES

✓ Sample collection and delivery
✓ Safe handling of specimens
✓ Initial macroscopic examination
✓ Initial microscopic investigation
✓ Further microscopic examinations
✓ Testing for antibody-coating of spermatozoa
Semen Collection & Delivery

- The sample should be collected after a minimum of 48 hours but not longer than seven days of sexual abstinence.
- To reduce the variability of semen analysis results, the number of days of sexual abstinence should be as constant as possible.
- Two samples should be collected for initial evaluation. The interval between the two collections should not be less than 7 days or more than 3 weeks.
- The results of a man's semen analysis can vary considerably.
EFFECT OF SEXUAL ABSTINENCE PERIOD

• WITH EACH OF DAY OF ABSTINENCE (UP TO 1 WEEK):
  – SEMEN VOLUME CAN RISE BY UP TO 0.4 ML,
  – SPERM CONCENTRATION CAN INCREASE BY 10-15 MILLION/ML
  – SPERM MOTILITY TENDS TO FALL WHEN THE ABSTINENCE PERIOD IS LONGER THAN 7 DAYS
Semen Collection & Delivery

- Ideally, the sample should be collected in a private room near the laboratory. If this is not possible, it should be delivered to the laboratory within 1 hour of collection.

- The sample should be obtained by masturbation. *Coitus interruptus* is not acceptable as a means of collection because the first portion of the ejaculate, which usually contains the highest concentration of spermatozoa may be lost. Moreover, there will be cellular and bacteriological contamination of the sample and the acid pH of the vaginal fluid adversely affects sperm motility.

- It is important to emphasize to the subject that the semen sample should be complete.
Initial macroscopic examination

✓ Liquefaction
✓ Appearance: A normal sample has a homogenous, grey-opalescent appearance.
✓ Volume: Ejaculate volume should be at least 1.5ml, as smaller volumes may not sufficiently buffer against vaginal activity. Low ejaculate volume may indicate retrograde ejaculation, ejaculatory duct obstruction, incomplete collection or androgen deficiency.
✓ Viscosity
✓ pH: If the pH is less than 7.0 in a sample with azoospermia, there may be obstruction of the ejaculatory ducts or bilateral congenital absence of the vasa.
Initial microscopic investigation

- **Concentration:** Sperm concentration should be >15 million sperm/ml, as lower concentrations may make pregnancy less probable.

- **Motility:** Sperm motility is the single most important measure of semen quality.

- **Agglutination of spermatozoa:** Frequent sperm agglutination is abnormal and suggests the presence of anti-sperm antibodies.

- **Cellular elements other than spermatozoa:** ‘Round Cells’: Include epithelial cells from the genitourinary tract, Prostate Cells, Spermatogenic cells, and Leukocytes.
Sperm Motility

The motility of each spermatozoon is graded as follow:

- **Progressive motility (PR):** spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- **Non-progressive motility (NP):** all other patterns of motility with an absence of progression, i.e. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
- **Immotility (IM):** no movement.
# Sperm Motility Grading

## WHO grading

a. **Grade A** (fast progressive) sperms swim forward and fast in a straight line-like guided missiles.

b. **Grade B** (slow progressive) sperms swim forward, but either in a curved or crooked line, or slowly (slow linear or nonlinear motility).

c. **Grade C** (non-progressive) sperms move their tails, but do not move forward (local motility only).

d. **Grade D** (immotile) sperms do not move at all. Sperms of grades C and D are considered poor.

## Hotchkiss and WHO grading

<table>
<thead>
<tr>
<th>Hotchkiss</th>
<th>WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 = Non-motile</td>
<td>WHO grade D</td>
</tr>
<tr>
<td>1 = Sluggish</td>
<td>WHO grade C</td>
</tr>
<tr>
<td>2 = Poor to fair motion</td>
<td>WHO grade C</td>
</tr>
<tr>
<td>3 = Good forward movement</td>
<td>WHO grade B</td>
</tr>
<tr>
<td>4 = Highly active progression</td>
<td>WHO grade A</td>
</tr>
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</table>
Sperm vitality by dye exclusion

- Cases in which the semen sample demonstrates all non-motile sperm or less than 5% to 10% motility may be due to ultra-structural defects, in which case the sperm are alive but have defects in the flagella. Although immotile, these sperm may appear morphologically normal
Further microscopic examinations

- Assessment of sperm morphology: By assessing the exact dimensions and shape characteristics of the sperm head, midpiece, and tail, sperm can be classified as “normal” or not. In the strictest classification system (Kruger morphology), only 14% of sperm in the entire ejaculate are truly normal looking. Sperm morphology is a sensitive indicator of overall testicular health, because the sperm morphologic characteristics are determined during spermatogenesis. The main role of sperm morphology assessment in the male infertility evaluation is to complement other information and to better estimate the chances of fertility.
Fig. 2.13 Schematic drawings of some abnormal forms of human spermatozoa

A. Head defects
- (a) Tapered
- (b) Pyriform
- (c) Round
  - No acrosome
  - Small
- (d) Amorphous
- (e) Vacuolated
- (f) Small acrosomal area

B. Neck and midpiece defects
- (g) Bent neck
- (h) Asymmetrical
- (i) Thick insertion
- (j) Thin

C. Tail defects
- (k) Short
- (l) Bent
- (m) Coiled
- (n) >1/3rd head

D. Excess residual cytoplasm

Adapted from Kruger et al., 1993 and reproduced by permission of MQ Medical.
OPTIONAL TESTS

1. Calculation of indices of multiple sperm defects; Teratozoospermia index (TZI) or Multiple Anomalies index (MAI)
2. Panleukocyte (CD45) Immunocytochemical Staining
3. Interaction between Spermatozoa & Cervical Mucus (P.C.T)
4. Biochemical assays for accessory sex organ function (Zinc, Fructose, α-glucosidase)
5. Computer-aided sperm analysis
ADDITIONAL SEMEN PARAMETERS

- The seminal vesicles produce fructose in an androgen dependent process. Normal semen fructose concentrations range from 120 to 450 mg/dl. Inflammation of the seminal vesicles, androgen deficiency, partial obstruction of the ejaculatory ducts, or incomplete ejaculation may result in fructose concentrations below 120 mg/dl.

- Patients with obstructed seminal vesicles or congenital absence of the seminal vesicles, which is usually associated with bilateral absence of the vas deferens, demonstrate azoospermic, acidic, fructose-negative semen that does not coagulate. In addition, their ejaculate volume is low (<1.0 ml).
Important chemical substances that are present in each of the contributions to the ejaculate

<table>
<thead>
<tr>
<th>Source</th>
<th>Important biochemical substances</th>
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<tbody>
<tr>
<td>Testes and excurrent ducts</td>
<td>Testosterone, Inhibin, LDH C4, L-Carnitine, Glycerophosphorylcholine</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>Fructose, Prostaglandines, Substrate for semen clotting, Acid phosphatase</td>
</tr>
<tr>
<td>Prostate</td>
<td>Citrates, Proteases, Peptidases, Hyaluronidase, Vesiculase, Spermine</td>
</tr>
<tr>
<td>Bulbourethral and urethral glands</td>
<td>Zn, Ca, Mg, Mucoproteins, IgG</td>
</tr>
</tbody>
</table>
RESEARCH TESTS

• Sperm function tests:
  – Reactive oxygen species
  – Human Sperm-Oocyte interaction tests
  – Human Zona Pellucida binding tests
  – Assessment of the acrosome reaction
  – Zona-free hamster oocyte penetration test
  – Assessment of Sperm Chromatin
<table>
<thead>
<tr>
<th>تعداد اسپرم در هر ویلد با بزرگنمایی ۱۰۰۰۰</th>
<th>رقت</th>
<th>(μ1 semen)</th>
<th>رقيق کننده (μ1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;۱۵</td>
<td>۱:۱</td>
<td>۲۰۰</td>
<td>۸۰۰</td>
</tr>
<tr>
<td>۱۵-۴۰</td>
<td>۱:۱</td>
<td>۱۰۰</td>
<td>۹۰۰</td>
</tr>
<tr>
<td>۴۰-۲۰۰</td>
<td>۱:۲</td>
<td>۵۰</td>
<td>۹۵۰</td>
</tr>
<tr>
<td>≥۲۰۰</td>
<td>۱:۴</td>
<td>۲۰</td>
<td>۹۸۰</td>
</tr>
</tbody>
</table>
Fig. 2.2 Nonspecific aggregation of spermatozoa in semen

Views of spermatozoa aggregated with an epithelial cell (a), debris (b) or spermatozoa (c, d).

Micrographs courtesy of C Brazil.
The major type of agglutination

- **Grade 1**: isolated < 10 spermatozoa per agglutinate, many free spermatozoa
- **Grade 2**: moderate 10–50 spermatozoa per agglutinate, free spermatozoa
- **Grade 3**: large agglutinates of >50 spermatozoa, some spermatozoa still free
- **Grade 4**: gross all spermatozoa agglutinated and agglutinates interconnected
Fig. 2.3 Schematic diagram of different extents of sperm agglutination

<table>
<thead>
<tr>
<th>Parts involved</th>
<th>Degree of agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Isolated (&lt; 10 sperm/agglutinate, many free sperm)</td>
</tr>
<tr>
<td></td>
<td>2. Moderate (10–50 sperm/agglutinate, free sperm)</td>
</tr>
<tr>
<td></td>
<td>3. Large (agglutinates &gt; 50 sperm, some sperm still free)</td>
</tr>
<tr>
<td></td>
<td>4. Gross (all sperm agglutinated, and agglutinates interconnected)</td>
</tr>
</tbody>
</table>

A. Head-to-head

B. Tail-to-tail (heads are seen to be free and move clear of agglutinates)

C. Tail-tip-to-tail-tip

D. Mixed (clear head-to-head and tail-to-tail agglutinations)

E. Tangle (heads and tails enmeshed. Heads are not clear of agglutinates as they are in tail-to-tail agglutination)

Reproduced from Rose et al. (1976) by permission of Wiley-Blackwell.
<table>
<thead>
<tr>
<th>رقيق کننده و semen</th>
<th>تعداد مربع شمارش شده</th>
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<tbody>
<tr>
<td>1+4</td>
<td>20</td>
</tr>
<tr>
<td>1+9</td>
<td>10</td>
</tr>
<tr>
<td>1+19</td>
<td>5</td>
</tr>
<tr>
<td>1+49</td>
<td>2</td>
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<table>
<thead>
<tr>
<th></th>
<th>100</th>
<th>50</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+4</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1+9</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1+19</td>
<td>2</td>
<td>0.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

شده
رش
شما
ربع
تعدادم
يق
ق
ر
كننده
و
رقيق کننده و semen

0.40.821+4
24101+9
48201+4
Fig. 2.5 Eosin-nigrosin smear observed in brightfield optics

Spermatozoa with red (D1) or dark pink heads (D2) are considered dead (membrane whereas spermatozoa with white heads (L) or light pink heads are considered alive (intact).
Fig. 2.6 Schematic representation of typical morphological changes in human spermatozoa subjected to hypo-osmotic stress

(a) no change. (b)–(g) various types of tail changes. Swelling in tail is indicated by the grey area.

Figure 5.4  Laboratory procedure described by the WHO to prepare high-quality sperm smears
Figure 5.2  Example of a good-quality sperm smear
Figure 5.3  Example of poor sperm smear, with debris and overlapping sperm
Figure 6.1  Normal spermatozoa with dimensions $5.0 \times 3.5 \mu m$
Figure 6.2  Normal spermatozoa with dimensions $5.0 \times 3.5\,\mu m$, but slightly tapered in the post-acrosomal region.
Figure 6.4  Normal spermatozoa with dimensions $5.0 \times 3.0 \mu m$, but slightly tapered in the post-acrosomal region.
Figure 6.5  Macrocephalic head
Figure 6.6  Round sperm head
Figure 6.7  Amorphous head
Figure 6.8  Irregular surface
Figure 6.9a  Tapered spermatozoon (mild form)
Figure 6.9b  Tapered head (moderate form)
Figure 6.10  Tapered: pyriform (dumbbell) (severe)
Figure 6.11  Tapered: pyriform (severe)
Figure 6.12  Large acrosomal area
Figure 6.13  Small acrosomal area (severe form)
Figure 6.14  Small acrosomal area (mild form)
Figure 6.16  Bent mid-piece
Figure 6.17  Mid-piece bent
Figure 6.18  Over-thick mid-piece. Arrowed sperm: pseudo-droplet defect
Figure 6.20  Cytoplasmic droplet
Figure 6.21  Cytoplasmic droplet
Figure 6.22  Coiled tail (Dag defect)
Figure 6.23  Coiled tail (Dag defect)
Figure 6.24  Tail-tip coiling
Figure 6.25  Double tail
Figure 6.26  Double tail
Figure 6.27  Tail bent than more than 90°. Arrowed sperm: normal curvature
Figure 6.28  Stumped tail
Figure 6.29  Abaxial implantation
Figure 6.30  Abaxial implantation
Figure 6.31  Flat implantation site
Figure 6.32  Acephalic cell (pinhead)
Fig. 2.11
Photomicrographs of Papanicolaou-stained immature germ cells and of cells of nontesticular origin found in semen. (From J. Suominen.)

(A) Squamous epithelial cells (~ 540×)
(B) Two neutrophils (leukocytes) and one monocyte (~ 2500×)
(C) Round spermatids (~ 1400×)
(D) Spermatids (~ 1400×)
(E) Macrophage (~ 1100×)
Thanks for your patience