Effect of active immunization of pre-partum and post-partum cows against prostaglandin F-2 α on lifespan and progesterone secretion of short-lived corpora lutea*

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Summary. Mature beef cows were actively immunized *pre partum* (N = 5) or *post partum* (N = 10) against a PGF-2 α -ovalbumin conjugate or against ovalbumin alone (control; N = 5). All cows in the control group exhibited first oestrous cycles which were of short duration (≤ 12 days). Mean specific serum binding to [3 H]PGF-2 α in the control group was consistently $< 1\%$. In the pre-partum PGF-2 α -immunized cows, lifespan and progesterone secretion of the first corpus luteum formed *post partum* was maintained for > 39 days. Specific serum binding to [3 H]PGF-2 α in pre-partum and post-partum PGF-2 α -immunized cows was elevated. Lifespan of the first corpus luteum formed in post-partum PGF-2 α -immunized cows was short (< 10 days; N = 1), normal (mean = 22 days; N = 4) or maintained (> 31 days; N = 5). Luteal lifespan was dependent upon serum PGF-2 α antibody titres, with cows exhibiting higher titres frequently having prolonged luteal lifespans after first ovulation. We conclude that active immunization of beef cows against PGF-2 α extends the lifespan and progesterone secretion of corpora lutea anticipated to be short-lived. These results support the concept that the shorter lifespan of some corpora lutea in post-partum cows is due to a premature release of PGF-2 α from the uterus.

Keywords: active immunization; PGF-2 α ; corpus luteum; cattle

Introduction

Corpora lutea having short lifespans (≤ 12 days) frequently form spontaneously before first oestrus in prepubertal (Berardinelli *et al.*, 1979, 1980; Keisler *et al.*, 1983) and post-partum cows and ewes (Corah *et al.*, 1974; LaVoie *et al.*, 1981; Lamming *et al.*, 1981; Sharpe *et al.*, 1986). Furthermore, corpora lutea having short lifespans occur after early weaning of calves from anoestrous cows (Odde *et al.*, 1980; Copelin *et al.*, 1987) and after ovulations induced by gonadotrophin-releasing hormone (GnRH) or human chorionic gonadotrophin during the early post-partum period in cattle (Lishman *et al.*, 1979; Pratt *et al.*, 1982; Garcia-Winder *et al.*, 1986). The physiological importance of short-lived corpora lutea is unknown. However, a transient increase in progesterone is followed by resumption of oestrous cycles of normal length in anoestrous sheep and cattle (Ramirez-Godinez *et al.*, 1981; Lamming *et al.*, 1981; Southee *et al.*, 1988a).

Mechanisms associated with subnormal luteal function may include: (1) inadequate preovulatory follicular maturation; (2) inadequate luteotrophic stimuli and/or (3) premature release of and/or increased responsiveness of luteal tissue to a luteolysin (Garverick & Smith, 1986). The

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premature release of a uterine luteolysin, presumably prostaglandin (PG) F-2 α , is a likely mechanism contributing to subnormal luteal function in post-partum cows for the following reasons: (1) short-lived corpora lutea do not have inherently short lifespans; rather the presence of the uterus results in premature luteolysis (Copelin *et al.*, 1987); (2) corpora lutea anticipated to have short lifespans were not more sensitive to the luteolytic effects of PGF-2 α than were corpora lutea anticipated to have normal lifespans (Copelin *et al.*, 1988) and (3) oxytocin-induced PGF-2 α release was greater on Day 5 of a short compared to a normal length oestrous cycle (Zollers *et al.*, 1988).

PGF-2 α is considered to be the primary uterine luteolysin in sheep and cattle (Goding, 1974; Horton & Poyser, 1976; Knickerbocker *et al.*, 1988). Both active and passive immunization against PGF-2 α lengthened the functional lifespan of corpora lutea in sheep (Scaramuzzi & Baird, 1976; Fairclough *et al.*, 1981), guinea-pigs (Horton & Poyser, 1974; Poyser & Horton, 1975) and cattle (Fairclough *et al.*, 1981; Chang *et al.*, 1987). Therefore, the objective of this study was to determine whether active immunization of pre-partum and post-partum cows against PGF-2 α would extend the lifespan and progesterone secretion of short-lived corpora lutea.

Materials and Methods

Animals. Twenty mature beef cows in moderate to good body condition and of various breeds (Angus, Simmental, Charolais and Hereford $\times$ Angus) and ages (3–13 years) were randomly allotted to three groups and actively immunized against a PGF-2 α –ovalbumin conjugate (Group PG_{pre} (N = 5), pre-partum PGF-2 α ; and Group PG_{post} (N = 10), post-partum PGF-2 α) or against ovalbumin alone (Group C (N = 5), control). Cows were observed for oestrus twice daily from calving until the end of the experiment. Only cows which stood to be mounted were considered in oestrus. In the absence of behavioural oestrus, day of oestrus (Day 0) was defined, based on concentration of progesterone in serum, as 3 days before the first post-partum increase in progesterone above 0.5 ng/ml. Calves were weaned from cows at 30–35 days *post partum*.

At the conclusion of the experiment, cows from each treatment group were randomly selected to be ovariectomized. The luteal ovary was removed via transvaginal incision to determine (gross and histological observation) whether the source of progesterone in the serum of PGF-2 α -immunized cows was a corpus luteum or a luteinized follicle.

Antigen preparation. Prostaglandin F-2 α was conjugated to chicken ovalbumin by the carbodiimide method (Bauminger & Wilchek, 1980): 200 mg PGF-2 α (5 mg Lutalyse/ml; Upjohn Co., Kalamazoo, MI, USA) were lyophilized and redissolved in 1.5 ml distilled water. Grade V chicken ovalbumin (164 mg; Sigma Chemical Co.) was dissolved in 8 ml distilled water and adjusted to a pH of 5.0–5.2. Then 400 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Sigma Chemical Co.) were added to the chicken ovalbumin while stirring. The PGF-2 α solution was added dropwise to the chicken ovalbumin and stirred for 3 h at 4°C. An additional 70 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were added and the solution stirred for another 4 h at room temperature and dialysed against distilled water overnight. After dialysis, the PGF-2 α –ovalbumin conjugate was lyophilized and stored at room temperature.

Immunization protocol. Primary immunizations consisted of 1 mg antigen (PGF-2 α –ovalbumin or ovalbumin alone) dissolved in 1 ml physiological saline (0.9% (w/v) NaCl; pH 7.2) and emulsified in 1 ml complete Freund's adjuvant (Sigma Chemical Co.). The emulsion was immediately injected intradermally in at least 6 sites in the axillary region. Two booster immunizations prepared as described for the primary immunizations were given at 2-week intervals after the primary immunization and consisted of 1 mg antigen, 1 ml physiological saline (0.9% (w/v) NaCl; pH 7.2), and 1 ml incomplete Freund's adjuvant.

Primary immunizations were given to cows in Group PG_{pre} on Day 270 or 271 of gestation. Cows in Groups C and PG_{post} received primary immunizations within the first 2 weeks *post partum*.

Antibody titration. Specific binding of [³H]PGF-2 α was measured in aliquants of serum samples collected weekly. The titre of the antisera was defined as the percentage of [³H]PGF-2 α specifically bound to serum at a 1:200 dilution. This dilution allowed comparison among all groups of the relative relationships of the percentage [³H]PGF-2 α specifically bound to serum. Binding of [³H]PGF-2 α in serum samples was measured in duplicate. Samples were incubated for 24 h at 4°C with 20 000 d.p.m. [5,6,8,9,11,12,14,15(n)-³H]PGF-2 α (100 μ l/tube; New England Nuclear, Boston, MA, USA). After incubation, a neutralized charcoal–Dextran suspension (500 μ l/tube; Sigma Chemical Co.) was used to separate free from antibody-bound [³H]PGF-2 α . After centrifugation at 1400 g for 10 min, the supernatant was decanted into 7-ml vials and 5 ml liquid scintillation cocktail were added. Vials were counted in a liquid scintillation counter for 2 min per sample.

Progesterone assay. Blood samples were collected via jugular venepuncture every other day from primary immunization until weaning and daily from weaning through two consecutive oestrous cycles or until at least 30 days after oestrus. Blood samples were allowed to clot at room temperature for 2 h before centrifugation. Serum was harvested and stored at -20°C until concentration of progesterone was measured by radioimmunoassay (Cantley *et al.*, 1975).

Serum samples (200 μl) were extracted in duplicate with 3 ml ethyl ether (anaesthesia grade) for 6 min on a mechanical shaker. Extraction efficiency averaged 88.8% in 12 assays. Crystalline progesterone dissolved in absolute ethanol was used as a standard. Progesterone was pipetted in triplicate into culture tubes in amounts of 20, 30, 50, 70, 100, 200, 300, 500, 750 and 1000 pg/tube and evaporated under air. Progesterone antiserum (200 μl , 1:1000 dilution) raised in sheep (RDR-9-P) was added to each tube with 100 μl (30 000 d.p.m.) of [1,2,6,7(n)- ^3H]progesterone (New England Nuclear) and contents were incubated overnight at 4°C . After incubation, 500 μl neutralized charcoal-Dextran in assay buffer were added to each tube to separate bound from free hormone. After a 10-min incubation in ice water, tubes were centrifuged at 1400 g for 10 min at 4°C . The supernatant was decanted into 7-ml plastic vials and 5 ml Scint-A scintillation cocktail (United Technologies Packard) were added to each vial and radioactivity measured in a liquid scintillation counter. Intra- and interassay coefficients of variation were 5.8 and 10.5%, respectively.

Statistical analyses. Interval from calving to first oestrus was analysed by one-way analysis of variance (Snedecor & Cochran, 1980). Differences among mean intervals to oestrus were analysed by the protected least significant difference procedure (Cochran & Cox, 1957). Oestrous cycle lengths for cows in Groups PG_{post} and C were analysed by Student's *t* test (Snedecor & Cochran, 1980). Progesterone data were analysed by analysis of variance for a split-plot design (Gill & Hafs, 1971). Percentages of [^3H]PGF-2a specifically bound within and among cows over time were subjected to arc-sin transformations and analysed by analysis of variance for a split-plot design (Gill & Hafs, 1971).

Results

The gestation length (mean $\pm$ s.e.) of cows in Group PG_{pre} (287 ± 3.9 days) was similar to the previously published gestation length (286 ± 0.5 days) for Simmental and Charolais cows (Notter *et al.*, 1978). Furthermore, cows in Group PG_{pre} did not experience any complications with parturition.

Oestrus was not observed in the majority of cows in the present study in conjunction with first ovulation. The interval (mean $\pm$ s.e.) from parturition to first oestrus, as determined by concentration of progesterone in serum, was shorter ($P < 0.01$) for cows in Group PG_{pre} (19.6 ± 2.7 days) compared to cows in Groups C and PG_{post} (31.4 ± 2.1 and 37.7 ± 3.0 days, respectively). Furthermore, all of the cows in Group PG_{pre} ovulated before weaning. Four cows in Group PG_{post} and 2 cows in Group C also ovulated before weaning.

Oestrous cycle lengths

All cows in Group C had first oestrous cycles which were short (Table 1). The subsequent oestrous cycle was of normal length (range: 17–22 days) in all but 1 cow, which had a second short oestrous cycle (7 days). Conversely, all cows in Group PG_{pre} maintained their corpora lutea for periods exceeding 39 days and 1 cow maintained a low concentration of progesterone in serum (~ 1 ng/ml) for over 42 days. At ovariectomy, the source of progesterone was determined to be a luteinized follicle. In Group PG_{post}, one cow had a short oestrous cycle, 4 cows had oestrous cycles of normal length, and 5 cows maintained the first corpus luteum formed *post partum* (Table 1).

Progesterone

Concentrations of progesterone in serum and PGF-2a antibody titres after calving for a representative cow from each treatment group are illustrated in Fig. 1. Included in this figure are examples of 2 cows in Group PG_{post}, one which had a first oestrous cycle of normal length (No. 74; PG_{post}-N), followed by maintenance of the subsequent corpus luteum and one cow which maintained the first corpus luteum formed *post partum* (No. 69; PG_{post}-M).

The concentration and pattern of secretion of progesterone was similar ($P > 0.10$) from Day 0 to 5 for cows in all 3 groups (Fig. 2a). Concentration of progesterone in plasma declined thereafter in Group C cows and continued to increase in cows in Groups PG_{pre} and PG_{post}. In Group PG_{post} there were 4 cows with first oestrous cycles of normal length (Group PG_{post}-N) and 5 (Group

Table 1. Effect of treatment on oestrous cycle length in cows

Group	No.	Oestrous cycle length (days)	
		Cycle 1	Cycle 2
C	5	8.4 ± 0.2	17.2 ± 2.7
PG _{pre}	5	> 39	—
PG _{post}			
Short	1	10	—
Normal	4	22.0 ± 0.9	—
Maintained	5	> 31	—

Values are mean ± s.e.

Fig. 1. Representative profiles of concentrations of progesterone in serum and percentage of [³H]PGF-2α specifically bound for cows immunized against PGF-2α before parturition (Group PG_{pre}; N = 5) or after parturition with normal (Group PG_{post}-N; N = 4) or extended (Group PG_{post}-M; N = 5) luteal function after first ovulation *post partum* and cows not immunized against PGF-2α (Group C; N = 5).

PG_{post}-M) in which the first corpus luteum formed *post partum* was maintained. The concentration and pattern of secretion of progesterone from Day 0 to 20 were similar (*P* > 0.05) for cows in both these subgroups of Group PG_{post} and in Group PG_{pre} (Fig. 2a).

PGF-2α antibody titres

Mean PGF-2α antibody titres for cows in Group C were consistently <1% throughout the experiment. Similarly, 1 cow in Group PG_{post}, which had a first oestrous cycle which was short

Fig. 2. Profiles of mean concentrations of progesterone (a) and percentage (mean $\pm$ s.e.) of [3 H]PGF-2 α specifically bound (b) for cows immunized against PGF-2 α before parturition (Group PG_{pre}; N = 5) or after parturition with normal (Group PG_{post}-N; N = 4) or extended (Group PG_{post}-M; N = 5) luteal function after first ovulation *post partum* and cows not immunized against PGF-2 α (Group C; N = 5).

(10 days), did not respond to immunization (PGF-2 α antibody titre of <3%). Conversely, in Groups PG_{pre} and PG_{post}, individual serum PGF-2 α antibody titres ranged from 28 to 40% and 13 to 60%, respectively, at the end of the experiment. There was no difference in mean PGF-2 α antibody titre in weekly samples between cows in Group PG_{pre} and Group PG_{post}-M ($P > 0.05$). In contrast, PGF-2 α antibody titres were lower ($P = 0.08$) from Days 3 to 22 inclusive for cows in Group PG_{post}-N than for those in Groups PG_{pre} and PG_{post}-M (Fig. 2b).

Source of progesterone

At the end of the experiment 12 cows were ovariectomized and the ovaries examined (gross and histological observation) to determine whether the source of progesterone in serum was a corpus luteum or a luteinized follicle. At ovariectomy, a corpus luteum was found in all cows examined in Group C (N = 3) and Group PG_{post} (N = 6). In Group PG_{pre}, 4 cows had corpora lutea and 1 cow had a luteinized follicle.

Discussion

Prostaglandin F-2 α has been proposed as the uterine luteolysin in numerous species including cattle (Horton & Poyser, 1976; Knickerbocker *et al.*, 1988). Active or passive immunization against PGF-2 α maintained luteal function beyond the normal oestrous cycle length in normally cycling guinea-pigs (Horton & Poyser, 1974; Hildebrandt-Stark *et al.*, 1975; Poyser & Horton, 1975), sheep (Scaramuzzi &

Baird, 1976; Fairclough *et al.*, 1981) and cattle (Fairclough *et al.*, 1981; Chang *et al.*, 1987). In the present study, active immunization against PGF-2 α extended the lifespan and function of corpora lutea anticipated to be short-lived.

Prostaglandin F-2 α is also reported to be important in the mechanism of ovulation (Espey, 1980; Murdoch, 1985). The ability of indomethacin (PG synthetase inhibitor) to block ovulation is documented for rats (Tsafiri *et al.*, 1973), rabbits (O'Grady *et al.*, 1972), sheep (Murdoch & Dunn, 1983) and cattle (De Silva & Reeves, 1985). Intrafollicular injection of PGF-2 α antiserum blocked luteinizing hormone (LH)-induced ovulations in rabbits (Armstrong *et al.*, 1974). However, in the present study active immunization against PGF-2 α apparently did not inhibit follicular rupture in most of the cows. Only one animal immunized against PGF-2 α had a luteinized follicle, whereas all other cows had corpora lutea with evidence of stigma formation. Active immunization against PGF-2 α may affect only systemic concentrations of PGF-2 α and may have little or no effect on local concentrations of PGF-2 α synthesized by follicular cells (Scaramuzzi & Baird, 1976).

The first post-partum ovulation occurred earlier in cows in Group PG_{pre} than in those in the other groups. Injection of PGF-2 α during the early post-partum period decreases the post-partum interval to oestrus in cattle (Randel *et al.*, 1988). Furthermore, prostaglandin F-2 α has been implicated in resumption of ovarian activity in post-partum dairy cows (Gilbault *et al.*, 1987). A characteristic of active immunization is that it frequently increases circulating concentrations of the antigen against which the animal is immunized, presumably due to a decrease in the metabolic clearance rate associated with the antibody-bound molecule (Scaramuzzi *et al.*, 1973; Sheldrick *et al.*, 1980). Perhaps the decreased interval to oestrus for cows in these experiments was due to increased circulating concentrations of PGF-2 α (albeit bound PGF-2 α) by virtue of its increased half-life. However, this would not account for the extended luteal lifespans which occurred in all of the pre-partum PGF-2 α -immunized animals. Alternatively, a reduction in the tissue availability of PGF-2 α during the early post-partum period in the pre-partum PGF-2 α group may have decreased post-partum interval length.

As expected, the overall antibody response to PGF-2 α was variable among animals. Only one cow immunized against PGF-2 α did not develop a titre and this cow had a short oestrous cycle similar to those of cows in the control group. The effect of active immunization against PGF-2 α on gestation length and the mechanics of parturition could not be evaluated in the present study since PGF-2 α antibody titres did not increase in Group PG_{pre} until after parturition.

In this experiment, cows with higher PGF-2 α antibody titres were more likely to have prolonged luteal function. This is in agreement with other studies showing a correlation between PGF-2 α antibody titres and oestrous cycle length in guinea-pigs (Horton & Poyser, 1974; Poyser & Horton, 1975). In the present study, cows in the post-partum PGF-2 α group which responded to immunization exhibited either luteal maintenance or oestrous cycles of normal length after first ovulation. In either case luteal function was prolonged relative to control animals. Perhaps the lower PGF-2 α antibody titres were unable to neutralize all of the endogenous PGF-2 α present in cows in which cycles were prolonged but not maintained. All of the cows exhibiting luteal maintenance had significantly higher PGF-2 α antibody titres which presumably neutralized all of the endogenous PGF-2 α .

The results of this study and others support the concept that short-lived corpora lutea are the result of a premature release of PGF-2 α from the uterus (Garverick & Smith, 1986). Firstly, hysterectomy prevents regression of corpora lutea anticipated to have short lifespans in anoestrous ewes and post-partum cows (Copelin *et al.*, 1987; Lewis & Bolt, 1987; Southee *et al.*, 1988b). Secondly, intrauterine infusion of indomethacin following GnRH-induced ovulation prolongs luteal function in post-partum beef cows (Troxel & Kesler, 1984). Thirdly, short-lived corpora lutea were not more responsive to PGF-2 α than corpora lutea having normal lifespans (Copelin *et al.*, 1988). Fourth, oxytocin induces a release of PGF-2 α on Day 5 of a short but not of a normal oestrous cycle following the early weaning of calves from cows (Zollers *et al.*, 1988). Furthermore, the magnitude of the oxytocin-induced PGF-2 α release on Day 5 of a short oestrous cycle is similar to that

observed on Day 16 of a normal oestrous cycle. It has been proposed that oxytocin is involved in luteolysis in sheep and cattle (Flint *et al.*, 1986; Auletta & Flint, 1988). The increased responsiveness of the uterus to oxytocin on Day 5 of a short oestrous cycle may reflect an increase in oxytocin receptors and/or an increase in uterine PG synthesis.

The mechanism by which the timing of PGF-2a secretion is advanced, thus causing a short oestrous cycle, is unclear. A period of progesterone priming is reported to be necessary for uterine PGF-2a secretion (Homanics & Silvia, 1988; Lafrance & Goff, 1988). It is unclear whether progesterone affects PGF-2a secretion by a direct and/or indirect mechanism. Progestagen pretreatment of anoestrous cows reduced the incidence of short oestrous cycles following induction of ovulation or early weaning of calves from cows (Ramirez-Godinez *et al.*, 1981; Pratt *et al.*, 1982). Progestagen pretreatment may co-ordinate the timing of PGF-2a secretion during the subsequent oestrous cycle by enhancing follicular oestradiol secretion (Inskeep *et al.*, 1988). Progestagen-pretreated anoestrous cows had increased preovulatory follicular oestradiol secretion and longer luteal lifespans than did control animals (Garcia-Winder *et al.*, 1987; Garverick *et al.*, 1988; Inskeep *et al.*, 1988). Ottobre *et al.* (1984) proposed that high concentrations of oestradiol at oestrus may induce the synthesis of uterine progesterone receptors in sheep. Progesterone receptors in the endometrium (Miller *et al.*, 1977) and myometrium (Rexroad, 1981) of sheep were elevated at oestrus and decreased by the mid-luteal phase. Schams (1987) reported a similar change in uterine progesterone receptors during the oestrous cycle of the cow. Therefore, decreased preovulatory concentrations of oestradiol around the time of oestrus may result in decreased synthesis of uterine progesterone receptors and allow the timing of uterine PGF-2a secretion to be advanced (Inskeep *et al.*, 1988).

In conclusion, active immunization of pre-partum and post-partum cows against PGF-2a extended the lifespan and function of corpora lutea anticipated to be short-lived. These results, in combination with previous reports, support the concept that short-lived corpora lutea in post-partum cows are the result of a premature release of PGF-2a from the uterus.

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