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Review

RELATIONSHIP BETWEEN THE OCCURRENCE OF APOPTOSIS AND DISORDERS IN PREIMPLANTATION EMBRYO ENVIRONMENT

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ABSTRACT

Increased incidence of apoptosis in preimplantation embryos is considered to be an indicator of inadequate developmental environment and is used as a sensitive marker of embryo quality in both experimental and clinical conditions. This review summarizes data on physiological characteristics of apoptotic cell death in mouse, rabbit, cow and pig preimplantation embryos. Furthermore, it shows how apoptosis incidence can be regulated by various physiological and non-physiological external factors present during various stages of embryonic development *in vitro* and *in vivo*.

Key words: preimplantation embryo; apoptosis; *in vitro*; maternal environment

INTRODUCTION

Apoptosis (cell death by suicide) is a physiological process occurring spontaneously in the majority of cell populations including embryonic cells (Pampfer and Donnay, 1999). The typical signs of apoptosis are the final results of a complex cascade of biochemical events. It has been shown, that apoptosis plays an important role in cellular response to suboptimal developmental conditions and stress. Thus, increased incidence of cell death is considered to be an indicator of inadequate environment for the development of preimplantation embryos *in vivo* or *in vitro* (Betts and King, 2001; Huppertz and Herrler, 2005).

During preimplantation period, apoptosis is usually triggered to fulfill one of two functions: morphogenetic or reparatory. Morphogenetic function includes elimination of incorrectly differentiated cells, e.g. cells of inner cell mass (ICM) line translocated to trophectoderm (TE) line or cells with superfluous potential. Therefore, apoptosis can act as control mechanism for the regulation of total cell number in embryo. Reparatory function includes elimination of damaged cells. Cell damage can be caused by internal (intracellular) factors, such as non-reparable disorders of DNA replication or cell division, or by external (extracellular) factors. Specific external factors represent the presence of environmental ligands triggering apoptotic process after binding to appropriate receptor (e.g. cytokines, glucocorticoids, etc.); non-specific external factors represent various physical and chemical impacts, which can cause irreversible damage of essential cellular systems (oxidative stress, radiation, chemotherapy, etc.), or non-invasive defects, such as absence of growth factors or other important (e.g. nutritional) substances in the environment of developing embryo (Levy *et al.*, 2001; Fabian *et al*., 2005b; Penaloza *et al*., 2006).

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Physiological characteristics of apoptosis in preimplantation embryos

We have shown that blastocysts of four evaluated mammalian species (mouse, rabbit, cow and pig) spontaneously display the presence of apoptotic cell death in relatively high number of obtained embryos (see Table 1). Incidence of spontaneous apoptosis differs among species and it always reaches higher values in blastocysts produced *in vitro*, when compared to *in vivo* delivered ones (Fabian *et al.*, 2005a,b; Gjørret *et al*., 2007; Fabian *et al*., 2007b, 2009b).

Apoptotic cells are located in the both early cell lineages – in the embryoblast and in the trophoblast (Figure 1F), and after reaching terminal stages of apoptotic process they are phagocyted by neighboring cells or extruded to the blastocoele or the perivitelline space. The extruded cells usually undergo secondary necrosis (Fabian *et al*., 2005b). Besides, mammalian blastocysts show high sensitivity to apoptosis induction by chemical treatment, i.e. supplementation of TNFα, actinomycin D or staurosporine during *in vitro* culture (Gjørret *et al*., 2007; Fabian *et al*., 2007a, 2007b).

Early embryos at stages following the embryonic genome activation (EGA) display sporadic incidence of apoptotic cell death (Figure 1A-E). They are able to respond to apoptotic induction; however, the effect is lower when compared to blastocysts. Embryos at stages preceding EGA do not show the presence of spontaneous apoptosis and their ability to response to apoptosis induction is extremely low (Gjørret *et al*., 2003; Fabian *et al.*, 2007b). Interestingly, the timing of the onset of spontaneous apoptotic wave overlaps with the onset of passive DNA cytosine demethylation in embryonic genome, even when embryonic development is arrested (Fabian *et al*., 2009a).

Our results proved that initiated apoptosis can be regulated by specific external impacts. For example, the addition of growth factor IGF-I into culture media had significant anti-apoptotic effect on both spontaneous and induced apoptotic processes in mouse embryos developing *in vitro* (Fabian *et al*., 2004).

Based on our *in vitro* experiments, we can conclude that inappropriate environmental conditions might affect the occurrence and the incidence of apoptosis and subsequently the quality of produced embryos more significantly at the later stages of preimplantation development than at the earlier ones.

Relationship between apoptosis incidence and maternal environment

In the following *in vivo* studies, performed on mouse animal model, we used apoptosis incidence as one of the main markers of embryo quality during the evaluation of the effect of various disorders of maternal homeostasis on preimplantation development.

Toxicological study on mouse females showed that maternal sub-chronic intoxication by relatively low doses of herbicide BASTA 15 might strongly affect developmental abilities and quality of preimplantation embryos, represented by a significant reduction in cell numbers in blastocysts and a significant elevation of the percentage of apoptotic cells per blastocysts (Fabian *et al*., 2011).

The other experimental study has shown that the presence of acute non-specific inflammation (hapten-induced colitis or paw *oedema*), accompanied by systemic biochemical changes (but not hyperthermia) in maternal organism during the preimplantation period would probably not affect the development and growth of embryo. Significant increase in the apoptosis

Table 1: Average incidence of apoptosis in blastocysts of four animal species produced *in vivo* or *in vitro*

		Embryonic day blastocyst	Average cell number per blastocysts	Average incidence of apoptotic cells in evaluated apoptotic cell	Average percentage of blastocyst with at least 1
Pig	*in vivo*	ED 5	± 100	0.8 %	50 %
Rabbit	*in vivo*	ED 4.5	± 300	0.2 %	50 %
	in vitro	ED 4.5	± 200	1.4 %	80 %
Mouse	*in vivo*	ED 4	± 50	1.2 %	25 %
	in vivo	ED 5	± 120	4.5 %	70 %
	in vitro	ED 5	± 90	6.0 %	85 %
Cow	*in vitro*	ED 7	± 175	8.9 %	100 %

Values were extrapolated from results of several previously published studies (Fabian *et al.*, 2005a, 2005b; Gjørret *et al*., 2007; Fabian *et al*., 2007b, 2009b).

incidence was recorded only in expanded blastocysts obtained from additional culture *in vitro*. Thus, we hypothesized that the presence of inflammatory mediators in the environment of developing preimplantation embryos might negatively affect physiology of cellular processes at sub-morphological level, however, embryos are able to manage with these changes using standard reparatory mechanism (Fabian *et al*., 2010).

Results obtained on mouse model simulating natural development of obesity and leanness in mammals showed that alterations in maternal body condition might have impact on reproductive process even at the time of ovulation, fertilization and early preimplantation development *in vivo* (Fabian *et al*., 2015). Both elevated and decreased body fat deposits were accompanied by increased incidence of blastocysts containing at least one apoptotic cell and increased percentage of apoptotic cells per blastocyst. In case of obesity, the effect was dependent on the stage of its development (Kubandová *et al*., 2014).

Based on our *in vitro* and *in vivo* experiments, we can conclude that apoptosis is a sensitive marker of the quality of naturally produced preimplantation embryos, and it can be used in both experimental and clinical conditions, involving the improvement of biotechnological techniques.

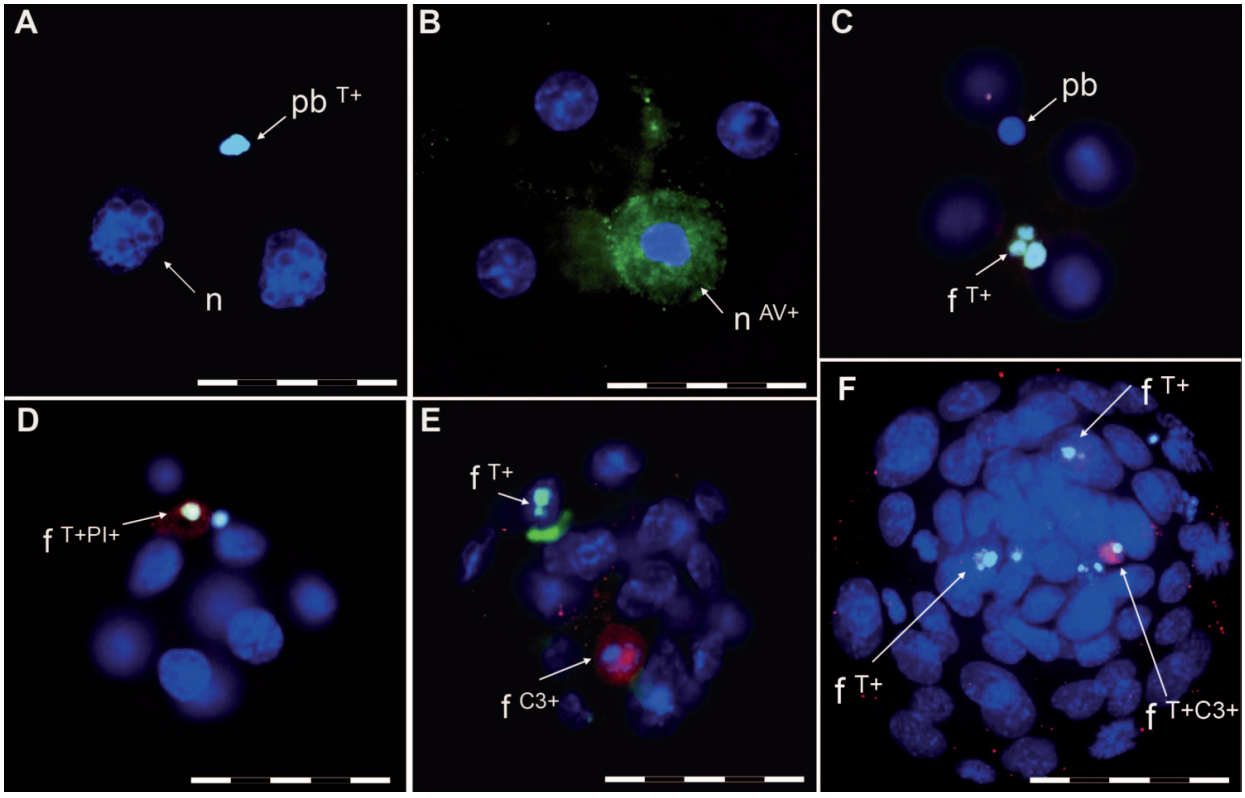

Fig. 1: Illustrative microphotographs of preimplantation embryos with dead cells displaying various features of apoptosis: A, 2-cell; B, 4-cell; C, 5-cell; D, 9-cell embryo; E, morula; F, blastocyst; pb, polar body; n, cell with normal nuclear morphology; f, cell with fragmented nuclear morphology. AV+, presence of phosphatidylserine flip on cell membrane (annexin V staining); T+, presence of specific DNA degradation in the nucleoplasm (TUNEL assay); C3+, presence of active caspase-3 in the nucleoplasm (immunohistochemistry); PI+, positive vital staining with propidium iodide illustrating corruption of nuclear membrane (Fluorescence microscopy, scale bar 50 µm.)

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