LUTALYSE® INDUCES UTERINE-OVARIAN $PGF_{2\alpha}$ RELEASE IN SHEEP: A CRITICAL COMPONENT OF INDUCED LUTEOLYSIS

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(ABSTRACT)

Exogenous $PGF_{2\alpha}$ (see Appendix I for definitions of abbreviations) is luteolytic in midluteal (i.e., d 9 of a 17 d estrous cycle) sheep. However, the pharmacokinetic responses to $PGF_{2\alpha}$-induced luteolysis are not known. This study (Exp. 1 and 2) was conducted to determine several pharmacokinetic responses to two dosing regimens of Lutalyse® ($PGF_{2\alpha}$). Experiment 1 was a 2 x 2 factorial design, with Lutalyse and H/Ox as main effects. Lutalyse (15 mg) was injected i.m., and blood samples were collected, relative to the time of injection, from the vena cava at points cranial and caudal to the uteroovarian vein. Progesterone and $PGF_{2\alpha}$ were measured in blood plasma. The $PGF_{2\alpha}$ concentrations were greater in H/Ox and sham H/Ox ewes treated-with Lutalyse® than in control ewes. Peak concentrations of $PGF_{2\alpha}$ were greatest in sham H/Ox Lutalyse-treated ewes, indicating that the uterus and(or) ovaries secrete $PGF_{2\alpha}$ in response to exogenous $PGF_{2\alpha}$. In Lutalyse-treated ewes, progesterone concentrations decreased by 50% within 8 h after treatment. The design of Exp. 2 was also a 2 x 2 factorial, with Lutalyse (2 x 5mg at 3 h intervals) and H/Ox as main effects. Prostaglandin $F_{2\alpha}$ and PGFM were measured in blood plasma collected, relative to the time of injections, from the vena cava at points cranial and caudal to the uteroovarian vein. The $PGF_{2\alpha}$ concentrations were greater in sham H/Ox ewes treated-with Lutalyse than in control ewes. Peak concentrations of $PGF_{2\alpha}$ were greater in sham H/Ox than in ewes in all other treatment groups, indicating again that the uterus and(or) ovaries secrete $PGF_{2\alpha}$ in response to exogenous $PGF_{2\alpha}$. In general, PGFM concentrations increased in a pattern similar to that of $PGF_{2\alpha}$ after Lutalyse injection; although there was a short delay of

approximately 2 min. Caudal vena caval $PGF_{2\alpha}$ concentrations in H/Ox Lutalyse-treated ewes were greater than that after saline injection, which indicates that metabolism may depend on the presence or absence of the uterus and(or) ovaries. In Exp. 1, caudal $PGF_{2\alpha}$ concentrations were greater in H/Ox ewes injected with 15 mg of Lutalyse than in ewes in all other treatments . However, in response to 5 mg Lutalyse, caudal $PGF_{2\alpha}$ concentrations were greater in sham H/Ox ewes than in all other treatment groups. This indicates that the larger dose in H/Ox ewes supersedes the capacity of the lung and kidney to dispose of $PGF_{2\alpha}$, and $PGF_{2\alpha}$ is more tightly regulated in intact ewes. The lungs and kidney are capable of metabolizing the smaller dose of Lutalyse but not the resulting $PGF_{2\alpha}$ production in intact ewes.

A short validation experiment was conducted to determine the effects of sampling location on progesterone, $PGF_{2\alpha}$, and PGFM concentrations. Sampling location did not affect the mean concentration of progesterone or PGFM. However, location affected the mean $PGF_{2\alpha}$ concentration. The concentration of $PGF_{2\alpha}$ was greater ($P < .05$) in saphenous vein and caudal vena caval blood plasma than in jugular plasma.

In summary, the uterus and(or) ovaries produce and regulate $PGF_{2\alpha}$ concentration in response to Lutalyse. It is speculated that a threshold $PGF_{2\alpha}$ concentration or duration of the $PGF_{2\alpha}$ peak concentration exists because these $PGF_{2\alpha}$ responses differed in intact Lutalyse-treated and saline-treated ewes in both experiments. The mean cranial $PGF_{2\alpha}$ concentration, peak concentration, duration of the peak, increase in $PGF_{2\alpha}$, and AUC were greater in response to 15 mg of Lutalyse, although the two smaller doses have been shown to be more efficacious in inducing luteolysis. The second dose of $PGF_{2\alpha}$ may act by mimicking pulses of $PGF_{2\alpha}$, and initiate the luteolytic cascade two times.

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STATEMENT OF THE PROBLEM

Lutalyse® ($PGF_{2\alpha}$) is luteolytic in sheep. Currently, two dosing regimens are used, with different efficacies. A bolus i.m. injection of 15 mg of Lutalyse is effective approximately 75% of the time, whereas, two 5 mg i.m. doses given 3 to 4 h apart have an efficacy value of approximately 90% (Hawk and Cooper, 1977; Hawk et al., 1978; Hackett and Robertson, 1980). In either case, the dose administered is rapidly metabolized; as much as 99% of the $PGF_{2\alpha}$ is metabolized within the first pass through the lungs (Samuelsson et al., 1971; Davis et al., 1980). Thus, if a 5 mg dose is uniformly absorbed, approximately .05 mg would remain and then be diluted in 5 to 7 L of blood, i.e., an estimated concentration of 7 to 10 ng/mL of $PGF_{2\alpha}$. It is feasible that the 7 to 10 ng/mL of $PGF_{2\alpha}$ reaching the uterus and ovaries would be sufficient to induce luteolysis. A similar concentration (12.1 ± 4.6 ng/ml) was found in the uterine vein during the periovulatory period (Land et al., 1976). The $PGF_{2\alpha}$ seems to be transferred from the uterus to the ovarian artery via a countercurrent exchange mechanism with a transfer efficiency of approximately 1% (Land et al., 1976). One percent of the uterine venous concentration (70 to 100 pg/mL) in response to a 5 mg Lutalyse® injection is similar to the periovulatory ovarian arterial concentrations of $PGF_{2\alpha}$ (158 ± 40 pg/mL; Land et al., 1976). However, many tissues utilize $PGF_{2\alpha}$; so, the actual amount of $PGF_{2\alpha}$ reaching the uterus and ultimately the ovary should be substantially less than 7 to 10 ng/mL.

The uterus is thought to release significant quantities of $PGF_{2\alpha}$ in response to endogenous $PGF_{2\alpha}$ (McCracken, 1980; McCracken et al., 1984; Silvia et al., 1991). Quantitative responses of the uterus to exogenous $PGF_{2\alpha}$ are unknown. Douglas and Ginther (1973) found luteolysis to be dependent on the dose of $PGF_{2\alpha}$. However, this does not explain how the smaller doses of $PGF_{2\alpha}$ are more efficacious than a single larger dose. This is especially intriguing when considered mathematically. Assuming uniform absorption and distribution, .15 mg of $PGF_{2\alpha}$ would remain after the 15 mg injection and

.05 mg from a 5 mg injection. Even if a 5 mg injection of Lutalyse® is given twice, the total amount of $PGF_{2\alpha}$ that may actually reach the uterus would be less than that with a 15 mg injection. One may speculate that the uterus responds to the first dose of $PGF_{2\alpha}$, and uterine $PGF_{2\alpha}$ secretion is markedly enhanced. The second dose may amplify this response. Around the time of luteolysis, pulsatile $PGF_{2\alpha}$ secretion is initiated and luteolysis occurs. Schramm et al. (1983) showed that pulsatile $PGF_{2\alpha}$ was more efficacious in inducing luteolysis than was a continuous infusion of $PGF_{2\alpha}$. Thus, a second dose of exogenous $PGF_{2\alpha}$ may act as a pulse of $PGF_{2\alpha}$. Based upon this information, the purpose of the study reported in this thesis was to characterize the response to one 15 mg (Exp. 1) and two 5 mg (Exp. 2) i.m. injections of Lutalyse®.

REVIEW OF LITERATURE

Exogenous $PGF_{2\alpha}$ Is Luteolytic

Based on earlier experiments showing that ovarian activity is dependent on uterine activity (Loeb, 1923; Wiltbank and Casida, 1956) and that $PGF_{2\alpha}$ is found in high concentrations in the endometrium (Pickles, 1967) and in menstrual fluid (Pickles, 1957), Pharriss and Wyngarden (1969) examined the role of $PGF_{2\alpha}$ in reproduction. Their experiment, in which infusion of $PGF_{2\alpha}$ into the uterus or right heart shortened pseudopregnancy in rats from 14 to 7 d and decreased progesterone concentrations, was the first to indicate that exogenous $PGF_{2\alpha}$ could influence reproduction. Exogenous $PGF_{2\alpha}$ is now used for estrus synchronization, induction of abortion, treatment of chronic endometritis, and induction of parturition in some livestock species, including sheep. Several experiments indicate that intrafollicular, intraarterial, intrauterine, i.v., and i.m. administration of $PGF_{2\alpha}$ is luteolytic in sheep (Inskeep et al., 1975, McCracken et al., 1970; Barrett et al., 1971; Thorburn and Nichol, 1971; Douglas and Ginther, 1973; Inskeep 1973). These $PGF_{2\alpha}$-induced luteolysis experiments show estrus synchronization to be dose and route of administration dependent in sheep. In sheep, two i.m. dosing regimens are usually used for estrus synchronization in midluteal phase ewes; either one 15 mg dose of $PGF_{2\alpha}$ or two 5 mg doses of $PGF_{2\alpha}$ given 3 to 4 h apart are used. However, the two 5 mg doses of $PGF_{2\alpha}$ seem to be more efficacious, i.e., inducing luteolysis and estrus, than a single 15 mg bolus dose of $PGF_{2\alpha}$ (Hawk and Cooper, 1977; Hawk et al., 1978; Hackett and Robertson, 1980). Even though the role of $PGF_{2\alpha}$ in spontaneous luteolysis and hormonal regulation of endogenous uterine $PGF_{2\alpha}$ secretion during luteolysis have been investigated, the mechanisms involved in exogenous $PGF_{2\alpha}$-induced luteolysis remain unknown.

$PGF_{2\alpha}$ As a Luteolysin

Uteroovarian Relationships: Anatomical and Physiological

Loeb (1923) first proposed the concept that ovarian activity was dependent on uterine function. In completely hysterectomized guinea pigs, the CL is "preserved"; in partially hysterectomized guinea pigs, luteolysis is delayed (Loeb, 1923). Thus, the uterus must be producing a "luteolytic" substance. This response also occurs in sheep (Wiltbank and Casida, 1956; Ginther, 1967; Anderson et al., 1969; Hunter, 1970). In sheep, removing the uterine horn adjacent (ipislateral) to the ovary with the CL will delay luteolysis; however, removing the contralateral uterine horn does not affect the timing of luteolysis (Inskeep and Butcher, 1966; Ginther, 1967; McCracken and Caldwell, 1969). Thus in sheep, the uterine-dependent luteolytic effect must be exerted locally. Autotransplant experiments also provide evidence for a local uteroovarian relationship. Luteolysis does not occur if the ovary is relocated to the neck (Goding et al., 1967). However, if the uterus and ovaries are autotransplanted to the neck, the CL regress normally (Harrison et al., 1968; McCracken et al., 1971). A similar local effect has also been observed in rats, hamsters, and cows but not in horses or rabbits (Scott and Rennie, 1970; Ginther and First, 1971).

Inskeep and Butcher (1966) postulated three plausible mechanisms for uterine control of luteal function: venoarterial, lymphatic, or by diffusion through the broad ligament. There are anatomical bases for these local pathways (Figure 1). The broad ligament is inundated with blood vessels and nerves that support the reproductive tract. Morris and McIntosh (1971) showed that the lymphatic system of the uterus and ovaries join near the ovarian branch of the uteroovarian vein. Complex branching and close contact between arterial and venous blood supplies are evident in the reproductive vasculature (Del Campo and Ginther, 1973). The highly tortuous ovarian artery, which

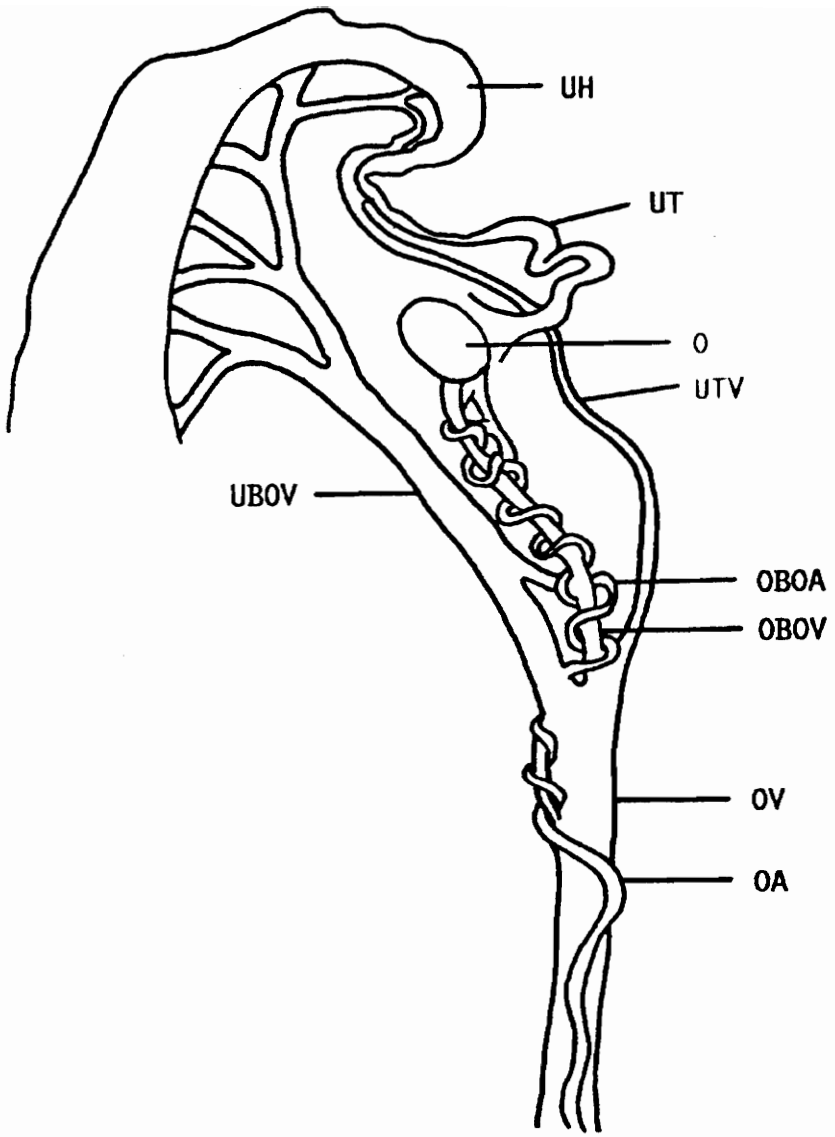

Figure 1. Local uterine and ovarian venoarterial pathways in the ewe (OA=ovarian artery, OV=ovarian vein, UBOV=uterine branch of ovarian vein, UBOA=uterine branch of ovarian artery, OBOV=ovarian branch of ovarian vein, OBOA=ovarian branch of ovarian artery, UTV=uterine tubal veins, O=ovary, UT=uterine tube, i.e., oviduct, UH=uterine horn). Permission for use of figure granted by E. Stephens and S. Fortin.

branches and supplies the ovary, uterus, and broad ligament rests upon the uteroovarian vein. The uteroovarian vein pools venous blood from the uterine branch of the ovarian vein, ovarian branch of the ovarian vein, and oviducts. Thus, arterial and venous blood are separate but in close proximity to each other.

Evidence that the vascular system is involved in luteolysis was first supplied by Kiracofe et al. (1966). Unilateral ligation of the uterine vein, but not uterine artery, prevented luteolysis. Barrett et al. (1971) showed that separation of the uteroovarian vein from the ovarian artery also blocked luteolysis (Barrett et al., 1971; McCracken et al., 1971). Baird and Land (1973) showed that luteolysis is dependent on the uterine vein and its anastomoses. Studies reported from Ginther's laboratory indicated that a uterine luteolysin may be transferred from the uterine branch of the ovarian vein to the ovarian branch of the ovarian artery, and that the luteolysin is then transported to the ovary (Ginther et al., 1973). Thus, the transfer was from the venous supply to the arterial system.

Uterine Venous Luteolysin

McCracken and colleagues, as well as Caldwell and Moor (1971), provided evidence of a uterine venous luteolytic factor. Plasma collected from the uterine and jugular veins of d 1 (d 0 is the day of estrus) ewes and uterine venous plasma from d 8 ewes, freeze dried, and then injected into the ovarian artery of d 8 luteal phase ewes did not induce luteolysis. In contrast, uterine venous plasma collected on d 14 shortened the length of the estrous cycle or reduced progesterone concentrations when infused into the ovarian artery. McCracken et al. (1970), using uteroovarian transplant donor ewes and ovarian transplant recipient ewes, demonstrated the presence of a luteolysin in uteroovarian venous blood from d 15 ewes. Plasma from d 2 and 10 donor ewes did not induce luteolysis as measured by progesterone concentration and estrus. Thus, the uterus

around the time of luteolysis secretes a luteolytic substance that must somehow reach ovarian arterial blood.

$PGF_{2\alpha}$: "**The**" Uterine Luteolysin

Attempts to isolate the uterine "luteolytic" substance were futile until 1969, when Pharriss and Wyngarden showed $PGF_{2\alpha}$ to shorten rat pseudopregnancy (Pharriss and Wyngarden, 1969). Although, Pharriss and Wyngarden incorrectly asserted that $PGF_{2\alpha}$ acted through venoconstriction on the uteroovarian vein, they are credited with providing evidence for $PGF_{2\alpha}$ as the luteolysin. However, a prostaglandin-like substance was first detected in fluid from the prostate gland (Goldblatt, 1933; von Euler, 1936) and in the human ovary in 1937 (von Euler and Hammartröm, 1937). Later experiments in humans detected prostaglandins in menstrual fluid (Pickles, 1957) and in endometrial tissue (Pickles, 1967). Was this substance produced by the uterus? Was it luteolytic?

Pharriss et al. (1972) provided an excellent review of prostaglandins in luteal function. According to Pharriss and associates, $PGF_{2\alpha}$ was "**the**" uterine luteolysin based upon the following criteria:

1. $PGF_{2\alpha}$ is luteolytic in all species in which luteolysis is dependent on the uterus.
2. $PGF_{2\alpha}$ induces luteolysis when administered systemically and locally.
3. $PGF_{2\alpha}$ is found in or released from the endometrium around the time of uterine- induced luteolysis.
4. Pregnancy involves maintenance of the CL; therefore, either $PGF_{2\alpha}$ is in low concentrations or inactive during pregnancy.
5. Functional, biochemical, and structural luteolysis should be similar in spontaneous and $PGF_{2\alpha}$-induced luteolysis.

In rats (Pharriss and Wyngarden, 1969; Labhsetwar, 1972), hamsters (Labhsetwar, 1972), guinea pigs (Blatchley and Donovan, 1969), and sheep (McCracken, et al., 1971),

in which luteolysis is dependent on uterine activity, $PGF_{2\alpha}$ is luteolytic. In humans (Wiqvist et al., 1970) and rhesus monkeys (Kirton et al., 1970), in which luteolysis is independent of the uterus, systemic administration of $PGF_{2\alpha}$ is not luteolytic. However, $PGF_{2\alpha}$ receptors are present in human luteal tissue indicating that local $PGF_{2\alpha}$ production by the ovary may contribute to luteal regression (Powell et al., 1974b).

Numerous experiments provide evidence for the presence of $PGF_{2\alpha}$ in uterine venous blood around the time of luteolysis (Bland et al., 1971; Harrison et al., 1972; Thorburn et al., 1973). Bland et al. (1971) reported an increase from 3 ng/mL in uterine venous blood on d 13 of the cycle of ewes to greater than 8 ng/mL on d 14 from the same collection site. Also, $PGF_{2\alpha}$ was detected in sheep endometrium (Wilson et al., 1972).

The CL is maintained for the first 50 d of pregnancy in ewes (Rowson and Moor, 1967). Therefore, either $PGF_{2\alpha}$ is in low concentrations or inactive during pregnancy. According to Pexton et al. (1975), luteolysis in pregnant ewes (d 15) is not prevented by changes in the amount of $PGF_{2\alpha}$ transported to or taken up by ovarian and luteal tissue. Therefore, the actions of $PGF_{2\alpha}$ must be prevented by either an additional luteotrophic or antiluteolytic factor. Rowson and Moor (1967) showed an antiluteolytic effect of the embryo on the ipislateral CL (1966). Thus, the CL is maintained during the early stages of pregnancy due to the prevention of $PGF_{2\alpha}$-induced luteolysis.

Additional evidence for $PGF_{2\alpha}$ as a luteolysin is provided by comparing $PGF_{2\alpha}$-induced and spontaneous luteal regression in rats. There is a shift from the production of progesterone to 20-α-dihydroprogesterone early in luteal regression in both cases (Lindner and Shelesnyak, 1967; Pharriss and Wyngarden, 1969). In hamsters, regressing luteal tissue, whether induced or spontaneous, show similar structural disorganization. Cortell (1975) and McClellan et al. (1977) showed $PGF_{2\alpha}$-induced luteolysis to be similar to natural luteolysis in ewes.

If $PGF_{2\alpha}$ produced by the endometrium can be detected in uterine venous blood during the periovulatory period and can be used to induce luteal regression in a

biochemical, structural, and morphologically similar fashion as spontaneous regression, could it be "**the**" luteolytic agent involved in CL regression? Indomethacin, a prostaglandin synthesis inhibitor, prevents luteolysis (Armstrong and Grinwich, 1972). Also in sheep, immunization against $PGF_{2\alpha}$ results in CL maintenance (Fairclough et al., 1976; Scaramuzzi and Baird, 1976). Thus, $PGF_{2\alpha}$ seems to be "**the**" uterine luteolytic agent involved in the demise of the CL.

Countercurrent Exchange of $PGF_{2\alpha}$

If complete hysterectomy and ipislateral hysterectomy can delay luteolysis, then the uterus must be producing a substance, which when delivered from uterine venous blood to the ovary, influences luteolysis. If periovulatory venous plasma can induce luteolysis in midluteal phase ewes and if separation of the uteroovarian vein from the ovarian artery prohibits luteolysis (Barrett, et al., 1971), then an exchange of this luteolytic substance must occur between the shared surface area and not systemically. McCracken et al. (1971) proposed a countercurrent mechanism to explain the dependency of ovarian function on venous blood.

Oviductal transfer and direct diffusion from the uterus to the ovary have been discounted (McCracken et al., 1971). Lymphatic drainage was initially discounted, but more recent evidence indicates that some $PGF_{2\alpha}$ is exchanged through the lymphatic system (Staples et al., 1982; Abdel Rahim, 1984). According to McCracken (1971), uterine venous blood empties into the uteroovarian vein where it is transferred to the ovarian artery and ultimately to the ovary. Diffusion from the uterine vein (high $PGF_{2\alpha}$ concentrations) to the ovarian artery (low $PGF_{2\alpha}$ concentrations) represents the most logical explanation for the exchange. In fact, the walls of these vessels are thinner wherever they exist in direct apposition to each other (Del Campo and Ginther, 1974). Thus, either countercurrent exchange or shunting occurs. Coudert et al. (1974) showed

no direct uteroovarian pathway (shunt) for transfer from the uterus to the ovary using both xenon-labeled gas and iodinated human serum albumin. Albumin is a large poorly diffusible molecule; labeled albumin did not appear in the ovarian artery after injection into the uterine vein. However, the detection of labeled xenon, a highly diffusible gas, in the ovarian arterial blood 20 s after infusion into the uterine vein (Coudert et al., 1974) shows that a local transfer mechanism must exist. Transfer efficiency of xenon is approximately 1%. Periovulatory arterial plasma $PGF_{2\alpha}$ (158 ± 40 pg/mL) is approximately 1% of periovulatory uterine venous $PGF_{2\alpha}$ (12,147 ± 4,583 pg/mL; Land et al., 1976). These experiments indicate that the countercurrent mechanism of $PGF_{2\alpha}$ transfer proposed by McCracken represents the most feasible mechanism of transfer of $PGF_{2\alpha}$ from the uterus to the ovary. Thus, $PGF_{2\alpha}$ is the uterine luteolysin that is transferred to the ovary via a countercurrent mechanism, and $PGF_{2\alpha}$ is involved in the control of the estrous cycle in ewes.

Regulation of the Ovine Estrous Cycle: Overview

Four organs are principally involved in the control of reproduction in the female: the hypothalamus, pituitary, ovary, and uterus. Figure 2 shows the relationships among the hormones from these tissues during the estrous cycle of ewes. Briefly, the estrous cycle has been divided in two phases: follicular and luteal. In ewes, luteal regression begins around d 13 of a 17-d cycle. As progesterone concentrations decrease, the frequency of LH pulses increase, as does estradiol. After the LH surge, ovulation occurs and a CL develops at the site of ovulation and secretes progesterone throughout the luteal phase.

The vasculature carrying signals among these organs, known as the hypothalamo-hypophyseal-gonadal axis, is quite complex. In females, two neurosecretory centers in the hypothalamus are ultimately responsible for ovarian activity. The tonic center is responsible for the episodic release of GnRH from the arcuate-median eminence region of the hypothalamus in response to ovarian steroid feedback mechanisms. Gonadotropin-releasing hormone secretion is then responsible for the release of the gonadotropins, FSH and LH, from the pituitary and thus, in part, controls the estrous cycle. Estrogen production is dependent on the aromatization of androgens produced in the thecal cells under LH stimulation to estradiol under FSH stimulation in the granulosal cells (Dorrington et al., 1975; Hoyer and Niswender, 1985). Tonic LH secretion is controlled by progesterone; low progesterone concentrations permit tonic LH secretion (Baird and Scaramuzzi, 1976; Karsch et al., 1979; Karsch et al., 1980). Increased tonic LH secretion increases estradiol secretion from the preovulatory follicle (McCracken et al., 1971; Hauger et al., 1977; Karsch et al., 1979, Baird, 1978; Baird et al., 1981). Increasing estradiol concentrations then stimulate the second center, known as the surge center, which ultimately induces the LH surge required for ovulation. Progesterone inhibits the surge of LH. Tonic FSH secretion, which stimulates follicular growth, is controlled by

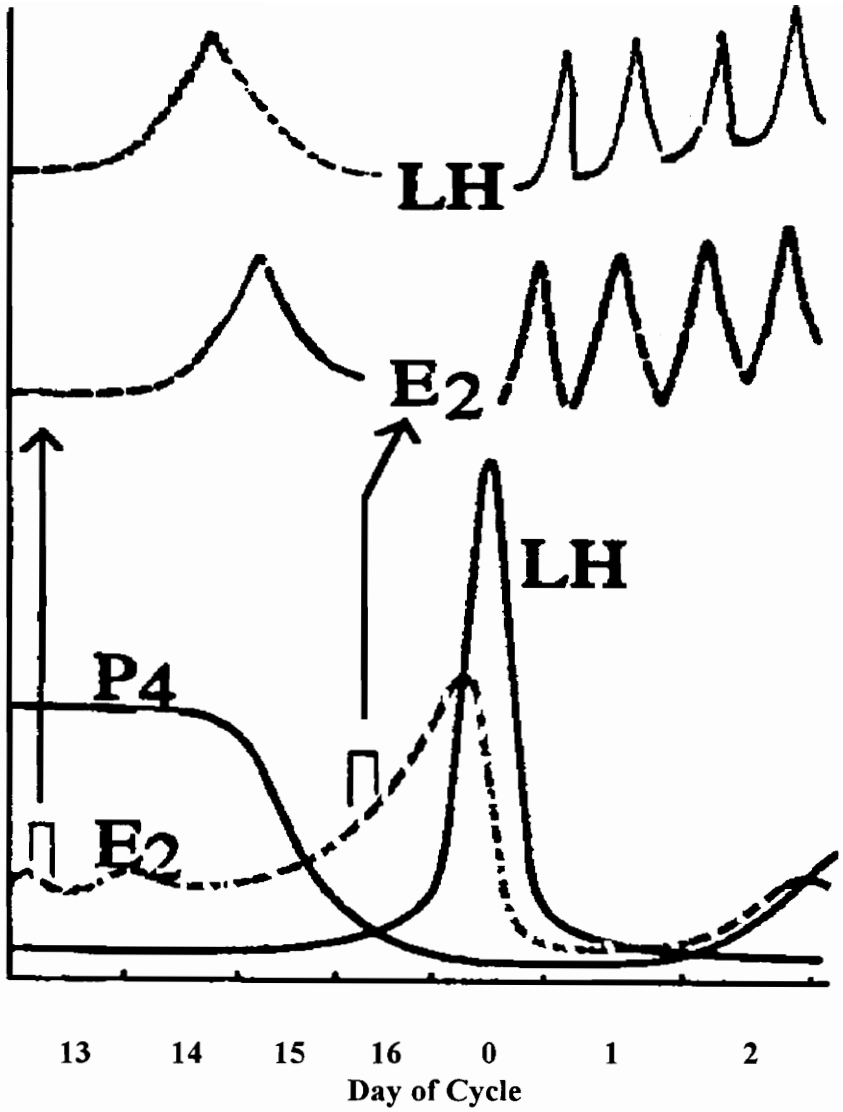

Figure 2. Relative changes in progesterone, estradiol, and LH secretion around the time of luteolysis in the ewe. The top half of the diagram illustrates changes in pulse frequency of LH and estradiol during the luteal and follicular phase.

GnRH and ovarian feedback (Goodman et al., 1981; Clarke et al., 1983). The FSH surge that coincides with the preovulatory LH surge is initiated by estradiol and inhibited by progesterone (Cumming et al., 1974; Reeves et al., 1974; Pant, 1977, Goodman et al., 1981). Miller et al. (1979) and Welschen et al. (1977) demonstrated that inhibin from ovarian follicular fluid inhibited FSH secretion and delayed ovulation, even though a LH surge was apparent. Thus, LH and FSH regulate ovarian activity and are, in turn, regulated by ovarian activity.

The secretory patterns of progesterone and estradiol indicate different roles in regulation of the estrous cycle. As the CL develops, progesterone concentrations increase and are maintained for several days. Progesterone concentrations rapidly decrease around d 14. In contrast, estradiol concentrations increase as the progesterone concentrations decrease. Early studies on the initiation of estrus indicate that progesterone and estradiol are regulators of cyclicity and not simply a result of cyclicity (Selye et al., 1936; Phillips, 1937; Dutt and Casida, 1948; Foote, 1964; Woody et al., 1967; Lewis et al., 1968). In ovariectomized ewes, pharmacological doses of estradiol can initiate estrus although pretreatment with progesterone significantly decreases the dose of estradiol required (Robinson, 1954a, b; Caldwell et al., 1972). It is the duration of the progesterone replacement therapy, which mimics the luteal phase, rather than the dose of progesterone that affects the estradiol dose required (Robinson et al., 1956; Scaramuzzi et al., 1971). Both estradiol and progesterone are vital to estrus synchronization, but how do they exert their effects on luteolysis?

Cyclic Luteolysis

The follicular phase of the estrous cycle in ewes is characterized by increasing estradiol, FSH, and LH and decreasing progesterone secretion. Uterine $PGF_{2\alpha}$ secretion seems quiescent from d 2 through 10 when compared with both the amplitude and frequency of $PGF_{2\alpha}$ secretion during the periovulatory period (Bland et al., 1971; Baird et al., 1976; Nett et al., 1976). Uterine $PGF_{2\alpha}$ is responsible for luteolysis (Inskeep and Murdoch, 1980). After ovulation, the CL slowly develops and is the predominant ovarian structure during the luteal phase. The luteal phase has been considered as the quiescent period in reproduction, when compared with the follicular phase, because less dramatic events are occurring. Instead, the luteal phase represents a preparatory period filled with numerous control mechanisms vital to ovine reproduction.

Several experiments indicate that estradiol and progesterone are involved in the regulation of uterine $PGF_{2\alpha}$ secretion (Barcikowski et al., 1974; Ford et al., 1975; Baird et al., 1976; Scaramuzzi et al., 1977; Ottobre et al., 1980). Progesterone from the newly developed CL is responsible for priming the uterus for subsequent $PGF_{2\alpha}$ secretion, although this response is maximized when supplemented with estrogen therapy (Scaramuzzi et al., 1977). Ford et al. (1975) indicate that progesterone priming is required for $PGF_{2\alpha}$ secretion because estradiol-17β increased $PGF_{2\alpha}$ concentrations in uterine venous plasma and endometrium on d 9 and 10 but not on d 4 or 5. However, if estradiol-17β is given after pretreatment with progesterone on d 1 to 5, $PGF_{2\alpha}$ concentrations in uterine venous plasma and endometrium increase. Brinsfield and Hawk (1973) indicated that progesterone increased endometrial lipid stores in ovariectomized ewes, and estradiol administration decreased this pool. Progesterone may also regulate $PGF_{2\alpha}$ secretion through influencing cyclooxygenase activity (Raw et al., 1988). Proper progesterone secretion is also essential for proper follicular development through its control over estradiol receptor concentrations (McCracken, 1980). Estradiol may

stimulate the synthesis of uterine oxytocin receptors (Hixon and Flint, 1987) and(or) stimulate the synthesis of precursors for $PGF_{2\alpha}$ production (Brinsfield and Hawk, 1973) while enhancing progesterone-primed cyclooxygenase activity (Raw et al., 1988). Estradiol may also be converted in the uterus into catechol estrogens, which are more potent $PGF_{2\alpha}$ stimulators (Kelly and Abel, 1980; Silvia et al., 1991).

Oxytocin has also been implicated in the regulation of $PGF_{2\alpha}$. Concomitant pulses of oxytocin are detected with $PGF_{2\alpha}$ pulses around the time of luteolysis, indicating that oxytocin may be involved in CL demise (Fairclough et al., 1980, Webb et al., 1981; Flint and Sheldrick, 1983). Uterine responsiveness to oxytocin seems to be regulated by estrogen and(or) progesterone because the uterus is responsive to oxytocin only during a short period of time. Oxytocin can stimulate uterine $PGF_{2\alpha}$ secretion on d 3 or 14 but not d 8 or 13 (Roberts et al., 1975, Roberts and McCracken, 1976; Burgess et al., 1990). In response to oxytocin on d 14, pulsatile $PGF_{2\alpha}$ secretion is initiated (Sharma and Fitzpatrick, 1974; Roberts et al., 1975; Burgess et al., 1990). A few small $PGF_{2\alpha}$ peaks occur before any decrease in progesterone secretion. Follicular estradiol increases, and luteal oxytocin secretion is initiated. After the uterus becomes responsive to oxytocin, uterine $PGF_{2\alpha}$ is enhanced. At luteolysis, several large pulses of $PGF_{2\alpha}$ are secreted 6 to 8 h apart (Silvia et al., 1991) It seems that uterine refractoriness to oxytocin develops and lasts for approximately 6 h. Luteal sensitivity to uterine $PGF_{2\alpha}$ also occurs. Silvia et al. (1991) indicate that the feedback mechanisms between uterine $PGF_{2\alpha}$ and luteal oxytocin determine the timing of the uterine $PGF_{2\alpha}$ pulses. Pulsatile $PGF_{2\alpha}$ is more effective in inducing luteolysis than is a continuous infusion of $PGF_{2\alpha}$ (Schramm et al., 1983), which indicates that both uterine responsiveness to oxytocin and the feedback mechanism between oxytocin and $PGF_{2\alpha}$ are vital to luteolysis. Does Lutalyse induce luteolysis through similar mechanisms?

Induced Luteolysis

Luteolysis in midluteal phase sheep can be induced with $PGF_{2\alpha}$. If a single 200 μg dose of $PGF_{2\alpha}$ is injected into the largest follicle on an ovary with an active CL, the ewe will return to estrus within 72 h (Inskeep, et al., 1975). Intrafollicular doses of 150 or 100 μg were less effective. Intrauterine administration of $PGF_{2\alpha}$ is more efficient than i.m. administration; CL collected from d 8 luteal phase ewes weighed significantly less than those from ewes given the same dose of $PGF_{2\alpha}$ i.m. (Douglas and Ginther, 1973). The dose of $PGF_{2\alpha}$ is also important. Corpora lutea from ewes given 6 mg of $PGF_{2\alpha}$ i.m. at d 8 of the estrous cycle weighed significantly less than those from ewes treated with 2 mg of $PGF_{2\alpha}$ (Douglas and Ginther, 1973). Luteolysis, measured by progesterone secretion, in intact and ovarian autotransplanted ewes, also occurs in a dose-dependent fashion. In ovarian autotransplanted ewes, intraarterial $PGF_{2\alpha}$ infusion (25 μg/h for 1 h) results in rapid CL breakdown; the decrease in progesterone secretion is slower with .01 and 1.0 μg/h intraarterial infusion rates (McCracken et al., 1970). McCracken et al. (1970) reported that progesterone secretion rate decreased to 50% of control within 1 h after interarterial infusion of $PGF_{2\alpha}$ at 50 μg/h for 6 h; the progesterone secretion rate then decreased to 5% of control values over 24 h. Barrett et al. (1971) reported a similar effect using a lower infusion rate (40 μg/h for 3 h). The minimum luteolytic intraarterial dose of $PGF_{2\alpha}$ in ovarian autotransplanted ewes lies between 2 and 10 μg/h (Chamley et al., 1972). In intact ewes, interarterial infusion (10 μg/h for 3 h) and uterine vein infusion (40 μg/h for 3 h) of $PGF_{2\alpha}$ also resulted in luteolysis (Thorburn and Nicol, 1971). Thus, exogenous $PGF_{2\alpha}$ is luteolytic in midluteal phase sheep, but how does $PGF_{2\alpha}$, endogenous or exogenous, induce luteolysis?

Mechanism of Action of $PGF_{2\alpha}$

Proposed Mechanisms

Proposed mechanisms for the luteolytic activity of $PGF_{2\alpha}$ include direct feedback on the pituitary gland, antigonadotropic effects, direct toxicity, altering steroidogenesis via enzyme activation including enhanced progesterone metabolism, interfering with cAMP or phospholipase C-activated second messenger systems, decreased LH receptor number, and decreased uteroovarian blood flow.

$PGF_{2\alpha}$ Acts on LH Activity

Denamur et al. (1966), using hypophysectomized sheep, showed that pituitary secretion of LH is essential for progesterone secretion, thus suggesting that $PGF_{2\alpha}$ by inactivating LH secretion may account for decreased progesterone secretion. However, progesterone secretion relies on CL functionality but does not in itself direct CL maintenance. According to Pharriss and his colleagues (1972), it seems unlikely that $PGF_{2\alpha}$ completely blocks the hypothalamo-hypophyseal axis or that $PGF_{2\alpha}$ can block the different luteotropins known to exist in different species. Additional evidence is provided by the fact that less $PGF_{2\alpha}$ is required to induce luteal regression when administered by infusion into the uteroovarian supply than systemically (McCracken et al., 1970; Goding et al., 1972), which indicates that $PGF_{2\alpha}$ is acting through a more direct mechanism rather than by inactivating the hypothalamo-hypophyseal axis.

Can $PGF_{2\alpha}$ be interfering with LH action? This seems likely, because $PGF_{2\alpha}$ inhibits the LH-induced increase in progesterone production in luteal cell cultures (Fletcher and Niswender, 1982). In rats (Grinwich et al., 1976) and sheep (Diekman et al., 1978), $PGF_{2\alpha}$ decreased the number of LH receptors. However, endogenous $PGF_{2\alpha}$ does not seem to regulate the number of LH receptors in sheep; progesterone

concentrations decrease before LH receptor numbers decrease. Thus, the decrease in LH receptor number seems to be a result of luteolysis and not the mechanism.

$PGF_{2\alpha}$ Acts through Venoconstriction of the Uteroovarian Vein

Prostaglandin $F_{2\alpha}$ is a potent venoconstrictor (DuCharme et al., 1968). Pharriss (1970) hypothesized that $PGF_{2\alpha}$ exerts its luteolytic effects by suffocating the CL through constriction of the uteroovarian vein. Several other experiments seem to support this hypothesis. During spontaneous luteolysis, capillary flow is decreased (Bruce and Moor, 1976). Decreased blood flow during luteolysis also occurs in cows (Wise et al., 1982). Thorburn and Hales (1972), as well as Niswender et al. (1975, 1976), have shown $PGF_{2\alpha}$ to reduce blood flow to the CL. However in sheep, $PGF_{2\alpha}$ can inhibit progesterone secretion, which is used as an indicator of CL activity, without a decrease in ovarian blood flow (Baird, 1974). Therefore, reduction in ovarian blood flow is rather a result of luteolysis and not an effector itself (Janson et al., 1974).

Prostaglandin $F_{2\alpha}$ administration to ovarian tissue first seemed to support the hypothesis about reduced blood flow, because $PGF_{2\alpha}$ actually stimulated progesterone secretion in vitro (Pharriss et al, 1968; Speroff and Ramwell 1970). These first reports were discredited in light of the overwhelming evidence that $PGF_{2\alpha}$, at the proper dose, inhibits in vitro and in vivo luteal progesterone secretion (Behrman et al., 1971; Pharriss et al., 1972; Henderson and McNatty, 1975; McNatty et al, 1975; Lahav et al., 1976; Henderson et al., 1977).

$PGF_{2\alpha}$ Acts through Cyclic Adenosine Monophosphate

A biochemical review of functional luteinization is provided by Henderson and McNatty (1975). Progesterone production from the CL relies on the activities of the adenylate cyclase system activated after LH receptor saturation (Marsh, 1971). Once synthesized in the plasma membrane, cAMP can induce granulosal cell progesterone production through a protein kinase mechanism. Oversimplified, LH binds to its receptor molecule in the plasma membrane, which consists of a regulatory unit, a coupling unit, and a catalytic unit. Once bound to the regulatory unit, a signal sent by the coupling unit is perceived by the catalytic unit which results in activation of the adenyl cyclase unit and production of cAMP. Protein kinases are then activated as long as sufficient cAMP is present. Through activation of these kinases, cAMP may affect steriodogenesis. Marsh (1976) outlined the mechanisms by which cAMP may direct steroidogenesis; the mechanism includes the 1) increase in the cholesterol pool via an increase in its concentration or availability in the mitochondria, 2) activation of enzymes, 3) activation of cofactors, such as NADPH, required in steriodogenesis, and 4) decreased inhibitor concentrations, such as end products that inhibit steriodogenesis.

Protein kinases may also phosphorylate enzymes resulting in their activation. The cholesterol pool is dependent on both cholesterol esterase, which is responsible for the freeing of esterified cholesterol, and on cholesterol ester synthetase, which esterifies free cholesterol for storage. Activation of protein kinase A by cAMP increases cholesterol esterase activity. Cholesterol esterase activity increases in response to LH administration in vivo (Behrman and Armstrong; 1969). It is interesting to note, however, that when cAMP is added to culture media, cholesterol esterase activity is not affected (Behrman and Armstrong, 1969). According to Marsh (1976), enhanced cholesterol esterase activity may be due to an increase in the conversion of cholesterol to pregnenolone thus removing some sort of negative feedback on the enzyme. The conversion of cholesterol to

pregnenolone in progesterone production is also dependent on cAMP (Hermier et al. 1971). Another mechanism by which cAMP may be mediating progesterone production is through inhibiting cholesterol ester synthetase and thereby increasing the available cholesterol pool. Flint et al. (1973) have shown that LH (in vivo) and cAMP (in vitro) inhibit cholesterol ester synthetase activity. However, these results seem to be a result of progesterone production and not by actually initiating cholesterol ester synthetase activity (Flint et al., 1973; Marsh, 1976). Activation of protein kinase A (through cAMP activity) also facilitates transport of cholesterol to the inner mitochondrial membrane for side chain cleavage (by P450scc) of cholesterol for conversion to pregnenolone (Leung, 1992). Pregnenolone is then secreted and enzymatically converted into progesterone by 3β-hydroxy-Δ^5-steroid dehydrogenase Δ^5,Δ^4-isomerase. However, in ewes, neither 3β-hydroxy-Δ^5 -steroid dehydrogenase Δ^5,Δ^4-isomerase nor P450scc activity is influenced by kinase activation (Wiltbank and Niswender, 1991) indicating that another mechanism must exist for $PGF_{2\alpha}$-induced changes in steroidogenesis. However, a decrease in 3β-hydroxy-Δ5-steroid dehydrogenase Δ^5,Δ^4-isomerase occurs in response to $PGF_{2\alpha}$ (Dwyer and Church, 1979; Hawkins et al., 1993). In rats, $PGF_{2\alpha}$ enhances 20α-hydroxysteroid dehydrogenase activity, thus decreasing progesterone concentration by degradation of progesterone (Jones and Hsueh, 1981). Another mechanism whereby cAMP may be involved in luteolysis is through regulating phosphodiesterase activity, which is responsible for the degradation of cAMP (Marsh, 1976).

Progesterone production requires NADPH as a cofactor because it is required in many side-chain cleavage steps in steriodogenesis. High concentrations of NADPH dehydrogenase are present in the bovine CL (Savard et al., 1963). The addition of NADPH to bovine luteal cell cultures results in an increase in progesterone production (Mason et al., 1962; Savard et al., 1963). Cyclic AMP, acting through activation of protein kinase, is responsible for this increase in NADPH (Marsh, 1976).

Thus, any disruption in the adenyl cyclase system can block progesterone production. Interference with cAMP-activated protein synthesis can also influence CL morphology leading to structural luteolysis (Marsh, 1976).

$PGF_{2\alpha}$, LH, and Cyclic Adenosine Monophosphate Interactions

In sheep (Powell et al., 1974a), humans, and cows (Powell et al., 1974b), $PGF_{2\alpha}$ receptors are found in the plasma membrane of the CL, the same site of the adenyl cyclase system. Can $PGF_{2\alpha}$ disrupt cAMP-related mechanisms in progesterone production? Does $PGF_{2\alpha}$ disrupt LH activation of the adenylate cyclase system? Does $PGF_{2\alpha}$ interfere with the coupling unit, prevent signal transmission, and reduce cAMP production? Does $PGF_{2\alpha}$ interfere with the catalytic unit signal reception or response? All of these represent feasible points where $PGF_{2\alpha}$ may interfere with luteal activity via adenyl cyclase disruption.

Prostaglandin $F_{2\alpha}$ is thought to interfere with LH activation of the adenyl cyclase system (Henderson and McNatty, 1975; Grinwich et al., 1976). Andersen et al. (1974) have shown $PGF_{2\alpha}$ to disrupt normal LH-induced adenylate cyclase activation in pigs, based upon decreased adenyl cyclase response to LH on d 16 and 17 when $PGF_{2\alpha}$ concentrations are increased. The coupling component appears to be the site of $PGF_{2\alpha}$ attack resulting in disruption of the transmission signal and thus, decreasing cAMP production (Henderson and McNatty, 1975). If $PGF_{2\alpha}$ disrupts LH activation, then cAMP production should be markedly decreased. However, because each hormone has its own regulatory unit, if the addition of a second hormone known to activate the adenylate cyclase system replenishes the decreased cAMP, then the coupling unit may be the site of $PGF_{2\alpha}$ attack. Prostaglandin E_2 activates the adenylate cyclase system (Marsh, 1971). If the addition of PGE_2 can overcome the inhibitory effect of $PGF_{2\alpha}$, the coupling unit may be the site of $PGF_{2\alpha}$ attack. In vitro, PGE_2 enhances progesterone production in human Graafian follicles, and $PGF_{2\alpha}$ inhibits progesterone production (Henderson and McNatty,

1975). When administered together to the culture, progesterone production matches that of PGE_2 alone. Thus, $PGF_{2\alpha}$ through interfering with the coupling unit is inhibiting LH activation of the adenyl cyclase system.

Why is $PGF_{2\alpha}$ able to induce luteolysis only at certain times? What protects the system from $PGF_{2\alpha}$? Henderson and McNatty (1975) stated that the LH surge saturates plasma membrane receptors, and dissociation is a slow process. In granulosal cell cultures, LH exposure has a profound effect on $PGF_{2\alpha}$ inhibition. Progesterone production in control cell cultures was 18.3 ± 1.7 μg, in LH- and FSH-treated cultures it was 48.0 ± 1.3 μg, and in LH-, FSH-, and $PGF_{2\alpha}$-treated cultures it was 19.4 ± 2.1 μg. Prostaglandin $F_{2\alpha}$ seems to agonize LH and FSH activity, because the combined treatment of LH, FSH, and $PGF_{2\alpha}$ did not differ from control values. In such cultures, 6-d exposure to FSH and LH before $PGF_{2\alpha}$ addition required significantly more $PGF_{2\alpha}$ to inhibit progesterone secretion. Thus, LH and FSH must be protecting the adenyl cyclase system. After the preovulatory LH surge, dissociation of LH from the receptor is slow (Channing and Kammerman, 1973; Haour and Saxena, 1974), and not all binding sites need to be filled for cAMP production (Koch et al., 1974). Henderson and McNatty (1975) proposed that in the latter stages of the luteal phase fewer receptors are occupied and that this enables $PGF_{2\alpha}$ to block cAMP production. A second explanation is offered by Rao et al. (1979). Receptor affinity for $PGF_{2\alpha}$ increases late in the luteal phase (Rao et al., 1979), thus enabling $PGF_{2\alpha}$ to act.

$PGF_{2\alpha}$-Induced Phospholipase C-Activated Second Messenger System

According to Knickerbocker et al. (1988), changes in enzyme activity are not initiated until after functional luteolysis and are thus, an effect and not the mechanism of action of $PGF_{2\alpha}$ in inducing luteolysis. Instead, Knickerbocker et al. (1988) and Davis et al. (1987) suggested that phopholipase C-activated second messenger systems under the

direction of $PGF_{2\alpha}$ are responsible for initiating luteolysis. Briefly, phopholipase C hydrolyzes phosphatidylinositol 4, 5-bisphosphate (PIP_2) into inositol 1,4,5,-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). Binding affinity between calcium and protein kinase C is enhanced by DAG, and IP_3 increases intracellular calcium release. Phospholipase C activity increases in ovine luteal tissue in response to $PGF_{2\alpha}$ (Jacobs et al., 1991). Prostaglandin $F_{2\alpha}$, by increasing phospholipase activity, triggers a cascade of events, including an increase in intracellular calcium, followed by activation of protein kinase C. Protein kinase C then activates numerous enzymes via phosphorylation which then contribute to luteolysis (Knickerbocker et al., 1988; Jacobs et al., 1991).

$PGF_{2\alpha}$-Induced Cytotoxicity

The concept of $PGF_{2\alpha}$ inducing cytotoxicity toward the CL had been discarded because administration of $PGF_{2\alpha}$ in vitro stimulated progesterone secretion (Pharriss et al. 1972; Behrman et al., 1971; Speroff and Ramwell, 1970). However, conflicting data have been provided to support $PGF_{2\alpha}$ inhibiting progesterone production in luteal cell cultures (O'Grady et al., 1972; Henderson and McNatty, 1975). Pate and Condon (1984) using bovine luteal cells provided evidence that no significant change was noted in progesterone production by bovine luteal cells when cultured with $PGF_{2\alpha}$. Instead, $PGF_{2\alpha}$ inhibited both LH- and cAMP-induced progesterone production.

More recently, data have reimplicated the possibility of cytotoxicity in luteolysis. Fitz et al. (1984) showed a cytotoxic effect of $PGF_{2\alpha}$ on the large luteal cells but not on small luteal cells in vitro. In vivo, a small decrease in the number of small luteal cells precedes the cytotoxic effect on the large luteal cell (Braden et al., 1988). However, $PGF_{2\alpha}$ is not completely responsible for inducing this reaction. Instead, the immune system, which is upregulated under the influence of estradiol, may direct cytotoxic actions against the luteal cells. Prostaglandin $F_{2\alpha}$ may accentuate this effect; $PGF_{2\alpha}$-treated

luteal cell cultures attract more eosinophils, thus enhancing structural degradation of the luteal tissue (Murdoch, 1987). According to Fairchild Benyo et al. (1991), $PGF_{2\alpha}$ may also accentuate this immune response by increasing the number of antigen-positive labeled luteal cells. These MHC class II molecules are typically only expressed on lymphocytes and macrophages and had not yet been identified on luteal (nonlymphoid) cells. Major histocompatibility complex class II molecules, acting through T-cell activation can upregulate the entire immune system. It is noteworthy that the expression of these MHC class II molecules increase with the age of the CL (Fairchild Benyo et al., 1991). This evidence indicates that luteolysis also evokes the immune system and that the immune reaction can be modulated by its hormonal environment.

Thus, $PGF_{2\alpha}$ may be acting through several direct and indirect mechanisms in inducing and conducting biochemical luteolysis (Figure 3). Research to date indicates the following possible actions of $PGF_{2\alpha}$ in luteolysis: 1) $PGF_{2\alpha}$ influences the phosphatidylinositide/calcium/protein kinase C second messenger systems; 2) the first pulse of $PGF_{2\alpha}$ above some threshold concentration may enhance uterine secretion of $PGF_{2\alpha}$ through an oxytocin feedback mechanism that triggers uterine smooth muscle contractility; 3) $PGF_{2\alpha}$ interacts with cAMP-dependent activities, thus changing the hormonal environment; and (4) $PGF_{2\alpha}$ may upregulate immune function by increasing the expression of antigens on luteal cells thereby facilitating structural luteolysis.

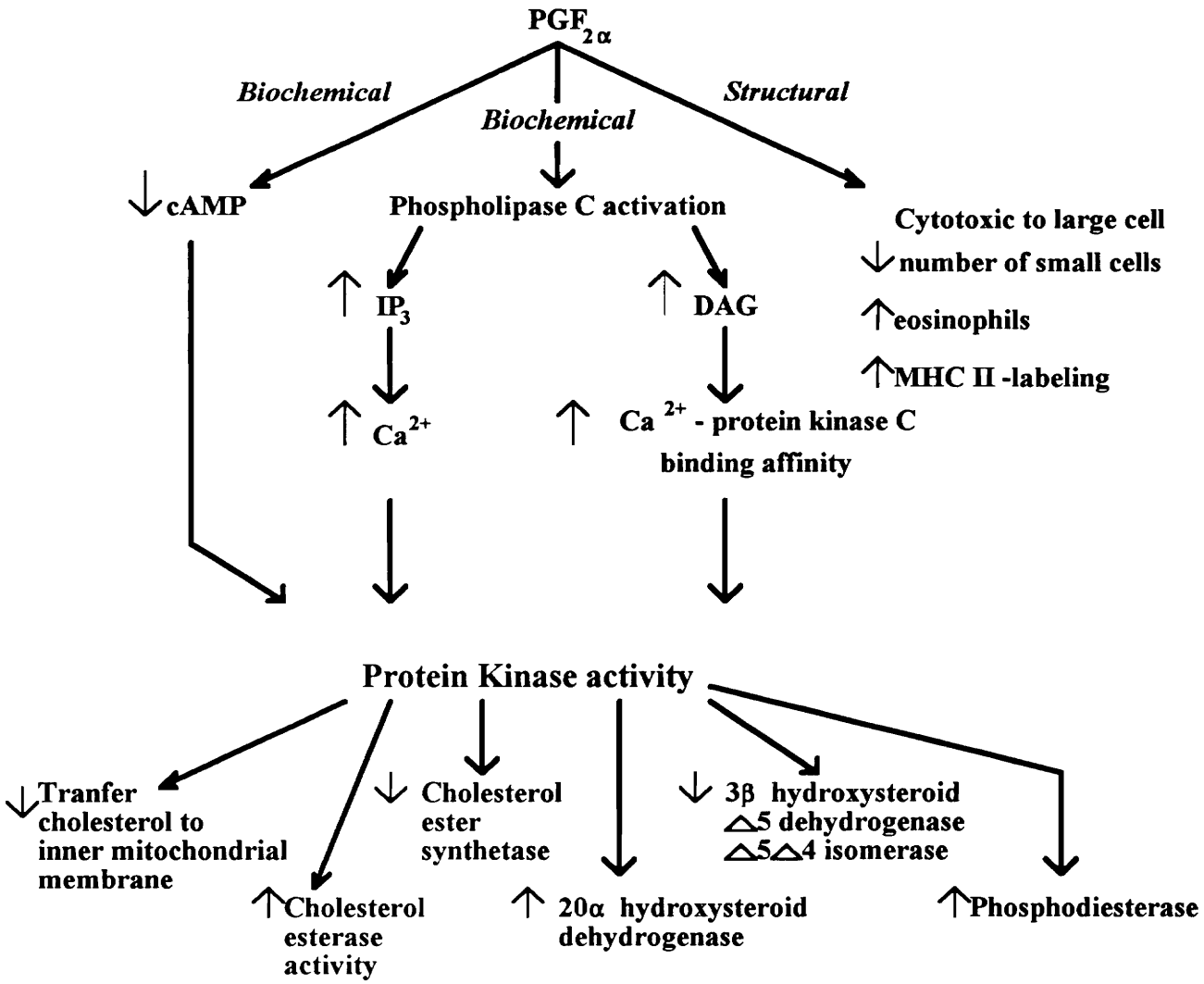

Figure 3. Proposed mechanisms of action of $PGF_{2\alpha}$ in luteolysis.

Gaps in Our Understanding of How Exogenous $PGF_{2\alpha}$ Works

How does exogenous $PGF_{2\alpha}$ work? When injected i.m., does $PGF_{2\alpha}$ affect the CL directly? Is the amount of $PGF_{2\alpha}$ that reaches the CL and(or) uterus greater than that in spontaneous luteolysis? Does $PGF_{2\alpha}$ simply modify the hormonal environment, thus triggering luteolysis? If uterine $PGF_{2\alpha}$ secretion can be enhanced by oxytocin secretion, which was stimulated by $PGF_{2\alpha}$, does the exogenous $PGF_{2\alpha}$ initiate luteal oxytocin secretion? Does it, i.e., $PGF_{2\alpha}$, override the mechanisms of progesterone and estradiol in regulating uterine responsiveness to this oxytocin? Does it stimulate uterine production of $PGF_{2\alpha}$? If so, how much reaches the uterus? How much $PGF_{2\alpha}$ does the uterus produce in response to an exogenous dose of $PGF_{2\alpha}$? Does it stimulate other tissues to secrete $PGF_{2\alpha}$? If so, how is it absorbed and delivered to these tissues? How much $PGF_{2\alpha}$ do they produce? Does exogenous $PGF_{2\alpha}$ during the midluteal phase turn on systems that are not involved in spontaneous luteolysis? Does exogenous $PGF_{2\alpha}$ upregulate immune function? Does it activate protein kinases? Does exogenous $PGF_{2\alpha}$ interfere with cAMP-related activities? How exogenous $PGF_{2\alpha}$ is picked up by the circulatory system and distributed to various tissues remains unknown. Earlier studies have shown the luteolytic activity of $PGF_{2\alpha}$ to be dose-dependent. However, in practice, a single larger bolus dose is less effective than two smaller doses? If pulsatile $PGF_{2\alpha}$ is more effective than continuous infusion, do two doses act as pulses and a single injection acts as a short-term continuous infusion of $PGF_{2\alpha}$? These questions remain unanswered at this time.

Prostaglandin Biosynthesis

An understanding of the biosynthesis and metabolism of $PGF_{2\alpha}$ may provide insight into the regulation of $PGF_{2\alpha}$ secretion during spontaneous and $PGF_{2\alpha}$-induced luteolysis. Prostaglandins represent a class of highly lipophilic compounds that evoke numerous biological actions in a variety of tissues and organs. These highly potent compounds are involved, for example, in reproduction in males and females, digestion, vasoconstriction and dilation, antiinflammatory responses, platelet activation, and immune reactions.

Prostaglandins were "discovered" in the early 1930's by Maurice Goldblatt (1933) and U.S. von Euler (1935) in fluid from the prostate gland, hence the name, prostaglandins. On the basis of prostanoic acid, Bergström (1968) classified the several classes as PGF, PGE, PGA, and PGB. Since then, many more prostaglandin classes have been identified. These classes vary in the cyclopentane ring structure of prostanoic acid, a 20-carbon fatty acid. Within these classes, numerous isomers exist due to saturation and stereochemistry. Subscripted numbers and Greek symbols are used to describe these isomers, e.g., $PGF_{2\alpha}$ vs $PGF_{1\alpha}$. In prostaglandin nomenclature, the subscripted number represents the number of double bonds, and the Greek symbol denotes the substituents stereochemical position on the cyclopentane ring. Prostaglandins of the F and E classes are vital to reproduction. Prostaglandins of the F class are readily identified by the hydroxyl groupings on carbons 9 and 11. On PGE, a ketone group replaces the hydroxyl group on carbon 9.

Arachidonic acid is considered the major substrate for two-series prostaglandin synthesis. However, arachidonic acid is acquired from the diet or through desaturation and chain elongation from linoleic acid (C18:2). Partial saturation of linoleic acid forms linolenic acid (C18:3), which undergoes chain elongation to form 8,11,14-eicosantrienoic acid, the precursors for the one-series prostaglandins. Further desaturation results in

arachidonic acid (5,8,11,14-eicosatetraenoic acid). Dienoic prostaglandins (two-series) are formed from this acid. Further desaturation of arachidonic acid forms timnodonic acid (5,8,11,14,17-eicosatetraenoic acid), the precursor for the three-series prostaglandins such as PGE_3.

"Free" arachidonic acid is rapidly esterified via fatty acid CoA ligase and acyl transferase into phospholipids, triacylglycerols, and cholesterol esters. Liberation of arachidonic acid from these storage forms via phospholipases or esterases leads to arachidonic acid enzymatic oxidation. Two oxidative pathways have been identified: cyclooxygenase and lipoxygenase (Figure 4). The primary products of the lipoxygenase pathway are the antiinflammatory, immunogenic leukotrienes. The cyclooxygenase pathway results in the formation of the prostaglandins. The cyclooxygenase pathway involves the conversion of arachidonic acid to endoperoxide intermediates. Prostaglandin endoperoxide synthase directs the oxidation of arachidonic acid to the intermediate diperoxide PGG_2. Prostaglandin H_2 is rapidly formed via PG hydroxperoxidase, releasing the free oxygen radical on carbon 15. Thus, PGH_2 represents the common precursor for biosynthesis of the prostaglandins.

Several unique enzymes chemically convert PGH_2 into potent biological molecules. Prostaglandin F synthetase and 9-keto-isomerase are two such enzymes. Prostaglandin F synthetase replaces the peroxide moiety with hydroxl groups on carbons 9 and 11 to form $PGF_{2\alpha}$, a potent vasoconstrictor, uterine tone controller, and luteolysin. The enzyme 9-keto-isomerase converts PGH_2 into PGE_2 which stimulates uterine contractions and vasodilation. Numerous other enzymes are involved in prostaglandin and thromboxane synthesis.

The arachidonic acid cascade is influenced by numerous factors including arachidonic acid stores, enzyme availability, free calcium, hormone concentrations, glucocorticoids, and pharmaceutical products. Substrate availability is dictated initially by diet and biosynthesis from essential fatty acids (EFA). Once arachidonic acid has been

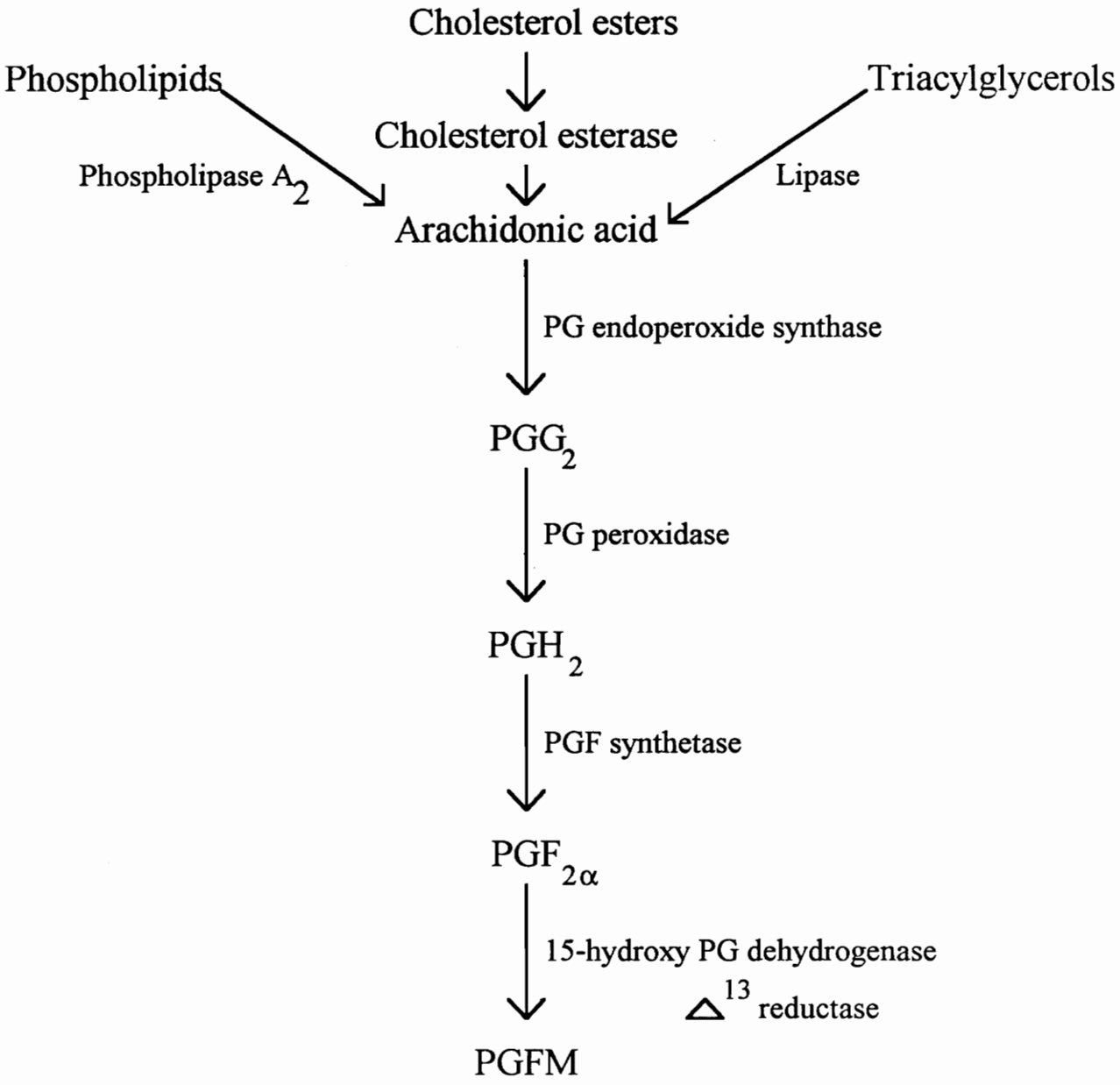

Figure 4. Biosynthesis and metabolism of $PGF_{2\alpha}$.

stored, liberating enzymes are required and govern the free arachidonic acid pool. The production of prostaglandins is also directed by glucocorticoids, which inhibit phospholipase activity, and nonsteriodal antiinflammatory agents, which inhibit cyclooxygenase activity.

Metabolism of $PGF_{2\alpha}$ and PGE_2 is as rapid as is their synthesis from PGH_2. The first step in metabolism of these prostaglandins involves the oxidation of the 15-hydroxyl group to a keto-moiety. Prostaglandin $F_{2\alpha}$ is metabolized via 15-hydroxy PG dehydrogenase and Δ^{13} reductase into 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (Hansen, 1976; Lands, 1979). High concentrations of this enzyme must be present in sheep lungs, because in a single passage up to 99 % of labeled $PGF_{2\alpha}$can be metabolized (Samuelsson et al., 1971; Davis et al., 1980). There are further steps for the metabolism of the prostaglandins to more water-soluble forms that are excreted in urine; however, once the hydroxyl group has been oxidized the associated prostaglandin loses its biological activity.

The production and metabolism of $PGF_{2\alpha}$ is regulated by several enzymes located throughout the body. Therefore, $PGF_{2\alpha}$ and PGFM concentrations from blood plasma samples may depend on the site of sampling.

Sampling Sites for Studying Prostaglandins

Studying $PGF_{2\alpha}$ secretion and metabolism in vivo is complicated. First, $PGF_{2\alpha}$ is produced by most cells and tissues. Therefore, many tissues contribute to the $PGF_{2\alpha}$ concentration measured at any given time. Uteroovarian sampling provides the best estimate of uterine $PGF_{2\alpha}$ secretion during the estrous cycle. However, these samples will also contain $PGF_{2\alpha}$ that has been produced by endothelial cells throughout the body as well as $PGF_{2\alpha}$ that has not been metabolized and continues to circulate.

Second, prostaglandins are short-lived; $PGF_{2\alpha}$ has a half-life of only a few minutes. Except during lambing and calving (Guilbault, 1988), sheep lungs are capable of metabolizing up to 99% of the $PGF_{2\alpha}$ presented to them (Samuelsson et al., 1971; Davis et al., 1980). Once $PGF_{2\alpha}$ enters the venous supply, the circulatory system then carries the $PGF_{2\alpha}$ to where it is immediately catabolized. Jugular sampling does not accurately reflect the actual $PGF_{2\alpha}$ produced, because most of the $PGF_{2\alpha}$ has been metabolized or utilized by other tissues. Some studies have focused on measuring PGFM for this reason.

Third, many samples are necessary to create an accurate secretion profile, because $PGF_{2\alpha}$ is secreted in a pulsatile fashion throughout the estrous cycle (Thorburn et al., 1973; Baird et al., 1976; Kindahl et al., 1976) The secretory pattern of $PGF_{2\alpha}$ changes toward the end of the luteal phase. Before progesterone concentrations decrease, the duration and magnitude of these $PGF_{2\alpha}$ pulses are enhanced (Ottobre et al., 1984). Thus, the pattern of the pulsatile release of $PGF_{2\alpha}$ changes throughout the estrous cycle.

Figure 5 shows the fate of $PGF_{2\alpha}$ produced by the uterus during luteolysis. According to this scheme, the most appropriate sites for measuring uterine $PGF_{2\alpha}$ production during luteolysis, whether induced or spontaneous, are within the uteroovarian vein. Comparison of sampling cranial and caudal to the uteroovarian vein should also reflect uterine $PGF_{2\alpha}$ contribution. The caudal samples in this technique would estimate

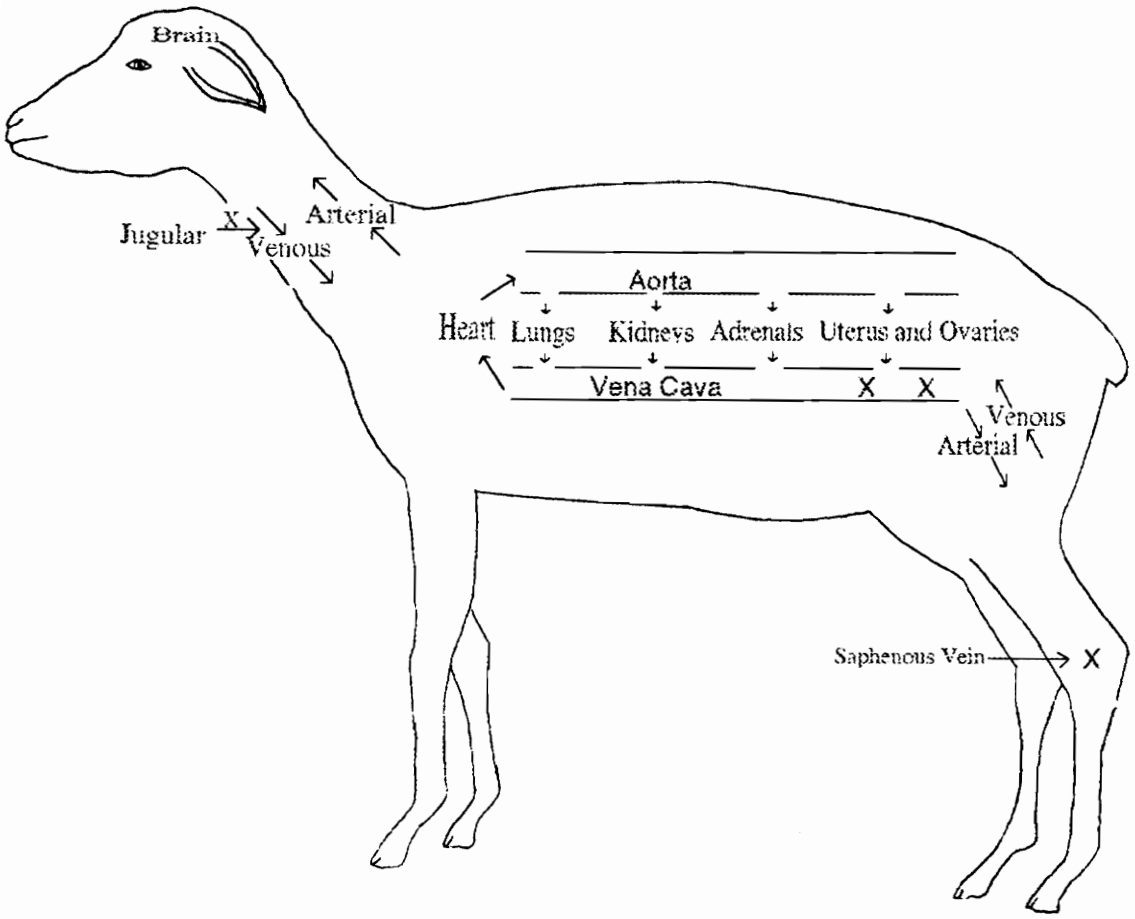

Figure 5. Schematic model for locations of $PGF_{2\alpha}$ synthesis and metabolism in the ewe. "X" indicates sampling locations used in this study.

the amount of $PGF_{2\alpha}$ produced by all other tissues before uterine $PGF_{2\alpha}$ contribution and that remaining after metabolism. The cranial samples would reflect the uterine contribution plus the caudal concentration.

Summary

Spontaneous ovulators have alternating periods of follicular and luteal development. As CL regress and ovarian follicles develop, progesterone concentrations decrease and estradiol concentrations increase. Progesterone and estradiol are responsible for priming the uterus for subsequent $PGF_{2\alpha}$ secretion, increasing the $PGF_{2\alpha}$ precursor pool, and influencing enzyme activity. Estradiol receptor concentrations are regulated in part by progesterone, and uterine oxytocin receptors are regulated by estradiol. Around luteolysis, concomitant pulses of oxytocin and $PGF_{2\alpha}$ are detected. Once the uterus becomes responsive to oxytocin, uterine $PGF_{2\alpha}$ secretion is initiated. Therefore, luteolysis in sheep may be controlled by the actions and interactions of estradiol, progesterone, and oxytocin on $PGF_{2\alpha}$ secretion. Prostaglandin $F_{2\alpha}$ induces luteolysis through several interacting mechanisms, including cAMP-related protein kinase activation and phoshatidylinositide/calcium/protein kinase C second messenger systems. The mechanism of induced luteal regression in ewes remains unknown. Exogenous $PGF_{2\alpha}$ causes a rapid decrease in progesterone, which is followed by an increase in estradiol within 24 h after the exogenous $PGF_{2\alpha}$. Thus, exogenous $PGF_{2\alpha}$ bypasses the need for the initial changes in estradiol, progesterone, and oxytocin. One may speculate that exogenous $PGF_{2\alpha}$ acts as the initial $PGF_{2\alpha}$ pulse, which initiates the cascade of events that lead to luteolysis, and two smaller doses of $PGF_{2\alpha}$ are more effective than one larger dose because two doses may mimic the recurring $PGF_{2\alpha}$ pulses that occur during spontaneous luteal regression.

OBJECTIVES

Based upon a review of the literature which reveals clear gaps in our understandings of the mechanism of action of exogenous $PGF_{2\alpha}$, the purpose of this study was to determine the uterine-ovarian response of luteal-phase ewes to two dosing regimens of $PGF_{2\alpha}$ (Lutalyse®; dinoprost tromethamine; generously donated by J.R. Chenault, The Upjohn Co., Kalamazoo, MI). In Exp. 1, a 2 x 2 factorial design with Lutalyse and H/Ox status as main effects and time as a split plot was used to determine the effects of a 15 mg dose of $PGF_{2\alpha}$ on uterine-ovarian $PGF_{2\alpha}$ secretion. Experiment 2 was conducted to determine the response to two 5 mg doses of $PGF_{2\alpha}$ given 3 h apart; the experimental design was similar to that of Exp. 1; however, the two injections added another split plot into the design. These experiments were designed to answer three major questions: 1) Does Lutalyse in intact and H/Ox ewes affect the mean $PGF_{2\alpha}$ concentration?; 2) Are the response curves to Lutalyse and saline in these ewes similar?, i.e., does Lutalyse or the act of injecting induce the response? 3) Does the total amount of $PGF_{2\alpha}$ present over the time analyzed, using area under the cranial response curve as the best estimate, vary with treatment? Thus, the uterine-ovarian responses were evaluated by comparing mean concentrations, shape of the response curves, and area under the response curves. Several pharmacokinetic variables were also estimated based on these responses. A validation experiment was also performed to determine whether $PGF_{2\alpha}$, progesterone, and PGFM in jugular blood from venipuncture differed from that in caudal vena caval blood and saphenous vein blood obtained via catheterization.

MATERIAL AND METHODS

General

Mature nonpregnant Suffolk, Dorset, Finn, and Hampshire crossbred ewes were penned twice daily during the breeding season with vasectomized rams. Ewes standing firmly for mounting were considered to be in estrus; the first day of estrus was designated d 0 of the estrous cycle. Ewes with at least two consecutive estrous cycles of 16 to 17 d in duration were used in this study. To minimize the effects of stress on prostaglandin production, ewes were handled extensively before and after surgery to accustom them to bleeding procedures. Thus, blood was obtained with a minimum of excitement to the ewes.

Experiment 1

Experimental Design

The aim of Exp. 1 was to study the dynamics of the changes in $PGF_{2\alpha}$ concentrations in response to one injection of Lutalyse into ewes on d 9 of the estrous cycle, i.e., d 9 ewes. This experiment was designed to determine the effects of exogenous $PGF_{2\alpha}$ ($PGF_{2\alpha}$ vs saline) and H/Ox (H/Ox vs sham H/Ox) on $PGF_{2\alpha}$ and progesterone concentrations. Ewes were assigned randomly to one of four treatment groups: 1) H/Ox, $PGF_{2\alpha}$-treated (H/Ox-Lutalyse); 2) H/Ox, saline-treated (H/Ox-saline); 3) sham-H/Ox, $PGF_{2\alpha}$-treated (Sham-Lutalyse); 4) sham-H/Ox, saline-treated (Sham-saline). Five ewes were used for each treatment group. The $PGF_{2\alpha}$-treated ewes were injected i.m. in the neck muscles with 3 mL (5 mg/mL; 15 mg) of Lutalyse, and control ewes received 3 mL of sterile saline. This experiment was conducted from October until December.

Blood Sampling

Frequent sampling for the first 2 h was indicated based upon studies by Granström and Kindahl (1982) and Kindahl (1985). These studies indicate that after an i.m. injection of 1.0 mg of $PGF_{2\alpha}$ in saline solution, PGFM concentrations in jugular blood increase within a few minutes. The increased concentrations were maintained for approximately 1 h, and then they return to pretreatment values within 3 to 4 h. A larger dose of $PGF_{2\alpha}$ (15 mg) was given in this experiment; therefore, blood samples were collected at 0, 5, 10, 15, 30, 60, 90, and 120 min relative to the injection of saline or Lutalyse. Samples were taken every 2 h for the next 20 h. To determine the luteolytic efficacy of the Lutalyse injection, progesterone concentrations in two samples taken 60 min before beginning the experiment were compared to the concentrations in two samples taken 36 and 48 h after injection.

For each blood collection, a 2 mL sample was taken from each catheter and discarded (catheter locations are described in detail in a subsequent section). Immediately after, a 12 mL sample was taken from a catheter placed caudal to the site where the uteroovarian blood enters the vena cava, and a 12 mL sample was taken with a separate syringe from a cranially placed catheter . Blood (6 mL) from each syringe was then transferred into the appropriately labeled, cranial or caudal, borosilicate glass tube and allowed to clot. Samples for serum were stored at room temperature for 4 h and checked for clotting before processing. The remaining 6 mL in each syringe was transferred into tubes containing 100 USP units of heparin (Sigma Chemical, St. Louis, MO) in 100 μL of physiological saline. Plasma collection tubes were capped and inverted several times to mix the heparin solution with the blood. Plasma samples were cooled in an ice water bath until processing. For processing, samples were centrifuged at 1,700 x g at 40° C for 25 min. Serum and plasma samples were then decanted into separate polypropylene tubes and stored at -20° C.

To prevent clotting, 3 mL of heparinized saline (50 USP units of heparin/mL of .9% saline) was injected into each catheter after each sampling time. After the 2-h bleeding schedule, a more concentrated heparinized saline solution (100 USP units/mL) was used to prevent clotting.

Changes in $PGF_{2\alpha}$ and progesterone concentration in the cranial and caudal vena cava were evaluated in response to each treatment. Progesterone concentration was used also to evaluate luteal regression, catheter location, and confirm the completeness of H/Ox.

Experiment 2

Experimental Design

The aim of Exp. 2 was to study the dynamics of the changes in $PGF_{2\alpha}$ concentrations in response to two 5 mg i.m. injections of Lutalyse given 3 h apart in d-9 ewes. The experimental design was similar to that of Exp. 1 with the exception that the two injections introduced a period effect into the design. Ewes were assigned randomly to one of four treatment groups: 1) H/Ox-Lutalyse; 2) H/Ox-saline; 3) Intact, $PGF_{2\alpha}$-treated (Intact-Lutalyse); 4) Intact, saline-treated (Intact-saline). The results of Exp. 1 indicated that a sham H/Ox surgery was unnecessary. The $PGF_{2\alpha}$-treated ewes were injected i.m. in the neck muscles with 1 mL (5 mg/mL; 5mg) of Lutalyse, and control ewes received 1 mL of sterile saline at each treatment time. This experiment was conducted from February until April, which is the latter part of the breeding season.

Blood Sampling

The PGFM profile from Exp. 1 indicated that $PGF_{2\alpha}$ is rapidly absorbed. Many blood samples are needed to obtain an absorption profile for $PGF_{2\alpha}$. However, pharmacokinetic analysis may be invalid if more than 5% of the total blood volume is

removed because it may represent a route of elimination(McGuill and Rowan, 1989). Extensive blood loss may also induce physiologic effects. Based upon these factors, blood samples were collected at 0, 2, 4, 6, 8, 10, 12, 24, 36, 60, 90, 120, and 150 min relative to Lutalyse or saline injection. Three hours after the first injection, when the second dose was given, the sampling schedule was repeated. The luteolytic efficacy of $PGF_{2\alpha}$ was determined by comparing progesterone concentrations in blood collected 1 h before to that in blood 36 to 50 h after treatment.

A 2-mL sample was taken from each catheter and discarded before each sample collection. Two 6 mL samples were taken simultaneously from the caudally and cranially placed catheters. For this experiment, only blood for plasma was collected. Blood was transferred immediately after collection into the appropriately labeled borosilicate glass tube containing 100 USP units of heparin/100 µl of .9% saline. Processing of the blood was identical to that in Exp. 1. To prevent clotting, 3 mL of heparinized saline (10 USP units/mL of .9% saline) was injected into the catheter after each sampling time with the exception of between the 2-min bleedings; no heparin solution was required during this time.

Changes in $PGF_{2\alpha}$ concentrations in the cranial and caudal vena cava, as well as PGFM concentrations in the cranial vena cava, were evaluated in response to each treatment. Progesterone concentration in several samples were used to evaluate luteal regression, catheter location, and confirm the completeness of H/Ox.

Validation Experiment

A short experiment was performed to test the effects of sampling location on progesterone, $PGF_{2\alpha}$, and PGFM concentrations. A second reason for this effort was to determine whether caudal distance from the uteroovarian-vena caval junction influenced the prostaglandin pattern. Simultaneous blood samples (5 mL) were collected from the jugular vein, the vena cava caudal to the uteroovarian vein, and the saphenous vein (Figure 5) in four ewes. These samples were transferred into borosilicate glass tubes containing 100 USP units of heparin in 100 μL of physiological saline. After complete mixing, the samples were placed on ice and processed for plasma as described previously.

Surgeries

Vena Caval Catheterization

After 24 h without feed and water, ewes were anesthetized with sodium pentobarbital (65 mg/mL of saline; Sigma Chemical) and the vena cava in each ewe was catheterized (Benoit and Daily, 1991). In this procedure, the saphenous vein dorsal to each hock was surgically exposed. A 90-cm long polyvinyl catheter (i.d. .42 mm, o.d. .74 mm, ICO-Rally Corp., Palo Alto, CA) was inserted into each vein through a small incision and then passed up through the saphenous vein and into the vena cava. The catheters were marked at 40, 45, 50, 55, 60, and 65 cm from the external end. One 4-mL blood sample was taken at each marked position on one of the catheters as it was passed up the vein. A second sample was taken at each position after several minutes, and the catheter was positioned temporarily. For each blood collection, a 2-mL sample was taken and discarded. Catheter placement was determined according to progesterone concentrations; high progesterone concentration marked the region where uteroovarian blood entered the vena cava. The two catheters, one cranial and one caudal to the uteroovarian vein (Figure

5), were used to collect vena caval blood with and without contributions from the uterus and ovaries. The caudal position was set to 10 cm less than the cranial position. Eight intact ewes were slaughtered after the end of Exp. 1 to visually assess catheter position relative to the junction of the uteroovarian vein and the vena cava. To prevent blood from clotting in the catheters, 3 mL of heparinized saline (100 USP units of heparin in 1 mL of .9% saline) were injected into each catheter. Catheters were checked for clotting 4 h before blood collection. If the catheters were blocked, they were removed and replaced, without changing placement distance. In Exp. 1, six catheters were blocked before the experiment began. These catheters were replaced using minimal sodium pentobarbital, and ewes were rested for 3 h before being used in the experiment.

There are limitations to this procedure. In several ewes with saphenous veins that were catheterized earlier in the year or during the previous year, the vein had collapsed, deteriorated, or developed scar tissue excluding catherization. This represented a limitation for this experiment, because these ewes had to be culled from this and other vena caval catheterization studies. It seems that the frequency of blood collection may also affect catheter patency, because several catheters failed during the latter stages of blood collection. In two ewes, necrotic or fibrous tissue was in close apposition to the catheter and in the catheter itself in two other ewes. Thus, the necrotic or fibrous tissue may have either blocked blood flow into the catheter or restricted blood flow through the catheter. Once the catheter and necrotic/fibrous tissue were removed, the catheterization procedure was performed using minimal anesthetic. Sodium pentobarbital has a direct effect on the hypothalamus, and thus LH secretion (Lewis et al., 1985). Therefore, when catheters were replaced, minimal sodium pentobarbital (< 6 mL; 65mg/ml) was given to minimize any hypothalamic effect. The longest time that a ewe was sedated for catheter replacement was 20 min.

In several ewes in Exp. 2, Silastic® medical grade catheters were used for vena caval catherization (i.d. .062 in, o.d. .125 in ; Dow Corning, Midland, MI). No evidence

of blood clotting was detected when this material was used. One shortcoming of the Silastic® tubing was the slight difficulty in stabilizing catheter location without occluding the catheter. A heparin solution of 100 USP units/mL of saline was used to prevent clotting within the catheters before the start of the experiment. Catheters were positioned at least 36 h before blood collection. All catheters were checked 3 h before blood collection.

Hysterectomy-Ovariectomy Procedures

In Exp. 1, all ewes were housed without feed and water for 24 h before H/Ox or sham H/Ox surgery. Ewes were anesthetized and prepared for surgery, and the H/Ox and sham H/Ox procedures were performed through a midventral laparatomy. The sham H/Ox procedure included several minutes of massage of the reproductive tract to simulate the effects of surgery. After H/Ox, at least two cervical rings determined visually on the excised portion of the reproductive tract confirmed completeness of H/Ox. These procedures were completed at least 36 h before blood collection. For Exp. 2, ewes were H/Ox 7 to 10 d before vena caval catherization.

Assay Procedures and Results

Progesterone

Two RIA were used to determine progesterone concentrations. A [^{125}I] progesterone kit (Diagnostic Products Corp., Los Angeles, CA) and a [^{3}H]progesterone (Gengenbach, et al., 1977) RIA were used to determine progesterone concentration for catheter position. For samples obtained after catheter placement, the [^{3}H]progesterone RIA was used to quantify progesterone. All samples were assayed in duplicate. The intraassay CV for Exp. 1 was 4.6%, and the interassay CV was 12.8%. For Exp. 2, all

samples were assayed in a single assay. The intraassay CV was 8.9%. For the validation experiment, only one assay was required; and the intraassay CV was 2.3%.

Prostaglandins

Using EIA developed by Del Vecchio et al. (1991) and Fortin et al. (1994), PGFM and $PGF_{2\alpha}$ concentrations were measured in the same extract of a given sample. Del Vecchio et al. (1991) and Fortin et al. (1994) used ethyl acetate (Fisher Scientific, Fairlawn, NJ) as the extraction solvent. However, acidified anesthesia grade ether (Mallinckrodt, Paris, Kentucky) was used for this study, because ether evaporates quicker than ethyl acetate. Extraction efficiencies for PGFM and $PGF_{2\alpha}$ did not differ for these two solvents ($P > .05$; intraassay CV = 3.7 and 18.1%, respectively). The interassay and intraassay CV for $PGF_{2\alpha}$ were 27.7 and 8.3%, respectively. The interassay CV for PGFM was 26.8%, and the intraassay CV was 7.5%.

Statistical Analysis

The Statistical Analysis System (SAS, 1985) was used for all statistical analyses. Slidewrite 2.0 Plus (1992, Advanced Graphics Software, Inc., Carlsbad, CA) for Windows 3.1 (Microsoft Corp., Redmond, WA), Microsoft Excel 4.0 (Microsoft Corp.), and Quattro Pro 1.0 (1992, Borland Int., Scotts Valley, CA) for Windows 3.1 were used to produce graphic representations of the data and to perform calculations.

Experiment 1

To determine whether H/Ox or Lutalyse, or their interaction significantly affected the mean concentration and if the cranial $PGF_{2\alpha}$ concentration changed over time, the effects of the fixed variables H/Ox, Lutalyse (main effects) and time (T), as well as the random effect of ewe, on mean $PGF_{2\alpha}$ and progesterone concentration in the cranial and caudal vena cava were determined using the following GLM statements:

```
PROC GLM; CLASSES H/OX LUTALYSE EWE T;
MODEL HORMONE = H/OX LUTALYSE H/OX*LUTALYSE
EWE(H/OX*LUTALYSE) T H/OX*T LUTALYSE*T H/OX*LUTALYSE*T ;
LSMEANS H/OX LUTALYSE H/OX*LUTALYSE/PDIFF;
```

where HORMONE was defined as either cranial $PGF_{2\alpha}$, caudal $PGF_{2\alpha}$, cranial progesterone, or caudal progesterone.

As the mean concentration increased for $PGF_{2\alpha}$, the standard deviation increased. Therefore, Hartley's test for homogeneity of variance was performed by comparing the variance in the Lutalyse-treated groups (TRT 1 & 3) to the variance in the saline-treated groups (TRT 2 & 4) using the following test:

H_0: $\sigma^2_{TRT\ 1\ \&\ 3} = \sigma^2_{TRT\ 2\ \&\ 4}$
H_a: $\sigma^2_{TRT\ 1\ \&\ 3} \neq \sigma^2_{TRT\ 2\ \&\ 4}$
Reject H_0 if $F_{\alpha=.05} > F_{crit}$ with DF_{max}, DF_{min}
where $F_{crit} = MSE_{max}/MSE_{min}$

If H_0 is rejected, the variances are heterogeneous and a transformation on the data is required. A natural logarithm transformation stabilized the variance on all datasets where heterogeneity of variance was indicated. Analysis between treatment groups was then performed on the transformed data with homogeneous variance. Reported means are calculated from untransformed data.

If time was a significant variable, i.e., if concentration changed over time, the overall shape of the curve for each hormone in response to treatment (TRT) was determined. The model, which included the greatest power ("n") of T (tested as a linear [n = 1], quadratic [n = 2], cubic [n = 3], quartic [n = 4], etc. polynomial) that was significant, was selected as the best overall curve for describing the changes in hormone concentration. To determine the overall shape of each curve, the following SAS statements were used:

```
  PROC GLM; CLASSES EWE TRT;
MODEL HORMONE = TRT EWE(TRT) T1...Tⁿ/SOLUTION SS1 SS2 SS3
  SS4;
```

Using the overall shape of the curve for each hormone, SAS models were then developed to describe the changes within treatments. To test for coincidence, the individual treatment profiles were based upon the overall shape of the curve. Profiles were compared for each treatment and variable as described previously (Gill and Hafs, 1971; Allen, 1983). This statistical test is used to answer the question, "Do the curves deviate from parallelism?". Thus,

H_0:Trt A = Trt B H_a:Trt A≠Trt B

where A and B represent two treatment groups

F_{crit}= MSE(overall for TRT A&B-sum of A+B) / MSE(sum of A+B)

with DF_1 = DF(overall for TRT A&B-sum of A+B)-DF (sum of A+B)

and DF_2 = DF(sum of A+B)

Reject H_0 if $F_{crit} > F_{\alpha=.05, DF_1, DF_2}$

The following SAS statements were used to calculate the MSE, the error associated with the model for that ordered polynomial for the overall treatment and individual treatment contributions:

```
PROC GLM; CLASSES TRT EWE;
MODEL HORMONE = TRT EWE(TRT) T1...Tⁿ/SS1 SS2 SS3 SS4;
PROC SORT; BY TRT:
PROC GLM; BY TRT; CLASSES EWE;
MODEL HORMONE = EWE T1...Tⁿ/SS1 SS2 SS3 SS4;
```

where "n" was consistent with the order of the overall curve determined for that hormone. The following table was designed for these computations and is provided as an example comparing the cranial $PGF_{2\alpha}$ response curve in H/Ox-saline vs H/Ox-Lutalyse treatments.

TABLE 1. SAMPLE CALCULATION FOR COMPARISON OF PROFILES

Treatment	DF	SSE	MSE	F value	P>F	H_0
Overall	191	122.0945044				
ΣIndividual	187	101.7400273	.54406565			
Overall -Σ Individuals	4	20.3542281	5.08557025			
				9.35	.001	Reject

Peak responses were estimated for each ewe, because this approach should reflect true physiological responses to the treatments. Analyses of the cranial $PGF_{2\alpha}$ peak detected in this experiment included: peak $PGF_{2\alpha}$ concentration, $PGF_{2\alpha}$ response, duration of the peak, and area under the cranial response curve (AUC). The effect of H/Ox status and Lutalyse treatment as well as their interaction on these pharmacokinetic variables was estimated by using the following SAS statements:

```
PROC GLM; CLASSES EWE H/OX LUTALYSE;
MODEL PGF2α PEAK PGF2α RESPONSE DURATION AUC =
  H/OX LUTALYSE H/OX*LUTALYSE;
LSMEANS H/OX*LUTALYSE/PDIFF;
```

To determine if the catheters were positioned correctly, the effect of sample location on $PGF_{2\alpha}$ and progesterone (P_4) concentration was determined using the following SAS statements:

```
PROC SORT; BY TRT;
PROC GLM; BY TRT; CLASSES EWE LOCATION;
MODEL PGF2α P4 = LOCATION EWE(LOCATION);
LSMEANS LOCATION/PDIFF;
```

For Exp. 1, an additional question was asked. Is progesterone a significant covariant for the $PGF_{2\alpha}$ responses, i.e., do the changes account for a significant amount of the changes in $PGF_{2\alpha}$? Both cranial and caudal progesterone concentrations were tested as covariants in cranial and caudal $PGF_{2\alpha}$ analyses using the following SAS statements:

```
PROC GLM; CLASSES TRT EWE;
MODEL CRANIAL PGF2α CAUDAL PGF2α = TRT EWE(TRT)
  CRANIAL P4 CAUDAL P4 T1...Tn/SS1 SS2 SS3 SS4;
```

Experiment 2

The statistical approaches, including testing for hetergenous variances and tranforming data, for determining the effect of treatment was similar to Exp. 1. In addition to determining the effects of treatment on $PGF_{2\alpha}$ concentrations, treatment effects were also determined for PGFM. The effect of period was first tested for all analyses because two injections were given at specific times. If period was significant, then analyses were performed separately on each period; otherwise, periods were combined. Did H/Ox status or Lutalyse or an interaction between them affect these concentrations? Did these concentrations change over time? If so, were these changes

over time dependent on H/Ox status, Lutalyse treatment, or their intereaction? To estimate these effects, the overall model was:

```
PROC GLM; CLASSES H/OX LUTALYSE EWE T;
MODEL CRANIAL PGF2α CAUDAL PGF2α PGFM = H/OX LUTALYSE
  H/OX*LUTALYSE EWE(H/OX*LUTALYSE) T H/OX*T LUTALYSE*T
  H/OX*LUTALYSE*T T*EWE(H/OX*LUTALYSE) PERIOD
  PERIOD*H/OX PERIOD*LUTALYSE PERIOD*H/OX*LUTALYSE
  PERIOD*EWE(H/OX*LUTALYSE) PERIOD*T PERIOD*H/OX*T
  PERIOD*LUTALYSE*T PERIOD*H/OX*LUTALYSE*T;
```

In addition, the question of whether $PGF_{2\alpha}$ was a significant covariant for PGFM concentrations was asked. Several orders of $PGF_{2\alpha}$ (L1 = log(CRAN), L2 = $(L1)^2$, L3 = $(L1)^3$, ...Ln = $(L1)^n$) were tested for significance using:

```
PROC GLM; CLASSES TRT EWE;
MODEL PGFM = TRT EWE(TRT) T1..Tn L1..Ln/SS1 SS2 SS3 SS4;
```

Validation Experiment

The effect of sample location on progesterone, $PGF_{2\alpha}$, and PGFM concentrations was examined. The effect of sample location was determined using the following SAS statements:

```
PROC GLM; CLASSES LOCATION SAMPLE EWE;
MODEL PGF2α PGFM P4 = LOCATION EWE(LOCATION)
  SAMPLE SAMPLE*LOCATION;
LSMEANS LOCATION/PDIFF;
```

RESULTS

Experiment 1

Cranial $PGF_{2\alpha}$ Response

The effect of treatment on mean cranial $PGF_{2\alpha}$ concentration for the duration of Exp. 1 is shown in Table 2. The SAS analyses for the entire duration of the experiment indicated that cranial $PGF_{2\alpha}$ concentrations were affected by Lutalyse ($P < .025$), time ($P < .0001$), and their interaction ($P < .0001$), but not by the interaction between H/Ox status and Lutalyse ($P > .05$). However, visual examination of the data indicated that the $PGF_{2\alpha}$ response was complete within 240 min after $PGF_{2\alpha}$ injection (Figure 6). Therefore, the data were divided into two subsets: $T < 240$ min and $T > 240$ min. After 240 min, cranial $PGF_{2\alpha}$ concentrations were not affected ($P > .05$) by any of the variables. However before 240 min, H/Ox ($P < .05$) and Lutalyse ($P < .0001$) affected cranial $PGF_{2\alpha}$ concentrations (Table 2). Results for all datasets are provided, although the discussions will pertain only to the $T < 240$ min data. Cranial $PGF_{2\alpha}$ concentrations were greatest ($P < .05$) in sham-H/Ox ewes treated with Lutalyse (3,654.3 pg/mL). Concentrations in Lutalyse-treated H/Ox ewes (1,069.3 pg/mL) were greater ($P < .05$) than those in saline-treated H/Ox and sham-H/Ox ewes (283.0 pg/mL and 320.8 pg/mL, respectively).

The $PGF_{2\alpha}$ concentration in the cranial vena cava changed over time ($P < .05$). Therefore, regression analysis using Type III sum of squares and time as a covariant was used to determine that the shape of the overall cranial $PGF_{2\alpha}$ response curve could best be described by a quartic equation. Individual regression equations were derived to describe the changes in $PGF_{2\alpha}$ concentration for each treatment based upon this quartic time component. The effects of treatment on the cranial $PGF_{2\alpha}$ profiles are shown in Table 4. The cranial $PGF_{2\alpha}$ profile did not differ between H/Ox-saline and sham-saline

ewes. However, the cranial $PGF_{2\alpha}$ profile in these ewes differed from H/Ox Lutalyse-treated and sham-Lutalyse ewes ($P < .05$). Cranial $PGF_{2\alpha}$ profiles in response to Lutalyse were similar between H/Ox and sham H/Ox ewes.

Peak $PGF_{2\alpha}$ concentration, increment in $PGF_{2\alpha}$ concentration (response), and duration of the peak provide insight into individual responses to treatment (Table 5). Basal concentrations of $PGF_{2\alpha}$ can not be estimated accurately, because only one sample was taken before treatment and due to the pulsatile nature of $PGF_{2\alpha}$ secretion. However, because cranial $PGF_{2\alpha}$ concentrations after 240 min were unaffected by any of the fixed effects, these concentrations were used to determine a resting cranial $PGF_{2\alpha}$ concentration for each ewe.

Peak concentration was greater ($P < .0001$) in sham-Lutalyse treated ewes (10,163.0 pg/mL) than in H/Ox Lutalyse-treated ewes (2,332.4 pg/mL). Peak concentrations were even less ($P < .001$) in sham-saline treated (680.9 pg/mL) and H/Ox saline-treated ewes (319.2 pg/mL). Peak concentrations were 21-fold greater than resting concentrations in sham Lutalyse-treated and nine-fold greater in H/Ox Lutalyse-treated ewes.

The cranial $PGF_{2\alpha}$ response, measured as the increase in $PGF_{2\alpha}$ concentration from resting concentrations, in H/Ox saline-treated ewes (91.6 pg/mL) was less ($P < .01$) than that in sham saline-treated ewes (299.2 pg/mL). This response was greater ($P < .001$) in H/Ox Lutalyse-treated ewes (1,927.9 pg/mL). An interaction between H/Ox status and Lutalyse treatment was detected; the increase in cranial $PGF_{2\alpha}$ concentration in sham Lutalyse-treated ewes (9,473.2 pg/mL) more than quadrupled that in H/Ox Lutalyse-treated ewes.

The duration of the detected peak, measured as the time interval that the cranial $PGF_{2\alpha}$ concentration was two standard deviations greater than the resting concentration for each ewe, was also affected by treatment ($P < .0001$). In one H/Ox saline-treated and two intact saline-treated ewes, peaks were detected. The durations of these peaks were

similar ($P > .05$). These increments were minor and of short duration ($T < 8$ min) when compared to the peaks detected in Lutalyse-treated ewes (Table 5), and probably represented typical spontaneous $PGF_{2\alpha}$ release. In H/Ox Lutalyse-treated ewes, the mean duration of the peak (77 min) was less ($P < .02$) than in sham-Lutalyse treated ewes (132 min).

Any analysis on the area under the cranial $PGF_{2\alpha}$ response curve (AUCCRAN) is somewhat biased because no true baseline can be calculated, and the ewe to ewe variation is great. Analysis of the $T < 240$ min data could be performed using the average concentration after 240 min as a resting concentration to correct for ewe to ewe variation. For each ewe, the AUC was calculated by subtracting the resting value for that ewe from each of the cranial $PGF_{2\alpha}$ concentrations in the $T < 240$ min dataset. Differences among these areas depended on H/Ox status ($P < .05$), Lutalyse ($P < .0001$), and their interaction ($P < .05$). The AUC for the cranial response did not differ between H/Ox saline-treated or sham saline-treated ewes. However, the areas for both groups of saline-treated ewes were less ($P < .05$) than those for H/Ox and sham Lutalyse-treated ewes ($P < .05$). An interaction between Lutalyse and the reproductive tract was detected; the AUC for sham Lutalyse-treated ewes was greater ($P < .005$) than the AUC for H/Ox Lutalyse-treated ewes. The effect of treatment on mean area under the cranial response curve is provided in Table 5.

Caudal $PGF_{2\alpha}$ Response

The effects of treatment on mean caudal $PGF_{2\alpha}$ concentrations for the entire duration of Exp. 1 are shown in Table 6. Plots of the mean caudal $PGF_{2\alpha}$ concentration over time for each treatment group indicated that, as the cranial response, the caudal response was complete within 240 min (Figure 7). After 240 min, caudal $PGF_{2\alpha}$ concentrations were not affected ($P > .05$) by time, H/Ox status, or Lutalyse treatment.

However before 240 min, Lutalyse affected ($P < .01$) caudal $PGF_{2\alpha}$ concentrations . The effects of treatment on mean caudal $PGF_{2\alpha}$ concentration are provided in Table 7. Caudal $PGF_{2\alpha}$ concentrations are similar ($P > .05$) in saline-treated ewes. Sham and H/Ox Lutalyse-treated ewes differed from each other and saline-treated ewes. The mean concentration of $PGF_{2\alpha}$ from the caudal location in H/Ox Lutalyse-treated ewes (1,111.5 pg/mL) was twice that in sham Lutalyse-treated ewes (562.5 pg/mL).

Caudal $PGF_{2\alpha}$ concentrations changed ($P < .0001$) over time in response to treatment. The overall regression equation that best described changes in caudal $PGF_{2\alpha}$ concentrations indicated that these responses were quartic in shape. Individual regression equations were derived to describe these changes in response to treatments. Table 4 shows the results from caudal $PGF_{2\alpha}$ profile comparisons between treatment. Changes in $PGF_{2\alpha}$ concentrations in the caudal vena cava were similar in saline-treated and sham H/Ox Lutalyse-treated ewes. The caudal $PGF_{2\alpha}$ profile in H/Ox Lutalyse-treated ewes differed from all other treatment groups.

Cranial Progesterone Response

The SAS analysis (Table 8) indicated a significant ($P < .0001$) effect of H/Ox status on cranial progesterone concentrations as well as an interaction between time and H/Ox, and Lutalyse. Mean progesterone concentrations in H/Ox-ewes, regardless of treatment did not differ (.1 ng/mL). The mean progesterone concentration in sham-operated ewes differed ($P < .05$) between saline and Lutalyse treatment and from H/Ox ewes. In intact ewes, the overall mean progesterone concentration for Lutalyse-treated ewes for the entire duration of the experiment was 3.1 ng/mL and 4.4 ng/mL for saline-treated ewes. Luteolytic efficacy was determined by comparing progesterone concentrations in two samples taken 60 min before and 36 to 48 h after the beginning of the experiment. In saline -treated sham H/Ox ewes, the cranial vena cava progesterone

concentration was greater than 2 ng/mL. However, in sham H/Ox Lutalyse-treated ewes, the progesterone concentration in the cranial vena cava 36 or 48 h after Lutalyse treatment was less than .3 ng/mL.

The purpose of using Lutalyse in midluteal phase ewes was to induce luteolysis, which would modify the luteal progesterone secretory pattern. Thus, the shape of the progesterone curve in intact ewes after Lutalyse should differ from that in saline-treated ewes. Analyses from SAS indicated that progesterone concentrations changed over time and with treatment. Therefore, individual regression equations were derived to describe these changes (Figure 8). Comparison of cranial progesterone profiles (Table 9) indicated that the cranial progesterone curve in Lutalyse-treated ewes differed ($P < .001$) from all other treatments. However, among H/Ox-saline and H/Ox Lutalyse-treated ewes and sham saline-treated ewes, changes in progesterone concentration were similar ($P > .05$).

The progesterone data was not split at 240 min, because in sham Lutalyse-treated ewes progesterone concentrations decreased slowly (Figure 8). According to the SAS-generated equation for sham-Lutalyse ewes, cranial progesterone concentration decreased to concentrations measured in H/Ox ewes within 24 h. The time to luteolysis, measured as changes in progesterone concentrations, varied from ewe to ewe. However, in all sham-Lutalyse ewes, a small peak of progesterone was detected after the initial decrease in progesterone concentration.

Caudal Progesterone Response

Caudal progesterone concentrations were affected by H/Ox status ($P < .0001$), time ($P < .0033$), and their interaction ($P < .05$; Table 8; Figure 9). Caudal progesterone concentrations in sham Lutalyse-treated (.9 ng/mL) ewes were greater ($P < .05$) than those in H/Ox Lutalyse-treated ewes (.1 ng/mL) and sham saline-treated ewes (.7 ng/mL). Mean progesterone concentrations in H/Ox ewes were similar regardless of treatment.

Individual regression equations were derived to describe the changes in caudal vena caval progesterone concentration. Caudal progesterone profiles did not differ ($P > .05$) among H/Ox ewes. However, caudal progesterone profiles in sham saline-treated ewes differed at $P = .06$ from those in H/Ox saline-treated ewes, but not from H/Ox Lutalyse-treated ewes. Profiles in sham-Lutalyse ewes differed ($P < .025$) from all other treatment groups. These results are provided in Table 9.

Verification of Catheter Location

The SAS analyses confirmed that the catheters were positioned correctly with respect to the uteroovarian vein. The effects of catheter location on mean $PGF_{2\alpha}$ concentration for the entire duration of the experiment are shown in Table 10. In sham Lutalyse-treated ewes, cranial $PGF_{2\alpha}$ and progesterone concentrations were greater ($P < .05$) than the corresponding caudal concentration. Progesterone concentrations in sham Lutalyse-treated ewes were 3.1 and .7 ng/mL for cranial and caudal samples, respectively, In sham saline-treated ewes, cranial and caudal $PGF_{2\alpha}$ concentrations were similar ($P > .05$). Progesterone concentration, cranial and caudal, was used to verify catheter position in sham saline-treated ewes. In this treatment group, the cranial progesterone concentration (4.4 ng/mL) was greater ($P < .05$) than the caudal concentration (.9 ng/mL). Eight intact ewes were slaughtered after the end of Exp. 1 to visually assess catheter position relative to the junction of the uteroovarian vein and the vena cava. In these ewes, the cranial catheter was located within the vena cava cranial to the uteroovarian vein, and the caudal catheter was caudal to this junction. In H/Ox ewes, catheter location did not affect ($P > .05$) either $PGF_{2\alpha}$ or progesterone concentrations. In H/Ox Lutalyse-treated and saline-treated ewes, cranial and caudal progesterone concentrations were .1 ng/mL.

Progesterone As A Covariant For $PGF_{2\alpha}$

Does progesterone influence the $PGF_{2\alpha}$ profile? The SAS analyses indicated that cranial and caudal progesterone concentrations changed as the cranial $PGF_{2\alpha}$ profile changed ($P < .05$). When cranial and caudal progesterone concentrations were included in the ANOVA model, the overall shape of the curve, determined as the order of time as a significant covariant, changed. A linear effect of time was defined for the cranial $PGF_{2\alpha}$ response curve rather than a quartic response. Approximately 3% of the variation in cranial $PGF_{2\alpha}$ concentration was explained by changes in cranial and caudal progesterone concentration. Progesterone was not a significant covariant for explaining the variation in $PGF_{2\alpha}$ concentration in the caudal vena cava.

TABLE 2. EFFECT OF TREATMENT ON CRANIAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT I

	Entire duration[a]			T < 240 min[a]			T > 240 min[b]		
Variable	DF	F value	P>F	DF	F value	P>F	DF	F value	P>F
H/OX	1	1.33	ns	1	4.55	.0500	1	.00	ns
LUTALYSE	1	7.22	.0250	1	31.68	.0001	1	.12	ns
H/OX*LUTALYSE	1	.66	ns	1	.97	ns	1	.36	ns
EWE(H/OX*LUTALYSE)	16			16			16		
T	20	13.12	.0001	8	10.42	.0001	12	1.25	ns
H/OX*T	20	2.04	ns	8	.78	ns	12	1.27	ns
LUTALYSE*T	20	14.18	.0001	8	7.40	.0001	12	1.59	ns
H/OX*LUTALYSE*T	20	2.00	ns	8	2.39	.0198	12	1.34	ns

[a]Indications of significance are based upon ANOVA using transformed data; n = 5 ewes/group.

[b]Indications of significance are based upon ANOVA on untransformed data; n = 5 ewes/group.

TABLE 3. EFFECT OF TREATMENT ON MEAN CRANIAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 1[a]

	Cranial $PGF_{2\alpha}$, pg/mL		
Treatment group	Entire duration[b]	T < 240 min[b]	T > 240 min[c]
H/Ox-Lutalyse	605.9[d]	1,069.3[d]	286.0[d]
H/Ox-saline	268.4[e]	283.0[e]	254.6[d]
Sham-Lutalyse	1,762.1[f]	3,654.3[f]	314.1[d]
Sham-saline	319.3[g]	320.8[g]	323.7[d]
Error MS (n = 5 ewes/group)	1.447	1.578	1.252

[a]Means were calculated from untransformed data.

[b]Indications of significance are based upon ANOVA using transformed data. Error MS reported has been untransformed.

[c]Indications of significance and Error MS reported are based upon ANOVA on untransformed data.

[d,e,f,g]Within a column, different superscripts differ (P < .05).

TABLE 4. COMPARISONS OF CRANIAL $PGF_{2\alpha}$ PROFILES FOR T < 240 MIN

EXPERIMENT 1[a]

	Cranial response[b]			Caudal response[b]		
Treatment Group	DF	F_{crit}	P>F	DF	F_{crit}	P>F
H/Ox-Lutalyse vs H/Ox-saline	67	5.11	.001	66	2.85	.025
H/Ox-Lutalyse vs sham-Lutalyse	69	1.56	ns	68	1.95	ns
H/Ox-Lutalyse vs sham-saline	67	2.47	.05	68	4.53	.001
H/Ox-saline vs sham-saline	66	1.23	ns	68	1.89	ns
H/Ox-saline vs sham-Lutalyse	68	4.81	.001	68	.86	ns
Sham-Lutalyse vs sham-saline	68	8.24	.001	70	2.32	ns

[a]Indications of significance were based upon ANOVA using transformed data; n = 5 ewes/group.

[b]For cranial and caudal comparisons, $DF_1 = 4$.

TABLE 5. EFFECT OF TREATMENT ON PEAK CRANIAL $PGF_{2\alpha}$ CHARACTERISTICS

EXPERIMENT 1[a]

Treatment Group	Peak[b], pg/mL	Response[b], pg/mL	Duration[c], min	Area under curve[c]
H/Ox-Lutalyse	2,332.4[d]	1,927.9[d]	77.0[d]	149,518.6[d]
H/Ox-saline	319.2[e]	91.6[e]	7.5[e]	18,698.9[e]
Sham-Lutalyse	10,163.0[f]	9,473.2[f]	132.0[f]	362,132.4[f]
Sham-saline	680.9[g]	299.2[g]	2.0[e]	15,223.4[e]
Error MS (n = 5 ewes/group)	1.230	1.355	1144.333	8.648E+9

[a]Means were calculated from untransformed data.

[b]Indications of significance are based upon ANOVA using transformed data. Error MS reported has been untransformed. For definitions of characteristics, see pages 45 and 46.

[c]Indications of significance and Error MS reported were based upon ANOVA on untransformed data. For definitions of characteristics, see pages 45 and 46.

[d,e,f,g]Within a column, different superscripts differ ($P < .05$).

TABLE 6. EFFECT OF TREATMENT ON CAUDAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 1

	Entire duration[a]			T < 240 min[a]			T > 240 min[b]		
Variable	DF	F value	P>F	DF	F value	P>F	DF	F value	P>F
H/OX	1	.23	ns	1	.62	ns	1	.00	ns
LUTALYSE	1	2.35	ns	1	8.52	.0100	1	.03	ns
H/OX*LUTALYSE	1	1.55	ns	1	2.73	ns	1	.30	ns
EWE(H/OX*LUTALYSE)	16			16			16		
T	20	4.87	.0001	8	4.78	.0001	12	1.00	ns
H/OX*T	20	.97	ns	8	.73	ns	12	1.20	ns
LUTALYSE*T	20	3.75	.0001	8	3.91	.0004	12	.61	ns
H/OX*LUTALYSE*T	20	1.15	ns	8	1.09	ns	12	.94	ns

[a]Indications of significance were based upon ANOVA using transformed data; n = 5 ewes/group.

[b]Indications of significance were based upon ANOVA on untransformed data; n = 5 ewes/group.

TABLE 7. EFFECT OF TREATMENT ON MEAN CAUDAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 1[a]

Treatment group	Caudal $PGF_{2\alpha}$, pg/mL		
	Entire duration[b]	T < 240 min[b]	T > 240 min[c]
H/Ox-Lutalyse	624.9[d]	1,111.5[d]	287.0[d]
H/Ox-saline	285.3[e]	323.1[e]	266.3[d]
Sham-Lutalyse	391.4[f]	565.5[f]	286.0[d]
Sham-saline	316.0[e]	337.0[e]	304.8[d]
Error MS (n = 5 ewes/group)	1.560	1.572	1.413

[a]Means were calculated from untransformed data.

[b]Indications of significance were based upon ANOVA using transformed data. Error MS reported has been untransformed.

[c]Indications of significance and Error MS reported are based upon ANOVA on untransformed data.

[d,e,f]Within a column, different superscripts differ (P < .05)

TABLE 8. EFFECT OF TREATMENT ON CRANIAL AND CAUDAL PROGESTERONE CONCENTRATIONS

EXPERIMENT 1[a]

Variable	DF	Cranial Progesterone		Caudal Progesterone	
		F value	P>F	F value	P>F
H/OX	1	73.11	.0001	20.32	.0001
LUTALYSE	1	2.13	ns	.46	ns
H/OX*LUTALYSE	1	2.06	ns	.45	ns
EWE(H/OX*LUTALYSE)	16				
T	20	3.49	.0001	2.15	.0033
H/OX*T	20	3.42	.0001	1.91	.0113
LUTALYSE*T	20	2.39	.0009	.67	ns
H/OX*LUTALYSE*T	20	2.40	.0008	.71	ns

[a]Indications of significance are based upon ANOVA using transformed data; n = 5 ewes/group.

TABLE 9. COMPARISONS OF CRANIAL AND CAUDAL PROGESTERONE PROFILES

EXPERIMENT 1[a]

	Cranial response[b]			Caudal response[b]		
Treatment group	DF	F_{crit}	P>F	DF	F_{crit}	P>F
H/Ox-Lutalyse vs H/Ox-saline	193	.14	ns	193	.37	ns
H/Ox-Lutalyse vs sham-Lutalyse	194	35.72	.001	195	15.00	.001
H/Ox-Lutalyse vs sham-saline	195	.14	ns	195	3.07	.06
H/Ox-saline vs sham-saline	192	.13	ns	192	3.09	.05
H/Ox-saline vs sham-Lutalyse	191	34.28	.001	192	14.20	.001
Sham-Lutalyse vs sham-saline	193	14.77	.001	194	4.33	.025

[a]Indications of significance are based upon ANOVA using transformed data; n = 5 ewes/group.

[b]For cranial and caudal comparisons, $df_1 = 2$.

TABLE 10. EFFECT OF CATHETER LOCATION ON $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 1[a]

	Mean $PGF_{2\alpha}$, pg/mL			
Catheter Location	H/Ox-Lutalyse	H/Ox-saline	Sham-Lutalyse	Sham-saline
Cranial	606.9[b]	265.2[b]	1,762.1[b]	331.8[b]
Caudal	628.8[b]	281.8[b]	391.4[c]	328.8[b]
Error MS (n = 5 ewes/group)	499,195.91	30,758.30	466,9631	53,629.04

[a]Means are calculated from untransformed data. Indications of significance and Error MS are based upon ANOVA using transformed data.

[b,c]Within a column, different superscripts differ (P < .05).

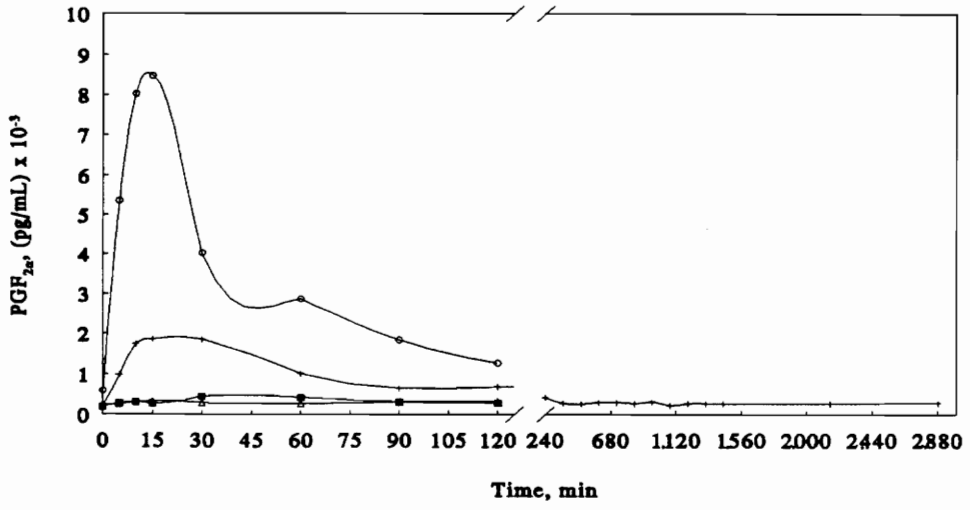

Figure 6. Cranial $PGF_{2\alpha}$ Profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Sham-Lutalyse (o), and Sham-saline (■) treated ewes- Experiment 1. Note: After 240 min, cranial $PGF_{2\alpha}$ concentrations were not affected by Lutalyse or H/Ox status; therefore, treatment groups were combined. Error MS = 1.45 from transformed data, and n = 5 samples per observation.

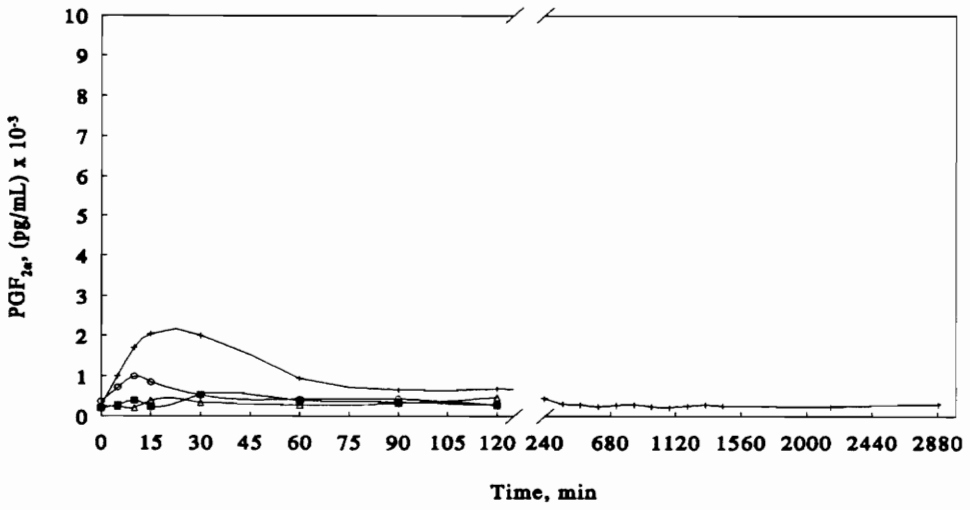

Figure 7. Caudal $PGF_{2\alpha}$ profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Sham-Lutalyse (o), and Sham-saline (■) treated ewes-Experiment 1. Note: After 240 min, caudal $PGF_{2\alpha}$ concentrations were not affected by Lutalyse or H/Ox status; therefore, treatment groups were combined. Error MS = 1.56 from transformed data, and n = 5 samples per observation.

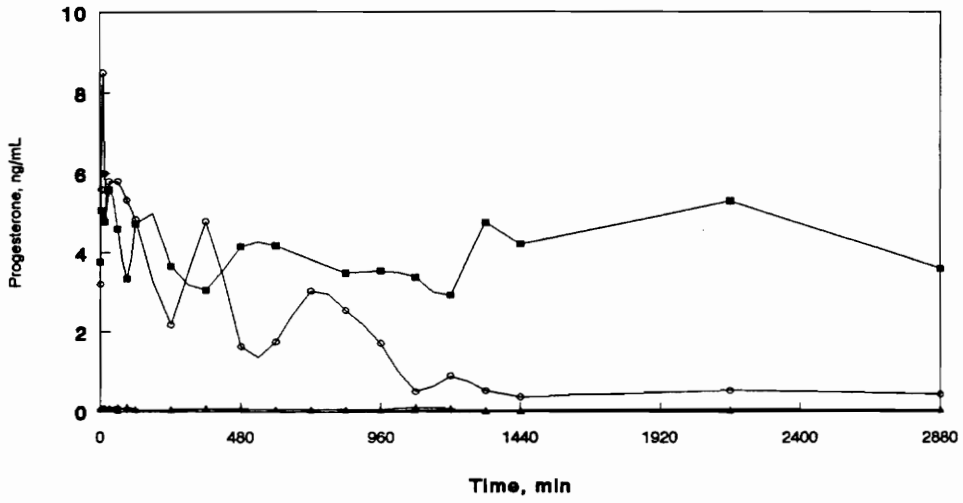

Figure 8. Cranial progesterone profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Sham-Lutalyse (o), and Sham-saline (■) treated ewes-Experiment 1. Error MS = 3.17 from untransformed data, and n = 5 samples per observation.

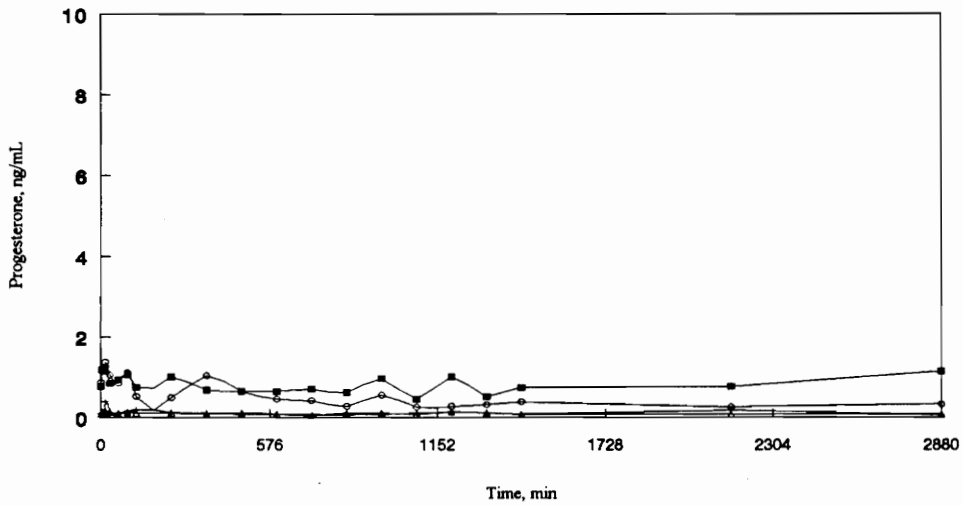

Figure 9. Caudal progesterone profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Sham-Lutalyse (o), and Sham-saline (■) treated ewes-Experiment 1. Error MS = .18 from untransformed data, and n = 5 samples per observation.

Experiment 2

Cranial $PGF_{2\alpha}$ Response

The effects of treatment and period on mean concentrations of cranial $PGF_{2\alpha}$ are shown in Table 11. Period did not affect ($P > .05$) mean cranial $PGF_{2\alpha}$ concentrations. Thus, each ewe was considered to have two independent responses to Lutalyse or saline treatments. Two H/Ox saline-treated ewes and one intact saline-treated ewe had greater initial cranial and caudal ($P < .05$) concentrations of $PGF_{2\alpha}$ than all other ewes, even after their data were transformed. Basal and resting concentrations were not estimable, because only one sample was taken before the experiment, of the short duration of the experiment, and the pulsatile nature of $PGF_{2\alpha}$ secretion. Therefore, for all analyses involving the comparison of concentrations, data for these three ewes were excluded. Results for both datasets, i.e., with and without the three ewes, are provided, although the discussion will pertain only to the homogenous dataset.

There was an interaction ($P < .05$) between H/Ox status and Lutalyse treatment on mean cranial $PGF_{2\alpha}$ concentrations (Table 11). Table 12 shows the effect of treatment on mean cranial $PGF_{2\alpha}$ concentration for the entire dataset and for the homogenous dataset. The mean cranial $PGF_{2\alpha}$ concentration for saline-treated H/Ox ewes (24.2 pg/mL) differed from Lutalyse-treated H/Ox ewes (57.7 pg/mL). Neither group differed from intact saline-treated ewes (53.0 pg/mL). Intact Lutalyse-treated ewes (417.5 pg/mL) had greater ($P < .05$) cranial $PGF_{2\alpha}$ concentrations than ewes in all other treatments.

Cranial $PGF_{2\alpha}$ concentrations changed over time (Figure 10). Therefore, individual regression equations were derived to describe the changes in the cranial $PGF_{2\alpha}$ profile. The overall shape of the response curve was determined to be quartic. Changes in cranial $PGF_{2\alpha}$ concentrations for saline-treated ewes, regardless of H/Ox status, are similar ($P > .05$). Thus, these datasets were combined for cranial analyses. Changes in cranial $PGF_{2\alpha}$

concentration in saline-treated ewes differed ($P < .05$) from changes in Lutalyse-treated ewes. Lutalyse also induced changes in $PGF_{2\alpha}$ concentrations that differed ($P < .05$) within H/Ox status. Results from profile comparisons for cranial $PGF_{2\alpha}$ are provided in Table 13.

Analyses of peak responses include peak concentration, $PGF_{2\alpha}$ response, and duration of the peak (Table 14). The first sample taken was used as the best estimate of a resting concentration. Results for analysis of treatment on peak responses of cranial $PGF_{2\alpha}$ are shown in Table 18. Period did not affect any of the peak analyses.

The mean peak cranial $PGF_{2\alpha}$ concentration in H/Ox saline-treated ewes did not differ from that in intact saline-treated ewes. Peak cranial $PGF_{2\alpha}$ concentration in H/Ox ewes did not differ ($P> .05$) between saline and Lutalyse treatment (266.7 and 246.6 pg/mL, respectively). However, the $PGF_{2\alpha}$ peak in intact ewes in response to Lutalyse (1,140.4 pg/mL) was greater ($P < .001$) than for all other treatment groups.

The actual $PGF_{2\alpha}$ response, measured as the difference between peak concentration and initial concentration, shows similar results. The cranial $PGF_{2\alpha}$ response did not differ ($P > .05$) within saline treatment. The cranial $PGF_{2\alpha}$ response did not differ ($P> .05$) between H/Ox or intact control ewes. The response in intact Lutalyse-treated ewes was five times greater than the intact saline response.

In one H/Ox and two intact saline-treated ewes, spontaneous $PGF_{2\alpha}$ peaks were detected. These increments were minor and of short duration ($T < 5$ min) when compared to induced peaks detected in Lutalyse-treated ewes. In intact Lutalyse-treated ewes, the duration of the $PGF_{2\alpha}$ peak (44.1 min) was greater ($P < .05$) than in H/Ox Lutalyse-treated ewes (27.2 min).

Lutalyse treatment and H/Ox status affected ($P < .05$) the area under the cranial $PGF_{2\alpha}$ response curve. A slight interaction ($P =. 06$) between H/Ox status and Lutalyse treatment was detected. The mean AUC for H/Ox control ewes did not differ ($P > .05$) from that measured in H/Ox Lutalyse-treated or intact saline-treated ewes. The AUC for intact Lutalyse-treated ewes was six times greater than for all other treatments.

Caudal $PGF_{2\alpha}$ Response

Period did not affect ($P > .05$) mean caudal $PGF_{2\alpha}$ concentrations. Caudal $PGF_{2\alpha}$ concentrations were affected ($P < .01$) by an interaction between H/Ox status and Lutalyse ($P < .01$). The effects of treatment and period on mean concentration of caudal $PGF_{2\alpha}$ are shown in Table 15. The mean caudal $PGF_{2\alpha}$ concentration for each treatment group is provided in Table 16. The mean concentration for H/Ox saline-treated ewes did not differ ($P > .05$) from that in intact saline-treated, or Lutalyse-treated H/Ox ewes. However, in intact ewes, caudal $PGF_{2\alpha}$ concentrations were greater ($P < .0001$) in response to Lutalyse than saline (278.2 pg/mL vs 39.7 pg/mL, respectively).

Time was a significant factor for determining the caudal $PGF_{2\alpha}$ concentration (Figure 11). Therefore, individual response equations to describe the changes in caudal $PGF_{2\alpha}$ concentration were derived for each treatment group. Comparison of the changes in $PGF_{2\alpha}$ concentration in the caudal vena cava indicate that saline-treated groups respond to the injection in a similar fashion. Therefore, as in the cranial analyses, these treatment groups were combined. Lutalyse, however, affected ($P < .05$) the shape of the overall caudal $PGF_{2\alpha}$ response curve. The caudal $PGF_{2\alpha}$ profile in H/Ox ewes treated with Lutalyse differed ($P <. .001$) from that in saline-treated ewes. This response also depended on H/Ox status; the caudal $PGF_{2\alpha}$ response curve in H/Ox Lutalyse-treated ewes differed ($P < .025$) from that in intact Lutalyse-treated ewes. The effects of treatment on changes in $PGF_{2\alpha}$ concentrations in the caudal vena cava are provided in Table 13.

PGFM Response

The PGFM concentrations were affected ($P < .05$) by H/Ox status, Lutalyse, time, and period, as well as several interactions (Table 17). Therefore, analysis of mean concentration was performed for each period. In both periods, PGFM concentrations were affected ($P < .0001$) by H/Ox status and Lutalyse treatment. No interaction between

Lutalyse and H/Ox was indicated for the first period; a slight interaction (P = .0595) was noted for the second period. Mean PGFM concentrations are shown in Table 18. The mean concentration differed between periods only in intact Lutalyse-treated ewes.

In response to the first injection, mean PGFM concentration did not differ between H/Ox saline-treated ewes and intact saline-treated ewes. Lutalyse affected (P < .05) PGFM concentrations; PGFM concentrations were greater in H/Ox Lutalyse-treated ewes than in saline-treated ewes. The mean PGFM concentration in intact Lutalyse-treated ewes was greater (P < .0001) than for all other treatment groups combined. The mean PGFM concentration in H/Ox control ewes did not differ from intact control ewes, even after the second saline injection. The mean concentration of PGFM in H/Ox Lutalyse-treated ewes was greater (P < .05) than that in control ewes and differed (P = .06) from concentrations in intact Lutalyse-treated ewes.

Comparisons of the relative changes in each treatment group were performed for each period separately. For each period, the overall regression equation was pentic (Figures 12 and 13). Changes in PGFM concentration within saline treatments were similar (P >..05); however, changes in H/Ox Lutalyse-treated ewes differed (P< .005) from those in ewes treated with saline, whether intact, H/Ox, or intact Lutalyse-treated. This response was detected in both periods. During period 1, changes in PGFM concentrations in H/Ox saline-treated ewes differed from those in intact Lutalyse-treated ewes. However, similar changes in PGFM concentrations were noted between these treatment groups after the second injection of Lutalyse. These results are provided in Table 19.

Verification of Catheter Location

Cranial and caudal $PGF_{2\alpha}$ concentrations were different (P < .005) only in intact Lutalyse-treated ewes (Table 20). Cranial and caudal $PGF_{2\alpha}$ concentrations in intact-saline ewes tended to be differ at P < .1. Cranial and caudal progesterone concentrations (4.2 and

.7 ng/mL, respectively) differed ($P < .05$) in intact saline-treated ewes. In H/Ox ewes, catheter location did not affect ($P > .05$) either progesterone or $PGF_{2\alpha}$ concentrations. For H/Ox ewes, whether saline or Lutalyse-treated, the mean cranial and caudal progesterone concentration was .1 ng/mL.

Two samples collected before the beginning of the experiment on d 9 were compared to samples collected 36 to 50 h after the end of the experiment to evaluate the efficacy of the Lutalyse injection. In all saline-treated ewes, progesterone concentrations were similar; however, in all intact Lutalyse-treated ewes, progesterone concentration had decreased to less than .3 ng/mL. Progesterone concentrations did not differ in H/Ox ewes, regardless of Lutalyse treatment.

$PGF_{2\alpha}$ As A Covariant For PGFM

In general, PGFM concentrations increased in a pattern similar to that of $PGF_{2\alpha}$ after Lutalyse injection. Increases in PGFM concentration followed the increases in $PGF_{2\alpha}$ concentration after a short delay of approximately 2 min, although PGFM concentrations were increased for a longer time than were $PGF_{2\alpha}$ concentrations. In intact Lutalyse-treated ewes, the initial PGFM concentration was greater ($P < .05$) in the second period. Figures 14 and 15 show the $PGF_{2\alpha}$ and PGFM responses, respectively, for intact and H/Ox Lutalyse-treated ewes. A quadratic function of the cranial $PGF_{2\alpha}$ concentration was a significant covariant for PGFM; however, this function explained only 2.5% of the variation in PGFM concentration. Most of the variation (64%) in PGFM concentration was explained by treatment, ewe to ewe variation, and time. Prostaglandin $F_{2\alpha}$ was a more useful covariant for changes in PGFM concentration in H/Ox Lutalyse-treated ewes than for ewes in all other treatment groups. Approximately 10% of the change in PGFM concentration was explained by changes in $PGF_{2\alpha}$ concentration. In all other treatment groups, less than

2.8% of the variation in PGFM was explained by changes in the cranial $PGF_{2\alpha}$ concentration.

TABLE 11. EFFECT OF TREATMENT ON CRANIAL $PGF_{2\alpha}$ CONCENTRATIONS EXPERIMENT 2[a]

	Entire dataset[b]			Homogenous dataset[c]		
Variable	DF	F value	P>F	DF	F value	P>F
H/OX	1	2.50	ns	1	4.96	.05
LUTALYSE	1	.19	ns	1	2.59	ns
H/OX*LUTALYSE	1	8.67	.01	1	5.84	.05
EWE(H/OX*LUTALYSE)	16			13		
T	11	3.65	.001	11	2.73	.025
H/OX*T	11	1.29	ns	11	1.04	ns
LUTALYSE*T	11	2.92	.001	11	2.07	.025
H/OX*LUTALYSE*T	11	.98	ns	11	.86	ns
EWE(H/OX*LUTALYSE*T)	176			143		
PERIOD	1	.55	ns	1	.33	ns
H/OX*PERIOD	1	.01	ns	1	.01	ns
LUTALYSE*PERIOD	1	1.55	ns	1	1.21	ns
H/OX*LUTALYSE*PERIOD	1	.84	ns	1	.60	ns
T*PERIOD	11	1.68	ns	11	1.58	ns
H/OX*T*PERIOD	11	.92	ns	11	.90	ns
LUTALYSE*T*PERIOD	11	.76	ns	11	.85	ns
H/OX*LUTALYSE*T*PERIOD	11	.74	ns	11	.76	ns

[a]Indications of significance are based upon ANOVA using transformed data.

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

TABLE 12. EFFECT OF TREATMENT ON MEAN CRANIAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 2[a]

Treatment group	Entire dataset[b], pg/mL	Homogenous dataset[c], pg/mL
H/Ox-Lutalyse	57.7[d]	57.7[d]
H/Ox-saline	103.9[e]	24.2[de]
Intact-Lutalyse	417.5[f]	417.5[f]
Intact-saline	61.4[g]	53.0[dg]
Error MS	523.167	4,251.385

[a]Means are calculated from untransformed data; indications of significance are based upon ANOVA using transformed data. Error MS reported has been untransformed.

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

[d,e,f,g]Within a column, different superscripts differ ($P < .05$).

TABLE 13. COMPARISON OF $PGF_{2\alpha}$ PROFILES

EXPERIMENT 2[a]

	Cranial[b]			Caudal[b]		
Treatment group	DF	F_{crit}	P>F	DF	F_{crit}	P>F
H/Ox-Lutalyse vs saline-treated[c]	334	7.18	.001	332	9.10	.001
H/Ox-Lutalyse vs intact-Lutalyse	219	2.91	.025	219	3.65	.025
H/Ox-saline vs intact-saline	219	.43	ns	218	.29	ns
Saline-treated[c] vs intact-Lutalyse	331	3.12	.025	331	2.84	.025

[a]Indications of significance are based upon ANOVA using transformed data; n = 5 ewes/group.

[b]For cranial and caudal comparisons, $DF_1 = 4$.

[c]H/Ox saline and intact saline treatment groups combined.

TABLE 14. EFFECT OF TREATMENT ON PEAK CRANIAL $PGF_{2\alpha}$ CHARACTERISTICS

EXPERIMENT 2[a]

Treatment Group	Peak[b], pg/mL	Response[b], pg/mL	Duration[c], min	Area under curve[c]
H/Ox-Lutalyse	246.6[d]	127.0[d]	27.2[d]	6,299.4[d]
H/Ox-saline	266.6[d]	149.8[d]	10.5[e]	4,313.9[d]
Intact-Lutalyse	1,140.4[e]	1,037.2[e]	44.1[f]	47,626.6[e]
Intact-saline	247.0[d]	204.8[d]	6.4[e]	8,126.7[d]
Error MS (n = 5 ewes/group)	1.391	1.343	255.692	7.7299E+8

[a]Means are calculated from untransformed data.

[b]Indications of significance are based upon ANOVA using transformed data. For definition, see page 59.

[c]Indications of significance are based upon ANOVA on untransformed data. For definition, see page 59.

[d,e,f]Within a column, different superscripts differ ($P < .05$).

TABLE 15. EFFECT OF TREATMENT ON CAUDAL $PGF_{2\alpha}$ CONCENTRATIONS EXPERIMENT 2[a]

	Entire dataset[b]			Homogenous dataset[c]		
Variable	DF	F value	P>F	DF	F value	P>F
H/OX	1	.22	ns	1	1.42	ns
LUTALYSE	1	1.60	ns	1	4.60	.10
H/OX*LUTALYSE	1	15.19	.001	1	9.24	.01
EWE(H/OX*LUTALYSE)				13		
T	11	2.84	.005	11	2.69	.005
H/OX*T	11	2.47	.01	11	2.60	.005
LUTALYSE*T	11	4.02	.001	11	3.46	.0001
H/OX*LUTALYSE*T	11	.83	ns	11	1.15	ns
EWE(H/OX*LUTALYSE*T)	176			143		
PERIOD	1	.03	ns	1	.09	ns
H/OX*PERIOD	1	.10	ns	1	.00	ns
LUTALYSE*PERIOD	1	2.20	ns	1	1.36	ns
H/OX*LUTALYSE*PERIOD	1	1.20	ns	1	1.71	ns
T*PERIOD	11	1.17	ns	11	1.17	ns
H/OX*T*PERIOD	11	.74	ns	11	.52	ns
LUTALYSE*T*PERIOD	11	.69	ns	11	.51	ns
H/OX*LUTALYSE*T*PERIOD	11	.88	ns	11	.85	ns

[a]Indications of significance are based upon ANOVA using transformed data:

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

TABLE 16. EFFECT OF TREATMENT ON MEAN CAUDAL $PGF_{2\alpha}$ CONCENTRATIONS

EXPERIMENT 2[a]

Treatment Group	Entire dataset[b], pg/mL	Homogenous dataset[c], pg/mL
H/Ox-Lutalyse	58.4[d]	58.4[d]
H/Ox-saline	92.7[e]	27.6[df]
Intact-Lutalyse	278.2[f]	278.2[e]
Intact-saline	39.8[d]	39.8[dg]
Error MS	1,712.484	6,692.883

[a]Means are calculated from untransformed data; indications of significance are based upon ANOVA using transformed data. Error MS reported has been untransformed.

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

[d,e,f,g]Within a column, different superscripts differ ($P < .05$).

TABLE 17. EFFECT OF TREATMENT ON CRANIAL PGFM CONCENTRATIONS EXPERIMENT 2[a]

	Entire dataset[b]			Homogenous dataset[c]		
Variable	DF	F value	P>F	DF	F value	P>F
H/OX	1	2.96	ns	1	9.36	.01
LUTALYSE	1	49.67	.001	1	64.27	.001
H/OX*LUTALYSE	1	.18	ns	1	.64	ns
EWE(H/OX*LUTALYSE)	16			13		
T	11	21.98	.001	11	17.47	.001
H/OX*T	11	2.74	.005	11	1.83	.05
LUTALYSE*T	11	16.26	.001	11	17.18	.001
H/OX*LUTALYSE*T	11	.61	ns	11	1.06	ns
EWE(H/OX*LUTALYSE*T)	176			143		
PERIOD	1	21.86	.0001	1	20.77	.0001
H/OX*PERIOD	1	12.86	.0004	1	13.67	.0003
LUTALYSE*PERIOD	1	22.15	.0001	1	12.55	.0005
H/OX*LUTALYSE*PERIOD	1	1.57	ns	1	.23	ns
T*PERIOD	11	3.43	.0002	11	2.93	.0016
H/OX*T*PERIOD	11	.85	ns	11	.42	ns
LUTALYSE*T*PERIOD	11	3.74	.0001	11	2.65	.0040
H/OX*LUTALYSE*T*PERIOD	11	.75	ns	11	.68	ns

[a]Indications of significance are based upon ANOVA using transformed data.

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

TABLE 18. EFFECT OF TREATMENT ON MEAN CRANIAL PGFM CONCENTRATIONS

EXPERIMENT 2[a]

Treatment group	Period 1 PGFM, pg/mL		Period 2 PGFM, pg/mL	
	Entire dataset[b]	Homogenous dataset[c]	Entire dataset[b]	Homogenous dataset[c]
H/Ox-Lutalyse	4,292.8[d]	4,294.8[d]	5,828.2[d]	5,828.2[d]
H/Ox-Saline	364.3[e]	172.5[e]	375.7[e]	192.8[e]
Intact-Lutalyse	7,052.1[f]	6,052.1[f]	7,381.9[f]	7,381.9[f]
Intact-saline	445.9[g]	515.0[g]	395.1[g]	447.7[e]
Error MS	3.228	5.452	3.224	2.915

[a]Means are calculated from untransformed data; indications of significance are based upon ANOVA using transformed data. Error MS reported has been untransformed.

[b]Five ewes were used for each treatment group.

[c]Refers to ewes with similar initial $PGF_{2\alpha}$ concentrations after transformation (n = 5 for H/Ox-Lutalyse and intact-Lutalyse treatments; n = 3 for H/Ox-saline; n = 4 for intact-saline).

[d,e,f,g]Within a column, different superscripts differ ($P < .05$).

TABLE 19. COMPARISON OF CRANIAL PGFM PROFILES BY PERIOD

EXPERIMENT 2[a]

Treatment group	Period 1[b]			Period 2[b]		
	DF	F_{crit}	P>F	DF	F_{crit}	P>F
H/Ox-Lutalyse vs H/Ox-saline	99	18.82	.001	99	5.95	.001
H/Ox-Lutalyse vs intact-Lutalyse	98	3.31	.01	97	3.96	.005
H/Ox-Lutalyse vs intact-saline	98	24.91	.001	97	11.00	.001
H/Ox-saline vs intact-saline	99	0.57	ns	98	1.09	ns
H/Ox-saline vs intact-Lutalyse	99	20.62	.001	98	1.92	ns
Intact-Lutalyse vs intact-saline	98	29.04	.001	96	3.95	.005

[a]Indications of significance are based upon ANOVA using transformed data; n = 5 ewes/group.

[b]For periods 1 and 2, $DF_1 = 5$.

TABLE 20. EFFECT OF CATHETER LOCATION ON $PGF_{2\alpha}$ CONCENTRATIONS EXPERIMENT 2[a]

Catheter Location	Mean $PGF_{2\alpha}$ Concentration, pg/mL			
	H/Ox-Lutalyse	H/Ox-saline	Intact-Lutalyse	Intact-saline
Cranial	53.7[b]	109.2[b]	260.1[b]	60.5[d]
Caudal	57.0[b]	95.6[b]	173.6[c]	42.6[e]
Error MS (n=5 ewes/group)	2,259.86	23,223.54	41,061.91	5,515.95

[a]Means are calculated from untransformed data. Indications of significance and Error MS are based upon ANOVA using untransformed data.

[b,c]Within a column, different superscripts differ ($P < .05$).

[d,e]Within a column, $P = .06$.

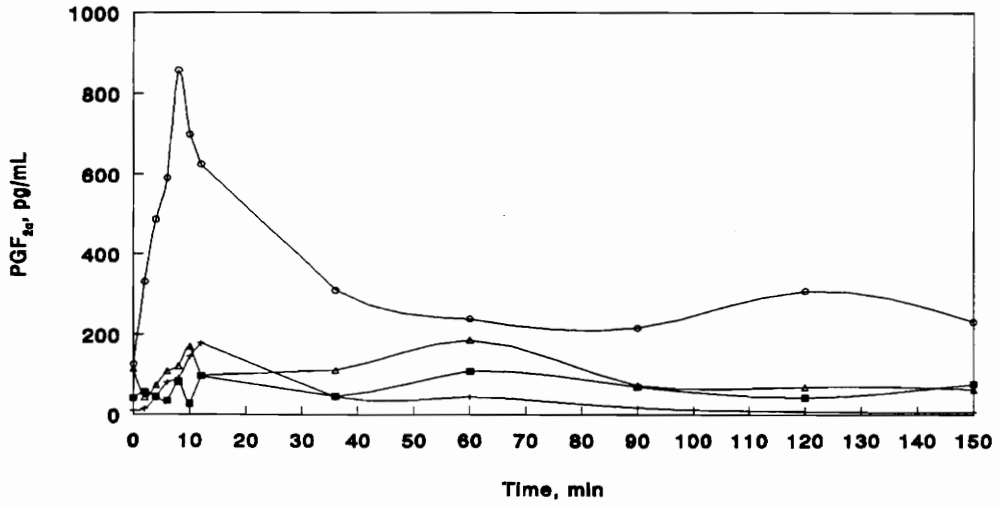

Figure 10. Cranial $PGF_{2\alpha}$ profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Intact-Lutalyse (o), and Intact-saline (■) treated ewes-Experiment 2. Error MS = 116.87 from transformed data, and n = 5 samples per observation.

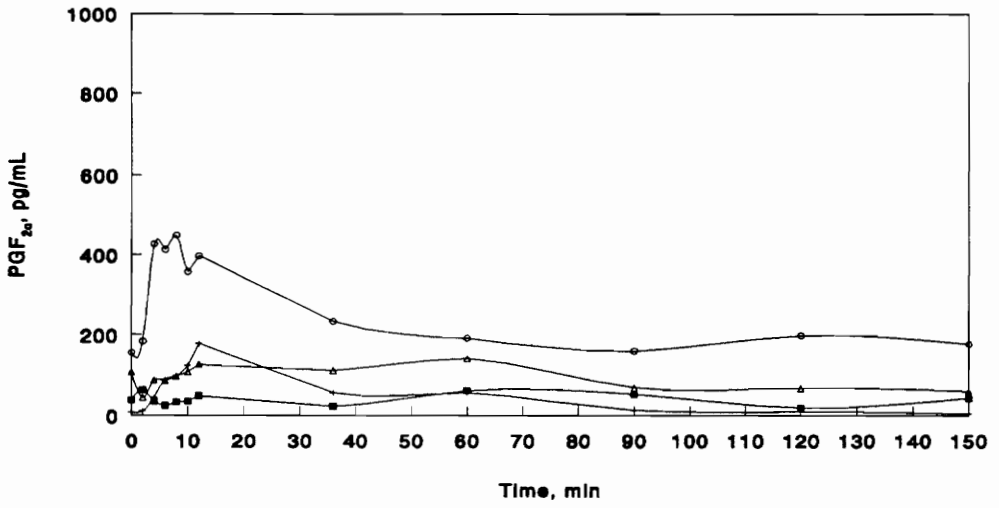

Figure 11. Caudal $PGF_{2\alpha}$ profiles in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Intact-Lutalyse (o), and Intact-saline (■) treated ewes-Experiment 2. Error MS = 394.14 from transformed data, and n = 5 samples per observation.

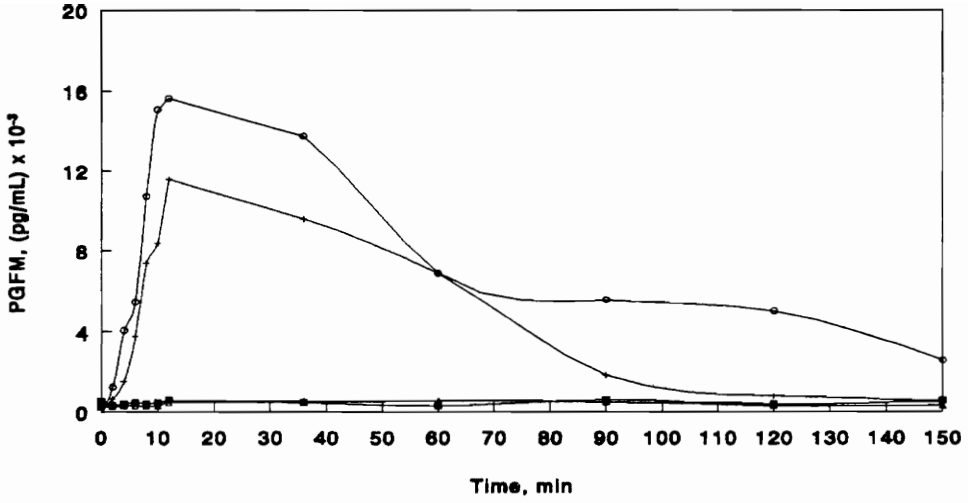

Figure 12. Cranial PGFM profiles after the first injection in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Intact-Lutalyse (o), and Intact-saline (■) treated ewes-Experiment 2. Error MS = 1.45 from transformed data, and n = 5 samples per observation.

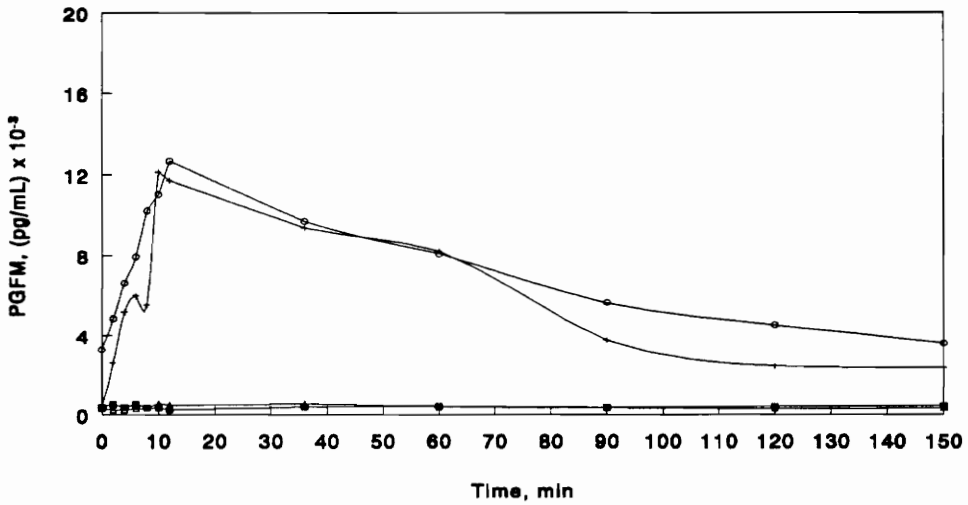

Figure 13. Cranial PGFM profiles after the second Lutalyse or saline injection in H/Ox-Lutalyse (+), H/Ox-saline (Δ), Intact-Lutalyse (o), and Intact-saline (■) treated ewes-Experiment 2. Error MS = 1.45 from transformed data, and n = 5 samples per observation.

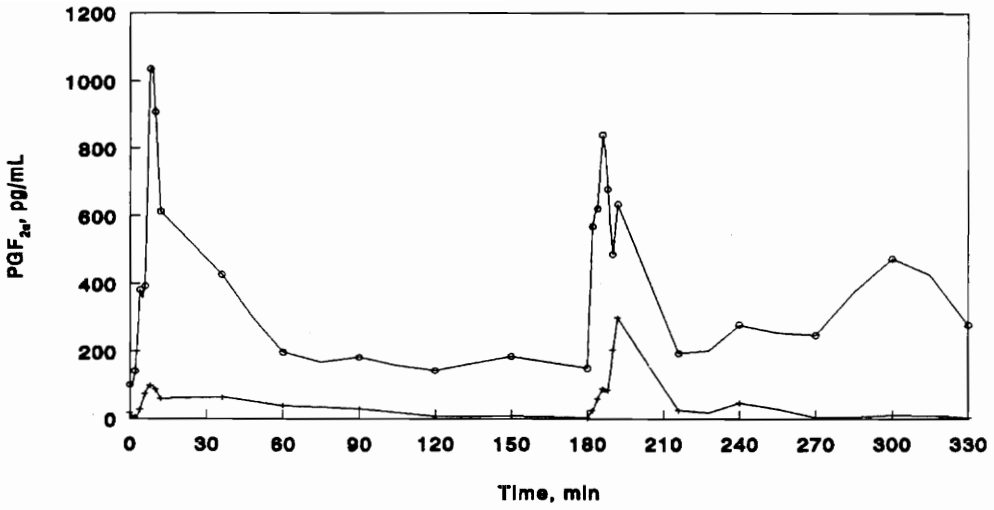

Figure 14. Cranial $PGF_{2\alpha}$ profiles in H/Ox Lutalyse (+), and Intact Lutalyse (o) treated ewes-Experiment 2. Note: The first Lutalyse injection was given at 0 min and the second Lutalyse injection was given at 180 min. Error MS = 116.87 from transformed data, and n = 5 samples per observation.

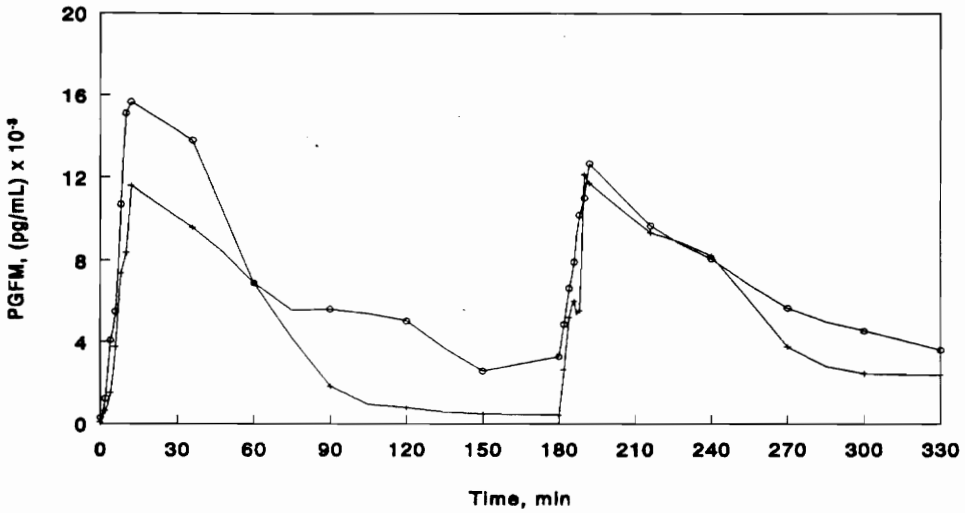

Figure 15. Cranial PGFM in H/Ox Lutalyse (+), and Intact Lutalyse (o) ewes-Experiment 2 . Note: The first Lutalyse injection was given at 0 min and the second Lutalyse injection was given at time 180 min. Error MS = 1.45 from transformed data, and n = 5 samples per observation.

Validation Experiment

The effect of sampling location on mean $PGF_{2\alpha}$, PGFM, and progesterone concentrations is shown in Table 21. Sampling location did not affect the mean concentration of progesterone or PGFM. However, sampling location affected ($P < .05$) the mean $PGF_{2\alpha}$ concentration. The concentration of $PGF_{2\alpha}$ was greater ($P < .05$) in saphenous vein and caudal vena caval blood plasma than in jugular plasma. A slight effect of caudal location was evident; caudal vena cava and saphenous vein samples tended to differ ($P < .1$).

Table. 21 EFFECT OF SAMPLING LOCATION ON MEAN CONCENTRATION OF PGFM, $PGF_{2\alpha}$, AND PROGESTERONE[a]

	$PGF_{2\alpha}$, pg/mL	PGFM, pg/mL	Progesterone, ng/mL
Jugular	82.9[b]	288.8	.86
Saphenous Vein	137.6[cd]	294.3	.81
Caudal Vena Cava	153.0[ce]	274.5	.75
Error MS (n=4/group)	4095.4	10,314.9	.05

[a]Means are calculated from untransformed data; indications of significance and Error MS are based upon ANOVA using untransformed data.

[b,c]Within a column, different superscripts differ ($P < .05$).

[d,e]Within a column, different superscripts tended to differ at $P < .1$

DISCUSSION

Evidence That Lutalyse Induces Uterine-Ovarian $PGF_{2\alpha}$ Secretion

The reproductive tract of ewes produces $PGF_{2\alpha}$ in response to an i.m. injection of Lutalyse (Table 22). In both experiments, the cranial $PGF_{2\alpha}$ concentration was greater in uterine-ovarian intact, Lutalyse-treated ewes than in ewes in all other treatment groups. Peak analyses also reflected this response; peak cranial $PGF_{2\alpha}$ concentrations in intact Lutalyse-treated ewes were several fold greater and the peak duration was longer than measured in saline-treated intact ewes. In intact Lutalyse-treated ewes in both experiments, the area under the cranial $PGF_{2\alpha}$ response curve was greater than that measured after saline injection. Thus, the reproductive tract seems to amplify $PGF_{2\alpha}$ concentrations through uterine and(or) ovarian $PGF_{2\alpha}$ production in response to Lutalyse injections .

The 15 mg injection of Lutalyse (Exp. 1) can be detected by comparison of mean $PGF_{2\alpha}$ concentration, peak $PGF_{2\alpha}$ concentration, $PGF_{2\alpha}$ response, duration of the $PGF_{2\alpha}$ peak, and AUC. In H/Ox Lutalyse-treated ewes, these variables were greater than they were after saline injection (Table 22). The 5 mg injection of Lutalyse did not increase the mean cranial $PGF_{2\alpha}$ concentration, peak concentration, or $PGF_{2\alpha}$ response in H/Ox ewes. However, the peak was longer in duration in H/Ox Lutalyse-treated than in saline-treated ewes. In both experiments, the cranial $PGF_{2\alpha}$ response curve in saline-treated H/Ox ewes was relatively flat when compared to that of Lutalyse-treated ewes.

The injection per se , i.e., stress, did not stimulate uterine and(or) ovarian $PGF_{2\alpha}$ production (Table 22). Although, in the first experiment, intact saline-treated ewes had greater cranial $PGF_{2\alpha}$ concentrations than did H/Ox saline-treated ewes; the difference in mean concentration was 37.8 pg/mL. Although the cranial $PGF_{2\alpha}$ peak concentration and $PGF_{2\alpha}$ response were also greater in the intact group, the duration of the $PGF_{2\alpha}$, AUC, and shape of the response curves were similar. Other experiments with frequent sampling

TABLE 22. SUMMARY FOR $PGF_{2\alpha}$ COMPARISONS FOR EXPERIMENTS 1 AND 2[a]

	Two 5 mg doses of $PGF_{2\alpha}$ 3 h apart			One 15 mg dose of $PGF_{2\alpha}$		
Variable	Trt 1 vs Trt 2	Trt 1 vs Trt 3	Trt 2 vs Trt 4	Trt 1 vs Trt2	Trt 1 vs Trt 3	Trt 2 vs Trt 4
Mean Cranial Concentration	DO NOT DIFFER	DIFFER (3>1)	TENDED TO DIFFER[b]	DIFFER (1>2)	DIFFER (3>1)	DIFFER (4>2)
Peak Cranial Concentration	DO NOT DIFFER	DIFFER (3>1)	DO NOT DIFFER	DIFFER (1>2)	DIFFER (3>1)	DIFFER (4>2)
Peak Response	DO NOT DIFFER	DIFFER (3>1)	DO NOT DIFFER	DIFFER (1>2)	DIFFER (3>1)	DIFFER (4>2)
Peak Duration	DIFFER (1>2)	DIFFER (3>1)	DO NOT DIFFER	DIFFER (1>2)	DIFFER (3>1)	DO NOT DIFFER
Area Under the Curve	DO NOT DIFFER	DIFFER (3>1)	DO NOT DIFFER	DIFFER (1>2)	DIFFER (3>1)	DO NOT DIFFER
Cranial Shape	DIFFER	DIFFER	DO NOT DIFFER	DIFFER	DO NOT DIFFER[c]	DO NOT DIFFER
Mean Caudal Concentration	DO NOT DIFFER	DIFFER (3>1)	DIFFER (2>4)	DIFFER (1>2)	DIFFER (1>3)	DO NOT DIFFER
Caudal Shape	DIFFER	DIFFER	DO NOT DIFFER	DIFFER	DO NOT DIFFER[c]	DO NOT DIFFER

[a]Trt 1=H/Ox-Lutalyse; Trt 2=H/Ox-Saline; Trt 3=Intact-Lutalyse; Trt 4=Intact-Saline

[b] Indicates $P < .1$.

[c] Profiles differ graphically, but not statistically.

schedules have shown $PGF_{2\alpha}$ secretion to be pulsatile in nature during the luteal phase (Thorburn et al., 1973; Baird et al., 1976; Kindahl et al., 1976); indicating that the uterus is not completely quiescent even in the midluteal phase. The progesterone secretion profile in intact saline-treated ewes indicated that these peaks did not induce luteolysis. In Exp. 2, the mean concentration, peak concentration, $PGF_{2\alpha}$ response, duration of the $PGF_{2\alpha}$ peak, shape of the cranial $PGF_{2\alpha}$ response curves and AUC were similar in H/Ox saline-treated and intact ewes. The CL did not regress after saline injection. Thus, the act of giving an injection does not stimulate $PGF_{2\alpha}$ secretion by the uterus and(or) ovaries.

In intact cows, PGF ($PGF_{2\alpha}$ and $PGF_{1\alpha}$) concentrations increased and decreased rapidly after i.m. $PGF_{2\alpha}$ administration (Hafs et al., 1974). Kindahl (1985) indicated that PGFM concentrations increase almost immediately after an i.m. injection of $PGF_{2\alpha}$. Results from this study also indicate that $PGF_{2\alpha}$ is rapidly absorbed and metabolized. Hafs et al. (1974) also reported that endogenous $PGF_{2\alpha}$ could be produced in response to the exogenous dose, because the estimated half-life of $PGF_{2\alpha}$ is only minutes and concentrations continue to increase long after the estimated half-life. Results from this study also indicate that the uterus and(or) ovaries secrete $PGF_{2\alpha}$ because in the uterine-ovarian intact ewe, the mean peak $PGF_{2\alpha}$ concentration and duration of the peak was greater than in H/Ox ewes.

Although these two experiments were conducted independently, with different ewes and sampling schedules, it seems that the uterine-ovarian response to the exogenous $PGF_{2\alpha}$ is dose-dependent. In response to the 15 mg Lutalyse injection, the $PGF_{2\alpha}$ peak was approximately 15 fold greater than that measured after saline injection. The peak concentration after a 5 mg injection of $PGF_{2\alpha}$ was five times greater than after saline injection. A similar trend was detected with duration of the peak, AUC, and the cranial $PGF_{2\alpha}$ response; the larger dose seemed to amplify the $PGF_{2\alpha}$ response. Based upon those arguments, these experiments indicate an i.m. injection of Lutalyse increases $PGF_{2\alpha}$ production by the reproductive tract in a dose-dependent fashion. Indeed, intrafollicular,

intrauterine, i.m., and i.v. experiments using exogenous $PGF_{2\alpha}$ to induce luteolysis, show a $PGF_{2\alpha}$ dose-dependent effect on luteolysis (Inskeep et al., 1975, McCracken et al., 1970; Thorburn and Nichol, 1971; Douglas and Ginther, 1973; Inskeep 1973).

Why then are two smaller doses of Lutalyse more efficacious in inducing luteolysis than a single larger dose of Lutalyse? Were both doses of Lutalyse luteolytic? Luteolysis, as measured by progesterone concentration, occurred in all ewes injected i.m. with two 5 mg doses of $PGF_{2\alpha}$ 3 h apart and in 4 of 5 ewes after a single 15 mg injection of $PGF_{2\alpha}$. Although based on only a limited number of ewes, these data indicate that the two-dose regimen is at least as efficacious as a single dose of $PGF_{2\alpha}$. Thirty minutes after ewes were injected with 15 mg of Lutalyse, progesterone concentrations began to decrease; decreasing to 50% at 8 h. In cows, progesterone concentrations began to decrease within 10 min after 30 mg of $PGF_{2\alpha}$ i.m., and they decreased from 3.9 ± .2 to 2.1 ± .2 ng/mL within 6 h (Hafs et al., 1974). In sheep given 10 to 40 μg/h of $PGF_{2\alpha}$ (Chamley et al. 1972) and cows given 1 mg of $PGF_{2\alpha}$ (Inskeep, 1973) intrauterine, $PGF_{2\alpha}$ reduced progesterone concentrations by half within 4 h. It is expected that progesterone concentrations decrease earlier after intrauterine $PGF_{2\alpha}$ administration than after i.m. injection, because i.m. $PGF_{2\alpha}$ must be picked up by the circulation and transported to the reproductive organs before inducing luteolysis. Progesterone concentrations did not decrease after saline treatment. In H/Ox ewes, progesterone concentrations did not change after Lutalyse or saline treatment because no CL was present. This also indicates that Lutalyse does not affect progesterone secretion elsewhere in the body.

In all intact Lutalyse-treated ewes, a small increase in progesterone concentration was detected after the initial decrease. Sellner and Wickersham (1970) and McCracken et al. (1970) also reported an increase in progesterone concentrations after the initial decrease in response to $PGF_{2\alpha}$. It is speculated that this may represent a period when the CL is rapidly regressing, thus dumping its progesterone into the general circulation.

Mechanism Of Action Explaining Differences In Dose Efficacy

Based upon the results of this study and mathematics, the 15 mg dose of Lutalyse should be more efficacious if $PGF_{2\alpha}$ causes luteolysis directly. However, $PGF_{2\alpha}$ is thought to induce luteolysis through several mechanisms; it may be that a $PGF_{2\alpha}$ threshold exists which when exceeded initiates several biochemical luteolytic pathways (Figure 3). Suggested pathways for $PGF_{2\alpha}$-induced luteolysis include: enhancement of phospholipase C activity; increased intracellular Ca^{2+}, which may influence enzyme activity (Knickerbocker et al., 1988; Jacobs et al., 1991) and cytotoxicity toward the luteal cell (Fitz et al., 1984; Braden et al., 1988; Murdoch, 1987; Fairchild Benyo et al., 1991). Interference with the hypothalamo-hypophyseal axis and venoconstriction have been discredited (Pharris et al., 1972; McCracken et al., 1970; Goding et al., 1972; Baird, 1974, Janson et al., 1974), although venoconstriction may play an auxiliary role in completing luteolysis. It may not be the amount of $PGF_{2\alpha}$ above the threshold that determines the efficacy of the dose of Lutalyse, but rather how many times $PGF_{2\alpha}$ sends its signals to the appropriate pathway. Schramms et al. (1983) also indicated that low pulses of $PGF_{2\alpha}$ were sufficient to induce luteolysis.

Results of these experiments show Lutalyse to induce uterine and(or) ovarian $PGF_{2\alpha}$ production through some mechanism (Figure 16). Prostaglandin $F_{2\alpha}$ production is dependent on arachidonic acid stores, enzyme availability, free calcium, the hormonal environment, and inhibitors (Silvia et al., 1991). During spontaneous luteolysis, as the hormonal environment shifts from a progesterone-protected stage to an increasingly estrogenic stage, $PGF_{2\alpha}$ production increases. The removal of progesterone alone is insufficient to account for the increase in $PGF_{2\alpha}$ secretion in this study, because after progesterone priming, premature withdrawal of progesterone resulted in a gradual increase in $PGF_{2\alpha}$ (Ottobre et al., 1984; Silvia et al., 1991). The initial $PGF_{2\alpha}$ from the Lutalyse injection may inhibit progesterone secretion. However, Pratt et al. (1977) showed that after $PGF_{2\alpha}$ administration, 17β-estradiol concentrations did not increase until after 8 h. In the

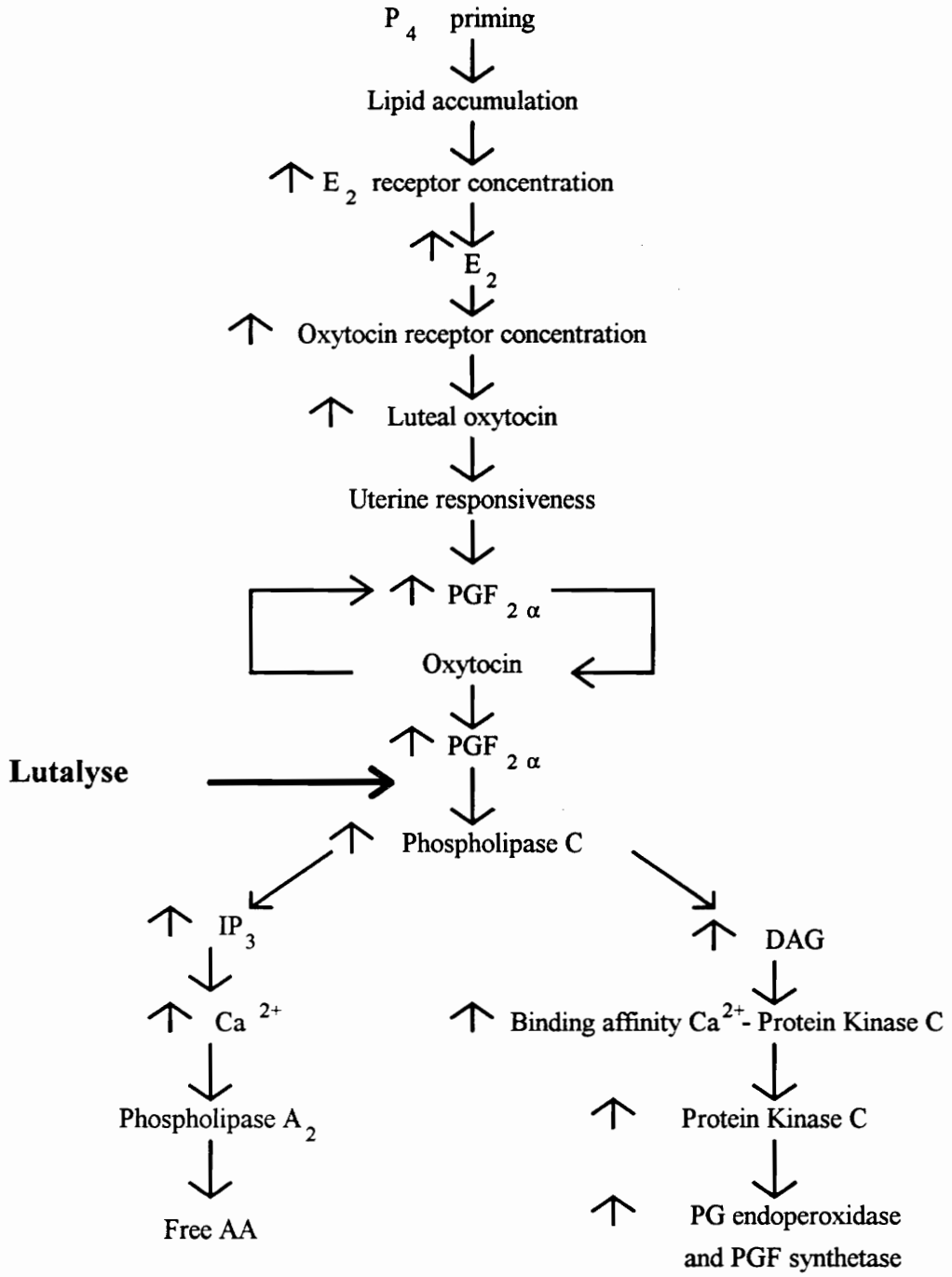

Figure 16. Proposed difference between spontaneous and Lutalyse- induced luteolysis.

present study, the $PGF_{2\alpha}$ produced by the reproductive tract was almost immediate. The progesterone concentration also changed within minutes after Lutalyse treatment. Thus, the initial dose of $PGF_{2\alpha}$ may decrease CL progesterone secretion, but the initial increase in $PGF_{2\alpha}$ secretion by the uterus did not seem to be due to a changing steroid hormone environment.

In the present study, the CL was fully functional for 4 d before ewes were injected with Lutalyse. A lipid pool accumulates in the endometrium under progesterone (Brinsfield and Hawk, 1973) and thus, once the appropriate enzymes free these lipid stores, $PGF_{2\alpha}$ secretion is initiated. Phospholipase C activity is enhanced by $PGF_{2\alpha}$ (Jacobs, et al., 1992). The result is an increase in IP_3 and DAG. The IP_3 then increases intracellular calcium release; thereby turning on numerous other enzymes (Knickerbocker et al., 1988). Phospholipase A, which enhances arachidonic acid release from its esterified storage form (Kunze and Vogt, 1971), is upregulated by free calcium (Knickerbocker et al., 1988). The initial dose of $PGF_{2\alpha}$ may increase uterine and(or) ovarian $PGF_{2\alpha}$ secretion by increasing free calcium. Diacylglycerol increases the binding affinity between Ca^{2+} and protein kinase C (Knickerbocker et al., 1988). Activation of protein kinase C may then trigger a cascade of events leading to the activation of numerous enzymes including prostaglandin endoperoxide synthase, which converts arachidonic acid to the unstable endoperoxides, and prostaglandin F synthetase that converts the endoperoxides to $PGF_{2\alpha}$. Prostaglandin concentrations are determined by the balance between production and catabolism. Prostaglandin Δ^{13}-reductase and 15-hydroxy-prostaglandin-dehydrogenase are responsible for $PGF_{2\alpha}$ metabolism to PGFM (Hansen, 1976; Lands, 1979). Activation of the production enzymes, or inhibition of the catabolic enzymes may result from activation of protein kinase C and free calcium. Lutalyse may trigger $PGF_{2\alpha}$ production or inhibit degradation through this mechanism. The resultant is a mass influx of $PGF_{2\alpha}$ into the system, which then triggers luteolysis.

This still does not explain why two smaller doses of Lutalyse are more effective in inducing luteolysis than one bolus injection (Hawk and Cooper, 1977; Hawk et al., 1978;

Hackett and Robertson, 1980). Experiments have shown $PGF_{2\alpha}$ to interfere with LH-induced cAMP related enzyme activities (Henderson and McNatty, 1975; Grinwich et al., 1976; Andersen et al., 1974). The coupling component appears to be the site of $PGF_{2\alpha}$ attack resulting in disruption of the transmission signal and decreasing cAMP production (Henderson and McNatty, 1975). Biochemical luteolysis results from decreased cAMP production, because enzyme activity is dependent on cAMP (Marsh, 1971). Knickerbocker et al. (1988) and Jacobs et al. (1991) suggested that $PGF_{2\alpha}$ activates phopholipase C-activated second messenger systems, which result in increased binding affinity between calcium and protein kinase C and increased intracellular calcium. Protein kinase C activation inhibits progesterone secretion. Prostaglandin $F_{2\alpha}$, then, by increasing phospholipase activity, triggers a cascade of events, including activation of luteolytic enzymes or down regulation of luteotrophic enzymes (Knickerbocker et al., 1988; Jacobs et al., 1991). Inhibition of progesterone secretion also results from free Ca^{2+} (Hoyer and Marion; 1989).

Biochemical luteolysis is completed before structural luteolysis. Lutalyse and its subsequent uterine-ovarian $PGF_{2\alpha}$ production may be structurally luteolytic through cytotoxicity toward the small and large cells. The number of eosinophils on and MHC labeling of luteal cells increase in response to $PGF_{2\alpha}$ (Murdoch, 1987; Fairchild Benyo et al., 1991) Thus, $PGF_{2\alpha}$ invokes both functional and structural luteolysis. It seems likely that these mechanisms may work jointly to induce luteolysis. Also, numerous feedback mechanisms exist in enzyme regulation. Once one enzyme is activated, the endocrine environment changes, thus triggering several other luteolytic, luteostatic, or luteotrophic activities. Thus, because $PGF_{2\alpha}$ is rapidly produced and metabolized, a portion of a single injection of Lutalyse interferes for one period of time. The CL may begin to regress, but because cAMP is inhibited for only a short time, the CL may "recover" due to diminishing $PGF_{2\alpha}$ inhibition. The two doses of Lutalyse may act by interfering twice with these processes.

Uterine-Ovarian Regulation of $PGF_{2\alpha}$ Metabolism

Analyses of caudal $PGF_{2\alpha}$ concentrations provide some insight into the regulation of $PGF_{2\alpha}$ in response to Lutalyse, because mean caudal $PGF_{2\alpha}$ concentrations are dependent on the presence of the reproductive tract. The injection per se did not influence caudal $PGF_{2\alpha}$ concentrations. In both experiments; the shapes of the caudal $PGF_{2\alpha}$ response curves in saline-treated intact and H/Ox ewes are similar. In the first experiment (15 mg), mean caudal $PGF_{2\alpha}$ concentrations were similar. In the second experiment, these concentrations differed, but by less than 12 pg/mL.

In both experiments, caudal $PGF_{2\alpha}$ concentrations increased shortly after Lutalyse injection; this response was not detected in saline-treated ewes. Caudal vena caval $PGF_{2\alpha}$ concentrations in H/Ox Lutalyse-treated ewes were greater than those after saline injection, indicating that metabolism is dependent on the presence or absence of the uterus and(or) ovaries. In response to the 15 mg Lutalyse injection, H/Ox ewes had greater mean caudal $PGF_{2\alpha}$ concentrations than for all other treatments. In the intact 5 mg Lutalyse-treated ewes, caudal $PGF_{2\alpha}$ concentrations were greater than for all other treatment groups. This is an interesting point that highlights the importance of the reproductive organs in the regulation of $PGF_{2\alpha}$. Prostaglandin $F_{2\alpha}$ is produced by most cells and tissues, while metabolism is primarily controlled by the lungs, kidney (Pace-Asciak and Granström, 1983) and endometrium (Casey et al., 1980). Two enzymes, 15-hydroxy-prostaglandin-dehydrogenase and Δ^{13}-reductase, are responsible for the metabolism of $PGF_{2\alpha}$ to PGFM (Hansen, 1976; Lands, 1979; Pace-Asciak and Granström, 1983), thus decreasing $PGF_{2\alpha}$ concentrations. High concentrations of these enzymes are present in the lungs, kidney, and endometrium, and appear to be regulated by $PGF_{2\alpha}$ concentration (Casey et al., 1980; Pace-Asciak and Granström, 1983). Several enzymes are responsible for the production of $PGF_{2\alpha}$ and are also present in these tissues (Pace-Asciak and Granström, 1983). The results of this experiment give some indication to what extent $PGF_{2\alpha}$ is regulated and by

what tissues. The 15 mg dose of Lutalyse resulted in greater caudal $PGF_{2\alpha}$ concentrations in H/Ox Lutalyse-treated ewes than in intact ewes injected with Lutalyse; the 5 mg dose resulted in greater $PGF_{2\alpha}$ concentrations in the intact Lutalyse-treated ewes. This indicates that the larger dose in H/Ox ewes somewhat exceeds the capacity of the lungs and kidney to dispose of $PGF_{2\alpha}$, and $PGF_{2\alpha}$ is more tightly regulated in intact ewes. The lungs and kidney are capable of metabolizing the smaller dose of Lutalyse but not the resulting $PGF_{2\alpha}$ production in intact ewes.

Sampling Sites

Results from the validation experiments in this study indicate that sample location affected the mean concentration of $PGF_{2\alpha}$, but not progesterone or PGFM. Therefore, jugular and caudal vena caval sampling to represent cranial vena caval $PGF_{2\alpha}$ concentrations may be inaccurate. The caudal sampling location in these experiments should reflect the total $PGF_{2\alpha}$ in the venous circulation before uterine-ovarian contribution. The $PGF_{2\alpha}$ concentrations in blood samples collected from the cranial vena cava represent the caudal $PGF_{2\alpha}$ concentration plus uterine-ovarian $PGF_{2\alpha}$. Thus, sampling jugular or caudal vena caval blood, rather than blood containing uterine-ovarian $PGF_{2\alpha}$, may lead to mistakes in interpretation of data.

Approximately 3% of the variation in the cranial vena cava $PGF_{2\alpha}$ concentration is explained by changes in progesterone concentration in the vena cava, cranial and caudal to the uteroovarian vein. However, the overall shape of the curve determined by the order of time as a significant covariant changed; a linear effect, rather than quartic effect of time was defined for the cranial $PGF_{2\alpha}$ response curve. These data support the concept that progesterone is important in regulating uterine $PGF_{2\alpha}$ secretion (Hawk and Bolt, 1970; Wilson et al., 1972; Barcikowski et al., 1974; Ford et al., 1975; Hixon et al., 1975; Baird et al., 1976; Scaramuzzi et al., 1977). These experiments from the 1970's show that

progesterone from the newly developed CL is responsible for priming the uterus for subsequent $PGF_{2\alpha}$ secretion induced by estradiol. Ford et al. (1975) indicated that progesterone priming is required for $PGF_{2\alpha}$ secretion because estradiol-17β increased $PGF_{2\alpha}$ concentrations in uterine venous blood and endometrium on d 9 and 10 but not on d 4 or 5 of the cycle of sheep. However, if estradiol-17β is given after pretreatment with progesterone on d 1 to 5, $PGF_{2\alpha}$ concentrations in uterine venous blood and endometrium increase. Raw et al. (1988) found that progesterone may regulate $PGF_{2\alpha}$ secretion through influencing cyclooxygenase activity (Raw et al., 1988). Progesterone also controls estradiol receptor concentrations (McCracken et al., 1980), and thus $PGF_{2\alpha}$ secretion. Progesterone was not a significant covariant for explaining the variation in $PGF_{2\alpha}$ in the caudal vena.

In Exp. 2, 2.5% of the variation in cranial PGFM concentration was accounted for by the changes in cranial $PGF_{2\alpha}$ concentration. Prostaglandin $F_{2\alpha}$ was more indicative of changes in PGFM concentration in H/Ox Lutalyse-treated ewes than in ewes in all other treatment groups. This indicates that changes in PGFM concentrations in the cranial vena cava do not reflect uterine-ovarian $PGF_{2\alpha}$ secretion, and thus, are not good indicators of $PGF_{2\alpha}$ secretion by these organs during induced-luteolysis. Several experiments have used peripheral PGFM concentrations as a measure of uterine-ovarian $PGF_{2\alpha}$ activity (Webb et al., 1981; Sheldrick and Flint, 1986; Zarco et al., 1988); results from this study, along with Fortin et al. (1994), provide evidence that PGFM accounts for a limited amount of the variation in cranial vena caval $PGF_{2\alpha}$ concentration.

Summary

The uterus and(or) ovaries produce and regulate $PGF_{2\alpha}$ concentration in response to Lutalyse. In both experiments, saline-treated ewes tended to have similar responses. In H/Ox ewes in both experiments, the Lutalyse injection could be detected with at least one $PGF_{2\alpha}$ response variable when compared to control ewes. The magnitude of the cranial

responses seemed to be greater in response to the 15 mg Lutalyse dose, although the two smaller doses have been shown to be more efficacious in inducing luteolysis (Hawk and Cooper, 1977; Hawk et al., 1978; Hackett and Robertson, 1980). A threshold of $PGF_{2\alpha}$ concentration or duration of the $PGF_{2\alpha}$ peak concentration may exist, because these $PGF_{2\alpha}$ responses differed in intact Lutalyse-treated and saline-treated ewes in both experiments. A single 15 mg injection of Lutalyse results in $PGF_{2\alpha}$ production by the uterus and(or) ovaries in sufficient quantities to trigger luteolysis. The 5 mg Lutalyse injection when administered twice at 3 h intervals, is also sufficient to induce uterine-ovarian $PGF_{2\alpha}$ production and luteolysis. It is speculated that the two doses mimic pulses of $PGF_{2\alpha}$, thereby initiating the luteolytic cascade twice.

IMPLICATIONS

In midluteal (d 9) phase ewes, exogenous $PGF_{2\alpha}$ is luteolytic. Results from this study indicate that the uterus and(or) ovaries secrete $PGF_{2\alpha}$ in response to an exogenous dose of Lutalyse ($PGF_{2\alpha}$) in a dose-dependent fashion. The response of these organs occurs within minutes, indicating that $PGF_{2\alpha}$-induced luteolysis differs from spontaneous luteolysis in ewes. Uterine-ovarian $PGF_{2\alpha}$ secretion is initiated within minutes and then progesterone concentrations decrease. In response to exogenous $PGF_{2\alpha}$, estradiol concentrations do not increase until after 8 h, thus precluding a change in the steroid hormone environment as responsible for the early stages of luteolysis. It is speculated that $PGF_{2\alpha}$ secretion is enhanced because the progesterone-primed uterus is triggered by the exogenous $PGF_{2\alpha}$. The mechanism of action of $PGF_{2\alpha}$ on the CL in spontaneous luteolysis is currently being examined; exogenous $PGF_{2\alpha}$ may act similarly. A single 15 mg dose of $PGF_{2\alpha}$ is less effacious than two smaller (5 mg) doses of $PGF_{2\alpha}$ given 3 h apart possibly because these two smaller doses mimic the pulsatile nature of $PGF_{2\alpha}$ during spontaneous luteolysis. The exact mechanisms of action of $PGF_{2\alpha}$ in spontaneous and $PGF_{2\alpha}$-induced luteolysis remain unknown.

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APPENDIX I

The following abbreviations were used throughout this thesis:

AA	Arachidonic acid
AUC	Area under the curve
cAMP	Cyclic adenosine 3',5'-monophosphate
CL	Corpus luteum or corpora lutea
CV	Coefficient of variation
d	Day
DAG	Diacylglycerol
DF	Degrees of freedom
EIA	Enzymeimmunoassay
Error MS	Error mean square
F_{crit}	Critical F value (calculated)
FSH	Follicle-stimulating hormone
GLM	General Linear Models
GnRH	Gonadotropin-releasing hormone
h	Hour(s)
H/Ox	Hysterectomized-ovariectomized
IP_3	Inositol 1,4,5-trisphosphate
LH	Luteinizing hormone
min	Minute(s)
MSE	Mean Square Error
ns	Not significant ($P > .05$)
P	Probability
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGFM	13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$
PIP_2	Phosphatidylinositol 4,5-bisphosphate
RIA	Radioimmunoassay
s	Second(s)
SAS	Statistical Analysis System
SSE	Sum Square Error
SAS	Statistical Analysis System

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Married Michael R. Wade	May 2, 1992

Schools Attended:

Livingston High School	1976-1978
Livingston, New Jersey	
Buckeye High School	1979-1980
Buckeye, Arizona	
Winter Haven High School	1980-1980
Winter Haven, Florida	
Hanover Park Regional High School.	1980-1981
East Hanover, New Jersey	
Virginia Polytechnic Institute and State University	1989
Blacksburg, Virginia	

Honors and Activities

Phi Sigma-Honor Society
Alpha Phi Omega-National Service Fraternity
National Service Award Recipient