

RELATIONSHIPS AMONG PROGESTERONE, ESTRADIOL-17 β , 13, 14-
DIHYDRO-15-KETO-PROSTAGLANDIN F₂ α AND PROSTAGLANDIN F₂ α IN
INTACT EWES AROUND THE TIME OF LUTEOLYSIS

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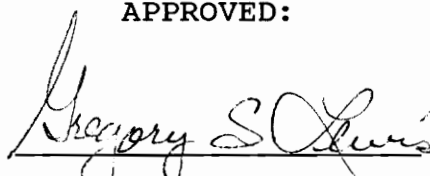
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Thesis submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirement for the degree of
MASTER OF SCIENCE

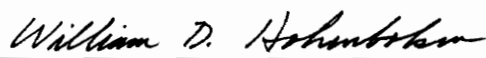
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Animal Science
(Physiology of Reproduction)

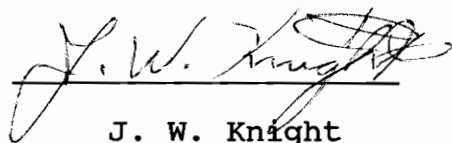
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September, 1991

Blacksburg, Virginia

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RELATIONSHIPS AMONG PROGESTERONE, ESTRADIOL-17 β , 13, 14-DIHYDRO-15-KETO-PROSTAGLANDIN F₂ α AND PROSTAGLANDIN F₂ α IN INTACT EWES AROUND THE TIME OF LUTEOLYSIS

(ABSTRACT)

The exact mechanisms controlling uterine secretion of prostaglandin F₂ α (PGF₂ α) are not known. This study (Experiments 1, 2 and 3) was conducted to evaluate the relationships of progesterone and estrogen to changes in 13,14-dihydro-15-keto-prostaglandin F₂ α (PGFM) and PGF₂ α in ewes. Experiment 1 was designed to determine whether a radioimmunoassay (RIA) for progesterone would detect pessary-released 6 α -methyl-17 α -hydroxy-progesterone (MPA; n=3) and oral 17 α -acetoxy-6-methyl-16-methylene-4,6-pregnadiene-3, 20 dione (MGA; n=3) in blood plasma of ovariectomized ewes. Neither progestogen treatment interfered with the RIA. Experiment 2 was conducted to answer the question: Do MPA-containing pessaries delay luteolysis in intact ewes? Ewes were treated with MPA-containing (n=10) or blank pessaries (controls; n=8) from d 7 and until d 18 of the estrous cycle for control and until d 22 for MPA-treated ewes; d 0 was the day of estrus. Blood samples were collected from the jugular vein throughout the experiment. Pessaries containing MPA did not affect the timing of luteolysis (d 15.4 $\pm$.2), but they prolonged (P < .05) the interestrus interval (17.5 d for control vs 24.1 d for MPA-treated ewes). Experiment 3 was designed to study

the relationships among progesterone, estrogen, PGFM and $\text{PGF}_2\alpha$ in ewes. Ewes were treated with MPA-containing (n=7; 60 mg), progesterone-containing (n=8; 45 mg) or blank pessaries (n=8) from d 7 until d 20 of the estrous cycle. From d 14 and continuing until 24 h after estrus, jugular and vena caval blood samples were collected during two sampling periods daily. Plasma was assayed for progesterone, estrogen, PGFM and $\text{PGF}_2\alpha$. Treatment did not affect the profiles of change in concentration of progesterone, PGFM and jugular $\text{PGF}_2\alpha$, but treatment affected ($P < .05$) estrogen and vena caval $\text{PGF}_2\alpha$ profiles. Overall, treatment affected ($P < .05$) the mean concentrations of estrogen, progesterone, PGFM and $\text{PGF}_2\alpha$. Sampling site (jugular vs. vena cava) affected ($P < .0001$) the mean concentration of progesterone, estrogen and $\text{PGF}_2\alpha$, but site did not affect PGFM concentrations. Hormonal relationships associated with changes in release of $\text{PGF}_2\alpha$ were evaluated. Estrogen seemed to be the primary hormone controlling $\text{PGF}_2\alpha$ release. In conclusion, MPA treatment did not delay the timing of luteolysis, but it increased the interestrus interval. Of the compounds measured, estrogen accounted for the greatest proportion of the variation in $\text{PGF}_2\alpha$ release in ewes around the time of luteolysis.

ACKNOWLEDGMENTS

The author would like to thank the following:

Dr. G. S. Lewis for his guidance, assistance and understanding throughout my research and class work.

Dr. W. D. Hohenboken and Dr. J. W. Knight for serving on my research committee.

Dr. R. P. Del Vecchio for always having time to answer my questions, his guidance and friendship.

Wimpy Williams and Phil Keffer for all their help and for putting up with my Thursday excursions to the Moore Farm.

Lee Johnson for his willingness to always help and for listening to my never-ending complaints.

Mrs. Ellie Stephens for her invaluable help putting this thesis together.

Irma Litchfield, Stacey Hall, Clara Diaz and Han Swinkels for there unconditional friendship and support.

Dr. R. A. Nugent III, for his friendship and guidance every time I was lost.

Jeff Kearnan, Sharbel Tarraf, Ed Custer and Melissa Hajdu, for being good office partners.

Brian Sayre and Ahmed Ramadan for their valuable help in my research projects and lab work.

Gene Ball for his friendship, patience, understanding, support, love and for helping me remain "sane" until the end.

Last, and most important of all to my parents, without your love, support and financial assistance one of my dreams would have never come true. Gracias.

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STATEMENT OF THE PROBLEM

The exact mechanisms that control uterine secretion of $\text{PGF}_2\alpha$ (abbreviations are defined in Appendix I) around the time of luteolysis in livestock are not yet known. The classical literature on sheep first indicated that complete hysterectomy prolonged the life span of the corpus luteum in livestock; therefore, a "substance" produced in the uterus was thought to be responsible for luteolysis (Wiltbank and Casida, 1956). Later studies indicated that concentrations of $\text{PGF}_2\alpha$ in sheep were higher around the time of luteolysis than on other days of the estrous cycle. Also, it was shown that exogenous $\text{PGF}_2\alpha$ would induce luteolysis. Thus, $\text{PGF}_2\alpha$ was called the luteolytic agent in sheep (McCracken et al., 1970; Thorburn et al., 1972; Wilson et al., 1972a).

In ewes, uterine secretion of $\text{PGF}_2\alpha$ may be mediated by the ovarian steroids estrogen and progesterone, and by pituitary and ovarian oxytocin. Overall, the literature indicates that during the estrous cycle, progesterone must decline and estrogen must increase before the uterus can secrete $\text{PGF}_2\alpha$ in amounts adequate to cause complete luteolysis (McCracken et al., 1984). However, the evidence to support that theory is not entirely convincing.

The hormonal control of uterine secretion of $\text{PGF}_2\alpha$ is difficult to study in ovarian intact ewes; exerting fine control over the hormonal environment in intact ewes is

impossible. Thus, a model system must be developed to study the relationships among ovarian steroids, pituitary and ovarian oxytocin and uterine $\text{PGF}_{2\alpha}$ around the time of luteolysis. The experiments described in this thesis addressed some fundamental questions about the hormonal relationships associated with changes in $\text{PGF}_{2\alpha}$ release in ewes. These experiments established a foundation for building a model to understand the control of uterine secretion of $\text{PGF}_{2\alpha}$ in ewes.

REVIEW OF LITERATURE

OVERVIEW OF ESTROUS CYCLE OF SHEEP

Marshall (1904) first described the estrous cycle in sheep. The length of the estrous cycle is usually 16 to 17 d, but it varies somewhat with breed and age of ewe. Most breeds of sheep have estrous cycles during autumn and winter months; this period is referred to as the breeding season. During spring and summer months, most ewes are anestrus.

The day that estrus is first detected is commonly designated as d 0 of the estrous cycle. Estrus lasts 24 to 48 h, and ewes ovulate near the end of estrus. The ewe's estrous cycle is endocrinologically regulated by ovarian steroids (estrogen and progesterone), $\text{PGF}_2\alpha$, oxytocin and hypothalamic and pituitary hormones.

The ewe's estrous cycle can be divided into two phases, the follicular phase and the luteal phase. During the follicular phase, a rapid decrease in progesterone and a marked increase in estradiol are characteristic events. These changes in progesterone and estradiol are followed by estrus and ovulation. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations also increase during the follicular phase. Follicle-stimulating hormone and LH are secreted from the pituitary in response to hypothalamic gonadotropin-releasing hormone (GnRH) and

positive and negative feedback mechanisms involving estrogen and progesterone. Follicle-stimulating hormone begins to increase at d 4 to d 6, and it continues to increase throughout the follicular phase; the increase may stimulate follicular growth. Concentrations of LH peak at the beginning of estrus, and LH causes ovulation (Sorensen, 1979; Hafez, 1987).

Following ovulation, the corpus luteum (CL) develops; this marks the beginning of the luteal phase. The CL secretes progesterone, and progesterone concentrations are high during the luteal phase. The functional life span of the ovine CL is usually from about d 4 to d 14 (Hafez, 1987). Uterine $\text{PGF}_2\alpha$ and pituitary and luteal oxytocin appear to act in a positive feedback loop to cause the regression of the corpus luteum (McCracken et al., 1984).

UTERINE CONTROL OF LUTEAL FUNCTION

Loeb (1923) found that complete hysterectomy "preserved" the CL in guinea pigs; partial hysterectomy caused a short delay in luteolysis. He hypothesized that the life span of the CL and ovulation were linked. In 1940, Hatcher et al. suggested that the uterus produced a hormone that caused luteolysis. Wiltbank and Casida (1956) showed that complete hysterectomy in ewes prolonged the life span of the CL in all cases, which indicated that the uterus

controls CL life span in ewes. Other studies indicated that the uterus controls CL life span in cows, sows and mares (Melampy and Anderson, 1968). But, how was the uterus acting on the corpus luteum?

Inskeep and Butcher (1966) determined that there is a local utero-ovarian relationship in ewes. Unilateral hysterectomies adjacent to (ipsilateral) the ovary with the CL lengthened the estrous cycle (34 to 37 d); cycle length was 16.5 to 18.5 d for controls. Unilateral hysterectomies opposite (contralateral) to the ovary with a CL did not change cycle length (17 d). Ginther (1967) and Hawk (1968) showed that intrauterine devices (IUD) ipsilateral to the ovary containing CL shorted the estrous cycle in ewes. Hawk hypothesized that IUD caused endometrial inflammation. These results indicated that luteal life span in ewes is regulated through local, rather than systemic, pathways. Later studies indicated that uteri of sows and cows control luteolysis through local pathways, but luteolysis in mares is controlled through a systemic pathway (Del Campo and Ginther, 1973a; Ginther, 1974).

Once it was determined that the uterus exerted local control over CL life span in ewes, the next step was to determine how the uterus causes luteal regression. Intrauterine injections of benzyl alcohol cause endometrial irritation. Intrauterine infusion of benzyl alcohol ipsilateral to the CL shortened the estrous cycle in ewes,

but intrauterine infusions of benzyl alcohol contralateral to the CL did not affect cycle length (Woody et al., 1969). These data were used to formulate the hypothesis that the uterine luteolytic factor in sheep is produced in the endometrium.

Exogenous $\text{PGF}_2\alpha$ was shown to be luteolytic in sheep (McCracken et al., 1970). Wilson (1972) reported that ovine endometrium produced prostaglandins and that endometrial concentrations of $\text{PGF}_2\alpha$ were higher on d 14 of the estrous cycle than on d 3, 5 and 11; luteolysis is first detected on about d 14 in sheep. Prostaglandin $\text{F}_2\alpha$ concentrations in utero-ovarian venous plasma were higher in ewes during luteal regression than during the luteal phase of the estrous cycle (Thorburn et al., 1972).

In summary, studies reported from the late 1950's to the mid-1970's indicated that the uterus controls luteal life span in sheep. Uterine control of luteal function is communicated through a local, rather than systemic, pathway, and $\text{PGF}_2\alpha$ may be the endometrial compound that exerts primary control over CL life span. Endocrine control of the uterine secretion of prostaglandins will be discussed later.

OVARIAN AND UTERINE VASCULAR ANATOMY

After determining that uterine $\text{PGF}_2\alpha$ may be causing luteal regression in ewes, the question became: How is $\text{PGF}_2\alpha$ transported from the uterus to the ovaries? Various luteolytic pathways were suggested; some of these hypothetical pathways included vascular and lymphatic components.

ANATOMY

In ewes, the uterus and ovaries share common arteries and veins (Ginther, 1974). The ovarian artery originates at the aorta and divides into three branches: uterine, ovarian and tubal (Del Campo and Ginther, 1973a). Blood is mainly supplied to the uterus by the uterine branch of the ovarian artery and to the ovaries by the ovarian branch of the ovarian artery. The ovarian vein, commonly called the utero-ovarian vein, is a large common venous trunk that drains the ovary, most of the uterus and oviducts. The utero-ovarian vein is also composed of three branches; uterine, ovarian and tubal. The ovarian artery and the utero-ovarian vein form the vascular pedicle. It is important to note that the ovarian artery is in close apposition and coils around the utero-ovarian vein from its origin to its divisions (Del Campo and Ginther, 1973a; Del Campo and Ginther, 1973b; Figure 1).

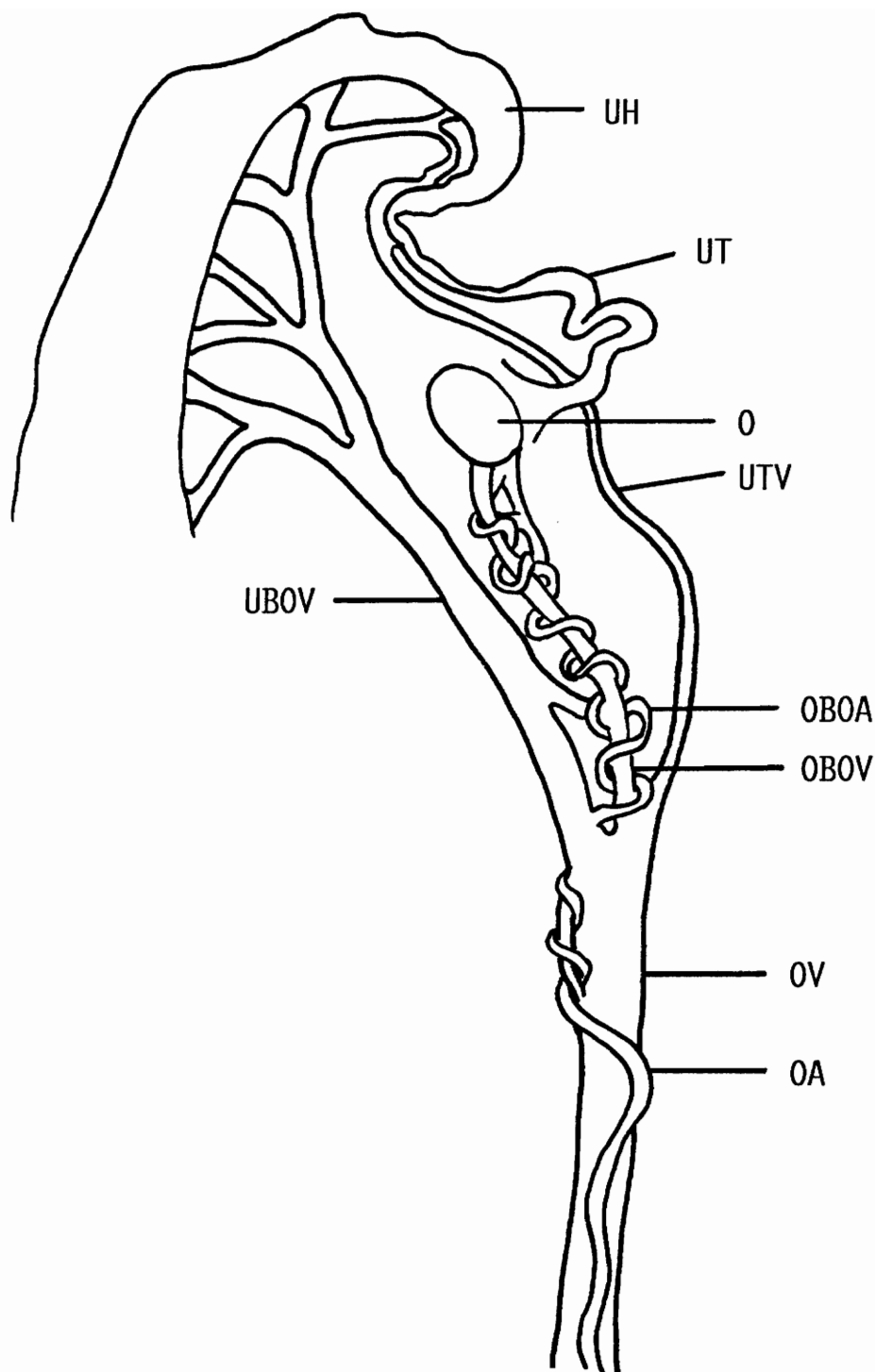


Figure 1. Local uterine and ovarian venoarterial pathways in the ewe (OA=ovarian artery, OV=ovarian vein, OBOV=uterine branch of ovarian vein, OBOA=uterine branch of ovarian artery, UTV=uterine tubal veins, O=ovary, UT=uterine tube ,i.e., oviduct, UH=uterine horn)

VENOARTERIAL PATHWAYS

Ginther (1974) reviewed evidence supporting the idea that the uterus causes luteolysis in ewes through a venoarterial pathway. Ginther et al. (1973a) unilaterally hysterectomized ewes and surgically anastomosed uterine veins and ovarian arteries. Venous blood from the intact uterine side was drained into the main uterine vein of the hysterectomized side. Weight of the CL on the hysterectomized side was less in ewes with patent anastomoses than in controls or in ewes with occluded anastomoses. Therefore, local luteolytic effects of the uterus may involve the veins that drain the uterus and the ovarian artery.

Ginther and Del Campo (1973b) studied the areas of blood vessels where uterine venous blood was in close proximity to ovarian arterial blood. There were three areas of extensive surface contact: 1) the initial one third of the ovarian branch of the ovarian artery; 2) the area at the junction of the uterine and ovarian veins; 3) throughout the utero-ovarian vein where the ovarian artery coils. Del Campo and Ginther (1974) also found that the average thickness of the walls of arteries and veins in the areas of apposition was less than in other areas. The circular layer of smooth muscle in the arteries was also thinner. All of

these studies led to the idea that luteolytic substance passed directly from uterine venous blood into ovarian arterial blood.

In summary, Ginther hypothesized that the luteolytic substance is produced in the uterus and is transported by the veins that drain the uterine horns. In the areas of close veno-arterial apposition, the substance is passed from the uterine vein into the ovarian artery. It is then carried by the ovarian artery directly into the ovary to cause luteolysis.

LYMPHATIC INVOLVEMENT

Some details of Ginther's hypothesis about veno-arterial exchange of luteolysin have been questioned. Even though vascular connections between the uterus and ovary were accounted for in Ginther's studies, the lymphatic system was not. Lymphatics draining the ovary are associated with the utero-ovarian vasculature pedicle, and they enlarge during the luteal phase (Morris and Sass, 1966). Staples et al. (1982) and Abdel Rahim et al. (1984) showed that the lymphatic system in ewes accounted for some of the transport of prostaglandin from the uterus to the ovaries. However, the importance of the lymphatics in the control of luteal lifespan in ewes is not clear.

SYNTHESIS AND METABOLISM OF PROSTAGLANDINS

The discovery of prostaglandins has led to many important breakthroughs in the understanding of reproductive physiology. Prostaglandins (PG) were first discovered in human semen (von Euler, 1936) and later in human menstrual fluid (Pickles, 1957). During the 1960's, many PG were isolated, and their chemical structures were determined (Bergström and Samuelsson, 1967). The nomenclature and synthesis of PG will be described below.

Prostaglandins are 20-carbon fatty acids. They have a cyclopentane ring with two aliphatic chains attached (Poyser, 1981). Nomenclature for prostaglandins includes the abbreviation PG, a letter, a number and often a Greek symbol. For example, in $\text{PGF}_2\alpha$, the letter following the PG abbreviation describes the moieties attached to the cyclopentane ring. The numerical subscript relates to the number of double bonds in the molecule, and the Greek symbol refers to the stereo-isometric form of the molecule. Known prostaglandins range from PGA through PGJ.

Prostaglandins are produced and released throughout an animal's body in response to a variety of stimuli (Wolfe, 1982). In mammals, PG are not stored appreciably in tissues, instead they seem to be released soon after synthesis. Arachidonic acid (AA) is a major substrate for PG production. Products produced from AA are given the general name, eicosanoids. There are two types of

eicosanoids, those produced from the cyclooxygenase pathway and those produced from the lipoxygenase pathway. Products of the cyclooxygenase pathway include prostaglandins, prostacyclin and thromboxanes, and products from the lipoxygenase pathway are called leukotrienes (Tepperman, 1987).

The fatty acid linoleate is the precursor for the monoenoic prostaglandins, such as PGE_1 and PGH_1 . The dienoic types of prostaglandins ($\text{PGF}_2\alpha$, PGE_2 , PGI_2 , etc.) are synthesized from arachidonic acid, and the trienoic PG are synthesized from timnodonic acid (Pace-Asciak and Granstöm, 1983). The synthesis of all prostaglandins is complex. The synthesis of $\text{PGF}_2\alpha$ will be used as a general model for the synthetic pathways (Figure 2).

The first step in the synthesis of prostaglandins is the liberation of AA from cellular phospholipids, triacylglycerols or cholesterol esters. Phospholipase A_2 liberates AA from phospholipids. Esterases and lipases liberate AA from triacylglycerols and cholesterol esters.

The second step for PG synthesis consists of the conversion of AA into endoperoxides (PGG and then PGH) by the enzymes PG endoperoxide synthetase (cyclooxygenase) and PG hydroperoxidase, respectively. Oxygen and heme are required for the completion of this step. Inhibitors for

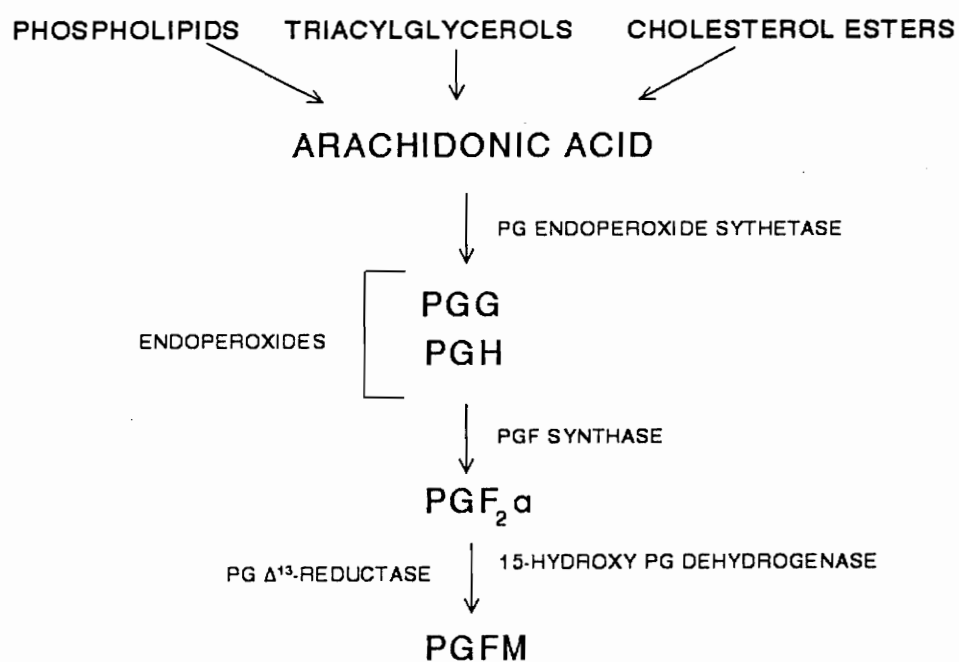


Figure 2. Synthesis and metabolism of PGF₂α.

this step include aspirin and indomethacin. The first and second stages for PG synthesis (i.e., liberation of AA and production of endoperoxides) are not tissue specific; the remaining stages of the pathway are tissue dependent (Tepperman, 1987).

Prostaglandin H_2 is not a stable compound, and it is transformed rapidly into $PGF_2\alpha$, PGE_2 , prostacyclin (PGI_2) and thromboxane (TXA_2). These conversions are completed through enzymatic activities. Prostaglandin H_2 is converted into $PGF_2\alpha$ by PGF synthase (Tepperman, 1987). Prostaglandin $F_2\alpha$ is then converted into 13,14-dihydro-15-keto- $PGF_2\alpha$ (PGFM) by 15-hydroxy PG dehydrogenase and PG Δ^{13} -reductase (Pace-Asciak and Granstöm, 1983).

Prostaglandins are metabolized rapidly. The major site for PG metabolism is the lungs. In sheep, as much as 99 % of tritium-labeled $PGF_2\alpha$ can be metabolized during a single passage through the lungs, regardless of the stage of the estrous cycle (Davis et al., 1980).

ENDOCRINE REGULATION OF PGF₂ α SECRETION

Several physiological stimuli affect the release of PGF₂ α from the uterus of nonpregnant ewes around the time of luteolysis. The first type of stimulus is produced by ovarian steroidal hormones (progesterone and estrogen). The second type of stimulus is created by oxytocin. The relationship between these stimuli will be discussed.

OVARIAN STEROIDS

Progesterone.

Progesterone was first related to ovarian and uterine activities when it was used to synchronize estrus. Dutt and Casida (1948) showed that the timing of estrus and ovulation could be altered in intact ewes by progestogen injections starting on d 0 and continuing to d 4, 8 or 12 of the estrous cycle. Ewes treated with progestogens were in estrus 3 to 4 d after the end of the treatment (O'Mary et al., 1950). Progesterone was then thought to be one of the main agents affecting the time of ovulation, since the onset of estrus could now be controlled (Foote, 1964).

Once it was determined that progesterone played an important role in the timing of events in the estrous cycle, it was important to determine when and how these changes occurred. Intact ewes injected with progesterone early in the cycle (d 0 to 6) had shorter cycles (12.7 d) than did controls (Woody et al., 1967). Hysterectomized ewes treated

with progesterone and hysterectomized control ewes had longer cycles (60 d) than did intact ewes with no progesterone treatment. Therefore, Woody (1968) hypothesized that progesterone exerted part of its influence on the CL through an action on the uterus.

After the linkage of prostaglandins to luteal regression in the early 1970's, Wilson et al. (1972b) hypothesized that progesterone itself was not responsible for the elevated levels of $\text{PGF}_2\alpha$ in the endometrium on d 14. Higher endometrial concentrations and contents of $\text{PGF}_2\alpha$ were detected in ovariectomized ewes than in ewes treated with progesterone on d 4 to 8 after estrus. In contrast, in intact ewes, an increase in endometrial $\text{PGF}_2\alpha$ content was detected in progesterone-treated ewes (d 0 and 1) than in controls. Wilson also hypothesized that progesterone may be luteolytic early in the cycle through an interference with CL formation.

Baird et al. (1976) autotransplanted the ovary, uterus and anterior vagina to the neck in ewes. Uterine $\text{PGF}_2\alpha$ secretion increased on d 12 to 14 of the estrous cycle, at the same time as the decline in progesterone. In contrast to other studies, daily progesterone injections administered to ovariectomized ewes from d 0 to 7 after estrus increased uterine $\text{PGF}_2\alpha$ concentration (Scaramuzzi et al., 1977). With this information, authors concluded that the uterus will only release $\text{PGF}_2\alpha$ in amounts adequate to cause luteolysis

after it has been primed with progesterone for 7 to 10 d, and then after the progesterone concentrations decline.

Ottobre et al.(1980) treated ewes with progesterone on d 0 of the estrous cycle. They showed that in uterine blood peak concentrations of $\text{PGF}_2\alpha$ were measured earlier in the cycle (d 8) in progesterone-treated than in control ewes. It was also determined that a peak in $\text{PGF}_2\alpha$ was detected in treated ewes before the decline in luteal progesterone. In contrast to other studies, Ottobre et al. (1980) found that progesterone treatments caused the early release of $\text{PGF}_2\alpha$.

Progestogens have also been administered in MPA pessaries to control the length of the estrous cycle (Echternkamp et al., 1976). Progestogenated pessaries had no effect on development and regression of CL when they were inserted on d 2, 4, 6, 8, 10 and 12 of the estrous cycle and left for 12 d. There were no increases in estrogen until termination of the progestogen treatment (Schoombe et al., 1988). This was not observed with treatments involving MGA and oral MPA.

In summary, progesterone is needed for uterine secretion of $\text{PGF}_2\alpha$. Progesterone injections given early in the estrous cycle will reduce CL life span in ewes; one progesterone injection given on the day of estrus is sufficient to cause early CL regression (Ottobre et al., 1980). The uterus must be primed with progesterone for a finite period of time before it is capable of synthesizing

enough $\text{PGF}_2\alpha$ to cause luteolysis. Progestogenated pessaries should have no effect on CL life span, but they will affect the timing of estrus. The mechanisms controlling the effect of progesterone on uterine secretion of $\text{PGF}_2\alpha$ in ewes will be discussed next.

How does progesterone control uterine secretion of $\text{PGF}_2\alpha$? There are many explanations for progesterone's involvement in synthesis and secretion of $\text{PGF}_2\alpha$. Boshier and Holloway (1973) found that uterine epithelial lipids were higher during diestrous and pro-estrous in rats. The increase in lipid concentration was thought to be due to an increase in plasma progesterone and a decrease in estrogen. Progestogen (MPA) injections given to ovariectomized rats increased uterine lipid storage. Thus, progesterone facilitates the uterine storage of lipids.

In endometrial epithelial cells in ewes, lipid droplet concentrations were lowest on d 4 to 6 of the estrous cycle, started to increase on d 10 and were maintained high until d 4 of the next cycle (Brinsfield and Hawk, 1973). Lipid droplets appear to increase as the CL is formed. Progesterone injections given to ovariectomized ewes increased endometrial lipid content (Brinsfield and Hawk, 1973).

Progesterone may also affect the synthesis of $\text{PGF}_2\alpha$. In the rat uterus, progesterone regulates the acylation and deacylation cycles of phospholipids, triacylglycerols and

other compounds. Progesterone treatments inhibited phospholipase A₂ activity in ovariectomized rats pretreated with estradiol (Toth and Hertelendy, 1986). In ewes, progesterone may also play a key role in regulating the ability of the uterus to secrete PGF₂α by increasing cyclooxygenase activity (Raw et al., 1988). Finally, in ovariectomized ewes progesterone treatments for 10 d decreased oxytocin receptors, oxytocin being an important part of the uterine secretion of PGF₂α (Vallet et al., 1990).

Estrogen.

Foote (1964) first showed that in ewes a single injection of estradiol-17β (E₂-17β) early in the estrous cycle induced ovulation. In intact ewes, E₂-17β treatment on d 5 of the cycle caused a new ovulation within 56 h after treatment (Howland et al., 1968). Injections of estradiol on d 11 and 12 of the cycle caused early CL regression (Stormshak et al., 1969). An increase in LH activity and a decrease in progesterone were also detected (Howland et al., 1968).

In contrast to those studies, Piper and Foote (1968) found that daily injections of estradiol beginning on d 4 of the estrous cycle in ewes prolonged the life span of the CL. In intact ewes, daily injections of estradiol on d 1 to 6 had no effect on the weight of the CL compared to injections

given on d 9 to 12 of the cycle (Hawk and Bolt, 1970). One may conclude that, in ewes, the effect of estradiol on the CL depended upon the stage of the cycle at which the treatment was given. Injections of estradiol during the last week of the ewe's cycle initiated CL regression. The dose of $E_2-17\beta$ administered was also important.

After determining that estradiol causes CL regression in ewes, the next question was, what mechanisms are triggered by estradiol to cause the CL to regress? Stormshak (1969) showed that estradiol failed to cause regression of the CL in hysterectomized ewes. Estradiol administered on d 11 and 12 to unilaterally hysterectomized ewes caused a decrease in size of the CL adjacent to the intact uterine horn but no significant change in the size of the CL located on the hysterectomized side (Akbar et al., 1971). The effect of estradiol on the CL was through a uterine-mediated mechanism.

In intact ewes, estradiol peaks occur throughout the cycle, but higher concentrations of $PGF_2\alpha$ and $E_2-17\beta$ usually only occur coincidentally on around d 15 of the estrous cycle. Infusion of estradiol on d 14 of the cycle caused a peak in $PGF_2\alpha$ secretion; infusions on d 6 to 10 of the cycle did not cause a significant increase. The ovine uterus was responsive to estradiol only in the late luteal phase (Barcikowski et al., 1974). Therefore, it can be concluded

that estradiol triggers CL regression through an increase in uterine production of $\text{PGF}_2\alpha$.

In rats, estradiol injections increased uterine phospholipase A_2 activity. Estradiol also seems to regulate the rate of arachidonic acid turnover in lipids (Toth and Hertelendy, 1986). In the uterus of ovariectomized rats, E_2 - 17β injections caused the rapid disappearance of lipids and stimulation of lipid synthesis (Boshier and Holloway, 1973). Similar results have been reported for ewes.

Injections of E_2 - 17β decreased lipid droplets in the uterus of ovariectomized ewes (Brinsfield and Hawk, 1973). Cyclooxygenase concentrations were increased in ovariectomized ewes pretreated with progesterone and then treated with estradiol (Raw et al., 1988). Estradiol benzoate administered to ewes on d 9 and 10 of the cycle will not only increase $\text{PGF}_2\alpha$ secretion and decrease progesterone, but it also increases the number of oxytocin receptors in the endometrium and myometrium within 24 h after treatment (Hixon and Flint, 1987). The interactions between estrogen, progesterone, oxytocin and $\text{PGF}_2\alpha$ will be discussed later.

Roles of Progesterone and Estrogen in $\text{PGF}_2\alpha$ secretion.

As established before, both progesterone and estrogen play important roles in controlling uterine secretion of $\text{PGF}_2\alpha$ in sheep. How do these two hormones interact to

elicit uterine secretion of $\text{PGF}_2\alpha$? Using ovariectomized ewes, Caldwell et al. (1972) tried to mimic the normal patterns of hormone secretion found in intact ewes. Levels of $\text{PGF}_2\alpha$ increased on d 13 and 14 of the "artificial" estrous cycle when ewes were primed with progesterone for 13 or 14 d and given an injection of estradiol benzoate at the end of the treatment. No increase in $\text{PGF}_2\alpha$ secretion was detected in ewes treated only with progesterone. Similar results were reported by Silvia and Raw (1988). Prostaglandin $\text{F}_2\alpha$ pulses were not detected in ovariectomized ewes treated with progesterone or estrogen alone. Pulses were found in ovariectomized ewes treated with a combination of progesterone and estrogen; these pulses did not differ from those measured in intact ewes (Silvia and Raw, 1988).

Some studies indicate that estrogen is the most important hormone controlling uterine secretion of $\text{PGF}_2\alpha$. In ovariectomized and intact ewes, estradiol injections caused the actual release of $\text{PGF}_2\alpha$ from the uterus (Caldwell et al., 1972; Warren et al., 1973; Ford et al., 1975). In contrast, other studies indicate that estrogen is not needed for the release $\text{PGF}_2\alpha$. Louis et al. (1977) found that in bilaterally ovariectomized ewes estrogen infusions for 9 d had no effect on the concentration of $\text{PGF}_2\alpha$ in uterine tissue. Progesterone infusions significantly increased uterine $\text{PGF}_2\alpha$ concentration in ewes. Similar results by Baird et al. (1976), Scaramuzzi et al. (1977) and Ottobre et

al. (1984) showed that estrogen alone had no effect on uterine PGF₂ α secretion.

A period of progesterone priming may be necessary for increasing uterine secretion of PGF₂ α (Roberts et al., 1975). Estradiol administered alone to intact ewes on d 5 and 6 of the estrous cycle and progesterone administered alone on d 1 to 4 of the cycle did not regress the CL. Treatment with both hormones can cause early regression of the CL by d 9 of the estrous cycle (Warren et al., 1973). In intact ewes, E₂-17 β injections on d 9 and 10 of the cycle increased uterine PGF₂ α concentration, but injections on d 4 and 5 were not effective. However, exogenous progesterone administered to ewes on d 1 to 5 and estrogen on d 4 and 5 increased PGF₂ α concentrations (Ford et al., 1975). These studies indicated that a period of progesterone influence is necessary before estrogen can act as a luteolytic substance in ewes. Also, progesterone only needs to be present in ewes until d 9 of the estrous cycle to stimulate PGF₂ α release on d 12 and 13 (Ottobre et al., 1984).

Both estrogen and progesterone affect uterine secretion of PGF₂ α in sheep. Although studies have been equivocal in determining which of the two hormones is more important in this process, a general scenario can be described. Apparently, progesterone needs to be present in high amounts in ewes for a finite period of time to prime the uterus. Progesterone then needs to decline and estrogen increase for

luteolysis to occur. High concentrations of estrogen are then followed by increased concentrations of $\text{PGF}_2\alpha$, and finally the corpus luteum regresses. Oxytocin also plays an important role in uterine $\text{PGF}_2\alpha$; its role will be described next.

Oxytocin.

Roberts et al. (1976) studied uterine tissue in ewes at different stages of the estrous cycle. They found that ovine endometrium is receptive to oxytocin and that the synthesis of prostaglandins may be related to oxytocin and oxytocin receptors. Ovarian steroids could affect uterine prostaglandin synthesis by regulating the availability of endometrial oxytocin receptors. Immunization of sheep against oxytocin prolongs luteal life span for 3 to 7 d (Sheldrick et al., 1980). But, oxytocin injections given to ewes on d 1 to 6 or d 3 to 6 of the estrous cycle did not decrease the length of the cycle (Milne, 1963).

Jugular venous concentrations of oxytocin in ewes decreased at the time of luteal regression (d 11 to 14) and increased again when the new CL was formed. One of the major sources of oxytocin during the end of the luteal phase is the large luteal cells (Rodgers et al., 1983). Luteal oxytocin is secreted in response to prostaglandin stimulation (Flint and Sheldrick, 1983).

The luteolytic action of estradiol-17 β could be mediated by oxytocin and the number of endometrial oxytocin receptors in the ovine endometrium (Sheldrick et al., 1980). Anestrous ewes treated with either estradiol-17 β and oxytocin or estradiol alone exhibited a dramatic increase in PGF₂ α release. No effect on PGF₂ α release was detected in ewes treated only with oxytocin (Sharma and Fitzpatrick, 1974). Injections of estradiol benzoate into ewes on d 9 and 10 of the estrous cycle increased oxytocin concentrations, which was followed by an increase in PGF₂ α secretion and a decrease in progesterone (Hixon and Flint, 1987).

Depending upon the experimental conditions, progesterone may inhibit or stimulate oxytocin production. Ewes pretreated with progesterone have lower numbers of oxytocin receptors compared to ewes treated with estradiol (Vallet et al., 1990). Homanics and Silvia (1988) treated ovariectomized ewes with progesterone and estrogen either alone or in combination. Ewes were then given an injection of oxytocin on d 5, 10 and 15 of the treatment regimen. No change in PGFM concentrations were detected on d 5 and 10. On d 15, PGF₂ α increased in ewes treated with progesterone. Exogenous progesterone administered to intact ewes at the end of the luteal phase did not affect oxytocin production; oxytocin increased as expected. Therefore, a decrease in

progesterone is not necessary for an oxytocin surge to occur (Fairclough et al., 1983).

A general hypothesis has been established for the overall hormonal regulation of uterine secretion of $\text{PGF}_2\alpha$ from the ovine uterus (McCracken, 1980; McCracken et al., 1984). At the end of the luteal phase and once progesterone concentrations begin to decline, estradiol concentrations begin to increase. Progesterone is thought to inhibit estradiol receptor production. Estradiol receptors begin to increase in number, and high concentrations of estradiol stimulate the formation of endometrial oxytocin receptors. Next, ovarian and pituitary oxytocin interact with these newly formed oxytocin receptors. This stimulates uterine secretion of $\text{PGF}_2\alpha$, and prostaglandin concentrations increase. After the increase in $\text{PGF}_2\alpha$, progesterone declines; the CL begins to regress. Oxytocin from the CL is being secreted in response to uterine $\text{PGF}_2\alpha$ stimulation, and uterine $\text{PGF}_2\alpha$ is secreted in response to luteal oxytocin stimulation, i.e., a positive feedback loop. Finally, uterine oxytocin receptors become desensitized and the feedback loop is terminated.

Even though this hypothesis is complete, many critical questions about its validity remain unanswered. The role of progesterone, estradiol and oxytocin in relation to prostaglandin secretion are controversial. Does progesterone have to decline and estrogen increase for there

to be increased synthesis of $\text{PGF}_2\alpha$ in the ovine uterus
around the time of luteolysis ?

MATERIALS AND METHODS

GENERAL

Twice daily during the breeding season (September to March), vasectomized rams were used to check mature ewes in the Virginia Tech flock for signs of estrus. As ewes were detected in estrus, they were assigned to experiments. Pessaries, prepared in-house, were used in the experiments described in this thesis. Expanded polyether foam test tube plugs (Jaece Industries, North Tonawanda, NY), monofilament fishing line (8 kg test), 6 α -methyl-17 α -hydroxy-progesterone acetate (MPA; Sigma Chemical Co., St. Louis, MO) and 4-pregnene-3,20-dione (progesterone; Sigma Chemical Co.) were used to prepare pessaries. Monofilament line was sewn through the foam plugs, and the line was secured with an over-hand knot. Progesterone (45 mg) or MPA (60 mg) in ethanol (10 ml) was added to the pessaries in two 3 ml and one 4 ml aliquots. Ethanol was allowed to evaporate between additions of MPA or progesterone. Pessaries were stored in plastic bags until they were used. Before pessary insertion, the ewe's vaginal area was washed and disinfected. Pessaries were pushed into lubricated applicators made from 12 ml syringes and inserted into ewes. After each insertion, applicators were disinfected with Betadine solution. The monofilament line was used to remove

pessaries from ewes. For these studies, blood samples were collected with 12 ml syringes and transferred into 16 mm x 100 mm borosilicate glass tubes containing 100 USP units of heparin (Sigma Chemical Co.) in 100 μ l of .9 % saline. Blood samples were centrifuged at 1600 g at 4°C for 25 min. Plasma was decanted into 9 ml polypropylene tubes and stored at -20° C until assayed.

EXPERIMENT 1

The purpose of this experiment was to determine whether a radioimmunoassay (RIA) for progesterone would detect pessary-released MPA and oral 17 α -acetoxy-6-methyl-16-methylene-4,6-pregnadiene-3,20-dione (MGA; The Upjohn Co., Kalamazoo, MI) in blood plasma from ovariectomized ewes. Ewes (n=6) were anesthetized with sodium pentobarbital (65 mg/ml saline; Sigma Chemical Co.) and bilaterally ovariectomized through a midventral laparotomy using standard aseptic procedures. The ewes were used for three trials and assigned randomly to different treatment groups each time. Ewes were rested for 10 d between trials. In trial one, jugular blood samples were collected at 0900 h daily from d 4 until d 13 after surgery (d 0). On d 7, either MPA-containing or blank pessaries were inserted into the ewes (n=3/group), pessaries were removed on d 13. For trials 2 and 3, jugular blood samples were collected at 0900

h daily for 10 d. Beginning 3 d and continuing until 10 d after the first blood sample was collected for trial 2, ewes were fed either corn meal containing MGA (0.5 mg MGA/0.45 kg corn meal/ewe/day; n=3 ewes) or corn meal only (0.45 kg/ewe/day; n=3 ewes). One-half of the daily ration of corn meal, with or without MGA, was fed in the morning and the other half was fed in the evening. For trial 3, progesterone-containing or blank pessaries were inserted into the ewes (n=3 ewes/group) on d 3 and removed on d 10 of the blood-sampling regimen. Blood plasma was assayed for progesterone.

EXPERIMENT 2

This experiment was designed to answer the question: Do MPA-containing pessaries delay luteolysis in intact ewes? The day of estrus was considered d 0. On d 7 of an estrous cycle, either MPA-containing or blank pessaries were inserted into intact ewes (n=10 MPA-treated and 8 control ewes). Pessaries were removed from control ewes 24 h after first detection of estrus and from MPA-treated ewes on d 22. From control ewes, jugular blood samples were collected daily from d 7 to d 16 and then at 8-h intervals from d 16 until 24 h after first detection of estrus. From MPA-treated ewes, jugular blood samples were collected daily

from d 7 until d 22 and then at 8-h intervals until 24 h after first detection of estrus.

EXPERIMENT 3

This experiment was designed to answer the question: Does exogenous progesterone and MPA alter blood plasma concentrations of progesterone, estrogen, $\text{PGF}_2\alpha$ and PGFM around the time of luteolysis in ewes? On d 7 of an estrous cycle, MPA-containing, progesterone-containing or blank pessaries (controls) were inserted into intact ewes (n=8/group). New progesterone-containing pessaries were inserted into the progesterone-treated ewes on d 14. Pessaries were removed from control ewes 24 h after first detection of estrus and from MPA- and progesterone-treated ewes on d 20.

SURGERY

Venae cavae in all ewes were catheterized on d 11 of an estrous cycle. Catheterization procedures have been described in detail (Benoit and Daily, 1991). Briefly, ewes were anesthetized with sodium pentobarbital. A surgical site was prepared on the right hind leg of each ewe, and the saphenous vein was located just dorsal to the hock. The skin covering the vein was incised, and blunt dissection was used to expose the saphenous vein. A small hole was cut in

the vein, and a 100 cm catheter (i.d. .42 mm, o.d. .74 mm; polyvinyl chloride tubing; ICO-Rally Corporation, Palo Alto, CA), marked at 45, 50, 55 and 60 cm, was inserted into the saphenous vein. The catheter was passed up the length of the saphenous vein and into the vena cava. Two sets of blood samples (6 ml for each sample) were collected at 10 min intervals; samples were collected at each distance marked on a catheter. Progesterone was measured in plasma from each blood sample. On d 13, vena caval catheters were positioned at the marked distance where progesterone concentrations were the highest, and jugular veins were catheterized with polyvinyl chloride tubing (i.d. .34 mm, o.d. .66 mm; ICO-Rally Corporation). High vena caval progesterone concentrations were important because they indicated the point where ovarian and uterine blood mixed with vena caval blood. Of the 24 ewes in the experiment vena caval catheters were placed at 45 cm in one ewe, at 50 cm in two ewes and between 55 and 60 cm in 21 ewes. Vena caval catheter placement was verified visually in one ewe. If vena caval catheters failed to deliver blood, a quick surgery was performed. For this surgery, ewes were anesthetized, the failed catheter was removed and replaced with a new catheter. Placement distance was not changed.

BLOOD COLLECTION SCHEDULE

From d 14 until 24 h after first detection of estrus in control ewes, and from d 14 until d 20 or 24 h after first detection of estrus in MPA and progesterone-treated ewes, jugular and vena caval blood samples were collected during two sampling periods daily. During each sampling period (0700 to 0900 and 1800 to 2000 h), jugular and vena caval blood samples (12 ml) were collected at 30 min intervals; five samples were collected from each vein during each sampling period. At each sampling time, the vena caval blood sample was collected first. Immediately after the vena caval sample was collected, the jugular blood sample was collected. After each blood sample was collected and again at the end of each collection period, catheters were flushed with 15 ml of sterile .9% physiological saline solution and 5 ml of heparinized saline (either 10 or 50 USP units heparin/ml).

BLOOD HANDLING

Blood samples were collected and handled as described in the General section, except that samples were stored on ice until the last sample was collected during a sampling period. A portion (800 μ l) of samples collected during each period were pooled, and a combined sample for the morning and one for the afternoon were used to measure progesterone, estrogen, PGFM and PGF₂ α for each ewe.

HORMONE ASSAYS

PROGESTERONE

A radioimmunoassay was used to quantitate progesterone in jugular and vena caval plasma (Lewis et al., 1990). For experiments 1 and 2, the intraassay and interassay coefficients of variation (CV) were 3.6 % and 7.4 %, respectively. For Experiment 3, the intraassay and interassay CV were 5.1 % and 8.2 %, respectively. Cross-reactivity of MPA with the antiserum used in the progesterone assay was evaluated. One microgram of MPA cross-reacted less than .4 % with the progesterone antiserum.

ESTROGEN

A RIA was used to quantitate estrogen in jugular and vena caval plasma (Guthrie and Bolt, 1983; Lewis et al., 1989). The intraassay and interassay CV were 8.5 % and 24.6 %, respectively.

PGFM AND PGF₂ α

Enzymeimmunoassays (EIA) were used to quantitate PGFM and PGF₂ α in jugular and vena caval plasma (Del Vecchio et al., 1991). The intraassay and interassay CV for PGFM were 4.8 % and 24.6 %, respectively. A variation of the EIA for PGFM was used to quantify PGF₂ α . The intraassay and

interassay CV for $\text{PGF}_2\alpha$ were 14.8 % and 16.1 %, respectively.

For the $\text{PGF}_2\alpha$ assay, two separate standard curves were compared. The first was a normal standard curve for the assay. The second standard curve was created by spiking charcoal-stripped plasma with the same concentrations as $\text{PGF}_2\alpha$ contained in the normal standard curve for the assay. Prostaglandin $\text{F}_2\alpha$ was extracted from these charcoal-stripped plasma samples using procedures described for a PGFM assay (Del Vecchio et al., 1991). With a 77 % extraction efficiency, both curves were parallel.

STATISTICAL ANALYSES

GENERAL

The Statistical Analysis System (SAS, 1985) was used for all data analyses. For all experiments, the effects of treatment on average hormone concentrations and profiles of changes in hormone concentrations were determined (Allen et al., 1983). First, the General Linear Models (GLM) procedure was used to determine the overall shape of the hormonal profiles. The general SAS statements were:

```
PROC GLM; CLASS TREATMENT EWE;  
MODEL HORMONE = TREATMENT EWE(TREATMENT) DAY DAY2 DAY3 etc.  
/ SOLUTION SS1 SS3;  
LSMEANS TREATMENT;
```

Treatment (fixed effect) and ewe (random effect) were considered independent variables and day of blood sampling (Day) was considered a covariant. The SAS models were processed individually with linear (DAY), quadratic (DAY²), cubic (DAY³), etc. components included. The model that included the highest power of DAY in which all DAY components were significant (based upon Type III sums of squares) was considered the model that best described the overall changes in hormonal concentrations. Type I sums of squares were used to determine the effects of treatment on average hormonal concentrations. For this determination, the EWE(TREATMENT) mean square was used as the error term for the F-test, which is appropriate for a mixed model.

Next, SAS models that described hormonal changes within treatments were processed. The DAY component in these models was consistent with the power of DAY from the model that best described overall changes in concentrations of the hormone being evaluated. The general SAS statements were:

```

PROC SORT; BY TREATMENT;
PROC GLM; BY TREATMENT; CLASS EWE;
MODEL HORMONE = EWE DAY DAY2 DAY3 etc. / SOLUTION SS1 SS3;

```

The hypothesis was tested that residual variances for combined treatments were equal to the residual variance from the overall model. Evidence to support the rejection of that hypothesis was interpreted to mean that the hormonal profiles differed with treatment. An example of the calculations used to test the hypothesis about equality of residual variances is shown in Table 1.

TABLE 1. CALCULATIONS FOR EQUALITY OF RESIDUAL VARIANCE

SOURCE	ERROR DEGREES OF FREEDOM	ERROR SUMS OF SQUARES	ERROR MEAN SQUARE	F-TEST
Overall	106	.9833		
Treatment 1	79	.7515		
Treatment 2	<u>25</u>	<u>.2306</u>		
Sum	104	.9822	.0095	
Difference	2	.0011	.0005	F _{2,104} =.0586

The value of 2 for Difference in Table 1 indicates that a quadratic equation best described the overall changes in concentrations of the hormone used for this example; the value for Difference will equal the power of DAY used in the overall model. Results of this example indicate that residual variances were equal, the hypothesis should be accepted, and treatment did not affect hormonal profiles.

EXPERIMENT 1

Mean concentrations of progesterone in jugular blood and progesterone profiles were evaluated as described above.

EXPERIMENT 2

General Linear Models procedures were used to evaluate the effects of treatment on day of luteolysis (DOL) and interestrous interval. The DOL for each ewe was the first day during the experimental period that progesterone was ≤ 1 ng/ml of plasma. The CL were defined as regressed when progesterone concentrations in a ewe were ≤ 1 ng/ml. Interestrous intervals were determined using visual observations for signs of estrus.

Concentrations of progesterone in jugular blood and progesterone profiles were evaluated as described in the General section. For analysis of hormonal profiles, progesterone data for d 7 through d 18 were used. No blood samples were taken after d 18 for the control group. Progesterone concentrations in naturally-cyclic (control) ewes increase after luteolysis (Lewis et al., 1981). Therefore, comparisons between treated and control ewes after d 18 would be biased.

EXPERIMENT 3

Day of luteolysis and interestrous intervals were evaluated as described for Experiment 2. Also, daily

changes in mean hormone concentrations and profiles were evaluated for progesterone, estrogen, PGFM and PGF₂ α in jugular and vena caval blood as described before. To determine the relationship between various independent (estrogen, progesterone and day) and dependent variables (PGFM and PGF₂ α), PROC STEPWISE was used. PROC STEPWISE is a model-building procedure that uses all independent variables specified to determine variables that should be included in a regression model (SAS, 1985). In addition to PROC STEPWISE, another model-building procedure was used in which powers, e.g., EJ (jugular estrogen), EJ², PJ (jugular progesterone), etc., of each independent variable were included. An example model is:

```
PROC SORT; BY TREATMENT;  
PROC GLM; BY TREATMENT; CLASS EWE;  
MODEL PGFMJ = EWE EJ EJ2 EJ3 PJ PJ2;  
/ SOLUTION SS1 SS3;
```

Information from regression procedures were used to develop flow charts to describe the relationships among the compounds evaluated. General linear models procedures were used to determine the effect of sample site (jugular vs. vena cava) on mean hormone concentrations.

RESULTS

EXPERIMENT 1

Pessary-released MPA and oral MGA treatments did not interfere with the progesterone RIA used for the studies described in this thesis. Average progesterone concentrations (ng/ml) in ovariectomized ewes treated with MPA-containing pessaries (.2) and oral MGA (.1) did not differ from progesterone (.1) concentrations in control ewes, all of which were less ($P < .001$) than progesterone concentrations in progesterone-treated (.4) ewes.

Progesterone profiles for MPA-treated, MGA-treated and control ewes did not differ. The RIA detected authentic progesterone delivered from pessaries. Profiles of progesterone concentrations for ewes treated with authentic progesterone differed ($P < .001$) from those in control, MPA-treated and MGA-treated ewes (Tables 2,3, and 4; Figure 3). Figure 3 shows the mean daily concentrations of progesterone for ewes in each treatment group. Progesterone profiles for MPA-treated, MGA-treated and control groups were linear; progesterone concentrations did not exceed about .2 ng/ml for the duration of the experiment. For the authentic progesterone-treated ewes, progesterone was less than .2 ng/ml until d 2. On d 4, 1 d after pessary insertion

progesterone peaked at 1.2 ng/ml, then it declined to .3 ng/ml by d 6 and remained at about .3 ng/ml throughout the remainder of the blood sampling period (Figure 3).

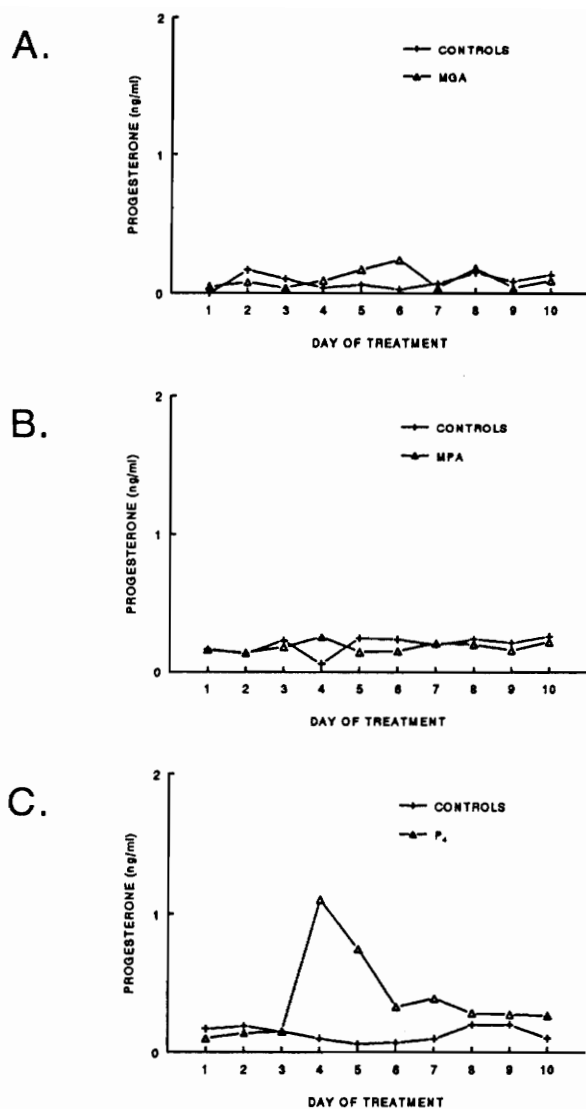


Figure 3. Progesterone concentrations in ovariectomized ewes without or with MGA (A), MPA (B) or authentic progesterone (C).

TABLE 2. EFFECT OF MPA ON PROGESTERONE PROFILES IN
OVARIECTOMIZED EWES

Source	Error df	Error SS	F
Overall	106	.9833	ns
MPA-treated	25	.2306	
Control	79	.7515	

TABLE 3. EFFECT OF MGA ON PROGESTERONE PROFILES OF
OVARIECTOMIZED EWES

Source	Error df	Error SS	F
Overall	106	1.0814	ns
MGA-treated	25	.3009	
Control	79	.7515	

TABLE 4. EFFECT OF ALL TREATMENTS ON PROGESTERONE PROFILES
OF OVARIECTOMIZED EWES

Source	Error df	Error SS	F
Overall	106	5.3393	***
Control	79	3.3436	
Progesterone	25	1.3127	

***P < .0001

EXPERIMENT 2

Pessaries containing MPA did not affect the interval from estrus to the first day of luteolysis, i.e, the first day that progesterone was ≤ 1 ng/ml of plasma (Table 5). Overall, the first day of luteolysis was detected an average of $15.4 \pm .2$ d after the ewes were found in estrus (Table 5). Interestrous intervals differed ($P < .05$) with treatment; interval was $23.9 \pm .1$ d in MPA-treated and $17 \pm .2$ d in control ewes (Table 5).

Treatment with MPA-containing pessaries did not affect the average concentration of progesterone ($1.6 \pm .04$ ng/ml) or the profiles of changes in concentrations of progesterone (Table 6; Figure 4). Progesterone data collected until d 18 of the estrous cycle were used to determine the effects of treatment on means and profiles. Thus, because profiles did not differ with treatment, a single regression equation was derived to best describe changes in progesterone concentrations from d 7 (first day of blood sampling) until d 18 of the cycle. Figure 4 shows mean daily concentrations of progesterone for ewes in each treatment group and progesterone values predicted with the overall regression equation; the equation was $Y = 24.44 - 9.9 X + 1.3 X^2 - .08 X^3 + .002 X^4$. Overall, progesterone concentration (ng/ml) was about 1.8 on d 7, peaked at 2.6 on d 11 to 12, declined to 1 by d 15 and remained less than 1 until d 18 (Figure

4). For MPA-treated ewes, progesterone was less than 1 ng/ml from d 19 through d 25 (Figure 4).

TABLE 5. EFFECT OF MPA-CONTAINING PESSARIES ON LUTEOLYSIS
AND ESTRUS IN INTACT EWES

	n	d 1 of Luteolysis ^d	Interestrous interval (d)
MPA-treated	10	15.3	23.9 ^b
Control	8	15.6	17.0 ^c
SE ^a		.3	.3

^aSE = overall standard error from the SAS model used to analyze the data.

^{bc}Means with different superscripts differed ($p < .05$).

^dd 1 of luteolysis = first d when progesterone concentrations in a ewe were ≤ 1 ng/ml.

TABLE 6. EFFECT OF MPA ON PROGESTERONE PROFILES OF INTACT
EWES

Source	Error df	Error SS	F
Overall	251	94.9027	ns
MPA-treated	106	26.1658	
Control	141	66.3022	

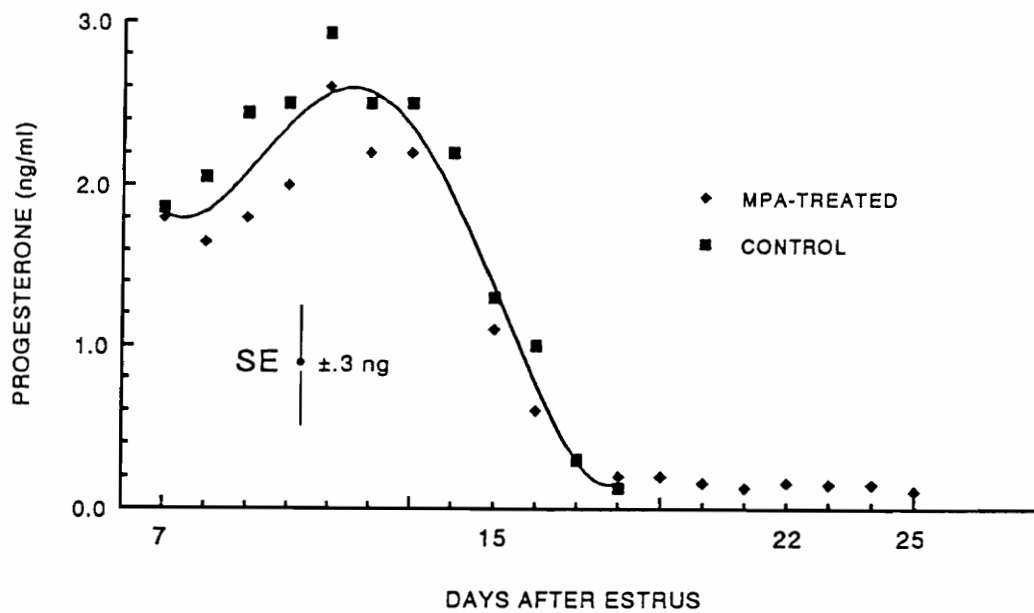


Figure 4. Progesterone concentrations in control ewes and ewes treated with MPA. SE = overall standard error from the SAS model.

EXPERIMENT 3

Pessaries containing MPA and authentic progesterone did not affect the intervals from estrus to, as defined, the first day of luteolysis. Therefore, authentic progesterone-containing pessaries did not maintain high progesterone concentrations, as was expected. Overall, the first day of luteolysis was detected an average of $15.3 \pm .05$ d after ewes were found in estrus (Figure 5).

The effects of treatment on means for each compound and sampling site are shown in Table 7. Treatment with MPA-containing pessaries did not affect the average concentration (pg/ml) of jugular estrogen (EJ) and vena cava estrogen (EVC), but treatment with progesterone-containing pessaries did ($P < .001$; Table 7; Figure 7). Mean concentrations of jugular progesterone (PJ), vena caval progesterone (PVC) and vena caval PGFM (PGFMVC) differed ($P < .001$) between MPA- and progesterone-treated ewes and between MPA-treated and control ewes, but it did not differ for each compound between progesterone-treated and control ewes (Table 7; Figures 6 and 8). Average concentrations (pg/ml) of jugular PGFM (PGFMJ) differed among all treatments ($P < .001$). It is important to note that PGFMJ and PGFMVC concentrations (pg/ml) in MPA-treated ewes (160.4 and 185.2, respectively) were considerably lower

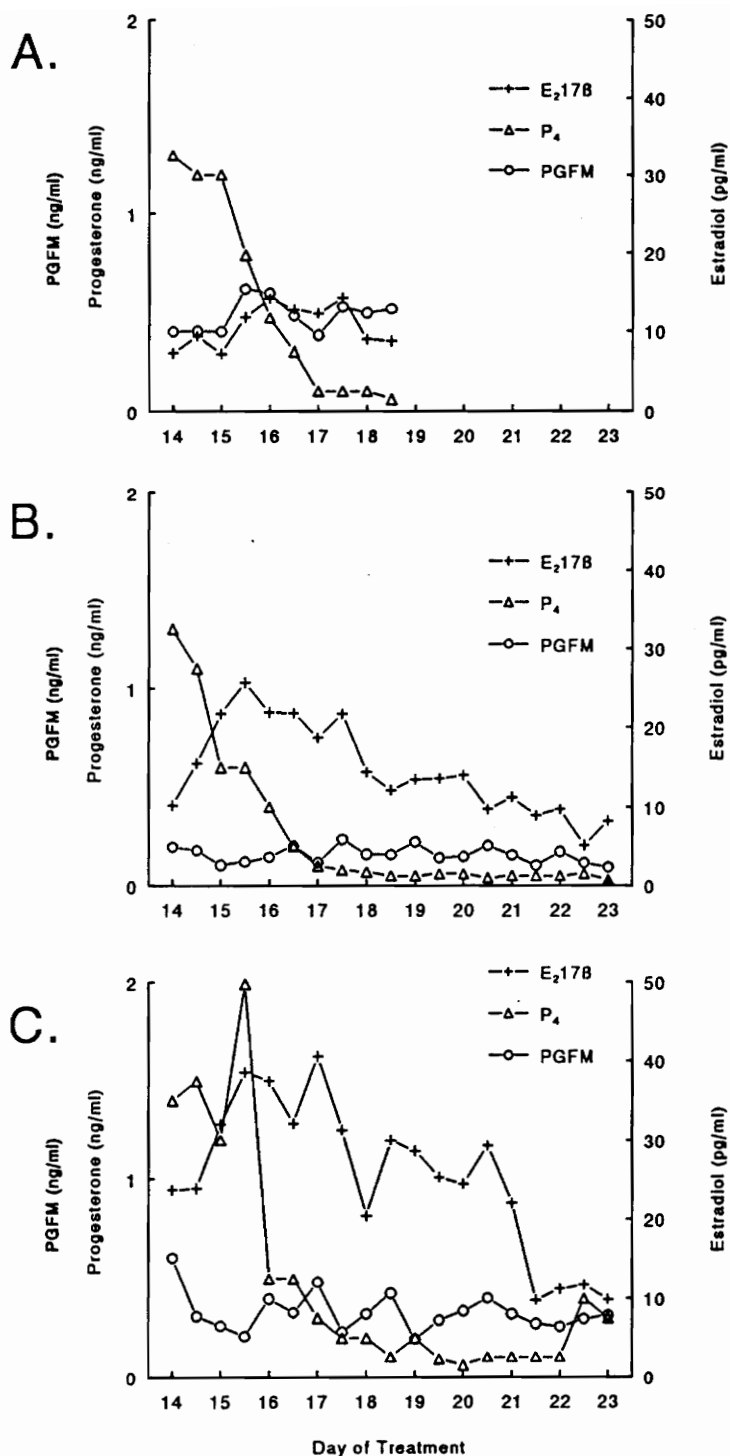


Figure 5. Jugular progesterone, estrogen and PGFM concentrations in control (A), MPA-treated (B) and authentic progesterone-treated ewes (C). Note: jugular PGF₂ α is not shown.

TABLE 7. LEAST-SQUARES MEANS AND OVERALL STANDARD ERRORS
FOR PLASMA CONCENTRATIONS OF COMPOUNDS MEASURED IN

EXPERIMENT 3

Compound measured	Treatment			SE
	Control	MPA	P4	
Progesterone (ng/ml)				
Jugular	.6 ^{ad}	.3 ^{bd}	.5 ^{ad}	.04
Vena Cava	2.3 ^{ae}	.9 ^{be}	2.1 ^{ae}	.15
Estrogen (pg/ml)				
Jugular	10.9 ^{ad}	15.2 ^{ad}	26.5 ^{bd}	1.2
Vena Cava	28.1 ^{ae}	24.1 ^{ae}	35.0 ^{be}	1.6
PGFM (pg/ml)				
Jugular	478.9 ^{ad}	160.4 ^{bd}	330.8 ^{cd}	18.3
Vena Cava	433.3 ^{ad}	185.2 ^{bd}	493.8 ^{ad}	33.9
PGF ₂ α (pg/ml)				
Jugular	21.1 ^{ad}	12.4 ^{bd}	22.6 ^{ad}	.9
Vena Cava	188.1 ^{abe}	19.2 ^{ae}	400.7 ^{be}	36.6

a,b,c Values within a row that do not have any superscript letters in common differed (P < .05).

d,e For each compound measured, values within a column with different superscript letters differed (P < .05).

than in control (478.9 and 433.3, respectively) and progesterone-treated ewes (330.6 and 493.85, respectively). Vena caval $\text{PGF}_2\alpha$ concentrations ($\text{PGF}_2\alpha\text{VC}$; pg/ml) differed between control (188.9) and progesterone-treated ewes and between MPA-treated (19.4) and progesterone-treated (400.7) ewes ($P < .0001$). Jugular $\text{PGF}_2\alpha$ concentrations ($\text{PGF}_2\alpha\text{J}$) were less than 23 pg/ml in ewes in all groups (Table 7).

The effect of treatments on hormonal profiles are shown in Table 8. Hormonal profiles for PJ, PVC, PGFMJ, PGFMVC and $\text{PGF}_2\alpha\text{J}$ did not differ with treatment (Tables 9, 10, 13, 14, 15, and 16; Figures 5,6,8 and 9). Hormonal profiles for EJ and EVC did not differ between MPA-treated and control ewes, but they differed ($P < .05$) between authentic progesterone-treated ewes and ewes in the other two groups (Tables 11 and 12). Vena caval $\text{PGF}_2\alpha$ profiles differed ($P < .0001$) between MPA- and progesterone-treated ewes, between control and progesterone-treated ewes, but not between control and MPA-treated ewes.

For control ewes, jugular progesterone concentrations (ng/ml) were about 1.3 on d 14, declined to 1 by d 15 and remained less than 1 until d 18. Vena caval progesterone concentrations (pg/ml) followed the same pattern starting at about 5 on d 14 and declining to less than 1 on d 15 (Figure 6). For MPA-treated ewes, PJ concentrations (ng/ml) were about 1.3 on d 14, declined to 1 by d 15 and remained less

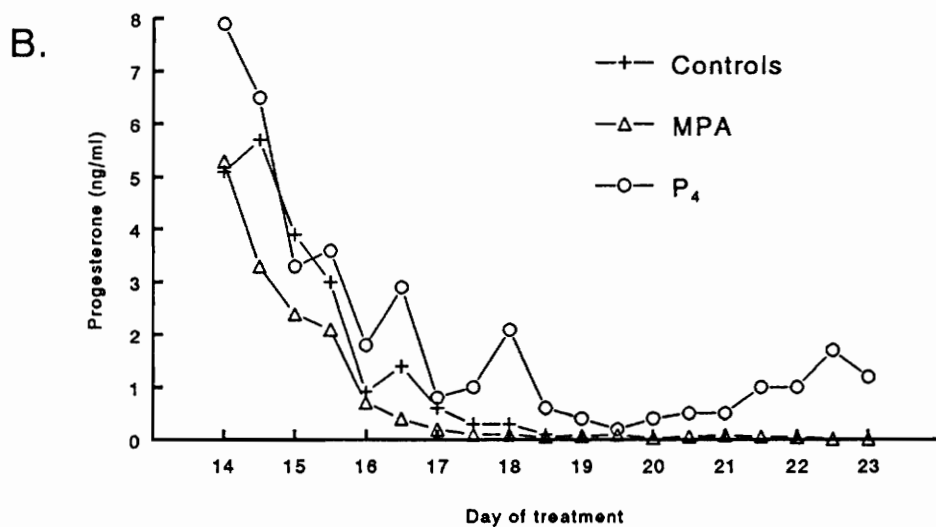
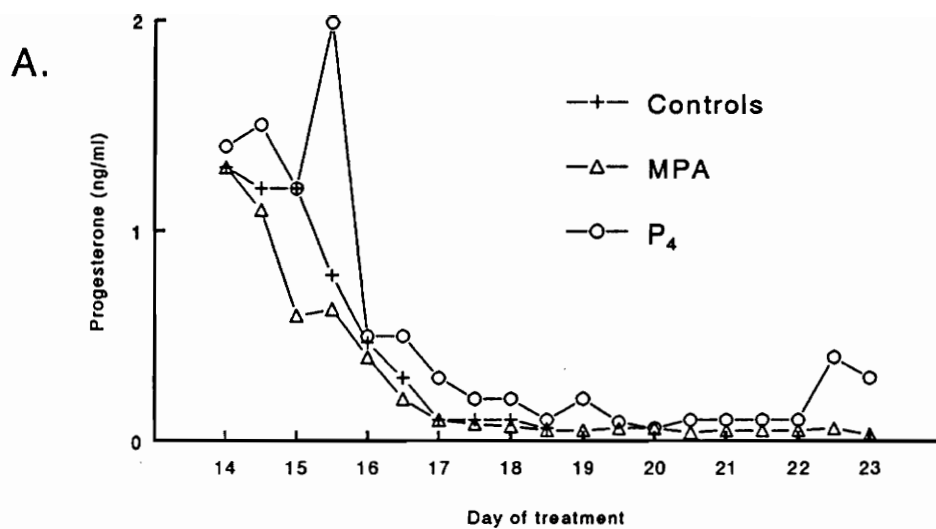


Figure 6. Jugular (A) and vena caval (B) progesterone concentrations in control, MPA-treated and authentic progesterone (P₄)-treated ewes.

TABLE 8. EFFECT OF TREATMENT ON PROFILES OF CONCENTRATIONS
OF COMPOUNDS MEASURED IN EXPERIMENT 3

Compound measured	Treatment		
	Control	MPA	P4
Progesterone (ng/ml)			
Jugular	a	a	a
Vena Cava	a	a	a
Estrogen (pg/ml)			
Jugular	a	a	b
Vena Cava	a	a	b
PGFM (pg/ml)			
Jugular	a	a	a
Vena Cava	a	a	a
PGF ₂ α (pg/ml)			
Jugular	a	a	a
Vena Cava	a	a	b

a,b,c Within a row, compounds that do not have any letters in common differed ($P < .05$).

TABLE 9. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON
JUGULAR PROGESTERONE PROFILES

Source	Error df	Error SS	F
Overall	179	17.25	ns
Control	61	5.51	
MPA	113	11.62	
Overall	198	123.2	ns
Control	64	5.9	
Progesterone	132	116.3	
Overall	250	130.2	ns
MPA	116	13.7	
Progesterone	132	116.3	

TABLE 10. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON VENA
CAVAL PROGESTERONE PROFILES

Source	Error df	Error SS	F
Overall	179	579.48	ns
Control	61	284.76	
MPA	113	273.62	
Overall	198	1072.6	ns
Control	64	295	
Progesterone	132	766.5	
Overall	250	130.2	ns
MPA	116	301.9	
Progesterone	132	766.5	

TABLE 11. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON
JUGULAR ESTROGEN PROFILES

Source	Error df	Error SS	F
Overall	182	15818.2	ns
Control	64	3331.3	
MPA	116	12485.9	
Overall	199	44218.7	*
Control	65	3436.8	
Progesterone	133	39870.9	
Overall	251	54229.3	*
MPA	117	14143.4	
Progesterone	132	766.5	

*P < .05

TABLE 12. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON VENA
CAVAL ESTROGEN PROFILES

Source	Error df	Error SS	F
Overall	182	54111.9	ns
Control	64	9527.8	
MPA	116	44251.6	
Overall	199	95890.8	*
Control	65	10329.9	
Progesterone	133	83549.2	
Overall	251	130805.9	*
MPA	117	46057.4	
Progesterone	133	83549.2	

*P < .05

TABLE 13. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON
JUGULAR PGFM PROFILES

Source	Error df	Error SS	F
Overall	183	8881334	ns
Control	65	6535318	
MPA	117	2333083	
Overall	197	14589660.9	ns
Control	63	5927676	
Progesterone	131	22510223	
Overall	251	10660913	ns
MPA	117	2333083	
Progesterone	133	8313233	

TABLE 14. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON VENA
CAVAL PGFM PROFILES

Source	Error df	Error SS	F
Overall	183	10001183	ns
Control	65	6913735	
MPA	117	2920271	
Overall	199	90512004.4	ns
Control	65	6913735.3	
Progesterone	133	83545486	
Overall	249	86061812.8	ns
MPA	117	2920271.8	
Progesterone	133	83545486	

TABLE 15. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON
JUGULAR PGF₂ α PROFILES

Source	Error df	Error SS	F
Overall	183	32908.6	ns
Control	65	24416	
MPA	117	8086.1	
Overall	199	70287.6	ns
Control	65	24416.1	
Progesterone	133	45182.5	
Overall	251	53402.5	ns
MPA	117	8086.1	
Progesterone	133	45182.5	

TABLE 16. EFFECT OF MPA AND PROGESTERONE TREATMENTS ON VENA
CAVAL PGF₂ α PROFILES

Source	Error df	Error SS	F
Overall	183	626720	ns
Control	65	569152	
MPA	117	57388.3	
Overall	199	128110440	*
Control	65	569152.8	
Progesterone	133	127193405	
Overall	251	53402.5	*
MPA	117	8086.1	
Progesterone	133	45182.5	

*P < .05

than 1 until d 23. Vena caval progesterone started at about 5, declined to ≤ 1 by d 15 and remained at ≤ 1 until d 23 (Figure 6). Jugular progesterone concentrations (ng/ml) for the progesterone-treated ewes were about 1.4 on d 14, peaked at about 2 on d 15.5, declined sharply to .5 by d 16 and remained at ≤ 1 until d 23. Vena caval progesterone concentrations (ng/ml) were about 8 on d 14, declined to ≤ 1 by d 18.5 and gradually increased to about 1.8 by d 23 (Figure 6).

In control ewes, jugular estrogen concentrations (pg/ml) were about 7 on d 14, peaked at about 14 on d 15, remained higher than 12 until d 17 and then fell to about 8 on d 18. Vena caval estrogen concentrations were about 20 on d 14 and 15, peaked at around 45 on d 16, declined to about 25 by d 17.5 and then increased to about 35 on d 18.5 (Figure 7). For the MPA-treated ewes, jugular estrogen concentrations (pg/ml) were around 8 on d 14, peaked at about 26 on d 15.5, remained higher than 15 until d 18 and decreased gradually to about 8 by d 23. Jugular estrogen decreased after d 20.5, even though these ewes came into estrus 36 to 48 h after sponge removal (d 20). Vena caval estrogen concentrations were about 17 on d 14, peaked at about 35 on d 15.5, decreased gradually to about 12 on d 21.5, peaked again at nearly 43 on d 22 (estrus) and then decreased to about 12 on d 23 (Figure 7). In progesterone-treated ewes, jugular estrogen concentrations (pg/ml) were

around 23 on d 14, peaked at about 40 on d 17 and decreased gradually to 10 on d 23. Vena caval estrogen was nearly 34 on d 14, peaked at about 50 by d 18 and declined to 10 by d 23 (Figure 7).

In control ewes, jugular PGFM concentrations (pg/ml) were around 400 on d 14 and 15, peaked at about 600 on d 16, declined to 400 on d 17 and increased again to about 540 on d 18.5. Vena caval PGFM fluctuated between about 220 and 580 pg/ml during the entire sampling period (Figure 8). Jugular PGFM concentrations for MPA-treated ewes fluctuated constantly, ranging from about 100 to 240 pg/ml. Vena caval PGFM concentrations also fluctuated constantly, averaging about 200 pg/ml throughout the sampling period. Jugular and vena caval PGFM and vena caval $\text{PGF}_2\alpha$ concentrations for progesterone-treated ewes fluctuated considerably throughout the entire sampling period; concentrations ranged (pg/ml) from around 200 to 600 for PGFMJ, 200 to 1600 for PGFMVC (Figure 8) and 10 to 1300 for $\text{PGF}_2\alpha$ (Figure 9).

In control ewes, jugular $\text{PGF}_2\alpha$ concentrations did not exceed about 25 pg/ml during the sampling period. Vena caval $\text{PGF}_2\alpha$ concentrations (pg/ml) were about 180 on d 14, peaked at about 230 on d 16.5, returned to near 180 by d 17 and remained at around 180 until d 18.5 (Figure 9). In MPA-treated ewes, jugular $\text{PGF}_2\alpha$ was about 11 pg/ml and $\text{PGF}_2\alpha\text{VC}$

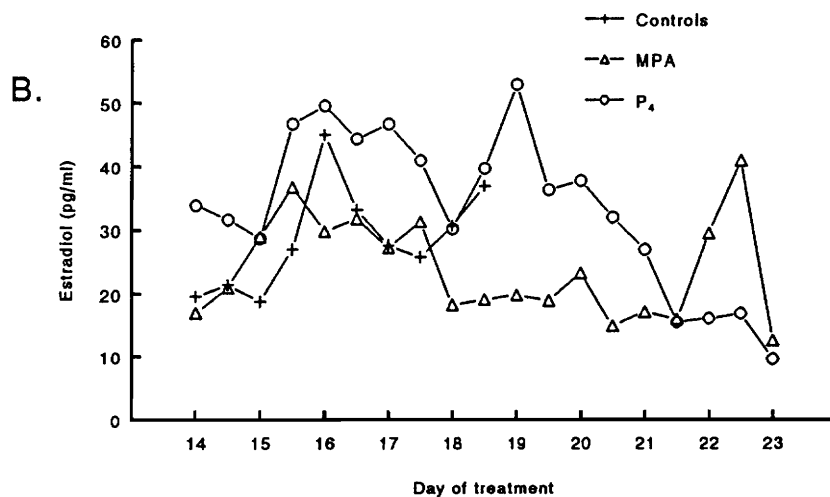
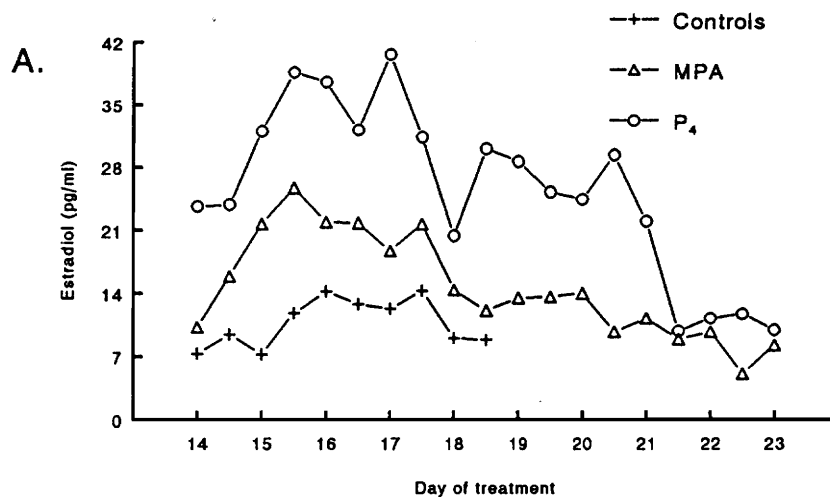


Figure 7. Jugular (A) and vena caval (B) estrogen concentrations in control, MPA-treated and authentic progesterone-treated ewes. Progesterone-containing pessaries affected ($P < .0001$) estrogen profiles.

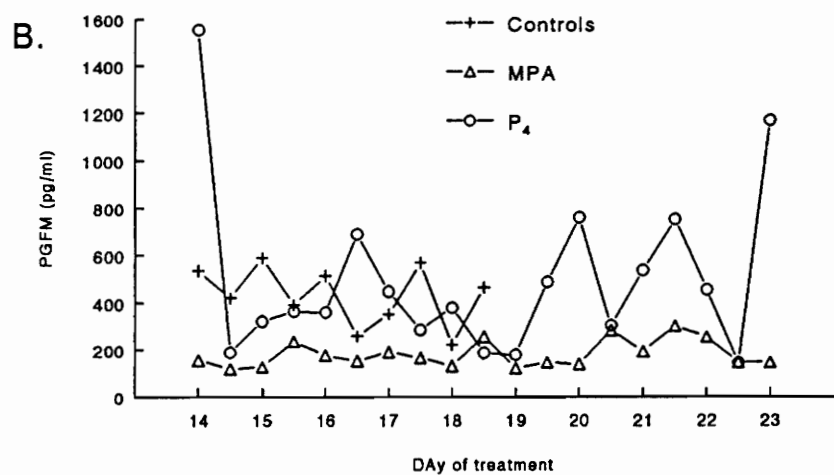
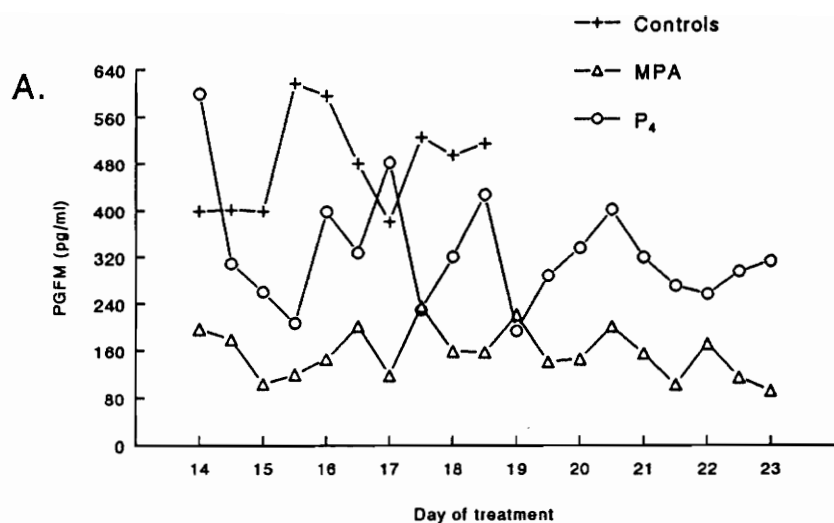


Figure 8. Jugular (A) and vena caval (B) PGFM concentrations in control, MPA-treated and authentic progesterone-treated ewes.

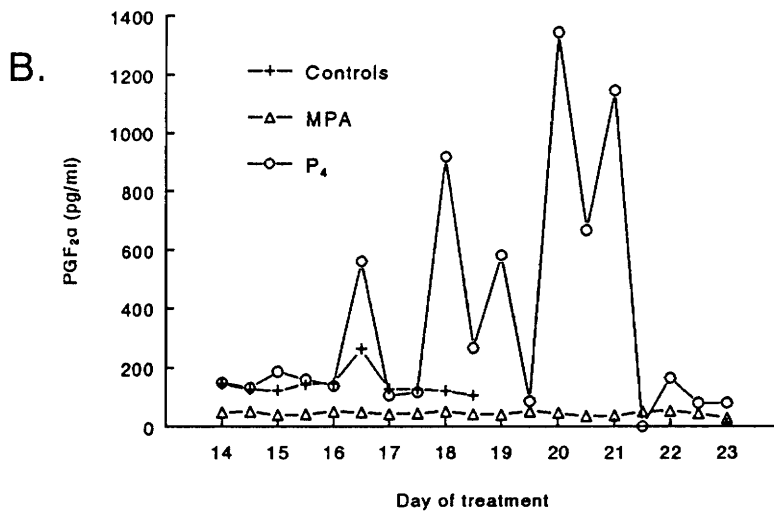
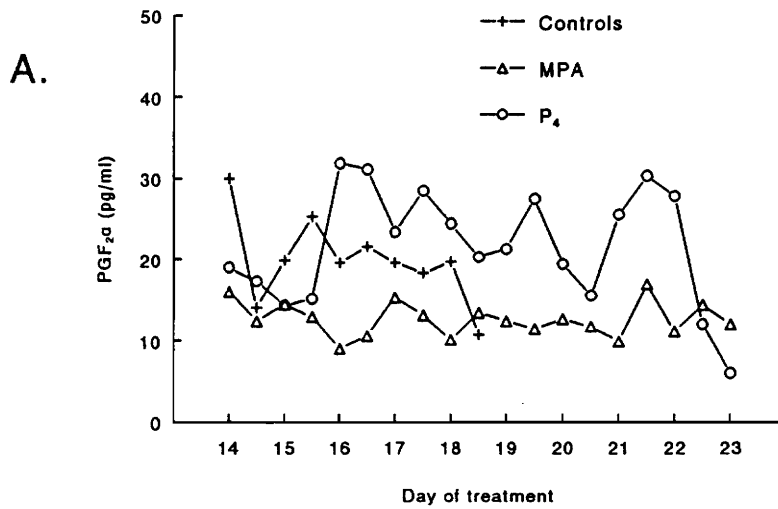


Figure 9. Jugular (A) and vena caval (B) PGF_{2α} concentrations in control, MPA-treated and authentic progesterone-treated ewes.

was about 35 pg/ml throughout the sampling period (Figure 9). Jugular $\text{PGF}_2\alpha$ concentrations for the progesterone treated ewes remained at about 22 pg/ml during the entire sampling period.

Site of blood sampling affected the mean concentration of some compounds (Table 7). Overall, concentrations of estrogen, progesterone and $\text{PGF}_2\alpha$ for all treatment groups were higher ($P < .001$) in vena caval than in jugular blood plasma. Average vena caval and jugular concentrations of PGFM did not differ.

Flow charts were developed to illustrate compound relationships during the experiment. Figures 10, 11 and 12 illustrate these relationships. For the three treatment groups, day did not account for a significant amount of the variation. For control ewes, jugular and vena caval estrogen and progesterone accounted for significant amounts of the variation in concentrations of each other. Vena caval PGFM accounted for a significant amount of the variation in PGFMJ. Next, progesterone jugular and PVC accounted for significant amounts of the variation in EVC, and vice versa. Progesterone vena cava accounted for part of the variation in EJ. Then, both EJ and EVC explained a significant amount of the variation in PGFMVC. Jugular estrogen accounted for some of the variation in $\text{PGF}_2\alpha\text{VC}$. And, $\text{PGF}_2\alpha\text{VC}$ accounted for some of the variation in PGFMVC. Finally, PGFMVC accounted for a significant amount of the

variation in PGFMJ. These models indicate that estrogen and PGF₂ α VC accounted for a significant amount of the variation in PGFMVC, progesterone affected PGFM through estrogen, and PGFM in the vena cava accounted for much of the variation in PGFM in jugular blood (Figure 10). The model-building procedure that used powers of the independent variables indicated that the quadratic effect of estrogen and the cubic effect of progesterone accounted for a significant amount of the variation in PGFMJ. In other words, an increase in estrogen and an increase and then a decrease in progesterone were associated with a release of PGFM.

For the MPA-treated ewes, a flow chart was also developed (Figure 11). Again, jugular and vena caval estrogen, progesterone and PGFM accounted for a significant amount of the variation in changes in concentration of each other. Jugular estrogen accounted for a significant amount of the variation in PGF₂ α VC. Estrogen jugular, EVC and PGF₂ α VC accounted for a significant amount of the variation in PGFMVC. In turn, PGFMVC explained a significant amount of the variation in PGFMJ. Jugular and vena caval progesterone tended to account for some of the variation in PGFMJ. Again, estrogen accounted for a significant amount of the variation in PGFMVC, which in turn accounted for a significant amount of the variation in PGFMJ. The relationship between PJ, PVC and PGFMJ is not easy to understand physiologically. Progesterone may be having a

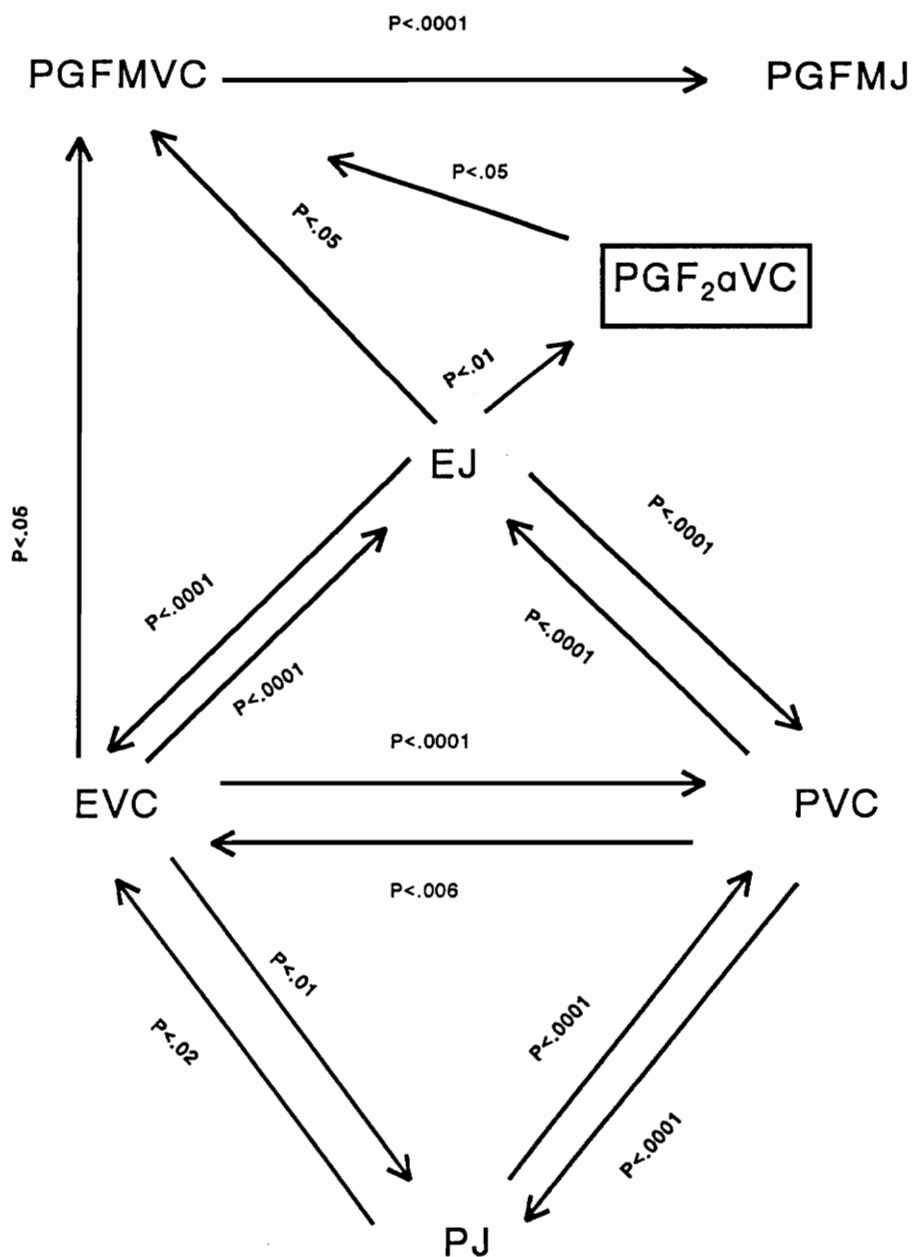


Figure 10. Relationships among progesterone, estrogen, PGF₂α and PGFM in control ewes. P-values were derived from the PROC STEPWISE analysis.

direct negative effect on PGFM (Figure 11). The second model-building procedure indicated that the quartic effect of estrogen accounted for a significant amount of the variation in PGFMVC.

Progesterone pessaries did not maintain high progesterone concentrations for the entire sampling period, but they did have some effect on jugular and vena caval estrogen and PGFM concentrations in the ewes. Vena caval estrogen accounted for a significant amount of the variation in PGFMJ and $\text{PGF}_2\alpha\text{VC}$. Vena caval progesterone accounted for some of the variation in PGFMJ and PGFMVC. The second model-building procedure indicated that a significant amount of the variation in PGFMJ and PGFMVC was explained by the cubic and linear effects of progesterone, respectively (Figure 12). Since the effects of progesterone pessaries on the ewes was different than expected, it is difficult to explain physiologically some of these relationships.

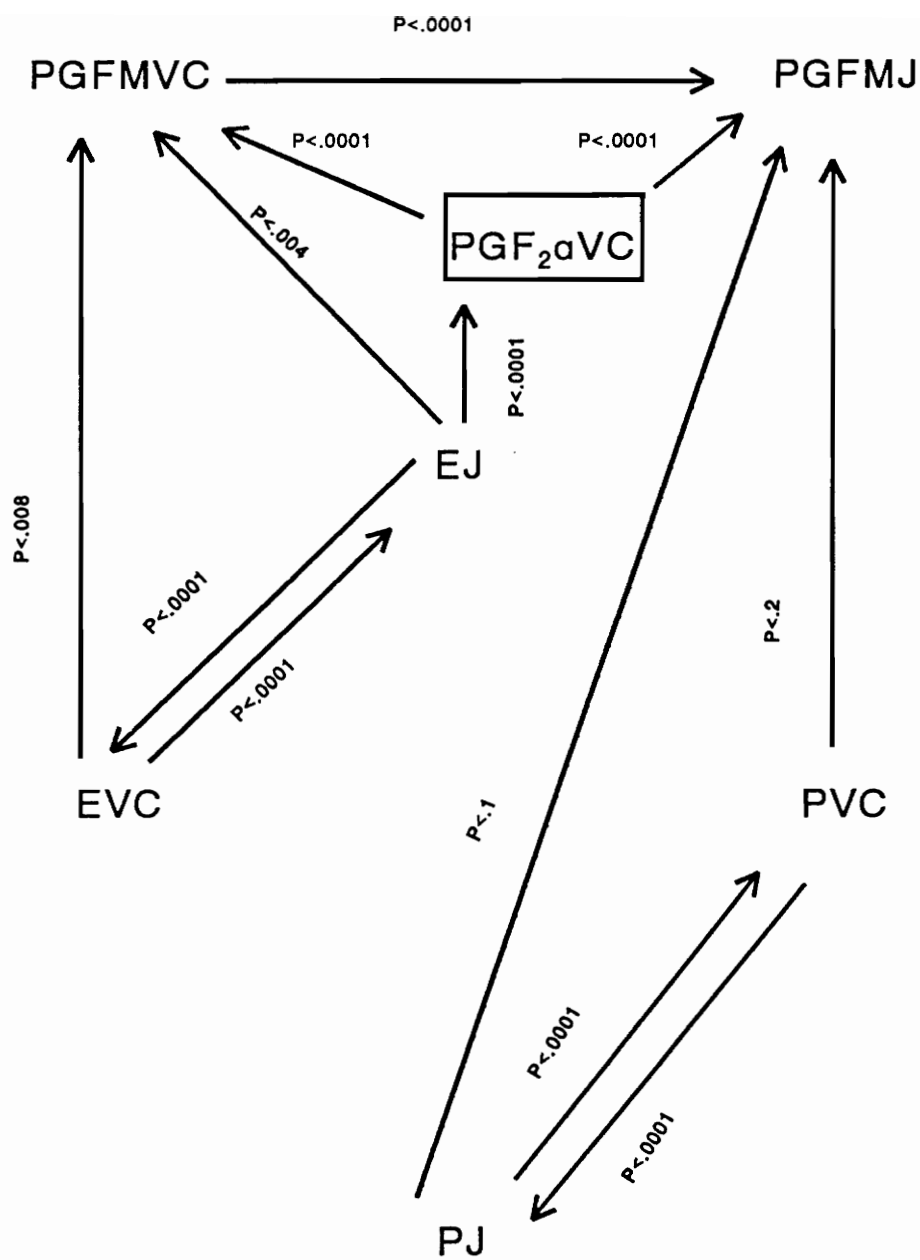


Figure 11. Relationships among progesterone, estrogen, PGF₂α and PGFM in intact MPA-treated ewes. P-values were derived from the PROC STEPWISE analysis.

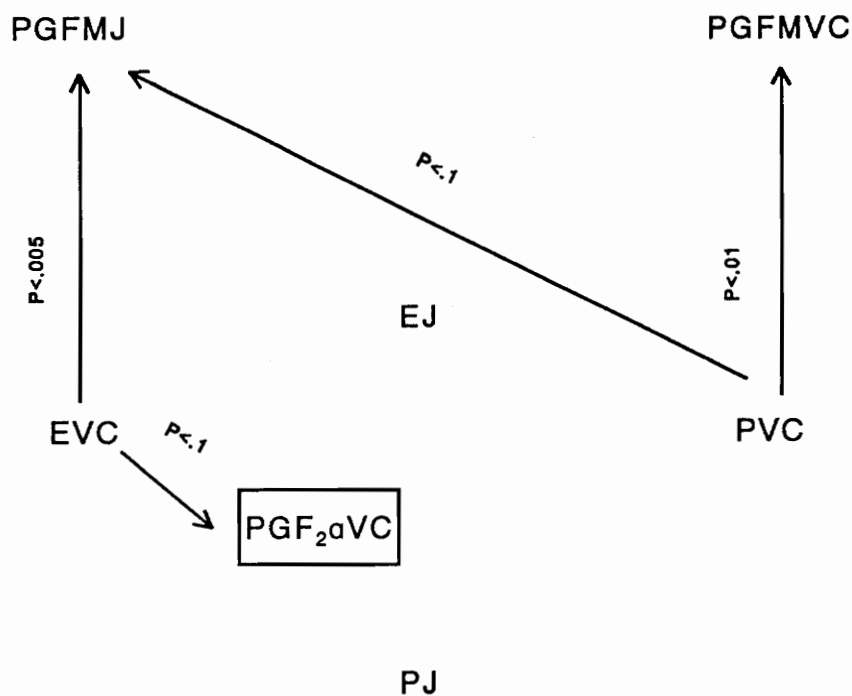


Figure 12. Relationships among progesterone, estrogen, PGF₂α and PGFM in intact authentic progesterone-treated ewes. P-values were derived from the PROC STEPWISE analysis.

DISCUSSION

Progestogen treatments did not delay luteolysis in ewes. This agrees with a study (Schoombe et al., 1988) that was published shortly after the experiments for this thesis were initiated. Even though progestogen treatments did not delay luteolysis, they increased the interestrus intervals, which agrees with previous studies on the use of progestogens to synchronize estrus in ewes (Dutt and Casida, 1948; O'Mary et al., 1950; Echternkamp et al., 1976; Schoombe et al., 1988). Treatments with MPA did not affect progesterone concentrations or profiles. Because in Experiment 1 only basal amounts of progesterone could be detected in jugular blood of ovariectomized ewes treated with MPA, which indicated that MPA did not interfere with the progesterone RIA, progesterone concentrations and profiles for MPA-treated ewes in Experiments 2 and 3 were considered "normal" (Lewis et al., 1981).

As shown in Figure 7, estrogen concentrations in MPA-treated ewes were maintained high and did not decrease until after pessary removal. These data may indicate that large ovarian follicles were maintained in the MPA-treated ewes. Pessaries were inserted on d 7; progestogen treatments started on other days may not have similar effects. Similar studies on follicular development in cattle indicated that large follicles were maintained in cows treated with

progestogens late in the estrous cycle (Driancourt, 1991; Rajamahendran and Talor, 1991).

Overall, in the control ewes, progesterone concentrations decreased as estrogen concentrations increased. An increase in PGFM occurred at about the same time as the increase in estrogen. Also, an increase in vena caval $\text{PGF}_2\alpha$ was observed during the same time period. Previous studies also showed an increase in PGFM and $\text{PGF}_2\alpha$ concentrations as progesterone decreased and estrogen increased (Caldwell et al., 1972; Baird et al., 1976; Louis et al., 1977; McCracken et al., 1984). In MPA-treated ewes, PGFM and $\text{PGF}_2\alpha$ concentrations remained basal; in spite of the evidence that luteal progesterone decreased, estrogen increased, and the assumption that progestogen (MPA) levels were probably maintained high.

Relationships among compounds measured in authentic progesterone-treated ewes were not clear. The pessaries did not maintain high concentrations of progesterone, as was expected. Pessaries should have been changed more often or they should have contained greater amounts of progesterone. Also, the CL regressed on time in all of these ewes. Estrogen, PGFM and $\text{PGF}_2\alpha$ concentrations were generally higher and more variable than in the other treatment groups. Even though progesterone levels were not maintained high, the pessaries had some effect on the hormonal environment of the ewes. Therefore, it is difficult to produce a valid

physiological explanation for the relationships affecting uterine secretion of $\text{PGF}_2\alpha$ in progesterone-treated ewes in this study.

Flow charts were developed to depict relationships among compounds measured, but relationships among compounds does not imply cause and effect. Also, one compound may account for a significant amount of the variation in another compound but, this represents only a portion of the variation accounted for in the overall model.

Flowcharts indicated a significant effect of estrogen on changes in PGFM and $\text{PGF}_2\alpha$ concentrations. In contrast to some reports (Baird et al., 1976; Louis et al., 1977; Scaramuzzi et al., 1977; Ottobre et al., 1984), data from this and two other studies indicate that estrogen, and not progesterone, is the prevailing hormone in the control of uterine secretion of $\text{PGF}_2\alpha$ in ewes (Hixon et al., 1975; Zhang et al., 1991). However, data in this thesis seem to support the idea that a period of progesterone priming of the uterus is needed before estrogen can increase uterine secretion of $\text{PGF}_2\alpha$ (Ford et al., 1973; Warren et al., 1973; Roberts et al., 1975).

In control and MPA-treated ewes vena caval estrogen accounted for a significant amount of the variation in jugular estrogen, and jugular estrogen accounted for a significant amount of the variation in vena caval estrogen. The same was true for jugular and vena caval progesterone.

These results agree with Benoit and Daily (1991). Similar results were found for jugular and vena caval PGFM, and vena caval $\text{PGF}_2\alpha$ and PGFM. Neither jugular and vena caval $\text{PGF}_2\alpha$ nor jugular PGFM and vena caval $\text{PGF}_2\alpha$ accounted for a significant amount of the variation in each other; probably because $\text{PGF}_2\alpha$ is rapidly metabolized in the lungs (Davis et al., 1980). Therefore, one can conclude that jugular estrogen, progesterone and PGFM may be good indicators of their vena caval counterparts. Also, changes in PGFM in the vena cava may fairly represent overall concentration, but not minute-to-minute, changes in vena caval $\text{PGF}_2\alpha$. Jugular and vena caval PGFM do not appear to be good representations of vena caval $\text{PGF}_2\alpha$. Vena caval $\text{PGF}_2\alpha$ may prove to be a good representation of uterine $\text{PGF}_2\alpha$, but the possibility has not yet been tested. So, is jugular PGFM a good representation of uterine $\text{PGF}_2\alpha$? The concentration changes in jugular PGFM have often been used as estimators of changes in uterine $\text{PGF}_2\alpha$, but the validity of this practice must be questioned.

For control ewes, and as indicated in Figure 10, EVC, EJ and $\text{PGF}_2\alpha\text{VC}$ accounted for a significant amount of the variation in PGFM in the vena cava. Estrogen also accounted for a significant amount of the variation in $\text{PGF}_2\alpha$. Since $\text{PGF}_2\alpha$ is metabolized into PGFM, and vena caval blood is transported throughout the body, it is only logical that $\text{PGF}_2\alpha$ accounted for some of the variation in PGFM (Pace-

Asciak and Granstöm, 1983). Estrogen may be controlling PGF₂ α production and secretion through increasing phospholipase A₂ and cyclooxygenase activity and increasing the number of oxytocin receptors (Brinsfield and Hawk, 1973; Hixon and Flint, 1987; Raw et al., 1988).

Progesterone seemed to exert its control on uterine secretion of PGF₂ α through estrogen, since both are closely associated. In this case, progesterone may be acting as a "time-controlling hormone" for the initiation of luteolysis and secretion of uterine PGF₂ α by keeping levels of estrogen and oxytocin receptors low and inhibiting enzymatic activity necessary for PGF₂ α synthesis (Toth and Hertelendy, 1986; Hixon and Flint, 1987; Raw et al., 1988).

It is important to note that most of the literature indicates that progesterone has to decrease and estrogen increase for there to be increased uterine secretion of PGF₂ α around the time of luteolysis (McCracken et al., 1984). And, most of the progesterone is produced by the CL. If PGF₂ α causes luteolysis, and it will only be secreted in sufficient amounts to cause luteolysis when progesterone is already decreasing, i. e., the CL is already regressing, then what is initiating the regression of the CL?

The control of uterine secretion of PGF₂ α in the MPA-treated ewes seems somewhat different than that in the controls. Again, jugular and vena caval estrogen seem to account for a significant amount of the variation in PGFMVC;

estrogen also affects $\text{PGF}_2\alpha$ production. Prostaglandin $\text{F}_2\alpha$ affects PGFM, since $\text{PGF}_2\alpha$ is metabolized into PGFM. In this case, progesterone tends to directly account for some of the variation in PGFM. This may be because these ewes received progestogen treatments throughout most of the sampling period. The progestogens may have affected uterine estrogen receptors, which may affect the ability of the uterus to secrete $\text{PGF}_2\alpha$.

The control of uterine secretion of $\text{PGF}_2\alpha$ in progesterone-treated ewes is not clear due to the problems stated before. Although definite conclusions cannot be made, estrogen seems to be the most important hormone affecting $\text{PGF}_2\alpha$ release.

In summary, one can conclude that progestogen treatments do not delay luteolysis or affect progesterone concentrations and profiles, but they increase the interestrus intervals. Estrogen seems to be the most important hormone affecting uterine secretion of $\text{PGF}_2\alpha$. Progesterone may be affecting $\text{PGF}_2\alpha$ through an indirect mechanism. Jugular estrogen, progesterone and PGFM concentrations seem to be a reasonable indicator of changes in vena caval concentrations of each compound. Also, vena caval PGFM may be related to concentrations of vena caval $\text{PGF}_2\alpha$.

IMPLICATIONS

Even though there is an extensive amount of literature, there are no concrete answers regarding the control of uterine secretion of $\text{PGF}_2\alpha$ around the time of luteolysis in livestock species. The need to develop a model system to answer this and many other implied questions regarding $\text{PGF}_2\alpha$ secretion is evident. From this thesis, estrogen seems to be the key hormone controlling the release of $\text{PGF}_2\alpha$ from the ovine uterus. The precise control of uterine secretion of $\text{PGF}_2\alpha$ and luteolysis in livestock species still largely remains a mystery.

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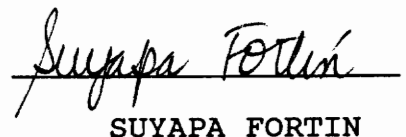
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SUYAPA FORTIN

APPENDIX I

The following abbreviations were used throughout this thesis:

AA	Arachidonic acid
CL	Corpus luteum or corpora lutea
CV	Coefficient of variation
E ₂ -17 β	Estradiol 17 β
EIA	Enzymeimmunoassay
EJ	Jugular estrogen
EVC	Vena caval estrogen
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
IUD	Intrauterine device or devices
LH	Luteinizing hormone
MGA	17 α -acetoxy-6-methyl-16-methylene-4, 6-pregnadiene-3, 20 dione
MPA	6 α -methyl-17 α -hydroxy-progesterone acetate
ns	not significant (P > .1)
PGF ₂ α	Prostaglandin F ₂ α
PGF ₂ α J	Jugular PGF ₂ α
PGF ₂ α VC	Vena caval PGF ₂ α
PGFM	13,14-dihydro-15-keto-prostaglandin F ₂ α
PGFMJ	Jugular PGFM
PGFMVC	Vena caval PGFM
PJ	Jugular progesterone
PVC	Vena caval progesterone
RIA	Radioimmunoassay