SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2022v43n2p573

In vitro and *in vivo* parameters for identification of landrace pigs with low reproductive performance

Parâmetros *in vitro* e *in vivo* para detecção de machos suínos Landrace com baixo desempenho reprodutivo

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Highlights

Evidence of subfertile individuals with the same conditions of seminal quality.
Ineffectiveness of correlating seminal quality patterns with reproductive outcome.
Data from homospermic insemination used for decision making.

Abstract

In pig farming, measurements of production parameters play a fundamental role in the success of the activity. Minimal differences in fertility between breeders can lead to less reproductive efficiency and, less productivity. However, assessing the fertility of each male and the early identification of subfertile males is a difficult task to be performed. Thus, the aim of this study was to evaluate the use of in vitro and in vivo parameters in the identification of subfertile males of the Landrace breed, aiming to collaborate with genetic improvement programs, routine optimization in the Genetic Diffusion Units (GDUs) and the results of performance. In experiment 1, an approach to identify males with subfertility was evaluated based on retrospective data. For this, the results (averages of birth rates, number of total births and average percentages of female and male piglets per litter) were evaluated for a total of 996 matings and 847 parturitions. The inseminations came from ejaculates of 32 males, who had at least 19 females inseminated with homospermic doses in the concentration of 2.5 x 10^9 total sperm from the same male. As for the birth rate (BR), an average of 85.47% ± 6.05 was observed with a group of median males, seven males that stood out and one individual (M32) with a performance of 58.06% ± 9.0. For the total number of piglets born (PB) the average was 13.41 ± 0.56, with three males with better performance and one (M32) with very poor performance (8.62 ± 0.59). In experiment 2, it was verified whether evaluations of inseminating doses (ID)

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Received: June 25, 2021 - Approved: Nov. 22, 2021

of semen in vitro (motility and sperm morphology) after 96 hours of storage had correlations with fertility in vivo, which can be used to identify subfertile males. The evaluations were performed on 30 ejaculates regarding the means of BR and PB, considering only those who had at least 7 females inseminated. There were no correlations between the motility assessments and semen morphological changes and the reproductive parameters evaluated. The results obtained in vivo, referring to BR and PB, demonstrated that it was possible to identify differences between males, the individual (M32) had the worst results for the percentages of BR and PB. It is concluded that there are males of high and low fertility and that only the in vitro analyzes carried out in this study are not enough to categorize them, however, the evaluation of retrospective data was efficient for this purpose.
Key words: Genetic improvement. Pigs. Semen. Subfertility.

Resumo

Na suinocultura moderna, as mensurações de parâmetros de produção têm papel fundamental para o sucesso da atividade. No entanto, a avaliação da fertilidade de cada macho e a identificação precoce de machos subférteis é uma tarefa difícil de ser realizada. O objetivo deste estudo foi avaliar a utilização de parâmetros in vivo e in vitro na identificação de machos subférteis da raça Landrace, visando colaborar com os programas de melhoramento genético, otimização da rotina nas Unidade de Difusão Genética (UDGs) e dos resultados a campo. No experimento 1, foi proposta uma abordagem de identificação dos machos subférteis tendo como base dados retrospectivos. Para isso, foram avaliados os resultados (médias das taxas de parto, número de nascidos totais e média das porcentagens de leitões fêmeas e machos por leitegada) de um total de 996 coberturas e 847 partos. As inseminações foram oriundas de ejaculados de 32 machos, que tiveram ao menos 19 fêmeas cobertas com doses homospérmicas na concentração de 2,5 x 10^9 de espermatozoides totais e obrigatoriamente do mesmo macho. Quanto a taxa de parto (TP) obtivemos uma média de 85,47% ± 6,05 e observou-se um grupo de machos medianos, sete machos que se destacaram positivamente e um indivíduo (M32) com um desempenho 58,06 ± 9,0. Para número de leitões nascidos totais (NT) obtivemos uma média de 13,41 ± 0,56 e notou-se três machos com melhor desempenho e um (M32) com péssimo desempenho (8,62 ± 0,59). No experimento 2, foi verificado se as avaliações das doses inseminantes (DI) de sêmen in vitro (motilidade e morfologia espermática) após 96 horas de armazenamento apresentaram correlação com a fertilidade in vivo. As avaliações foram realizadas em 30 ejaculados quanto às médias de TP e NT, considerando apenas ejaculados que tiveram ao menos 7 fêmeas inseminadas. Não foram verificadas correlações entre as avaliações de motilidade e alterações morfológicas do sêmen com os parâmetros produtivos avaliados. Os resultados obtidos in vivo, referentes a TP e NT, mostrou que foi possível identificar diferença entre os machos, onde o indivíduo (M32) apresentou os piores resultados para as porcentagens de TP e NT. Desta forma, pode-se concluir que existem machos de alta e baixa fertilidade e que somente as análises in vitro realizadas neste estudo não são suficientes para categorizá-los, no entanto, a avaliação de dados retrospectivos foi eficiente para esta finalidade.
Palavras-chave: Melhoramento genético. Sêmen. Subfertilidade. Suínos.

Introduction

Genetic diffusion units (GDU) and artificial insemination (AI) play a very important role in modern production systems (Knox, 2016). GDUs comprise males with the highest genetic merits, and they contribute to biosecurity and the production of insemination doses (IDs) under strict quality controls (Maes, Soom, Appeltant, Arsenakis, & Nauwynck, 2016). The demand for higher production efficiency requires the use of genetically superior males in terms of relevant production traits, lower sperm numbers per ID, and fewer inseminations per female. It also increases the need that sows give birth to piglets from these males (Knox, 2013).

Despite the existence of promising molecular approaches for predicting male fertility such as evaluation of chromosomal defects, chromatin integrity, and proteomics, they are not yet available in routine GDUs (Andrade, Passarelli, Torres, Monteiro, & Martins, 2017; Rahman, Kwon, & Pang, 2017; Roca, Broekhuijse, Parrilla, Rodriguez-Martinez, & Bolarin, 2015). Sperm motility and morphology are important in routine evaluations to ensure that ID production meets the quality standards. Some studies have shown significant correlations of these characteristics with farrowing rates and total number of births per litter (Broekhuijse, Feitsma, & Gadella, 2011; Jung, Rüdiger, & Schulze, 2015).

Therefore, this study aimed to identify subfertile Landrace males using in vivo and in vitro evaluation parameters in order to contribute to breeding programs, optimize the routines in GDUs, and improve reproductive results.

Materials and Methods

Animals, housing, and semen collection

We used retrospective data from a GDU located in Guarapuava/PR and a nucleus farm of Landrace females located in the municipality of Chapecó/SC. Although the database used in experiment 2 is the same as in experiment 1, the number of males is smaller.

Landrace males aged 8-24 months from nucleus farms were transported to the GDU and were quarantined in an isolated facility. The health program included two vaccines: a circovirus1 and a reproductive vaccine (against Leptospira, parvovirus, and erysipelas)2. Males were fed an exclusive diet for males, and they consumed approximately 2.5 kg/day, depending on their body status. The diet guaranteed 3,192 Kcal/kg metabolizable energy (ME), 0.79% digestible lysine, 0.83% calcium, and 0.44% available phosphorus.

After mating training on a semi-automatic mannequin3, the males were translocated to the housing shed so that they could be integrated in the farm's collection routine. After the animals were ready, ejaculates were collected at intervals of seven days. When the animal entered the collection room, they were evaluated for the presence of injuries. If there were none, the process began with dry cleaning of the foreskin and fixation of the penis to the equipment's artificial vagina. After obtaining the ejaculate in the collection cup, it was examined by microscopy.

The ejaculate was evaluated in the laboratory using the CASA4 system with

regard to motility, vigor, agglutination, concentration, and morphology. Based on these data, the ejaculate was diluted using a long-acting diluent5 to obtain a concentration of 2.5 □ 109 total sperm cells and a volume of 90 mL per ID. Subsequently, the doses were transported in a vehicle with a refrigeration system to the nucleus farm located in Chapecó/SC, where they were kept refrigerated at a temperature between 15 and 18 ºC, and they were stored for a maximum of six days.

Females were separated from piglets at approximately 26 days of lactation, and the females were then housed in individual boxes where they received nutritional flushing (3,185 Kcal/kg EM, 0.68% digestible lysine, 0.75% calcium, and 0.36% available phosphorus). An adult male was presented to the females twice per day for stimulation and estrus detection. After estrus detection, the females were inseminated intracervically. The AI protocol was as follows: the first AI was performed 24 hours after estrus detection, the second AI was performed 24 hours after the first AI, and if necessary, further AIs were performed subsequently (at 24-hour intervals), provided the sow responded to the human tolerance-reflex behavior in the presence of the male.

After insemination, the diet was adjusted to gestation requirements (2,900 Kcal/kg ME, 0.52% digestible lysine, 0.8% calcium, and 0.29% available phosphorus), based on three feed curves: 0-49 days of gestation: 2.2 kg; 50-84 days of gestation: 2.5 kg; 85-110 days of gestation: 2.9 kg. Reproductive events were recorded using S2®6 software, and the data analyzed subsequently.

Experiment 1 - Evaluation of in vivo parameters

Retrospective data of inseminations from ejaculates of 32 Landrace males from 01/04/2017 to 12/18/2018 (totaling 24 months), whose doses were used for AI of at least 19 females, were evaluated. All doses used for AI during female estrus were homospermic and originated exclusively from the same male and the same ejaculate, as the study was conducted in a nucleus farm. Only sows between the second and fourth reproductive cycle and which had received two or three AIs per estrus were considered for analysis. In total, data from 996 inseminated females and 847 births were examined. The following parameters were evaluated: mean farrowing rates per male, mean total births from inseminations per male, and mean proportions of female and male piglets per litter.

Experiment 2 - Evaluation of in vitro parameters

The doses were analyzed to identify whether in vitro evaluations during ID storage were correlated with male fertility and thus whether these data could be used for early detection of subfertile males. The doses were evaluated for sperm motility and morphology after 96 hours of storage. The evaluations were carried out using the CASA4 system. IDs were used to perform homospermic inseminations as described in experiment 1. The evaluations were carried out per ejaculate, and only ejaculates used to inseminate at least seven females were considered. Mean farrowing rates and total number of piglets per litter were assessed.

Statistical analyses

Results were described as means ± standard deviation of the mean or percentage, according to the type of variable. The Shapiro-Wilk test was used to test normality of data. The GLM (Statistical Analysis System - SAS Inst. Inc., Cary, NC) analysis of variance test and comparison of means using the Tukey test at a significance level of 5% were used for data meeting parametric assumptions. Mean farrowing rates per male deviated significantly from normal distribution, thus a Kruskall-Wallis was used, with Dunn's test post hoc. Correlations were tested using the GraphPadInstat® statistical package, with Pearson's correlation test at a significance level of 5%.

Results and Discussion

Experiment 1 - Evaluation of in vivo parameters

Thirty-two males were evaluated, and the mean number of inseminated females per male was 31.12 ± 13.60, totaling 996 coverages and 847 births. The values for each male are shown in Figure 1. The overall mean farrowing rate was 85.47% ± 6.05%. There were differences between the boars; several males showed average results, seven males produced good results, and one male had poor results ($P < 0.05$) (Figure 2).

Figure 3 shows the mean total number of births per litter of each male (M1 to M32). The overall mean was 13.41 ± 0.56 births. A similar result was observed for farrowing rates; three males had good results, some males had average results, and one male had poor results ($P < 0.05$), which was the same male (M32) that had produced an unsatisfactory farrowing rate. The means of female and male piglets per litter were 49.15 ± 2.87 and 51.29 ± 1.98, respectively, with no difference between boars for this parameter ($P = 0.81$).

Experiment 2 - Evaluation of in vitro parameters

Thirty ejaculates from 25 males were evaluated, and the mean number of females inseminated with the ID prepared from each ejaculate was 8.9 ± 1.77, totaling 267 coverages. The values for each ejaculate are shown in Figure 4.

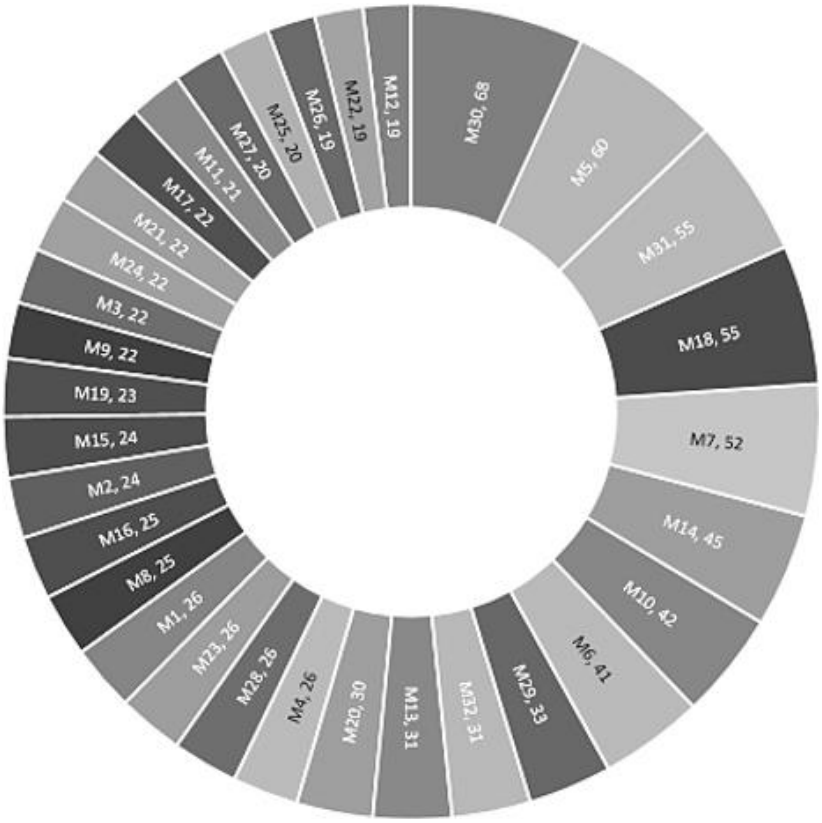

Figure 1. Number of females inseminated per evaluated male (M1 to M32) for *in vivo* parameters.

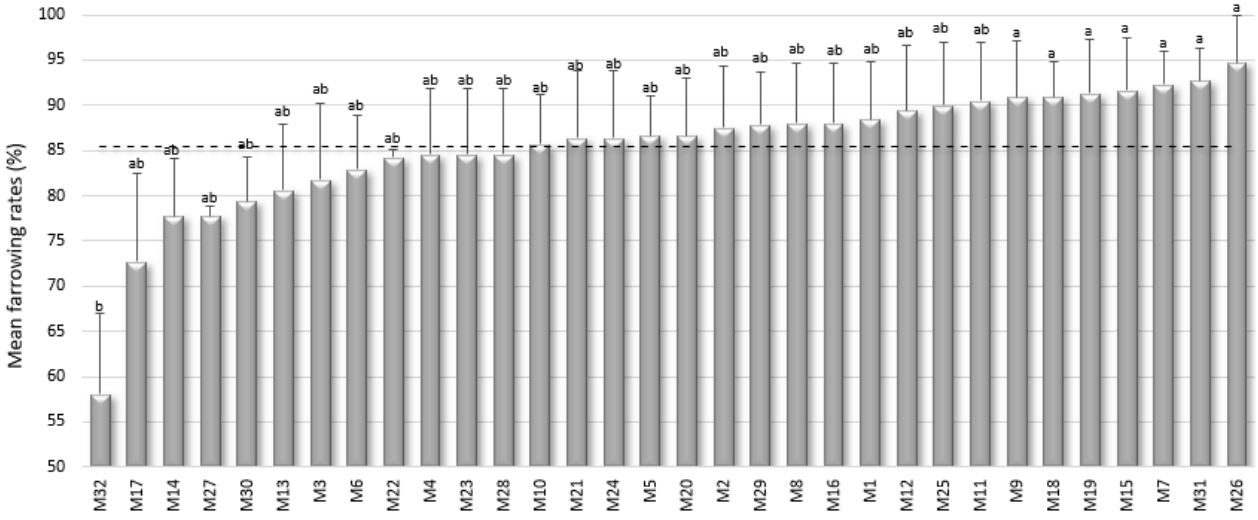

Figure 2. Mean farrowing rates after insemination with homospermic doses of each evaluated male (M1 to M32). The dotted line indicates the overall mean [a, b] Different superscript letters between means indicate significant differences (P <0.05).

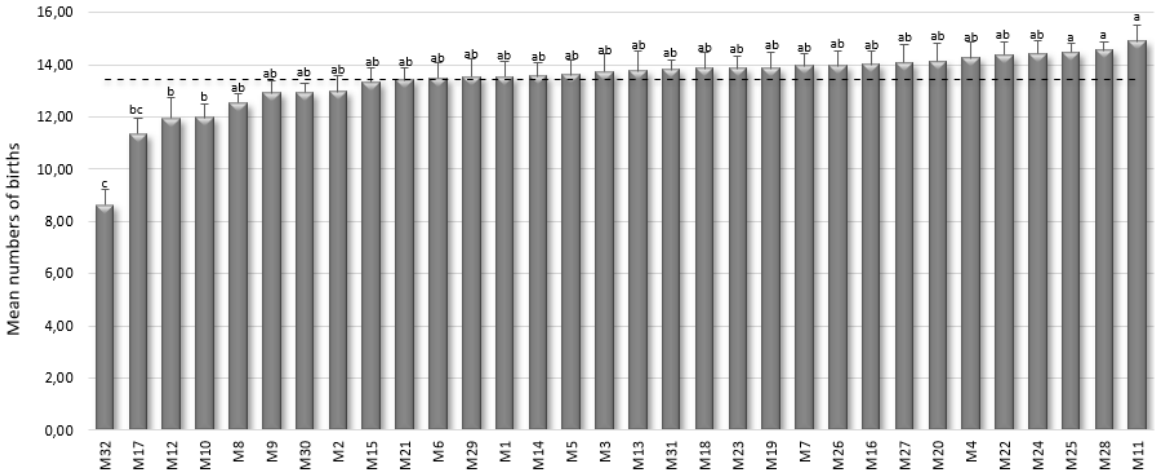

Figure 3. Mean numbers of births from farrowing females after insemination with homospermic doses of each evaluated male (M1 to M32). The dotted line indicates the overall mean. [a,b,c] Different superscript letters between means indicate significant differences (P <0.05).

Figure 4. Numbers of inseminated females per ejaculate of evaluated males (E1 to E30) for in vitro parameters.

There was no correlation of the mean percentage of sperm motility evaluated after 96 hours of cooling with mean farrowing rates and the number of total births after insemination, as shown in Figure 5 and Table 1.

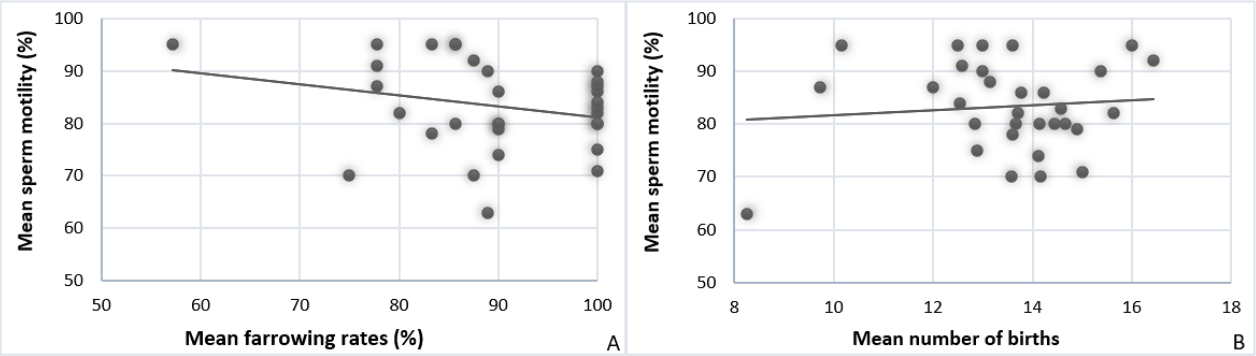

Figure 5. Graphic representation of the correlations between the mean percentages of sperm motility measured after 96 hours of cooling and mean farrowing rates (A) and mean number of total births (B).

Table 1
Correlations (r) between farrowing rate and total number of births and sperm motility and sperm defects, and respective significance levels (P)

	Farrowing rate (%)	Total number of births
motility	-0.2552 (P = 0.1659)	0,0952 (P = 0.6081)
sperm defects	0,2242 (P = 0.2253)	-0,2169 (P = 0.2431)

There was no correlation of the percentage of sperm defects evaluated after 96 hours of storage with mean farrowing rates and the number of total births after insemination, as shown in Figure 6 and Table 1. Table 1 shows the correlations of sperm quality variables (motility and sperm defects) with the total number of births and farrowing rates, which were not significant (P > 0.05).

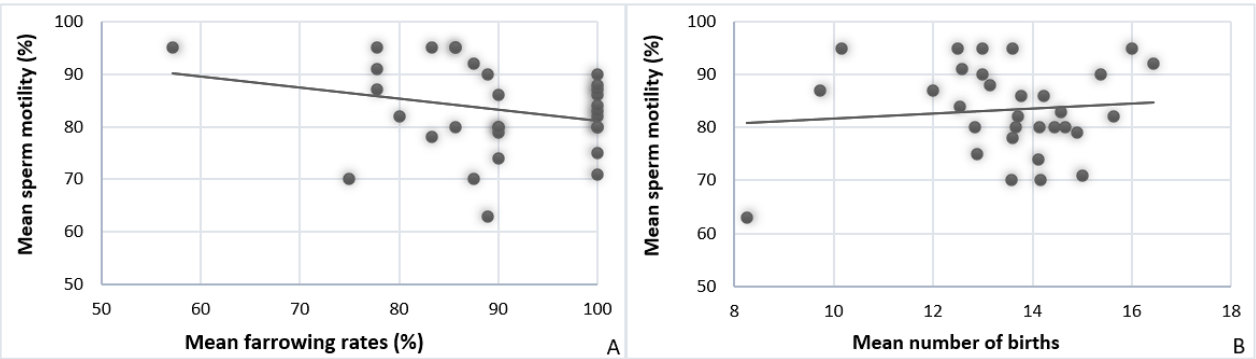

Figure 6. Graphic representation of the correlations between the mean percentages of sperm defects measured after 96 hours of cooling and mean farrowing rates (A) and mean number of total births (B).

The results of farrowing rates and total number of births showed differences between males. Male 32 had the worst results regarding farrowing rate and number of born piglets, i.e., 58.06% ± 9.0% and 8.62 ± 0.59, respectively. All males were considered suitable according to the CASA system and to the routine evaluations at the GDU. The inadequacy of male 32 was only identified after in vivo evaluation.

These results were incompatible with the expected minimum, considering the current reproductive performance parameters obtained in Brazil. Nevertheless, this boar was selected for collection in a GDU. Using the same number of sperm cells for different males may result in different litter sizes, confirming that seminal characteristics affect the in vivo result (Moreira, Ferreira, Panzardi, & Corcini, 2013).

The evaluated system used homospermic doses, that is, IDs from a single boar. In Brazil, it is common to prepare heterospermic doses from ejaculates of two or more males, which makes it even more difficult to identify subfertile males (Ferreira et al., 2014).

The male's genetic lineage is a fertility-affecting parameter which best explains the variation in farrowing rates and total number of births (Broekhuijse et al., 2011). Although all animals in this study were of the same lineage, subfertile males were identified. Even though all evaluated males met the seminal quality requirements at the GDU (in vitro), one individual negatively affected the in vivo results. We suggest that genetic disorders may not affect semen quality but may affect fertility (Arruda et al., 2015).

The correlation between in vitro data (motility and sperm defects) and the data observed on the farm (farrowing rates and total numbers of births) was weak (< 20%) for the analyzed variables. In routine ID production, ejaculates containing sperm with morphological abnormalities > 30% are discarded.

Genetic problems are difficult to identify in conventional evaluations of ejaculate quality conducted at GDUs because males may show similar behavior and seminal quality. For example, defective chromatin structures affect sperm functioning during the later stages of fertilization and embryo development, thus it is considered a non-compensable characteristic (Yeste, 2016), whereas lacking membrane integrity can be compensated by insemination with high numbers of sperm cells, thus it is classified as a compensable characteristic. Apparently, the number of cells per IDs may compensate for membrane, acrosome, or mitochondrial deficiencies, as observed in a previous study (Menezes et al., 2020).

The farrowing rate and litter size results showed that the effect of ejaculate quality characteristics on the fertility of breeding males was small (Broekhuijse et al., 2011), which was mainly due to the production processes of GDUs which have minimum quality parameters for IDs.

The analysis of morphology, concentration, and motility are consolidated basic parameters to determine sperm quality (Andrade et al., 2017). However, recent studies characterized the seminal plasma proteome of pigs and investigated its association with reproductive traits: Kwon et al. (2015) described litter size biomarkers for pig

sperm and Pérez-Patiño et al. (2018) found a correlation of porcine seminal plasma protein expression with weaning rate and litter size.

Thus, in vitro evaluation of the quality parameters of the ejaculate (motility and spermatic defects) are imprecise, corroborating the findings of a previous study (Dyck et al., 2011). For better accuracy, in vitro tests must be combined with the use of genomic and in vivo monitoring (Dyck et al., 2011), or they must be combined with other in vitro parameters such as sperm motility, vigor, sperm concentration, and sperm morphology. Although the effects seem small, the correlating economic result in the swine industry is considerable. Thus, optimizing the fertilizing capacity of males is critical as it allows for increased productivity, resulting in a smaller gap between genetically superior and inferior animals.

Conclusions

There was no correlation between the analyzed in vivo and in vitro data, and the analyses did not identify males with altered fertility. However, retrospective evaluations of homospermic inseminations considering farrowing rates and litter sizes indicated subfertile males, which revealed variation in fertility.

Manufacturers

[1] Circunvent® PCV, MSD Saúde Animal, United States.

[2] FarrowSure® GOLD. Zoetis Saúde Animal, United States.

[3] Equittec®. Brazil.

[4] AndroVision®. Minitube, Germany.

[5] Vitasen LD®. Magapor, Spain.

[6] Agriness. Brazil.

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