A180 Physiology of Reproduction in Male and Semen Technology

Effect of different melatonin concentration in integrity of acrosomal membrane in swine sperm cryopreserved

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Boar semen is extremely vulnerable to temperature variation, and sensitive to lipoperoxidation damage, because its membrane contains high concentration of polyunsaturated fatty acids (BONDAN, C. Am J of Biochm Biotech. in press. 2016). The structural differences in the lipid layers and the process of "cryo-capacitation" can explain the sensitivity of the swine sperm cells to cryopreservation (BAILEY, J.L. J Androl. v. 21, p. 1-7, 2000). Melatonin is an amphiphilic antioxidant capable of penetrating in cellular compartments, protecting the cells from oxidative damage (REITER, R.J. News Physiol. Sci. v.15, p.246-250, 2000). Therefore, the objective of this study was to evaluate whether different concentrations of melatonin have protective effect to the acrosome membrane of cryopreserved swine sperm. For this, the rich fraction of 5 ejaculates from 3 boars was diluted with commercial extender in the ratio 1:1. Samples were kept at 20°C for 120 min and then conditioned at 15°C for 180 min. Then, the ejaculate was centrifuged (1600xg/5min/15°C) and the sediment was resuspended in 1:2 semen:cooling extender (CE - 80% lactose solution 11% and 20% of egg yolk) and kept at 5°C for 90 minutes. At the end of this period, the same volume of freezing extender (FE - 89.5% CE, 1.5% Ex Orvus Paste and 9% glycerol) was added to reach a final sperm concentration of 5x109/ml. Melatonin was added to extenders CE and FE, with a final concentration of 0; 1.25; 2.5 to 5 mM. Semen was then transferred to a 0.5ml straw and kept for 20min in nitrogen vapor and then immersed in liquid nitrogen. Samples were thawed at 37°C for 20 seconds. Further, semen was diluted to 1:4 with commercial extender and centrifuged (800xg/3 min). The pellet was resuspended again in commercial extender. Samples were evaluated for motility (under phase contrast microscopy) and acrosomal membrane integrity using FITC-Pisum sativum (FITC – PSA, 11.7 mg / mL) by flow cytometry (BD-Accuri). Data were analyzed using PROC MIXED (SAS® Institute Inc., Cary, NC, EUA). The Tukey test was used to compare the mean of the minimum squares of the group and the data are presented as mean of percentage ± SD. Regression analysis was performed in Instat® (Graphpad Instat: GraphPad Software Oberlin, San Diego – CA, USA). There was no difference between melatonin concentrations in percentage of motility after thawing [(32.66% ± 2.69 (0); 35.00% ± 2.69 (1.25 mm); 39.50% ± 2.8 (2.5 mM) and 37.3% ± 2.69 (5 mM)]. No effect was identified on acrosome integrity, being the percentage of post-thaw acrosomes integrity of 51.96% ± 3.64 (0); 49.13% ± 3.64 (1.25 mM); 47.48% ± 3.64 (2.5 mM) and 48.80% ± 3.78 (5 mM). There was no correlation between melatonin concentrations and motility ($r = -0.072$, $p = 0.58$) and the percentage of post-thaw intact acrosomes ($r = 0.1419$, $p = 0.283$). We concluded that the addition of melatonin in the concentrations used did not have a protective effect on the acrosomal membrane for cryopreservation of boar semen.