Boar Semen Evaluation Using CASA and Its Relation to Fertility

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Summary

For artificial insemination in sow, using optimal dose of spermatozoa is crucial. Dilution rate of raw semen can be established based on various parameters, best characterized using CASA. In this paper, CASA is used for evaluation of younger and adult boar semen, in relation to subsequent fertility and also, for emphasizing special sperm features such as hyperactivation or presence of sperm subpopulations. Based on our findings, we conclude that CASA system provides accurate data for semen evaluation, concerning motility and velocity parameters; also it can be established if semen is hyperactivated or not. The analyzed semen parameters, at least in the frame of this paper, do not support a differentiation of semen subpopulations. Semen parameters described using CASA can serve as predictive values for fertility parameters, such as conception rate and litter size.

Artificial insemination in swine represents a powerful tool for genetic improvement, enhanced selection and economic profitability. In order to achieve all these goals, each ejaculate has to be well managed, in order to generate good fertility parameters, and the crucial problem is represented by the dilution rate. The optimal dilution rate ensures good fertility parameters with minimal sperm concentration. Nowadays, computerized analyzing systems (CASA) provide the best way for semen assessment and ensuring the optimal dilution rate. Accuracy and precision of CASA systems has allowed detection of subtle changes in sperm motion and the subsequent improvement of differentiation among treatments in laboratory studies of new seminal extenders, cryoprotectants, or other steps of aliquots processing (e.g. centrifugation) of a given sperm suspension.

The CASA-subjected study of motility can be included in the group of functional tests that can be considered as useful for in vivo predictive evaluation of an ejaculate (Aparicio et al., 2005).

CASA can be regarded as a useful quality assurance technique for boar semen regardless of the precise predictive power with respect to fertility.

CASA measurements, with the exception of VCL, made in artificial insemination centers on the day of semen collection are valid indicators of semen quality 24 hours later, when usually the semen is used on farms (Holt C et al., 1997).
The aim of this paper is represented by evaluation of CASA system in order to assess semen parameters in younger and adult boars, in relation to subsequent fertility and also, to appreciate if CASA system allows a differentiation between hyperactivated and non-hyperactivated sperm, or to characterize sperm subpopulations.

Materials and methods

Semen samples were obtained from Duroc boars, with age ranging between 14 and 18 months (Group 1, n = 5) and between 28 and 32 months (Group 2, n = 5). Considering the age of the boars as an important factor for spermatogenesis, we have compared the semen characteristics and fertility traits between the two groups. Sperm-rich ejaculate fractions were collected using the gloved-hand method, extended (1:1, vol/vol) in Zoosperm ND4 (Importvet), and after evaluation of conventional semen characteristics, diluted in order to obtain 3 x 10^9 spermatozoa per dose (included in a total volume of 80 ml). Further, in the laboratory of Reproduction department in the Faculty of veterinary medicine Timisoara, semen samples were assayed using CASA equipment, following the technique used by Frunza et al (2007). Briefly, samples were loaded in a Leja standard count 4 chamber slide (IMV USA, Maple Grove, MN, USA) and sperm concentration and characteristics of sperm motility determined using a computer-assisted sperm analysis system (CASA; Integrated Visual Optical System, Version 12; Hamilton Thorne Research, Beverly, MA, USA). Starting CASA values for boar sperm analysis were consistent with manufacturer recommendations. The following characteristics of sperm motility were determined: percentages of motile and progressively motile spermatozoa (with fast, medium and slow movements), percentage of immotile spermatozoa, percentage of viability, path velocity of the smoothed cell path (VAP), average velocity measured in a straight line from the beginning to the end of the sperm track (VSL), average velocity measured over the actual point to point track followed by the sperm cell (VCL), straightness (STR - average value of the ratio VSL/VAP) which measured the departure of the sperm cell path from a straight line, linearity (LIN - average value of the ratio VSL/VCL) which measured the departure of the cell track from a straight line, and wobble (WOB).

Wobble is the expression of the relationship between the average and curvilinear paths, calculated as (VAP/VCL) x 100. WOB would be low for a track with a wide trajectory (high ALH), but high for a circling track, since the curvilinear and average paths would be similar. A high linearity trajectory is one where the curvilinear path has a relatively low amplitude of lateral head displacement, and the general direction of movement is the same as that of the straight-line path (Mortimer, 1997).

We focused on sperm motility because it indicates active metabolism and integrity of membranes, and is of great importance for fertilizing capacity.
Measures of semen quality performed in the laboratory were compared with conception rates and litter sizes obtained when the semen samples were used in on-farm artificial insemination. Sows (Landrace x Large White) with age ranging between 12 and 16 months were inseminated in relation to their receptivity to boar. For instance, sows immobile to man in the morning were inseminated at midday, and insemination was repeated next morning. Third insemination was carried after another 24 hours if sow was still immobile to man. Correlations between the mean measurements derived from extended semen and regarding reproductive parameters were examined using Spearman’s rank test.

Results and discussions

The obtained results are depicted in table 1 and 2. Table 1 includes semen features regarding motility and also their relation to reproductive parameters. It can be observed that the average number of diluted semen doses was similar for both groups (27.4) and also the average number of the inseminated sows with semen obtained from a single boar (12.4 for G1 and 12.6 for G2). The percentage of motility ranged in the well accepted values for a good semen, and there were not registered significant differences among groups.

There should be mentioned that percentage of progressive spermatozoa from G1 is significant larger than for G2, but, in G2 were registered significantly more viable spermatozoa than in G1. In this respect, it could be speculated that viability has a more important prevalence than motile progressive movement regarding ability to fertilize, as proved by the conception rate (82.6% for G1, very significant different – for p = 0.01, compared to 93% for G2). These features could also have an important role to the resistance of individual sperm to the action of extenders and cryoprotectants.

Regarding the motile spermatozoa, it could be observed that the percentage of fast moving spermatozoa was greater for G1, when the spermatozoa displaying medium or slow movements were equally represented for both groups. From this point of view, taking in consideration only subcategories of motile spermatozoa, we could not identify sperm subpopulations.

Abaigar et al. (1999) identified in their study three sperm subpopulations. Group 1 represented those sperm with highly progressive movement (high VSL) and with vigorous flagellar action (high BCF); group 2 represented spermatozoa showing an active type of movement (high VCL) but with considerably reduced forward progression (lower VSL and higher ALH). Group 3 appeared to represent a slow and possibly degenerate class of cells, for which all motion parameters were significantly lower than in either group 1 or 2.

In a study from 2005, Cremades et al reported the presence of three semen populations. P1 contained the largest number of spermatozoa (90.4%) and depicted the most vigorous (highest VCL and ALH and high BCF) and/or progressive (highest VSL and VAP) movement, spermatozoa whose forward
swimming line followed either rectilinear or parabolic tracks. Population 2 (P2) included those spermatozoa whose movement was defined as less vigorous (low VCL and ALH), although they exhibited a high frequency of flagellar beat (the highest BCF), yielding 8.3% of the total motile population. They were poorly progressive (low STR and LIN), following irregular trajectories (the VCL being much higher than the VAP) and covering very short distances (very low VSL).

Population 3 (P3) contained the lowest number of spermatozoa (1.3%) and included those spermatozoa that moved their tails slowly (low BCF) and whose sperm heads had a low ALH, following a curved line (low VCL and very low VAP) without obvious forward motility (undetectable VSL, LIN, and STR).

There were not registered significant different values between groups regarding immotile spermatozoa, or the average litter size.

Table 2 contains data regarding sperm velocity and reproductive parameters. There could be observed the significant differences between G1 and G2 regarding VAP, VSL and VCL (all the parameters defining the average distance covered by spermatozoa in straight line and progressive movement, taking into consideration the sperm head as a marker), while ALH and BCF did not displayed significant differences.

The first definition of hyperactivated motility based solely upon head movement characteristics was developed for mouse spermatozoa. For 30 Hz analysis of the trajectory of the head-midpiece junction, hyperactivated spermatozoa were defined as having WOB <56% and VCL >169 mm/s. Definitions for hyperactivated motility have also been developed for rabbit spermatozoa: WOB < 69% and VCL > 55 mm/s when analysed at 30 Hz. The common factor in all of these studies of the sperm head kinematics of hyperactivated spermatozoa is the high VCL and ALH and the low linearity, or wobble, of the trajectories. These properties are common to hyperactivated spermatozoa of all the species studied, and reflect the similarity in the flagellar movement patterns which cause hyperactivation. That is, the high amplitude flagellar bends associated with increased flexibility of the proximal midpiece.

Mammalian spermatozoa have unique cAMP-signaling cascades that are connected to protein tyrosine phosphorylation through the action of the serine/threonine kinase PKA, and these signaling systems are apparently activated during the process of fertilization (Harayama, 2003).

The accumulating evidence shows that the state of proteintyrosine phosphorylation is dramatically enhanced in sperm flagellum during capacitation in a number of animal species.

This high phosphorylation in the whole flagellum has been considered to be associated with sperm hyperactivated motility that is required for successful fertilization including penetration through cumulus and zona pellucida.

Hyperactive boar spermatozoa showed mean lateral head displacement >3.5mm, curvilinear velocity >97mms−1, linearity <32% and wobble <71% (Schmidt and Kamp, 2004).
Hyperactive mammalian spermatozoa have been characterized by a vigorous and non-linear movement caused by an increased amplitude of flagellar beats (whiplash movement). This form of motility has been observed in sperm at the site and the time of fertilization and appears, therefore, to be essential for fertilization. It has been suggested that the mechanical thrust due to hyperactive motility is vital for spermatozoa to penetrate the zona pellucida of the oocyte. Additional functions, such as releasing spermatozoa that are attached to the oviductal epithelium and improving sperm movement through the mucus of the oviduct and cumulus matrix of the egg are also discussed.

The hyperactivated sperm is characterized by a decrease in VCL and LIN, and an increase of LHD. For a multiparametric definition of hyperactive boar spermatozoa, the following threshold values were used in combination: VCL > 97µm/s, LHD mean >3.5µm, LIN < 32% and WOB < 71% (Schmidt and Kamp, 2004).

Analyzing the ejaculates from all the boars, it could be emphasized that only boar nr. 471458 covered all the criteria for hyperactivation (VCL, ALH, LIN and WOB), while the rest of the ejaculates registered threshold values only for VCL, ALH and/or WOB. Semen obtained from the above mentioned boar (471458) generated the lowest conception rate (75%) in both groups, fact that allowed us to presume that its semen was partially hyperactivated from the collection moment, phenomenon associated with premature acrosome reaction. This boar displayed also the highest VCL, compared to all other assessed boars. In group 2, none of the boars displayed LIN values fitted to hyperactivation threshold.

If the acrosome reaction occurs prematurely, smaller numbers of intact spermatozoa will be available for interaction with oocytes at the site of fertilization (Holt C. et. al, 1997).

Freshly ejaculated semen usually contains only a minor fraction of hyperactive spermatozoa. Rather, hyperactivity develops during capacitation either in vivo or in vitro. Capacitation is accompanied by changes in membrane structure thus increasing Ca\(^{2+}\) influx which triggers hyperactivity and, finally, the acrosome reaction.

The commonly used parameters to identify hyperactive spermatozoa are a decrease in curvilinear velocity (VCL) and linearity (LIN) as well as an increase in lateral head displacement (LHD). For boar spermatozoa a decrease in VSL and an increase in VCL, resulting in a reduced linearity, were reported upon hyperactivation (Mortimer 1997).

Hyperactivated motility occurs when the flagellum develops high-amplitude waves in the proximal, rather than the distal, region (Mortimer, 2000). The kinematics of hyperactivated spermatozoa is very different from spermatozoa in seminal plasma and from noncapacitated spermatozoa. The kinematic definition for hyperactivated motility is VCL ± 150 µm/s and LIN < 50% and ALHmax ± 7.0 µm.
Our results in semen assessment using CASA and the correlation established with subsequent fertility indicate the relevance of this process, although examination of the recent CASA literature underlines the persistent inadequacy of statistical analyses presented in support of various hypotheses (Holt W et al., 2007). The use of purely automated systems required several compromises in the methods used for trajectory analysis, since only mathematical methods of analysis could be used.

Development of sperm sorting technology is species specific with efficiency of the overall process highly dependent on the ability of spermatozoa to withstand a suite of stressors including dilution, high pressure, centrifugation, cryopreservation and thawing.

Regarding the importance of CASA system in sperm assay, Estienne et al. (2007) concluded that reproductive performance was reduced, however, in sows inseminated with semen the sperm motility of which was less than 60%, suggesting that subjective appraisals of the percentage of sperm cells displaying motility can at least be used to identify ejaculates of overtly poor quality.

Holt et al. (1997) demonstrated that up to 24% of the variance in litter size due to boars on commercial swine farms could be explained by differences in sperm motion characteristics determined using CASA technology. Boar spermatozoa that exhibited increased VSL and track linearity were associated with larger litter sizes.

Litter size is expressing the conjunction of various factors, related more or less to fertility but underlying the organism ability to ensure optimal medium for establishing, maintaining and finishing the pregnancy. Average values for litter size even in G1 (10.46) or in G2 (11.18) ranged in the limits wide accepted for intensive exploitation systems (Xu et al., 1998, Behan – 2002, Satake et al., 2006) Xu et al. (1998) using semen with motility varying from 62.5 to 75% obtained litter size ranging from 7.26 to 10.55.

Concerning the number of spermatozoa per inseminated dose, Behan (2002) reported farrowing rates of 91.1, 91.8 and 65.8 % for insemination with 3, 2 and 1 billion spermatozoa, and the mean litter size was 12.3, 12.3 and 12.1.

Frang ez et al. (2005) reported that their study on in vivo indices showed significant differences in pregnancy rates (83% versus 70%), when sows were inseminated with 2.5 × 10^9 motile sperm obtained from boars on 24 h and 72 h collection intervals, respectively.

In a study from 2002, Flowers observed that increasing the number of sperm inseminated generally has a positive effect on the number of pigs born alive, especially between the range of 1 to 3 × 10^9 cells. Also, the range in mean litter size was between 10.2 and 11.5 pigs when the insemination dose contained 3 × 10^9 spermatozoa and between 9.1 and 10.1 pigs when 2 × 10^9 sperm cells were used. Furthermore, boars whose spermatozoa exhibited increased straight-line velocity and track linearity were associated with large litter sizes.
Conclusions

CASA system provides accurate data for semen evaluation, concerning motility and velocity parameters.
Based on depicted parameters, it can be established if semen is hyperactivated or not.
The analyzed semen parameters, at least in the frame of this paper, do not support a differentiation of semen subpopulations.
Semen parameters described using CASA can serve as predictive values for fertility parameters, such as conception rate and litter size.

Acknowledgments

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References

1. Abaigar Teresa, W. V. Holt, Robin A.P. Harrison and G. Del Barrio - Sperm Subpopulations in Boar (Sus scrofa) and Gazelle (Gazella dama mhorr), 1999.


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