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Short communication

Effects of supplemental seminal plasma on cryopreserved boar sperm quality

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Abstract

To analyse the effects of supplemental autologous seminal plasma on boar semen quality before freezing and after thawing, thirty ejaculates were collected from six Pietrain boars. The main factors of a 2 x 2 x 2 factorial arrangement of treatments were Beltsville thawing solution (BTS), seminal plasma before freezing, and seminal plasma after freezing-thawing. The percentage of acrosome-intact sperms was reduced by semen dilution. There were no interactions of main factors. The addition of seminal plasma to semen before freezing did not affect semen quality, but the addition of seminal plasma after freezing-thawing increased the percentage of acrosome-intact sperm. This approach indicates that the addition of seminal plasma to boar semen after freezing-thawing improves semen quality.

Keywords: Acrosome integrity, freezing, thawing, semen
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Freeze-thawed boar semen has limited commercial use because during the freezing-thawing process, 50% of the spermatozoa lose their fertilizing capacity (Qian *et al*., 2016) as a result of thermal shock, which compromises viability and integrity of DNA and increased lipid peroxidation of membrane phospholipids (Fraser *et al*., 2017). Cryodamage is not restricted to sperm membrane, but affects the integrity of sperm chromatin (nucleoprotein structure and DNA) and mitochondrial function, involves degradation of mRNAs, and impairs the function of certain proteins (Yeste, 2016). Seminal plasma protects the plasmatic membrane of the spermatozoa, providing resistance to cold shock, capacitation and acrosome reaction (Gómez-Fernández *et al*., 2012). Evidence suggests that supplemental seminal plasma increases the percentage of viability (motility, membrane integrity and acrosome) of spermatozoa (Alkmin *et al*., 2014), which is attributed to proteins, antioxidant enzymes, and organic or inorganic components (González-Cadavid *et al*., 2014), although others have reported a decrease in the integrity of acrosome (Fernandez-Gago *et al*., 2013). The differences among these studies could be explained by the time and proportion of the addition of seminal plasma. The authors of the current study hypothesized that adding 20% seminal plasma to diluted or undiluted boar semen before or after cryopreservation could improve sperm viability. Therefore, the main objective of this study was to evaluate the effects of supplemental seminal plasma on boar semen quality before and after cryopreservation and thawing.

Thirty ejaculates were taken from six Pietrain boars (1095–1460 days old). Boars were housed in individual pens, and fed twice daily with 1.5 kg of concentrate, which contained 15% crude protein, and 1.2 Mcal metabolizable energy. Semen samples were collected once a week using the gloved hand method into 100 mL pre-warmed (38 °C) tubes. The sperm-rich portions of the ejaculate were evaluated for conventional semen characteristics (volume, sperm concentration and percentage of motile spermatozoa) using standard laboratory techniques (Gutiérrez-Pérez *et al*., 2009). Ejaculates (five each boar) with ≥200 × 10^6 sperm/mL, ≥85% sperm with normal morphology, and with ≥75% and ≥80% of motile and viable spermatozoa, respectively, were selected for cryopreservation (Hernández *et al*., 2015). Half (50 mL) of the total semen sample was extended (1 : 1) with Beltsville thawing solution (BTS) (Sigma-Aldrich Co., St. Louis, MO, USA)

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and cooled at 16 °C for 24 hours before freezing. Semen subsamples with and without BTS were or were not supplemented with 20% seminal plasma (1 : 4) before freezing and after freezing-thawing.

Seminal plasma was obtained from the same boars at the time that the semen samples were collected and processed according to the strategies of boar sperm cryopreservation of Okazaki & Shimada (2012). Plasma was obtained by centrifuging the ejaculate (800 g x 10 min), collecting supernatant, re-centrifuging, and filtering (10 µm nylon filter) to remove sperm cells. Filtered samples were observed under a microscope to confirm the absence of spermatozoa. As a freezing extender, semen samples were dissolved (1 : 1 (v/v)) in a distilled water preparation containing Tris (111 nM), citric acid (31.4 mM), glucose (185 mM), egg yolk (20% (v/v)), and kanamycin sulphate (100 µg/mL) (Sigma-Aldrich Co., St. Louis, MO, USA). Diluted semen was centrifuged at 800 × *g* 15 °C for 10 min. The supernatant was discarded and the sperm pellet was re-suspended in the same freezing medium to a concentration of 1.2×10^9 sperm/mL and cooled from 15 °C to 4 °C for 180 min. The processed semen was loaded intro 0.5 mL straws and sealed with polyvinyl alcohol. Straws were exposed to liquid nitrogen vapour (4 cm) for 20 min and then plunged into liquid nitrogen (-196 °C). After two weeks, all frozen semen straws were thawed in a warm water thermo (37 °C, 30 s). Half of the straws with thawed semen were diluted with 20% (1 : 4) of the seminal plasma described above and incubated at 25 °C for one hour. The other half of the straws were not diluted with seminal plasma. Thawed semen was evaluated for sperm acrosome integrity using the triple stain protocol (Bismarck brown/rose Bengal/trypan blue, Sigma-Aldrich Co., St. Louis, MO, USA). One hundred spermatozoa were examined in randomly selected fields at 1000x magnification with a bright-field microscope and assessed for percentages of sperm that were alive with normal and damaged acrosome (Talbot & Chacon, 1981).

Data were analysed as a completely randomized design with a 2 x 2 x 2 factorial arrangement of treatments using the GLM procedure (SAS, 1999), in which the main factors were the use or not of BTS as semen extender, with or without supplemental seminal plasma before freezing semen, and with or without supplemental seminal plasma after freezing-thawing semen.

Raw fresh semen had 85% viability, but thawed semen after freezing had 60% viability. This means that the freezing-thawing process reduced almost 24% of semen viability. Means of interactions were statistically not significant (Table 1). Figures 1 and 2 show the percentages of live sperm of thawed semen. The use of BTS as semen extensor reduced (P <0.05) the percentage of live sperm with acrosome-intact membrane and therefore increased (P <0.05) the percentage of sperm with damaged acrosome. The use of seminal plasma before freezing did not modify the percentage of live sperm with intact or damaged acrosome membrane. Supplemental seminal plasma after freezing-thawing increased (P <0.05) the percentages of live-sperm with acrosome-intact membrane and reduced those with acrosome-damaged membrane.

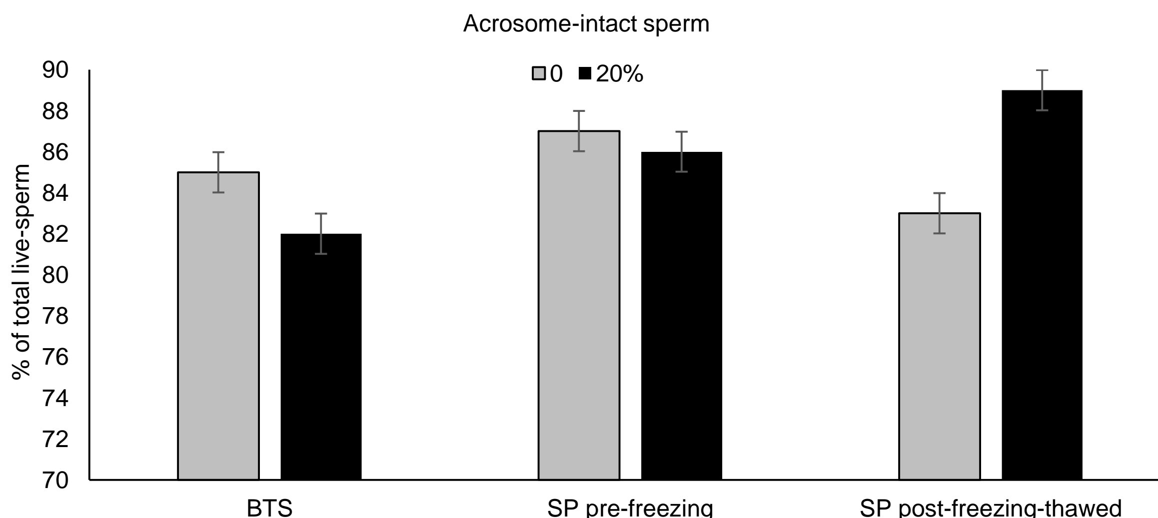

Figure 1 Percentages of live spermatozoa with acrosome-intact membrane in thawed semen influenced by semen extensor and seminal plasma before freezing or after freezing-thawing

Table 1 Effects of dilution and seminal plasma addition at pre- and post freezing on boar spermatozoa

	-Beltsville thawing solution				+Beltsville thawing solution				
	-SP pre-freezing		+SP pre-freezing		-SP pre-freezing		+SP pre-freezing		
	-SP thawed	+SP thawed	-SP thawed	+SP thawed	-SP thawed	+SP thawed	-SP thawed	+SP thawed	SEM
Live with intact acrosome	60.0	73.5	64.0	62.5	56.5	57.5	58.5	66.5	1.83
Live without acrosome	12.0	5.0	12.0	7.0	12.0	7.5	12.0	11.5	0.42
Dead with intact acrosome	19.0	14.5	18.0	24.5	20.5	20.0	19.5	12.0	0.51
Dead without acrosome	9.0	7.0	6.0	6.0	11.0	15.0	10.0	10.0	0.59

Values expressed as %
SP: seminal plasma; SEM: standard error of means

In this study, semen quality was evaluated before and after the cryopreservation process. The results confirmed that boar semen is affected negatively by the freezing-thawing process. The viability values found in the thawed semen are similar to those reported by Roca *et al.* (2006). Similar to the current results, decreased spermatozoa with intact acrosome after freezing-thawing have been reported by other authors (Trzcińska *et al*., 2015; Fraser *et al*., 2017). This damage was more evident in cells from ejaculates diluted with BTS compared with semen that was not diluted. These variations may be due to the wash effect induced by the semen extender (Maxwell *et al*., 1997).

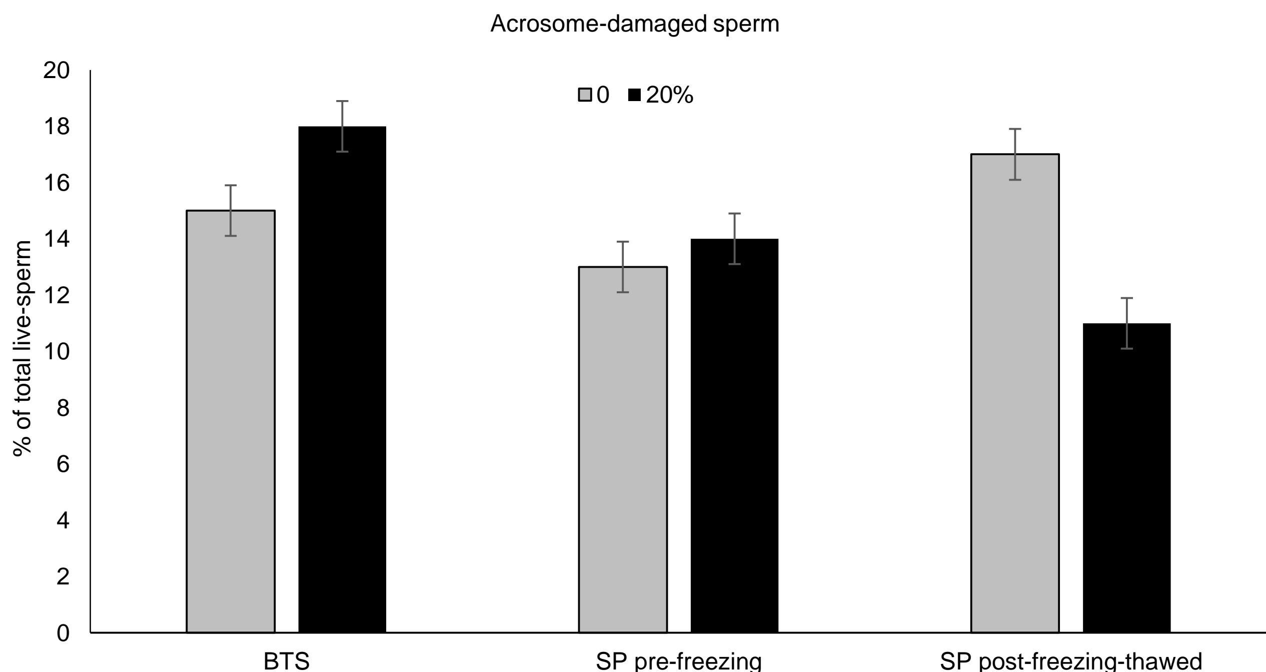

Figure 2 Percentages of live spermatozoa with damaged acrosome membrane in semen influenced by semen extensor and seminal plasma before freezing or after freezing-thawing

Contrary to previous studies (Muiño-Blanco *et al*., 2008) that reported beneficial effects on semen quality by seminal plasma as extender, the authors did not find any influence on percentage of live spermatozoa with intact acrosome from the addition of seminal plasma before freezing. These differences in

the effects of seminal plasma on sperm viability are dependent on the ejaculated quality and composition (amino acids, lipids, fatty acids, peptides, and proteins) of seminal plasma (Roca *et al*., 2006), especially sperm adhesins, which represent 90% of total proteins of seminal plasma (Dostàlovà *et al*., 1994). The beneficial effects found in live spermatozoa with acrosome intact from the addition of seminal plasma after freezing-thawing process were reviewed by Okazaki & Shimada (2012), who indicated that seminal plasma worked as a capacitation-inhibitory factor, probably through an important increase in membrane fluidity (Fernández-Gago *et al*., 2013).

It is concluded that the addition of 20% seminal plasma after freezing-thawing semen improves the percentages of live-spermatozoa with intact acrosome membranes.

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Authors' Contributions

MBM designed the experiment, and supervised and monitored the experimental work. MBV realized the experimental work, semen cryopreservation and viability assessment. BDM developed the statistical analysis. JMPR wrote and revised the manuscript for important intellectual content and approved the final version. MLJM revised the laboratory procedures and experimental design.

Conflict of Interest Declaration

None of the authors has any conflict of interest to declare.

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