

## CASA Systems Artifacts on Sperm Analysis for Artificial Insemination

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### DESCRIPTION

Artificial Insemination (AI) has benefited the pig industry by enhancing the efficiency of reproduction through increased fertility rates and a measure of yield, such as litter size. An advantage of using AI technology is that fewer boars are needed to inseminate the same number of sows. Thus, artificial insemination stations improve the reproductive efficiency of sire lines [1,2]. The increased use of AI worldwide has revolutionized the pig industry by reducing production costs and increasing the efficiency of commercial lines through heterosis and complementarity. However, fertility variables have multifactorial effects that are largely associated with age, nutrition, anatomy, and physiology, handling, season, genetic line, and variables related to the AI technique [3]. The male can be evaluated for infertility or subfertility making use of multiple of clinical or laboratory methods, and also from a laboratory evaluation of semen. Semen analysis is the best way to evaluate male fertility ability being centered on sperm concentration, motility, morphology and viability [4-7].

Currently, there are two approaches to perform the semen analysis: The subjective and the Computer Assisted Semen Analysis (CASA). The subjective method is being used from the middle of past century. Subjective analysis implies considerable variability in the results obtained, which seriously limits its diagnostic value, which is evident not only between observers, but also in the same technical analyst [8-10]. The main problem of subjective analysis is that introduces a big bias in the results with a high CV among repetitions of the same ejaculate analysis. CASA technology was developed to overcome these limitations of subjective methods, where the spermatozoa are analyzed computationally, which very briefly consists of the definition of their centroid and analyzing their displacement in the cartesian space, with the calculation of a whole series of kinematic parameters. Parameters related to concentration, morphology, DNA fragmentation and others follow specific image analysis algorithms in each case, always yielding quantitative values, whose meaning far exceeds those subjective classifications mentioned above [8,11-13].

Aspects such as total and progressive motility are closely linked to temperature, since cellular metabolism is altered by it. On the

other hand, it is common to talk about room temperature when that does not indicate at all what degrees one works at. It seems that the best way to evaluate semen is to use the temperature of the female genital tract where it should stay after ejaculation. In fact, in human samples, it has been found that temperature affects sperm kinematics very significantly, and this refers to both the incubation temperature and the analysis temperature [14]. Therefore, it was recommended to use both temperatures at 37°C in order to standardize the conditions and because it is the physiological temperature, although the highest values were obtained after incubating at 37°C and analyzing the samples at 22°C. For its part, the morphometric analysis showed that the sperm had a smaller size after being incubated at 37°C with respect to the values observed at 22°C, while the cells became less elliptical, which indicates that this reduction was greater in length than width cells. It should be noted that, in that same study, the subjective evaluation of the samples did not allow observing differences between the two temperatures, which reinforces the idea that this type of evaluation is not sufficient for a correct evaluation of sperm functionality [15].

Another element to consider is that referred to the counting chamber used. There are basically two types of chambers, those based on droplet displacement and those based on capillary diffusion. The first are based on the traditional use of a slide and cover. In any case, its use does not ensure a uniform distribution of the drop of semen since the cover remains floating on its surface which, in addition, produces passive movements of the fluid [16]. For this reason, specific chambers (Makler®, Spermtrack®) were developed that, following the same concept, comprise a cover with a certain weight, which ensures the expansion of the fluid on the base of the chamber. On the other hand, its design allows ensuring a height between the base and the cover that can be 10 or 20 µm, depending on the model.

### CONCLUSION

Particularly, the effect of frame rate and counting chamber use has been studied in a variety of species, showing that the sperm behavior is species-specific and need for accurate evaluation in each species and conditions. In recent years similar studies has been published in a variety of species to achieve a satisfactory sperm kinematic analysis defining the FRO, that was off to 300 fps

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in stallion, 250 fps in bull, donkey and salmon, 225 fps in boar and sturgeon and 200 fps in eel. Other innovations in CASA systems technology they have to do with improvements in camera acquisition and video capture time, 3D technology, or new approaches for sperm cell tracking.

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