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The effect on preimplantation embryo development of non-specific inflammation localized outside the reproductive tract

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Abstract

The aim of this study was to evaluate the possible effect of non-specific acute inflammation localized outside the reproductive tract on the quality of preimplantation embryos. In fertilized female mice two experimental models of inflammation were used—trinitrobenzene sulfonic acid colitis and carrageenan paw oedema. Inflammation was induced during the cleavage period of embryo development and embryos were collected at 92 h post hormonal synchronization. Stereomicroscopical evaluation of *in vivo* derived embryos showed that the presence of inflammation in the maternal body did not affect their basic developmental abilities, i.e. there were no significant differences in the proportion of early blastocysts, morulas, slowly developing embryos and degenerates between embryonic pools obtained from mothers with induced inflammation and control mothers. In the next step, non-degenerated embryos from all mothers were cultured *in vitro* under standard conditions for another 24 h, and the average cell number (fluorescence DNA staining) and the incidence of cell death (fluorescence viability staining combined with TUNEL assay) were evaluated. The majority of cultured embryos reached expanded blastocyst stage. There were no significant differences in the average cell numbers of blastocysts, but blastocysts derived from mothers with induced inflammation showed a significantly higher incidence of dead cells in both experiments. The majority of dead cells were of apoptotic origin. These results show that non-specific inflammation localized outside the reproductive tract has no detrimental effect on the preimplantation embryo growth; however it can affect the embryo quality.
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Keywords: Preimplantation development; Cell death; Colitis; Paw oedema

1. Introduction

Inflammation can cause various reproductive disorders (reviewed in [1]). It has been shown that even the activation of inflammatory or immune responses external to the reproductive tract (f.e. mastitis) can reduce pregnancy rates [2]. This reduction is usually caused by embryo loss. The unanswered question remains whether these restricted disorders can affect embryo even during the preimplantation period of its development.

Early embryos are highly sensitive to the environment in which they develop (reviewed in [3,4]). Previous *in vitro* studies showed that the presence of various inflammatory mediators in culture media can significantly affect their developmental capabilities [5–9]. However, no experiments have been performed to prove their harmfulness in *in vivo* conditions.

The aim of this study was to evaluate the possible effect on the quality of preimplantation embryos of two

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different acute inflammatory processes localized outside the reproductive tract (using two well-established rodent inflammation models).

Trinitrobenzene sulfonic acid (TNBS) colitis is a commonly-used model of hapten-induced inflammation which exhibits features comparable to T-helper cell type I mediated autoimmune process. Rectal administration of TNBS causes focal disruption of the epithelial barrier and activates intestinal immune cells. Inflammation is characterized by mucosal over-expression of tumor necrosis factor α (TNF-α), interferon-γ and interleukin-1β (IL-1β), which leads to recruitment of neutrophil granulocytes and production of proteases and pro-apoptotic factors [10,11]. This process is also accompanied by the activation of various enzymes (COX-2, iNOS [12]) and increased expression of other pro-inflammatory cytokines (interleukin-17, interleukin-18 [13,14]) or profibrinogenic growth factors (transforming growth factor β1, insulin-like growth factor 1), which stimulate proliferation of mesenchymal cells [15].

Carrageenan paw oedema is routinely used as a phlogistic tool for the investigation of anti-inflammatory or pro-inflammatory effects of various agents. In the first phase, carrageenan injection into the hindpaw of a rodent causes local over-production of histamine, serotonin, 5-hydroxytryptamine and platelet activating factor, which mediate vasodilatation, plasma extravasation and secretion of cytokines (TNF-α, IL-1β, interleukin-6 (IL-6)) in vascular endothelial cells [16,17]. Exudation elevates to a peak between 4 to 6 h after induction. The second phase of inflammation is characterized by the release of bradykinin, prostaglandins and kinins and polymorphonuclear neutrophil influx into the paw tissue [18]. Leukocyte migration is accompanied by the production of oxygen-derived free radicals and hydroxyl radicals inducing lipid peroxidation and cellular damage [19]. Again, expression of COX-2 and NOS is elevated [20,21].

Our study tests the effect of experimentally induced inflammation on developmental capacities and quality of preimplantation embryos. In both cases, inflammation was induced during the cleavage period of embryo *in vivo* development, and its clinical features persisted until embryo isolation at the early blastocyst stage. Embryos were subsequently cultured *in vitro* for another 24 h. Finally, two basic quality parameters – embryo growth and the incidence of cell death were evaluated in such obtained blastocysts.

2. Materials and methods

2.1. Experimental design

Sexually mature female mice (ICR strain, Velaz, Prague, Czech Republic; 4–5 wk old) underwent synchronization treatment with pregnant mare's serum gonadotropin (eCG 4 IU ip; Folligon, Intervet International, Boxmeer, Holland), followed 47 h later by administration of human chorionic gonadotropin (hCG 6 IU ip; Pregnyl, Organon, Oss, Holland). Females were mated with males of the same strain overnight and mating was confirmed by identification of a vaginal plug. At 68 h post hCG administration, experimental inflammation was induced in fertilized dams: A, trinitrobenzene sulfonic acid colitis, or B, carrageenan paw oedema.

Embryos were collected 24 h after the induction of inflammation– during the late morula/early blastocyst stage of embryonic development. This period, preceding the start of implantation, was chosen to avoid embryo loss. The dams were killed by cervical dislocation, embryos were recovered by flushing the oviduct and the uterus using a flushing–holding medium (FHM [22]) and subjected to stereomicroscopic classification (Olympus SZ51). In the next step, blastocysts, morulas and slowly-developing embryos (except degenerates) from all dams in one group were pooled and cultured *in vitro* under standard conditions for another 24 h to the expanded blastocyst stage [one embryo/1 μL KSOM (Specialty Media Group, Phillipsburg, USA) in a humidified atmosphere with 5% CO_2 at 37 °C]. The quality of such derived embryos was assessed by fluorescence microscopy. Three consecutive experiments were performed in each case.

All animal experiments were reviewed and approved by the Ethical Committee for animal experimentation of the Institute of Animal Physiology, approved by the State Veterinary and Food Administration of the Slovak Republic, and were performed in accordance with Slovak legislation based on EC Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes.

2.2. Colitis

All mice were first anaesthetized with a ketamine/xylasine mixture [42.5% of ketamine (Narkamon 5% inj., Spofa, Prague, Czech Republic) + 7.5% of xylasine (Rometar 2% inj., Spofa) + 50% of NaCl 0.9%; 60 μL/20 g body weight ip]. Colitis was induced by intrarectal administration of 120 mg/kg of the hapten reagent TNBS (Fluka, Steinheim, Germany) in 50%

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