ABSTRACT

Two experiments tested the effect of recombinant ovine and bovine interferon-\(\tau\) on corpus luteum lifespan, intersternal interval, and oxytocin-induced uterine secretion of prostaglandin F\(_{2\alpha}\). Cows received intrauterine injections of 100 \(\mu\)g of recombinant ovine interferon-\(\tau\) plus 1.4 mg of BSA or of 1.5 mg of BSA alone in Experiment 1 and 200 \(\mu\)g of recombinant bovine interferon-\(\tau\) plus 1.3 mg of BSA or 1.5 mg of BSA alone in Experiment 2. Twice daily injections (0700 and 1900 h) were split evenly between the uterine horns from d 14 to 24 of the experimental estrous cycle via an AI pipette in Experiment 1 and via intrauterine catheters in Experiment 2. On d 17, cows were injected with 100 IU of oxytocin, and plasma was collected for analysis of 13,14-dihydro-15-keto-prostaglandin F\(_{2\alpha}\). Recombinant ovine interferon-\(\tau\) extended the lifespan of the corpus luteum (27.5 vs. 19.2 d) and intersternal interval (30.5 vs. 20.6 d) and abolished the oxytocin-induced increase in 13,14-dihydro-15-keto-prostaglandin F\(_{2\alpha}\), which peaked at 30 min for the BSA control group (210.8 pg/ml). Recombinant bovine interferon-\(\tau\) also extended the lifespan of the corpus luteum (29.0 vs. 21.4 d) and intersternal interval (31.5 vs. 22.6 d) and abolished the oxytocin-induced increase in 13,14-dihydro-15-keto-prostaglandin F\(_{2\alpha}\), which peaked at 30 min for the BSA control group (205.6 pg/ml). In conclusion, recombinant ovine interferon-\(\tau\) and recombinant bovine interferon-\(\tau\) were effective antiluteolytic agents in cattle.

(Key words: bovine, corpus luteum, interferon, progesterone)

Abbreviation key: b = bovine, CL = corpus luteum, IFN = interferon, o = ovine, r = recombinant, PGFM = 13,14-dihydro-15-keto-prostaglandin F\(_{2\alpha}\).
bIFN-α (29). Recombinant (r) bIFN-α extends CL lifespan in cyclic cows (25, 26). However, i.m. injection of rbIFN-α causes an increase in body temperature, which is associated temporally with a decline in plasma progesterone and LH (1, 26). Moreover, i.m. injections of bIFN-α decreased conception rate in heifers, even when the treatment regimen was designed to reduce hyperthermia (2). It is important to determine whether bIFN-τ and the highly homologous ovine (o) IFN-τ (29) have side effects comparable with those of bIFN-α, which may limit their usefulness as possible fertility-enhancing agents for cows.

Ovine IFN-τ may be useful for cattle because its deduced amino acid sequence has about 80% homology with bIFN-τ (29). Natural and recombinant oIFN-τ extend CL lifespan in ewes (22). In addition, Heyman et al. (12) demonstrated that trophoblastic vesicles from ewes extended CL lifespan in cows when transferred in utero. Thus, roIFN-τ may extend CL lifespan in cows, lending support to the hypothesis that IFN-τ is the conceptus-derived protein responsible for maternal recognition of pregnancy in ruminants.

Purification of significant amounts of bIFN-τ requires a large number of conceptuses, making bIFN-τ impractical for studies with large numbers of cows. Because of this, to date, only one limited study has been conducted to investigate the effect of IFN-τ on CL lifespan and uterine PGF2α secretion of cows (11). This study used natural bIFN-τ, which is a glycosylated protein purified from conceptuses (10). Recombinant forms of bIFN-τ (14) and oIFN-τ (23), which are not glycosylated, have been expressed in quantities that allow a more extensive investigation of their role in regulating uterine secretion of PGF2α and its potential role as a fertility-enhancing molecule. These recombinant interferons have been shown to elicit some biological responses that are indistinguishable from IFN-τ purified from conceptuses (4, 14, 22, 23). Recombinant forms of bIFN-τ and oIFN-τ, however, may possibly have effects that are different from those of natural bIFN-τ on reproductive responses of cattle. In the present study, two experiments were conducted to determine whether intratrine injection of roIFN-τ and rbIFN-τ would extend CL lifespan and interestrous interval and whether this effect was associated with attenuated oxytocin-induced uterine secretion of PGF2α.

MATERIALS AND METHODS

Experiment 1

Cows. Thirty nonlactating Holstein cows were palpated per rectum for ovarian activity and for uterine and cervical condition. Cows that were considered to be reproductively sound received a subcutaneous ear implant of Synchromate-B® (Sanofi, Inc., Overland Park, KS). Lutalyse® (25 mg; Upjohn Co., Kalamazoo, MI) was administered i.m. 7 d later. Synchromate-B® implants were removed 2 d after the Lutalyse® injection. Cows were checked twice daily for estrus (0700 and 1900 h) for 30 min from 24 to 96 h after implant removal. Blue paint (Impervo; Benjamin Moore Company, Jacksonville, FL) and yellow crayon marker (Paint Stick; La-Co Industries, Chicago, IL) were applied to the tailhead to facilitate detection of estrus. Cows exhibiting estrus within 2 d of implant removal were allowed to progress through one estrous cycle and, upon return to estrus, were assigned randomly to receive BSA (n = 5) or roIFN-τ (n = 6).

Cows were administered IFN-τ or BSA into the uterus to mimic the secretion of bIFN-τ by the conceptus. Cows were administered intratrine injections of roIFN-τ or BSA by AI pipette twice daily (0700 and 1900 h) via the cervix from d 14 to 24 of the estrous cycle. Contents of the AI straws were allowed to thaw at room temperature (22°C) before being loaded into the AI pipette. A protective sheath was placed over the AI pipette and the insemination straw to protect against contamination within the vagina. The pipette tip was passed to the external cervical os. The pipette gun and straw were then pushed through the end of the protective sheath and passed into the cervix. One-half of the contents of the AI straw was allowed to thaw at room temperature (22°C) before being loaded into the AI pipette. A protective sheath was placed over the AI pipette and the insemination straw to protect against contamination within the vagina. The pipette tip was passed to the external cervical os. The pipette gun and straw were then pushed through the end of the protective sheath and passed into the cervix. One-half of the contents of the AI straw was placed into the lower one-third of each uterine horn, using a mark on the AI pipette as a guide. Cows were maintained on pasture and fed peanut hay and corn silage throughout the experiment.

Blood was collected via jugular venipuncture on alternate days from d 0 to 13 and daily thereafter until the cow returned to estrus.
Plasma was collected after centrifugation (20 min at 1200 × g at 4°C) and frozen until assayed for progesterone.

On d 17, all cows were fitted with indwelling jugular catheters (Angiocath®, 2.1 mm o.d., 1.3 mm i.d.; Becton Dickinson, Rutherford, NJ). Blood samples (10 ml) were collected into heparinized tubes every 15 min for 2 h prior to injection of oxytocin and at 5 min, 15 min, and subsequent 15-min intervals thereafter for 4 h after injection of oxytocin (100 IU, i.v.). Samples were kept on ice until centrifuged (20 min at 1200 × g at 4°C) within 30 min of collection. Plasma was frozen (-20°C) until assayed for 13,14-dihydro-15-keto PGF2α (PGFM).

Rectal temperatures were taken on the first 2 d of treatment at 0700 h just prior to infusion, and at 1400 h because i.m. administration of rbIFN-α to cows caused a peak pyrogenic response at approximately 6 h after administration (18).

Cows were observed for estrus twice daily (0800 and 2000 h) from d 14 until the last cow came into estrus.

Preparation of Materials for Intrauterine Infusion. Solutions for intrauterine infusion were prepared in 20 mM Tris·HCl and 150 mM NaCl buffer, pH 8.0, containing 100 U/ml of penicillin and 100 μg/ml of streptomycin (Sigma Chemical Co., St. Louis, MO) and sterilized by using a .45-μm filter. Bovine serum albumin (radioimmunoassay grade, fraction V; United States Biochemical Corp., Cleveland, OH) was prepared at concentrations of 1.5 mg/.8 ml of buffer. Recombinant rtIFN-τ (.1 mg; (23)) and BSA (1.4 mg) were prepared in .4 ml of buffer. Bovine serum albumin was added such that an equal mass of protein was infused into the uterus of each cow to reduce proteolysis of rtIFN-τ by endogenous proteases.

Individual doses of rtIFN-τ plus BSA and BSA alone (Experiment 1) were aspirated under a laminar flow hood into .5 ml A1 straws between air bubbles, sealed, and stored at -20°C until intrauterine infusion.

Experiment 2

Cows. Cows in Experiment 2 were managed according to the protocol of Experiment 1. Cows were assigned randomly to receive BSA (n = 5) or rbIFN-τ (n = 6) during the estrous cycle immediately following the synchronized estrus. Both IFN-τ and BSA were administered via intrauterine catheters to reduce the induction of pyometra. Cows underwent midventral laparotomy and were fitted with a sterile intrauterine catheter (Tygon, .51 mm i.d., 1.53 mm o.d., .51 mm wall; Norton Performance Plastics, Akron, OH) that was inserted into each uterine horn on d 9 or 10 of the experimental cycle and fixed in place as described by Knickerbocker et al. (15). Biotal® (Boehringer-Engelheim, St. Joseph, MO) was used to induce anesthesia, which was maintained by Halothane (Halocarbon Laboratories, River Edge, NJ) during surgery. Catheters were inserted approximately 45 mm into the lumen of the anterior end of each uterine horn and secured by suture and a polyvinyl cuff (V/10; Bolab Inc., Lake Havasu City, AZ). Catheters were exteriorized via a small incision in the left flank, placed in a plastic bag wrapped in tape (Zonas; Johnson & Johnson Products, Inc., New Brunswick, NJ), and sutured to the skin. Sterile 22-gauge blunt needles were inserted into the exterior end of the catheters and sealed with injection caps (Becton Dickinson) to facilitate ease of injection and maintain a sterile environment inside the catheter. The catheters were stored in iodine-soaked gauze sponges inside the bags. Antibiotics (Polyflex®, 167 mg/ml; Bristol Laboratories, Syracuse, NY) were administered (18 ml) following surgery, and catheters were flushed with a solution of infusion buffer containing antibiotics to check for patency. Intrauterine injections, split evenly between the two uterine horns, were administered through intrauterine catheters at 0700 and 1900 h (1.5 mg of total protein per infusion) from d 14 to 24 of the estrous cycle.

Blood samples, rectal temperatures, and oxytocin challenge on d 17 were conducted according to the protocols in Experiment 1. Cows were observed for estrous behavior from d 14 to 35 of the experimental estrous cycle.

Preparation of Materials for Intrauterine Infusion. Solutions for intrauterine infusion were prepared in the same buffer as in Experiment 1. Recombinant rtIFN-τ (.2 mg; (14)) and BSA (.3 mg) were prepared in .8 ml of buffer. Individual
doses of rbIFN-τ plus BSA and BSA alone were divided into aliquots into sterile 1.0-ml polypropylene tubes under a laminar flow hood and stored at −20°C until used.

The 100 μg of roIFN-τ per infusion in Experiment 1 (100 μg/infusion) and 200 μg of rbIFN-τ per infusion in Experiment 2 (200 μg/infusion) were based on differences in antiviral activity in a plaque inhibition assay in which Madin-Darby bovine kidney cells were challenged with vesicular stomatitis virus (28). Specific antiviral activity of the preparations were 112 I.U. for roIFN-τ and 23 I.U. for rbIFN-τ. Thus, the amount of rbIFN-τ infused was increased to 200 μg per infusion because the antiviral activity was 85% less than the roIFN-τ used in Experiment 1, and insufficient amounts of rbIFN-τ were available to apply equal amounts of antiviral activity (e.g., 487 μg of rbIFN-τ per infusion).

Radioimmunoassay of Plasma Hormones

Progesterone concentrations in heparinized plasma samples were measured by radioimmunoassay (15). A 2:1 ratio of hexane to benzene was used to extract 100 or 200 μl of plasma. A 1:30,000 dilution of antisera achieved an assay sensitivity of 31.2 pg per tube. Intraassay and interassay coefficients of variation were 8.2 and 5.3%, respectively.

The PGFM concentrations in plasma (200 μl) were measured as described by Guilbault et al. (8). At an antisemil dilution of 1:6000, sensitivity of the assay was 5 pg per tube or 25 pg/ml of plasma. Intraassay and interassay coefficients of variation were 9.5 and 13.7%, respectively. Some plasma samples with low concentrations of PGFM were reanalyzed using a more sensitive assay, validated in our laboratory with antisera to PGFM developed by immunizing rabbits against PGFM conjugated to keyhole limpet hemocyanin. Plasma samples (200 μl) were measured in duplicate without extraction. Prostaglandin-free plasma was obtained from a cow at d 38 postpartum that had been injected twice (1 g/Lm injection) at a 12-h interval with the prostaglandin synthetase inhibitor, flunixin meglumine (Banimine 50 mg/ml; Schering Corp., Kenilworth, NJ). The cow was bled 4 h after the second injection. The prostaglandin-free plasma had undetectable concentrations of PGFM when assayed utilizing standard curves in 0.5 M Tris-HCl buffer, pH 7.5. The PGFM standard solutions were made by serial dilutions in buffer of a stock solution (10 μg/ml in Tris-HCl buffer) of authentic PGFM. Final PGFM standard concentrations were 0, 1.5, 3, 5, 10, 25, 50, 100, 250, 500, and 1000 pg/100 μl in duplicate. Each tube contained 200 μl of prostaglandin-free plasma and 100 μl of a PGFM standard solution. Experimental plasma samples (200 μl) were added to 100 μl of buffer. A 100-μl aliquot of human gamma globulins (Sigma Chemical Co.) was added to each tube and incubated at 22°C for 15 min. A 100-μl aliquot of 1:20,000 rabbit antiserum to PGFM was added to each tube and incubated at 22°C for 30 min. Approximately 18,000 dpm of [5, 6, 8, 9, 11, 12, 14-3H]PGFM (178 Ci/mM; Amersham Corp., Arlington Heights, IL) in 100 μl of buffer was added to give a final assay volume of 600 μl. Tubes were incubated for 1 h at 22°C and then 12 h at 4°C. Separation of free and bound PGFM was accomplished by precipitation of proteins (plasma protein, human gamma globulins, and rabbit anti-PGFM) with 750 μl of a cold 40% solution of polyethylene glycol-8000 in distilled water. Following centrifugation at 3000 × g for 30 min at 4°C, the supernatant was discarded, and the pellet was resuspended in 750 μl of buffer and 750 μl of polyethylene glycol. A second centrifugation was performed as before, and the pellets were resuspended in 1 ml of buffer and then transferred to a scintillation vial. Scintiverse-BioHP (4 ml; Fisher Scientific, Fair Lawn, NJ) was added to each scintillation vial.

Accuracy of the assay procedure was determined by measuring PGFM previously added to prostaglandin-free plasma (10, 50, 100 pg/ml). Recoveries (least squares means ± SEM) were 9.19 ± 1.65, 56.29 ± 1.65, and 112.35 ± 1.65 pg/ml, respectively. Recovery of added (x) versus measured (Y) PGFM concentration was described by linear regression (Y = −1.79 + 1.14x; R² = .99). Parallelism was shown by comparing 50, 100, and 200 μl of plasma containing 1000 pg/ml to the PGFM standard curve, giving regression equations of Y = 2.33 − 2.14x and Y = 2.67 − 2.37x, respectively. Sensitivity of the assay was established at 7.5 pg/ml. Crossreactivity of the antibody with arachidonic acid, PGG2α, PGF1α, and prostaglandin E2 was <1%.
INTERFERON-τ ALTERS CORPUS LUTEUM LIFESPAN

Statistical Analysis

Changes in concentrations of progesterone and PGFM in plasma were analyzed by using the general linear models procedure of SAS (31) for a split-plot analysis of variance with repeated measurements over time. The mathematical model included variability because of treatment (roIFN-τ vs. BSA in Experiment 1; rbIFN-τ vs. BSA in Experiment 2), cow nested within treatment, time, treatment by time interaction, and residual error.

RESULTS

Effects of Intrauterine Injections on Estrous Cycle Responses

Experiment 1. Two cows from the roIFN-τ treatment group were diagnosed with pyometra on d 23 and 24 of the experimental treatment cycle and were not included in the analyses of CL lifespan and interestrous interval (Table 1). The CL lifespan was defined as the number of days from estrus (d = 0) to completion of CL regression (plasma progesterone ≤1.0 ng/ml). Treatment with roIFN-τ extended CL lifespan (P < .01; Table 1 and Figure 1) and interestrous interval (P < .01; Table 1) for cyclic cows compared with those of controls. The control and treated cows had similar interestrous intervals in the cycle preceding injections. Regression analysis of progesterone profiles indicated that progesterone response curves differed (Figure 1a) between roIFN-τ and BSA treatment groups (P < .01). The CL lifespans ranged from 24 to 29 d for cows treated with roIFN-τ, but cows treated with BSA had all completed luteolysis by d 22 of the experimental cycle.

Experiment 2. Cows received intrauterine injections immediately following synchronization of estrus because maintaining intrauterine catheters for an extended period over two estrous cycles was not desirable. Intrauterine catheters were used in Experiment 2 to prevent the incidence of pyometra that occurred in Experiment 1. No cows developed pyometra in Experiment 2. Based on the same criterion as in Experiment 1, cows treated with rbIFN-τ had longer CL lifespans (P < .05; Table 1, Figure 1b) and interestrous intervals than did controls (P < .05; Table 1). Regression analysis of progesterone profiles indicated that progesterone response curves differed (Figure 1b) between treatments (P < .01). Corpus luteum lifespans ranged from 27 to 35 d in cows treated with rbIFN-τ, except for one cow in the group with an 18-d CL lifespan (Figure 2d). Two cows in the rbIFN-τ treatment group had not undergone complete luteolysis by d 34 of the estrous cycle. Examinations per rectum of both cows revealed no evidence of pyometra. In contrast, cows treated with BSA completed luteolysis by d 25 (Figure 2c).

Profiles of PGF2α

Experiment 1. Plasma samples collected from jugular blood were assayed for PGFM by radioimmunoassay as an indicator of oxytocin-induced PGF2α secretion from the uterus at d 17 (15, 33). The two cows that developed

Figure 1. Mean (± SEM) plasma progesterone concentrations of cows infused with a) recombinant ovine interferon-τ (●) or BSA (○) and b) recombinant bovine interferon-τ (__) or BSA (○) from d 14 to 24 of the estrous cycle.

Journal of Dairy Science Vol. 78, No. 9, 1995
TABLE 1. Corpus luteum (CL) lifespan and interestrous interval for recombinant ovine interferon-τ (roIFN-τ), recombinant bovine interferon-τ (rbIFN-τ), and control groups.

<table>
<thead>
<tr>
<th>Response</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows, no.</td>
<td>Control roIFN-τ</td>
<td>Control rbIFN-τ</td>
</tr>
<tr>
<td></td>
<td>X  SEM</td>
<td>X  SEM</td>
</tr>
<tr>
<td>Interestrous interval, d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preexperimental cycle</td>
<td>21.4 .8</td>
<td>21.3 .9**</td>
</tr>
<tr>
<td>CL Lifespan, d</td>
<td>19.2 1.1</td>
<td>27.5 1.2</td>
</tr>
</tbody>
</table>

1Two cows diagnosed with pyometra were not included in experimental responses.
2Not measured. Cows were used in the estrous cycle following synchronization of estrus.

*p < .05.
**p < .01.

Figure 2. Individual plasma progesterone profiles for cows infused with a) BSA or b) recombinant ovine interferon-τ in Experiment 1 and c) BSA or d) recombinant bovine interferon-τ in Experiment 2 from d 14 to 24 of the estrous cycle.
pyometra were included in the PGFM analysis because the condition became evident at a later stage of the experiment (d 23 and 24). The PGFM response between treatments was significant \((P < .01)\) when the two cows with pyometra were excluded from the PGFM analysis. Mean basal concentrations of plasma PGFM, calculated from samples collected every 15 min for 2 h prior to oxytocin injection, were 37.1 ± 3.9 (SEM) pg/ml for rolIFN-\(\tau\) and 31.3 ± 4.2 pg/ml for BSA. Cows receiving BSA injections had an acute rise in plasma PGFM concentrations to approximately 210 pg/ml at 30 min after oxytocin injection (Figure 3a). Treatment with rolIFN-\(\tau\) abolished oxytocin-induced increase of plasma PGFM at 30 min (rolIFN-\(\tau\): 34.4 ± 17.0 vs. BSA: 210.8 ± 18.6 pg/ml; \(P < .01\)). A treatment by time interaction \((P < .01)\) indicated that the peak PGFM response, which occurred at 30 min in the BSA-treated cows, was attenuated in the cows treated with rolIFN-\(\tau\). Individual profiles for plasma PGFM of cows treated with rolIFN-\(\tau\) showed little response during the first 30 min to oxytocin injections with concentrations of PGFM not exceeding 100 pg/ml (Figure 4b). In contrast, BSA-treated cows had concentrations of PGFM that peaked over 100 pg/ml in all five cows (Figure 4a).

Experiment 2. One cow in the rbIFN-\(\tau\) treatment group had undergone luteal regression by d 18 and was not included in the PGFM analysis. For this cow, plasma PGFM concentrations were nearly 200 pg/ml 2 h prior to and almost 800 pg/ml 45 min after oxytocin injection. Basal concentrations of PGFM were 37.6 ± 4.4 pg/ml with rbIFN-\(\tau\) and 29.2 ± 4.4 pg/ml with BSA. Treatment with rbIFN-\(\tau\) abolished oxytocin-induced increase of plasma PGFM at 30 min (rbIFN-\(\tau\): 38.5 ± 37.9 pg/ml for rbIFN-\(\tau\) vs. 205.6 ± 37.9 pg/ml for BSA; \(P < .01\); Figure 3b). Variances differed between treatments for plasma PGFM concentrations \((P < .01)\). Plasma PGFM concentrations were reanalyzed as a reciprocal of the plasma PGFM concentration, and a treatment by time interaction was detected \((P < .01)\).

Individual plasma PGFM profiles showed little response (not exceeding 100 pg/ml) to oxytocin injection during the first 30 min in cows treated with rbIFN-\(\tau\) with concentrations of PGFM (Figure 4d). In contrast, cows treated with BSA had concentrations of PGFM that peaked over 100 pg/ml for three of the five cows (Figure 4c).
DISCUSSION

The experiments in this study were not designed to test for differences in efficacy between rolIFN-τ and rbIFN-τ but rather to examine the effects of IFN on CL lifespan, the interestrous interval, and the oxytocin-induced secretion of uterine PGF$_{2α}$. Intrauterine injection of 100 μg of rolIFN-τ or 200 μg of rbIFN-τ twice daily from d 14 to 24 of the estrous cycle extended CL lifespan and interestrous intervals and attenuated oxytocin-induced secretion of uterine PGF$_{2α}$. Intrauterine injection of BSA had no effect on interestrous interval when preexperimental and experimental cycles of Experiment 1 were compared. Intrauterine injections of 35 μg of natural bIFN-τ twice daily from d 15 to 21 of the estrous cycle extended CL lifespan and reduced PGF$_{2α}$ in cows (11). These studies support the hypothesis that the bIFN-τ secreted by the conceptus extends CL lifespan through an antiluteolytic mechanism.

Luteolytic pulses of PGF$_{2α}$ did not occur in pregnant cows (24), and oxytocin-induced secretion of PGFM was reduced in pregnant cows (33). Oxytocin-induced increases in plasma concentrations of PGFM were abolished by IFN-τ, thus supporting IFN-τ as the agent responsible for eliminating luteolytic pulses of PGF$_{2α}$ in early pregnancy. It is hypothesized that the antiluteolytic effect of IFN-τ occurs through induction of an endometrial prostaglandin synthesis inhibitor in the bovine endometrium (3, 7) or that an inhibition in the induction of endometrial oxytocin receptors occurs in cyclic cows (13).

Two cows from the BSA-treated group in Experiment 2 did not respond to oxytocin on d 17 of the estrous cycle with increased concentrations of PGFM. One cow had a CL lifespan of 25 d and possibly had not yet developed the ability to respond to oxytocin. The other cow had a CL lifespan of 21 d. Why this cow did not respond to oxytocin is not known. One cow in the rbIFN-τ treatment group had a CL lifespan of only 18 d, which is not surprising, because previous studies of cows treated with rbIFN-α or rbIFN-τ also showed that a small percentage of cows did not respond to treatment with extended CL lifespans (2, 11, 25, 26). Lafrance et al. (16) described an abnormal heifer that was not able to maintain pregnancy even after receiving normal embryos from donor cows. This heifer was challenged with oxytocin on d 17 of one estrous cycle and on d 17 to 19 of seven consecutive cycles after being inseminated, but an attenuated PGFM response was not detected even when the heifer was pregnant. Embryos recovered from the heifer and transferred to normal heifers exerted an antiluteolytic effect, i.e., attenuated PGFM response to oxytocin on d 17 to 19. These observations indicate the possibility of an abnormal uterine environment in the abnormal heifer. This study did not determine whether the uterus was responsive to bIFN-τ secreted by the conceptuses or whether the uterine environment in this heifer was not able to support normal conceptus development. However, results from this study and from experiments using bIFN-τ (11) or rbIFN-α (2, 25, 26) suggest that a subpopulation of cows may be unable to respond to the antiluteolytic effects of bIFN-τ, perhaps because of a deficiency in receptors or postreceptor mechanisms.

| TABLE 2. Least squares means for rectal temperature on d 14 and 15 immediately prior to (0700 h) and 6 h after (1300 h) injections of BSA, recombinant bovine interferon-τ (rolIFN-τ), and recombinant bovine interferon-τ (rbIFN-τ). |
|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Treatment                      | 0700 h | 1300 h | 0700 h | 1300 h | 0700 h | 1300 h |
|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                 |       |       |       |       |       |       |       |       |
| **Experiment 1**               |       |       |       |       |       |       |       |       |
| Control                        | 38.3 ± .1 | 38.6 ± .1 | 38.1 ± .1 | 38.7 ± .1 |       |       |       |       |
| rolIFN-τ*                      | 38.3 ± .1 | 38.9 ± .1 | 38.4 ± .1 | 38.7 ± .1 |       |       |       |       |
| **Experiment 2**               |       |       |       |       |       |       |       |       |
| Control                        | 38.7 ± .1 | 39.3 ± .1 | 38.7 ± .1 | 39.0 ± .1 |       |       |       |       |
| rbIFN-τ                        | 38.6 ± .1 | 39.2 ± .1 | 38.5 ± .1 | 39.0 ± .1 |       |       |       |       |

*P < .05; treatment by day by time interaction in Experiment 1.

Journal of Dairy Science Vol. 78, No. 9, 1995
Rectal temperatures were recorded on the first 2 d of infusions in both experiments to observe whether either roIFN-τ or rbIFN-τ caused a hyperthermic response similar to that caused by i.m. (1, 2, 26) or intrauterine (26) administration of rbIFN-α. Although rbIFN-α extended CL lifespan (25, 26), the hyperthermic response caused by rbIFN-α is a concern because i.m. injection of rbIFN-α decreased pregnancy rate in heifers (2). Neither roIFN-τ nor rbIFN-τ caused a hyperthermic response when administered into the uterus in this study. Also, a low dose of 1 mg of rbIFN-τ, injected i.m. in cows, did not cause a hyperthermic response (17). Uterine infusion of 125I-labeled oIFN-τ into nonpregnant ewes showed that very little intact protein passed into the maternal circulation (6), explaining why intrauterine injections of rbIFN-τ and roIFN-τ may not have a pyrogenic effect. Prostaglandins appear to play a role in the hyperthermic response caused by IFN, because blocking of prostaglandin synthesis during the administration of rbIFN-α drastically reduced the hyperthermic temperature response (1).

Bovine IFN-τ mRNA was detected in bovine embryos by d 12 of pregnancy; however, maximal gene expression and secretion of blFN-τ occurred around d 15 to 16 (29). The critical time for the presence of a conceptus (embryo plus extraembryonic membranes) to exert its antiluteolytic effect is d 16 (21). Therefore, administration of roIFN-τ or rbIFN-τ was initiated on d 14 to allow IFN-τ to attenuate PGF2α secretion and maintain the CL. Injections of IFN were continued through d 24 to ensure that experimental differences in CL lifespans and interestrous intervals could be detected.

Two cows from the roIFN-τ treatment group developed pyometra during the latter stages of the experiment (d 23 and 24), probably resulting from contamination introduced into the uterus during twice daily IFN-τ injections via passage of the insemination pipette through the vagina and cervix. Both oIFN-τ
and bIFN-τ inhibited proliferation of lymphocytes (5, 19, 20, 30, 32) and other cell types (27). An immunosuppressive effect of roIFN-τ in the uterus might have contributed to the induction of pyometra in these cows. Cows in Experiment 2 were fitted with indwelling uterine catheters to avoid contamination of the uterus, and none developed pyometra.

Recombinant oIFN-τ (23) and rbIFN-τ (14) are not glycosylated, yet both molecules extended CL lifespan and interestrous interval and abolished oxytocin-induced increase in plasma concentrations of PGFM. These results indicate that glycosylation of bIFN-τ is not essential for its antiluteolytic activity. Because glycosylation does not appear to be necessary to extend CL lifespan in cattle, roIFN-τ may be used to study mechanisms by which bIFN-τ prolongs maintenance of the CL during early pregnancy.

In summary, rbIFN-τ and roIFN-τ were effective in extending CL lifespan and interestrous interval and eliminating oxytocin-induced secretion of PGF2α. A small percentage of cows might not be able to respond to bIFN-τ, but IFN-τ shows promise as a means to extend fertility in cows by allowing conceptuses more time to develop because of extended CL maintenance. Studies are underway to test this possibility.

ACKNOWLEDGMENTS

This is Journal Series Number R-04473 of the Florida Agricultural Experiment Station. Research was supported by USDA Grant Number 89-37240-4583 and NIH Grant HD21896. The authors acknowledge the assistance of N. Drost and E. Schmitt in animal preparation and M. E. Hissem in manuscript preparation.

REFERENCES