EVALUATION OF INSEMINATION DOSES FROM BOARS BY FLOW CYTOMETRY

Frydrychova S., Lustykova A., Seifert J., Kucharova S., Rozkot M.

Institute of Animal Science, Prague Uhříněves, Czech Republic

Abstract

The objective of this study was to assessed the sperm viability and acrosome integrity in boar insemination doses (ID) prepared in short-term and long-term extender by flow cytometry. Fifty-eight ejaculates from 6 healthy and fertile AI boars were used for this study. Fresh boar semen was diluted in long-term commercial extenders Androhep (A), Androstar plus (AS+) and short-term extender VIP 5 in a semen-dilution ratio of 1+2. ID were stored at a 17°C. Sperm viability, acrosome integrity and progressive sperm motility were evaluated in ID after 24h and 48h storage time. According results, there were not found differences between extenders during storage time in ID in sperm viability, acrosome integrity and progressive motility ($p>0.05$). In conclusion, the present study did not find a significant difference between long and short-term extender in progressive sperm motility, viability and acrosome integrity in ID for 24 h to 48 h of storage time.

Key Words: Boar semen; flow cytometry; semen evaluation

Artificial insemination (AI) in pigs is a common biotechnological method used in pig reproduction. The effective use of semen in AI depends upon the ability of extender to provide a suitable environment for spermatozoa during storage. Extenders can be classified as short-term (3 days), mid-term (4-5 days) and long-term (7 days) (Gadea, 2003). The progressive motility of spermatozoa during the storage period is influenced by the type of extender used in the production of insemination doses. There are currently a large number of boar sperm extenders on the market, but there is a high variability between different diluents in terms of sperm viability and fertilizing ability (Karunakaran et al., 2017). Monitoring the effect of extenders during storage of boar sperm is important because sperm with an intact plasma membrane and acrosome are able to fertilize an oocyte in vivo (Waterhouse et al., 2004). The use of new accurate and objective technologies such as CASA (computer-assisted sperm analysis) or flow cytometry can improve the quality control of boar semen. Flow cytometry belongs to the important methods for evaluation of the functional and morphological properties of sperm, as the method allows the evaluation of several indicators simultaneously in a sperm population as a whole or for each sperm individually. It also informs us about the selected sperm quality indicator in the sample by examining the integrity of the membrane, DNA, mitochondria, acrosome, oxidative stress and other properties (Dolník et al., 2019).

Therefore, the objective of this study was to assess the viability and integrity of acrosomes in boar insemination doses (ID) prepared in short-term and long-term extender by flow cytometry.

Material and Methods

Fifty-eight sperm ejaculate from eight fertile AI boars of Přeštice black-pied pig aged 3.5 to 5 years were used in this study. Ejaculate were collected using the gloved-hand technique and the gel portion was removed by using double gauze.

The boars were kept in the same housing, feeding and breeding conditions.

The following parameters were evaluated in the fresh native boar semen: semen volume, sperm motility, sperm concentration and morphologically abnormal spermatozoa (MAS). Sperm motility was evaluated by SCA software (Sperm Class Analyzer, version 5.4. Microptic S.L., Spain). Evaluation was performed using a 2 µl sample placed in a Leja 20 chamber and 500 sperm were evaluated by negative phase contrast microscopy with a heating stage (38 °C) at 160x magnification. Progressive sperm motility (%) expressed as VCL> 25 µm/s (curvilinear velocity) and STR≥45% (straightness) was used for this study. Sperm concentration was measured with IMV AccuRead (manufactured in USA for Biochrom Ltd. Cambridge, UK). Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500× magnification. The boar semen was diluted in dilution ratio 1+2 in long-term extender Androhep (A), Adrostar plus (AS+) (Minitüb, Germany) and short-term extender VIP 5 (Hema Malšice, Czech Republic) and ID (90 ml) was stored at a 17 °C. Sperm viability, acrosome integrity and progressive sperm motility were evaluated in ID after 24h or 48h storage time before using them. Sperm viability was assessed using SYBR-14 and propidium iodide (Live/DeadTM Sperm Viability Kit, InvitrogenTM, TermoFisher, USA) and acrosome integrity with using lectin PNA (Lectin PNA From Arachis hypogaea (peanut), Alexa Fluor™ 488 Conjugate, InvitrogenTM, TermoFisher, USA). Samples were prepared according Partyka et al. (2010) and determined by flow cytometry Guava easyCyte TM5 (Merck, Czech Republic).

Basic statistical characteristics of the results of arithmetic means, standard deviations (SD) and significance (p) were calculated by the QC Expert program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). The data were analysed by statistical analysis of variance ANOVA followed by the Fisher test ($p<0.05$).

Results and Discussion

The initial quality of native semen was as follows: semen volume 332.46±132.34 ml, progressive sperm motility 77.88±16.43%, sperm concentration 369.42±159.72×10^3/mm^3 and MAS 25.61±17.88%.

The percentage of progressive sperm motility decreased with storage time without significant differences ($p>0.05$) between extenders when individual measurement days were compared. These results are recorded in Table 1.

Also, the percentage of sperm viability decreased with storage time without significant differences ($p>0.05$) between extenders during storage time. These results are noted in Table 2.

Waberski et al. (2011) and Henning et al. (2012) also noted that sperm viability, which is expressed as a measure of boar sperm membrane integrity at insemination doses, was not affected by the extender during the first 48 hours of storage time.

In the percentage of acrosome integrity, no was found significant differences ($p>0.05$) between extenders during storage time however, there was a reduction in acrosome integrity during follow-up. These results are shown in Table 3.

Teixeira et al. (2015) noted that in their study that sperm motility, viability, and acrosome integrity were not significantly affected by the type of extender up to a storage time of 96 h at 17 °C. Pinart et al. (2015) also determined motility, viability or acrosome integrity in the short-term and extra long-term extender during 8 months period where they found that these doses used at 48 hours after collection not differ in ability to preserve and fertilization. On the contrary, Waterhouse et al. (2004) found that long-term extenders increased sperm longevity but also appeared to preserve structural integrity plasma membrane and acrosome than short-term extender.

Table 1. Mean values and standard deviation (SD) percentage of progressive sperm motility in different extenders at 24h and 48h storage time.

Extender	Storage time		
	24h	48h	Total
A	86.55±14.32	76.32±14.68	81.43±14.52
AS+	77.37±11.31	75.02±21.54	76.20±16.42
VIP5	83.65±12.99	82.36±8.18	83.01±10.35

p>0.05

Table 2. Mean values and standard deviation (SD) percentage of viability in different extenders at 24h and 48h storage time.

Extender	Storage time		
	24h	48h	Total
A	64.36±13.19	55.18±19.50	59.77±16.77
AS+	59.60±11.86	50.54±28.77	55.07±20.31
VIP5	63.65±8.69	58.91±12.78	61.28±10.73

p>0.05

Table 3. Mean values and standard deviation (SD) percentage of acrosome integrity in different extenders at 24h and 48h storage time.

Extender	Storage time		
	24h	48h	Total
A	75.86±9.97	71.91±11.74	73.88±10.85
AS+	75.05±9.82	73.73±16.11	74.39±12.96
VIP5	81.72±0.93	68.48±12.29	75.10±13.22

p>0.05

Conclusion

The present study did not find a significant difference between long and short-term extender in progressive sperm motility, viability and acrosome integrity in ID for 24 h to 48 h of storage time.

References

ČEŘOVSKÝ J. (1976): Metoda barvení kančích spermií pro morfologické hodnocení. Živočišná Výroba, vol. 21, pp. 361-366.

DOLNÍK M., MUDROŇOVÁ D., POŠIVÁK J., LAZAR G., MUDROŇ P. (2019): Flow cytometry in assessment of sperm integrity and functionality – a review. Acta Vet. Brno, vol. 88, pp. 169–175. doi.org/10.2754/avb201988020169

GADEA J. (2003): Review: semen extenders used in the artificial insemination of swine. Spanish Journal of Agricultural Research, vol. 1, pp. 17 -27. doi: https://doi.org/10.5424/sjar/2003012-17

HAUGAN T., GAUSTAD A., REKSEN O., GRÖHN Y., HOFMO P. (2007): Fertility results of artificial inseminations performed with liquid boar semen stored in X-CellTM vs BTS extender. Reprod Domest Anim, vol. 42, pp. 94-99. https://doi.org/10.1111/j.1439-0531.2006.00738.x.

HENNING H., PETRUNKINA A.M., HARRISON R.A., WABERSKI D. (2012): Bivalent response to long-term storage in liquid-preserved boar semen: a flow cytometric analysis. Cytometry, vol. 81, pp.576–587. doi: 10.1002/cyto.a.22058.

KARUNAKARAN M., CHAKURKAR E.B., RATNAKARAN U., NAIK P.K., MONDAL M., MONDAL A.& SINGH N.P. (2017): Characteristics of boar semen preserved at liquid state. Journal of Applied Animal Research, vol. 45:1, pp. 217-220. DOI: 10.1080/09712119.2016.1150848

PARTYKA A., NIŻAŃSKI W., ŁUKASZEWICZ E. (2010): Evaluation of fresh and frozen-thawed fowl semen by flow cytometry. Theriogenology, vol. 74, pp. 1019-1027. DOI: 10.1016/ j.theriogenology.2010.04.032

PINART E., YESTE M., PRIETO-MARTÍNEZ N., REIXACH J., BONET S. (2015): Sperm quality and fertility of boar seminal doses after 2 days of storage: does the type of extender really matter? Theriogenology, vol.83, pp.1428-1437. https://doi.org/10.1016/ j.theriogenology.2015.01.007.

TEIXEIRA S.M.P., CHAVEIRO A., MOREIRA DA SILVA F. (2015): The effects of three extenders on refrigerated boar semen. S. Afr. j. anim. sci., vol. 45, pp. 82-88. http:// dx.doi.org/10.4314/sajas.v45i1.10

WABERSKI D., HENNING H., PETRUNKINA A.M. (2011): Assessment of storage effects in liquid preserved boar semen. Reprod Domest Anim, vol. 46, pp. 45–8. doi: 10.1111/j.1439-0531.2011.01836.x.

WATERHOUSE K.E., DE ANGELIS P.M., HAUGAN T., PAULENZ H., HOFMO P.O., FARSTAD W. (2004): Effects of in vitro storage time and semen-extender on membrane quality of boar sperm assessed by flow cytometry.Theriogenology, vol. 62, pp. 1638-1651. doi: 10.1016/ j.theriogenology.2004.03.001.

Corresponding Address:
Ing. Soňa Frydrychová, Ph.D.
Institute of Animal Science Prague
Department of Pig Breeding Kostelec nad Orlicí
Komenského 1239, 51741 Kostelec nad Orlicí
Czech Republic
E-mail:frydrychova.sona@vuzv.cz

This study was supported by research project MZe-RO0718.