Luteal function after intrauterine infusion of recombinant bovine interferon- α_1 into postpartum beef cows expected to have short or normal luteal phases

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Summary. This study was conducted to determine whether intrauterine infusion of recombinant bovine interferon- α_1 (rboIFN- α_1), which has 70% sequence identity to bovine trophoblast protein-1, will prevent regression of corpora lutea anticipated to have a short lifespan.

Twenty-six beef cows in good body condition were allotted to four treatment groups at parturition in a 2×2 factorial design. Treatments were: group 1, saline; group 2, rboIFN- α_1 ; group 3, norgestomet-saline; and group 4, norgestomet-rboIFN- α_1 . Norgestomet implants were inserted on days 21–24 postpartum and removed 9 days later (before injection of human chorionic gonadotrophin (hCG)). Ovulation was induced 30 to 33 days postpartum with 5000 or 10 000 iu hCG. Groups 1 ($n = 7$) and 3 ($n = 5$) were given intrauterine infusions (rectocervical approach) twice daily with saline on days 1–12 or 13–24 after hCG injection, respectively. Cows allotted to groups 2 ($n = 8$) and 4 ($n = 6$) were given intrauterine infusions (rectocervical approach) of 2 mg rboIFN- α_1 twice daily on days 1–12 or 13–24 after hCG injection, respectively.

Treatment with both norgestomet and rboIFN- α_1 delayed ($P < 0.01$) luteolysis. Lengths of luteal phases (days; mean $\pm$ SEM) were 8.4 ± 0.7 (group 1, saline), 14.1 ± 1.0 (group 2, rboIFN- α_1), 18.6 ± 1.3 (group 3, norgestomet-saline) and 20.8 ± 1.2 (group 4, norgestomet-rboIFN- α_1). Concentration of progesterone in serum was similar among all groups the first 6 days following hCG-induced ovulation, but differed ($P < 0.01$) thereafter. Mean concentration of progesterone was lower ($P < 0.01$) in saline-treated cows (group 1) from days 8 to 13 than in cows infused with rboIFN- α_1 or pretreated with norgestomet. In summary, intrauterine infusion of rboIFN- α_1 delayed luteolysis of corpora lutea anticipated to have a short lifespan.

Keywords: corpus luteum; progesterone; norgestomet; recombinant bovine interferon- α_1 ; cow

Introduction

In domestic ruminants, pregnancy is established when the corpus luteum is rescued from the luteolytic effects of prostaglandin F $_{2\alpha}$ (PGF $_{2\alpha}$; Niswender & Nett, 1988). Presence of the conceptus prevents luteolysis in cattle unless the corpus luteum regresses prior to maternal recognition of pregnancy (approximately day 15; Betteridge *et al.*, 1978; Northey & French, 1980).

Intrauterine infusion of conceptus homogenates prolonged luteal function in sheep (Moor, 1968) and cattle (Northey & French, 1980). Two closely related conceptus proteins, ovine trophoblast protein-1 (oTP-1) and bovine trophoblast protein-1 (bTP-1), are transiently produced at about the time of maternal recognition of pregnancy and are associated with maintenance of luteal

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function (for review see Roberts, 1989). Recent studies provide evidence that oTP-1 and bTP-1 are structurally similar to interferons of the α_{II} subclass (Imakawa *et al.*, 1987, 1989; Stewart *et al.*, 1987; Charpigny *et al.*, 1988). Both oTP-1 and bTP-1 have antiviral activity (Roberts *et al.*, 1989) and oTP-1 can compete with interferon- α_1 for binding to interferon receptors in ovine endometrium (Stewart *et al.*, 1987; Hansen *et al.*, 1989).

Uterine infusion of oTP-1 at about the time of maternal recognition of pregnancy extended luteal function in sheep (Godkin *et al.*, 1984; Vallet *et al.*, 1988), while bTP-1 had a similar effect in cattle (Thatcher *et al.*, 1989). Similar results have been reported following intrauterine infusion of the more readily available recombinant bovine interferon- α_1 (rboIFN- α_1) into cows from days 15.5 to 21 post-oestrus (day 0 = oestrus; Plante *et al.*, 1988).

In cattle, the corpus luteum formed following the first spontaneous or induced ovulation at puberty or following parturition is short-lived (Corah *et al.*, 1974; Gonzalez-Padilla *et al.*, 1975; Lishman *et al.*, 1979; Odde *et al.*, 1980; LaVoie *et al.*, 1981; Pratt *et al.*, 1982), unless the cows have been pretreated with a progestogen. Luteal regression in cows with short luteal phases occurs prior to the time that the anti-luteolytic action of the conceptuses is manifested. Whether administration of rboIFN- α_1 will prevent subnormal luteal function is unknown. The present study was conducted to determine if uterine infusion of rboIFN- α_1 will prevent regression of corpora lutea anticipated to have a short lifespan.

Materials and Methods

Twenty-six anoestrous beef cows in good body condition were allotted at parturition to two intrauterine infusion groups (vehicle or rboIFN- α_1) pretreated with or without norgestomet (17 α -acetoxy-11 β -methyl-19-nor-preg-4-ene-3,20-dione; a progestin) implants in a 2 $\times$ 2 factorial design. Treatments were: group 1, saline; group 2, rboIFN- α_1 ; group 3, norgestomet saline; and group 4, norgestomet rboIFN- α_1 . Norgestomet implants were inserted on days 21–24 postpartum and removed 9 days later (prior to injection of human chorionic gonadotrophin (hCG)). Pretreatment of early postpartum cows with norgestomet for 9 days results in subsequent luteal phases of normal length (16–19 days) instead of short luteal phases.

Beginning at calving, ovarian activity was monitored until induction of ovulation with hCG by determination of progesterone from serum collected thrice weekly. Cows were considered to be anoestrous if the concentration of progesterone in the serum was below 0.5 ng/ml and no increase in concentration of progesterone was observed prior to injection of hCG. Ovulation was induced 30–33 days postpartum with 5000 or 10 000 iu hCG (day 0; 48 h after implant removal for groups 3 and 4). Doses were similarly distributed over all groups and there was no difference in ovulatory response between the two dose levels. Short-lived corpora lutea have been induced following injection of hCG early postpartum. The purpose of groups 1 and 2 was to determine if intrauterine infusion of rboIFN- α_1 on days 1–12 after hCG injection would prevent subnormal luteal function. Groups 3 and 4 served as control groups to verify that intrauterine infusions of rboIFN- α_1 on days 13–24 post hCG injection would prolong luteal function (Plante *et al.*, 1988). Groups 1 ($n = 7$) and 3 ($n = 5$) were given intrauterine infusions (recto-cervical approach) twice daily with saline (1 ml; containing bovine serum albumin in amounts similar to rboIFN- α_1 in groups 2 and 4) on days 1–12 or 13–24 after hCG injection, respectively. Cows allotted to groups 2 ($n = 8$) and 4 ($n = 6$) were given intrauterine infusions (recto-cervical approach) of 2 mg rboIFN- α_1 (1 ml) twice daily on days 1–12, or 13–24, post hCG injection, respectively. Sterile intrauterine infusion (stainless steel) catheters were used for infusions. Blood samples were collected twice daily during intrauterine infusions and daily throughout the remainder of the first oestrous cycle. Collection of blood samples was stopped following luteal regression. Concentrations of progesterone in serum were measured as described by Cantley *et al.* (1975). Luteal regression was judged to have occurred when concentrations of progesterone in serum had declined to < 0.5 ng/ml. Since length of luteal phase differed within treatments, a value of 0.05 ng/ml (minimum detectable concentration) was assigned to each sample period for each animal following luteal regression until luteolysis had occurred in all animals in that group. The length of the luteal phase was calculated from the time of injection of hCG to luteal regression.

Data were analysed using Statistical Analysis Systems' General Linear Models procedures (SAS, 1985). Differences in duration of luteal lifespan were determined by 2 $\times$ 2 factorial analysis of variance (Steel & Torrie, 1960). Differences in serum concentration of progesterone were determined by 2 $\times$ 2 factorial analysis of variance for repeated measurements (Gill & Hafs, 1971).

Results

The main treatment effects of norgestomet and rboIFN- α_1 on length of the luteal phase were significant ($P < 0.01$) and the interaction was nonsignificant (Fig. 1). Norgestomet pretreatment

increased ($P < 0.01$) oestrous cycle length by 10.2 days in saline-treated animals, while infusion of rboIFN- α_1 increased ($P < 0.01$) oestrous cycle length by 5.7 days in cows anticipated to have short oestrous cycles (groups 1 and 2) and 2.2 days ($P = 0.14$) in cows anticipated to have normal oestrous cycles (norgestomet pretreated; groups 3 and 4). Patterns of secretion of progesterone in serum differed within and among treatments ($P < 0.05$; Fig. 2). Concentrations of progesterone in serum were similar among groups during the first 6 days following hCG-induced ovulation. Thereafter, mean concentration of progesterone declined and was lower ($P < 0.05$) in saline-treated cows (group 1) from days 8 to 13 than in cows infused with either rboIFN- α_1 or pretreated with norgestomet (groups 2–4). In addition, concentrations of progesterone on days 17.5–19 of the oestrous cycle were higher ($P < 0.05$) in norgestomet-treated cows given intrauterine infusions of rboIFN- α_1 (group 4) than norgestomet-treated cows infused with saline (group 3). Concentrations of progesterone in group 2 were lower ($P < 0.05$) during the later stages of the oestrous cycle than those observed in groups 3 and 4.

Fig. 1. Length of luteal phase in anoestrous beef cows following induction of ovulation with human chorionic gonadotrophin on days 30–33 postpartum. Cows pretreated with norgestomet were anticipated to have normal length luteal phases, the others short luteal phases. Cows were given intrauterine infusions of either saline (S) or recombinant bovine interferon- α_1 (I) on days 1–12 (short luteal phase) or 13–24 (normal luteal phase), respectively.

Discussion

Our previous experiments have shown that short-lived corpora lutea in postpartum anoestrous cows are not due to (1) inadequate gonadotrophic stimulation during either the pre- or post-ovulatory period (Garverick *et al.*, 1988); (2) a decrease in occupied LH receptors, unoccupied LH receptors, and basal and agonist-stimulated adenylate cyclase activities preceding luteolysis (Smith *et al.*, 1986); (3) reduced ability of luteal cells to secrete progesterone (Copelin *et al.*, 1987, 1988); or (4) increased responsiveness of luteal tissue to PGF2 α (Copelin *et al.*, 1988).

The first corpus luteum formed postpartum does not have an inherently short lifespan. Reduced luteal lifespan is associated with a premature luteolytic influence of the uterus (presumably PGF2 α). Corpora lutea, for example, were maintained in cows (Copelin *et al.*, 1987) and ewes (Southee *et al.*, 1988) hysterectomized prior to first spontaneous oestrus and ovulation and in postpartum cows immunized against PGF2 α (Copelin *et al.*, 1989). Secretion of progesterone was similar to that observed in cows during a normal oestrous cycle (Copelin *et al.*, 1987, 1989). In other studies, pulse amplitude and basal concentrations of plasma PGF2 α were increased on days 4–9 for postpartum cows having a short rather than a normal length oestrous cycle (Cooper *et al.*, 1991), and secretion of PGF2 α *in vitro* from endometrial tissue from cows anticipated to have short

Fig. 2. Concentration of progesterone in serum of anoestrous beef cows following induction of ovulation with human chorionic gonadotrophin on days 30–33 postpartum. Cows represented on the lower panel were pretreated with norgestomet and were anticipated to have normal length oestrous cycles. Cows represented in the upper panel were anticipated to have short oestrous cycles. Intrauterine infusions of saline or recombinant bovine interferon- α_1 1 (rboIFN- α_1 1) were performed on days 1–12 for cows in the upper panel and on days 13–24 for cows in the lower panel.

oestrous cycles was greater than from cows anticipated to have normal oestrous cycles (Zollers *et al.*, 1991b). Injection of oxytocin on day 5 of the oestrous cycle induced a release of 13,14-dihydro-15-keto prostaglandin F₂ α (PGFM) in cows with a short, but not normal, oestrous cycle length (Zollers *et al.*, 1989). Similarly, Hunter *et al.* (1989) have demonstrated an association between pulses of oxytocin and PGFM on day 5 in ewes with short luteal phases similar to that observed during normal luteolysis. In addition, Zollers *et al.* (1991a) reported higher concentrations of oxytocin receptors on day 5 of the oestrous cycle in cows anticipated to have short compared with normal luteal phases (pretreatment with a progestogen). In cows pretreated with a progestogen, concentration of oxytocin receptors were lower and uterine responsiveness (PGFM release) to oxytocin was inhibited on day 5 following oestrus. Thus, previous experiments have led us to conclude that reduced luteal lifespan in anoestrous cows is due to a premature release of PGF₂ α ,

which may be associated with response of the uterus to oxytocin when concentrations of uterine oxytocin receptors are increased.

In the present study, luteal lifespan of cows anticipated to have short cycles was extended by intrauterine infusion of rboIFN- α_1 . Infusion of rboIFN- α_1 was initiated in all groups of cows 4–5 days prior to expected luteolysis. Similar results have been reported following intrauterine infusion of rboIFN- α_1 (days 15.5–21 postoestrus) into cows (Plante *et al.*, 1988; Thatcher *et al.*, 1989) and sheep (Stewart *et al.*, 1989; Schalue-Francis *et al.*, 1991) anticipated to have normal luteal phases. Similarly, luteolysis is delayed in sheep and cattle following intrauterine infusion of oTP-1 or bovine conceptus proteins (Knickerbocker *et al.*, 1986; Vallet *et al.*, 1988; Thatcher *et al.*, 1989). In each case endometrial secretion of PGF 2α was inhibited. Recombinant bovine interferon- α_1 has been shown to decrease PGF 2α secretion by bovine endometrium under *in vitro* conditions (Barros *et al.*, 1990). In cattle, bTP-1 may increase the activity of an endometrial prostaglandin synthetase inhibitor, thereby reducing endometrial PGF 2α synthesis (Gross *et al.*, 1988a, b). In addition, interferons may inhibit the rise in the concentration of uterine oxytocin receptors which may be required for controlling pulsatile secretion of PGF 2α (Flint *et al.*, 1991). Although the mechanism by which intrauterine infusion of rboIFN- α_1 extends luteal function, in short or normal luteal phases, is unclear, all data are consistent with the concept that secretion of PGF 2α by the endometrium is somehow inhibited and that this inhibition extends luteal function. Whether inhibition of release of PGF 2α is due to reduced concentrations of oxytocin receptors in the uterus by rboIFN- α_1 is unknown. Also, although inhibition of endometrial PGF 2α synthesis is a possible mechanism, an effect at the ovarian level cannot be eliminated. Further study is needed to examine the mechanism whereby rboIFN- α_1 extends luteal function in corpora lutea intended to be short-lived. Regardless, infusion of rboIFN- α_1 at a time prior to maternal recognition of pregnancy extended luteal function in cows anticipated to have short luteal phases. Development of a treatment to reduce the incidence of short cycles may result in increased fertility of postpartum beef cows. Increased conception rates have been observed in ewes following treatment with rboIFN- α_1 (Nephew *et al.*, 1990; Schalue-Francis *et al.*, 1991).

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