Effect of Intrauterine and Intramuscular Administration of Recombinant Bovine Interferon α1 on Luteal Lifespan in Cattle

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ABSTRACT

Intrauterine and intramuscular administration of interferon was tested for effectiveness in extending luteal lifespan in cattle. Intrauterine infusion of 1 mg of recombinant bovine interferon-alpha1, twice daily, to lactating dairy cows from d 14 to 21 after estrus extended interestrous interval (30.4 ± 1.91 d versus 24.8 ± .58 d) and functional lifespan of the corpus luteum (28.4 ± 2.01 d versus 23.6 ± .75 d). In another experiment, twice daily intramuscular injection of 20 mg interferon to Simmental heifers from d 15 to 19 extended interestrous intervals (24.6 ± 1.36 d versus 20.6 ± .49 d) and functional lifespan of the corpus luteum (23.2 ± .37 d versus 20.2 ± .73 d). In a third experiment, pubertal dairy heifers received twice daily intramuscular injections of 0, 2.5, 5.0, or 10.0 mg/injection of interferon from d 14 to 21 after estrus. The three interferon-treated groups had longer interestrous intervals and functional luteal lifespans than the control group. Interestrous intervals were 22.0 ± .68, 24.0 ± 1.14, 24.6 ± 1.17, and 25.4 ± .97 d, respectively. The present data strengthen the theory that an interferon-alpha-like molecule can regulate luteal function in cattle. Such a regulatory compound might prove useful in schemes to reduce embryonic mortality caused by aberrant secretion of embryonic interferon.

INTRODUCTION

The establishment of pregnancy in domestic ruminants requires embryonic signals to prevent luteolysis of the corpus luteum (CL) and secure continued progesterone secretion from the ovary. In cattle, one of the signals is produced around d 15 to 17 of gestation because embryo removal after d 15 and intrauterine infusion of embryonic homogenates or secretory products from this period extend CL function (3, 13, 16, 17). The molecule responsible for this action has been identified as a complex of proteins called bovine trophoblast protein-1 (bTP-1) (2, 7, 9). Infusion of bTP-1 into the uterine lumen of cyclic cows extends CL lifespan by inhibiting endometrial prostaglandin F2α (PGF2α) release in vivo (11, 26) and inhibiting PGF2α secretion in vitro (10, 26).

Recent studies have demonstrated that both bTP-1 and its ovine equivalent, oTP-1, have a high degree of sequence homology with interferon of the α class (5, 14, 15). In particular, bTP-1 has a 70% predicted amino acid sequence identity with bovine interferon-α class...
Both bTP-1 (8) and oTP-1 (19) possess antiviral activity. In addition, binding of radiolabelled human interferon-α to membrane receptors from uteri of cyclic ewes can be inhibited by purified oTP-1 (24). Salamonsen et al. (21) have shown that PGF2α and PGE2 secretion by cultured ovine endometrial cells can be inhibited by both oTP-1 and human interferon-α.

In a previous study (18), we showed that intrauterine infusion of recombinant bovine interferon-α1, type 1 (IFN) into cyclic cows from d 15.5 to 21 after estrus delayed luteolysis. This raises the possibility that interferon treatment might be a means of decreasing embryonic mortality by supplementing bTP-1 secretion of retarded or small conceptuses with exogenous interferon. For this to be practical, delivery routes more accessible and less damaging to embryos than intrauterine infusion must be found. Accordingly, the objective of the present experiment was to test whether systemic as well as intrauterine administration would extend CL lifespan.

MATERIALS AND METHODS

Preparation of Interferon

Recombinant bovine IFN (4) was supplied as a formulated lyophilized substance. In addition, a lyophilized placebo without IFN was utilized. Both of these materials were prepared by CIBA-GEIGY, Basel, Switzerland. The stock preparation of IFN had a specific antiviral activity of 1.4 x 10^7 units/mg protein determined by viral plaque inhibition of vesicular stomatitis virus grown on Madin-Darby bovine kidney (MDBK) cells (12).

For intrauterine infusions, a vial containing 125 mg of lyophilized IFN was reconstituted with 12.5 ml of a filter-sterilized solution (.45-μm filter) of 5 mg/ml protease-free bovine serum albumin, fraction V (BSA) (Boehringer Mannheim, Indianapolis, IN). The control vial containing the placebo formulation was also reconstituted in 12.5 ml of a sterile solution of 15 mg/ml BSA. For intrauterine infusions, a vial containing 125 mg of lyophilized IFN was reconstituted with 12.5 ml of a filter-sterilized solution (.45-μm filter) of 5 mg/ml protease-free bovine serum albumin, fraction V (BSA) (Boehringer Mannheim, Indianapolis, IN). The control vial containing the placebo formulation was also reconstituted in 12.5 ml of a sterile solution of 15 mg/ml BSA. Thus, these two solutions contained a final protein concentration of 15 mg/ml (10 mg/ml IFN and 5 mg/ml BSA for IFN infusions; 15 mg/ml BSA for control infusions). Infusion solutions were loaded into 25 ml AI straws (IMV, Minneapolis, MN). For each straw, 100 μl of the respective solution were aspirated into the middle of the straw. This bolus was bracketed between air bubbles that, in turn, were bracketed between 50 μl of phosphate-buffered saline (10 mM NaPO₄, pH 7.4, containing 154 mM NaCl, 100 IU/ml penicillin, and 100 μg/ml streptomycin). Straws were plugged and kept at 4°C until used (within less than 8 d).

For intramuscular administration, sterile water was added to a vial containing 125 mg IFN to produce a final concentration of 10 mg/ml (Experiment 2) or 2.5 mg/ml (Experiment 3). The placebo solution alone (Experiment 2) or a solution of BSA dissolved in the placebo solution to a final concentration of 2.5 mg/ml (Experiment 3) was used as control injections.

Administration of Interferon

Experiment 1. Fifteen lactating dairy cows maintained in an outdoor lot were palpated rectally, and those possessing a functional CL were injected with 25 mg of Lutalyse® (Upjohn Co., Kalamazoo, MI) to regress corpora lutea. Cows were checked twice daily for estrus. The first 10 cows (6 Holstein and 4 Jersey) exhibiting estrus were assigned randomly to receive control (1.5 mg of BSA/infusion; 5 cows) or IFN solutions (1.0 mg of IFN with .5 mg of BSA/infusion; 5 cows). The control and IFN solutions were introduced in utero, twice daily (0600 h and 1800 h) from d 14 (morning) through d 21 (evening) postestrus (day of estrus = d 0). The infusion technique was used as described previously (16) except that no epidural anesthesia was given.

Experiment 2. A pool of 25 dairy Simmental heifers, 18 to 20 mo of age and weighing 350 to 420 kg, were synchronized using a prostaglandin analogue (Estrumate®; Coopers Animal Health). The first 10 heifers showing signs of estrus were selected for the study. These animals were housed in an indoor pen and fed corn silage and hay. Animals were randomly assigned to receive IFN or the placebo solution. IFN (20 mg in 2 ml) was administered intramuscularly twice daily from d 15 to 19 after estrus at 0800 and 1600 h. Control animals received an equivalent volume of placebo solution.
Experiment 3. Fifty Holstein heifers 11 to 15 mo of age, 230 to 343 kg in weight, and kept in four different outdoor pens, were rectally palpated, and those possessing a functional CL injected with 18.5 mg of Lutalyse®. Estrous behavior was checked three times daily (0700, 0100, and 1900 h) and those detected in estrus \( n = 23 \) were assigned randomly within pens to one of four doses of IFN \( (0, 2.5, 5.0, \) and \( 10.0 \) mg/injection). Animals receiving no IFN were given a control injection of 4 ml of BSA \( (2.5 \) mg/ml) dissolved in placebo solution. Injections were given intramuscularly twice daily \( (0700, 1900 \) h) from d 14 to 21 of the estrous cycle. The experiment was performed on two different occasions, using different heifers to obtain the necessary numbers of animals per group \( (n = 6, 5, 5, \) and 7 for \( 0, 2.5, 5.0, \) and \( 10.0 \) mg, respectively). Therefore, the statistical analysis included effects of period.

Detection of Estrus and Blood Sampling

In Experiments 1 and 3 (Florida), animals were observed twice daily \( (0700\) and 1900 h) for estrus starting on d 13 of the estrous cycle. Detection of estrus was facilitated by the use of estrus detectors (KaMar, Steamboat Springs, CO) in Experiment 1. In both experiments, blood samples were collected from the coccygeal vein in a vacutainer tube (Becton Dickinson and Company, Rutherford, NJ) once daily (between 0600 and 0930 h) beginning on d 14 of the estrous cycle and continuing until detection of estrus. Blood was allowed to clot at room temperature for 2 to 3 h and stored an additional 2 to 6 h at 4°C. The serum was harvested by centrifugation for 10 min at 500 \( \times \) g and was then frozen and maintained at \(-20^\circ\)C until concentration of progesterone was determined.

In Experiment 2 (Switzerland), estrous behavior was observed for at least 5 min at 0700, 1200, 1600, and 1900 h, starting on d 15 of the estrous cycle. Blood samples were taken daily \( (0730 \) h) by jugular venipuncture from d 15 to 26 after estrus into heparinized tubes. Samples collected on d 20 and 21 were inadvertently lost. Plasma was harvested by centrifugation at 2000 \( \times \) g for 10 min at 4°C and then frozen and maintained at \(-20^\circ\)C until concentration of progesterone was determined. Personnel involved in administering injections, estrus detection, and progesterone assays were unaware of the treatment administered.

Progesterone Determination

For Experiment 1 and 3, concentrations of progesterone in serum were measured in duplicate, using 100- or 200-μl of samples, in a radioimmunoassay as described by Knickerbocker and coworkers (16). The intraassay and interassay coefficients of variation for four assays were 7.0 and 9.0%, respectively. Sensitivity of the assay was 31.2 pg/tube. In Experiment 2, progesterone concentrations in plasma were measured using a commercial enzyme linked immunosorbent assay kit from Cambridge Veterinary Sciences (Cambridge, UK). Detection limit of the assay was 0.5 ng/ml (intra-assay CV = 10%).

Interferon Assay

Plasma IFN titers were assessed by a plaque-inhibition antiviral assay (12). Results were expressed as the reciprocal of the dilution of plasma causing a 50% reduction in vesicular stomatitis virus inhibition of MDBK cell growth.

Statistical Analysis

Data analyzed were interestrous intervals and duration of functional luteal lifespan (the interval between the 1st d of estrus and the day when serum progesterone concentration first fell below 1 ng/ml). For Experiments 1 and 2, data were analyzed by one-tailed Student's \( t \) test. For Experiment 3, data were analyzed by the General Linear Models procedure of SAS (22). The model considered effects of period, pen, treatment, period \( \times \) pen, period \( \times \) treatment, and pen \( \times \) treatment. Since very little variation (F value <1) was associated with pen, period \( \times \) pen, period \( \times \) treatment, and pen \( \times \) treatment, these effects were subsequently removed from the model. Differences in treatment means were evaluated by orthogonal contrasts (0 versus 2.5, 5.0, and 10.0 mg; 2.5 versus 5.0 and 10.0 mg and 5.0 versus 10.0 mg). Furthermore, cycle lengths and functional CL lifespan were categorized as extended (>24 d for interestrous intervals and >23 d for dura-
Figure 1. Effect of intrauterine and intramuscular (i.m.) administration of various concentrations of interferon (IFN) on estrous cycle lengths. Circles represent observation for individual animals and the horizontal bar represents means. Cows infused in utero with IFN (panel A) had longer (P<.02) interestrous intervals than cows that received infusion of a control BSA solution. In the first i.m. experiment (panel B), interestrous intervals were longer (P<.01) for heifers treated with 20 mg IFN twice daily than for control heifers. In the second i.m. experiment (panel C), the three IFN-treated groups had a longer interestrous interval (P<.02) than the control group.

RESULTS

Effects of Intrauterine Infusion of Interferon on Interestrous Interval and Corpus Luteum Lifespan

Experiment 1. Lactating dairy cows receiving IFN in utero had estrous cycles of 25 to 36 d (Figure 1A). All but one of five cows had cycle lengths greater than 27 d. In contrast, all the control cows returned to estrus within 24 to 26 d after estrus. Overall, the mean interestrous interval was prolonged (P<.02) in the IFN-treated group compared within that in the control group (30.4 ± 1.91 versus 24.8 ± .58 d). Interferon also extended (P<.03) the functional lifespan of the CL (28.4 ± 2.01 d for IFN-treated cows versus 23.6 ± .75 d for control cows).

Concentrations of progesterone in serum at times preceding CL regression (i.e., from d 14 to 18) were also compared between the two treatment groups to determine whether IFN affected progesterone before luteolysis. Progesterone concentration, during these 5 d did not differ (P>.10) between treatments. The least squares means of progesterone for cows receiving a control solution in utero were 7.00, 6.97, 6.89, 7.52, and 7.74 ng/ml at d 14 to 18, respectively; whereas the IFN-treated cows had least squares means of 7.83, 6.71, 8.49, 8.25,
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\text{IFN} \rightarrow \text{Vehicle (Experiment 2). Data are expressed as the reciprocal of the dilution of plasma causing a 50\% reduction in vesicular stomatitis virus inhibition of Madin-Darby bovine kidney cell growth (X ± SEM).}
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and 8.38 ng/ml. The pooled standard error of the mean was .58 ng/ml.

Effects of Intramuscular Administration of Interferon on Interestrous Interval and Corpus Luteum Lifespan

Experiments 2 and 3. In Experiment 2, control heifers returned to estrus on either d 20 or 21 after estrus. In contrast, IFN-treated heifers had extended estrous cycles: one heifer returned to estrus on d 23, two on d 24, and one each on d 25 and 27 (Figure 1B). The mean interestrous interval was longer (P<.01) for the IFN-treated group (24.6 ± 1.36 d) than for the control group (20.6 ± .49 d). Interferon also extended (P<.01) the functional lifespan of the corpus luteum (23.2 ± .37 d versus 20.2 ± .73 d). In Experiment 3, the six heifers receiving 0 mg of IFN had an interestrous interval between 20 to 24 d, whereas groups receiving 2.5, 5.0, and 10.0 mg/injection included several heifers with prolonged estrous cycles (2 of 5, 5 of 6, and 6 of 7 heifers with a cycle length greater than 24 d; Figure 1C). Mean interestrous intervals were 22.0 ± .68, 24.0 ± 1.14, 24.6 ± 1.17 and 25.4 ± .97 d, respectively, for heifers receiving 0, 2.5, 5.0, and 10.0 mg of IFN. Interferon-treated heifers had prolonged cycles (P<.02) when compared with the control group. Cycle lengths did not differ between the 2.5, 5.0, and 10 mg/injection groups. Nonetheless, the length of estrous cycle increased slightly as dose of IFN increased. When the results were analyzed as categorical data, orthogonal contrasts confirmed dose-dependent effects of IFN. The number of animals in the IFN-treated groups (2.5, 5.0, and 10 mg) showing a prolonged cycle was higher (P<.04) when compared with the control group. Groups treated with 5.0 or 10.0 mg also contained more animals with an extended cycle than the 2.5 mg group treated with (P<.10). There was no difference between groups treated with 5.0 and 10.0 mg of IFN. Means for the period of functional lifespan of the corpus luteum were 20.5 ± .56, 23.0 ± 1.26, 23.6 ± 1.17, and 24.1 ± .74, respectively, for the groups treated with 0, 2.5, 5.0, or 10.0 mg IFN. Luteal lifespan was longer (P<.01) in the IFN-treated groups as compared with that in the control group, and there were no significant differences between groups treated with 2.5, 5.0, or 10 mg. When luteal lifespan was analyzed as categorical data, the same conclusions were found as for cycle lengths, with the same probabilities.

Concentrations of serum progesterone preceding luteolysis (i.e., from d 14 to 18) did not differ (P>.10) between the four treatment groups. The least squares means of progesterone for heifers receiving no IFN were 8.65, 8.08, 8.36, 9.36, and 7.50 ng/ml at d 14, 15, 16, 17, and 18, respectively. Interferon-treated heifers had similar values of 9.37, 6.97, 7.86, 9.02, and 8.24 for the 2.5-mg IFN group; 9.34, 8.18, 9.33, 9.88, and 7.90 for the 5-mg IFN group; and 9.06, 7.08, 8.54, 10.13, and 9.75 for the 10-mg IFN group. The pooled standard error of the mean was .86 ng/ml.

Pharmacokinetics of Interferon Administration

Blood samples in Experiment 2 were evaluated for antiviral activity. Each day, blood was obtained prior to administration of 20 mg IFN intramuscularly and antiviral activity in peripheral blood was elevated from d 16 to 19 (mean titer = 3 to 3.5 log dilutions; Figure 2).

DISCUSSION

Local or systemic administration of interferon-alpha1 can prolong functional lifespan of the CL and length of the estrous cycle. Intra-

uterine infusion of IFN prolonged the interestrous interval to an average of 30.4 d, an extension similar to results for in utero infusions of proteins from conceptus-conditioned culture medium from d 15.5 through 21 after estrus (16). Cows receiving an intrauterine infusion of BSA also had slightly extended interestrous intervals, possibly due to uterine irritation (23, 27).

Systemic administration of IFN by an intramuscular route can also prolong interestrous interval and the functional lifespan of the CL. Administration of IFN via intramuscular injection extended estrous cycle length by an average of 2 to 4 d. It thus appears that IFN can be given to cattle to regulate luteal function through easily accessible systemic routes that do not involve the cumbersome techniques of intrauterine administration.

As in our previous study (18), a few animals receiving IFN returned to estrus as rapidly as animals in the control groups. The reason for this is unclear. Inadequate dosage is not a plausible explanation because this phenomenon appeared in all doses in Experiment 3. Interferon may act in part through different mechanisms than bTP-1 since in vitro culture of endometrial explants from d 17 cyclic cows with bTP-1 reduced PGF production, whereas culture with IFN did not decrease PGF2α secretion but did increase secretion of prostaglandin E2 (26). Perhaps the mechanisms that are engaged to extend CL lifespan are more affected by IFN in some animals than in others.

In cattle, early embryonic mortality represents about a 30% decrease in the overall pregnancy rate in normal cows before d 35 to 42 (1). The period from d 16 to 21 of gestation may be critical for embryonic survival as the conceptus must be present to prevent luteolysis (3, 13, 17) by secreting bTP-1 (10, 26). A d-16 embryo measuring at least 25 mm can secrete detectable amounts of bTP-1, and secretion increases with conceptus development (6). An optimal bTP-1 concentration may not be reached when the embryo is retarded or in asynchrony with the endometrium. Embryonic secretion of bTP-1 was reduced in embryos that are heat-stressed in vitro (20), and such a condition in vivo would decrease embryonic survival. Thus, the application of exogenous bTP-1 or a closely related molecule such as recombinant bovine interferon-α1 may be beneficial for improving the survival of embryos secreting too little bTP-1 at the critical period for maintenance of the CL. The hypothesis that maintaining CL function pharmacologically during this critical period can be used to increase fertility is supported by data of Thatcher et al. (25), who administered human choric gonadotropin at d 15 of gestation to artificially maintain CL function; subsequent fertility rate was improved by approximately 7%. Therefore, systemic administration of IFN also has the potential of increasing fertility through its actions on blocking luteolysis.

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