Animal Reproduction

Physiology of reproduction in male and semen technology e biotechnology

Percoll® gradient induces sperm capacitation detected by intracellular Zinc signaling

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For the success in each step of IVP process (IVM, IVF and IVC) some requisites are needed. Concerning the sperm, cells need to be capacitated for fertilization by functional and structural alterations resulting in permeability changes in cell membrane, promoting the influx of calcium. Recent studies described zinc signatures as a method to assess sperm capacitation (Kerns et al., Nat Communi, 9:2061, 2018). Although sperm needs to be capacitated to fertilize, a premature capacitation is harmful. The manipulation, freezing/thawing, sperm preparation for IVP and cell sorting are involved with this premature capacitation. It is not yet clear if the submission of semen to a Percoll® gradient promotes complete sperm capacitation, reflecting on the success of the *in vtro* embryo production. The aim of this study was to evaluate if Percoll® gradient induces sperm capacitation, and its correlation with IVP rates. For that, semen of 10 Nelore bulls, used on in-vitro embryo production where submitted or not to Percoll® gradient. Sperm motility was evaluated by computer assisted sperm analysis (CASA) and sperm acrossomal/plasmatic integrity (FITC/PI), mitochondrial membrane potential (JC1) by Flow Cytometry. Sperm capacitation was evaluated by chlortetracycline fluorescence assay (CTC) (Ward and Storey., Develop Bio, 2:287-6, 1984) and zinc assay with fluorescence probe Fluozin™ -3 AM (FZ3). A correlation test (PROC CORR SPEARMAN RANK) was done in attempt to correlate the sperm capacitation variables with *in vtro* embryo production (cleavage rate: total of cleavage structures/oocytes, blastocyst rate: blastocyst/oocytes and embryo development rate: blastocyst/cleavage structures). Sperm zinc signature in samples submitted or not to Percoll® gradient were: signature 1 (non capacitated): 14.11% and 6.21%, signature 2 (undergoing capacitation): 45.32% and 33.38%, signature 3 (capacitated sperm): 1.95% and 4.86%, and signature 4 (remodeled plasma membrane): 38.62% and 55.55%, respectively. Semen not submitted to Percoll® gradient presented higher percentage of zinc signature 3 4.86%, p = 0.025, higher percentage of capacitated spermatozoa with reacted acrosome by CTC 82.98%, p = 0.002, and higher percentage of sperm rectilinearity 80.40%, p = 0.041 when compared to samples submitted to Percoll® gradient. In addition, sperm submitted to Percoll® gradient, had a higher percentage of non-capacitated cells in CTC evaluation 20.64%, p = 0.003, when compared to sperm not submitted to Percoll® gradient. We observed a relative correlation between sperm Zinc signature 3 (capacitated cells) and in-vitro embryo development rate (rho: -0.650, p 0.041), showing that the more capacitated cells, less embryos are produced. Percoll® gradient seems to remove sperm already capacitated from samples, confirmed by the presence of more non-capacitated cells after centrifugation. This condition seems to be important for bovine in-vitro fertilization and success of bovine IVP.