Sample preparation for CASA analysis

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When carefully validated, CASA systems provide valuable information for quality assurance of semen planned for sale, and for the understanding of the diversity of sperm responses to changes in the micro-environment in research (Amann P. and Waberski D., 2014).

Although the evaluation of semen by the CASA system has demonstrated the ability to give the best reproducibility, there still exists measurable variability among laboratories which may be due to various factors such as dilution rate and type of extender (Vizcarra JA, Ford JJ. 2006).

Other factors that influence the analysis conducted by CASA include the method of sampling, sample processing, and the time span between sampling and analysis. All consumables employed for semen sampling preparation should be handled aseptically and must also be tested for sperm toxicity. Therefore, a standardised routine is the premise for reliably obtaining reproducible results (De Alba Romero C., 2011).

The preparation and homogenization of the sample is the most important step towards obtaining a correct measurement and consistent and reproducible results (Nicolae M., 2006). The essential measures leading to a standardised CASA process are:
1. Consistent mixing of the ejaculate
2. Correct drawing and handling of the sample
3. Correct measurement settings of the software (classification parameters, calibration, and chamber depth)

1. Mix the ejaculate well by inverting 5 times (by 180 degrees), without shaking.
2. The best tool for correct semen sample dilution is an electronic pipette with a dilution function.
   - First, draw in the extender (must be held at the same temperature as the semen sample).
   - Secondly, draw in the sample from the ejaculate. It is very important to draw the semen sample correctly: to be representative it must be taken from the centre of the bag or container, at approx. 5 mm depth, immediately after mixing (see step 1).
3. Wipe the outside tip of the pipette and eject the sample into an appropriate vial.

A certain number of sperm per area or field should not be exceeded. Therefore, the ejaculate sample must be pre-diluted. It is important to control the temperature not only of the semen samples but also of the extender used for pre-dilution. The following dilution rates for boar semen are recommended, depending on the semen concentration of the ejaculate:

• Standard: 200-600 million/ml (1+9 = 90 µl sperm + 810 µl extender)
• High: > 600 million/ml (1+19 = 45 µl sperm + 855 µl extender)
• Low: < 200 million/ml (1+4 = 180 µl sperm + 720 µl extender)

Before starting the analysis, ensure the following actions have been taken:
• Pre-warming all materials to 38°C / 100°F
• Preparing and pre-warming the extender to 38°C / 100°F
• Pre-warming the microscope stage to 38°C / 100°F

The procedure for the analysis is as follows:
• Mix the pre-diluted sample in its vial by inverting it at least 5 times, without shaking.
• For the removal of a sample for CASA, use a volumetric pipette. Load the counting chamber with the appropriate volume according to the chamber size and without any air bubbles.
• After filling the chamber, perform the measurement with the CASA system immediately.
• Scientific studies have shown that the measurement with a CASA-system should start 15-20 seconds after filling the chamber (Nicolae M., 2006).

When considering the possible and common mistakes of a CASA analysis, it becomes evident how important the points described above are for correct sample preparation and homogenization.

Variations in sperm mobility caused by temperature differences, incompletely filled counting chambers or high and low concentrations of the same sample in two measurement fields are among the most frequent mistakes of measurement. These can be avoided by precisely observing the procedure: adequate pre-warming of all materials and solutions which will be in contact with the semen sample, the correct position of the pipette for removing the sample, a well-mixed sample before pipetting, and the correct filling of the counting chamber without any air bubbles.

References:
Nicolae M (2006): Untersuchung flussigkonservierten Eberspermas mittels computergestutzter Spermienanalyse und Bindung FITC-konjugierter Mannose an die Spermienmembran; Vizcarra JA, Ford JJ (2006): *Validation of the Sperm Mobility Assay in Boars and Stallions.* Theriogenology 66:1091-1097; De Alba Romero C (2011): *Evaluation of semen by using the CASA system (Computer Assisted Semen Analysis) Sperm Vision™ in semen production laboratories: Experience in Spain and Germany.* SINSUI 2011. Portoalegre, Brasil; Rupert P, Amann A, Waberski D (2014): *Computerassisted sperm analysis (CASA): Capabilities and potential developments.* Theriogenology 81: 5-17.