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Administration of peripheral blood mononuclear cells into the uterine horn to improve pregnancy rate following bovine embryo transfer

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ABSTRACT

Embryo transfer (ET) has been used to improve reproductive efficiency and genetic make-up in bovine species. However, the success rate of ET has not been improved since its inception. Here we examined whether administration of autologous peripheral blood mononuclear cells (PBMCs) into the uterine horn can improve pregnancy rates following bovine ET. First we determined that the abundance of interleukin (IL)-1α, IL-1β and IL-8 transcripts in PBMCs was greatest after 24 h of culture. PBMCs that had been cultured for 24 h were gently administered non-surgically to the uterine horn ipsilateral to the corpus luteum on day 4 of the estrous cycle. On day 7, the ET was carried out and the pregnancy rate in the PBMC-treated group was compared with that in the non-treated group. The pregnancy rate on day 60 in the PBMC-treated group (76.7%, 56/73) was significantly higher than that in the non-treated group (59.7%, 43/72, $p < 0.05$). These results indicate that administration of autologous PBMCs into the uterine horn improves pregnancy rates following bovine ET.

1. Introduction

In the farm animal industry, embryo transfer (ET) technology has been used to reproduce the most valuable females and to produce males of high genetic value. ET technology has been proposed as a means of improving the pregnancy rate of repeat-breeding cows (Tanabe et al., 1985; Dochi et al., 2008). However, the highest pregnancy rates following ET are reported to be 70% (Hasler, 2007). The 30% pregnancy failure may result from a lack of pregnancy recognition between the pre-implantation embryo and the uterine endometrium. Insufficient development of the endometrium during the first 2–3 weeks is thought to be a likely cause of pregnancy failure (Katagiri and Takahashi, 2004).

It has been thought that cytokines and chemokines from endometrial immune cells prepare the uterus for nurturing an embryo, which eventually forms the placental structure (Leung et al., 2000; Kammerer et al., 2004; Dimitriadis et al., 2005; Oliveira and Hansen, 2008). Cytokines and chemokines produced in the female reproductive tract after mating may enhance reproductive success, and have a role in evoking the local endometrial changes that are required for pregnancy. In fact, interleukin (IL)-1 partially controls the process of embryo implantation or establishment of placenta in different animal models (Simon et al., 1994; Paula-Lopes et al., 1999; Lee et al., 2005; Okuda and Sakumoto, 2006). The IL-1 system contains two agonists: IL-1α and IL-1β. IL-1α modifies the endometrial structure for embryo implantation in humans (Singer et al., 1997; Huang et al., 1998), while IL-1β modulates the growth of bovine embryos during the early stages of development (Paula-Lopes et al., 1998). IL-8 is a neutrophil chemoattractant and is a potent angiogenic factor (Koch et al., 1992; Desbaillets et al., 1997) produced by monocytes and

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Table 1
Primers for real-time PCR used in this study.

Gene	Primer	Sequence	Annealing temperature (°C)	Product (bp)
IL-1α	Forward	5′-GACCACCTCTCTCTCAATC-3′	52	146
	Reverse	5′-CCATCACCACATTCTCC-3′		
IL-1β	Forward	5′-AATGAACCGAGAAGTGG-3′	50	282
	Reverse	5′-TTCTTCGATTTGAGAAG-3′		
IL-8	Forward	5′-GACTTCCAAGCTGGCTGTTG-3′	55	304
	Reverse	5′-CATGGATCTTGCTTCTCAGCTC-3′		
GAPDH	Forward	5′-GAGATGATGACCCTTTTGGC-3′	55	356
	Reverse	5′-GTGAAGGTCGGAGTCAACG-3′		

endothelial cells (Matsushita et al., 1988; Baggiolini et al., 1989). Several studies revealed that chemokines including IL-8 play important roles in human embryo implantation (Caballero-Campo et al., 2002; Selam et al., 2002; Jones et al., 2004). These results suggest that local immune cells also play an essential role in the fate of embryos in ruminant ungulates (Hansen, 1995).

Endometrial lymphocytes constitute the maternal aspect of the maternal–embryo interface and are considered to contribute to successful pregnancy (Kearns and Lala, 1983; Bulmer and Peel, 1996). Administration of splenocytes (Takabatake et al., 1997) or thymocytes (Fujita et al., 1998) into the endometrium resulted in embryo implantation by regulating endometrial differentiation in mice. In addition, intrauterine administration of human peripheral blood mononuclear cells (PBMCs) has been shown to improve pregnancy rates in patients with repeated *in vitro* fertilization-ET failures (Yoshioka et al., 2006). These studies indicate that administration of immune cells into the uterus causes endometrial response that is necessary for the establishment of pregnancy. Thus, conditioning the uterine environments with immune cells could increase pregnancy outcome. However, little is known about the role of immune cells infused into the uterus in bovine species. The objective of the present study was to examine whether administration of bovine PBMCs into the uterus prior to the ET procedure improves the pregnancy rate.

2. Materials and methods

2.1. Animals

All animal procedures in the present study were approved by the Committee for Experimental Animals of Zen-noh Embryo Transfer (ET) Center. All recipient animals for ET were fed the same feed, and water was supplied ad libitum. We confirmed that development of the corpora lutea and genital organs of the heifers were normal by rectal examinations. The study was carried out at the Zen-noh ET Center from June to October 2008.

2.2. Experimental design

2.2.1. Experiment 1

Bovine PBMCs were isolated from whole blood of three heifers at day 3 of the estrous cycle (day 0 = estrus) and then cultured for 0, 24 or 48 h *in vitro*. mRNA expressions of IL-1α, IL-1β and IL-8 in the cells were examined using the quantitative reverse transcription-polymerase chain reaction (RT-PCR).

2.2.2. Experiment 2

To determine the effect of PBMCs on pregnancy rate following ET, autologous PBMCs cultured *in vitro* were administered to the uterine horn ipsilateral to the corpus luteum on day 4 of the estrous cycle. Three days later, bovine embryos were transferred into the uterine horn. The control group received no treatment. Pregnancy was determined on days 30 and 60 of gestation.

2.3. Preparation of bovine peripheral blood mononuclear cells (PBMCs)

Bovine PBMCs were isolated using Ficoll-Paque™ PLUS (GE Healthcare, Uppsala, Sweden) centrifugation as described previously (Hashi et al., 1998). After centrifugation, PBMCs were collected from the interphase layer and washed two times in Dulbecco's phosphate-buffered saline (DPBS, Invitrogen, Carlsbad, CA, USA). PBMCs (1×10^6 cells/ml) suspended in RPMI medium 1640 containing 2 mM L-glutamine and 25 mM N-2-hydroxyethylpoperazine-N′-2-ethane sulfonic acid (RPMI 1640, Invitrogen) and 10% (v/v) fetal bovine serum (FBS, Hyclone, Logan, UT, USA) were incubated for 0, 24 or 48 h in tissue culture dishes (Biocoat Collagen I Cellware 100 mm dish, BD Bioscience, Erembodegem, Belgium) at 39 °C under 5% CO_2 in air with high humidity.

2.4. RNA extraction and reverse transcription

Total RNA was isolated from PBMCs (2×10^6 cells) of three heifers using a RNeasy Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions and was stored at −80 °C. Total RNA (500 ng) was reverse transcribed using a QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions.

2.5. Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Gene transcripts were quantified by real-time quantitative RT-PCR. PCR experiments were conducted in triplicate for each transcript. Relative levels of each transcript and bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined for every sample. PCR was performed by adding a 2 μl aliquot of each sample to the PCR