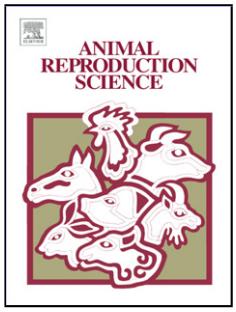

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Relationship between sperm motility characteristics and ATP concentrations, and association with fertility in two different pig breeds

Nina Hårdnes Tremoen[a,c], Ann Helen Gaustad[a,b], Ina Andersen-Ranberg[b], Maren van Son[b], Teklu Tewoldebrhan Zeremichael[a], Kirsten Frydenlund[a], Eli Grindflek[b], Dag Inge Våge[c], Frøydis Deinboll Myromslien[a,*]

[a]*Department of Biotechnology, Inland Norway University of Applied Sciences, NO-2318 Hamar, Norway*

[b]*Topigs Norsvin, Storhamargata 44, NO-2317 Hamar, Norway*

[c]*Centre for Integrative Genetics (CIGENE), Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway*

*Corresponding author: Inland Norway University of Applied Sciences, Department of Biotechnology, P.O. Box 400, N-2418 Elverum, Norway; Tel.: +47 62 51 78 68; E-mail address: froydis.myromslien@inn.no (F. D. Myromslien)

ABSTRACT

Boar fertility has a major impact on overall pig reproductive efficiency. Using accurate and objective *in vitro* sperm variables for predicting *in vivo* fertility from a single ejaculate, however, is challenging. Motility is the most widely used indicator of sperm quality, and a computer assisted sperm analysis (CASA) system is now available for objective assessment of sperm motility characteristics. In this study sperm motility characteristics and semen ATP concentrations were investigated and the effect of both were evaluated on total number of piglets born (TNB) when Norwegian Landrace (NL) and Norwegian Duroc (ND) boar semen was used for AI. In addition, breed differences for semen storage capacity were investigated. The results from CASA analysis indicated there were differences between NL and ND sperm motility variables. The percentage of motile sperm cells decreased in both NL ($P = 0.01$) and ND ($P<0.0001$) during storage. A large proportion of sperm cells with a hyperactive motility pattern were detected in ND semen on the day of collection, with no significant changes as a result of storage. Inconsistent with this finding, there was greater degree of hyper-activation in sperm motility pattern for NL because of semen storage. There was a significant decrease in semen ATP concentration during storage ($P<0.0001$) in both breeds. The linearity of sperm movement at the day of collection and the wobble after storage influenced TNB in NL, while the percentage of motile cells, curvilinear velocity and lateral head amplitude on the day of semen collection and linearity after storage influenced TNB in ND.

Keywords:

Boar sperm; Motility characteristics; CASA; ATP; Fertility

1. Introduction

Boar fertility has a major impact on overall pig reproduction efficiency. Selection of boars with relatively greater fertility is economically essential both for farmers and breeding companies. In artificial insemination (AI) centres, however, utilising accurate and objective variables for predicting field fertility results with use semen of a single ejaculate is challenging. Motility and morphology are the most widely used indicators of sperm quality and is the routine technique that is used for decision of approval or rejection of ejaculates for AI use. Motility has traditionally been manually and subjectively assessed using phase contrast microscopy, however, there is an objective computer assisted sperm analysis (CASA) available for evaluation of sperm motility characteristics (Amann and Waberski, 2014). The advantage of an objective analysis of sperm motility has led to an increased use of CASA for sperm quality assessment in mammals (Mortimer et al., 1997; Verstegen et al., 2002). To utilize the potential of CASA analysis at AI centres, however, there is a need for more studies of specific sperm motion characteristics within ejaculates and how these potentially correlate with male fertility.

The CASA default reports usually include mean values for hundreds of single sperm cell tracks presented as curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), amplitude of the lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN) and wobble (WOB) (Mortimer, 2000). In addition to these variables, evaluation of hyperactive motility is of interest. Hyper-activated sperm are characterized by a vigorous and non-linear movement, caused by an increased amplitude of flagellar beats (Schmidt and Kamp, 2004). This swimming pattern varies from species to species, and for boar sperm the thresholds have been related to VCL, ALH, LIN and WOB (Schmidt and Kamp, 2004). Sperm hyperactivity is reported to be important for fertilization of the oocyte, but it is a highly ATP-consuming process. If initiated too early, hyperactivity

poses a risk of depleting the energy store of the sperm cells before these cells reach the oocyte for fertilization (Mortimer et al., 1997; Suarez and Ho, 2003). Sperm motility variables and ATP content in semen, therefore, were analysed in this study for further evaluation of the relationship between hyperactive motility and ATP concentrations.

The fertility of liquid preserved sperm decreases gradually during storage, and differences in sperm storage capacity between individual boars has been reported (Waberski et al., 2011). In Norway, the liquid diluted semen used in pig production is recommended to be used within 96 hours after collection (Norsvin, 2017). Several farmers ordering semen for insemination, however, are not able to use the doses until two or more days after collection, due to factors such as long distance transport and a long shipment time. The sperm storage capacity, therefore, is also an important factor in pig production.

The aim of this study was to investigate specific sperm motility variables and ATP concentrations in ejaculates from Norwegian Landrace (NL) and Norwegian Duroc (ND) boars, and to evaluate the possible effect of these variables on field fertility measured as total number of piglets born (TNB). In addition, the influence of semen storage on these sperm traits and on consequent TNB, was investigated for possible breed differences.

2. Materials and methods

2.1. Animals, collection and processing of semen

This study was based on semen collected at the AI station managed by Norsvin at Hamar, Norway, between 21 February 2014 and 20 March 2015. Ejaculates from 103 purebred NL boars (n = 239) and 88 purebred ND boars (n = 179) were included in the study. The boars were housed in individual 6 m^2 pens, fed a standard commercial diet and had access to straw and sawdust as materials in which boars could express rooting behaviors. All animals were

cared for according to laws, internationally recognized guidelines and regulations for management of pigs in Norway (The Animal Protection Act of 20 December 1974, the Animal Welfare Act of 19 June 2009 and the Regulations for management of pigs in Norway of 18 February 2003). All boars were routinely used for AI. The age of the boars at the time of semen collections ranged from 241 to 1041 days (median age = 338 days).

The sperm-rich fraction of the ejaculates was collected using the gloved hand technique. At the AI station, sperm motility and morphology were subjectively evaluated using a phase contrast microscope (Leica DM 4000B, Leica Microsystems, Wetzlar, Germany) at 37 °C, and ejaculates with <70% motile and/or >20% morphologically abnormal sperm were discarded. The total concentration of sperm cells was assessed by NucleoCounter® SP-100TM (ChemoMetec, 3450 Allerød, Denmark). Ejaculates approved by the quality check were diluted to achieve a concentration of 25 x 10^6 cells/mL in Androstar® Plus extender (Minitube, 84184 Tiefenbach, Germany), transferred to airtight tubes containing doses of 89 mL, and stored at 18 °C until shipment. Only semen assessed to acceptable for use with AI was used in this study. All doses were marked with donor ID, breed, and the last day of recommended use, which is the fourth day after collection. Semen was delivered to customers either by overnight mail, including domestic and international air transport, courier cars or buses, or customers obtained the semen at a drop point near the AI centre. During the 15 minutes of transportation from the AI station to the laboratory, the samples were packed in a styrofoam box to ensure a stable temperature. At the laboratory, semen was transferred to 15 mL falcon tubes and the samples were procured for CASA and ATP analysis on the day of semen collection (Day 0) and after storage at 18 °C for 96 hours (Day 4). In the current study, one to five ejaculates were collected from each boar (Table 1) and different ejaculates from the same boars were treated as separate samples.

2.2. Assessment of sperm motility

Sperm motility analysis was performed using Sperm Vision CASA system (SpermVision, Minitube GmbH,, Tiefenbach,Germany), with Leja-4 standardized counting chambers (Leja products, Nieuw-Vennep, the Netherlands) and analysed using a phase contrast microscope (Axio Lab.A1, Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with a Basler avA1000-120km 1024 x 1024 pixels digital camera (Basler Vision Technologies, Ahrensburg, Germany). The Sperm Vision and the Leja-4 slides were pre-warmed at 38 °C. Boar semen, diluted in Androstar® Plus extender, as described previously in this manuscript, was incubated at 38 °C for 10 min prior to CASA analysis. The capillary flow chambers of the Leja counting slides were filled with 3 µL pre-warmed semen. Individual samples were analysed (one chamber filled, one sample analysed) on the day of collection and after 4 days of storage at 18 °C, each in two parallel analyses. Analysis was performed on eight microscope fields with a total of at least 500 cells analysed per sample. A mean of the eight fields was used for statistical analysis. The sperm motility variables measured were total motility, progressive motility, hyperactive motility, VAP, VCL, VSL, STR, LIN, WOB, ALH, BCF and average orientation change of the head (AOC). The manufacturer's microscope settings for boar semen were used with sperm cell detection based on head area (35 μm^2-100 μm^2), 60 Hz frame rate and 30 frames captured per object. Sperm cells were defined as motile if AOC>7^o (manufacturer's default setting for boar semen). In addition, motile cells with VSL<10 µm/s were defined as locally motile and cells with VSL>10 µm/s as progressive motile. The criteria for hyperactive motility for each single sperm cell track were VCL>97 µm/s, ALH>3.5 µm, LIN<32% and WOB<71% (Schmidt and Kamp, 2004).

2.3. Assessment of ATP concentrations in semen

The ATP content in semen was determined using the CellTiter-Glo® Luminescent Cell Viability Assay (Technical Bulletin, Promega, 2012). Repeated evaluation assessments were performed to determine the optimal sperm cell number for the analysis. The standard curve was generated from ATP disodium salt hydrate (Sigma, A7699-1G) by dilution in phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.76 mM KH_2PO_4, 8.1 mM Na_2HPO_4 x $2H_2O$, pH 7.4.) Boar semen, diluted in Androstar® Plus extender, as described previously in this manuscript, was diluted 1:10 in PBS, and 50 µL was transferred to a white 96-well microtiter plate (NUNC™, Denmark). Subsequently 50 µL CellTiter-Glo® Reagent was added to each well and the mixture was gently shaken for 2 min in a rotary shaker (IKA® MS 3 digital, USA) to induce cell lysis. After a further 15 min of incubation at room temperature, bioluminescence measurement was performed using FLUOstar OPTIMA multiwell plate reader (BMG LABTECH GmbH, Offenburg, Germany) with MARS data analysis software (Version 1.10, BMG LABTECH, Germany). Samples were analysed on the day of collection and after 4 days of storage at 18 °C, each in three parallel analyses. The bioluminescence value for each sample, measured in relative luminescence units (RLU), was converted to the corresponding ATP value in nM using the standard curve values. The average of the three parallel analyses was used for statistical analysis.

2.4. Fertility records

Insemination dates of gilts and sows with doses from specific ejaculates were collected through the national litter recording system "ingris" (https://ingris.animalia.no/IngrisWeb) and semen storage time was determined based on semen collection date of each individual boar. Only herds that were situated geographically close enough for courier car or self-service

would be able to use doses on the day of semen collection. Insemination records indicating the doses that had been used more than 4 days post-collection were omitted from the data set. The litter records were included in the dataset provided they matched the semen collection dates at which the CASA and ATP analyses were performed. In addition, only litter results from purebred litters were included in the dataset. The total number piglets born (TNB) for each litter was calculated as the sum of liveborn and stillborn piglets and mummified foetuses. Litter records with zero TNB or with >29 TNB were deleted from the dataset collected using ingris. Among the females that farrowed between 109 and 125 days after the latest insemination date, only 6.3% and 6.0% of the preceding inseminations in NL and ND respectively, were performed on the day of semen collection. In contrast, 24.9% and 28.3% in NL and ND respectively, were performed on the recommended last day of usage. The semen samples collected and analysed in this study resulted in 677 NL and 166 ND purebred litters.

2.5. Statistical analyses

The analyses of TNB were divided into Day 0 and Day 4 within breed due to estimate effects affecting TNB at the day of collection (Day 0) and after storage at 18 °C for 96 hours (Day 4). The difference of TNB between the two breeds were large (13.8 and 8.9 for NL and ND, respectively) and, therefore, the two breeds were separated into different analyses.
The statistical analyses were performed using the software package Statistical Analysis Software (SAS) version 9.4 for Microsoft Windows (SAS Institute Inc., Cary, NC, USA). The CASA and ATP data were normally distributed (tested by Shapiro-Wilk test), and the results were analysed using the paired t-test for assessing storage effects (Day 0 and Day 4) and the unpaired t-test for assessing breed effects (NL compared with ND). Correlations (Pearson) were tested using PROC CORR. The results were considered statistically significant when

P<0.05. Boxplots were made using the RStudio version 3.4.0 (RStudio, Inc., 250 Northern Avenue Suite 420, Boston, Massachusetts 02210, US). The possible effects on TNB were analysed using the General Linear Model (GLM) procedure. First, the type of insemination (single/double), age of the boar, parity (divided into three classes; 1, 2 and >2), herd number, the storage time of the semen before insemination occurred (0-4 days), season (divided into four classes based on the four seasons in Norway (winter (December, January, February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November)), batch number (a measure for the arrival of the boars to the AI station, meaning that every group wise intake of boars is given a specific number) and interval class (the interval in days since previous collection from each boar (<4, 4, >4)) were tested in the model. Further, models were constructed by including the semen quality variables, measured at the Day 0 and Day 4, with a significant effect on TNB upon a correlation analysis (Table 4). Following this, a backwards selection approach was used where all the variables of interest from the correlation analyses (Table 4) were fitted in a model. The variable with the greatest *P*-value in the GLM analyses was excluded, if the variable had no significant effect. This re-fitting of the model was continued until all the variables were statistically significant (*P*<0.1). The final models were as follows:

Model 1) NL, Day 0: TNB = herd + LIN

Model 2) NL, Day 4: TNB = herd + WOB

Model 3) ND, Day 0: TNB = parity + batch number + % motile cells + VCL + ALH

Model 4) ND, Day 4: TNB = parity + batch number + LIN

3. Results

3.1. Assessment of sperm motility and ATP concentrations in semen

There was a significant effect of storage (Day 0 and Day 4) and breed (NL and ND) for both ATP-concentrations and CASA-variables. The mean values (± SD) of the kinematic sperm motility variables (VAP, VCL, VSL, STR, LIN, WOB, ALH and BCF) obtained from CASA for the two breeds are included in Tables 2 and 3.

For NL boars (Table 2), there were differences between Day 0 and 4 for all CASA variables, except percentage of progressively motile cells ($P = 0.63$). The *P*-values associated with the differences were <0.001, except for the difference in percentage motile cells for which it was <0.01. There was a change in ATP concentrations in semen between Days 0 and 4 ($P<0.0001$; Figure 1). The ND boars had a slightly different pattern than NL boars for changes in ATP concentrations between Days 0 and 4. Percentage motile cells, percentage progressive cells, VAP, VCL, VSL, ALH and ATP concentration were different from Day 0 to 4 ($P<0.001$), while the values for the other variables were not significantly different (Table 3 and Figure 1).

All the CASA variables and ATP measurements were different between the two breeds ($P<0.0001$) with two exceptions: VSL ($P = 0.09$) and WOB ($P = 0.09$) on Day 4. Due to the statistically significant difference between the breeds, all further calculations and interpretations were performed for each breed separately. Also within breeds there were individual differences in the ejaculates as depicted in Figure 1.

To estimate the correlations, the motility variables analysed by CASA were associated with the concentrations of ATP in the semen. Both sperm motility and progressive motility were positively but weakly correlated with the concentrations of ATP on Day 0 and 4 in both breeds (range correlation coefficient: 0.09 - 0.20). Thus, for the greatest concentrations of

ATP quantified in the semen there was a greater percentage of motile sperm and progressive cells, as depicted in Figure 2. In addition, VSL on Day 0 and WOB and BCF on Day 4 was positively, however, weakly correlated with the concentrations of ATP in NL (range correlation coefficient: 0.12-0.17, $P<0.05$). For ND, the variables VSL, STR, LIN, WOB and BCF on Day 0 and VSL, STR, LIN and WOB on Day 4 were positively, however, weakly correlated with concentrations of ATP (range correlation coefficient: 0.15-0.26, $P<0.05$; data not shown).

3.2. Field fertility, CASA variables and ATP concentrations

The total number of piglets born per litter (TNB) on the farms, using commercial semen doses at 0 to 4 days post collection of ejaculates sampled for the CASA and ATP measurements, constitute the field fertility in this analysis. The mean of TNB in NL was greater compared with the mean of TNB in ND ($P<0.0001$). The average TNB per litter was 13.8 (SD = 3.49) for NL and 9.0 (SD = 3.01) for ND. The minimum and maximum TNB were 2 and 24, and 1 and 16 for NL and ND, respectively. The Pearson correlation and *P*-values between the CASA variables and ATP concentrations measured and TNB were calculated (Table 4). There were significant correlations for several of the variables, however, the correlations were weak (range correlation coefficient: -0.065 to -0.17 and 0.077 to 0.20, for NL and ND respectively).

For further evaluation of the effects of the semen quality variables on TNB, a separate GLM for each breed was constructed. The correlation analysis indicated that the ATP concentration in NL on Day 0 was weakly correlated to TNB and, therefore, it was tested in the GLM. The effect of ATP, however, was not significant in the model and was, therefore, excluded. The CASA variable, LIN, had an effect on TNB in NL semen used on day 0 (Table

5) and were included in Model 1. In Model 2, the CASA variable, WOB, was included (Table 6), in Model 3 the CASA variables for motile sperm cells (%), VCL and ALH, were included (Table 7) and in Model 4 the CASA variable LIN was included (Table 8).

The NL model on Day 0 (Model 1) and Day 4 (Model 2) explained 18% and 19%, respectively, of the variability in TNB. While for ND, the Day 0 model (Model 3) and Day 4 model (Model 4) explained 26% and 23% of the variability in TNB, respectively. The *P*-values and R-square for the model are listed in Tables 5 through 8.

4. Discussion

The aim of the present study was to investigate sperm motility variables and ATP concentrations of boar semen from two breeds, NL and ND, and to evaluate the possible effect on field fertility in terms of TNB. Using CASA for measurement of sperm variables is an important aspect of the study compared to the use of traditional subjective methods of microscopic assessment of sperm motility. These subjective methods have been successful in terms of characterising samples with poor sperm motility. The CASA instrument, however, provides for an objective and more repeatable assessment of the number of motile sperm cells in a sample, as well as for measuring several other variables. This will not only allow for separation of samples with poor sperm motility, but can also be a useful technique in predicting the most desirable boars to be used in AI based on fertility variables. In the present study, there were differences both in the sperm motility variables measured by CASA and ATP concentrations in semen of the two breeds. There were also differences between the breeds where prediction variables were associated with TNB. In addition, there were differences in prediction variables for semen quality between Day 0 and 4 of semen storage within the breeds.

The largest difference between NL and ND was the percentage of hyperactive sperm cells. On average, ND had a greater percentage of hyperactive sperm cells on Day 0, but the difference between Days 0 and 4 was not statistically significant. In contrast, the percentage of hyperactive sperm cells in NL was much less, compared to ND, and increased significantly with storage (6.3% increase). Breed differences have been reported previously, in terms of differences in sperm physiology and semen plasma composition. For example, there are differences among breeds in the composition of fatty acids in the sperm cell membrane, capacitation and capacity for cryopreservation (Waterhouse et al., 2004; Waterhouse et al., 2006). Furthermore, differences in sperm shape and dimensions among pig breeds, including Landrace and Duroc, have been reported (Saravia et al., 2007) and small differences in head size and morphology can result in large differences in sperm hydrodynamics, thus effects on sperm motility variables (Dresdner and Katz, 1981). Breed differences in semen volume, number of total sperm and number of viable sperm has also been detected (Smital et al., 2004). Duroc boars have a greater sperm cell concentration as well as lesser semen volume compared to Landrace (Smital, 2009). Thus, the sperm of Duroc boars has a lesser volume of seminal plasma per cell compared to Landrace. Plasma proteins in the seminal plasma have several roles and effects (Flowers et al., 2016). For example, one of the groups of plasma proteins in seminal plasma, spermadhesins, bind to the acrosome and function to preserve membrane integrity, motility and mitochondrial activity (Gonzalez-Cadavid et al., 2014). These effects will possibly be less in Duroc semen as the ratio between seminal plasma and sperm is less, compared to Landrace semen.

In the present study, the ATP concentrations in the semen is correlated with several of the motility variables of sperm cells, including motility and progressive motility on Day 0 and 4 in both breeds. The correlation indicated that greater concentrations of ATP in the semen was associated with a greater percentage of motile cells and progressive cells. The connection

between concentration of ATP and motility of boar sperm has previously been explained by the amount of calcium in the cells, which affects the motility through regulation of the ATP concentration in the cell (Li et al., 2016). Loss of motility may be due to lesser ATP production in the cells and a decrease in ATP has been observed as a result of storage in different media (Jones and Bubb, 2000; Fraser et al., 2001; Gogol et al., 2009). This supports the results in the current study indicating that the decrease in the number of motile cells could be related to the decrease in ATP concentrations as a result of semen storage.

Hyperactive motility requires ATP for the vigorous swimming pattern of sperm cells (Suarez and Ho, 2003; Li et al., 2016). In the current study, therefore, it was of interest to evaluate a possible correlation between the percentage of hyper-activated sperm cells and the concentrations of ATP in semen. Only a non-significant positive trend, however, was observed for this correlation. A reason for not detecting an association between ATP concentrations in semen and sperm motility might be that the cells are continuously synthesising and using ATP (Medrano et al., 2006). A previous study reported that even though the percentage of motile sperm cells decreased as a result of short-time storage, the sperm cells may still be able to maintain the potential to obtain a hyperactive motility (Henning et al., 2014). In addition, in the current study the ATP measurements were conducted in semen including both intracellular and extracellular ATP. The lack of relationship between the ATP content and the percentage of hyperactive cells in this study could, therefore, be concealed by a greater concentration in extracellular ATP. In previous studies, however, the ATP content in seminal plasma was negligible (Long and Guthrie, 2006).

Significant differences in most of the sperm motility variables and semen ATP concentrations were observed between Day 0 and Day 4. The difference between Day 0 and Day 4 is important because semen rarely is used for AI on the day of production. Semen is

frequently transported for large distances and will in most cases not be available for AI until the day after collection. The general recommendation is to use the semen within 96 hours, which was the reason for using the Day 4 time for semen quality analysis in the current study. Some farmers, however, use AI doses that have been stored for longer than the recommended 96 hours before use. Individual variation in boar semen storage capacity has been observed in both an *in vitro* study evaluating long-term semen-extenders (Waterhouse et al., 2004) and in an *in vivo* study using a short-term semen-extender (Haugan et al., 2005). The effect of storage time for semen on TNB, therefore, was assessed in the present study, however, there was no significant effect of storage time on TNB. In addition, the magnitude of the CASA variable-changes between Day 0 and 4 had no effect on TNB.

The percentage of motile cells has an effect on TNB (Vyt et al., 2008; Broekhuijse et al., 2012a). In the present study, a small positive effect of the percentage motile cells on TNB was detected. This effect, however, was only significant in ND at Day 0. In addition, the CASA variables, VCL and ALH, influenced TNB in ND at Day 0 with a negative and positive effect, respectively. In ND on Day 4, the CASA variable, LIN, had a significant positive effect on TNB. In addition, this variable had a significant positive effect in NL on Day 0 of semen storage. For NL on Day 4, only WOB had a significant positive effect on TNB. This indicates that there are differences in the motility patterns in NL and ND that affect TNB. In addition, it indicates that the specific sperm motion variables that were analysed by CASA are related to TNB.

Altogether, results of the present study indicate that sperm in ejaculates from NL boars had a lesser forward pattern at 96 hours of storage as evidenced by greater VCL and ALH values, and lesser LIN and WOB values. This has an unfavourable effect on TNB and, therefore, it is suggested that the management of the AI station should consider the LIN and WOB values in ejaculates from NL boars, in addition to general motility evaluation, for

decision of approval or rejection of ejaculates for use in AI. For ND, lesser LIN values and greater VCL and ALH values compared to NL indicate that the ND sperm are in transition to "hyperactive-like" motility already at the time of semen collection. In addition, results of the present study indicate that a more straightforward motility pattern has a positive effect on TNB. Thus, in addition to motility in general, the AI station should consider the values for LIN, VCL and ALH when assessing ejaculates from ND boars for deciding whether or not to use ejaculates for AI. Altogether, the variables VCL, ALH, LIN and WOB affected TNB when using one or more of the models for assessments and all these variables are among the threshold values defining the swimming pattern of hyperactive boar sperm (Schmidt and Kamp, 2004). This possible association between hyperactive sperm motility and TNB in pigs has to our knowledge, not been previously reported.

In total, the models using in the present study explained 18% to 26% of the variation in TNB. This association may be considered to be negligible. In a previous large-scale study, however, the direct boar effects explained only 6.6% of the variation in TNB, and semen quality variables were only a small part of the direct boar effect (Broekhuijse et al., 2012b). In addition, it has been estimated that a difference of only 0.2 piglets in TNB is of great economic relevance (Roca et al., 2015). In controlled studies for detection in fertility differences, a high degree of standardization is needed with respect to boars, breed, age, semen collection frequency, semen extender used, semen age and basis for exclusion of samples (Amann et al., 2018). In the present study, based on data from a full-scale commercial AI station and its customers, these aspects were considered.

In conclusion, in the current study there were differences between the NL and ND breeds in terms of the CASA variables and effect on TNB. Although motility is the most widely used sperm quality variable at AI stations, results in the present study indicate that, surprisingly, only in the ND ejaculates was sperm motility on Day 0 associated with TNB.

Several of the CASA variables with threshold values defining hyperactive sperm motility were associated with TNB in both breeds. It, therefore, is suggested that these variables should be taken into consideration when evaluating the fertility potential and approval of the ejaculates for AI use.

Conflict of interest

The authors have no conflicts of interest to declare.

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Table 1

Number of ejaculates per boar in the breeds Norwegian Landrace (NL) and Norwegian Duroc (ND)

No. of ejaculates per boar	1	2	3	4	5
NL (103 boars)	27	33	31	7	5
ND (88 boars)	33	27	22	4	2

Table 2

Means (± SD), minimum and maximum values for the kinematic sperm motility variables in ejaculates from 103 Norwegian Landrace boars (*n* = 239) on the day of collection (Day 0) and after 96 hours of storage (Day 4)

	Day 0			Day 4		
Variable	Mean ± SD	Min	Max	Mean ± SD	Min	Max
VAP (µm/s)	50.32 ± 6.11	35.09	71.83	54.53 ± 7.01	39.48	74.25
VCL (µm/s)	99.38 ± 15.00	66.83	154.58	114.14 ± 16.86	77.69	171.42
VSL (µm/s)	41.33 ± 4.52	29.57	57.14	42.57 ± 4.60	32.65	57.02
STR (%)	82.06 ± 3.24	73.00	89.00	78.09 ± 3.92	64.00	86.00
LIN (%)	41.73 ± 3.72	32.00	51.00	37.39 ± 3.10	28.00	46.00
WOB (%)	50.64 ± 2.72	44.00	58.00	47.66 ± 1.89	43.00	54.00
ALH (µm)	2.67 ± 0.48	1.80	4.43	3.24 ± 0.58	2.04	5.32
BCF (Hz)	32.07 ± 2.54	26.68	40.88	30.21 ± 2.82	23.13	37.69

Table 3

Means (± SD), minimum and maximum values for the kinematic sperm motility variables in ejaculates from 88 Norwegian Duroc boars (*n* = 179) measured on the day of collection (Day 0) and after 96 hours of storage (Day 4)

	Day 0			Day 4		
Variable	Mean ± SD	Min	Max	Mean ± SD	Min	Max
VAP (µm/s)	54.37 ± 6.32	38.14	67.17	57.14 ± 5.73	40.21	72.68
VCL (µm/s)	114.48 ± 15.50	76.57	146.10	120.29 ± 13.98	81.57	156.65
VSL (µm/s)	39.80 ± 3.94	29.72	49.34	41.85 ± 3.70	30.25	51.52
STR (%)	73.20 ± 3.68	64.00	84.00	73.21 ± 4.72	62.00	84.00
LIN %)	34.78 ± 2.95	25.00	44.00	34.80 ± 3.49	28.00	44.00
WOB (%)	47.40 ± 1.86	4.00	53.00	47.34 ± 1.95	41.00	52.00
ALH (µm)	3.80 ± 0.54	2.55	5.04	4.02 ± 0.60	2.34	5.51
BCF (Hz)	26.47 ± 1.88	22.74	31.55	26.28 ± 2.65	19.92	32.72

Table 4

The Pearson correlation coefficient (corr) and *P*-values for the correlations between the total number of piglets born (TNB), ATP and CASA variables for Norwegian Landrace and Norwegian Duroc on the day of collection (Day 0) and after 96 hours of liquid storage (Day 4)

	NORWEGIAN LANDRACE				NORWEGIAN DUROC			
	Day 0		Day 4		Day 0		Day 4	
Variable	Corr	*P*-value	Corr	*P*-value	Corr	*P*-value	Corr	*P*-value
ATP (nM)	-0.075	0.050[a]	-0.020	0.61	0.12	0.11	0.068	0.39
Motile (%)	0.049	0.20	0.082	0.034[a]	0.20	0.0089[a,b]	0.18	0.017[a]
Progressive (%)	0.078	0.043[a]	0.091	0.017[a]	0.16	0.044[a]	0.17	0.029[a]
Hyperactivated (%)	0.060	0.12	0.082	0.033[a]	-0.096	0.22	-0.13	0.11
VAP (µm/s)	0.033	0.39	0.050	0.19	-0.15	0.048[a]	-0.099	0.20
VCL (µm/s)	-0.030	0.44	0.019	0.62	-0.18	0.022[a,b]	-0.16	0.046[a]
VSL (µm/s)	-0.071	0.064[a]	-0.011	0.77	-0.11	0.16	0.010	0.90
STR (%)	0.013	0.73	0.044	0.25	0.16	0.036[a]	0.17	0.031[a]
LIN (%)	0.11	0.0049[a,b]	0.044	0.25	0.17	0.32[a]	0.19	0.012[a,b]
WOB (%)	0.13	0.001a	0.077	0.045[a,b]	0.15	0.060[a]	0.20	0.011[a]
ALH (µm)	0.12	0.0014[a]	0.11	0.0052[a]	-0.15	0.047[a,b]	-0.17	0.027[a]
BCF (Hz)	-0.065	0.092[a]	-0.023	0.55	0.070	0.34	0.16	0.044[a]

Note: Significance of correlation ($P<0.1$) is indicated by [a] and variables with a significant effect ($P<0.1$) in general linear models are indicated by [b]

Table 5

Degrees of freedom (DF), sum of squares (SS), mean squares, *F* values and *P*-values (*P*>*F*) for the variables with a significant effect in Norwegian Landrace on Day 0 on the total number of piglets born

Source	DF	SS	Mean Square	*F* Value	*P* > F
Herd	61	1392.58	22.83	2.09	<.0001
LIN	1	117.22	117.22	10.73	0.0011

Note: R^2 for the model is 0.19

Table 6

Degrees of freedom (DF), sum of squares (SS), mean squares, *F* values and *P*-values (*P*>*F*) for the variables with a significant effect in Norwegian Landrace on Day 4 on the total number of piglets born

Source	DF	SS	Mean Square	*F* Value	*P* > F
Herd	61	1392.58	22.83	2.09	<.0001
WOB	1	69.48	69.48	6.32	0.012

Note: R^2 for the model is 0.18

Table 7

Degrees of freedom (DF), sum of squares (SS), mean squares, *F* values and *P*-values (*P*>*F*) for the variables with significant effect in Norwegian Duroc on Day 0 on the total number of piglets born

Source	DF	SS	Mean Square	*F* Value	*P* > F
Parity	2	81.76	40.88	5.28	0.0061
Batch number	14	192.83	13.77	1.78	0.047
Motile sperm cells (%)	1	41.60	41.60	5.37	0.022
VCL	1	63.45	63.45	8.19	0.0048
ALH	1	34.45	34.45	4.45	0.037

Note: R^2 for the model is 0.26

Table 8

Degrees of freedom (DF), sum of squares (SS), mean squares, *F* values and *P*-values (*P*>*F*) for the variables with a significant effect in Norwegian Duroc on Day 4 on the total number of piglets born

Source	DF	SS	Mean Square	*F* Value	*P* > *F*
Parity	2	82.23	41.11	5.19	0.0066
Batch number	14	226.35	16.17	2.04	0.018
LIN	1	53.22	53.22	6.72	0.011

Note: R^2 for the model is 0.23

Figure legends:

Fig. 1. Percentage of motile sperm, progressive sperm, hyperactive sperm and concentrations of ATP measured in boars of two breeds, Norwegian Landrace (NL) and Norwegian Duroc (ND), at the day of collection (Day 0) and after 96 hours of storage (Day 4) Significance levels are indicated: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

Fig. 2. The relationship between measured ATP concentrations in semen and motility variables measured by computer assisted semen analysis (CASA) in boars of two breeds, Norwegian Landrace (NL) and Norwegian Duroc (ND) on the day of collection (Day 0) (A, C and E) and after 96 hours of storage (Day 4) (B, D, and F)