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Up-regulation of expression of interferon-stimulated gene 15 in the bovine corpus luteum during early pregnancy

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ABSTRACT

Interferon-τ (IFNT), the pregnancy recognition signal in ruminant species, is secreted by conceptus trophectoderm cells and induces expression of IFN-stimulated gene 15 (ISG15) in the uterus and corpus luteum (CL) in ewes. Expression of ISG15 in ovine CL is speculated to be through an endocrine pathway, but it is unclear whether expression of ISG15 in bovine CL is via such a pathway. In this study, CL were obtained from cows on d 16, 25, 60, 120, 180, and 270 of pregnancy, and endometrium, mammary gland, ovarian stroma, and CL were also collected from cows on d 18 of pregnancy and on d 15 and 18 of the estrous cycle. All tissue explants from d 15 of the estrous cycle were cultured in the absence or presence of 100 ng/mL of recombinant bovine IFNT for 24 h. The results indicated that ISG15 and conjugated proteins were expressed in CL of both cyclic and pregnant cows regardless of pregnancy status and were up-regulated during early pregnancy. The mammary gland from d 18 of pregnancy did not express ISG15, but explants of the mammary gland from d 15 of the estrous cycle did express ISG15 after being treated with IFNT. However, luteal explants from d 15 of the estrous cycle did not express ISG15 after being cultured for 24 h. In conclusion, ISG15 expression is up-regulated in the bovine CL during early pregnancy. Interestingly, cultured CL cells do not respond to IFNT, suggesting that the pregnancy-dependent stimulation of ISG15 expression is controlled by something other than IFNT in the bloodstream.

Key words: interferon-stimulated gene 15, corpus luteum, mammary gland, bovine

INTRODUCTION

Interferon-τ (**IFNT**) is a major product of mononuclear trophectoderm cells of ruminant conceptuses during early pregnancy before placental attachment (Roberts, 2007). Its expression is regulated by at least 2 uterine-derived factors, granulocyte-macrophage colony-stimulating factor and fibroblast growth factor 2, as well as multiple signaling pathways (Ealy and Yang, 2009). Its primary action is local to uterine epithelia as a paracrine signal for maternal recognition of pregnancy (Roberts et al., 1992; Bazer et al., 1997). On binding to its specific receptors, uterine epithelia, IFNT directly silences expression of estrogen receptor α, and therefore indirectly silences oxytocin receptor expression to prevent pulsatile secretion of $PGF_{2\alpha}$ that would otherwise be luteolytic (Bazer et al., 2009). Much of the present data are consistent with this hypothesis in the ovine, but the mechanism is unclear in the bovine (Roberts et al., 2008). Krishnaswamy et al. (2009) recently reported that oxytocin receptor down-regulation was not necessary to reduce the oxytocin-induced uterine release of pulsatile prostaglandin F by IFNT in a bovine endometrial epithelial cell line. This means that other mechanisms may exist in cattle. Arosh et al. (2004) suggested that IFNT directly or indirectly stimulates secretion of prostaglandin E_2, which may also participate in the prevention of luteolysis of the corpus luteum (**CL**) in the bovine.

Interferon-τ also induces synthesis and secretion of IFN-stimulated genes (**ISG**) in the uterus, such as ISG 15-kDa protein (**ISG15**; Johnson et al., 1999), a ubiquitin homolog (ubiquitin cross-reactive protein). In cows and sheep, synthesis of endometrial ISG15 coincides with the secretion of conceptus-derived IFNT (Austin et al., 1996; Johnson et al., 1998). Recently, it was shown that ISG15 is expressed in other tissues during early pregnancy in cattle and sheep. In bovine peripheral blood leukocytes, ISG15 mRNA levels were greater in pregnant cows than in nonpregnant cows on d 18 and 20 after the onset of estrus (Gifford et al., 2007). Han et al. (2006) also reported that ISG15 mRNA was up-regulated in blood cells from pregnant cows, compared with nonpregnant cows. In the ovine CL, ISG15 mRNA was elevated after intrauterine infusion of or intramuscular or subcutaneous injection with IFNT (Spencer et al., 1999b; Chen et al., 2006). Oliveira et al. (2008) reported that IFNT from the conceptus induces ISG15 expression in peripheral blood cells and in CL

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after being released into the uterine venous system during the period of pregnancy recognition in ewes.

Immune cell populations exist within bovine CL (Lobel and Levy, 1968). Niswender et al. (1997) reported that these immune cells are present primarily as a result of high blood flow to CL. These immune cells may have an active role in controlling the life span and function of the CL, and may have powerful local effects both on luteolysis and on prolonging the functional life span of CL with the assistance of their cytokine products during pregnancy (Mori, 1990; Pate, 2003). Coculturing bovine luteal endothelial cells with peripheral blood mononuclear cells (**PBMC**) increased monocyte chemoattractant protein 1 secretion by an average of 5-fold over values for either cell type alone. Luteal endothelial cells cocultured with concanavalin A-activated PBMC increased monocyte chemoattractant protein 1 secretion by an average of 12-fold compared with controls (Liptak et al., 2005). Fujiwara (2006) suggested that the PBMC receive several signals from the conceptus and to change their functions, which could, in turn, affect the functions or differentiation of nonimmune organs, such as the CL, during the early stages of pregnancy in humans.

It has been suggested that in sheep, in addition to the intrauterine paracrine actions of IFNT for pregnancy recognition signaling, conceptus-derived IFNT act on CL through an endocrine pathway (Oliveira et al., 2008). Interferon-τ also activates gene expression in components of the circulating immune system during early pregnancy in ewes (Yankey et al., 2001), but it is unclear whether such a mechanism is responsible for expression of ISG15 in bovine CL. In this study, we hypothesize that bovine conceptus-derived IFNT released into the uterine vein can act on organs other than the uterus, such as the mammary gland and ovary, in the same IFNT environment. If expression of mammary ISG15 is increased in early pregnancy, detection of ISG15 in milk somatic cells would be an ideal noninvasive diagnostic tool for early pregnancy detection. The objectives of the present study were to determine 1) expression of ISG15 in the mammary gland and ovarian stroma during early pregnancy; 2) changes in ISG15 protein expression in CL throughout pregnancy; and 3) effects of recombinant bovine IFNT on ISG15 expression in explant cultures of the mammary gland, ovarian stroma, and CL from nonpregnant cows.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise stated. All plasticware was from Nunc (Nunc, Nalge Nunc International, Roskilde, Denmark). Mouse anti-bovine ISG15 mAb (**5F10**) was generously provided by Thomas R. Hansen (Colorado State University, Fort Collins). Recombinant bovine IFNT (1.13×10^8 IU of antiviral activity/mg) was generously provided by R. Michael Roberts (University of Missouri, Columbia). Secondary goat anti-mouse IgG-horseradish peroxidase (**IgG-HRP**; 62-6520, Zymed) and a diaminobenzidine kit were from Invitrogen Corporation (Carlsbad, CA).

Animals and Experimental Design

Holstein cows between 6 and 8 yr of age were used in all the procedures described. They were managed, including being bred, on the experimental farm of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China), and were fed a TMR. Animal care and experimental procedures were approved and conducted under established standards of the Institute of Animal Sciences and Chinese Academy of Agricultural Sciences. Controlled internal drug-releasing devices (InterAg, Hamilton, New Zealand) were used to synchronize estrus, and estrous behavior was monitored 3 times per day. The day of AI was designated d 0 of pregnancy. Cows assigned to the nonpregnant group were not inseminated. The endometrium, mammary gland, ovarian stroma, and CL were sampled from pregnant and nonpregnant cows on d 18 after AI (n = 4 for each group) after slaughter, and these samples were also collected from nonpregnant cows on d 15. Pregnancy was confirmed by the presence of a conceptus in the uterus at slaughter or by the result of pregnancy diagnosis using transrectal ultrasound (d 28 to 30). Corpora lutea were also obtained from cows on d 16, 25, 60, 120, 180, and 270 of pregnancy (n = 4 for each group) either at the time of slaughter or by oophorectomy. Some tissues were used for explant cultures, some were fixed in 4% buffered paraformaldehyde for immunohistochemical analysis, and the remaining tissues were stored at −80°C for protein analysis.

Tissue Explant Culture

Tissue explants were cultured as described by Austin et al. (1996) with modifications. Briefly, tissue explants of the endometrium, mammary gland, ovarian stroma, and CL from nonpregnant cows on d 15 were placed in sterile PBS solution and immediately transported to the laboratory at room temperature. Tissues were placed in Dulbecco's modified Eagle's medium with F12 salts (**DMEM/F12**, Gibco BRL, Grand Island, NY) containing penicillin and streptomycin (100 IU/mL, 0.1 mg/mL). Approximately 200 mg of each tis-