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Expression and localization of interleukin 1 beta and interleukin 1 receptor (type I) in the bovine endometrium and embryo

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ABSTRACT

The interleukin-1 (IL1) system likely mediates mammalian embryo–maternal communication. In cattle, we have reported that the uterine fluid of heifers carrying early embryos shows downregulated IL1 beta (IL1B), which could lead to reduced NFkB expression and dampening of maternal innate immune responses. In this work, we assessed the expression of IL 1 beta (IL1B) and its receptor, interleukin 1 receptor type I (IL1R1) in the bovine endometrium and embryos by RT-PCR, immunohistochemistry and Western blot at the time of blastocyst development. Day 8 endometrium, both collected from animals after transfer of day 5 embryos (ET) and sham transferred (ST), showed *IL1B* and *IL1R1* mRNA transcription and protein co-localization. Similarly, day 8 blastocyst, from ET animals and entirely produced in vitro, showed *IL1R1* mRNA transcription and IL1B and IL1R1 protein co-localization. *IL1B* mRNA was detected in the analyzed blastocysts, but at very low levels that precluded its quantification. IL1B and IL1R1 immunostaining was observed in luminal epithelial cells, glandular epithelium and stromal cells. The presence of embryos increased endometrial IL1B protein locally, while no differences regarding IL1R1 protein and *IL1B* and *IL1R1* mRNA were detected. These results suggest that the early preimplantation bovine embryo in the maternal tract might interact with the maternal immune system through the IL1 system. Such a mechanism may allow the embryo to elicit local endometrial responses at early stages, which are required for the development of a receptive endometrium.

1. Introduction

Cytokines, initially identified as peptides and protein secretory products of immune cells, play important roles in the endometrial physiology and maternal regulation of embryonic development (Robertson et al., 1992; Singh et al., 2011). In mammals, deregulated expression of cytokines can lead to total or partial failure of implantation and abnormal placental formation (Guzeloglu-Kayisli et al., 2009).

Interleukin-1 (IL1), a major pro-inflammatory cytokine, locally regulates many endometrial functions at the

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foeto-maternal interface in mammalian and non-mammalian vertebrates (Paulesu et al., 2005). The IL1 system is composed of two agonists (IL1alpha, IL1beta), an antagonist (IL1 receptor antagonist, IL1RN), and a receptor family. The IL1 receptor family consists of the type I (IL1R1) and type II (IL1R2) IL1 receptors, and the IL1R accessory protein (IL1RAP) (Dinarello, 1994). Both IL1alpha (IL1A) and IL1beta (IL1B) bind to IL1R1 and to IL1R2 (Dower et al., 1985), whereas IL1RAP does not recognize the ligand, but increases the receptor affinity for interleukins (Colotta et al., 1993; Wesche et al., 1997). Only the IL1R1 transduces a signal in response to IL1, while the IL1R2 suppresses IL1 activity by competing for IL1 binding (Sims et al., 1994).

The IL1 system has been identified in mammalian embryos and in the reproductive tract. Thus, IL1B has been detected in human, mouse and bovine endometrium (McMaster et al., 1992; Paula-Lopes et al., 1999; Simón et al., 1993b; Tabibzadeh and Babaknia, 1995); in human, mouse, porcine and bovine embryos (De los Santos et al., 1996; Muñoz et al., 2012; Tuo et al., 1996); and in human, porcine and bovine uterine fluid (Davidson et al., 1995; Muñoz et al., 2012; Paula-Lopes et al., 1999; Ross et al., 2003; Simón et al., 1996). Similarly, the endometrium of humans, mice, pigs, and rabbits contains IL1A (McMaster et al., 1992; Tabibzadeh and Babaknia, 1995; Tuo et al., 1996; Yang et al., 1995). Among the receptors, IL1R1 has been detected in human, murine, porcine and bovine endometrium (Ross et al., 2003; Simón et al., 1994, 1993a,b; Tanikawa et al., 2005) and in preimplantation human and mouse embryos (De los Santos et al., 1996; Kruessel et al., 1997). In addition, IL1RAP has been found in human and porcine endometrium (Ross et al., 2003; Simón et al., 1996; Tabibzadeh and Babaknia, 1995), and in preimplantation human and mouse embryos (De los Santos et al., 1996; Kruessel et al., 1997).

Preimplantation embryos from various species produce and respond to IL1 (Austgulen et al., 1995; Pampfer et al., 1991; Sheth et al., 1991; Simón et al., 1994; Stewart and Cullinan, 1997; Tuo et al., 1996; Zolti et al., 1991). Moreover, in humans, preimplantation embryos release IL1B into culture medium in an amount proportional to blastomere number (Barañao et al., 1997; Taskin et al., 2012), suggesting that IL1B secretion might predict embryonic potential to establish pregnancy. In cattle, the addition of IL1B at 8–10 h after insemination increases the proportion of oocytes that develop to the blastocyst stage (Paula-Lopes et al., 1998). Furthermore, in endometrial cell cultures, IL1B inhibits growth of stromal cells, but not epithelial cells (Davidson et al., 1995), whereas IL1B increases the secretion of prostaglandins by epithelial and stromal endometrial cells (Betts and Hansen, 1992; Davidson et al., 1995).

We have recently reported a decrease in IL1B in bovine uterine fluid during early pregnancy, which could potentially lead to a reduction in NFkB expression and dampening of maternal innate immune responses (Muñoz et al., 2012). Such an observation, coupled with evidence of IL1B participating in early embryonic development and endometrial function in the cow (Betts and Hansen, 1992; Davidson et al., 1995; Paula-Lopes et al., 1998), suggests that the IL1 system might play an important role during early embryonic development in cattle.

The main hypothesis of this study was that the early pre-implantation bovine embryo in the maternal tract interacts with the maternal immune system through local modulation of the IL1 system. Through such a mechanism, the embryo could act during the early stages of pregnancy to elicit local responses, which are required for the development of a receptive endometrium. To test that hypothesis, changes in gene and protein expression of IL1B and IL1R1 during transient *in utero* development of embryos produced *in vitro* were evaluated in the bovine endometrium and embryos during the blastocyst stage. Caruncular and intercaruncular endometrial and embryonic expression of IL1B and IL1R1 was examined by RT-qPCR, immunohistochemistry and Western blot at the time of blastocyst development.

2. Materials and methods

All experimental procedures involving animals were performed according to the European Community Directive 2010/63/EU (Spanish Regulation, R.D.53/2013), and were sanctioned by the Animal Research Ethics Committee of SERIDA (Agreement date 08 February 2012).

All reagents were purchased from Sigma (Madrid, Spain) unless otherwise stated.

2.1. Animals and embryo transfer

Procedures involving animal feeding and management, oestrus synchronization, embryo production, embryo transfer (ET) and recovery, and progesterone (P4) blood sampling and analysis have been described elsewhere (Gómez et al., 2008; Hidalgo et al., 2004; Muñoz et al., 2012).

Embryos were produced *in vitro* (IVP) using oocytes collected from slaughterhouse ovaries as previously reported (Gómez et al., 2008). Briefly, cumulus–oocyte complexes (COCs) were matured in TCM199, $NaHCO_3$ (2.2 g/L), foetal calf serum (FCS, F-4135) (10% v/v), pFSH-LH (Stimufol, ULg FMV France) and 17β-oestradiol (1 μg/mL). In vitro fertilization (IVF) was performed with frozen/thawed sperm by using a swim-up procedure. Presumptive zygotes were cultured in synthetic oviduct fluid (SOF) with 6 g/L BSA at 38.7 °C, 5% CO_2, 5% O_2 and 90% N_2. Droplets with 50 μL SOF and 25–30 embryos per drop were layered under mineral oil. On day 5 after IVF, embryos (morulae) were temporarily transferred to recipients until day 8, or continued *in vitro* culture up to day 8. Under these conditions, approximately 85% of embryos cleaved by day 3 and produced >40% morulae on day 5.

Oestrus cycles were synchronized by using an intravaginal progestogen device (PRID ALPHA, Ceva, Barcelona, Spain) for 10 days combined with a prostaglandin analogue (Dynolitic, Pfizer, Madrid, Spain) injected 48 h before progestogen removal. Animals were observed at least 3 times per 30 min a day for oestrus detection, commencing 33 h after progestogen removal. Day 0 was considered a fixed time 48 h after progestogen removal, to coincide with the IVF onset in the laboratory. On day 0, ovaries were scanned