Polish Journal of Veterinary Sciences Vol. 27, No. 1 (2024), 147–150

DOI 10.24425/pjvs.2024.149345

Short communication

Post-thaw quality of boar spermatozoa is affected by ejaculate fractions and extenders

Ł. Zasiadczyk, K. Kurpanik, L. Fraser, W. Kordan

Department of Animal Biochemistry and Biotechnology, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland

Correspondence to: Ł. Zasiadczyk, e-mail: lukasz.zasiadczyk@uwm.edu.pl

Abstract

The aim of this study was to investigate the effect of different extenders on the post-thaw (PT) quality of sperm originating from the sperm-rich fraction (SRF) and post-sperm-rich fraction (PSRF) of boar ejaculate. Motility and velocity parameters, analyzed using a computer-assisted semen analysis (CASA) system, and membrane integrity parameters were markedly higher in frozen-thawed (FT) spermatozoa of the SRF in both the Belstville Thawing Solution (BTS) and Androhep Plus (AHP) extenders, irrespective of the post-thaw (PT) storage time. Furthermore, reduced cryo-survival was more marked in FT spermatozoa of the PSRF in both extenders following storage for 60 min. It was found that the SRF-stored samples in the AHP extender for 60 min exhibited significantly higher percentages of spermatozoa with total motility, mitochondrial function and acrosome integrity than those stored in the BTS extender. The findings of this study confirm that components of the ejaculate fractions and extender have varying effects on the cryo-survival of boar spermatozoa.

Keywords: boar, cryopreservation, ejaculate fraction, semen extender

Introduction

Cryopreservation compromises the functions of boar spermatozoa resulting in their reduced fertilizing ability (Yeste 2016). The freezability of boar semen is determined by many factors, such as the sperm source, biochemical composition of the seminal plasma (SP) and extender (Fraser and Strzeżek 2007, Kaeoket et al. 2011, Wasilewska and Fraser 2017). Boar ejaculate consists of two main fractions, the sperm-rich and post sperm-rich fraction (SRF and PSRF, respectively), which differ in terms of their biochemical composition due mainly to the contributions of the testicular and epididymal fluids, and secretions of the accessory sex glands (Rodríguez-Martínez et al. 2021). Generally, spermatozoa from the PSRF of boar ejaculate are characterized by reduced post-thaw (PT) quality compared with those from the SRF; however, modifications of the freezing protocol have been shown to improve the sperm cryo-survival (Rodriquez-Martinez et al. 2008, Saravia et al. 2009). In previous studies, it has been confirmed that the components of different extenders could exert varying effects on the cryo-survival of boar semen (Kaeoket et al. 2011, Wasilewska and Fraser 2017). For the PT quality analysis we used a short-term extender (Belstville Thawing Solution, BTS) and a long-term (Androhep® Plus, AHP) extender, which comprises a buffering system, antioxidants and enhance cell membrane protective components (Kaeoket et al. 2011, Weitze 2014). Moreover, the AHP extender has been shown to be superior to the BTS extender with respect to PT quality of boar semen (Kaeoket et al. 2011). This study investigated the effect of short-term and long-term extenders on the PT quality of spermatozoa, originating from the SRF and PSRF of boar ejaculate.

Materials and Methods

A total of twenty one ejaculates were collected from three Polish Large White boars stationed at the Cryopreservation Laboratory of the Department of Animal Biochemistry and Biotechnology, University of Warmia and Mazury in Olsztyn. Animal experiments were performed in accordance with the guidelines set out by the Local Ethics Committee. The two fractions of boar ejaculate, the SRF and PSRF, were collected and were processed according to a standard cryopreservation protocol (Fraser and Strzeżek 2007, Wasilewska and Fraser 2017). Briefly, sperm samples were suspended in the BTS extender at 17°C (200×10^6 spermatozoa/ml). Following a 2h incubation period and centrifugation, the sperm pellets were re-suspended in an extender containing 11% lactose (w/v) with 5% lipoprotein fraction isolated from ostrich egg yolk (LPFo). The samples were cooled for a 2h period at 5°C, and further diluted (2:1) with a lactose-LPFo extender containing glycerol and OEP (Orvus Es Paste). The cooled samples (500×10^6 spermatozoa/ml) were packaged in sterilized aluminum tubes and frozen in a controlled programmable freezer (Ice Cube 1810, SYLAB, Austria). Following a 2-week storage period in liquid nitrogen, the frozen samples were thawed and split into two equal portions. Each portion of the frozen-thawed (FT) sperm samples was diluted (50×10^6 spermatozoa/ml) with the BTS extender (Minitube, Germany) or AHP extender (Minitube, Germany), and stored for 15 min (0 min) and 60 min, prior to PT analysis.

Motility and velocity parameters were analyzed using a computer-assisted sperm analysis system (CASA, HTR-IVOS 12.3, Hamilton Thorne Biosciences, MA, USA). The parameters analyzed using the CASA system included total motility (TMOT, %), progressive motility (PMOT, %), velocity average path (VAP, μm/s), velocity straight line (VSL, μm/s) and velocity curvilinear (VCL, μm/s) (Wasilewska and Fraser 2017). Sperm mitochondrial membrane potential (MMP) was evaluated using dual fluorescent staining with JC-1 and propidium iodide (PI) (Molecular Probes, Eugene, OR, USA), according to a previously described method (Thomas et al. 1998). Cells were classified as viable spermatozoa with intact mitochondria and non-viable spermatozoa with damaged mitochondria. Sperm plasma membrane integrity (PMI) was assessed using a the Live/Dead Sperm Viability Kit (Molecular Probes, Eugene, OR, USA). Cells were classified as membrane-intact and membrane-damaged spermatozoa. Acrosome integrity (AI) was monitored using the isothiocyanate-labeled peanut agglutinin (FITC-PNA) staining protocol (Aquila et al. 2011). Spermatozoa without FITC-PNA/PI staining or exhibiting uniform bright staining over the acrosome region were classified as live cells with intact acrosome, whereas spermatozoa exhibiting disrupted fluorescence were classified as dead cells with damaged acrosome. All stained samples for the fluorescent assays were observed at 600× magnification under an epifluorescence microscope (Olympus CH 30, Tokyo, Japan), and approximately 200 sperm cells were examined for each slide. The data were analyzed using the Statistica software package, version 12.5 (StatSoft Incorporation, Tulsa, OK, USA). The Neuman-Keuls post hoc test was used to analyze significant differences among the means. All results are expressed as the mean ± standard error (SEM) and were considered significant at $p \leq 0.05$.

Table 1. Quality characteristics of frozen-thawed (FT) boar spermatozoa. Values represent the means (± SEM) of 21 ejaculates for each treatment.

Sperm	Beltsville Thawing Solution (BTS)				Androhep (AHP)			
	0 min		60 min		0 min		60 min	
parameters	SRF	PSRF	SRF	PSRF	SRF	PSRF	SRF	PSRF
TMOT (%)	50.6±2.3^{a}	27.3±3.0^{b}	28.9±1.9^{b}	14.3±2.1^{c}	54.1±2.4^{a}	32.0±3.1^{b,d}	38.0±3.1^{d}	16.3±2.3^{c}
PMOT (%)	42.9±2.0^{a}	18.9±2.3^{b}	21.9±1.6^{b}	8.5±1.5^{c}	45.9±2.1^{a}	23.5±2.6^{b}	29.3±2.7^{b}	9.8±1.8^{c}
VAP (μm/s)	85.0±1.3^{a}	60.7±4.7^{b}	67.3±1.9^{b}	48.6±4.9^{c}	87.7±1.6^{a}	68.1±4.0^{b}	71.8±2.3^{b}	48.4±4.9^{c}
VSL (μm/s)	70.6±1.6^{a}	50.9±4.1^{b,c}	59.1±1.7^{b}	41.1±4.2^{c}	73.5±1.7^{a}	57.9±3.7^{b}	61.6±2.2^{b}	40.4±4.4^{c}
VCL (μm/s)	135.6±2.4^{a}	98.8±7.4^{b}	102.7±3.3^{b}	80.0±8.1^{c}	138.1±2.5^{a}	111.8±6.3^{b}	112.1±2.7^{b}	82.2±7.9^{c}
MMP (%)	38.0±1.7^{a,d}	25.4±2.3^{b}	26.3±1.4^{b}	15.6±2.2^{c}	43.0±2.0^{a}	32.2±2.2^{d}	33.6±2.4^{d}	20.6±2.6^{b,c}
PMI (%)	41.9±1.7^{a}	29.2±2.4^{b,c}	29.9±1.6^{b,c}	17.8±2.5^{d}	46.2±1.9^{a}	34.2±2.1^{b}	35.7±2.5^{b}	23.3±3.0^{c,d}
AI (%)	60.1±2.2^{a}	39.3±3.1^{b}	40.9±1.3^{b}	26.3±2.2^{c}	62.2±2.8^{a}	44.9±4.0^{b,d}	52.6±3.5^{d}	25.8±2.4^{c}

SRF – sperm-rich fraction; PSRF – post sperm-rich fraction; TMOT – total motility; PMOT – progressive motility; VAP – velocity average path; VSL – velocity straight line; VCL – velocity curvilinear; MMP – mitochondrial membrane potential; PMI – plasma membrane integrity; AI – acrosome integrity. Values within the same row with different letters (a, b, c and d) are significant at $p \leq 0.05$.

Results and Discussion

Quality characteristics of FT boar spermatozoa from different ejaculate fractions following PT storage in different extenders are shown in Table 1. CASA-analyzed motility and velocity parameters, and membrane integrity parameters were markedly higher in the SRF-derived FT spermatozoa held in both the BTS and AHP extenders, irrespective of the storage time. There are significantly marked differences in the biochemical composition of the SRF and PSRF, which have varying effects on the sperm cryo-survival (Saravia et al. 2009, Rodríguez-Martínez et al. 2021). Evidence has been shown that proteins are differentially expressed in the SRF and PSRF, and that the overexpression of the SRF-derived proteins are associated with various sperm functions, such as membrane stability and capacitation (Rodríguez-Martínez et al. 2021). According to Saravia et al. (2009), spermatozoa collected from the SRF had higher percentages of motility parameters at different stages of the cryopreservation process than those from the remaining portion of ejaculate. Moreover, spermatozoa originating from the SRF exhibited increased cryo-tolerance (Saravia et al. 2009), and differences in the composition of the SP from fractionated ejaculate have been shown to affect the sperm susceptibility to cryo-damage (Wasilewska-Sakowska et al. 2019). It is noteworthy that the presence of a high portion of SP from the PSRF significantly compromised the PT quality of boar semen (Fraser and Strzeżek 2007, Saravia et al. 2009, Rodríguez-Martínez et al. 2021). Previous studies showed that spermadhesins (PSP-I, PSP-II, AWN, AQN-1 and AQN-3), membrane-bound proteins (VDAC2 and ACRBP) and fibronectin of the SP have a protective role in sperm functions (Yeste 2016, Rodríguez-Martínez et al. 2021). The findings of the current study confirm that such a protective role of the SP components was more accentuated in FT spermatozoa of the SRF, resulting in reduced susceptibility to cryo-damage, irrespective of the extender type.

Differences in the PT semen quality between the BTS and AHP extenders were more marked in the SRF following a 60-min storage period (Table 1). Furthermore, FT spermatozoa of the SRF exhibited significantly higher ($p \leq 0.05$) TMOT, MMP and AI after 60-min PT storage in the AHP extender compared with the BTS extender (Table 1). Notably, variations in the PT semen quality between the short-term and long-term extenders (BTS and Androstar® Plus, respectively) could be attributed to differences in the composition of the extenders (Kaeoket et al. 2011). Besides antioxidants, the AHP extender comprises an enhancing cell membrane protective component which confers protection to the sperm structures against cold shock (Kaeoket et al. 2011, Weitze 2014). In the current study it is likely that the different components of the AHP extender could be responsible for the higher cryo-survival of spermatozoa of the SRF compared with the BTS extender following PT storage.

It can be suggested that differences in the composition of the ejaculate fractions and extender affect the cryo-survival of boar spermatozoa; however, components of the SRF appeared to exert a more beneficial effect on the sperm cryo-survival, irrespective of the extender type.

Acknowledgements

This study was financed by the University of Warmia and Mazury in Olsztyn as part of statutory activity (No. 11.610.003-110) and by the Ministry of Education and

Science under the “Regional Initiative of Excellence” program for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

References

Aquila S, Giordano F, Guido C, Rago V, Carpino A **(2011)** Nitric oxide involvement in the acrosome reaction triggered by leptin in pig sperm. Reprod Biol Endocrinol 9: 133.

Fraser L, Strzeżek J **(2007)** Effect of different procedures of ejaculate collection, extenders and packages on DNA integrity of boar spermatozoa following freezing-thawing. Anim Reprod Sci 99: 317-329.

Kaeoket K, Chanapai P, Junchiyaphoom P, Chanapiwat P **(2011)** The effect of using long term and short term extenders during cooling process on the quality of frozen boar semen. Thai J Vet Med 41: 283-288.

Rodríguez-Martínez H, Martínez EA, Calvete JJ, Peña Vega FJ, Roca J **(2021)** Seminal plasma: relevant for fertility? Int J Mol Sci 22: 4368.

Rodríguez-Martínez H, Saravia F, Wallgren M, Roca J, Peña FJ **(2008)** Influence of seminal plasma on the kinematics of boar spermatozoa during freezing. Theriogenology 70: 1242-1250.

Saravia F, Wallgren M, Johannisson A, Calvete JJ, Sanz L, Pena FJ, Roca J, Rodríguez-Martínez H **(2009)** Exposure to the seminal plasma of different portions of the boar ejaculate modulates the survival of spermatozoa cryopreserved in MiniFlatPacks. Theriogenology 71: 662-675.

Thomas CA, Garner DL, DeJarnette JM, Marshall CE **(1998)** Effect of cryopreservation of bovine sperm organelle function and viability as determined by flow cytometry. Biol Reprod 58: 786-793.

Wasilewska K, Fraser L **(2017)** Boar variability in sperm cryo-tolerance after cooling of semen in different long-term extenders at various temperatures. Anim Reprod Sci 185: 161-173.

Wasilewska-Sakowska K, Zasiadczyk Ł, Fraser L **(2019)** Effect of fractionated seminal plasma on sperm characteristics following cryopreservation of boar semen. Ann Anim Sci 19: 695-712.

Weitze KF **(2014)** Benefits of Androhep®Plus and Androstar®Plus long-term extenders for boar semen. (Minitüb Gmbh) Technical Report 5: 1-6.

Yeste M **(2016)** Sperm cryopreservation update: cryodamage, markers, and factors affecting the sperm freezability in pigs. Theriogenology 85: 47-64.