Animal Reproduction

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Embryology, developmental biology, and physiology of reproduction

Metabolic effects of fetal bovine serum removal on in vitro culture of bovine embryos

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Supplementation with fetal bovine serum (FBS) renders embryo culture media as undefined. This can mask results of scientific research, specifically, studies of cell differentiation that are influenced by glucose metabolism, as well as by growth factors present in FBS. In this study, we tested the hypothesis that the removal of FBS during in vitro culture (IVC) does not reduce development rates, but alters aspects related to energy metabolism. Grade I oocytes were collected from commercial slaughterhouse ovaries and subsequently in vitro matured and fertilized. Zygotes were then placed in KSOM medium (Millipore) in an atmosphere of 5% CO2 and 5% O2 and randomly distributed in the following groups: group SFB, in which embryos were supplemented with 5 % (v / v) of SFB (Thermo Fisher) at the fourth day (D4) of IVC, Group KSOM- 30, in which 30% of the medium volume was removed and renewed at D4, and Group KSOM-zero, in which there was no supplementation with FBS and no renewal of the culture medium. In D9, rates of blastocyst formation (blastocysts/total zygotes) and development (blastocysts/cleaved) were recorded. Furthermore, blastocysts were stained with 2.5 mM CellROX (ThermoFisher) and 1 mM MitoTracker Red (ThermoFisher) for 30 minutes or subjected to measurement of NADH and FAD+ upon excitation with 360nm and 488nm, respectively, under an epifluorescence microscope. Fluorescence intensity was measured using Image J (NIH) *software*. Data were analyzed by ANOVA, considering replicate as a random variable, and the comparison of means was performed using Tukey's test in SAS 9.4 software. Results showed that the blastocyst rate (n = 8 replicates) in SFB group (33.61 ± 2.85%) was higher (p = 0.02) than in KSOM-zero group (21.57 ± 2.85%), which tended to be smaller (p = 0.06) than KSOM-30 group (31.48 ± 2.85%). Development rates (n = 8 replicates) tended to be higher in SFB (40.79 ± 3.94%, p = 0.07) and KSOM-30 (39.93 ± 3.94%, p = 0.09) groups than in KSOM-zero group (27.45% ± 2.85%). There were no significant differences in fluorescence intensities of CellROX and MitoTracker (n = 8 embryos per group) or NADH and FAD+ (n = 8 embryos per group). Thus, it was concluded that the removal of FBS reduced blastocyst rates, although this was not observed when there was 30% renewal of the medium in D4. It was also concluded that removal of SFB did not alter the observed metabolic variables. Studies are being carried out to verify the impact of SFB on cell differentiation of IVP bovine embryos. Financial support by FAPESP grants 2017 / 09576-3, 2017 / 25574-0, 2019 / 03014-9.