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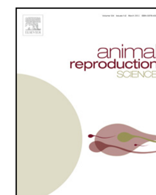

Early bovine embryos regulate oviduct epithelial cell gene expression during *in vitro* co-culture☆

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ABSTRACT

In mammals, the oviduct may participate to the regulation of early embryo development. *In vitro* co-culture of early bovine embryos with bovine oviduct epithelial cells (BOEC) has been largely used to mimic the maternal environment. However, the mechanisms of BOEC action have not been clearly elucidated yet. The aim of this study was to determine the response of BOEC cultures to the presence of developing bovine embryos. A 21,581-element bovine oligonucleotide array was used compare the gene expression profiles of confluent BOEC cultured for 8 days with or without embryos. This study revealed 34 differentially expressed genes (DEG). Of these 34 genes, *IFI6, ISG15, MX1, IFI27, IFI44, RSAD2, IFITM1, EPSTI1, USP18, IFIT5*, and *STAT1* expression increased to the greatest extent due to the presence of embryos with a major impact on antiviral and immune response. Among the mRNAs at least 25 are already described as induced by interferons. In addition, transcript levels of new candidate genes involved in the regulation of transcription, modulation of the maternal immune system and endometrial remodeling were found to be increased. We selected 7 genes and confirmed their differential expression by quantitative RT-PCR. The immunofluorescence imaging of cellular localization of STAT1 protein in BOEC showed a nuclear translocation in the presence of embryos, suggesting the activation of interferon signaling pathway. This first systematic study of BOEC transcriptome changes in response to the presence of embryos in cattle provides some evidences that these cells are able to adapt their transcriptomic profile in response to embryo signaling.

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Abbreviations: BOEC, bovine oviduct epithelial cells; DEG, differentially expressed genes; IVP, *in vitro* production; IVF, *in vitro* fertilization; IFNT, interferon tau; COC, cumulus-oocyte complexes; FCS, foetal calf serum; SOF, synthetic oviductal fluid; TCM-199, tissue culture medium-199; ISREs, interferon-stimulated response elements; ISG, interferon stimulated genes.

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1. Introduction

During preimplantation development, the embryo is exposed to the markedly changing environment of the female reproductive tract, the oviduct (Leese, 1988). In mammals, the oviduct provides an optimal environment for the final preparation, transport and survival of gametes, the fertilization process, and early embryonic development. During the preimplantation period, the embryo is free-living, moving continuously through the tract in a specific tubal microenvironment before implantation in the uterus. During the preimplantation phase, the fertilized zygote undergoes the first cell divisions, and then cells increase their intracellular contacts leading to compaction (morula stage), blastocoele formation and cell differentiation. These crucial events require an effective embryo-maternal dialogue involving oviduct fluid secretions and cellular interactions (Leese et al., 2008). Several studies reported cycle-dependent morphological, proteomic and transcriptomic changes in the oviduct, most likely related to the regulation of embryo development. For example, Bauersachs et al. (2003) reported that most of the bovine oviduct genes upregulated in the luteal phase are involved in the regulation of transcription and cell proliferation whereas the majority of those, upregulated during the follicular period, are involved in protein secretion.

In order to experimentally address and understand the complex interactions between the embryo and the maternal environment, appropriate *in vitro* models of these processes are necessary. In the first assays of *in vitro* production (IVP) of bovine embryos in conventional complex cell-culture media, embryos were systematically blocked between the eight-cell and 16-cell stages (First and Parrish, 1987), which correspond to maternal-to-embryonic transition (Wright and Bondioli, 1981). Furthermore, *in vitro* embryo production is associated with poor blastocyst quality and high embryonic mortality after transfer of these embryos to female recipients (Rizos et al., 2002; Rodriguez-Dorta et al., 2007). In later studies, oviducts from sheep (Eyestone et al., 1987) and rabbits (Ellington et al., 1990) were used as an *ex-vivo* culture system. Several co-culture systems of IVP of sheep and bovine embryos with oviduct epithelial cells were tested, and blastocyst rates were higher than with culture in medium alone (Gandolfi and Moor, 1987). Numerous reports have also demonstrated the beneficial effects of co-culture with oviductal cells on early embryo development in humans (Bongso and Fong, 1993). The mode of action of co-culture systems could be explained by several mechanisms. One of them is a negative conditioning role in which oviductal cells may avoid oxidative stress by regulation of O_2 concentration (Watson et al., 1994; Rizos et al., 2001) and by production of antioxidant enzymes. The second is a positive conditioning role in which oviduct epithelial cells may increase the formation of metabolites, which are required for optimal embryonic development, and may regulate embryo development through the secretion of embryotrophic factors such as growth factors (Mermillod et al., 1993) or other proteins (Lee et al., 2004; Buhi, 2002; Killian, 2004; Goncalves et al., 2007; Hao et al., 2008). Although significant efforts were employed to standardize and optimize defined culture media, it is generally accepted that *in vitro*-produced blastocysts are of lesser quality than *in vivo*-derived embryos. Thus, identifying the molecular mechanisms of preimplantation embryo development is of particular importance in the identification of reasons for poor quality in IVP embryos.

Despite the fact that embryo co-culture with oviduct cells is not the ideal system for producing bovine embryos for commercial purposes, it may nevertheless be a suitable method for studying embryo-maternal interaction. Complementary to available *in vivo* models, cell culture systems are essential for functional studies of candidate genes involved in the embryo-maternal dialogue. In this study, in order to identify genes potentially involved in embryo-maternal communication, we first described the effects of co-culture on blastocyst rate (percentage of cleaved embryos that develop to the blastocyst stage) and then focused on mRNA expression changes in bovine oviduct epithelial cells (BOEC) used for co-culturing the embryos. For this purpose, we used large-scale cDNA microarray hybridization to identify differentially regulated genes in BOEC at the end point of *in vitro* culture.

2. Materials and methods

Biological material has been collected at local slaughterhouse (Charal, Sablé sur Sarthe, France), with the permission of the direction of the slaughterhouse and the agreement of local sanitary services. The experimental design is summarized in Fig. 1.

2.1. Oviduct cell culture

The process of primary cell culture used in this study was already described and validated, indicating a high rate of epithelial cells (ciliated and secretory) expressing vimentin and showing epithelial type ultrastructure under electron microscope (Mermillod et al., 1993; Van Langendonckt et al., 1995). Briefly, bovine oviducts were collected from a local slaughterhouse without regard to oestrus cycle status of the donor. All the following procedures took place within 2–3 h post-mortem. The oviduct was rinsed with Hepes-Buffered Tissue culture medium-199 (Gibco, Invitrogen, Cergy Pontoise, France), cleaned from surrounding tissues and washed rapidly in ethanol 70%. Mucosae were mechanically expelled from the oviduct by gentle scraping with a sterile glass slide. Epithelial cells were then washed three times by sedimentation in 10 mL of Tissue culture medium-199 (TCM-199, Gibco, Invitrogen, Cergy Pontoise, France) supplemented with 10% heat-treated foetal calf serum (FCS) and 8 μL/mL gentamycin. The resulting cellular pellet was diluted 100 times in TCM-199 supplemented with 10% FCS and 80 μg/mL gentamycin before seeding in four-well culture plates (NUNC, Roskilde, Denmark) in a humidified atmosphere with 5% CO_2 at 38.5 °C. The medium was completely renewed at day 2. Then, half of the medium was removed at day 4 and replaced by TCM-199 supplemented with 10% heat-treated FCS + 60 μg/mL gentamycin. At day 5, the medium was replaced with SOF (synthetic oviductal fluid)

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