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Expression of apoptosis regulatory genes and incidence of apoptosis in different morphological quality groups of in vitro-produced bovine preimplantation embryos.

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INTRODUCTION

Apoptosis has been demonstrated in various species of mammalian preimplantation embryos and receives increasing attention for the potential role in early embryonic loss (Gjorret et al., 2001). The lower quality of in vitro-produced embryos can be attributed to the ICM having less viable cells because of a lower number of cells and a higher incidence of apoptosis (Knijn et al., 2003). During pre-implantation development, apoptosis is regulated by the activity of pro- and anti- apoptotic genes (Exley et al., 1999). Caspases act at two levels to trigger cell death, and Bcl-2 family proteins regulate the activity of caspases (Exley et al., 1999). However, the correlation between morphological quality of bovine preimplantation embryos and expression of apoptosis regulatory genes has not yet been established. This study is, therefore, aimed at investigating stage specific expression profiles of apoptosis regulatory genes in various morphological quality groups of in vitro-produced bovine preimplantation embryos; and analyzing the relationship between DNA fragmentation and the expression of major apoptosis regulatory genes in bovine preimplantation embryos.

Materials and Methods

In-vitro produced bovine preimplantation embryos were categorized into three groups (Good, Fair and Poor) based on the IETS criteria set by Merton (2002). Messenger RNA

was isolated from triplicate pools of 10-15 immature and mature oocytes, and embryos of 2-cell, 4-cell, 8-cell, morulae and blastocyst stages using oligo $(dT)_{25}$-attached magnet beads (Dynal, Oslo, Norway) according to the manufacturer's instructions. 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 analyzed by using real time PCR. Besides, differential cell staining was performed in good, fair and poor quality groups of bovine blastocysts. DNA fragmentation was detected by TUNEL labelling using TUNEL reagent (Roche Diagnostics GmbH, Mannheim, Germany). A protein kinase inhibitor, staurosporine (STS) was used to induce apoptosis, as a positive control. Western blot were done to analyze the protein expression of the major pro-apoptosis Bax and an anti-apoptosis Bcl-2.

Results and Discussions

Expression of pro- and anti-apoptosis genes

We analyzed the expression of apoptosis-related genes with respect to morphological quality of bovine preimplantation embryos. The proapoptotic Bax (Fig.1) was expressed in all preimplantation embryonic stages. Bax protein was expressed only in morphologically poor quality blastocysts. However, Bcl-2 protein was not expressed in any quality group. The relative abundance of caspase-3 was found to be significantly higher in morphologically poor quality 2-cell and 4-cell stage embryos than that of good quality embryos of the same developmental stages. The trend is similar in fair and poor quality groups. The effect of Bax and caspase-3 in earlier stages could precede the DNA fragmentation observed at the blastocyst stage. The tumour suppressor p53 is an important mediator of the responses to cellular stress, and is known to cause apoptosis. We found a significantly higher expression of p53 from oocyte to 4-cell stage as compared with the latter preimplantation stages. Anti-apoptosis Mcl-1 plays a major role in many cell death and survival regulatory programs. In our study, this gene has been found to promote survival of cells as the abundance of the transcript was significantly ($p<0.05$) higher in good quality oocytes (Fig.1) and 8-cell stage embryos compared with that of poor and fair quality groups of the same developmental stages. In this study,

TUNEL stained nuclei were found in each quality group of blastocysts, a higher number being observed in morphologically poor quality bovine blastocysts.

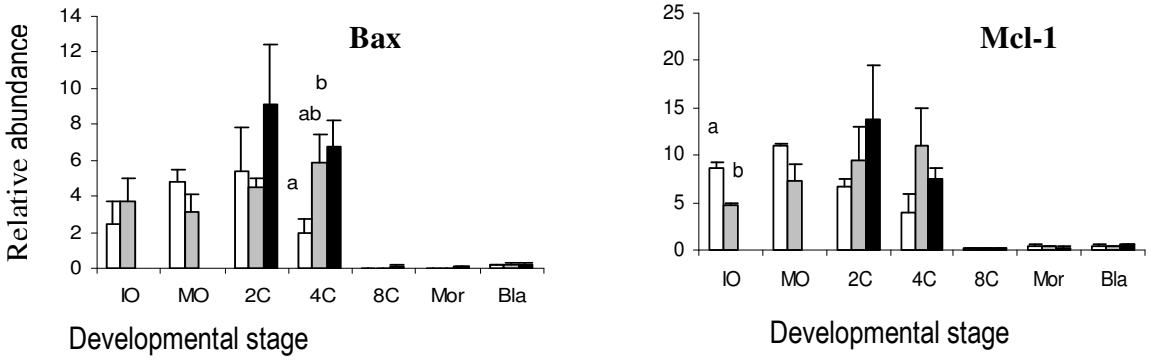

Fig.1 Relative abundance of mRNA of a pro-apoptosis gene Bax and an anti-apoptosis Mcl-1 in different developmental stages of in-vitro produced bovine embryos.

Differential cell staining

The numbers of inner cell mass (ICM) of good (31±7) and fair (30±4) quality blastocysts were significantly ($p<0.01$) higher than that of poor (25±5) quality group. Moreover, the trophectoderm cells of good quality blastocysts (106±18) were significantly ($p<0.01$) higher than those of fair (72±13) and poor (66±14) quality groups.

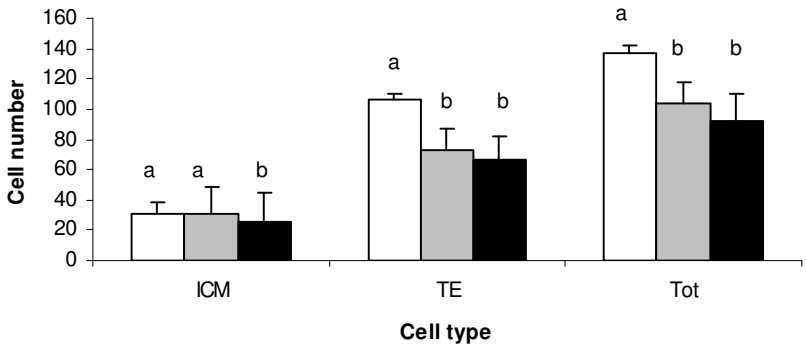

Fig.2 Number of cells versus cell type

TUNEL stained nuclei and Bax protein expression

There was no significant difference between the ratio of TUNEL stained nuclei and total cells in good (0.073±0.61) and fair (0.078±0.45) quality blastocysts. Differences were, however, significant ($p<0.01$) between good (0.73±0.61) and poor (0.18±0.13) quality as well as fair (0.078±0.45) and poor (0.18±0.13) quality bovine blastocysts. Western blot

analysis was performed using proteins extracted from pools of blastocysts of good, fair and poor quality. As a result, Bax protein expression was found in poor quality blastocysts while the protein was not detected in good and fair quality groups.

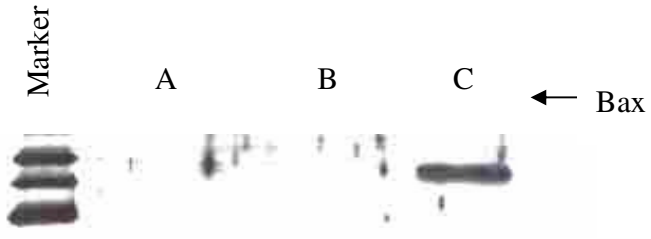

Fig.3 Expression of Bax protein (23 kDa) in morphologically poor quality (C) blastocysts compared to that of fair (B) and good (A) quality blastocysts.

In Conclusion, we demonstrate for the first time that morphological embryo quality differences are correlated with differences in stage specific expression of some apoptosis regulatory genes, number of ICM and TE cells, TUNEL stained nuclei and the expression of Bax protein altogether. A higher DNA fragmentation was evidenced in poor quality blastocysts.

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