PP-298

Protective Effects of Different Cryoprotectants on Post-Thawed Rabbit Epididymal Sperm Chromatin Condensation

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Aim of the study: Cryopreservation is a long-term storage technique with very low temperatures to preserve the sperm of various animal species for extended period of time at a low cost. This technique can contribute to the persistence of endangered species and hence biodiversity. To preserve the sperm of any animal species, various cryoprotectants are used in sperm freezing protocols. The aim of this study was to determine the protective effects of different cryoprotectants on chromatin condensation of the epididymal rabbit sperm after the freeze–thawing process using Toluidine Blue (TB) stain.

Material and Methods: Epididymal sperms were collected from rabbits (n=32) and evaluated at 37 °C. Pooled semen samples were diluted in Tris-based extender containing different cryoprotectants. Samples were divided into 12 groups as follows: Control, C (Control, C-C; L-Glutamin, C-LG; Basal Medium Eagle Amino Acids, C-BME); Paclitaxel, P (P-C; P-LG; P-BME); Resveratrol, R (R-C; R-LG; R-BME); Paclitaxel+Resveratrol, PR (PR-C; PR-LG; PR-BME). Diluted semen samples were aspirated into 0.25-ml (medium-sized). Straws, sealed with polyvinyl alcohol powder, and equilibrated at 5 °C for 2 h. After equilibration, the straws were frozen in liquid nitrogen vapour, 4 cm above the liquid nitrogen, for 15 min and plunged into liquid nitrogen for storage. Frozen straws were then thawed individually at 37 °C for 25 s in a water bath for evaluation. TB stain (stains phosphate residues of fragmented DNA) was used to assess the sperm chromatin structure. Obtained results for each dose were analysed statistically.

Results: No significant difference was observed in the mean percentage of damaged DNA of sperm cells among the groups. Further analyses are required to reveal the protective effects of different cryoprotectants on incomplete DNA structure and DNA packaging in the epididymal sperm of rabbit during cryopreservation. It is well known that storage of the sperm for a long time causes deterioration of the sperm quality, but supplementation of several cryoprotectants may provide the protection against damage of sperm DNA in cryopreservation. In this respect, the question is which cryoprotectants and doses are more effective. This and suchlike studies can enable researchers to plan the future efforts for the conservation and persistence of endangered animal species.

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