WHO 5th EDITION MANUAL FOR SEMEN ANALYSIS

COUNTING SPERM MOTILITY

Pages 21-26 (Extracted)

2.5 Sperm motility

The extent of progressive sperm motility (see Section 2.5.1) is related to pregnancy rates (Jouannet et al., 1988; Larsen et al., 2000; Zinaman et al., 2000). Methods of motility assessment involving computer-aided sperm analysis (CASA) are described in Section 3.5.2.

Sperm motility within semen should be assessed as soon as possible after liquefaction of the sample, preferably at 30 minutes, but in any case within 1 hour, following ejaculation, to limit the deleterious effects of dehydration, pH or changes in temperature on motility.

- Mix the semen sample well (see Box 2.3).
- Remove an aliquot of semen immediately after mixing, allowing no time for the spermatozoa to settle out of suspension.
- Remix the semen sample before removing a replicate aliquot.
- For each replicate, prepare a wet preparation approximately 20 μm deep (see Section 2.4.2).
- Wait for the sample to stop drifting (within 60 seconds).
- Examine the slide with phase-contrast optics at ×200 or ×400 magnification.
- Assess approximately 200 spermatozoa per replicate for the percentage of different motile categories.
- Compare the replicate values to check if they are acceptably close. If so, proceed with calculations; if not, prepare new samples.

Note 1: The procedure may be performed at room temperature or at 37 °C with a heated microscope stage, but should be standardized for each laboratory. If sperm motility is to be assessed at 37 °C, the sample should be incubated at this temperature and the preparation made with prewarmed slides and coverslips.

Note 2: The use of an eyepiece reticle with grid (see Fig. 2.4a) is recommended to limit the area viewed; this allows the same area of the slide to be assessed during both stages of scoring. Assess progressive motility first, then non-progressive motility and immotility (see Section 2.5.1). Limiting the area, and thus the number of spermatozoa assessed, ensures that several areas of the preparation are examined for motility.

2.5.1 Categories of sperm movement

A simple system for grading motility is recommended that distinguishes spermatozoa with progressive or non-progressive motility from those that are immotile. The motility of each spermatozoon is graded as follows:

- 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, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
- Immotility (IM): no movement.

Comment 1: The previous edition of this manual recommended that progressively motile spermatozoa should be categorized as rapid or slow, with a speed of >25 μm/sec at 37 °C defining "grade a" spermatozoa. However, it is difficult for technicians to define the forward progression so accurately without bias (Cooper & Yeung, 2006).

Comment 2: When discussing sperm motility, it is important to specify total motility (PR + NP) or progressive motility (PR).

2.5.2 Preparing and assessing a sample for motility

- If motility is to be assessed at 37 °C, turn the stage warmer on 10 minutes in advance, to allow the temperature to stabilize.
- Prepare a wet preparation 20 μm deep (see Section 2.4.2).
- Examine the slide with phase-contrast optics at ×200 or ×400 magnification.
- Wait for the sample to stop drifting.
- Look for spermatozoa in an area at least 5 mm from the edge of the coverslip (see Fig. 2.4b), to prevent observation of effects of drying on motility.

- Systematically scan the slide to avoid repeatedly viewing the same area. Change fields often. Avoid choosing fields on the basis of the number of motile sperm seen (field choice should be random).
- Start scoring a given field at a random instant. Do not wait for spermatozoa to swim into the field or grid to begin scoring.
- Assess the motility of all spermatozoa within a defined area of the field. This is most easily achieved by using an eyepiece reticle (see Fig. 2.4a). Select the portion of the field or grid to be scored from the sperm concentration, i.e. score only the top row of the grid if the sperm concentration is high; score the entire grid if the sperm concentration is low.
- Scan and count quickly to avoid overestimating the number of motile spermatozoa. The goal is to count all motile spermatozoa in the grid section instantly; avoid counting both those present initially plus those that swim into the grid section during scoring, which would bias the result in favour of motile spermatozoa.
- Initially scan the grid section being scored for PR cells (see Section 2.5.1). Next count NP spermatozoa and finally IM spermatozoa in the same grid section. With experience, it may be possible to score all three categories of sperm movement at one time, and to score larger areas of the grid.
- Tally the number of spermatozoa in each motility category with the aid of a laboratory counter.
- Evaluate at least 200 spermatozoa in a total of at least five fields in each replicate, in order to achieve an acceptably low sampling error (see Box 2.5).
- Calculate the average percentage and difference between the two percentages for the most frequent motility grade (PR, NP or IM) in the replicate wet preparations.
- Determine the acceptability of the difference from Table 2.1 or Fig. A7.2, Appendix 7. (Each shows the maximum difference between two percentages that is expected to occur in 95% of samples because of sampling error alone.)
- If the difference between the percentages is acceptable, report the average percentage for each motility grade (PR, NP and IM). If the difference is too high, take two new aliquots from the semen sample, make two new preparations and repeat the assessment (see Box 2.6).
- Report the average percentage for each motility grade to the nearest whole number.

Note 1: Assess only intact spermatozoa (defined as having a head and a tail; see Section 2.7.3), since only intact spermatozoa are counted for sperm concentration. Do not count motile pinheads.

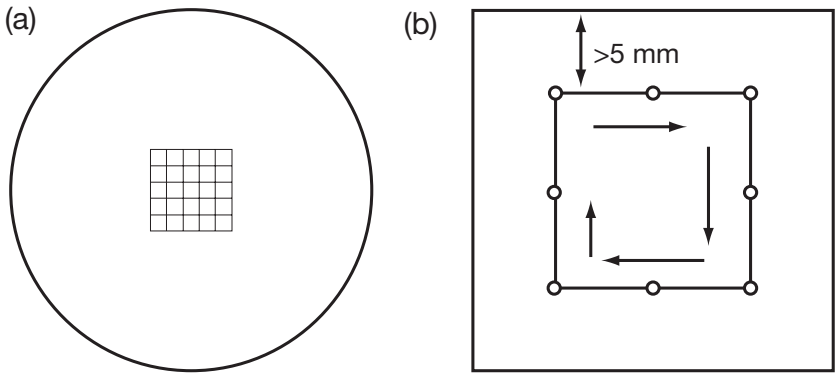
Note 2: If spermatozoa are being scored in two stages (i.e. PR first, followed by NP and IM from the same area) and a count of 200 spermatozoa is achieved before all motility categories from that area have been scored, counting must continue beyond 200 spermatozoa until all categories have been counted, in order to avoid bias towards the motility category scored first.

Note 3: It is common to overestimate sperm motility, but this can often be avoided by reversing the order of analysis (NP and IM first), using an eyepiece reticle, and being aware of, and avoiding, to the extent possible, potential sources of bias (see Section 7.13.3).

Note 4: On rare occasions, with inhomogeneous samples, even a third set of replicates may provide unacceptable differences. In this case, calculate the mean of all replicates and note this in the report.

Fig. 2.4 Aids to assessing sperm motility

(a) An eyepiece reticle makes it easier to count motile and immotile spermatozoa. (b) Systematic selection of fields for assessment of sperm motility, at least 5 mm from the edges of the coverslip.



Box 2.5 Errors in estimating percentages

How certain your estimate of a percentage is depends not only on the number (N) of spermatozoa counted but also on the true, but unknown, percentage (p) (binomial distribution). The approximate standard error (SE) is $\sqrt{((p(100–p))/N)}$ for percentages between 20 and 80. Outside this range, a more appropriate method to use is the angular transformation (arc sin square root), $z = \sin^{-1}\sqrt{(p/100)}$, with a standard deviation of $1/(2\sqrt{N})$ radians, which depends only on the number of spermatozoa counted and not the true percentage.

Table 2.1 Acceptable differences between two percentages for a given average, determined from replicate counts of 200 spermatozoa (total 400 counted)

Average (%)	Acceptable Difference*
0	1
1	2
2	3
3–4	4
5–7	5
8–11	6
12–16	7
17–23	8
24–34	9
35–65	10

Average (%)	Acceptable Difference*
66–76	9
77–83	8
84–88	7
89–92	6
93–95	5
96–97	4
98	3
99	2
100	1

**Based on the rounded 95% confidence interval.*

Box 2.6 Comparison of replicate percentages

Percentages should be rounded to the nearest whole number. The convention is to round 0.5% to the nearest even number, e.g. 32.5% is rounded down to 32% but 3.5% is rounded up to 4%. Note that the rounded percentages may not add up to 100%.

If the difference between the replicate percentages is less than or equal to that indicated in Table 2.1 for the given average, the estimates are accepted and the average is taken as the result.

Larger than acceptable differences suggest that there has been miscounting or errors of pipetting, or that the cells were not mixed well, with non-random distribution in the chamber or on the slide.

When the difference between percentages is greater than acceptable, discard the first two values and reassess. (Do not count a third sample and take the mean of the three values, or take the mean of the two closest values.)

For estimates of sperm motility, or vitality by eosin alone and for the hypo-osmotic swelling (HOS) test, prepare fresh replicates from new aliquots of semen. For estimates of vitality from eosin–nigrosin smears and sperm morphology, reassess the slides in replicate.

With these 95% CI cut-off values, approximately 5% of replicates will be outside the limits by chance alone (see Appendix 7, section A7.3). Exact binomial confidence limits can now be computer-generated, and these are used in this manual for the graphs and tables provided to assess agreement of replicates.

2.5.3 Worked examples

Example 1. Sperm motility estimates in replicate counts of 200 spermatozoa are: progressive, 30% and 50%; non-progressive, 5% and 15%; immotile, 65% and

35%. The most common category is immotile, with an average of 50% and a difference of 30%. From Table 2.1, it is seen that for an average of 50%, a difference of up to 10% would be expected to occur by chance alone. As the observed difference exceeds this, the results are discarded and two fresh slides are prepared and the sperm motility re-estimated.

Example 2. Sperm motility estimates in replicate counts of 200 spermatozoa are: progressive, 37% and 28%; non-progressive, 3% and 6%; immotile 60% and 66%. The most common category is immotile, with an average of 63% and a difference of 6%. From Table 2.1, it is seen that for an average of 63%, a difference of up to 10% would be expected to occur by chance alone. As the observed difference is less than this, the results are accepted and the mean values reported: PR 32%, NP 4%, IM 63%.

2.5.4 Lower reference limit

The lower reference limit for total motility (PR + NP) is 40% (5th centile, 95% CI 38–42).

The lower reference limit for progressive motility (PR) is 32% (5th centile, 95% CI 31–34).

Comment: The total number of progressively motile spermatozoa in the ejaculate is of biological significance. This is obtained by multiplying the total number of spermatozoa in the ejaculate (see Section 2.8.7) by the percentage of progressively motile cells.