



Increase of DNA fragmentation and apoptotic related gene expression in frozen-thawed bovine blastocysts

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Evaluation of apoptosis and expression level of apoptotic related genes is useful to examine the variation of embryo quality according to environmental change. The objective of this study was to investigate DNA fragmentation and apoptotic related gene expression patterns in frozen-thawed bovine blastocysts. In vitro produced day 7 blastocysts were frozen by two different vitrification methods (conventional 0.25ml straw or MVC straw). After thawing, DNA fragmentation of survived embryos was examined by TUNEL assay, and their apoptotic gene (survivin, Fas, Hsp 70 and caspase-3) expression patterns were evaluated using real time quantitative RT-PCR. In vitro survival rates of frozen-thawed embryos were higher in the MVC vitrification method (88.2% re-expanded at 24h, and 77.1% hatching at 48h) than in conventional (C) vitrification method (77.0% re-expanded at 24h, and 66.7% hatching at 48h). However, both vitrified methods presented a significantly higher apoptotic index (C vitrification method 11.9, and MVC vitrification method 11.0%) than in non-frozen embryos (3.0%). Expression levels of survivin, Fas, caspase-3, and Hsp 70 were also increased in the frozen-thawed embryos compared to non-frozen. These results indicated that cryopreservation procedure might cause damage resulted in an increase of DNA fragmentation and apoptotic related gene transcription to reduce developmental capacity of frozen-thawed embryos.

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