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Effect of a single intrauterine administration of recombinant bovine interferon-τ on day 7 of the estrous cycle on the luteal phase length and blood profile in dairy cows

S. Matsunaga [a,1], T. Osawa [a,*,1], M. Geshi [b], H. Takahashi [b], S. Inumaru [c], Y. Yokomizo [c,†], Y.-I. Miyake [d]

[a] Laboratory of Theriogenology, Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan
[b] National Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, 2 Ikenodai, Tsukuba, Ibaraki 305-0901, Japan
[c] National Institute of Animal Health, National Agriculture and Food Research Organization, Kannondai, Tsukuba, Ibaraki 305-0856, Japan
[d] Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

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ABSTRACT

This study tested the effect of recombinant bovine interferon-tau (rboIFN-τ) on the length of estrous cycle, luteal lifespan and side effects of rboIFN-τ in the cow. A normal estrous cycle in six non-lactating cycling Holstein cows was observed (non-treated cycle), and either 2.0 mg of liposomalized rboIFN-τ (treated cycle) or bovine serum albumin (BSA; placebo cycle) was infused in the uterus on day 7 of the estrous cycle (day 0 = day of ovulation). Rectal temperature, heart rate and respiratory rate were recorded and blood samples were collected before and after the treatments. The length of the estrous cycle and corpus luteum lifespan in rboIFN-τ treated cycles were not significantly different from those of the non-treated and placebo cycles. In contrast, the rboIFN-τ treatment caused a transient increase in rectal temperature and a decrease in the number of peripheral lymphocytes and neutrophils after the treatment.

1. Introduction

For successful pregnancy, it is essential that the maternal body accepts the embryo without rejection. Therefore, appropriate recognition of pregnancy by the mother must occur. It is generally considered that pre-implantation embryo secretes a number of factors related to the maternal recognition of pregnancy, and the mother receiving these signals prepares for implantation. If maternal recognition fails due to the causes related to embryo, mother or both, the embryo is rejected by the mother and dies.

Interferon-tau (IFN-τ) is an embryonic factor produced in ruminants that is secreted from the trophoblast during the period from day 16 to 24 of pregnancy (Bartol et al., 1985). Recently, Bott et al. (2010) have demonstrated that IFN-τ is released from the uterus into the uterine vein and acts through a local-endocrine mechanism on the corpus luteum (CL) and delay luteolysis in the ewe. The major function of IFN-τ is the inhibition of luteolysis. In the normal cow estrous cycle, luteolysis is triggered by the oxytocin signaling between the receptor on the endometrium and oxytocin secreted from the CL. The oxytocin signaling subsequently induces a rapid increase of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) that acts as a signal of luteolysis. IFN-τ inhibits the upregulation of oxytocin receptors in the endometrium at the end of the estrous cycle and thus prevents the luteolytic effect of $PGF_{2\alpha}$ (Horn et al., 1998).

IFN-τ is classified as a type I interferon that shares gene structure and function with other type I interferons (Demmers et al., 2001). Both IFN-τ and IFN-α inhibit luteolysis. For the purpose of prevention of early embryonic death and improvement of conception rate in the cow, several studies on interferon have been conducted. The studies using IFN-α showed extension of the CL lifespan, but it also showed side effects of IFN-α such as rise of body temperature (Plante et al., 1989, 1991). On the other hand, IFN-τ has been considered more useful for clinical applications because of its low cytotoxicity (Demmers et al., 2001). Klemann et al. (1990) first expressed recombinant bovine IFN-τ (rboIFN-τ) in *Escherichia coli* that has since allowed many studies using rboIFN-τ.

Meyer et al. (1995b) reported that the repeated intrauterine administration of 0.2 mg recombinant IFN-τ twice a day during the period of days 14 to 24 after ovulation had extended CL lifespan by approximately 8 days. They also reported the prevention of oxytocin-induced $PGF_{2\alpha}$ secretion by IFN-τ. However, a single administration of IFN-τ would be preferable for the convenience of clinical applications. Liposomal encapsulation is one of the drug delivery systems that has been used for sustained release of

* Corresponding author. Tel./fax: +81 19 621 6278.
E-mail address: osawa@iwate-u.ac.jp (T. Osawa).
[1] These authors equally contributed to this work.
[†] Deceased.

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proteins (Meyer et al., 1994; Angst and Drover, 2006). A single intrauterine infusion of liposomally encapsulated recombinant IFN-τ has been shown to extend CL lifespan in cyclic ewes treated on day 10 (L'Haridon et al., 1995) and heifers treated on day 13 (Geshi et al., 2002). To apply IFN-τ in recipient cows in the field, anti-luteolytic effect of rboIFN-τ on day 7 should be verified. Its potential side effects also should be determined.

The objective of this study was to elucidate the effect of a single intrauterine administration of rboIFN-τ on day 7 of the estrous cycle on the length of the luteal lifespan, rectal temperature, heart rate, respiratory rate and blood profile in dairy cows.

2. Materials and methods

2.1. Animals

Six non-lactating, cycling Holstein cows kept at the Takizawa Experimental Station, Iwate University, were used for this study. Average (±S.D.) age, body weight and parity of the animals were 6.4 (±1.3), 696.3 (±28.2) and 2.8 (±0.8), respectively. Throughout the study, the experimental animals were daily fed 15 kg of corn silage, 12 kg of hay (mixture of timothy and orchard grass) and 1.5 kg of concentrates. The concentrate mixture was composed of corn (52%), oil cake (16%), bran (12%), and beet pulp, alfalfa hay cube, vitamin and mineral supplements (20%). The total dietary requirement of each animal was divided into two halves and each half was fed at 07.30 h and 17.00 h.

2.2. Experimental design

Either 2.0 mg of rboIFN-τ (rboIFN-τ treated cycle) or 2.0 mg of bovine serum albumin (BSA, placebo cycle) was randomly infused in the anterior portion of the uterine horn ipsilateral to the CL on day 7 (day 0 = day of ovulation as determined by ultrasonography). By the end of the experimental period, all animals had both treatments (one rboIFN-τ treatment and one placebo treatment). To eliminate a possible effect of the order of treatment on the length of the subsequent estrous cycle, a non-treatment estrous cycle (non-treated cycles; $n = 5$) was imposed between rboIFN-τ treated ($n = 6$) and placebo cycles ($n = 6$) (Data of a non-treated cycle in one animal was not available).

2.3. Preparation of rboIFN-τ

The rboIFN-τ used for this study was derived from the cell culture medium of TN5/NIAH cells infected with recombinant baculovirus encoding the bovine interferon-τ gene (Watanabe et al., 2002). The rboIFN-τ (82% purity) was concentrated to 12.5 mg/ml in phosphate buffer solution (PBS) and the antiviral activity was 3.52×10^{10} U/ml. The antiviral activity of the rbIFN-τ was determined by measuring the inhibition of the cytopathogenic effect of the Vesicular stomatitis virus using MDBK cell line one (Eichmann et al., 1990). The assay was calibrated against a human IFN-τ reference standard (Interferon alpha 2b, human, second International Standard 1999) provided by the National Institute for Biological Standards and Control (NIBSC, UK). Into a vial of anionic liposome (COATSOME® EL-01-A, NOF Corporation, Tokyo, Japan), 20 mg of rboIFN-τ in 1.6 ml of PBS and 0.4 ml of purified water containing 400 U penicillin and 0.4 mg streptomycin were placed. The mixture in the vial was then gently shaken five times by hand and packaged in a 0.25 ml straw. The final concentration of rboIFN-τ in the straw was 8.0 mg/ml. The same amount of liposomalized BSA in PBS was used as a placebo. Straws were stored at −30 °C until the time of administration.

2.4. Examination of the reproductive organs

In each estrous cycle, ultrasonography per rectum was performed for monitoring the size of the CL everyday (during the follicular phase, day 17 to day 4 of the following cycle) or every other day (during the luteal phase, days 5 to 16). The CL cross-sectional area (mm^2) was calculated using the formula as described previously (Nishigai et al., 2001).

In the case of the cystic CL that had a cavity, the true CL cross-sectional area was calculated by subtraction of the cavity area from the total area.

2.5. Administration of rboIFN-τ

Administration of rboIFN-τ or placebo was performed on day 7 of each estrous cycle. Prior to the administration, 3.0 ml of 2% lidocaine hydrochloride (Xylocaine injection 2%, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was injected on the caudal vertebrae for epidural anesthesia. Rectal temperature, heart rate and respiratory rate were recorded and blood samples were collected from the coccygeal vein into the heparinized vacuum tubes at 24 h and 5 min before and 1, 3, 6, 12, 24, 48, 72 and 120 h after rboIFN-τ administration. Complete blood cell counts were performed at each sampling time with an automatic counter (Celltac α, MEK-6258, Nihon Kohden Corporation, Japan). Differential white blood cell (WBC) counts were performed on smear preparations stained with Wright/Giemsa stain. Plasma samples were obtained by centrifugation at 1500*g* for 15 min and stored at −30 °C until the hormone assays were performed.

2.6. Hormone assays

Plasma concentrations of progesterone were determined by a double-antibody radioimmunoassay (Taya et al., 1985). Ovine antiserum raised against progesterone (GDN#337) was provided by Dr. G.D. Niswender (Colorado State University, USA). The intra- and inter-assay coefficients of variation were 9.3% and 7.6%, respectively.

2.7. Statistical analysis

Estrous cycle length was defined as the time interval between the ovulations of the two subsequent cycles. The CL lifespan was defined as the number of days from functional CL formation (plasma progesterone $\geqslant 1.0$ ng/ml) to CL regression (plasma progesterone <1.0 ng/ml). Estrous cycle length, CL lifespan and the maximum cross-sectional area of CL were compared among the treatments (rboIFN-τ-treated, BSA-treated and untreated cycles) by one-way factorial analysis of variance (ANOVA). Data on rectal temperature, heart rate, respiratory rate and hematological parameters were analyzed across observation time-points using one-way repeated measures ANOVA for each group (rboIFN-τ treatment or BSA treatment). In case of statistical significance, post hoc paired *t*-test (two-tailed) was employed to compare pre-treatment value and post-treatment values. Two-way repeated measures ANOVA was performed to compare changes in rectal temperature, heart rate, respiratory rate and hematological parameters over time between rboIFN-τ and BSA treated groups. Treatment, time and their interaction were included in the model.

3. Results

3.1. Estrous cycle responses

The mean (±S.E.M.) length of the untreated cycle, rboIFN-τ-treated cycle, and BSA-treated (placebo) cycle were 22.0 ± 0.8,