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Physiology of reproduction in male and semen technology

Evaluation of Dithiothreitol and Glutathione antioxidants supplemented during cryopreservation of Large White boar semen and subsequent to fertilization of porcine oocytes

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Cryopreservation is the most practical approach for the long-term storage of sperm in boars. However, the freezing and thawing processes result in compromised sperm function and *in vitro* fertilization (IVF) success. The study aimed to evaluate the fertilizing ability of frozen-thawed semen on matured porcine oocytes following IVF. Semen was collected from three Large White boars and then transported to the laboratory for evaluation. Semen was diluted with Beltsville Thawing Solution then equilibrated at 17°C for 120 min, later centrifuged at 800 x g for 10 min at 15°C. Semen pellets were resuspended with egg yolk citrate base extender and placed back for 90 min at 5°C. Semen was supplemented with 4 different fraction B (control, 5 mM DTT, 5 mM GSH and a combination of 2.5 mM DTT + 2.5 mM GSH) extenders and loaded into 0.25 mL freezing straws, then placed in vapour for 20 min, later plunged into nitrogen tank (-196°C). Semen was thawed at 37°C then evaluated for sperm motility. Pig ovaries were collected from the local abattoir and transported to the laboratory within an hour in 0.9% saline water in a thermos flask at 38°C. The slicing method was used to retrieve the oocytes from the ovaries. Oocytes were washed three times in modified Dulbecco phosphate buffered saline and modified Medium 199. Only good quality oocytes were *in vitro* matured for 44 hrs in North California State University-23 medium supplemented with 10 ng/mL of follicle-stimulating hormone, 10 ng/mL of luteinizing hormone and 10% porcine follicular fluid. The oocytes were washed five times in pre-warmed (37°C) 100 µl of IVF medium drops and then distributed into 50 µl of the IVF drops. A drop of 50 µl of capacitated diluted (1 x 10^6) fresh and frozen-thawed sperm was used for IVF. Sperm and oocytes were co-incubated at 38.5°C in a moist atmosphere of 5% CO_2 in the air for 6 hrs. The fertilization rate was evaluated by the presence of a pronucleus with the aid of an inverted microscope using Hoechst 33342 staining. Data were analyzed using the GLM procedure. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at a 0.05 level of significance. Percentage data are presented as mean ± standard deviation values. The sperm total motility of frozen-thawed semen ranged from 22.4 to 32.0% for all treatments (P>0.05). The percentage of polyspermy differed significantly among the treatments (P<0.05). The total fertilization rate ranged from 31.9 to 48.7%. Raw semen (11.8±9.4) and combination of 2.5 mM DTT + 2.5 mM GSH (14.1±10.4) treatments recorded higher percentage of polyspermy as compared to the 5 mM GSH (0.8±1.9) and 5 mM DTT treatments (2.2±4.9), (P<0.05). The 5 mM DTT treatment (19.7±9.9) non-significantly had a high percentage of oocytes showing normal fertilization (2 PN) as compared to all the treatments. The 5 mM Glutathione treatment (31.9±8.6) non-significantly recorded the least percentage of total fertilization rate as compared to all the treatments (P>0.05). In conclusion, both Glutathione and Dithiothreitol did not have any effect on the fertilization rate by cryopreserved boar semen.

These results are already published https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9104872

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