Active immunization of ewes against prostaglandin $F_{2\alpha}$ to control ovarian function

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The hypothesis that pregnancy success could be improved in early *postpartum* ewes by prolonging the lifespan of the corpus luteum via active immunization against prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was tested. Further experiments in ewes immunized against $PGF_{2\alpha}$ investigated the effects of exogenous $PGF_{2\alpha}$ on the preovulatory follicle and the effects of $PGF_{2\alpha}$ and oestradiol benzoate on corpus luteum function. Four weeks *prepartum*, 39 ewes bred to lamb during seasonal anoestrus received either 5 mg $PGF_{2\alpha}$ –ovalbumin conjugate ($n = 20$; immunized) or ovalbumin ($n = 19$; control) in Freund's complete adjuvant. Treatments were repeated on day 5 *post partum* with reagents emulsified in Freund's incomplete adjuvant. On day 17 *post partum*, ewes received 500 iu pregnant mares' serum gonadotrophin (PMSG) and 48 h later 50 μ g gonadotrophin-releasing hormone (GnRH). Laparoscopy was performed 36 h after GnRH to assess ovarian activity and ewes with recent ovulations were inseminated into the uterus. No immunized ewes had ovulated, but ten had follicles that luteinized and secreted progesterone during the 8 weeks studied. Eighteen of 19 control ewes ovulated and 15 of 18 had increased progesterone concentration for at least 21 days. By day 70 *post partum*, progesterone had returned to basal values in all control ewes. In a second study, 24 immunized ewes bearing persistent corpora lutea, and for which the interval from the previous parturition was greater than 90 days, received 15 mg $PGF_{2\alpha}$ and 500 iu PMSG followed 48 h later by 50 μ g GnRH. $PGF_{2\alpha}$ induced corpus luteum regression in all ewes. PMSG and GnRH treatments resulted in oestrus in 21 of 24 ewes. Sixteen hours after GnRH, 10 ewes received a second injection of 15 mg $PGF_{2\alpha}$. $PGF_{2\alpha}$ induced follicular rupture in eight of ten immunized ewes, whereas only two of 14 ewes not receiving $PGF_{2\alpha}$ ovulated ($P < 0.01$). All anovular ewes had large cystic follicles that luteinized. In a third study, 22 ewes immunized against $PGF_{2\alpha}$ and having persistent corpora lutea, received, on two consecutive days, either oestradiol benzoate (750 μ g; $n = 11$) in oil or oil ($n = 11$). Laparoscopy was performed on all ewes immediately before injections and four of the 11 ewes in each group possessed uterine horns that were filled with fluid. No fluid was judged to be in the horns of the remaining seven ewes in each group. On the basis of serum concentrations of progesterone and laparoscopies, oestradiol benzoate induced luteal regression in those ewes with uterine fluid and failed to induce luteal regression in those ewes lacking uterine fluid. Luteal function was unaffected in ewes that received the oil vehicle. These data suggest that premature luteal regression was not the reason for failure of occurrence of pregnancy. Immunization against $PGF_{2\alpha}$ was effective in blocking ovulation, but not in inhibiting oestrous behaviour or the formation of persistent luteal tissue. Treatment of immunized ewes with exogenous $PGF_{2\alpha}$ restored the ability of ewes to ovulate, providing further evidence for the involvement of $PGF_{2\alpha}$ in ovulation. The ability of oestradiol to induce luteolysis in immunized ewes was associated with the presence of uterine fluid.

Introduction

Theoretically, ewes could lamb twice a year if they conceived within 35 to 40 days *post partum*. The duration of *postpartum*

anoestrus is a constraint in establishing accelerated lambing programmes. Factors influencing the duration of this period include: season of the year and breed (Whiteman *et al.*, 1972), degree of mammary stimulation (Kann and Martinet, 1975), gestation (Al-Gubory and Martinet, 1986) and nutritional status (Dunn *et al.*, 1969).

Although the first preovulatory surge of luteinizing hormone (LH) and first ovulation occur early *post partum* (Shirar *et*

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al., 1989), they are generally not accompanied by oestrous behaviour and are associated with inadequate luteal function (Hunter, 1968; Land, 1971). Corpora lutea formed at first ovulation *post partum* can develop and actively secrete progesterone, but regress prematurely as a consequence of the uterine luteolytic agent prostaglandin $F_{2\alpha}$ (PGF_{2 α}) (Shirar *et al.*, 1989). *Postpartum* luteal dysfunction appears to result from increased release of, or corpus luteum sensitivity to, PGF_{2 α} (Troxel and Kesler, 1984). The lifespan of corpora lutea can be extended by intrauterine administration of indomethacin (Lewis and Warren, 1977), hysterectomy (Anderson, 1977) or by immunization against PGF_{2 α} (Scaramuzzi and Baird, 1976), all of which support a role for the uterus and its luteolytic agent PGF_{2 α} in controlling luteal function. Although inadequate luteal support is a major factor determining embryo viability in early *postpartum* ewes (Land, 1971), fertilization failure and inappropriate uterine environment also contribute to the poor pregnancy rates observed when breeding early *postpartum* ewes (Dawe and Fletcher, 1976; Wallace *et al.*, 1989a).

In ewes, uterine involution has been described as complete between days 17 and 45 *post partum* (Call *et al.*, 1976; Honnold, 1977; Shirar and Martinet, 1982). The involuting uterus may disrupt sperm transport causing infertility. By depositing semen directly into the uterine horns via laparoscopy, very high fertilization rates have been achieved (McKelvey *et al.*, 1985). However, when this was done in ewes induced to ovulate on day 28 *post partum*, no pregnancies were maintained despite the fact that fertilization occurred (McKelvey *et al.*, 1989). Pregnancy failure also occurred in early *postpartum* ewes that served as recipients for embryo transfer (Wallace *et al.*, 1989a). In both cases, while premature luteal regression was obviously associated with embryo loss, ewes exhibiting normal luteal function also failed to maintain pregnancies beyond the time of a normal luteal phase (McKelvey *et al.*, 1989; Wallace *et al.*, 1989a). These observations support the hypothesis that inadequate luteal support is not the only factor involved in ensuring embryo viability in early *postpartum* ewes (Wallace *et al.*, 1989a, b). It has been suggested that early *postpartum* ewes fail to maintain pregnancy as a result of the inability of the embryo to prevent luteolysis or an inappropriate uterine environment, or both (Wallace *et al.*, 1989a, b).

Active immunization against PGF_{2 α} has been described as an efficient method to induce persistent corpora lutea in ewes and cows (Scaramuzzi and Baird, 1976; Copelin *et al.*, 1989). Since first *postpartum* ovulations in these species are frequently associated with short-lived corpora lutea, which compromise embryo survival, we tested the hypothesis that pregnancy success could be enhanced in ewes destined to form short-lived corpora lutea by prolonging the lifespan of the corpus luteum.

During the course of the first experiment, we observed that gonadotrophin treatment, applied to ewes immunized against PGF_{2 α} , resulted in the formation of large cystic follicles that subsequently luteinized and secreted progesterone during the experimental period. A second set of experiments was therefore conducted to determine the effects of exogenously administered PGF_{2 α} on the function of the pre-ovulatory follicle and the effects of exogenous PGF_{2 α} and oestradiol benzoate on corpus luteum function in ewes immunized against PGF_{2 α} .

Materials and Methods

Experiment 1

Animals. Thirty-nine crossbred Rambouillet ewes bred to lamb during seasonal anoestrus were managed on range conditions and supplemented with hay and grain as required to maintain body condition. All ewes lambed within one week without experiencing any type of difficulty and had a gestation of normal duration. All ewes were lactating throughout the period of study and lambs (1 or 2) had free access to ewes at all times.

Antigen preparation. Forty millilitres of Lutalyse (5 mg PGF_{2 α} ml⁻¹; Upjohn Co., Kalamazoo, MI) was lyophilized and redissolved in 1.5 ml of double distilled water. PGF_{2 α} was conjugated to chicken ovalbumin using the carbodiimide method described by Bauminger and Wilchek (1980). Briefly, 400 mg of 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Sigma Chemical Co., St Louis, MO) was added to PGF_{2 α} while stirring. Chicken ovalbumin (164 mg; Grade V; Sigma Chemical Co.) was then dissolved in 5 ml double-distilled water, adjusted to pH 5.1 and added, while stirring, to the PGF_{2 α} -EDC solution. Stirring continued for 3 h at 4°C after which an additional 70 mg of EDC was added. The solution was stirred for another 4 h at room temperature and dialysed against double-distilled water for 24 h. After dialysis, the PGF_{2 α} -ovalbumin conjugate was lyophilized and stored at room temperature.

Immunization protocol. Four weeks before the expected date of lambing, ewes received primary immunizations of either PGF_{2 α} -ovalbumin conjugate ($n = 20$; immunized) or ovalbumin ($n = 19$; control) emulsified in Freund's complete adjuvant (Sigma Chemical Co.). Treatments were repeated 5 days after parturition (secondary immunizations) with reagents emulsified in Freund's incomplete adjuvant. Each ewe was injected intradermally in at least six sites on the inside of the rear leg with an emulsion of 5 mg PGF_{2 α} -ovalbumin or ovalbumin dissolved in 0.5 ml of physiological saline and 1.5 ml of Freund's complete (primary immunization) or incomplete (secondary immunization) adjuvant.

Experimental procedures. All ewes received an i.m. injection of 500 iu of pregnant mares' serum gonadotrophin (PMSG 6000; Intervet Inc., Millsboro, Delaware) on day 17 *post partum* and 48 h later an injection of 50 µg GnRH i.m. (Cystorelin; Ceva Laboratories, Overland Park, KS). Ovarian structures (corpora lutea and follicles) were assessed by laparoscopy 36 h after injection of gonadotrophin releasing hormone (GnRH). All laparoscopies were performed under xylazine hydrochloride (10 mg; Rompun, Haver, Shawnee, KS) anaesthesia. Ewes with recent ovulations underwent intrauterine insemination by laparoscopy (McKelvey *et al.*, 1985) with 0.1 ml of neat semen. Laparoscopies of the immunized group were repeated 1 and 8 weeks later to assess ovarian function. Blood samples were collected via jugular venepuncture every three days to assess serum concentrations of progesterone and antibody titres against PGF_{2 α} .

Assay procedures. Concentrations of progesterone were determined in duplicate samples of 50 and 200 µl serum per

tube by radioimmunoassay procedures described by Sheffel *et al.* (1982) and established in our laboratory (Keisler and Keisler, 1989). Minimum detectable concentrations were 5 pg per tube; recoveries averaged 89% and inter- and intra-assay coefficients of variation were 6% ($n = 12$) and 11% ($n = 5$), respectively. Assay blanks were less than 1 pg per tube.

Antibody titration. Specific binding of [³H]PGF_{2α} was assessed in duplicate aliquots of serum samples taken every week and defined as the percentage of [³H]PGF_{2α} that was specifically bound by serum at a 1:200 dilution. 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_{2α} (100 µl per tube; New England Nuclear, Boston, MA). After incubation, a neutralized charcoal-dextran suspension (700 µl per tube; Sigma Chemical Co.) was used to separate free from bound [³H]PGF_{2α}. After centrifugation at 1400 g for 10 min, the supernatant was decanted into 7 ml vials and 5 ml scintillation cocktail was added. Radioactivity in vials was counted in a Beckman LS1275 liquid scintillation counter for 2 min per sample.

Crossreactivity of antisera with PGE₁, PGE₂ and 15-keto-13,14-dihydroprostaglandin F_{2α} (PGFM) was tested and defined as the capacity of a 100-fold mass addition of these compounds to displace [³H]PGF_{2α} at an amount equivalent to 50% displacement (Abraham and Odell, 1970). No significant displacement was observed (i.e. crossreactivity < 1%).

Statistical analysis. Where appropriate, differences among treatment groups were tested by one-way analysis of variance or χ^2 analysis.

Experiment 2

Twenty-four seasonally anoestrous ewes immunized against PGF_{2α} and having persistent, induced, functional luteal structures (as assessed by serum concentrations of progesterone and laparoscopy) received 15 mg PGF_{2α} (Lutalyse) and 500 iu PMSG congruently, followed 48 h later by 50 µg GnRH. Follicular rupture was expected to start 24 h after injection of GnRH (Roberts *et al.*, 1985). Ewes were given a second injection of either PGF_{2α} ($n = 10$; 15 mg) or saline ($n = 14$) 16 h after the injection of GnRH. On the basis of observations of Murdoch *et al.* (1986), exogenous PGF_{2α} when given 16 h after GnRH, could reverse the inhibition of ovulation induced by indomethacin. Vasectomized rams, with painted briskets, were joined with ewes for detection of oestrus. Serum concentrations of progesterone and laparoscopies, performed one week after injections of PGF_{2α}, were used to assess ovarian activity.

Follicular fluid and serum from immunized ($n = 5$) and control ewes ($n = 5$) were collected 1 h before injection of GnRH to assess antibody titres. Ovaries from five immunized ewes, which subsequently had serum concentrations of progesterone indicative of luteal function for 50 days, were collected for histology. Ovaries were fixed in 10% neutral buffered formalin and processed for standard haematoxylin and eosin histology. Eight serial sections from each ovary were individually examined under light microscopy for integrity of the follicle wall and presence of luteal tissue.

Fig. 1. Temporal changes in (mean $\pm$ SEM) [³H]PGF_{2α} bound to a 1:200 dilution of serum from ewes immunized against PGF_{2α} (●; $n = 20$) and from controls (■; $n = 19$). Primary immunizations were given at week 0 and lambing occurred between weeks 4 and 5.

Experiment 3

Twenty-two ewes, immunized against PGF_{2α} and having serum concentrations of progesterone indicative of luteal function for a period greater than 60 days, were subjected to laparoscopic ovarian examinations. All ewes examined had ovarian luteal tissue and eight of those had enlarged uterine horns filled with fluid. Ewes were then randomly assigned among ewes with or without uterine fluid, to receive either oestradiol benzoate (750 µg) in oil ($n = 11$), or oil ($n = 11$) via i.m. injections on two consecutive days. This dose of oestradiol benzoate has been reported to result in luteal regression in cycling ewes when administered on days 9 and 10 of the oestrous cycle (Hixon and Flint, 1987). Blood samples were collected on days -8 and -1 relative to the first injection of oestradiol (day 0), and twice a day thereafter for 6 days. A blood sample was obtained and a second laparoscopy was performed on day 10 to assess ovarian activity and appearance of the uterus.

Results

Animals

All ewes developed granulomas at the site of immunization. No other complications were observed as a result of the immunization procedure.

Antibody titres

Antibody titres to PGF_{2α} increased from basal values 2–3 weeks after primary injection in immunized ewes and maintained a plateau at 30–40% specific binding throughout the experimental period (Fig. 1). Antibody titres assessed in follicular fluid were similar to those found in serum ($P > 0.1$).

Fig. 2. Representative ovarian structures visualized on the ovary of an (a) immunized or (b) control ewe examined by laparoscopy 36 h after injection of gonadotrophin-releasing hormone (GnRH).

Experiment 1

None of the immunized ewes ($n = 20$) ovulated in response to the PMSG and GnRH treatments. However, ten ewes had large cystic follicles, occupying most of the ovarian surface, which appeared smooth, with prominent blood vessels and an absence of the ovulatory stigma (Fig. 2a). These unruptured follicles underwent subsequent luteinization, as determined from serum concentrations of progesterone throughout the period studied (Fig. 3), and by laparoscopies performed 1 and 8 weeks after the hormonal treatments. No significant ovarian structures were observed in the remaining ten immunized ewes.

Most of the ewes in the control group ovulated (18 of 19) as determined by laparoscopy performed 36 h after the GnRH (Fig. 2b). At that time, all ewes having ovarian follicles with ovulatory stigma were inseminated into the uterus. Mean ovulation rates were 3.3 ± 0.42 (mean $\pm$ SEM). Fifteen of the 18 ewes that ovulated had subsequent periods of at least 21 days duration of increased serum concentrations of progesterone, indicative of luteal function (Fig. 4). By seventy days *post partum*, serum concentrations of progesterone had returned to basal values in all ewes. Luteal function was characterized in control ewes by concentrations of progesterone which: (a) remained at basal values despite the presence of corpora lutea confirmed by laparoscopy ($n = 3$), (b) remained high for 21–23 days ($n = 4$) or (c, d, e) remained high for at least 30 days, but had reached basal values by day 70 (i.e. day 50 after insemination; $n = 4, 4$ and 3 , respectively; Fig. 4).

Fig. 3. Serum concentrations (mean $\pm$ SEM) of progesterone in ewes immunized against prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) ($n = 10$). Ewes were given pregnant mares' serum gonadotrophin (PMSG) (500 iu) and gonadotrophin-releasing hormone GnRH (50 μ g) on days 17 and 19 *post partum*, respectively.

At the time of insemination, the two uterine horns were similar in diameter and appeared to have returned to their normal nonpregnant position.

Experiment 2

Exogenous $PGF_{2\alpha}$ induced functional and structural luteal regression in all immunized ewes as determined from serum concentrations of progesterone and laparoscopy. Subsequent PMSG and GnRH treatments induced oestrus in 86% of treated ewes. Exogenous $PGF_{2\alpha}$, given 16 h after GnRH to immunized ewes, induced follicular rupture in eight of ten ewes, whereas only two of 14 ewes not receiving $PGF_{2\alpha}$ ovulated ($P < 0.01$). All ewes not ovulating formed large follicles, similar in appearance to those in Expt 1, which subsequently luteinized. The immunized ewes, which did not receive a second injection of $PGF_{2\alpha}$ and yet ovulated, had antisera titres of $PGF_{2\alpha}$ similar to those of ewes that formed unruptured follicles (i.e. 35–40% binding).

Ovaries from five ewes that had high serum concentrations of progesterone for 50 days without a defined ovulatory stigma were collected for histological examination. Integrity of the follicle wall and presence of luteal cells were observed. Two of these ewes had enlarged uterine horns filled with fluid.

Experiment 3

Serum concentrations of progesterone remained high in ewes that received oestradiol benzoate and lacked fluid-filled uterine horns as well as in those ewes that received the oil vehicle (Fig. 5). By the third day after the final injection of oestradiol, concentrations of progesterone had returned to basal values in all of four ewes that received oestradiol and possessed fluid-filled

Fig. 4. Serum concentrations of progesterone from day -4 to day 70 *post partum* (day 0 = parturition) in control ewes inseminated (intrauterine) on day 20 *post partum*. Serum concentrations of progesterone remained: (a) basal despite the presence of corpora lutea confirmed by laparoscopy ($n = 3$); (b) high for 21 days ($n = 4$); (c) high for at least 30 days but below 1 ng ml⁻¹ ($n = 4$); (d) between 1–3 ng ml⁻¹ ($n = 4$) or (e) above 3 ng ml⁻¹ ($n = 3$). By day 70 *post partum* serum concentrations of progesterone in all ewes returned to basal values.

uterine horns. Luteal regression and apparent reabsorption of the uterine fluid was verified by laparoscopy.

Discussion

The results obtained were unexpected in regard to the initial objectives of this study. First, early *postpartum* ewes were predicted to have a greater incidence of short-lived corpora lutea than observed in this study (Wallace *et al.*, 1989a; Shirar *et al.*,

1989). Second, immunization against PGF_{2α} was not expected to block ovulation but to result in corpora lutea with long life-spans (Scaramuzzi and Baird, 1976; Ronayne *et al.*, 1990). Both findings compromised the initial objective of this study which was to improve pregnancy rates in early *postpartum* ewes by extending the lifespan of corpora lutea that were anticipated to be short lived.

Short-lived corpora lutea in sheep have been associated with endocrine events that occur during the follicular phase or luteal phase of the oestrous cycle (Hunter 1991). Early *postpartum*

Fig. 5. Serum concentrations of progesterone in relation to the time of injection of (a, b) oil and (c, d) oestradiol benzoate injections. (a) ($n = 4$) and (c) ($n = 4$) refer to ewes with uterine horns filled with fluid and (b) ($n = 7$) and (d) ($n = 7$) refer to ewes without uterine fluid.

ewes induced to ovulate by administration of PMSG after a period of progestagen treatment will result in a high incidence of short-lived corpora lutea (McKelvey *et al.*, 1989; Wallace *et al.*, 1989a). In the studies conducted by McKelvey, Wallace, and co-workers the high incidence of short-lived corpora lutea in early *postpartum* ewes was observed after these ewes were used as recipients for transferred embryos or were not inseminated into the uterus. In Expt 1, we used a combination of PMSG and GnRH which was effective in inducing ovulation and a subsequent period of prolonged luteal function. In this experiment, intrauterine inseminations were followed by a period of at least 21 days of high progesterone secretion in 15 of the 18 ewes. We suggest that luteolysis was prevented from occurring at the expected time following ovulation owing to the antiluteolytic effects of the embryo. Premature luteal regression and embryo loss in early *postpartum* ewes has been associated with the inability of the embryo to prevent luteolysis (Wallace *et al.*, 1989a).

The role of prostaglandin in the mechanism of follicular rupture has been documented in several species including rats (Orczyk and Behrman, 1972), rabbits (O'Grady *et al.*, 1972), pigs (Ainsworth *et al.*, 1979), sheep (Murdoch and Dunn, 1983), monkeys (Wallach *et al.*, 1975) and women (Priddy *et al.*, 1990). In ewes, this role is supported by observations that (i) preovulatory follicles synthesize $\text{PGF}_{2\alpha}$, PGE_2 and progesterone in response to the surge of LH (Murdoch *et al.*, 1981; Murdoch and Dunn, 1982); (ii) ovulation can be prevented by administration of the cyclooxygenase inhibitor indomethacin (Murdoch and Dunn, 1983); and (iii) exogenous prostaglandins can reverse the blockade of ovulation by indomethacin (Murdoch *et al.*, 1986). Recently, it has been suggested that $\text{PGF}_{2\alpha}$ might not

be as essential to ovulation as was previously thought (Espey *et al.*, 1986; Murdoch and McCormick, 1991; Tanaka *et al.*, 1991). Follicular rupture can occur in the absence of a preovulatory rise in ovarian $\text{PGF}_{2\alpha}$ (Murdoch, 1988). Tanaka *et al.* (1991) suggested that products of the lipoxygenase pathway may be more important for ovulation than prostanoids; however, this could not be demonstrated in sheep, as an inhibition of leukotriene synthesis did not block ovulation (Carvalho *et al.*, 1989). Murdoch and McCormick (1991) suggested that the anti-ovulatory effects of indomethacin in sheep may be unrelated to its ability to inhibit prostaglandin synthesis, but may interfere with leucocyte chemoattraction by inhibition of follicular collagenolysis. A role for systemic $\text{PGF}_{2\alpha}$ in ovulation in sheep has also been questioned because active and passive immunization against $\text{PGF}_{2\alpha}$ resulted in the formation of persistent luteal structures that were morphologically similar to those that formed during the normal oestrous cycle (Fairclough *et al.*, 1981; Ronayne *et al.*, 1990). In our study, immunization against $\text{PGF}_{2\alpha}$ blocked ovulation as determined by the absence of ovulatory stigma and by histological examination of the luteal structures. The observed unruptured follicles were similar to those described by Murdoch and Dunn (1983) and underwent subsequent luteinization, which supports the concept that formation of luteal tissue is not dependent on $\text{PGF}_{2\alpha}$ (Murdoch and Dunn, 1983). Titres of antibodies against $\text{PGF}_{2\alpha}$ in follicular fluid were similar to those observed in serum, which was not surprising in light of the fact that leakage of proteins with molecular weights as large as 900 000 into the follicular fluid can occur (Edwards, 1974). The fact that other researchers failed to block ovulation in $\text{PGF}_{2\alpha}$ immunized cows (Copelin *et al.*, 1989) and ewes (Scaramuzzi and Baird, 1976; Ronayne *et al.*,

1990) may be related to antibody titres. In work conducted by Copelin *et al.* (1989), cows ovulated while PGF_{2α} antibody titres were increasing. It is possible that the titres achieved by the time that follicular rupture occurred were not sufficient to block ovulation but were adequate to prevent luteal regression. Ronayne *et al.* (1990) described the induced corpora lutea in immunized ewes as morphologically similar to those that formed during the normal oestrous cycle, and persisted for the approximate duration of the corpus luteum of pregnancy. They suggested that the corpora lutea may have a predetermined lifespan that is species specific.

In a different study, we observed that pregnancy could be established in ewes immunized against PGF_{2α} (two out of three ewes) when the inhibitory effects of immunization were reversed by administration of a second injection of PGF_{2α}, given as reported in Expt 2. These ewes did not experience any difficulties at parturition (C. Bettencourt and D. Keisler, unpublished).

The presence of fluid within the uterine horns of immunized ewes has been described by other investigators and could be due to a prolonged period of progesterone exposure (Fairclough *et al.*, 1981; Ronayne *et al.*, 1990). Studies performed in ovariectomized progesterone-treated ewes have identified PGF_{2α} as the primary prostaglandin present in uterine fluid (Moffatt *et al.*, 1987). Bazer *et al.* (1979) observed an accumulation of uterine fluid very rich in PGF_{2α} in the nonpregnant uterine horn of unilaterally pregnant ewes, when they restricted the conceptus to the contralateral uterine horn. Oestradiol benzoate, given to immunized ewes possessing persistent luteal structures and fluid-filled uterine horns, resulted in luteal regression and reabsorption of the uterine fluid. In contrast, the luteolytic effects of oestradiol were not observed in immunized ewes possessing persistent luteal structures and lacking fluid-filled uterine horns. Treatment of ewes possessing or lacking fluid-filled uterine horns with oil also did not affect luteal function. On the basis of these observations, it is suggested that the presence of uterine fluid may be associated with the ability of oestradiol to facilitate luteolysis.

Since short-lived corpora lutea were observed in three of 18 control ewes in Expt 1, we suggest that an inadequate uterine environment was the major cause of pregnancy failure. Inappropriate uterine environment has been described as a major factor influencing pregnancy rates in *postpartum* ewes (Wallace *et al.*, 1989a, b). In addition, first ovulation in early *postpartum* ewes was frequently associated with short luteal phases (Shirar *et al.*, 1989). In the present study, the incidence of short-lived corpora lutea was much lower than was reported by other researchers (Land, 1971; Shirar *et al.*, 1989). Because 83% of control ewes secreted progesterone for at least 21 days after intrauterine insemination, we suggest that fertilization may have occurred, but owing to an inappropriate uterine environment, no pregnancies were maintained. McKelvey *et al.* (1985, 1989) described uterine insemination as a good method for obtaining viable embryos and for achieving high fertilization rates in day 28 *postpartum* ewes. However, for those embryos to develop, they had to be transferred to the uterus of a cycling ewe (Wallace *et al.*, 1989b).

The syndrome of unruptured preovulatory follicles has been extensively investigated in sheep by Murdoch and co-workers who used nonsteroidal anti-inflammatory agents, such as

indomethacin, to block ovulation. To our knowledge, there are no publications reporting the same type of cystic follicles as a result of immunoneutralization against PGF_{2α}. Murdoch *et al.* (1986) reported that PGF_{2α} and PGE₂, given 16 h after GnRH, could reverse the inhibition of ovulation induced by indomethacin. As 99% of injected PGF_{2α} is metabolized during a single passage through the lungs (Davis *et al.*, 1980), the time of administration of PGF_{2α} with regard to the surge of LH may be critical to reverse the inhibitory effects of indomethacin. Because we used a combination of PMSG and GnRH in our experiments to induce ovulation, it was difficult to predict the time of the preovulatory surge of LH, as some ewes may have had a surge of LH prior to injection of GnRH. This could explain why the exogenous PGF_{2α} was not fully effective in inducing follicular rupture in immunized ewes in Expt 2.

The mechanism by which PGF_{2α} interferes with follicular rupture has not been fully elucidated (for review see Espey, 1980; Lipner, 1988) but it appears to involve activation of follicular collagenolysis (Murdoch *et al.*, 1986). Follicular progesterone (Murdoch and Dunn, 1982), PGE₂ (Murdoch *et al.*, 1981), histamine (Szego and Gitin, 1964), prolactin (Hamada *et al.*, 1980) and plasminogen activator (Beers *et al.*, 1975) also play roles in the mechanism of follicular rupture.

The development of luteinized unruptured follicles has been described in women treated with nonsteroidal anti-inflammatory agents in association with a decrease in the synthesis of ovarian prostaglandins (Priddy *et al.*, 1990). It has been suggested that a method of inhibiting follicular synthesis or ovarian actions of prostaglandins, but lacking the hazardous peripheral side effects of drugs such as indomethacin, could have potential use as a nonhormonal contraceptive method (Murdoch and Dunn, 1983; Killick and Elstein, 1987; Priddy *et al.*, 1990). Since the immuno-inactivation of PGF_{2α} is confined to the extracellular compartments (Scaramuzzi and Baird, 1976) and crossreactivity of antisera with other prostaglandins was less than 1%, we speculate that the peripheral side effects of immunization against PGF_{2α} may be minimal. The potential use of this method in terms of regulating fertility in other species deserves further research.

In conclusion, we suggest that pregnancy failure in early *postpartum* ewes may have resulted from an inappropriate uterine environment rather than from inadequate luteal support in these studies. Immunization against PGF_{2α} was effective in blocking ovulation but not in inhibiting oestrous behaviour or formation of persistent luteal tissue. Exogenous PGF_{2α} resulted in luteal regression and partially overrode the negative effects of immunization against PGF_{2α} upon follicular rupture, which provides further evidence of the involvement of PGF_{2α} in the mechanism of ovulation. Immunization against PGF_{2α} can be a useful method to control ovulation and luteal function in sheep. The potential use of this method in terms of regulating fertility in other species deserves further research.

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