Assessment of *in vitro* boar semen quality

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Abstract. Reproductive performances of the boars used in commercial artificial insemination depend on semen quality assessment. The aim of this study was to analyze *in vitro* quality of the main commercial swine breeds' (Large White, Landrace, Pietrain and Duroc) semen parameters including volume, concentration, motility, viability, acrosome integrity, functional integrity (hypo-osmotic swelling test and thermoresistance test) and DNA integrity (DNA fragmentation index) in order to compare them with fertility (conception rate). Significant differences were observed between volume, concentration, progressive motility, viability and morphology of sperm. No other differences were observed in the case of immature sperm, functional and DNA integrity of sperm and conception rate. Between swine breeds there are some differences regarding semen quality parameters, but it is difficult to establish a clear correlation in order to predict semen fertility.

Key Words: artificial insemination, *Sus scrofa domesticus*, commercial swine breeds, evaluation, semen parameters.

Introduction. Efficiency improvement of pork production is the result of implementation of several new biotechnological techniques and production practices (Gerrits et al 2005). The need to continually improve the efficiency of pork production, suggests that commercial artificial insemination (AI) practice should involve increased use of boars with the highest genetic merit for important production traits. Using sub-fertile boars and low quality ejaculates reduces production efficiency and lowers profit margins for the producer. The "predictors of useable semen" used in most commercial AI stations provide a very conservative estimate of the relative fertility of individual boars (Foxcroft et al 2008). Male factor infertility is commonly defined in terms of the conventional semen profile, which provides descriptive information on the number of sperm in the ejaculate, the percentage of progressive motility and morphologically normal sperm (Aitken 2006). AI stations as a part of "quality control" often includes only motility, which is a rather insensitive parameter for detecting sperm damage due to storage. Thus there is a need to apply more sophisticated testing (Waberski et al 2008). Other sperm parameters can contribute to better assessment of sperm quality. Both sperm and acrosome membrane integrity have been reported to be successfully associated with male *in vivo* fertility (Kordan et al 2013). Sperm DNA integrity can be used as an additional parameter to provide a more comprehensive description of semen quality (Perez-Llano et al 2006).

In Romania companies of pig genetic improvement sell valuable animals and semen for the large, medium or small size pig farms. The main commercial breeds include Large White, Landrace, Pietrain and Duroc (Petrescu-Mag et al 2017). Many factors play a role in boar semen fertility. Thus, boar age (Miclea et al 2008; Tăpăloagă et al 2013), collection frequency (Miclea et al 2007) or season (Tăpăloagă et al 2013), type of extenders (Bogdan et al 2018) or influence of prostaglandin F2α (Pandur & Păcală 2012) were well analyzed in order to identify the real influence of them on semen quality. The aim of this study was to analyze *in vitro* quality of the main commercial swine breeds' (Large White, Landrace, Pietrain and Duroc) semen parameters including volume, concentration, motility, viability, acrosome integrity, functional integrity (hypo-

osmotic swelling test and thermoresistance test) and DNA integrity (DNA fragmentation index) in order to compare them with fertility (conception rate).

Material and Method. Semen from eight commercial boars (2 Large White, 2 Landrace, 2 Pietrain and 2 Duroc) was available from the SC Semtest-BVN Târgu-Mureș, Romania. Ejaculates were collected once at 3 or 4 days using gloved-hand method (Zăhan et al 2014).

In vitro evaluation. Three ejaculates from each boar were used for *in vitro* evaluation. Sperm volume was determined by weighing, while for sperm concentration was used photometer method. For motility, samples were examined under a phase contrast microscope with a 37°C heated stage at 200x magnification. Progressive motility was expressed as the percentage of the total number of sperm.

Sperm viability. The percentages of viable sperm were determined by eosin-nigrosin staining as described by Dott & Foster (1972). Evaluation was undertaken using bright-field microscope (Olympus BX41) at 1000x magnification, 200 sperm being examined for each smear.

Sperm morphology. Evaluation of *sperm* morphology was made by examining formalin saline fixed samples (2.9 g tri-Natriumcitrat x $2H_2O$, 4 mL of 37% formalin and 96 mL of MilliQ water) with a phase-contrast microscopy at 1000x magnification (Olympus BX41). At least 200 sperm per sample were examined in order to establish head and tail normality and acrosome integrity by normal apical ridge (NAR) and classified using the scoring system reported by Pursel et al (1972).

Assessment of functional integrity. Hypo-osmotic swelling test (HOST) was accomplished by placing 0.1 mL semen sample into 1 mL of HOS (0.0375 mg/mL Natriumcitrate x $2H_2O$ and 13.5 mg/mL fructose (Roth), 100 mOsm/kg) in a water bath at 37°C for one hour (Perez-Llano et al 2001). After incubation a drop of the sperm suspension was placed on the slide and covered with a glass coverslip. A total number of 200 sperm cells were counted, those with any degree of coiled tail (HOST positive sperm) as well as those with straight tail (negative HOST sperm).

Thermoresistance test was used in order to establish semen ability to maintain motility during incubation time. Sperm progressive motility was analyzed at 15, 60, 120, 180 and 240 min of incubation at 37°C.

DNA integrity (DNA fragmentation index). For DNA fragmentation index (DFI) semen was processed according to the instruction of the Sperm-*Sus*-Halomax® kit (ChromaCell SL, Madrid, Spain). For a better examination, spermatozoa from each group were diluted in TL to give a final concentration of 5-10 x 10^6 spermatozoa per mL. After 5 min in a 90–100°C water bath, vials with agarose were left into a thermostatic water bath at 37°C to equilibrate for 5 min. When the agarose reached 37°C, 25 µL of semen were added to the vial and mixed with a pipette. Then, a drop of the cell suspension was placed on a previously treated and pre-cooled (5°C) slide and it was covered with a glass coverslip at 4°C for 5 min. The coverslip was smoothly removed and the slide was introduced into 10 mL of the lyses solution and maintained for 5 min at room temperature. The slide was then washed in MilliQ water for 5 min, dehydrated in sequential 70 and 100% ethanol baths for 2 min each and air dried. Just before analyzing under bright-field microscopy, the slides were immersed for 5-10 min in staining solution A and B. According to the kit instructions, spermatozoa showing a halo of dispersion equal or wider than the core minor diameter were considered positive for high DFI.

Fertility. Fertility is broadly defined as the ability to produce viable offspring, the most obvious endpoint being calving rate. There are several other fertility endpoints between the time of insemination and birth that include fertilization rate, non-return (to estrus)

rate, and conception rate (Utt 2016). Conception rate (CR) was used for *in vivo* evaluation of fertility. A total number of 161 sows were IA between March and August 2017. CR was established based on ultrasound evaluation of pregnancy.

Statistical analysis. GraphPad Prism v. 6.0 software (Graph Pad Software Inc., San Diego, CA, USA) was used for data analysis. The values were expressed as mean and standard error of mean (SEM). The results were statistically analyzed using one way analysis of variance (ANOVA) with Tukey's Multiple Comparisons Test. In all cases, $P<0.05$ was considered to be significant.

Results and Discussion. Values of *in vitro* and *in vivo* semen quality are shown in Table 1. The differences between breeds from every semen quality parameter were statistically analyzed. In the case of volume of ejaculate, the differences were significant between maternal and paternal breeds ($P<0.5$), with a higher volume of semen for maternal breeds (Large White and Landrace). The situation was similar in the case of concentration, but with higher percentage of semen for paternal ones. However, the mean number of doses obtained per ejaculate varied between 25.42 (Duroc) and 18.24 (Landrace), with significant differences between Duroc and Landrace ($P<0.01$) and Large White respectively ($P<0.05$).

Table 1

Boar semen quality and fertility (mean ± SEM)

Parameters	*Large White*	*Landrace*	*Pietrain*	*Duroc*
V (mL)	260.00±4.65[a]	248.70±10.63[a]	191.2±16.30	207.20±13.41
PM (%)	70.00±1.83	73.33±2.79	73.33±1.05	87.50±1.12[a]
C (n x 10^9/mL)	0.236±0.01[a]	0.220±0.01[a]	0.345±0.02	0.368±0.02
LS (%)	84.00±1.77	85.00±1.21	85.50±1.12	93.50±0.62[a]
NM (%)	96.00±0.52[a]	93.17±0.48	92.17±0.54	92.67±0.49
IM (%)	2.50±0.43	1.00±0.52	2.83±0.69	1.33±0.42
NAR (%)	96.00±0.86	93.33±0.80	96.67±0.67	96.50±0.56
HOST (%)	25.33±3.84	28.5±4.79	23.17±4.13	35.50±1.38
DFI (%)	2.00±0.58	1.83±0.40	1.33±0.49	0.83±0.40
CR (%)	86.59±4.42	86.30±3.40	77.78±5.66	86.09±5.99

V – volume, PM – progressive motility, C – concentration, LS – live sperm, NM – normal morphology, IM – immature, NAR – normal apical ridge, HOST – hypo-osmotic swelling test, DFI – DNA fragmentation index, CR – conception rate.

Related to progressive motility and live sperm, results showed high values of these parameters on Duroc in comparison with other tested breeds. Thus, for progressive motility the differences were significant between Duroc and Peitrain, Landrace and Large White ($P<0.001$). For viability the differences were significant between Duroc and Pietrain ($P<0.01$) and between Duroc and Landrace and Large White ($P<0.001$). Better semen morphology was observed at Large White boars. No other differences were observed in the case of immature, functional and DNA integrity of sperm. Regarding conception rate, there was no significant difference, even if the Pietrain semen had lower *in vivo* fertility. The dynamic of motility after thermoresistance test is presented in Figure 1. Although semen motility is considered to be an important parameter to validate the quality of the processed ejaculate, it is only minimally related to fertility (Broekhuijse et al 2012).

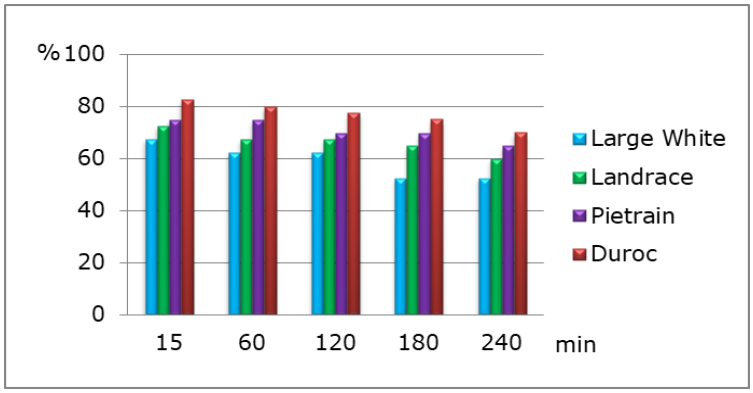

Figure 1. Thermoresistance test of boar semen.

In the case of three pure breeds (Large White, Pietrain and Hampshire) Gonzalez et al (2013) observed a good correlation between total motility and other parameters such as progressive motility, viability, membrane integrity, total motility after thermoresistance test and positive membrane integrity after thermoresistance test. In another study (Zaja et al 2016), semen samples of Swedish Landrace, German Landrace, Large White, Pietrain and PIC-hybrid boars were analyzed in order to determine the influence of breed and hybrid genetic composition of boars on semen quality. Conventional semen quality variables differed depending on breed and PIC-hybrid genetic composition, though these differences were typically insignificant. These results and many others strongly indicate that there are a lot of factors that influence the semen quality parameters.

Conclusions. There are some differences between swine breeds regarding semen quality parameters, but it is difficult to establish a clear correlation in order to predict semen fertility.

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