Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Physiology of reproduction in male and semen technology

Effect of equilibration time in plasmatic membrane integrity and motility of boar spermatozoa

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Among the techniques with the potential to decrease cryodamage in spermatozoa is the equilibrium time (ET). ET is defined as the period during which sperm cells are kept in contact with cooling/freezing media components at a temperature of 5°C, as this may provide a more proper osmotic balance between the intra and extracellular environment. An alternative that has already proved efficient in improving the cryotolerance of sperm from other species. Therefore, the experiment aims to evaluate the influence of the ET in the cryopreservation protocols of boar semen. For this, five boars of a commercial hybrid line are used. Four ejaculates of each animal (n= 20) were collected by the gloved-hand technique. After collection, semen samples were analyzed for total motility (TM) and Progressive motility (PM) (Computer-Assisted Sperm Analysis, SCA®) and sperm concentration in the Neubauer chamber. Only samples with more than 80% TM were cryopreserved. Posterior to the analysis, samples were destined for the two-step cryopreservation procedure. Each sample was extended 4-fold with Beltsville Thawing Solution. Semen was maintained at 17ºC/24 hours and centrifuged (2100xg/3min), sperm pellets were extended in cryopreservation medium without cryoprotectant (BotuSui Fração A®, Botupharma, Botucatu-SP). Subsequently cooling to 5°C, samples were remaining under this temperature for periods of 0, 2, and 4 hours of ET. Then samples were diluted with cryopreservation medium (BotuSui Fração B®, Botupharma, Botucatu-SP - 6% glycerol and 6% methylformamide) to $1x10^9$ sperm/mL. Extended sperm were packed into 0.5mL straws. The freezing curve from 5ºC to -120°C was -20°C/min, using a controlled-rate freezer (TK 3000®; TK Tecnologia em Congelação Ltda., Uberaba-Brazil). Samples were finally plunged into liquid nitrogen at -196°C and stored. So, four straws were thawed at 37 °C for 30 seconds for analyzes of TM and PM in CASA and integral plasma membrane (MI) by flow cytometry (Accuri C6®; Becton Dickinson and Company; San Jose, CA, USA) using the probes SYTO-59 (S10341, Molecular Probes Inc., Eugene, Oregon, EUA) and propidium iodide (PI, L0770, Sigma-Aldrich Co., Saint Louis, Missouri, EUA). The original or transformed data were submitted to PROC MIXED using the SAS program. We expected that the equilibration time would be beneficial to TM, PM, and MI. However, our results are contrary to expected, showing no changes in the value of the samples. There was no difference ($p<0.05$) for TM (15.32%±1.54 - 0h, 17.72±2.06 - 2h, and 15.97±1.67 - 4h) and for PM (6.26±0.87 - 0h, 6.57±0.90 - 2h and 6.26±0.84 - 4h). Concerning MI, the population of the treatments 2 and 4 hours (32.30±1.99 and 31.90±2,93, respectively) presents no statistical difference between the treatments ($p<0.05$). The implementation of equilibration time for periods of 0, 2, and 4 hours not shown to be beneficial for TM, PM and MI. Financial support by FAPESP - 2017/20796-5.