

THESIS





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thesis entitled

Involvement of Ovarian Follicles in Prostaglandin F2a Induced Luteal Regression in Cattle

presented by

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## INVOLVEMENT OF OVARIAN FOLLICLES IN

## PROSTAGLANDIN $F_2^{\alpha}$ INDUCED LUTEAL

## **REGRESSION IN CATTLE**

Ву

Trudy Lynn Hughes

## A THESIS

## Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

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## ABBREVIATIONS

CAMP	adenosine 3',5'-cyclic monophosphate
g	gram(s)
h	hour(s)
hCG	human chorionic gonadotrophin
ng	nanogram(s)
pg	picogram(s)
$PGF_2^{\alpha}$	prostaglandin $F_2^{\alpha}$
PGFM	13,14-dihydro-15 keto PGF, a PGF $_2^{\alpha}$ metabolite
SEM	standard error of the mean

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#### ABSTRACT

# INVOLVEMENT OF OVARIAN FOLLICLES IN PROSTAGLANDIN $F_2^{\alpha}$ INDUCED LUTEAL REGRESSION IN CATTLE

By

Trudy Lynn Hughes

Factors potentially involved in initiating luteolysis in cattle were inspected. To investigate changes in luteotropic support, frequency and amplitude of pulses of luteinizing hormone were characterized on days 8, 13 and 15 postestrus. Interactions between products from ovarian follicles and prostaglandin  $F_2 \alpha (PGF_2 \alpha)$  on luteal regression were also tested. Changes in serum concentrations of progesterone were monitored after an injection of saline or  $PGF_2^{\alpha}$  on day 14 to heifers whose ovarian follicles had been destroyed or had undergone sham destruction on day 9 postestrus.

Amplitude of pulses of luteinizing hormone was greater on day 15, whereas neither basal concentrations nor frequency of pulses changed with time. Thus, there was no reduction in availability of luteotropic support by day 15.  $PGF_2^{\alpha}$  was significantly less efficacious in causing luteolysis in animals whose follicles had been destroyed than in control animals. Products of ovarian follicles interact with  $PGF_2^{\alpha}$  to heighten responsiveness of corpora lutea to luteolytic factors.



### INTRODUCTION

Luteinizing hormone (LH) is the primary luteotropin in cows as presence of LH is required for development and maintenance of bovine corpora lutea (Hansel et al., 1973; Hoffman et al., 1974). Reduced availability of circulating LH achieved by immuninactivation results in early luteal regression in cattle (Snook et al., 1969; Hoffman et al., Mean concentrations of LH do not decrease near the 1974). time functional luteal regression begins as demonstrated by decreasing concentrations of progesterone in peripheral plasma (Spicer et al., 1981; Villa-Godoy et al., 1985). However, it is not known whether the pulsatile pattern of release of LH is altered during this period (Rahe et al., 1980; Walters et al., 1984). Though maintenance of developed corpora lutea was not investigated, McNeilly et al. (1984) demonstrated pulsatile secretion of LH is required for the formation of functional corpora lutea in ewes. In addition, in cows and primates there is a synchronous relationship between the pulsatile release of gonadotropins and the pulsatile release of progesterone from mature corpora lutea which suggests a functional relationship might exist between the two during diestrus (Walters et al., 1984; Healy et al., 1983). Thus a reduction in the frequency or



amplitude of pulses of LH during late diestrus could be responsible for the decline in luteal function.

Exogenous administration of estradiol-17  $\beta$  or prostaglandin  $F_2 \alpha$  (PGF<sub>2</sub>  $\alpha$ ) causes functional luteolysis in cows and ewes. Uterine synthesis and release of  $PGF_2 \alpha$  is stimulated by increasing concentrations of estradiol-17 $\beta$  in the uterine blood supply (Barcikowski et al., 1974). Concentrations of estradiol-17<sup> $\beta$ </sup> in serum increase during late diestrus before luteal function begins to decline in sheep and cows (Barcikowski et al., 1974; Fogwell et al., 1985). Thus, estradiol-17 $\beta$  may trigger an increase in uterine synthesis and release of  $PGF_2^{\alpha}$  which then in turn results in luteolysis. Indeed, luteal lifespan in heifers is extended following destruction of ovarian follicles (Fogwell et al., 1985; Villa-Godoy et al., 1985) which are the primary sources of estradiol-17 $\beta$  in cattle (Ireland and Roche, 1983a; Ireland et al., 1984). However, in addition to the positive effect of estradiol-17  $^\beta$  on uterine secretion of  $PGF_2 \alpha_r$  results from experiments with ewes (Gengenbach et al., 1977) and cows (Hixon et al., 1983) indicate that an additional role for estradiol-17  $\beta$  in luteal regression exists. There is evidence that estradiol-17  $^\beta$  and PGF  $_2^{\,\alpha}$ synergize during luteal regression since a combination of exogenous estradiol and  $PGF_2\alpha$  is a more efficacious luteolytic treatment than either hormone administered alone (Gengenbach et al., 1977; Hixon et al., 1983). However, it is not known if ovarian follicles, or their products



influence the ability of  ${\rm PGF}_2{}^\alpha$  to cause luteal regression in cows.

This experiment was designed to address the following two questions. Firstly, does the pattern of pulsatile secretion of LH change before the spontaneous decline of luteal function occurs? Secondly, does destruction of ovarian follicles alter the efficacy of exogenous  $PGF_2^{\alpha}$  to cause luteal regression in heifers? the second s



#### **REVIEW OF LITERATURE**

#### Development and Maintenance of Corpora Lutea

Two or three days before estrus, the ovulatory follicle of a cow is the largest follicle on either ovary (Dufour et al., 1972). As luteinization of the ovulatory follicle occurs during the periovulatory period of several species, the major end product of steroidogenesis within this follicle shifts from estradiol-17<sup> $\beta$ </sup> to progesterone (Channing, 1980; Murdoch and Dunn, 1982; Ireland and Roche, 1983b). In cows, hyperplasia of the corpus luteum continues until day 9 (day 0 = estrus; Donaldson and Hansel, 1965). Furthermore, changes in the type of cells comprising the corpus luteum also occur. Luteal cells may be divided into two steroidogenic types based on cell size and origin. Granulosa cells give rise to large luteal cells while thecal cells develop into small luteal cells, which in turn differentiate into large luteal cells as the corpus luteum ages (Donaldson and Hansel, 1965; Fritz et al., 1981; Alila et al., 1983). The importance of this differentiation to luteal function will be discussed in following sections.

Presence of LH is required for the functional and morphological differentiation of follicular cells to luteal cells as well as being required for the maintenance of



bovine luteal cells <u>in vitro</u> (Gospodarowicz and Gospodarowicz, 1972, 1975). Similarly, primate corpora lutea <u>in vivo</u> require LH (Hutchinson et al., 1984). Reduced availability of circulating LH causes decreased weights of corpora lutea and early luteal regression in cows (Snook et al., 1969; Hoffman et al., 1974). LH is the primary luteotropin in the cow (Hansel et al., 1973; Hoffman et al., 1974) though other factors such as PGI<sub>2</sub> (Milvae and Hansel, 1980), PGE<sub>2</sub> (Godkin et al., 1977), insulin (Veldhuis et al., 1984), and increased blood flow to the corpus luteum (Niswender et al., 1976; Wise et al., 1976) have luteotropic capabilities.

## Mechanism of Action of LH on Production of Progesterone

LH exerts luteotropic effects by a cascade of events: 1) LH binds to receptors for LH which are present in the luteal cell membrane (Rao et al., 1979; Hwang et al., 1983), 2) membrane bound LH activates the adenylate cyclase system, 3) adenylate cyclase catalyzes intracellular production of cAMP and, 4) cAMP acts at several control points in steroidogenesis to stimulate production of progesterone (Marsh, 1976; Williams et al., 1978; Darbon et al., 1980). Oxytocin is also present in high concentrations within the bovine corpus luteum (Wathes and Swann, 1982; Wathes et al., 1984) and though it is secreted concomitantly with progesterone from the ovary during late diestrus (Flint and Sheldrick, 1983; Walters et al., 1984) no role for LH in control of



ovarian production or release of oxytocin has been demonstrated.

## <u>Causes of Luteolysis in Non-</u> <u>Pregnant Animals</u>

Since LH is necessary for the development and maintenance of corpora lutea in cows, it may be hypothesized that control of luteal regression may depend upon several variables which influence luteotropic support. These variables include: 1) amount of LH which is available to the corpus luteum, defined hereafter as concentration of LH in serum, 2) number and affinity of receptors for LH in luteal tissue, 3) ability of the LH receptor complex to stimulate activity of the adenylate cyclase system, 4) ability of adenylate cyclase to increase production of cAMP, 5) effectiveness of cAMP to stimulate various control points in steroidogenesis, 6) interference by luteolytic factors at any of the previously mentioned control points. The objective of this review is to discuss the importance of the aforementioned variables to spontaneous luteolysis. A general model which summarizes and integrates this information will be presented in conclusion of this section and will introduce the objectives of my thesis research.

### Luteotropic Factors

Availability of LH. Low basal concentrations of LH are maintained throughout diestrus in the cow (Spicer et al., 1981; Villa-Godoy et al., 1985). However, Rahe et al. (1981), and Walters et al. (1984) found changes in the

pattern of pulsatile secretion of LH occurs between early and mid-diestrus. From day 3 to day 11 postestrus, frequency of pulses of LH decreases while amplitude of these pulses increases. Changes in secretory pattern of LH during diestrus are largely due to alterations in the concentrations of progesterone and estradiol-17 $\beta$  found in serum (Goodman and Karsch, 1980; Goodman et al., 1981), but other non-steroidal and uncharacterized follicular products may also have effects (Barraclough et al., 1979; Cummins et al., 1983). Matton et al. (1981) and Ireland et al. (1979) found both follicular inventories and concentrations of steroids within follicles are altered between days 11 to 17 of the estrous cycle, and it is probable these changes are reflected in serum concentrations of ovarian products to which the hypothalamus and pituitary are exposed. Thus, there is reason to believe that a change in the pulsatile pattern of release of LH occurs during late diestrus before a decline in secretion of progesterone from the bovine corpus luteum occurs. While mean daily concentration of LH does not change near the time luteal function declines, the pulsatile pattern of secretion may be altered and contribute to factors causing luteal regression in non-pregnant cows and heifers.

Dynamics of Luteal Receptors for LH During the Estrous Cycle. Affinity of luteal receptors for LH remains constant throughout diestrus and initiation of luteal regression (Diekman et al., 1978; Rao et al., 1979). However,

concentration of receptors for LH in corpora lutea decreases concomitantly with luteal functions (Diekman et al., 1978; Spicer et al., 1981). In addition, there is a decrease in binding capacity of corpora lutea for LH during mid-diestrus which precedes any significant decline in concentrations of progesterone in serum (Spicer et al., 1981). One factor which reduces the concentration of receptors for LH during diestrus is the differentiation of small luteal cells into large cells as the corpus luteum ages (Fitz et al., 1981; Fitz and Sawyer, 1982; Alila, 1983). Fitz et al. (1982) found the number of binding sites for LH are 10 fold greater in small luteal cells than in large cells. Thus, as the proportion of large luteal cells increases, the capacity of corpora lutea to bind LH declines and corpora lutea could become less responsive to stimulation by LH (Ursely and Leymarie, 1979; Koos and Hansel, 1980; Fitz et al., 1982; Hoyer et al., 1984). Basal secretion of progesterone from bovine luteal cells in vitro does decline between corpora lutea collected on day 10 postestrus and those collected on day 15 postestrus even though plasma progesterone levels were maintained during this interval. However, it is not clear if the ability to respond to LH is involved in this diminished ability to secrete progesterone. LH stimulated production of progesterone from corpora lutea also declined between day 10 and 15 postestrus through the percent of progesterone produced following LH treatment in comparison



to basal production of progesterone was maintained (Milvae and Hansel, 1983).

If reduced availability of LH to the luteal cell, which is caused either by depressed concentrations of LH in serum or by a decline in binding capacity of luteal tissue for LH, is involved in luteal regression an increase in concentrations of LH in serum would extend luteal function. In fact, injections of human chorionic gonadotrophin in cows (hCG; Wiltbank et al., 1961) and infusions of LH in ewes (Karsch et al., 1970) during diestrus prolong the lifespan of corpora lutea. However, these treatments do not block luteolysis from eventually taking place indicating that variables in addition to availability of LH are involved in luteal regression.

Steroidogenesis and Accumulation of CAMP. Increased proportion of large luteal cells during late diestrus (Fitz et al., 1981; Fitz and Sawyer, 1982) may cause a decrease in responsiveness to luteotropic support for reasons in addition to the fact that large cells contain fewer receptors for LH (Fitz et al., 1982). LH does not stimulate accumulation of cAMP in large luteal cells, nor can cAMP increase secretion of progesterone from large cells as it does from small cells (Fitz et al., 1982; Hoyer et al., 1984). Progesterone secretion is thus negatively modulated during late diestrus by decreased binding capacity of luteal cells for LH, decreased proportion of cells which are responsive to

LH, decreased production of LH stimulated production of CAMP, and decreased responsiveness of luteal cells to cAMP.

#### Luteolytic Factors

Evidence Suggesting PGF<sub>2</sub>  $\alpha$  is the Luteolytic Factor. Hansel et al. (1973) and Inskeep (1973) summarized numerous studies that determined uterine production of  $\text{PGF}_2\,^\alpha$  is involved in luteal regression in cows and ewes. The bovine corpus luteum is another source of prostaglandins including those with luteotropic, PGI<sub>2</sub>, and luteolytic, PGF<sub>2</sub> $\alpha$ , effects (Milvae and Hansel, 1983). Blockade of synthesis of endogenous prostaglandins achieved by injecting indomethacin (Smith and Lands, 1971) in ewes and heifers prevents normal luteal regression (Lewis and Warren, 1977). Additionally, in vitro addition of indomethacin to luteal tissue of ewes (Evard et al., 1978) and cows (Pate and Condon, 1984) increases the ability of LH to stimulate synthesis of progesterone. Immunogenic inactivation of endogenous  $PGF_2 \alpha$ (Scaramuzzi and Baird, 1976; Fairclough et al., 1981) or removal of uterine produced  $PGF_2 \alpha$  by hysterectomy (Brunner et al., 1969; Bolt and Hawk, 1975) prolongs the lifespan of corpora lutea in cows and ewes. Therefore, both ovarian and uterine production of  $PGF_2 \alpha$  have implicated roles in luteolysis. Short term treatment (1 h or less) of luteal cells with  $PGF_2^{\alpha}$  in vitro (Hixon et al., 1983; Heath et al., 1983) and injection of  $PGF_2 \alpha$  in vivo (Hixon and Hansel, 1974; Heath et al., 1983; Schallenberger et al., 1984) results in an immediate increase in secretion of

progesterone. This effect is probably due to massive exocytosis of progesterone and oxytocin containing granules which are found in both large and small luteal cells (Quirk et al., 1979; Sawyer et al., 1979; Heath et al., 1983). Following this period of increased secretion luteal concentrations of progesterone (Heath et al., 1983) and serum concentrations of progesterone (Hixon and Hansel, 1974; Schallenberger et al., 1984) decline rapidly. This sequence of events indicates that the ultimate action of  $PGF_2^{\alpha}$  is luteolytic and not luteotropic.

Large luteal cells contain more receptors for  $PGF_2^{\alpha}$ (Fitz et al., 1982) and are more responsive to  $PGF_2^{\alpha}$  than are small luteal cells (Heath et al., 1983). Rao et al. (1979) reported a progressive increase in number and affinity of receptors for  $PGF_2^{\alpha}$  in bovine corpora lutea during the estrous cycle. These findings are consistent with the concept that the proportion of large luteal cells increases during diestrus and that this change could be involved in luteal regression. Furthermore, a lower proportion of large luteal cells during early diestrus and luteal development could in part explain the failure of exogenous  $PGF_2^{\alpha}$  administered before day 4 postestrus to cause luteolysis (Saumande and Chupin, 1981; Battista et al., 1984). Since large cells are less responsive to LH than small cells, it is not surprising that an inverse relationship exists between ability of bovine luteal cells to produce progesterone and to bind  $PGF_2^{\alpha}$  (Henderson and McNatty,

1977). In addition to the intraluteal changes occurring during diestrus which increase the ability of corpora lutea to respond to  $PGF_{2}\alpha$ , distinct elevations of  $PGF_{2}\alpha$  in serum occur as luteal functions declines (McCracken, 1980; Auletta et al., 1984; Fogwell et al., 1985). Therefore, during late diestrus  $PGF_{2}\alpha$  becomes more available to luteal cells as concentrations of  $PGF_{2}\alpha$  in serum increase and ability of luteal cells to bind  $PGF_{2}\alpha$  peaks. These  $PGF_{2}\alpha$  related events are coincident with the decreased ability of luteal cells to respond to LH and the two factors in combination may result in luteal regression.

Mechanism of Action of  $PGF_{2}^{\alpha}$  in Luteolysis.  $PGF_{2}^{\alpha}$  acts at several control points in luteal cell function to exert its luteolytic effects. Decreased luteal function after treatment with  $PGF_2^{\alpha}$  occurs prior to a decrease in concentration or affinity of receptors for LH in luteal tissue (Grinwich et al., 1976; Thomas et al., 1978; Spicer et al., 1981). Thus, subsequent points in the LH stimulated release of progesterone are important. In vitro, PGF2a inhibits LH stimulated adenylate cyclase activity and subsequent production of cAMP by luteal tissue of rats (Grinwich et al., 1976; Thomas et al., 1978). However, when cAMP is supplied to luteal cells of rats by addition of dibutryl cAMP to culture media (Jordan, 1981) or by increasing intracellular levels of cAMP by treating bovine luteal cells with cholera toxin or forskolin (Pate and Condon, 1984), production of progesterone continues to be inhibited by  $PGF_2^{\alpha}$ . Therefore,



inhibitory effects of  $PGP_2 \alpha$  on LH stimulated production of progesterone occur at control points in steroidogenesis both before and after accumulation of cAMP.

In order for boyine luteal cells to remain functional in vitro they must remain in a morphological configuration resembling epithelial cells. Presence of LH is required to maintain this configuration while  $PGF_2 \alpha$  inhibits this configuration (Gospodarowicz and Gospodarowicz, 1975). Thus morphological differentiation of luteal cells is one area of luteal cell function that  $PGF_2\alpha$  influences. Also,  $PGF_2\alpha$  may affect viability of luteal cells. One of the earliest events in luteal regression is increased lysosomal formation and lysosomal enzyme activity which lead to autophagocytosis and degeneration of luteal cells (McClellan et al., 1977). Lysosomes may be a site of interaction between luteolytic and luteotropic hormones as binding sites for  $PGF_2\alpha$  and LH are present in lysosomal membranes of bovine corpora lutea (Mitra and Rao, 1978). Thus,  $PGF_2 \alpha$  may interfere with luteal function by affecting the morphology and viability of luteal tissue in addition to direct effects on LH stimulated steroidogenesis. Additionally, PGF2 a reduces the amount of blood flow to the ovary (Niswender et al., 1976). Thus  $PGF_2 \alpha$ may impair luteal function by diminishing the amount of luteotropic agents and required metabolites delivered to the corpus luteum.

Hormonal Control of PGF<sub>2</sub><sup>a</sup> Secretion. Increased synthesis (Huslig et al., 1979) and secretion (Lewis et al.,

1977; McCracken, 1980; Auletta et al., 1984; Fogwell et al., 1985) of  $PGF_{2}\alpha$  from the uterus during late diestrus is controlled by a complex interaction between progesterone, estradiol and oxytocin. Progesterone administered on days 0 to 3 postestrus results in premature luteal regression (Battista et al., 1984). The mechanism by which progesterone produces this effect may be by lowering mean concentration of LH in serum (Battista et al., 1984). However, an interaction with  $PGF_2^{\alpha}$  is implied as hysterectomy (Moor et al., 1966; Woody et al., 1968) or treatment with indomethacin (Lewis et al., 1977a) prevents progesterone induced luteolysis. To this end, it has been demonstrated that administration of progesterone to ewes increases uterine content of  $\mathrm{PGF}_2^{\alpha}$  (Wilson et al., 1972) and hastens the occurrence of the first peak of  $PGF_2^{\alpha}$  prior to luteal regression (Ottobre et al., 1980). Additionally, administration of 100 mg/day of progesterone before day 3 postestrus followed by exogenous  $PGF_2^{\alpha}$  does not shorten the length of estrous cycles (11.7 days) more than administration of progesterone alone (13.2 days; Battista et al., 1984). In contrast, exogenous progesterone decreases content and concentration of  $PGF_2\alpha$  in endometrial tissue of ovariectomized ewes (Wilson et al., 1972) and concentration of  $PGF_2^{\alpha}$  in plasma remains low (Ford et al., 1975) or is decreased (Fairclough et al., 1983). Therefore, Ford et al. (1975) suggest the stimulatory effect of progesterone on


uterine secretion of  $\text{PGF}_2\alpha$  is indirect and acts to prime the uterus to respond to estradiol-17ß.

Injections of estradiol into corpora lutea of monkeys cause increased concentrations of luteal  $PGF_2\alpha$  (Auletta et al., 1978). Similarly, infusions of estradiol-17ß into uterine arterial blood of ewes results in increased uterine synthesis and release of PGF2 (Barcikowski et al., 1974). These effects of estradiol on uterine secretion of  $\text{PGF}_2\alpha$  are influenced by presence of progesterone however. Injections of estradiol-17 $\beta$  in intact ewes on days 9 and 10 postestrus, increase uterine secretion of  $PGF_2\alpha$  but the same dose of estradiol-17 $\beta$  is ineffective on days 4 and 5 unless the animals receive injections of progesterone on days 1 through 5 (Ford et al., 1975). Given alone, 10 mg of progesterone on days 1 through 5 did not alter secretion of uterine  $\text{PGF}_2\alpha$ (Ford et al., 1975). Likewise, injections of estradio1-178 cause early luteal regression in intact animals but injections of estradiol on day 5 or 6 were only luteolytic if preceded by injections of progesterone on days 1 through 4 (Warren et al., 1973).

The scenario of progesterone priming followed by increased serum concentrations of estradiol-17 $\beta$  to cause uterine release of PGF<sub>2</sub><sup> $\alpha$ </sup> fits well with the profiles of concentrations of these hormones in blood during diestrus. During this time, secretion of progesterone begins to decline as secretion of estradiol-17 $\beta$  and PGF<sub>2</sub><sup> $\alpha$ </sup> increases (Barcikowski et al., 1974; Fogwell et al., 1985).

Alterations in numbers of endometrial binding sites for progesterone and estradio1-176 occur during the cycle in parallel to the changes occurring in concentrations of these steroids in serum (Kimbal and Hansel, 1974; Zelenski et al., 1982). On days 13 to 14 postestrus, concentrations of progesterone declines and peaks of estradio1-17ß become associated with peaks of  $\text{PGF}_2\,\alpha$  and the amplitude of peaks of  $PGF_2 \alpha$  increase with time (Barcikowski et al., 1974). A decline in progesterone may facilitate the stimulatory action of estradiol-17 $\beta$  on the uterus as progesterone diminishes translocation of the estradiol/receptor complex and retention of this complex within the nucleus (Okulicz et al., 1981; Smanik et al., 1982). Indeed, presence of exogenous progestogens during late diestrus in ewes decreases the amplitude of pluses of the prostaglandin F metabolite 13, 14-dihydro-15-keto-PGF (PGFM; Fairclough et al., 1983). Within 18 h following removal of progestogen impregnated vaginal sponges from cows, a marked increase in PGFM was detected in plasma (Smith et al., 1979).

<u>Role of Oxytocin in Luteal Regression</u>. In addition to the ovarian steroids previously discussed, a role for oxytocin in luteolysis has been suggested (Hansel and Wagner, 1960; Armstrong and Hansel, 1959; Sheldrick et al., 1980). However, luteolysis induced by oxytocin may be due to oxytocin stimulated release of  $PGF_2^{\alpha}$  from the uterus rather than a direct effect of oxytocin on luteal tissue since

hysterectomy completely blocks oxytocin induced luteolysis (Armstrong and Hansel, 1959; Brunner et al., 1969).

Oxytocin stimulates synthesis and release of  $PGF_2^{\alpha}$  from endometrial tissue (Roberts et al., 1976) and increases plasma concentrations of PGFM in ewes (Fairclough et al., 1984). Effectiveness of oxytocin to increase release of  $PGF_2^{\alpha}$  or its metabolite during late diestrus (Roberts et al., 1976; Fairclough et al., 1984) is correlated with increased concentrations of estradiol-17 $\beta$  in plasma (Barcikowski et al., 1974). In ovariectomized ewes infusion of estradiol-17  $^\beta$  for 6 h increases oxytocin induced  $\text{PGF}_2^\alpha$ release from the uterus. Infusion of progesterone for 2 to 6 days blocks the stimulatory interaction between estradiol- $17^{\beta}$  and oxytocin on PGF<sub>2</sub><sup> $\alpha$ </sup> release. However, following 10 days of infusion of progesterone, estradiol augmented oxytocin induced release of  $PGF_{2}\alpha$  was increased 50 to 100 fold above the release induced without previous exposure to progesterone (McCracken, 1980). McCracken (1980) suggests estradio1-178 and progesterone alter uterine responsiveness to oxytocin through inducing increased receptors for oxytocin in the endometrium.

During mid-to-late diestrus there is an increased number of receptors for oxytocin in the endometrium (Roberts et al., 1976), increased concentration of oxytocin within corpora lutea (Schams et al., 1984) and in peripheral blood (Schams et al., 1980; Flint and Sheldrick, 1983; Walters et al., 1984). Therefore, ability of oxytocin to cause

increased uterine release of  $PGF_2 \alpha$  is temporally correlated with luteal regression. A cycle of positive feedback may be generated during late diestrus between ovarian secretion of oxytocin and uterine secretion of  $PGF_2 \alpha$ . An injection of  $PGF_2 \alpha$  causes increased release of oxytocin from the ovary (Flint and sheldrick, 1982). Similarly, vaginal distention causes early luteal regression (Hansel and Wagner, 1960) and increased secretion of oxytocin (Roberts and Share, 1968) possibly due to increased levels of  $PGF_2 \alpha$  secreted following vaginal stimulation (McCracken et al., 1980). Estradiol enhances while progesterone blocks the ability of vaginal distention to increase plasma concentrations of oxytocin (Roberts and Share, 1969).

Evidence for a Direct Luteolytic Action of Estradiol-176 on Luteal Cells. Destruction of ovarian follicles, the primary source of circulating estradiol-176, results in prolonged lifespan of corpora lutea in ewes (Karsch et al., 1970) and cows (Fogwell et al., 1985; Villa-Godoy et al., 1985). Following destruction of ovarian follicles, administration of estradiol benzoate results in early luteal regression in ewes (Hixon et al., 1975) as do injections of estradiol in intact animals (Hansel et al., 1973; Cook et al., 1974; Karsch and Sutton, 1976). Estradiol induced luteal regression is not, however, entirely dependent upon presence of the uterus. While hysterectomy completely blocks the ability of exogenous oxytocin and progesterone to cause luteal regression (Brunner et al., 1969), exogenous



estrogen significantly depresses luteal content and plasma concentrations of progesterone in hysterectomized cows and ewes although estrogen's ability to cause luteal regression is greatly diminished (Brunner et al., 1969; Gengenbach et al., 1977). Also, destruction of ovarian follicles which results in diminished levels of estradiol blocks PGF2 a induced luteal regression in ewes (Hixon et al., 1975). One proposed mechanism of action for estradiol is a synergism with  $PGF_2^{\alpha}$  on corpora lutea, though a direct interaction in cows has not yet been demonstrated in vitro (Hixon et al., 1983). In contrast, results from studies conducted with sheep support the hypothesis. Injection of low doses of estradio1-17<sup> $\beta$ </sup> and PGF<sub>2</sub> $\alpha$  in hysterectomized ewes whose follicles had been destroyed resulted in complete luteal regression in 3 of 4 ewes, while either treatment alone was only marginally effective (Gengenbach et al., 1977).

Neither are the luteolytic effects of an injection of estradiol due to the ability of estradiol-176 to depress concentrations of LH in serum when concentrations of progesterone are high (Beck et al., 1976; Goodman et al., 1981; Karsch et al., 1980). After ovarian follicles were destroyed, injection of estradiol in ewes results in an increase in basal concentration of LH before concentrations of progesterone are depressed (Hixon et al., 1975; Gengenbach et al., 1977). In addition, infusion of LH does not block estrogen induced luteolysis (Cook et al., 1974). Thus estradiol-17<sup>B</sup>'s luteolytic effects are not entirely



dependent on the effects of estradiol-17 $^{\beta}$  on the uterus and concentrations of LH in serum. As will be discussed in a following section, estradiol-17 $^{\beta}$  can act directly on luteal cells to affect their steroidogenic function.

Luteolytic Effects of Estradiol-17 <sup>β</sup> During the Estrous Cvcle. Luteal cells of cows (Kimball and Hansel, 1974) and ewes (Glass et al., 1984) contain binding proteins for estradiol-17 $\beta$  indicating that the corpus luteum is a target tissue for estradiol-178. Concentrations of cytosolic binding proteins for estradiol-17 $\beta$  increase between early and mid-diestrus in corpora lutea of ewes (Sheridan et al., 1975; Glass et al., 1984) and cows (Kimball and Hansel, 1974) before luteal function declines. Also, the concentration of binding proteins is highly correlated with concentrations of estradiol-17  $\beta$  found in the plasma of cows during this time (Kimball and Hansel, 1974). Additionally, large steroidogenic cells contain 3.5 fold higher concentration of cytosolic estradiol- $17\beta$  receptors than do small luteal cells collected from ewes on day 10 postestrus (Glass et al., 1984). The combination of increased availability of estradiol-176 in serum (Barcikowski et al., 1974; Fogwell et al., 1985) and increased concentration of binding proteins for estradiol-17 $\beta$  in luteal cells during late diestrus, in part, explains why the luteolytic effectiveness of exogenous estradiol is increased between early to late diestrus (Warren et al., 1973).



Intraluteal Effects of Estradiol-17 $\beta$  and Other Follicular Products. Estradio1-178 depresses the ability of LH to stimulate synthesis and release of progesterone (Moody and Hansel, 1971; Williams and Marsh, 1973) from both large and small bovine luteal cells (Urselv and Leymarie, 1979). Luteolytic actions of estradiol-17 $\beta$  occur in part after LH activation of adenylate cyclase since estradiol-17 $\beta$  does not significantly affect cAMP accumulation in LH stimulated luteal cells, and treatment of luteal cells with dibutvrl cAMP or cholera toxin only partially overcomes the inhibitory effects of estradiol- $17\beta$  (Williams and Marsh, 1978). Conversion of pregnenolone to progesterone is catalyzed by  $3\beta$ -hydroxysteroid dehydrogenase. The activity of this enzyme is inhibited by estradiol- $17\beta$  (Akbar et al., 1972; Caffrey et al., 1979) and thus represents one site in the steroidogenic pathway influenced by estradio1-178.

Antagonistic interaction between cAMP and estradiol-17ß may also influence luteal cell function. Bodwin et al. (1981) demonstrated that inverse relationships exist between cAMP in the cytosol and nuclear uptake of the estrogen/ receptor complex and between cytosolic estradiol-17ß and nuclear binding of cAMP in mammary tissue of rats. As nuclear translocation of the receptor is critical for steroid hormone action (Yamamoto and Alberts, 1976), I suggest that decreased ability of corpora lutea to respond to LH as the luteal tissue ages either due to diminished number of receptors for LH or alterations in proportion of



small to large luteal cells, would result in diminished accumulation of cAMP within luteal cells. Decreased concentrations of cAMP would increase the ability of corpora lutea to respond to estradiol-17 $\beta$ . In addition to the effects of estradiol-17 $\beta$ , follicular fluid diminishes adenylate cyclase activity and cAMP accumulation in gonadotropin stimulated ovarian tissue (Amsterdam et al., 1979; Ledwitz-Rigby, 1980) and results in depressed production of progesterone (Shemesh, 1979). Karsch et al. (1970) observed that destruction of ovarian follicles decreases by four fold the amount of exogenous LH that is required to maintain corpora lutea in intact animals.

In summary, due to alterations in the type of luteal cells present, the responsiveness of corpora lutea to LH may decline while responsiveness to estradiol-17<sup>β</sup> may increase during late diestrus. These alterations in binding capacity for LH and estradiol-17<sup>β</sup> in combination with increased serum concentrations of estradiol-17<sup>β</sup> can result in the initial decline in production and release of progesterone from corpora lutea. Diminished serum concentrations of progesterone in combination with high levels of oxytocin and estradiol-17<sup>β</sup> and increased concentration of binding proteins for estradiol-17<sup>β</sup> and oxytocin in the uterus, causes increased uterine secretion of PGF<sub>2</sub><sup>α</sup> and luteal regression ensues.

### MODEL OF INITIATION OF LUTEAL REGRESSION AND EXPERIMENTAL OBJECTIVES

### Early Development of the Corpus Luteum

#### Luteotropic Factors

#### Luteal tissue contains increased receptors for LH. Steroidogenic cells are primarily small in size which in turn are the most responsive cells to stimulation by LH. Pulses of LH are of low amplitude but high frequency.

## Luteolytic Factors

Concentrations of luteal receptors for PGP<sub>2</sub> a and estradiol-178 are low as are concentrations of PGP<sub>2</sub> a and oxytocin in blood. A transitory increase in estradiol occurs. The uterus is unresponsive to estradiol and oxytocin stimulation and contains a low concentration of receptors for estradiol and oxytocin.

### Maturation of the Corpus Luteum

Luteal tissue is still quite responsive to LH stimulation but a decrease in specific binding for LH occurs as the proportion of steroidogenic cells which are large increase. Pulses of LH are of high amplitude and low frequency. Concentration of luteal receptors for PGF<sub>2</sub> and estradiol-17<sup> $\beta$ </sup> are increased but levels of these hormones in blood are low. The uterus has increased concentration of receptors for estradiol-17<sup> $\beta$ </sup> and oxytocin though high levels of progesterone blocks their ability to stimulate release of PGF<sub>2</sub>a.



## Initiation of Luteal Regression: Decline of Luteal Function Begins

# Luteotropic Factors

Proportion of steroidogenic cells which are large peaks. Large cells contain a lower concentration of receptors for LH and are less responsive to LH stimulation for accumulation of CAMP and production of progesterone. While basal secretion of LH is unchanged, it is not known if the pattern of pulses is altered.

# Luteolytic Factors

Increased proportion of large luteal cells results in increased concentration of receptors for estradiol- $17\beta$  and PGF<sub>2</sub><sup> $\alpha$ </sup>. Increased concentrations of estradiol- $17\beta$  in peripheral plasma are found due to increased secretion of estradio1-178 from ovarian follicles. The ability of estradio1-178 and oxytocin to stimulate uterine secretion of PGF2a is increased. Whether or not ovarian follicles of cows are necessary for the luteolytic effects of PGF<sub>2</sub>  $\alpha$ to be manifested is not known.

### Luteal Regression

Luteal receptors for LH steadily decline. As secretion of progesterone declines basal concentrations of LH in plasma increase. Pulses of LH demonstrate decreased amplitude and increased frequency.

Previous exposure of the uterus to high levels of progesterone during the preceeding stages of luteal function followed by declining levels of progesterone in serum during this stage allows estradio1-17 ß from ovarian follicles to stimulate increased release of PGF $_{2}^{\alpha}$  from the uterus. Increased PGF2 a secretion is also due to effects mediated by oxytocin from the corpus luteum which are augmented by estradio1-17β.

The corpus luteum is responsive to  $PG_{\Gamma_{\alpha}}^{\alpha}$  and estradiol-17.8 since if contains an increased proportion of large luteal cells. Initially  $PG_{\Gamma_{\alpha}}^{\alpha}$  causes increased refease of progesterone and oxytocin due to a massive exocytosis of luteal



Luteal Regression (Continued)

## Luteotropic Factors

#### Luteolytic Factors

granules. Meanwhile PGF<sub>2</sub><sup> $\alpha$ </sup> blocks LH induced cAMP accumulation and production of progesterone. Increased secretion of oxytocin in turn further stimulates uterine secretion of PGF<sub>2</sub> $\alpha$ . Long term exposure of luteal cells to PGF<sub>2</sub> $\alpha$  results in loss of luteal receptors for LH and autophagocytosis.

# Experimental objectives

Parts of this model are well documented as described in the Review of Literature. Other aspects are suppositional and require further investigation. Of particular interest to this investigation are those factors involved in the stage I have termed "initiation of luteal regression." In order to clarify factors that are involved with the initial decline in function of the bovine corpus luteum the following questions were posed:

- Is an alteration in the pattern of pulsatile release of LH associated with initiation of luteal regression?
- (2) Do follicular products affect response of corpora lutea to exogenous PGF<sub>2</sub>a?

# MATERIALS AND METHODS

After exhibiting two or more estrous cycles of 17 to 24 days, twenty Holstein heifers were assigned at random to a 2 x 2 factorial experiment. Main effects were: 1) destruction of ovarian follicles on day 9 (estrus = day 0), and 2) injection of  $PGF_2 \alpha$  on day 14 postestrus. Visible follicles were destroyed in 10 heifers by electrocautery then ovaries were x-irradiated (x-irrad) as described by Villa-Godoy et al. (1985). The remaining 10 heifers (control) experienced similar surgical manipulations except no follicles were cauterized and ovaries were not x-irradiated (Villa-Godoy et al., 1985; Fogwell et al., 1985). On day 14 postestrus, heifers received an intramuscular injection of 15 mg PGF2 al (n=10) or 3 ml of 2 percent Tham<sup>2</sup> buffered saline (Saline; n=10). The dosage of  $PGF_2^{\alpha}$  was selected as the minimal effective level required to cause luteolysis in cows as determined by Lauderdale (1979). Therefore, the four groups (5 heifers/group) were: x-irrad PGF2α, x-irrad saline, control PGF2 a, and control Saline.

<sup>&</sup>lt;sup>1</sup>Lutylase<sup>•</sup>, the Upjohn Company.

<sup>&</sup>lt;sup>2</sup>Tris (Hyroxy methyl) Aminomethane, Fisher Scientific Company.



To evaluate success of destruction of follicles twenty days following estrus, eleven days after surgery, x-irrad heifers were ovariectomized supravaginally. Corpora lutea were isolated from ovarian stroma and weighed. The ovarian stroma was then sliced into 1 to 2 mm sections and each section was examined macroscopically for follicles  $\geq$  2 mm. Only animals whose ovaries were free from visible follicular development were accepted as x-irrad heifers.

From day 8 through day 20 postestrus, jugular venous blood was collected from indwelling cannulas every 8 h to determine concentrations of progesterone in serum and thus monitor luteal function. To examine pulsatile release of LH, blood was sampled every 15 min from 0800 to 2000 h on days 8 and 13 postestrus from all animals and on day 15 from heifers injected with saline. Day 15 was selected as the last day of monitoring the pulsatile pattern of secretion of LH based on the observation by Villa-Godoy et al. (1985) that a decline in luteal function in similarly treated control heifers can be detected as early as day 16 postestrus. Since our first experimental objective was to determine if a change in the pulsatile pattern of release of LH occurred before luteal function declined sampling subsequent to day 15 was not conducted. For the same reason animals receiving PGF<sub>2</sub> $\alpha$  on day 14 were not sampled on day 15 for LH. Samples of blood were allowed to clot at room temperature before refrigeration at 4°C for 24 to 48 h. After centrifugation, serum was decanted and stored at -20°C



until assayed for concentrations of LH (Convey et al., 1976), estradiol-17ß (Carruthers and Hafs, 1980), and progesterone (antibody against progesterone as validated by Convey et al., 1977; assay as described by Louis et al., 1973). Due to insufficient volume of serum in samples taken every 8 h, assays for estradiol-17ß were limited to pools of samples collected every 15 min on days 8, 13 or 15 postestrus. Coefficients of variation within and among assay of serum pools were 3.9% and 11.0% for LH, 6.4% and 17.4% for progesterone, and 11.5% and 9.8% for estradiol-17ß. Sensitivities of assays, defined as the first point on the standard curve lower than the 95% confidence interval of the buffer controls, were 0.075 ng for LH, 0.04 ng for progesterone, and 0.8 pg for estradiol-17ß.

Samples with undetectable concentrations of LH were assigned a value equivalent to assay sensitivity. Pulses, amplitude, frequency and baseline LH were determined using the following criteria. A pulse of LH was defined as an increase in concentration of LH which exceeds another value within the preceding 30 min by twice the within assay standard deviation  $(2 \times SD = 0.96 \text{ ng})$ . Amplitude of peaks equals the difference between maximal value reached during a pulse and the preceding nadir. Frequency of pulses refers to the mean number of pulses experienced per animal during the 12 h sampling period. Baseline was defined as the mean of values equal to interpulse nadirs  $\pm$  sensitivity of the assay.

Concentrations of estradiol-17 $\beta$ , progesterone and baseline LH as well as frequency and amplitude of pulses of LH were analyzed using error terms generated from linear regression analyses for split plot designs (Alvey et al., 1980). Specific contrasts among data on LH were examined with the Bonferroni <u>t</u> test (Gill, 1978), while data on estradiol-17 $\beta$  were contrasted with Scheffe's test (Gill, 1978).

To reduce heterogeneity of variance, daily mean concentrations of progesterone were calculated and used in the analysis of variance for treatment effects. Progesterone data were divided into two periods. Period 1 included day 8 to day 13 and was used to examine differences between surgical groups before injection of  $PGF_2\alpha$  or saline on day 14. Period 2 included samples collected between the time of injection and day 20. Dunnet's t test was used to determine when a significant decrease in concentration of progesterone occurred following injection of  $PGF_2\alpha$  or saline. Concentrations of progesterone before surgery or before injection were used as covariates in analyses of variance for Periods 1 and 2 respectively. Weight of corpora lutea were contrasted using Welch's approximate t test (Gill, 1978). During Period 2 concentrations of progesterone which fell and remained below 2 ng/ml for two or more consecutive samples was used as a limit to monitor impaired luteal function in this study rather than 1 ng/m1 which is frequently used. This was a conservative measure designed to



increase sensitivity to detect diminished luteal function in individual animals.

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#### RESULTS

Before injection of PGF<sub>2</sub>  $\alpha$  on day 14 postestrus, one control heifer had begun spontaneous luteal regression on day 13 postestrus as assessed by a 70% reduction in concentration of progesterone (ll.5 mg/ml to 3.8 ng/ml), increased concentrations of estradiol-17 $\beta$  (7.9 gg/ml), and increased frequency of pulses of LH (6 pulses/5 hours). Data from this heifer were excluded from all analyses.

No ovaries from x-irrad heifers contained follicles  $\geq$  2 mm on day 20 postestrus. Thus, electrocautery of follicles and x-irradiation of ovaries destroyed existing follicles and prevented growth of follicles. Furthermore, concentrations of estradiol-17ß in these heifers tended (P < 0.1) to be lower on day 15 (1.9  $\pm$  0.4 pg/ml) than on day 8 (4.0  $\pm$  0.5 pg/ml). In contrast, concentrations of estradiol-17ß in control heifers did not differ between day 8 and 15 (Figure 1).

Basal concentrations of LH did not differ among days sampled or between surgical groups. Yet on days 13 and 15, frequency (p < 0.05) and amplitude (p < .01) of pulses of LH were greater than on day 8 in x-irrad heifers (Figure 2). In control animals, frequency of pulses did not vary across days though amplitude of pulses of LH was increased





Figure 1. Concentrations of estradiol-17<sup>8</sup> in serum of heifers. During surgery on day 9 postestrus, ovarian follicles were destroyed (hatched bars), or not destroyed (open bars). Data are means ± SSM. Only animals receiving saline on day 14 are reported for day 15. Numbers within brackets represent number of heifers sampled.

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\* Concentrations of estradio1-17  $\beta$  in heifers whose follicles were destroyed were less on day 15 than on day 8 (P < 0.1).









- Figure 2. Characteristics of pulsatile secretion of LH in serum of heifers.<sup>a</sup> During surgery on day 9 postestrus, ovarian follicles were destroyed (hatched bars), or not destroyed (open bars). Animals included for day 15 received an injection of saline on day 14. Data are mean <u>+</u> SEM. Only animals receiving saline on day 14 are reported for day 15. Numbers within brackets represent number of heifers sampled.
  - <sup>a</sup> See <u>Materials and Methods</u> for criteria defining pulses of LH.
  - \* Greater than the comparable value for day 8 (P < 0.05).
  - \*\* Greater than the comparable value for day 8 (P
    < 0.01).</pre>



Figure 2
(p < 0.01) on day 15 in control heifers and did not differ from amplitude of pulses found in x-irrad heifers on day 15. Thus availability of LH in serum, if defined as either increased basal concentration of LH without change of pulsatile secretion or constant basal concentration of LH with increased frequency and/or amplitude of pulses of LH, was increased between day 8 and 15 postestrus in both control and x-irrad heifers.

As expected, there were no differences in profiles of concentrations of progesterone in serum between control and x-irrad heifers from day 8 to day 13 postestrus (Figure 3). From day 14 through day 20, concentrations of progesterone were above 2 ng/ml in all heifers injected with saline. However, all control heifers injected with PGF<sub>2</sub> $\alpha$  demonstrated concentrations of progesterone in serum below 2 ng/ml within 32 h after injection. In contrast, in x-irrad heifers injected with PGF<sub>2</sub> $\alpha$  mean concentration of progesterone did not decline between injection of PGF<sub>2</sub> $\alpha$  and day 20 postestrus and weights of corpora lutea (5.4 ± 1.1 gm) did not differ from x-irrad heifers injected with saline (5.0 ± 0.1 gm). However, by day 20 postestrus three of five x-irrad heifers injected with PGF<sub>2</sub> $\alpha$  had concentrations of progesterone in serum fall and remain below 2 ng/ml.



Figure 3. Concentrations of progesterone in serum of heifers. During surgery on day 9 postestrus ovarian follicles were destroyed (X-IRRAD), or not destroyed (CONTROL). On day 14 postestrus, heifers were injected with Tham buffered saline (open circles) or  $PGF_2^{\alpha}$  (closed circles). Days of surgery and time of injection ( $T_0$ ) are indicated by arrows. Data are daily means <u>+</u> SEM. Numbers within brackets represent numbers of heifers sampled.



Figure 3

## DISCUSSION

Stable levels of progesterone continued through day 20 in saline injected control heifers indicating luteal regression had not yet occurred in this group. In experiments with animals of the same breed and approximate age and using the same surgical procedures, Villa-Godoy et al. (1985) reported control animals experienced luteal regression between 17 and 22 days postestrus. Thus, failure to detect luteal regression by day 20 in saline injected control heifers in this study is apparently due to random variation in the length of estrous cycles (Bartol et al., 1981) rather than to surgical manipulations.

In heifers, passive immunization against LH reduces weights of corpora lutea (Snook et al., 1969) and decreases concentrations of progesterone in serum (Hoffman et al., 1974). Injection of hCG or LH in heifers (Wiltbank et al., 1961; Brunner et al., 1969) and infusion of LH in ewes (Karsch et al., 1970; Karsch et al., 1971) prolongs luteal function. Decreased baseline concentrations, frequency or amplitude of pulses would decrease luteotropic support and could initiate luteal regression. However, we did not detect any changes in the pulsatile secretory pattern for LH on the days sampled which would lower availability of



concentrations of LH in serum to corpora lutea. On the contrary, amplitude of pulses of LH increased 86% between days 13 and 15 postestrus. Increased amplitude of pulses is surprising since concentrations of progesterone and estradiol- $17^{\beta}$ , which are thought to be the primary factors involved in altering the episodic pattern of release of LH (Goodman and Karsch, 1980; Karsch et al., 1980; Beck et al., 1976; Hausler and Malven, 1976), did not change in control heifers during these days. Since luteal regression was not detected during the sampling period of this study it is still unclear if the pulsatile secretion of LH changes immediately before function luteal regression begins however.

Reduced concentrations of estradio1-17 $\beta$  in serum (Beck et al., 1976; Goodman et al., 1981) and the possible reduction in other non-identified follicular products (Cummins et al., 1983; Barraclough et al., 1979) in x-irrad heifers possibly accounts for the increased frequency of pulses of LH. Destruction of ovarian follicles cause increased lifespan of corpora lutea in heifers (Fogwell et al., 1985; Villa-Godoy et al., 1985) because luteolytic factors are attenuated and potentially because the availability of LH in serum is increased. Additionally, increased secretion of LH may explain the maintenance of corpora lutea in x-irrad heifers treated with  $PGF_2 \alpha$  in this study. This is unlikely however, since a 10 fold increase in concentration of LH cannot block luteal regression in-



duced by a 25 mg injection of  $PGF_2^{\alpha}$  in cows (Gonzalez-Mencio et al., 1977), nor will exogenous LH prolong lifespan of corpora lutea in cows or ewes indefinitely (Wiltbank et al., 1961; Karsch et al., 1971). Therefore, failure of  $PGF_2^{\alpha}$  to induce luteal regression in x-irrad heifers is most likely due to absence of follicular products and thus their effects on luteal tissue rather than increased availability of LH in serum.

As diestrus advances, corpora lutea are more responsive to the luteolytic effects of estradiol-17 $\beta$  (Warren et al., 1973) and PGF<sub>2</sub> $\alpha$  (Inskeep et al., 1973; Battista et al., 1984) suggesting that the increase in plasma concentrations of both hormones in late diestrus (Fogwell et al., 1985; Barcikowski et al., 1974) may be involved in normal luteal regression. On day 14 postestrus, 15 mg PGF $_2\alpha$  caused a rapid decline in serum concentrations of progesterone in control heifers but not in x-irrad heifers. Thus, it is evident that follicular products alter responsiveness of bovine corpora lutea to exogenous  $PGF_2\alpha$  as was observed previously in ewes (Gengenbach et al., 1977). Ovarian follicles are important in normal luteal regression in heifers because: 1) estradiol- $17^{\beta}$  stimulates uterine secretion of PGF<sub>2</sub> $\alpha$  (Barcikowski et al., 1974), 2) estradiol-17 $\beta$  potentiates and may be required for maximal luteolytic effectiveness of PGF<sub>2</sub> $\alpha$ , and 3) estradiol-17 $\beta$  has direct luteolytic actions on luteal function. Indeed estradio1-178 depresses LH stimulated synthesis and release



of progesterone from boyine luteal cells (Williams and Marsh, 1978; Urselv and Levmarie, 1979) past the point of LH induced cAMP accumulation (Williams and Marsh, 1978). Activity of 3<sup>β</sup>-hydroxysteroid dehydrogenase which converts pregnenolone to progesterone is one point in the steroidogenic pathway that estradiol-17 $\beta$  inhibits (Akbar et al., 1972; Caffrey et al., 1979). In addition, nonsteroidal components of follicular fluid reduce luteal function in primates (Stouffer et al., 1983), and depress basal and LH induced secretion of progesterone in luteinized bovine follicles (Shemesh et al., 1979) possibly by inhibiting LH-sensitive adenylate cyclase activity (Amsterdam et al., 1979; Ledwitz-Rigby, 1980). Attempts to demonstrate an interaction between estradiol-17  $\beta$  and PGF<sub>2</sub>  $\alpha$  directly on luteal tissue in vitro have proven unsuccessful to date (Hixon et al., 1983).

In summary, though no changes in the concentrations of progesterone or estradiol- $17\beta$  in serum were noted the amplitude of pluses of LH increased while frequency of pulses and baseline concentrations of LH remained the same in control heifers. Therefore, there was no reduction of luteotropic support by day 15 postestrus. Additionally, removal of ovarian follicles resulted in increased amplitude and frequency of pulses of LH prior to any detectable changes in serum concentrations of progesterone and estradiol- $17\beta$ . Increased luteotropic support is not, however, believed to to be the reason that destruction of ovarian follicles



results in the lengthening of the lifespan of corpora lutea of cows. It is suggested that products of ovarian follicles, such as estradiol-17 g, act directly on corpora lutea to affect the luteolytic efficacy of exogenous PGF<sub>2</sub> a and are thus required for spontaneous regression of bovine corpora lutea.



## SUMMARY AND CONCLUSIONS

The study presented examines two factors that could be involved in initiation of luteal regression. Based on results from this study it is suggested that availability of LH in serum is not diminished between mid-to-late diestrus though it is unknown if a change in the pulsatile release of LH is altered immediately before luteal function declines. However, as suggested in the model, ability to respond to luteotropic support may decline during this time and thus corpora lutea require large increases in LH in serum to offset this declined responsiveness.

The presence of ovarian follicles clearly facilitates the ability of exogenous  $PGP_2^{\alpha}$  to cause luteal regression. This suggests products of ovarian follicles negatively affect luteal function in ways other than the ability of estradiol-17<sup> $\beta$ </sup> to stimulate increased synthesis and release of  $PGP_2^{\alpha}$  from either the uterus or ovary. This luteolytic action of ovarian follicular products is possibly directly on luteal cells and interferes with LH induced stimulation of progesterone secretion. Estradiol-17<sup> $\beta$ </sup> and other follicular products may synergize with the luteolytic effects of  $PGP_2^{\alpha}$  on luteal cell function at the same or different sites of LH stimulated steroidogenesis but this synergism directly on luteal tissue remains to be elucidated.

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