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Roles of cell death in sexual dimorphism during preimplantation development

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Female bovine embryos progress at lower rates and originate smaller blastocysts than male counterparts. However, when and how sex dimorphism starts to occur is not clear. The knowledge of singularities among female and male embryos can be useful for human assisted reproductive medicine, when X-linked disorders risk is detected, and for livestock sex-specific breeding programs. The aim of this study was to characterize the roles of cell death in development of female and male embryos. Using sex-sorted semen from three different bulls for fertilization, we compared bovine sex-specific embryos at 96, 120 and 144 hpi, assessing quality parameters. For that, embryos with more than 4 cells at 96 and 120 hpi; or more than 8 cells at 144hpi were fixed in 4% PFA and stained for caspase 3 (apoptosis marker) by immunofluorescence. Nuclei were counterstained using HOECHST. Cell fragmentation was estimated by number of enucleated cytoplasm fragments inside zona pellucida. Results were grouped as Female and Male, since consistency among bulls 1, 2 and 3 data was detected. The analysis was performed as follows: I. Total cell number; II. Apoptosis (rate of apoptotic cells in embryos); III. Fragmentation (rate of fragmented cells in embryos). The effect of time over each embryo sex (Kruskall-Wallis/ Dunn, F96xF120xF144; M96xM120xM144) and the effect of sex over each moment (Mann Whitney, F96xM96; F120xM120; F144xM144) were analysed using GraphPad InStat ($p=0.05$). In this study, 379 embryos (65-93 per group) were evaluated, obtained in three replicates. As expected, mean cell numbers increased from 96 to 144 hpi (F: 11.88 ± 0.53^{a}, 15.42 ± 1.04^{a}, 28.1 ± 2.44^{b}; M: 11.33 ± 0.64^{A}, 16.62 ± 1.12^{B}, 40.19 ± 2.86^{C}). Comparing Female vs Male, decreased cell numbers was detected at 144hpi (F: 28.1±2.44, M: 40.19±2.86*). Regarding apoptosis, in female groups the higher rate was detected at 96hpi (23.08 ± 2.54^{a}, 14.62 ± 2.0^{b}, 14.46 ± 1.94^{b}). For male embryos, at 144 hpi the lowest rate was detected (21.40 ± 2.68^{A}, 15.23 ± 1.63^{A}, 9.71 ± 1.43^{B}). Female embryos presented higher apoptosis rates at 144 hpi (F: 14.46±1.94, M: 9.71±1.43*), in reflex to a cell number decrease and to a tendency ($p=0.07$) of increase in number of apoptotic cells (F: 2.91±1.50, M: 2.38±1.52). Cell fragmentation remained unaltered for female embryos (17.19±1.67, 15.55±1.55, 14.97±1.34), and for male embryos decreased at 144 hpi (15.76 ± 1.36^{A}, 13.11 ± 1.01^{A}, 10.98 ± 1.19^{B}). Female embryos presented higher fragmentation rates comparing to male group at 144 hpi (F: 14.97±1.34, M: 10.98±1.19*), and this increase was also due to a numeric increase in fragmented cell numbers (3.47±0.22, 2.73±0.17*). These new results lead us to propose that sex dimorphism is established at 144hpi in bovine, during morula-blastocyst transition, and cell death is involved in this process.

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