THE EFFECTS OF RECOMBINANT BOVINE INTERFERON-α ON FERTILITY IN EWES

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ABSTRACT

Recombinant bovine interferon-α1 (rBolFN-α) may be useful for enhancing fertility in sheep because it has extensive sequence homology with ovine trophoblast protein-1. To test the effectiveness of rBolFN-α, several experiments were performed in which bred females were given intramuscular injections of rBolFN-α around the time of maintenance of the corpus luteum. Treatment with rBolFN-α enhanced the fertility of ewes that were bred via natural service or embryo transfer of whole or demi-embryos. Interferon treatment was successful in enhancing lambing rate if injections were given twice daily from Days 11 to 18, 12 to 14, 12 to 15 or 12 to 16. Overall, the lambing rate for ewes bred via natural service was 94/126 (74.6 %) for control ewes and 101/126 (80.2 %) for rBolFN-α treated ewes. Litter size was not affected by treatment. Interferon treatment was not successful in increasing the lambing rate if given as a single injection on Day 12 or as a series of once-daily injections from Days 11 to 16. These results demonstrate that rBolFN-α can increase the lambing rate in ewes.

Key words: interferon, fertility, ewe, embryo transfer

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INTRODUCTION

Ovine trophoblast protein-1 (oTP-1) is an antiluteolytic type I interferon that is produced by the developing conceptus of sheep (1,2). Earlier studies have shown that interferon-α molecules that are related but not identical to oTP-1 can exert effects on the reproductive tract that are similar to that of oTP-1, including binding to ovine endometrial oTP-1 receptors (3,4), altering the secretion of prostaglandins and protein from the endometrium (5,6) and extending the luteal lifespan (7). Collectively, these studies suggest interferon-α may be used to reduce early embryonic mortality resulting from decreased or inappropriately-timed production of oTP-1 or bTP-1. Such a treatment might also be useful in embryo transfer, particularly for demi-embryos or frozen embryos, since associated damage to the trophoblast may make these embryos deficient in trophoblast proteins such as oTP-1. Two experiments (6,9) have indicated beneficial effects of one of these related interferons, bovine interferon-α1, on the lambing rate of ewes (8). The objectives of our current experiments were to evaluate the effects of rBoIFN-α on fertility of ewes bred via natural service or by the transfer of whole or demi-embryos.

MATERIALS AND METHODS

Materials

The interferon used in these studies, recombinant bovine interferon-α1 (rBoIFN-α) was produced in E. coli using recombinant DNA technology (10), purified to homogeneity as determined by SDS-PAGE and formulated as a lyophilized dry substance. Prior to use, rBoIFN-α was dissolved in carrier solution (a buffered mannitol). This same carrier solution was used as a placebo. The levels of endotoxin in stock preparations were similar to that found in placebos and were always less than 4 units per milligram as determined by the Limulus test (11). Other products were Lutalyse (Upjohn, Kalamazoo, MI); Chronogest intravagal sponges (polyurethane sponges containing 40 mg flugestone acetate) and pregnant mare’s serum gonadotropin (PMSG) (Chrono-Gest Intervert International, Boxmeer, The Netherlands); and follicle stimulating hormone-P (FSH-P) (Schering Corporation, Kenilworth, NJ).

Fertility in Ewes Bred Via Natural Service

To synchronize estrus, crossbred Swiss White Alpine X Ile de France ewes were treated with Chronogest intravaginal sponges for 14 days. At the time of removal, 400 IU PMSG was injected intramuscularly. Mating took place 48 to 60 hours later by individually exposing ewes to rams (one ram/eight ewes). The ewes were assigned randomly to receive treatment of either a placebo or rBoIFN-α. In Experiment 1, ewes received a single injection of 5 mg rBoIFN-α or an equivalent volume of placebo (2 ml) on Day 12 after mating (day of mating = Day 0). In Experiment 2, ewes received once-daily injections of 2 mg, i.m., of rBoIFN-α or
similar volume (1 ml) of placebo from Days 11 to 16 after mating. In Experiment 3, ewes received twice-daily injections of either 2 mg rBolFN-α, i.m., or a similar volume (1 ml) of placebo from Days 12 to 14 after mating. Experiments 4 and 5 were performed similarly except that treatments were given on Days 12 to 15 (Experiment 4) or Days 12 to 16 (Experiment 5). The number of lambs born was determined at parturition.

Fertility after Embryo Transfer

Mature Suffolk and Dorset ewes served as superovulated donors. Beginning on Day 12 of a natural estrous cycle (estrus = Day 0) or on Day 12 after receiving a Chronogest intravaginal pessary, ewes began superovulatory treatments with FSH-P. Injections of FSH-P consisted of a 5 mg dose of FSH-P on the morning of Day 12, a 2.5 mg injection on the afternoon of Day 12 and 2.5 mg injections on the morning and afternoon of Days 13 and 14. Ewes were injected with 5 mg of Lutalyse on the morning and afternoon of Day 14. At estrus, donors were artificially inseminated with semen from sires of their respective breeds, and embryos were collected surgically on Day 7 after estrus. A portion of the embryos were split into demi-embryos as described previously (12). Estrus was synchronized in recipient ewes by inserting Chronogest pessaries for 12 or 13 days and injecting 10 mg Lutalyse, i.m., on the day of pessary removal. Each recipient received one (one ewe), two (two ewes), three (four ewes) or four (31 ewes) embryos on Day 7, with all embryos being placed in a uterine horn on the side of a corpus luteum. An effort was made to balance the number of Suffolk and Dorset embryos placed in each recipient. The recipient ewes received twice-daily injections of 2 mg rBolFN-α, i.m., or a similar volume (1 ml) of placebo twice daily from Days 11 to 18 after estrus. The number of offspring was recorded at birth.

Statistical Analysis

Data on the lambing rate were analyzed several ways. Data from experiments in which rBolFN-α was given once-daily (Experiments 1 and 2) were analyzed by least-squares analysis of variance using the General Linear Models program of the Statistical Analysis System (13). Model components were the experiment, interferon and interferon X experiment. Additionally, effects of interferon treatment on the lambing rate were tested using the Mantel-Haenszel-Peto procedure described by Yusuf et al. (14). For the Mantel-Haenszel-Peto procedure, hypothesis testing was based on one-tailed tests of significance. Data from experiments in which rBolFN-α was given twice-daily (Experiments 3 to 6) were analyzed as in Experiments 1 and 2. Data for whole embryos in Experiment 6 were considered separately than data collected for demi-embryos in Experiment 6.

Data from Experiment 6 were also analyzed separately from the data of other experiments. Data on the lambing rate were analyzed by the Mantel-Haenszel-Peto procedure (14) and by least-squares analysis of variance. For analysis of variance, the model components were type of embryo (whole vs demi-embryo), interferon
treatment, and type of embryo X interferon. Additionally, the embryonic survival rate (number of lambs born/number of embryos transferred) was analyzed by the Mantel-Haenszel-Peto procedure and by least-squares analysis of variance using the embryo as the experimental unit and the model components of the type of embryo, interferon, type of embryo X interferon and ewe(type of embryo X interferon). Probability levels were calculated two ways by assuming the ewe was both a fixed effect and a random effect. Other model components were always considered as fixed effects.

The effect of interferon on the number of ewes born per ewe lambing was analyzed by Chi-square analysis for pooled data from Experiments 1 and 2 and separately for pooled data from Experiments 3 to 5.

RESULTS

Results of experiments in which rBolFN-α was administered once at Day 12 after estrus or once-daily from Days 11 to 16 are shown in Table 1. Neither treatment enhanced the pregnancy rate or affected litter size of the lambing ewes.

Table 1. Effect of once-daily administration of bovine interferon-α1 on pregnancy rate of ewes

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambing Rate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1 (5 mg, Day 12)</td>
<td>38/44 (86.4 %)</td>
<td>35/42 (83.3 %)</td>
</tr>
<tr>
<td>Experiment 2 (2 mg, Days 11 to 16)</td>
<td>44/56 (78.6 %)</td>
<td>39/55 (70.9 %)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>82/100 (82.0 %)</td>
<td>74/97 (76.3 %)</td>
</tr>
<tr>
<td><strong>Litter Size</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1 (5 mg, Day 12)</td>
<td>2.0 ± 0.10</td>
<td>1.9 ± 0.12</td>
</tr>
<tr>
<td>Experiment 2 (2 mg, Days 11 to 16)</td>
<td>2.3 ± 0.16</td>
<td>1.8 ± 0.14</td>
</tr>
</tbody>
</table>

a Number of ewes lambing/number of ewes mated (% lambing). According to analysis of variance, lambing rate was affected by experiment (P=0.085) but not by interferon or the interferon X experiment interaction. Similarly, interferon had no effect when determined by the Mantel-Haenszel-Peto test.

b Number of lambs born/ewe lambing (mean ± SEM). The overall number of lambs born per ewe lambing was 175/82 (2.1 lambs/ewe) for ewes given a placebo and 138/74 (1.9 lambs/ewe) for ewes treated with interferon. The number of lambs per ewe was not affected by interferon treatment.

In contrast, twice-daily administration of rBolFN-α caused an increase (P=0.10 by analysis of variance and P<0.08 by Mantel-Haenszel-Peto test) in lambing rate but had no effect on number of lambs born per ewe lambing (Table 2). Though
the interaction was not significant, the beneficial effect of rBoIFN-α on lambing rate was more pronounced in experiments in which rBoIFN-α was given for extended periods (Table 2). Thus, treatment with rBoIFN-α from Days 12 to 14 increased lambing rate by 3.8 %, treatment from Days 12 to 15 increased lambing rate by 5.3 % and treatment from Days 12 to 16 increased lambing rate by 7.3 %. The relationship between days of interferon treatment and overall increase in lambing rate for Experiments 3 to 5 could be described using linear regression analysis by the following equation: Increase in lambing rate = -1.53 + 1.75(days of interferon treatment), r = 0.997 (P = 0.05).

Table 2. Effect of twice-daily administration of 2 mg bovine interferon-α11 on the lambing rate of ewes

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambing Rates</strong></td>
<td></td>
</tr>
<tr>
<td>Experiment 3, Days 12 to 14</td>
<td>35/47 (74.5 %)</td>
</tr>
<tr>
<td>Experiment 4, Days 12 to 15</td>
<td>29/38 (76.3 %)</td>
</tr>
<tr>
<td>Experiment 5, Days 12 to 16</td>
<td>30/41 (73.2 %)</td>
</tr>
<tr>
<td>Experiment 6, whole embryos</td>
<td>5/11 (45.4 %)</td>
</tr>
<tr>
<td>Experiment 6, demi-embryos</td>
<td>1/8 (12.5 %)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100/145 (69.0 %)</td>
</tr>
</tbody>
</table>

**Litter Size**

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 3, Days 12 to 14</td>
<td>2.0 ± 0.14</td>
</tr>
<tr>
<td>Experiment 4, Days 12 to 15</td>
<td>1.9 ± 0.14</td>
</tr>
<tr>
<td>Experiment 5, Days 12 to 16</td>
<td>1.8 ± 0.15</td>
</tr>
<tr>
<td>Experiment 6, whole embryos</td>
<td>1.8 ± 0.38</td>
</tr>
<tr>
<td>Experiment 6, demi-embryos</td>
<td>2.0</td>
</tr>
</tbody>
</table>

a Number of ewes lambing/number of ewes bred (% pregnant). According to analysis of variance, the lambing rate was affected by experiment (P < 0.001) and interferon (P = 0.10) but not by the interferon X experiment interaction. An effect of interferon on the lambing rate (P < 0.08) was also apparent when data were analyzed by the Mantel-Haenszel-Peto procedure.

b Number of lambs born/ewe lambing (mean ± SEM). The overall number of lambs born per ewe lambing (Experiments 3 to 5 only) was 179/94 (1.9 lambs/ewe) for ewes given a placebo and 193/101 (1.9 lambs/ewe) for ewes treated with interferon. The number of lambs per ewe lambing was not affected by interferon treatment.

Treatment of recipients of whole or demi-embryos also increased the lambing rate (Tables 2 and 3). This effect was not significant if data were analyzed by least-squares analysis of variance, but it approached significance (P < 0.07) when
analyzed by the Mantel-Haenszel-Peto procedure (14). Additionally, an effect of rBolFN-α on the lambing rate in embryo transfer recipients was indicated by the absence of an experiment X interferon interaction when data from embryo transfer recipients were analyzed with data from other ewes treated twice-daily with rBolFN-α (Table 2). From one to four embryos (generally four) were transferred into each ewe. The rate of embryonic survival was determined by evaluating the difference between the number of embryos transferred and number of lambs born. By this criterion, the survival of embryos was enhanced by treatment with rBolFN-α (Table 3; P<0.03 by analysis of variance with ewe as fixed effect; nonsignificant by analysis of variance with ewe as random effect; P<0.02 by Mantel-Haenszel-Peto procedure). As expected, the survival of whole embryos was significantly greater (P<0.025) than for demi-embryos, but rBolFN-α did enhance survival of both types of embryos (embryo type X interferon, nonsignificant).

Table 3. Lambing rate and embryonic survival of whole and demi-embryos transferred to ewe recipients treated with placebo or 2 mg bovine interferon-α11 twice-daily from Days 11 to 18 after estrus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lambing ratea</th>
<th>Embryonic survivalb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole embryo/placebo</td>
<td>5/11 (45.4 %)</td>
<td>9/40 (22.5 %)</td>
</tr>
<tr>
<td>Whole embryo/rBolFN-α</td>
<td>5/9 (55.6 %)</td>
<td>13/31 (41.9 %)</td>
</tr>
<tr>
<td>Demi-embryo/placebo</td>
<td>1/8 (12.3 %)</td>
<td>2/32 (6.3 %)</td>
</tr>
<tr>
<td>Demi-embryo/rBolFN-α</td>
<td>4/10 (40.0 %)</td>
<td>5/38 (13.2 %)</td>
</tr>
</tbody>
</table>

a Number of ewes lambing/number receiving embryos (% lambing). According to analysis of variance, the lambing rate was not significantly affected by type of embryo, interferon or the type of embryo X interferon interaction. There was an effect of interferon (P<0.07) detected by the Mantel-Haenszel-Peto procedure.

b Number of lambs born/number of embryos transferred (% survived). According to analysis of variance, embryonic survival was affected by embryo type (P<0.025 for ewe as random effect and P<0.01 for ewe as fixed effect) and interferon treatment (P<0.03 for ewe as fixed effect and nonsignificant for ewe as random effect) but not by the type of embryo X interferon interaction. According to the Mantel-Haenszel-Peto procedure, there was an effect of interferon (P<0.02).

DISCUSSION

Several experiments utilizing cattle as the experimental model have established the concept that altering luteolysis, using either GnRH analogues (15), hCG (16) or trophoblastic vesicles (17) can enhance the pregnancy rate. Presumably, these treatments rescue a portion of pregnancies that would otherwise be lost because...
the conceptus either is producing inadequate amounts of trophoblast protein-1 or
doing it at an inappropriate time. Accordingly, it is to be expected that
administration of trophoblast protein-1 itself or molecules such as rBo1FN-α that
can exert similar biological effects would also increase the pregnancy rate. As with
earlier reports (8,9), the present data confirm the beneficial effect of rBo1FN-α on
the pregnancy rate in ewes. Additionally, it was shown that rBo1FN-α can enhance
the survival of whole and demi-embryos in embryo transfer systems and that the
frequency and duration of rBo1FN-α treatment is important to achieving optimal
results.

Interferon treatment was only effective in enhancing the pregnancy rate if given
twice-daily. Furthermore, prolonged administration of rBo1FN-α (Days 12 to 16)
appeared to be more effective than administration over a shorter period. These
differences likely reflect differences in the efficacy of the interferon treatment
regimen for affecting luteal function. Because of the difficulties associated with
repeated, frequent administration of drugs under farm conditions, a practical
system for using rBo1FN-α to improve the pregnancy rate of sheep will probably
require a slow-release formulation of rBo1FN-α.

The greatest increase in the lambing rate achieved for naturally-mated ewes in
the present experiment was 7.3 %. This is a smaller increase than reported by
Nephew et al. (8), in whose study the lambing rate was 92 % for ewes treated with
rBo1FN-α was 92 % and 76 % for control ewes. In a study with a group of ewes
with low fertility, Schalue-Francis et al. (9) found that the lambing rate was 71 % for
interferon-treated ewes and 50 % for the controls. In another experiment by
Schalue-Francis et al. (9), in which the fertility of control ewes was similar to that
of our current experiments, the lambing rate was 85 % for ewes receiving interferon
and 74 % for the control ewes.

Although the number of ewes was small, the greatest benefit afforded by
treatment with rBo1FN-α in our study was for embryo transfer recipients. This is
not unexpected since the potential damage to the trophoblast or the retardation
of embryos used in embryo transfer might result in a large frequency of embryos
that are not capable of adequate trophoblast protein-1 production. It was thought
that demi-embryos would receive a greater benefit from treatment with rBo1FN-α
than whole embryos because of the reduction in cell numbers before embryo
transfer. While present results confirmed earlier findings about the lower
survivability of demi-embryos (12), the number of embryos and recipients were too
low to adequately test whether rBo1FN-α would be more effective in recipients
carrying demi-embryos.

Twice-daily administration of rBo1FN-α consistently increased the lambing rate
but had no effect on litter size at birth, a result similar to that of previous
experiments (8,9). In fact, one would not expect interferon administration treatment
to enhance the proportion of embryos surviving in a pregnant ewe. Rather, since
there is evidence that ewes with single embryos are less likely to maintain the
corpus luteum than ewes with twin embryos (12,18), and are therefore potentially
more likely to benefit from interferon, it might be expected that the proportion of ewes lambing with singletons would be increased by interferon. Embryonic survival in the embryo transfer experiment was increased by treatment with rBoIFN-α, but this effect reflects the ability of rBoIFN-α to increase the number of ewes that became pregnant rather than the number of embryos that survived per pregnant ewe.

In conclusion, these results indicate that proper administration of rBoIFN-α can increase the lambing rate of ewes made pregnant by natural mating or embryo transfer. Further work is needed to determine whether a release formulation of rBoIFN-α can be devised that would allow for simple administration and cause an increase in fertility large enough to make administration of rBoIFN-α practical under farm conditions.

REFERENCES


