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Correlação entre as características espermáticas avaliadas por um sistema computadorizado (CASA) e o desempenho reprodutivo de fêmeas suínas

Correlation between the sperm characteristics evaluated by a computerized system (CASA) and the reproductive performance of sows

Correlación entre las características espermáticas evaluadas por un sistema computadorizado (CASA) y rendimiento reproductivo de cerdas

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Resumo

Os sistemas de avaliação computadorizada de sêmen (CASA) têm sido uma das ferramentas mais utilizadas para avaliar a cinética dos espermatozoides. O objetivo deste estudo foi

estimar a correlação entre as características de movimento espermático avaliadas pelo CASA durante 72 horas de refrigeração com a taxa de parição (FR) e número total de leitões nascidos (TNB) após inseminação artificial. Fêmeas multíparas (n=464) foram inseminadas com sêmen de sete machos (19,6 ± 1.3 ejaculados/macho). Os parâmetros de movimento espermático foram determinados logo após a diluição e após 24, 48 e 72 horas de refrigeração a 15°C: motilidade total (TM-%), motilidade progressiva (PM-%), velocidade curvilínea (VCL-µm/s), velocidade linear progressiva (VSL-µm/s), velocidade média da trajetória (VAP-µm/s), amplitude de deslocamento lateral de cabeça (ALH-µm), frequência de batimento flagelar cruzado (BCF-Hz), retilinearidade (STR-%) e linearidade (LIN-%). O coeficiente de correlação de Pearson foi aplicado para análise dos dados e a comparação das médias das características entre os cachaços foi realizada pelo teste de Tukey a 5% de probabilidade. TM e PM no momento zero (T0) foram significativos e tiveram correlação moderada a alta com a FR e TNB. Após 72 horas de refrigeração, a qualidade do sêmen foi reduzida e mostrou significativa e baixa correlação com TM e PM com estes mesmos parâmetros. O cachaço que apresentou menor valor de TM e PM após a diluição obteve menor FR e TNB. Conclui-se que a análise computadorizada de sêmen logo após a diluição pode ser usada para predizer a fertilidade de cachaços.

Palavras-chave: Fertilidade; Leitões nascidos totais; Movimento espermático; Taxa de parição.

Abstract

Computer-assisted semen analysis (CASA) systems have been one the most used tools to evaluate sperm kinetics. The objective of this research was to estimate the correlation between sperm motility characteristics evaluated by CASA during 72 hours of cooling with the farrowing rate (FR) and total number of piglets born (TNB) after artificial insemination. Multiparous sows (n=464) were inseminated with semen from seven boars (19.6 ± 1.3 ejaculates/male). Sperm motility parameters were determined immediately after dilution and after 24, 48 and 72 hours of cooling at 15°C: total motility (TM-%), progressive motility (PM-%), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), average path velocity (VAP-µm/s), amplitude of lateral head displacement (ALH-µm), flagellar beat cross frequency (BCF-Hz), straightness (STR-%) and linearity (LIN-%). Pearson's correlation coefficient was applied to analyze the data and the comparison of the means of the sperm characteristics between the boars was done by Tukey's test at 5% probability. TM and PM at time zero (T0) were significant and had a moderate to high correlation with FR and TNB. After 72 hours of refrigeration, the semen quality was reduced and showed a significant and low correlation of the TM and PM with these same parameters. The boar presenting the lowest value of TM and PM after

dilution obtained lower FR and TNB. In conclusion, computer-assisted semen analysis soon after dilution can be used to predict fertility of boars.

Keywords: Farrowing rate; Fertility; Sperm movement; Total born piglets.

Resumen

Los sistemas computarizados de evaluación de semen (CASA) han sido una de las herramientas más utilizadas para evaluar la cinética de los espermatozoides. El objetivo de este estudio fue estimar la correlación entre las características de movimiento de esperma evaluadas por CASA durante 72 horas de refrigeración con la tasa de parto (RF) y el número total de lechones nacidos (TNB) después de la inseminación artificial. Las hembras multíparas (n = 464) fueron inseminadas con semen de siete machos (19.6 ± 1.3 eyaculado/macho). Los parámetros del movimiento de los espermatozoides se determinaron inmediatamente después de la dilución y después de 24, 48 y 72 horas de refrigeración a 15°C: motilidad total (TM-%), motilidad progresiva (PM-%), velocidad curvilínea (VCL-µm/s), velocidad (VAP-µm/s), velocidad media de la trayectoria (VAP-µm/s), amplitud de desplazamiento lateral de cabeza (ALH-µm), frecuencia de latido flagelar cruzado (BCF-Hz), rectilinealidad (STR-%) y linealidad (LIN-%). El coeficiente de correlación de Pearson se aplicó para el análisis de datos y la comparación de las medias de las características entre los verracos se realizó mediante la prueba de Tukey con una probabilidad del 5%. TM y PM en el tiempo cero (T0) fueron significativas y tuvieron una correlación moderada a alta con RF y TNB. Después de 72 horas de refrigeración, la calidad del semen se redujo y mostró una correlación significativa y baja con TM y PM con estos mismos parámetros. El verraco que presentó menor valor de TM y PM después de la dilución obtuvo FR y TNB más bajos. Se concluye que el análisis computarizado de semen inmediatamente después de la dilución puede usarse para predecir la fertilidad de los verracos.

Palabras clave: Fertilidad; Lechones nacidos totales; Movimiento espermático; Tasa de parición.

1. Introdução

The use of artificial insemination (AI) contributes to the success of the swine industry as it assists in the dissemination of genetic progress. The selection of young breeders is based on their genetic values of production characteristics (Marques, et al., 2017).

The breeder fertility can be affected by several factors, such as factors inherent to sperm production, ejaculate processing in the laboratory, genetic factors and factors related to matrix quality and insemination process (Kummer, et al., 2013).

Semen quality is extremely important as each breeder participates in a large number of services throughout the year. Thus, a good assessment of semen quality should be reliable. In addition, this assessment may help identify causes of low fertility (Petrocelli, et al., 2015).

The routine assessment of ejaculates used to prepare the insemination doses (ID), such as sperm motility and morphology, has made it possible to discard ejaculates of low quality and reduced fertilization capacity (Popwell & Flowers, 2004).

The reduction in the number of spermatozoa per dose or per inseminated female associated with the use of homospermic AI may show both male with low fertility and those with superior fertility that would go unnoticed using heterospermic AI and doses with the traditional sperm count (Dyck, et al., 2011).

When evaluating the farrowing rate and the litter size from homospermic AI, it is possible to retrospectively analyze reproductive performance of the boar (Foxcroft, et al., 2010). However, the high cost of testing *in vivo* fertility and the delay in obtaining fertility information from males has driven studies that seek *in vitro* evaluation methods (Kummer, et al., 2013).

Computer-assisted semen analysis (CASA) systems have been shown to be a useful tool in the kinetic assessment of spermatozoa, since they have great potential to predict male fertility by correlating the movement speed of the cells with the capacity to fertilize oocytes (Cox, et al., 2006).

In this context, the aim of this study was to identify the correlation between the sperm motility characteristics assessed by CASA system, determined in *in vitro* tests during semen storage with reproductive fertility, evaluated *in vivo*.

2. Metodologia

This is a quantitative, experimental and applied research with explanatory purpose. According Pereira, et al. (2018), a research has a quantitative approach when it uses the collection of data that are later analyzed by statistical methods to verify the relationships between variables. In this case, the seminal samples of each boar were analyzed and compared using statistical methods.

In addition, it can be classified as an experimental field research of an applied nature, as it was carried out in a pig farm in which the conditions were adapted to verify the sperm characteristics related to the reproductive performance of the gilts. According to Gerhardt & Silveira (2009), experimental field research creates conditions for manipulating subjects in

their own organizations, communities or groups. Furthermore, research is applied when it aims to generate knowledge for practical application, aimed at solving specific problems. In this study, the research is aimed at solving problems of dissemination of boar genetics that can bring losses in the farrow rate and number of piglets born.

The purpose of the explanatory research is concerned with identifying the factors that determine or contribute to the occurrence of the phenomena under study (Gil, 2017). In this case, we tried to connect the sperm characteristics measured by CASA with the individual fertility of the boars.

The research project was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Goiás, under the protocol number 028/14.

Local, Animals, Housing and Food

The experiment was carried out at the Artificial Insemination Center (AIC) of the company BRF S.A. in the city of Rio Verde, State of Goiás, Brazil, at a farm of the integrated pig production system of BRF S.A., with approximately 2,500 females ± 100, located in the southwest region of the State of Goiás, latitude 18°17'04.11"5 and longitude 51°05'45.47"0. The period experiment took place in the months of July to November 2013.

Seven boars of PIC® (PIC, Hendersonville, TN, USA) with an average age (±standard deviation) of 11 ± 1 month of age, kept in the AIC of BRF S.A. were housed in individual cages in a shed with controlled average temperature (±standard deviation) of 23 ± 5°C, receiving water *ad libitum* and corn-soy diet formulated to meet specific nutritional needs for reproduction (13.5% crude protein and 3210 kcal of metabolizable energy/kg), according

For the *in vivo* fertility test, performed in an integrated piglet production system (SPL) farm of BRF S.A., 464 multiparous females of PIC® (PIC, Hendersonville, TN, USA) were housed in individual cages (2.4 x 0.86 m) during gestation and farrowing in the farm of the piglet production system. The temperature control was done with a duct fan system and curtains management. Females had access to water *ad libitum* and were fed a corn-soy diet formulated to meet nutritional needs during reproduction, gestation and lactation (13.5% crude protein and 3210 kcal of metabolizable energy/kg).

Semen Collection and Processing

The boars selected for the experiment had a maximum of 4% of morphologically abnormal spermatozoa and a minimum 80% of total motility, according to the protocol established by the partner company for the use of ejaculates.

Semen collections were carried out for twenty-two weeks, once a week.

The semen was collected through the gloved hand technique with exposure of the penis of the animal which was directed to a disposable plastic cup preheated at 36°C, covered by a double filter to remove the gel fraction from the ejaculate.

Soon after the collection, the weight, the total motility and sperm concentration of the ejaculate was evaluated to calculate the number of insemination doses (ID) to be produced. The percentage of total motility (TM) and sperm concentration were obtained by the CASA system (Sperm Vision® 3.7, Minitüb GmbH, Tiefenbach, Germany). The semen sample was prepared in a microcentrifuge tube using an electronic pipette with 1:9 dilution (90 µL fresh semen and 810 µL preheated diluent). After dilution, the semen was agitated 10 times and a 3 µL aliquot containing 90000 sperm was taken for analysis in the CASA System, using counting chambers (Leja-4, Minitüb GmbH, Tiefenbach, Germany). Soon afterwards, eight fields along the central line of the slide were analyzed at 200X magnification.

The semen was diluted in Beltsville Thawing Solution diluent (BTS®, Minitüb GmbH, Tiefenbach, Germany) to obtain the insemination doses with 1.5 billion spermatozoa in 50 mL. These doses were placed in plastic tubes, sent to the SPL farm, and stored at 15°C for up to 72 hours for the in vivo assessment of semen fertility.

Three semen samples, of each ejaculated, after dilution, were conditioned in a microcentrifuge tube (2 mL) for further analysis.

Semen analysis

The sperm motility characteristics were evaluated after dilution (T0) after 24 (T24), 48 (T48) and 72 (T72) hours of refrigeration at 15°C. Before any analysis, samples were incubated for 10 minutes in a water bath at 37°C.

The analysis of sperm motility characteristics was performed using computer assisted semen analysis using the CASA system (Sperm Vision® 3.7, Minitüb GmbH, Tiefenbach, Germany). The following parameters of sperm motility were analyzed: total motility (TM-%), progressive motility (PM-%), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), average path velocity (VAP-µm/s), amplitude of lateral head displacement (ALH-µm), flagellar beat cross frequency (BCF-Hz), straightness (STR-%) and linearity (LIN-%).

Fertility test

Only females without reproductive problem, from the second to sixth farrow were used, ranging from three to four days between weaning and estrus.

After weaning, estrus was evaluated daily, once daily through the reflex of tolerance to the man in the presence of the male.

Females were randomly inseminated using the intrauterine method with semen from a single boar (homospermic), using doses produced from the same ejaculate, containing 1.5 billion spermatozoa in 50 mL. The first insemination was performed 12 hours after estrus detection and the other inseminations were performed at 24-hour intervals. Only females inseminated two- or three-times during estrus were considered in the experiment. Inseminations were performed with semen doses refrigerated for 24, 48 or 72 hours, depending on the number of doses used for each female, as a consequence of estrus duration. The farrowing rate and the total number of piglets born were evaluated using the Pig Champ Care® data program (PIC, Hendersonville, TN, USA) for data capture.

Data analysis

For statistical analysis of the data were used the software R 3.0.1, 2013 (R Core Team, 2013).

Pearson correlation coefficient was used to estimate the association between the sperm motility characteristics analyzed and farrowing rate and total number of piglets born after dilution, 24, 48 and 72 hours of refrigeration.

The means of sperm motility characteristics between the boars, after dilution and after 24, 48 and 72 hours of refrigeration were compared by Tukey's test at 5% probability.

3. Results and Discussion

A total of 137 ejaculates were collected form seven males, with an average semen collections per boar (±standard deviation) of 19.6 ± 1.3. The mean number of females inseminated per boar (±standard deviation) was 66 ± 12.

The main finding of this study was that the fertility of boars can be effectively predicted by means of computer-assisted semen analysis soon after dilution. In the current pig industry, the in vitro assessment of total motility and progressive motility, besides allowing the discard of low-quality ejaculates, is related to the farrowing rate and number of piglets born. Thus, the use of evaluations by computer systems eliminates the subjective effects of the analysis, allowing the identification of high fertility breeders to plan, optimize and maximize the use of material of higher genetic value, increasing productivity.

Table 1 shows the Person correlation between the parameters of sperm characteristics with farrowing rate (FR) and total number of piglets born (TNB) evaluated immediately after dilution (T0), 24 (T24), 48 (T48) and 72 hours (T72) of cooling of the insemination dose at 15°C.

The Pearson correlation coefficient in the present study revealed that, after dilution (T0), TM and PM parameters presented highly significant moderate to high correlation with the farrowing rate and the total number of piglets born (Table 1). After 72 hours of refrigeration, the semen quality reduced and showed a low correlation of the TM and PM with these same parameters (Table 1). However, at times T24, T48 and T72, was found low correlation for the parameters evaluated with farrowing rate and total number of piglets born (Table 1).

Table 1. Pearson correlation between the parameters of total motility (TM-%), progressive motility (PM-%), average path velocity (VAP-µm/s), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), straightness (STR-%), linearity (LIN-%), amplitude of lateral head displacement (ALH-µm) and flagellar beat cross frequency (BCF-%) with farrowing rate (FR) and total number of piglets born (TNB) evaluated immediately after dilution (T0), 24 (T24), 48 (T48) and 72 hours (T72) of cooling of the insemination dose at 15°C.

Parameter	T0		T24		T48		T72	
	FR	TNB	FR	TNB	FR	TNB	FR	TNB
TM	0.6906**	0.7143**	0.2834	0.3533	0.2555**	0.3489	0.1874*	0.2936**
PM	0.6515**	0.6620**	0.2432	0.31111	0.2602*	0.3159	0.2130*	0.2832**
VAP	0.2783**	0.3169	0.0521	0.0185	-0.0485	-0.1000	0.0106	0.0342
VCL	0.1654*	0.0227	-0.0644	-0.1000	-0.1180	-0.1366	-0.0283	0.0086
VSL	0.3073**	0.2775	0.1470	0.1244	0.0469	-0.0199	0.0739	0.1034
STR	0.1197	0.0074	0.3723	0.3789	0.2795**	0.2350	0.1880*	0.2123*
LIN	0.1939*	0.0825	0.3312	0.3294	0.2311*	0.1554	0.1688*	0.1396
ALH	0.2212*	0.3221	-0.1968	-0.2551	0.2838**	0.0287**	-0.1765*	-0.0172*
BCF	0.0667	0.0229	0.1619	0.1291	0.0429	0.0478	0.0832	0.1274

*P <0.05; **P <0.01.

A similar result was reported by Lima, et al. (2015), who observed a low correlation between TM and the total number of fetuses born and the number of live-born piglets, evaluating semen samples in which sperm viability reduced with the passage of time of refrigeration. It is known that increase in refrigeration time, result in a reduction in semen

quality which may be due to decrease in semen adenosine triphosphate (ATP) concentration during storage, due to sperm metabolism (Tremoen, et al., 2018). Thus, as in this study there was a highly significant positive correlation between the sperm motility characteristics (TM and PM) on the FR and TNB only at time zero (T0), it is believed that there is no need for several moments (T24, T48, T72) of semen assessment to predict the fertility of the male.

Table 2 shows mean values and standard deviation of sperm parameters of boars shortly after dilution (T0).

Table 2. Mean values and standard deviation of sperm parameters: total motility (TM-%), progressive motility (PM-%), average path velocity (VAP-µm/s), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), straightness (STR-%), linearity (LIN-%), amplitude of lateral head displacement (ALH-µm) and flagellar beat cross frequency (BCF-Hz) of boars shortly after dilution (T0).

BOAR	A	B	C	D	E	F	G
TM	96.39±1.05^{a}	96.31±1.19^{a}	86.89±3.66^{b}	95.13±1.61^{a}	96.70±1.67^{a}	95.15±2.18^{a}	96.27±2.07^{a}
PM	91.75±2.32^{a}	91.25±2.11^{a}	77.57±5.34^{b}	89.18±3.35^{a}	91.54±2.80^{a}	89.56±4.13^{a}	90.76±3.08^{a}
VAP	92.36±9.91^{a}	93.47±10.86^{a}	78.82±9.78^{c}	95.00±7.95^{a}	89.11±10.34^{ab}	81.21±11.48^{bc}	92.30±10.14^{a}
VCL	157.70±22.38^{a}	155.37±19.81^{ab}	137.80±16.38^{bc}	153.71±14.34^{ab}	153.68±20.45^{ab}	129.70±18.28^{c}	159.44±20.37^{a}
VSL	63.33±8.00^{a}	61.05±7.50^{a}	52.70±7.76^{b}	64.72±6.23^{a}	52.92±5.60^{b}	59.05±8.64^{ab}	60.79±8.20^{a}
STR	68.09±0.02^{b}	64.95±0.02^{c}	65.59±0.03^{bc}	67.67±0.04^{bc}	58.62±0.02^{d}	72.23±0.02^{a}	65.49±0.04^{bc}
LIN	40.09±0.03^{bc}	39.05±0.02^{bc}	38.18±0.04^{c}	41.95±0.03^{b}	33.81±0.02^{d}	44.91±0.02^{a}	37.86±0.03^{c}
ALH	4.40±0.41^{b}	4.42±0.28^{b}	3.74±0.29^{c}	4.55±0.61^{ab}	4.97±0.51^{a}	3.54±0.39^{c}	4.73a±0.51^{b}
BCF	34.71±2.25^{ab}	34.51±1.70^{ab}	34.60±2.73^{ab}	34.60±2.19^{ab}	32.56±2.28^{b}	35.59±2.54^{a}	33.68±2.24^{ab}

*Different letters in the same row indicate significant differences at P <0.05.

It was observed that the boar C presented lower TM (86.89±3.66) and PM (77.57±5.34) at time zero (T0, Table 2) and had lower FR (20.37±38.87) and TNB (9.45±5.02) than others males. Variability in the relation between semen quality and fertility estimates may be due to individual boar differences reason (Popwell & Flowers, 2004). Kommisrud, et al. (2002) identified this individual influence on concentration and motility several years ago.

In this study, the AI was evaluated with homospermic doses, independent of cooling time, in order to evaluate the actual reproductive potential of each breeder and to identify subfertile breeders, since low fertility performance in the field cannot be tolerated in commercial production.

At the other moments (T24 – Table 3, T48 – Table 4 and T72 – Table 5), there was a difference in the parameters evaluated among all males. However, there was no association of

these results with the farrowing rate and the total number of piglets born. Some boars had low TM and PM at these sampling periods without interference in the farrowing rate and total number of piglets born.

Table 3 reveals mean values and standard deviation of sperm parameters of boars after 24 hours (T24) of cooling at 15°C.

Table 3. Mean values and standard deviation of sperm parameters: total motility (TM-%), progressive motility (PM-%), average path velocity (VAP-µm/s), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), straightness (STR-%), linearity (LIN-%), amplitude of lateral head displacement (ALH-µm), flagellar beat cross frequency (BCF-Hz), farrowing rate (FR-%) and total number of piglets born (TNB) of boars after 24 hours (T24) of cooling at 15°C.

BOAR	A	B	C	D	E	F	G
TM	88.63±3.80^{a}	78.34±9.94^{b}	64.37±7.52^{d}	82.33±6.73^{ab}	88.08±4.63^{a}	68.03±13.72^{cd}	73.68±11.46^{bc}
PM	79.56±6.57^{a}	63.04±15.56^{bc}	47.09±10.46^{d}	68.5±9.93^{ab}	77.67±8.05^{a}	51.11±18.46^{cd}	57.78±17.14^{bcd}
VAP	59.28±13.10^{a}	49.57±12.01^{ab}	47.89±6.07^{b}	49.83±10.45^{ab}	53.98±12.52^{ab}	43.91±11.38^{b}	44.73±11.73^{b}
VCL	91.95±17.86^{a}	81.01±22.13^{ab}	85.22±12.64^{ab}	79.39±16.75^{ab}	91.09±19.40^{a}	72.58±22.08^{b}	72.14±19.38^{b}
VSL	45.52±10.99^{a}	35.16±7.23^{b}	33.07±5.68^{b}	37.73±8.18^{ab}	38.53±8.67^{ab}	32.40±7.63^{b}	33.85±8.77^{b}
STR	75.13±0.04^{a}	71.09±0.07^{abc}	67.80±0.05^{c}	74.53±0.03^{ab}	71.48±0.04^{bc}	73.64±0.04^{ab}	75.10±0.03^{ab}
LIN	48.26±0.07^{a}	44.05±0.09^{abc}	38.35±0.06^{c}	47.10±0.04^{ab}	42.52±0.05^{bc}	45.32±0.06^{ab}	46.55±0.03^{ab}
ALH	2.85±0.49^{ab}	2.74±0.77^{ab}	2.97±0.39^{a}	2.48±0.39^{ab}	2.75±0.47^{ab}	2.39±0.53^{b}	2.34±0.54^{b}
BCF	30.62±3.38^{a}	25.77±4.37^{bc}	24.62±3.97^{bc}	27.19±5.26^{abc}	28.98±3.69^{ab}	23.13±5.86^{c}	24.47±5.14^{c}
FR	93.51±14.02^{a}	89.55±14.49^{a}	20.37±38.87^{b}	92.59±13.48^{a}	91.55±17.52^{a}	94.44±9.60^{a}	96.51±3.80^{a}
TNB	12.54±2.22^{a}	12.68±2.19^{a}	9.45±5.02^{b}	13.26±2.14^{a}	12.23±1.90^{a}	12.38±2.59^{a}	13.15±2.36^{a}

*Different letters in the same row indicate significant differences at P <0.05.

In this study, male C presented the lowest TM and PM after dilution and 24 hours of storage, having presented smaller FR and TNB compared to others males. This fact confirms the high correlation found in this study between TM and PM after dilution (Table 3).

In addition to male inherent factors, gilts also influence fertilization success, since as there is a great variation duration of estrus and ovulation in swine (Soede, 1993; Knox & Rodriguez-Zas, 2001) and viability of the oocytes (Soede, et al., 1992). Consequently, reproductive performance of sows is also affected by the number of inseminations (Niyiragira, et al., 2018). Thus, AI protocols consider insemination until 24h before ovulation as the optimal time to achieve a good fertility (Kemp & Soede, 1996; Bennemann, et al., 2005).

Although recent research reports that single AI and double AI protocol give relatively similar conception rates and litter size, and does not influence the sex in offspring (Natumanya, et al., 2018). Nevertheless, in Japan, approximately 90% of the herds use artificial insemination with two or three inseminations per female during the estrous period (Iida & Koketsu, 2016). For this reason, in this experiment, females were inseminated two- or three-times during estrus.

Mean values and standard deviation of sperm parameters of boars after 48 hours (T48) and 72 hours (T72) of cooling at 15°C are presented at Table 4 and Table 5, respectively.

Table 4. Mean values and standard deviation of sperm parameters: total motility (TM-%), progressive motility (PM-%), average path velocity (VAP-µm/s), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), straightness (STR-%), linearity (LIN-%), amplitude of lateral head displacement (ALH- µm), flagellar beat cross frequency (BCF-Hz), farrowing rate (FR-%) and total number of piglets born (TNB) of boars after 48 hours (T48) of cooling at 15°C.

BOAR	A	B	C	D	E	F	G
TM	86.06±5.60^{ab}	78.71±11.18^{abc}	62.74±8.04^{d}	79.18±9.65^{ab}	87.93±4.33^{a}	68.54±13.90^{cd}	75.99±12.16^{bc}
PM	74.58±8.93^{a}	65.88±15.36^{ab}	44.56±10.53^{c}	64.13±13.58^{ab}	74.98±6.35^{a}	55.26±16.28^{bc}	58.13±16.65^{bc}
VAP	50.50±16.19^{a}	50.17±13.61^{a}	45.66±6.92^{a}	48.31±14.25^{a}	46.42±10.41^{a}	45.61±12.48^{a}	41.21±12.56^{a}
VCL	83.63±25.88^{a}	80.26±23.09^{a}	80.05±16.83^{a}	78.24±25.29^{a}	79.06±13.43^{a}	70.00±24.57^{a}	69.85±24.86^{a}
VSL	37.07±12.54^{a}	36.35±9.06^{a}	30.52±4.72^{a}	35.21±8.92^{a}	33.47±8.29^{a}	34.47±9.66^{a}	29.82±8.04^{a}
STR	72.28±0.05^{ab}	72.41±0.06^{ab}	66.15±0.05^{b}	72.71±0.06^{a}	71.15±0.05^{ab}	75.95±0.09^{a}	73.17±0.05^{ab}
LIN	43.61±0.07^{ab}	45.77±0.07^{ab}	38.15±0.06^{b}	45.81±0.08^{ab}	41.56±0.06^{b}	52.24±0.13^{a}	44.89±0.06^{ab}
ALH	2.65±0.65^{a}	2.60±0.71^{a}	3.00±0.43^{a}	2.49±0.76^{a}	2.54±0.61^{a}	2.35±0.78^{a}	2.31±0.74^{a}
BCF	28.31±4.98^{a}	25.11±5.05^{abc}	22.26±3.96^{bc}	25.15±5.28^{abc}	25.62±3.16^{ab}	20.67±4.99^{c}	22.94±4.90^{bc}
FR	93.51±14.02^{a}	89.55±14.49^{a}	20.37±38.87^{b}	92.59±13.48^{a}	91.55±17.52^{a}	94.44±9.60^{a}	96.51±3.80^{a}
TNB	12.54±2.22^{a}	12.68±2.19^{a}	9.45±5.02^{b}	13.26±2.14^{a}	12.23±1.90^{a}	12.38±2.59^{a}	13.15±2.36^{a}

*Different letters in the same row indicate significant differences at P <0.05.

However, after 48 hours of refrigeration, we observed an increase in the number of males exhibiting TM and PM below 70% (Table 4), and only two boars showed TM and PM above 70% after 72 hours of storage, without interference at FR and TNB (Table 5). Differently, Alkmin, et al. (2014) found that percentages of mobile and viable sperm decreased with increasing semen storage time of all boars evaluated. However, these percentages remained above 70% in all males after 48 hours of storage.

Table 5. Mean values and standard deviation of sperm parameters: total motility (TM-%), progressive motility (PM-%), average path velocity (VAP-µm/s), curvilinear velocity (VCL-µm/s), straight line velocity (VSL-µm/s), straightness (STR-%), linearity (LIN-%), amplitude of lateral head displacement (ALH-µm), flagellar beat cross frequency (BCF-Hz), farrowing rate (FR-%) and total number of piglets born (TNB) of boars after 72 hours (T72) of cooling at 15°C.

BOAR	A	B	C	D	E	F	G
TM	84.68±7.51^{a}	76.71±12.07^{abc}	63.94±9.74^{d}	81.00±7.67^{abc}	83.87±13.66^{ab}	69.47±15.71^{cd}	72.05±16.06^{bcd}
PM	74.65±8.21^{a}	60.57±16.42^{abc}	45.79±11.55^{d}	62.34±14.63^{abc}	71.93±13.86^{ab}	53.86±20.86^{cd}	58.22±16.87^{bcd}
VAP	51.51±13.96^{a}	50.45±20.27^{a}	45.48±10.33^{a}	50.31±17.44^{a}	48.22±14.75^{a}	46.35±15.07^{a}	48.62±15.14^{a}
VCL	82.84±21.76^{a}	84.62±37.63^{a}	76.45±2.54^{a}	82.40±24.64^{a}	80.82±21.80^{a}	73.43±28.40^{a}	75.50±26.77^{a}
VSL	38.41±10.18^{a}	33.70±10.41^{a}	31.12±4.79^{a}	36.20±13.01^{a}	34.69±10.22^{a}	34.14±10.57^{a}	36.60±11.97^{a}
STR	73.96±0.04^{ab}	68.36±0.07^{b}	68.95±0.06^{ab}	71.70±0.03^{ab}	72.00±0.04^{ab}	74.32±0.06^{ab}	74.81±0.08^{a}
LIN	46.09±0.06^{ab}	41.41±0.07^{b}	41.45±0.07^{ab}	43.60±0.04^{ab}	42.90±0.05^{ab}	48.05±0.10^{ab}	48.73±0.10^{a}
ALH	2.63±0.56^{a}	2.81±0.80^{a}	2.97±0.43^{a}	2.86±0.73^{a}	2.62±0.56^{a}	2.45±0.72^{a}	2.44±0.74^{a}
BCF	27.82±3.88^{a}	24.30±5.67^{ab}	21.78±4.50^{b}	25.27±4.91^{ab}	25.94±4.30^{ab}	22.22±6.99^{b}	23.33±6.24^{ab}
FR	93.51±14.02^{a}	89.55±14.49^{a}	20.37±38.87^{b}	92.59±13.48^{a}	91.55±17.52^{a}	94.44±9.60^{a}	96.51±3.80^{a}
TNB	12.54±2.22^{a}	12.68±2.19^{a}	9.45±5.02^{b}	13.26±2.14^{a}	12.23±1.90^{a}	12.38±2.59^{a}	13.15±2.36^{a}

*Different letters in the same row indicate significant differences at P <0.05.

Motility is the most widely used sperm quality variable in AI centers (Tremoen, et al., 2018), and in this study only the total and progressive motility of the ejaculates at T0 after dilution were associated with the farrowing rate and the total number of piglets born.

Nevertheless, Kadirvel, et al. (2019) found that semen cooling at 17°C maintain sperm motility and viability until the third day without a significant decline in sperm quality and fertility. By the second day, the membrane integrity did not differ, but on the 72h day it was significantly reduced. After insemination, the farrowing rate was 77.7%, 80.76%, 73.07% and 69.8%, respectively from day 0 to day 3 of storage and no difference were observed in pregnancy rate, farrowing rate and litter size between the different days of storage.

In this study, there was a positive and significant correlation between the percentage of progressive motility and a negative correlation of curvilinear velocity (VCL) and flagellar beat cross frequency (BCF) with farrowing rate. On the other hand, the total number of piglets born was positively affected by the percentage of TM and VAP and negatively by VSL and ALH. According to Broekhuijse, et al. (2012), reproductive fertility can be related to sperm parameters evaluated by the CASA system.

The results of the present study showed positive correlation of TM and PM with the litter size. However, Vyt, et al. (2008) verified a positive effect only on the percentage of total

sperm motility by the CASA system, on litter size and number of live-born piglets, however, no other parameter was related.

In the present study, there was no relationship of any of the sperm motility parameters evaluated with the farrowing rate at T24, T48 and T72 hours refrigeration. However, Ruiz-Sánchez, et al. (2006) observed positive and significant correlation between boar sperm motility with birth rate and litter size at seven and ten days of semen storage when compared to three days. Thus, the motility of boar sperm was significantly affected by the storage time.

The results obtained in different studies are constantly controversial (Ruiz-Sánchez, et al., 2006; Vyt, et al., 2008; Alkmin, et al., 2014; Tremoen, et al., 2018; Kadirvel, et al., 2019). Perhaps because the predicted values of sperm characteristics explain a small total variation of fertility. It is known that the highest degree of spermatic deterioration in a sample is associated with the lowest fertility; and the functional integrity of spermatozoa can be assessed by flow cytometry (Broekhuijse, et al., 2015).

In the present study, only males with a maximum of 4% of sperm morphological defects were used, and it was not possible to evaluate the effect of the percentage of sperm pathology on farrowing rate and total number of piglets born. However, Tsakmakidis, et al. (2010) observed that males with a high percentage of morphologically normal spermatozoa had no significant correlation with the total number of piglets born and the number of piglets alive per litter.

Matabane, et al. (2017) reported a weak positive correlation between normal sperm morphology and conception rate (r=0.11) when evaluating the correlation between sperm plasma membrane morphology and integrity and fertility after AI. In addition, a low and positive correlation was observed between normal sperm morphology and litter size (r=0.37) and total number of piglets alive (r=0.03). However, negative correlations were found between conception rate and morphological defects of spermatozoa.

For Foxcroft, et al. (2010), the productivity results were positive both for inseminations with heterospermic doses and with homospermic doses. However, a 10% increase in fertilization rate resulted in the addition of 2.5 embryos at the 30th day of gestation. Heterospermic AI is commonly used in pigs breeding but prevents a more accurate assessment of the individual fertility of boars. However, reproductive performance may be similar with homospermic and heterospermic inseminations (Ferreira, et al., 2015). In the present study, with homospermic insemination, male B had a lower farrowing rate compared to male C, but there were no other differences in the reproductive performance of the males

tested, which presented similar ejaculate quality. Thus, it was also possible to identify the subfertile male through the lower FR and TNB of male C.

4. Conclusion

Total motility and progressive motility soon after dilution assessed by computer-assisted semen analysis (CASA System) can be used to predict the fertility of boars, due to the association of these characteristics with the farrowing rate and total number of piglets born. The other parameters of sperm kinetics evaluated were not effective to predict the actual fertility of boars. The use of boars that have semen with a low percentage of total motility (less than 87%) and progressive motility (less than 78%), soon after the dilution, reduce the reproductive efficiency of piglet producing farms.

However, additional studies with CASA are still needed correlating seminal characteristics with fertility, whose semen quality is essential for AI and also to predict the reproductive potential of males, aiming at the genetic dissemination of high genetic value breeders in insemination centers, resulting in an adequate birth rate and total number of piglets born.

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