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Expression of Apoptosis Regulatory Genes and Incidence of Apoptosis in Different Morphological Quality Groups of Ivp Bovine Preimplantation Embryos

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Abstract

Apoptosis occurs during early development in both in vivo- and vitro- produced embryos, and may cause embryonic loss. In order to resolve the mechanisms and reasons of cellular fragmentation it is crucial to understand what genes may be responsible for regulation of this process. Despite the fact that apoptosis plays an important role in preimplantation embryo development, the correlation among morphological embryo quality, expression of apoptosis regulatory genes and the incidence of apoptosis has not yet been established. The objectives of this study were, therefore, investigating stage specific mRNA and protein expression profiles of apoptosis regulatory genes in three quality groups of in vitro-produced bovine preimplantation embryos; analysing the relationship between DNA fragmentation and morphological quality of embryos; and investigating the mRNA expression of Ped gene in bovine embryos of different cleavage rates. The relative abundance of mRNA of 9 pro- (Bax, caspase-9, Bcl-xs, P53, caspase⁻³, Fas) and anti- (Bcl-w, Bcl⁻² and Mcl⁻¹) apoptotic genes was analysed by using real time PCR. Moreover, differential cell staining, TUNEL labelling and western blot were done to analyse the variation in cell numbers, detect apoptotic nuclei and protein expression, respectively. The expression of Bax, caspase⁻³ and caspase-9 genes was found to be significantly ($p < 0.05$) higher in poor quality preimplantation embryos as compared with that of morphologically good embryos of the same stage of development. Moreover, the anti-apoptosis Mcl⁻¹ expression was significantly higher in good quality groups of immature oocytes, 8-cell and Morula stage embryos than that of their poor quality counterparts. Bax protein was detected only in morphologically poor quality blastocysts. Bcl⁻² protein was not detected in quality 1, 2, & 3 blastocysts. In conclusion, a higher incidence of apoptosis was evidenced in morphologically poor quality blastocysts and this study demonstrates that Bax, caspase⁻³ and Mcl⁻¹ can be used as potential markers of embryo quality to evaluate in vitro produced bovine preimplantation embryos.

Keywords: Apoptosis, bovine embryo, embryo quality