ULTRASTRUCTURE AND CELL DEATH OF VITRIFIED PORCINE BLASTOCYSTS

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Cryopreservation of porcine embryos by the simple open pulled straw (OPS) method was recently reported to result in live offspring (Berthelot \textit{et al.}, 2000, Cryobiology 41: 116–124), and is evaluated in the present study by light (LM) and transmission electron microscopy (TEM) as well as by TUNEL staining in order to detect morphological and molecular signs of cell death and subsequent regeneration. Blastocysts were collected from gilts on Day 5 (Day 0 = 1st AI) and were randomly assigned to one of three groups: Fresh controls (FC) were fixed immediately after collection, and vitrified embryos were fixed either immediately after vitrification and warming (V0) or after 24 h of culture upon warming after vitrification (V24). In each of the three groups, embryos were fixed and processed for LM/TEM (FC, n = 13; V0, n = 20; V24, n = 18) or TUNEL staining (counter staining with propidium iodide) and confocal laser scanning microscopy (FC, n = 32; V0, n = 31; V24, n = 33). At LM, the FC embryos displayed a well-defined trophoblast (Tb) and inner cell mass (ICM), expanded blastocoel cavity and a narrow or no perivitelline space. In V0 embryos, collapse of the blastocoel cavity and cell swelling was detected. At the TEM level, the V0 embryos showed extensive injuries including a general distension or shrinkage of mitochondria and massive increase in the amount of membrane-bound vesicles, vacuoles and secondary lysosomes. In both FC and V0 embryos, the presence of dead or phagocytosed cells in the ICM and Tb was occasional. A few extruded cells were often noticed in the perivitelline space or in the blastocoel cavity, and such cells ranged from being rather normal to showing typical morphological features of apoptosis. TUNEL staining confirmed the presence of a few apoptotic cells in both groups of embryos. Approximately 2/3 of the V24 embryos had, as evaluated by LM, partially recovered, re-expanded or even hatched whereas the remaining 1/3 had degenerated. At the TEM level, the recovered embryos displayed almost normal blastocyst morphology, except for a widening of the perivitelline space, accumulation of debris, increased electron-lucidity of the ICM and partial distension of mitochondria. The degenerated embryos had disintegrated into a poorly defined mass of cells and debris including cells with either decreased or increased electron-density of the cytoplasm and with abundant degeneration of mitochondria and other organelles. Both recovered and degenerated embryos displayed persistent abundant presence of small membrane-bound vesicles, vacuoles and secondary lysosomes. All V24 embryos displayed increased occurrence of dead or phagocytosed cells in the ICM and Tb as well as increased occurrence of extruded cells showing typical morphological features of apoptosis or secondary necrosis. TUNEL staining confirmed the increased occurrence of apoptotic cells in this group of embryos. In conclusion, immediately after vitrification and warming, porcine embryos displayed severe subcellular damages, but during 24 h of culture the majority of the embryos were able to regenerate. Along with the regenerative process, apoptosis became evident. Supported by CRAFT B contract no. QLK5-CT-2002-70983.