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Influence of sperm processing on IVP of bovine embryos

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The IVP rates of bovine embryos are within acceptable levels, although several factors contribute to reduce the percentage of transferable embryos. The effect of the bull used as sperm donor is one of such factors, but information about the processing of the sperm used in IVF is still scarce. The present study aimed to determine the influence of cryoprotectants and sperm selection gradient on sperm recovery and integrity, and the IVP rate of bovine embryos. Initially, sperm of a bull with known fertility for IVF was split in two fractions for cryopreservation: 5% glycerol (GLY); and 3% ethylene glycol (EG). At sperm selection, each group was centrifuged at 5000 g for 5 min in discontinuous gradients of Percoll® (90 and 45%; Per) or OptiPrep® (40 and 26%; OP) with reduced volume (600µl), forming four treatments: Per + GLY, Per + EG, OP + GLY, and OP + EG. Grade I and II cumulus-oocyte complexes (COCs) obtained from ovaries collected in slaughterhouse were randomly divided in 4 groups for IVM, in TCM 199 supplemented with gonadotropins and 10% estrus mare serum (EMS). Each IVM routine (n = 7) used one semen straw from each treatment (GLY and EG). Sperm concentration, vigor, motility and membrane integrity were evaluated after thawing and after selection. Each COCs group was inseminated (I0) with 1x10⁶ spermatozoa / ml. After 18 hours of incubation in TALP-Fert, presumptive zygotes were denuded and transferred (n = 725) to SOFaaei medium supplemented with 5% of EMS and cultured at 39 °C under humid atmosphere with 5% O₂ + 5% CO₂ + 90% N₂ in a bag-system. The results were evaluated by Statistix9®. Cryoprotectant did not influence (P > 0.05) recovery and sperm membrane integrity (GLY = 26.0% and 62.7%, EG = 24.9% and 67.5%, respectively). However, the selection gradient affected (P < 0.001) recovery rates and sperm membrane integrity (Per = 37% and 79.6%, OP = 13% and 50.6%, respectively). Embryo development (D8 blastocysts) was similar (P > 0.05) for Per + GLY (19.3%) and Per + EG (25.4%), but both rates were higher than those observed for OP + GLY (11.1%) and OP + EG (11.0%) (P < 0.001). The tested cryoprotectants did not affect the evaluated sperm parameters and the rate of embryo development. However, the Percoll gradient was more efficient than the OptiPrep for sperm selection, providing greater recovery rate and sperm integrity and higher efficiency in IVP of bovine embryos.