Animal Reproduction

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Physiology of reproduction in male and semen technology

The effect of cryoprotectants combination at different concentrations during cryopreservation of semen from windsnyer boars.

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Boar sperm is associated with the post-thawed sperm quality reduction due to the low cholesterol and high polyunsaturated fatty acid contents in their sperm plasma membrane (Li J, 2017, Cryobiology, 80, 119-125). The objective of the study was to investigate the efficacy of the cryoprotectants combination at different concentrations on the Windsnyer boars sperm quality during cryopreservation. A total of 18 ejaculates (6 replications/boar) were collected from three Windsnyer boars of proven fertility with the use of the hand-gloved technique method, twice per week. Boars semen were pooled and was extended with Beltsville Thawing Solution [(BTS) IMV Technologies, France], held at 18°C for 3 hours and centrifuged. The sperm pellet was re-suspended with Fraction A (20% egg yolk + BTS) and cooled at 5°C for 1 hour. Following cooling, semen was divided and diluted into the combination of the cryoprotectants [Ethylene glycol {(EG) Sigma-Aldrich®, Munich, United State of America} + Glycerol {(GLY) Laboratory Consumables & Chemicals Supplies cc, Johannesburg, South Africa} + Propanediol {(PDO) Rochelle Chemicals & Lab Equipment, Johannesburg, South Africa}] at equal contribution to make the total concentrations of 4, 8, 12 and 16% and the 0% (control; without cryoprotectant) and loaded into 0.25 mL straws (Embryo Plus, Brits, South Africa). The semen straws were placed on liquid nitrogen (LN_2) vapour for 20 minutes and then transferred to the LN_2 tank. However, the conventional boar semen cryopreservation protocol (4% GLY + 20% egg yolk + BTS) was tested, and the results were compared with the current experiment. Thawing was accomplished by immersing the semen straws in water at 40°C for 30 seconds. Sperm motility, viability and morphology characteristics were evaluated following thawing. Sperm motility was evaluated with the use of the Sperm Class Analyser® (Microptin, Spain) system. Eosin-Nigrosin staining was used to evaluate sperm viability and morphology at 100X magnification under a phase-contrast microscope (Olympus, BX 51FT, Tokyo, Japan). A total of 200 sperm per slide/treatment was counted for sperm viability and morphology characteristics. The data were analyzed using the analysis of variance (general linear model) and statistical analysis system (SAS®). Treatment means were separated using Fisher's protected t-test and the significant differences were determined by P-value at a significant level of $P<0.05$. The highest post-thawed sperm total motility (21.4±7.2) percentage was recorded in the treatments supplemented with 16% combination of EG + GLY + PDO, significantly different from 4% GLY (18.0±8.2). The least post-thawed sperm progressive motility percentage was recorded in the treatments supplemented with 0% (1.1±1.3) and 8% (4.7±3.3) combination of EG + GLY + PDO, significantly different from 4% GLY (8.0±6.4). However, semen samples supplemented with a 4% (28.8±4.4) and 16% (30.4±6.2) combination of EG + GLY + PDO recorded the highest post-thawed live normal sperm percentage which was significant to 4% GLY (32.1±6.7). There was no post-thawed sperm abnormality percentage recorded in the semen supplemented with the 0% combination of EG + GLY + PDO ($P<0.05$). The 16% combination of EG + GLY + PDO maintained boars sperm survival during cryopreservation.

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