The semen analysis and sperm preparation

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World Health Organization reference values for human semen characteristics 1

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WHO Laboratory manual for the Examination and processing of human semen
SEMEN ANALYSIS

Evaluation of male fertility

Testis function and male genital tract
Accessory sex glands (prostate and seminal vesicles)

Under given conditions of collection
A complete medical history and physical examination
It is impossible to characterize a man's semen quality from evaluation of a single semen sample

SEMEN PRODUCTION

The results of laboratory measurements of semen quality will depend on:
- whether a complete sample is collected
- the activity of the accessory sex glands
- the time since the last sexual activity
- the penultimate abstinence period
- the size of the testis

SEMEN ANALYSIS (WHO)
PREPARATION

Private room near the laboratory
A minimum of 2 days and a maximum of 7 days of sexual abstinence
A complete sample

Should be reported:

- Man’s name, birth date and personal code number
- The period of abstinence
- The date and time of collection
- The completeness of the sample
- Any difficulties in producing the sample
- The interval between collection and start of the semen analysis

PREPARATION

- The sample should be obtained by masturbation and ejaculated into a clean container made of glass or plastic (non toxic)
- The specimen container is placed on the bench or in an incubator (37°C) while the semen liquefies
- For ART or microbiological analysis, specimen containers and pipettes must be sterile
- Analyze ASAP
- Collection of semen at home: NO
- Collection of semen by non-toxic condom during sexual intercourse
- Coitus interrupts: NO
- Safe handling of specimen: infectious agents (HIV...)

Evaluation

Initial macroscopic examination

- Liquefaction (30 minutes)
- Semen viscosity
- Appearance of the ejaculate
- Semen volume
- Semen pH
Evaluation

Initial microscopic examination

- Thorough mixing and representative sampling of semen
- Making a wet preparation
- Aggregation of spermatozoa
- Agglutination of spermatozoa
- Cellular elements other than spermatozoa

Evaluation

- Sperm motility
- Sperm vitality
- Sperm numbers
- Counting of cells other than spermatozoa
- Sperm morphology
- Leukocytes
- Immature germ cells
- Antibody coating of spermatozoa
Sperm motility 1H and 4H

At least 200 spermatozoa in a total of at least 5 fields in each replicate

WHO (IV edition)
- a > 25µm/s: progressive (rapid)
- b 5-25µm/s: progressive (slow)
- c<5µm/s: non progressive
- d: immotile

Motility a + b ≥ 50% ; a ≥ 25%

WHO (V edition)
- progressive PR a + b
- non-progressive NP
- Immotile IM

Motility a + b + c ≥ 40% ; PR a+ b ≥ 32 %

Vitality

Eosin – Nigrosin

Evaluate 200 spermatozoa in each replicate

Spermatozoa
- With red or dark pink heads are considered dead (membrane damaged)
- With white heads or light pink heads are considered alive (membrane intact)

WHO (IV edition) ≥ 75% live
WHO (V edition) ≥ 58% live
Vitality

Hypo-osmotic swelling (HOS) test (if ICSI)

Evaluate 200 spermatozoa in each replicate

- Swollen spermatozoa are identified by changes in the shape of the cell
- Live cells are distinguished by evidence of swelling of the sperm tail: all forms of swollen tails = live spermatozoa

WHO (IV edition) ≥ 75% live
WHO (V edition) ≥ 58% live

Sperm numbers

Evaluate 200 spermatozoa in each replicate

**OLIGOZOOSPERMIA**

WHO (IV edition)
≥ 20M/ml ; ≥ 40M/ejaculate

WHO (V edition)
≥ 15M/ml ; ≥ 39M/ejaculate

Low sperm numbers: examination of the sediment of a centrifugated sample (two slides)

- cryptozoospermia: the presence of spermatozoa in either replicate
- suspected azoospermia: the absence of spermatozoa from both replicates
Sperm numbers

Low sperm numbers: examination of non-centrifugated samples to detect motile spermatozoa (when an accurate assessment of low sperm numbers is not required)

Assessing low sperm numbers in the entire improved Neubauer chamber

Counting of cells other than spermatozoa

Calculation of the concentration of round cells in $10^6$ per ml in semen (C)

N = the number of round cells counted in the same number of fields as 400 spermatozoa

S = the concentration of spermatozoa in $10^6$ per ml

C = S x (N/400)

If the estimated concentration exceeds $10^6$ per ml, their nature should be assessed:

- Peroxidase activity or leukocyte markers
- Identify immature germ cells
WHO (IV edition) Leukocytes <1M/ml
WHO (V edition) Leukocytes <1M/ml

LEUKOCYTOPERMIA

Cells other than spermatozoa (1)
- Spermatogony
- Spermatocyte I
- Spermatocytes II

Cells other than spermatozoa (2)
- Round spermatid
- Elongated spermatid
Sperm morphology
Shorr-stained spermatozoa

Papanicolaou-stained spermatozoa

Sperm morphology
Semen smearing method for sperm morphology

Computer-aided sperm morphometric assessment

TERATOZOOSPERMIA

WHO (IV edition) ≥ 14% (Kruger)
WHO (V edition) ≥ 4% (Kruger)

Normal spermatozoa
Acrosome 40-70% head
Midpiece 2 - 3µm
Head 3 - 5µm Tail 50µm
WHO according to Kruger

Unadjusted probability of pregnancy per month of attempt at pregnancy, based on normal morphology using World Health Organization (WHO) strict method

In this study, thresholds were found by both approaches: 19% with the WHO method and 39% with the method of David; there was no threshold for MAI.

Auger et al. 2010
MORPHOLOGY AND FERTILITY

- Each spermatozoon is evaluated as normal or abnormal.
- The % of normal sperm cells in the ejaculate is evaluated and related to fertility according to a threshold.

![Graph showing the distribution of normal sperm cells across different categories.]

**Table 2. Comparison of WHO World Health Organization (WHO) manual for normal morphology values with recently published literature.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>% of Normal</th>
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<td>Score 1</td>
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<tr>
<td>Score 4</td>
<td>Various</td>
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<td>25</td>
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</tbody>
</table>

Figure 2. Spermatogonial count, normal maturity assessed with various methods (WHO Manual, 2010).

Reference:
- Gomes et al. (2010).
- Some et al. (2012).
Anti-sperm antibodies (ASA)

- 10% infertile (3-20%) / 2% fertile men

When the blood – testis barrier is violated:
- Surgical, infectious (50%), testicular trauma
- Vasectomy
- Torsion
- Unexplained infertility
- Agglutination, mobility, vitality, MAR test +,
- + shaking phenomenon = abnormal PCT

% ASA

Spontaneous pregnancy:
- 10-49%
- 50-90%
- >90%

Abshagen 1998

ASA and fertility

FIV %Fertilization <<<

ICSI :++++

SPERM PREPARATION

- Simple washing
- Direct swim-up
- Discontinuous density gradients
Simple washing

- This simple washing procedure provides the highest yield of spermatozoa.
- Adequate only if semen samples are of good quality.
- It is often used for preparing spermatozoa for intrauterine insemination (donor).

Direct swim-up

- Spermatozoa may be selected by their ability to swim out of seminal plasma and into culture medium = the “swim-up” technique.
- The semen should preferably not be diluted and centrifuged prior to swim-up, because this can result in peroxidative damage to the sperm membranes.
- The direct swim-up technique can be performed either by layering culture medium over the liquefied semen or by layering liquefied semen under the culture medium.
- Motile spermatozoa then swim into the culture medium.
- Gives a lower yield of spermatozoa than washing, but selects them for their motility and is useful where the percentage of motile spermatozoa in semen is low, e.g. for IVF and ICSI.
Discontinuous density gradients

- The best selection of good quality spermatozoa, giving good separation from other cell types and debris.
- Used to recover and prepare spermatozoa for use in IVF and ICSI.
- Centrifugation of seminal plasma over density gradients consisting of colloidal silica coated with silane which separates cells by their density. Motile spermatozoa swim actively through the gradient material to form a soft pellet at the bottom of the tube.
- A simple two-step discontinuous density gradient preparation method is most widely applied, typically with a 40% (v/v) density top layer and an 80% (v/v) density lower layer.
- Sperm preparation using density gradient centrifugation usually results in a fraction of highly motile spermatozoa, free from debris, contaminating leukocytes, non-germ cells and degenerating germ cells.

**Sperm Preparation: Gradient centrifugation**

1. Prepare gradient
2. Add semen and centrifuge
3. Dead cells
4. Intact cells

**Semen preparation techniques for intrauterine insemination (Review)**

Boomsma CM, Heijsman MJ, Cohen JJ, Sargeant C

[Khalil’s conclusion]

There is insufficient evidence to recommend any specific preparation technique. Large high-quality randomized controlled trials comparing the effectiveness of a given method with a control are essential to determine the optimal semen preparation technique for clinical use.
CONCLUSION (1)

One ejaculate: No conclusion!
≥ 2 or 3 samples – 3 months

If necessary, complete with:
- Microbiological analysis
- Computer-aided sperm analysis: motility, concentration, morphology
- Biochemical assays for accessory sex organ function: citric acid, zinc, fructose, acid phosphatase, neutral α-glucosidase in seminal plasma
- Electron microscopy

CONCLUSION (2)

Research:
- Reactive oxygen species (ROS)
- Sperm–mucus interaction or a post-coital test (PCT)
- The sperm penetration assay (SPA)
- The sperm capacitation index (SCI)
- Sperm chromatin: TUNEL, COMET, SCSA
- Birefringence
- MSOME
- Emerging technologies: microarray, metabolomics, atomic force microscopy,....

CONCLUSION (3)

If azoospermia, complete with:
- A complete medical history
- Physical examination
- Measurement of selected hormones
- Genetic tests
- Testicular biopsy,....