

Quality control of boar semen motility

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Quality control (QC) of boar semen samples aims to evaluate holding samples that were stored at temperatures of $+15^{\circ}$ C to $+20^{\circ}$ C. Sperm motility is a typical parameter that is evaluated during QC and represents the fertilizing capacity of a semen sample.

A high sperm motility of a holding sample indicates not only a functional sperm tail for sperm movement. A high sperm motility also indicates a functional sperm membrane which is vital for a competent spermatozoon and for the fertilization of an oocyte. With this, sperm motility is an important characteristic of a functional sperm cell. The evaluation of sperm motility is relatively easy to perform and aims to gain information on the percentage of functional sperm cells.

During storage of semen doses, the functionality of sperm cells must be conserved. This is assured by several components in the semen extender. These extender components conserve the sperm membranes, but also and most importantly, slow down the metabolism of the spermatozoa. This will save energy and prevents the production of toxic metabolites.

However, the effect of the reduction of the sperm metabolism must be removed to perform sperm motility evaluation. Otherwise, sperm motility of a holding sample would be largely underestimated.

To assure that a semen sample regains its full motility potential it must be incubated for at least 20 minutes at a temperature of $+38^{\circ}$ C. Only then, sperm motility is fully re-established after semen storage at $+17^{\circ}$ C.

Graph 1 shows typical curves of sperm motility [%] at different periods of incubation at $+38^{\circ}$ C after storage at $+17^{\circ}$ C for Androstar[®] Plus as a high-quality semen extender and a control extender.



Total and progressive motility of the ejaculates after different periods of incubation





These graphs show, how the motility of spermatozoa, which were conserved in a high-quality, long-term extender is regained. Such a slow and sustainable increase of motility after semen storage is indicative for an excellent semen extender that preserves sperm motility and function very well. Sperm cells that are conserved in a high-quality semen extender regain their semen motility slowly and not fast. Most importantly, motility is maintained at a high level and does not decrease after 20 minutes of incubation. These facts must be considered when performing a comparison of semen extenders. Not all semen extenders can conserve semen motility in the same way as Minitube extenders.

Therefore, sperm motility of holding samples should only be evaluated and compared after 20 minutes of incubation at $+38^{\circ}$ C. Evaluation after, e.g., 5 minutes would bias the QC of a boar semen sample, due to the fact that conservation capabilities of a high quality semen extender are ignored.

The following points should be considered during QC of boar semen holding samples:

Preparations

- Semen tubes/bags maintained as holding samples must be clearly identified.
- Ensure optimal storage conditions of holding samples: +15°C to +20°C refrigerator, good hygiene.
- Prewarm all materials which will be used during evaluation to +38°C on a heating plate or in an incubator: counting chambers, pipette tips and sampling tubes. If an incubator is used, the material should be stored on a heating plate when evaluation work starts.
- Take semen tubes/bags out of the +15°C to +20°C refrigerator only shortly before measurement is started.
 Do not store semen at room temperature!
- Before taking an evaluation sample out of the semen tube/bag, gently turn the tube upside down several times (approx. 5 times) to achieve homogenous sperm concentration within the tube. To avoid formation of air bubbles and foam, do not shake!
- If the tubes must be used for more examinations on the following days, close them quickly after removing the sample and store them again in the semen refrigerator. Avoid storage at room temperature!
- Fill sampling tubes to about 2 thirds with the semen and keep them for minimum of 20 minutes at +38°C on the heating plate. The samples should not be incubated for more than 30 minutes before evaluation. A too short as well as a too long incubation time will have a negative effect on the analysis results!

Semen analysis

- Before putting a sample on a microscope slide and cover slip or filling of the counting chamber (Leja or Minitube) with the semen sample, the AndroVision[®] analysis window has to be opened. An optimal preparation is important, as delays will negatively influence the analysis result!
- Turn the sample tube upside down 3 times, then take a semen sample from the center of the tube using a pipette.
- Pipet 5 µl of semen on a microscope slide and put a coverslip on the droplet, or fill the counting chamber with 3 µl of the semen sample.
- Start the analysis within 20 seconds after preparing the slide/chamber. Analysis should be finished within 60 seconds. If the sample stays for too long on the heated stage and especially if exposed to the microscope light, motility will decrease and eventually cease. The sample will no longer be representative.
- Evaluate a minimum of 5 microscope fields or 500 sperm in total. Avoid fields at the margins of the chamber.
- If a large number of holding samples is to be evaluated, there should only be as many samples incubated at +38°C as can be evaluated within 30 minutes. If samples stay on the heating plate for too long, the analysis results can be influenced negatively!

