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Sperm quality of post-thawed boar semen using different freezing curves

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The boar sperm is susceptible to thermal shock during freezing and thawing being these events responsible for the low viability post-thawing. The objective of this study was to evaluate the effect of different freezing methods on sperm characteristics of boar semen. Twenty ejaculates from 20 animals were diluted in commercial extender and cooled at 17°C for 20 hours. The doses were centrifuged (1600g for 5 min. at 15 °C) to remove the supernatant. In methodology A (control - Westendorf et al., 1975, Deutsche Tierarztliche Wochenschrift, 82, 261-267), the pellet was resuspended in 1:3 semen: extender cooling (EC) (80% lactose solution 11% and 20% of egg yolk). Then, the samples were cooled to 5°C for 90 min. and then added two parts of freezing extender (EC 89.5%, 1.5% Ex Orvus Paste and 9% glycerol). The filling was done in 0.5 mL straws which were subjected to liquid nitrogen vapor (-90 °C) for 20 min. and kept in liquid nitrogen at -196 °C. In methodology B (biofreezer), the freezing extender was added just after the cooling extender, both in the same proportions described above. Doses were packaged into straws 0.5 ml and subjected to curve freezing ranging 15 °C to 5°C at 0.25°C/min., 5°C to -80°C at 10°C/min., and -80°C to -120°C at 5 °C/min. Thawing occurred in a water bath at 37°C for 20 seconds. The contents of each straw was diluted in 2.0 ml of ALMUS extender at 37°C and subjected to analysis of motility, vigor, membrane integrity (eosin nigrosin) and acrosome integrity (Pope staining). The data were submitted to paired t test, and statistical analyzes were performed in SAS statistical software (1996) at 5% probability. The motility (37.25 ± 2.98, 3.63 ± 37), the vigor (2.4 ± 0.14, 2.3 ± 0.105) and membrane integrity (61% ± 2.89, 60.25% ± 2.45) did not differ (P> 0.05) among the methodologies. The methodology A (45.9% ± 3.34) showed (P<0.05) higher acrosome integrity than the method B (40.45% ± 3.73), that can be explained by the fact that in the methodology A semen was only exposed to crioprotector at the temperature of 5 °C and not at 15 °C as in methodology B, in which can be occurred a cytotoxic effect. It is believed that the cooling curve of the methodology B was very slow, exposing spermatozoa for a long time in cryoprotectant prior to freezing. Thus, the method of freezing A is recommended, since it provided the best acrosome integrity.