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APOPTOSIS IN BOVINE EMBRYOS PRODUCED WITH OOCYTES MATURED IN SERUM-FREE MEDIUM UNDER LOW OXYGEN TENSION

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*In vitro*maturation (IVM) is a critical step for *in vitro* embryo production (IVP) and several factors can influence its efficiency, such as protein supplementation and atmospheric tension. The current study aimed to investigate the effect of serum and oxygen tension (O_2) during *in vitro* maturation (IVM) on incidence of apoptosis in bovine blastocysts.Immature COCs were distributed randomly in the following IVM groups:G1 (10% estrus cow serum [ECS] with 20% O_2); G2 (0.1% polyvinyl alcohol [PVA] with 20% O_2), G3 (10% ECS with 5% O_2) and G4 (0.1% PVA with 5% O_2). Basal maturation medium was TCM199 (Invitrogen, Caifornia, USA) and CO_2 tension was 5%. Maturated oocytes were subjected to *in vitro* fertilization in 100-µl drops of Fert-TALP supplemented with heparin and $2x10^6$ spermatozoa/mL for 21 h in a humidified atmosphere of 5% CO_2 and 38.8 ºC in air. Presumptive zygotes were denuded by vortexing in 0.1% hyaluronidase solution and cultured in CR2aa medium (Wilkinson *et al*. 1996; Theriogenology, 45: 41:49) with 2.5% fetal calf serum (FCS) (Nutricell, Campinas, SP, Brazil)under 5% CO_2, 5% O_2, 90% N_2 at 38.5°C. Blastocysts at eight day post-fertilizationfrom G1 (n=22), G2 (n=18), G3 (n=19) and G4 (n=18) were fixed and permeabilized for TUNEL assay (DeadEnd™ Florimetric TUNEL System-PROMEGA), according to the manufacturer instructions. Total cell number, apoptotic cell number and apoptotic cell index (calculated by dividing the apoptotic cell number by total cell number) were analyzed by analysis of variance and mean compared by Student Newman Keuls. Significance was estimated at the level of P= 0.05. The total cell number were not affected (P>0.05) when the ECS (G3: 112.73±2.87) was replaced by PVA (G4: 111.11±2.67) under 5% O_2, whereas higher (P<0.05) total cell number were found with ECS (G1: 116.90±2.60) compared to PVA (G2: 85.77±2.49), both under 20% O_2. Blastocysts from G4 showed lower (P<0.05) number of apoptotic cells (10.72±1.25)than those from G1 (20.95±1.29), G2 (19.50±1.42) and G3 (21.73±1.29), and lower (P<0.05) apoptosis index (G4: 0.09±0.01) than blastocysts from other groups(G1: 0.18±0.01; G2: 0.22±0.01and G3: 0.19±0.01). In conclusion maturation with PVA and 5% O_2 provides an in vitro maturation that results in blastocysts with low apoptosis index. Financial support: Fapemig.

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EVALUATION OF MORPHOLOGY CHANGES DURING THE DEVELOPMENT OF BOVINE EMBRYOS FERTILIZED *IN VITRO* WITH SEMEN EXPERIMENTALLY CONTAMINATED WITH *ESCHERICHIA COLI* SHIGA TOXIN-PRODUCING STX2 (STEC)

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Although increasingly used, assisted reproduction techniques still need researchs to asses the health risks of oocytes, embryos and sperm, because embryonic and fetal mortality have a major impact on the profitability of any livestock system. The aim of this study was to evaluated by optical microscopy and electron on transmission, changes in morphology during the development of bovine embryos, fertilized with semen experimentally contaminated with *Escherichia coli* producing the Shiga toxin stx2. Oocytes were aspirated from ovaries of slaughtered cows and ones with the intact zona pellucida were selected and matured. After 20 hours, the oocytes were divided into control group (n=418), fertilized with semen control and group infected (n=415), fertilized with sperm exposed to 200 UFC *E. coli* shiga toxin-producing stx2. Each semen was treated by the technique of discontinuous Percoll gradient, and the sperm concentration was adjusted to approximately 100 thousand sperm for each oocyte. After the period of fertilization, the embryos were evaluated for their morphology by optical microscopy and electron on transmission. The oocytes fertilized with contaminated semen (52,8%; n=219/415) showed cytoplasmic shrinkage, blastomere division failures, asymmetry of blastomeres, granular ooplasm with dark brown color, formation of vacuoles, degeneration and disruption of the zona pellucida. The thin sections of the same group showed granular cells and vacuolated, with structures similar to *E. coli,* that have been confirmed by transmission electron microscopy (70,3%; n=294/418). The presence of *E. coli* shiga toxin-producing stx2 cause morphological changes during the development embryo. Good hygiene of the prepuce of the bull and materials used during the collection, with the appropriate disinfectants, are extremely important, and the use of antibiotics effective for the dilution of semen, to ensure the descontamination, since the procedure used during IVF (Percoll) was not effective.