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THE EFFECT OF β-MERCAPTOETHANOL ON CLEAVAGE RATES, DEVELOPMENTAL COMPETENCE AND QUALITY OF *IN VITRO* PRODUCED BOVINE EMBRYOS.

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The production of excessive levels of reactive oxygen species can be a major problem during *in vitro* embryo culture. While studies have shown that supplementation with exogenous antioxidants can improve embryo quality, the results are controversial among researchers. In this study, we examined the effects of different concentrations of β-mercaptoethanol (β-ME) added to the culture media, on cleavage rates, the quality and developmental competence of *in vitro* produced bovine embryos. *In vitro* embryo production was completely serum-free as described previously (Van Hoeck *et al*., 2013). Briefly, in total 753 grade I oocyte cumulus complex (COCs) from 2-6mm diameter follicles were matured in groups of 50 in 500µl TCM with 20ng/ml EGF for 24h, fertilized in groups of 100 in 500µl fertilization medium for 20h (5% CO_2, 38.5^{o}C). Presumptive zygotes were denuded and randomly assigned to 4 treatments with different concentrations of β-ME: 0µM (control), 50µM, 100µM and 150µM and cultured in groups of ±25 in 50µl SOF supplemented with ITS (10µg/mL insulin; 5,5µg/mL transferrin; 6,7ng/mL selenium) and 2% BSA (Essentially fatty acid free) and covered with mineral oil (5% O_2, 5% CO_2, 38.5^{o}C). At 48 hour post insemination cleavage rate was evaluated, expressed as the number of embryos cleaved on the total number of embryos cultured. Blastocysts rates was evaluated at 7d after fertilization, blastocysts were fixed with 4% paraformaldehyde and total cell number and apoptotic cell ratio were determined by using DAPI and TUNEL staining. Data were analysed by ANOVA and Post Hoc Test (PHT). Comparable cleavage rates were obtained in treated groups: control (80.8%), 50µM (77.7%), 100µM (77.9%) and 150µM (73.6%) ($P>0.05$). Also, no significant effect of treatment could be found on blastocyst rates: control (36%), 50µM (36.5%), 100µM (38.4%) and 150µM (30.4%). The total cell number per blastocyst increased significantly ($P< 0.05$) using 100µM of β-ME as compared with the controls (158.0 ± 24.3 vs 123.2 ± 9.72, respectively). These results suggest that the inclusion of 100µM β-ME during *in vitro* embryo culture could be used for production of high quality bovine blastocysts in a serum-free IVC systems.