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LUTEAL REGRESSION OF CATTLE

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ROLE OF OVARIAN FOLLICLES IN LUTEAL REGRESSION OF CATTLE

By

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ABSTRACT

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LUTEAL REGRESSION OF CATTLE

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An experiment was conducted to determine if ovarian follicles, present after midcycle, influence luteal regression in cattle. Four cows had their ovarian follicles destroyed by electro-cautery and X-irradiation on days 10 or 11 of the estrous cycle. Six cows, (Sham-irradiated) served as controls. Experiment was terminated on day 22 postestrus at ovariectomy. Absence of visible follicles in X-irradiated ovaries on day 22 postestrus and lower concentrations of estradiol in blood compared to controls led to the conclusion that treatment was efficatious. Basal concentrations of LH in plasma did not differ between groups on any day. By day 22 postestrus concentrations of progesterone in plasma declined in control cows but remained elevated in treated cows. Weights of corpora lutea removed on day 22 postestrus were greater in treated cows compared to controls. Secretion of estradiol-17β was observed to increase prior to decline in secretion of progesterone in blood of control cows. Based on these results, it is concluded that ovarian follicles, present after midcycle are critical to the process of luteal regression in cattle.

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INTRODUCTION

Efficient production is a prime goal in agriculture. In animal agriculture, reproduction is critical to production. Animal scientists have provided many tools necessary to improve reproduction, yet much remains to be learned.

Genetic improvement, a hallmark of the dairy industry for 30 years, is based on selection for desireable traits the most important being high milk production. Although reproduction is a prerequisite for milk production, reproductive performance is not one of the traits generally selected for. Yet, failure to reproduce is a primary reason for culling. Often the high milk producing cows are retained in herds in spite of failure to conceive within a desired period of time. Incentives to maintain these cows in the milking herd are great. Better understanding of the physiological process of reproduction is the means by which animal scientists will devise the tools necessary to deal with infertility in todays dairy industry.

Critical to fertility in cows are expression of estrus and time ovulation related to estrus. These occur following cessation of production of progesterone by the corpus luteum (luteolysis). Defects in the luteolytic process could influence fertility. Important to understanding the process of luteal regression (luteolysis) is determining factors which initiate this event. It is the intent of this study to provide further

insight into factors influencing luteal regression.

REVIEW OF LITERATURE

Factors associated with regression of corpora lutea in cattle and sheep will be discussed in this review. Although cows are the intended focus, the abundance of literature concerning reproduction in ewes and many apparent similarities between ewes and cows justify using data from ewes to support the bovine model. Data concerning other species will not be discussed except to clarify or support important concepts not tested in cows or ewes.

With each estrous cycle in cows there is opportunity during estrus for conception. In the absence of pregnancy cyclicity continues providing more opportunities for conception. Most cows undergo estrous cycles every 17 to 25 days with an average cycle of 21 days (Moeller and VanDemark, 1951; Asdell et al., 1949). A corpus luteum (CL) forms with each estrous cycle and as a transient endocrine organ is a major component affecting estrous cycles. Continued luteal function is necessary for maintenance of pregnancy; however, in the absence of pregnancy, renewal of cyclicity is dependant upon regression of corpora lutea. The primary function of corpora lutea is secretion of progesterone a major reproductive hormone. Progesterone, in concert with other hormones of reproduction, influences the progess of reproduction.

Major Hormones of Reproduction

Progesterone: As luteal tissue forms during metestrus and diestrus

concentrations of progesterone circulating in the blood increase. Herriman et al. (1979) reported plasma concentrations of progesterone to rise in a linear fashion from day 2 postestrus through day 10. Wetteman et al. (1972) reported circulating concentrations of progesterone to increase between days 3 or 4 and day 11 postestrus. From day 10 postestrus through day 16 concentrations of progesterone in blood fluctuate but remain elevated. By day 18, concentrations of progesterone decline as the corpus luteum regresses (Wetteman et al., 1972; Chenault et al., 1975; Garverick et al., 1971). Absolute concentrations of progesterone observed by various groups often differ most likely due to variation between assays, however, the patterns of secretion of progesterone were similar. On day 2 postestrus progesterone in blood increases through days 8 to 10, is maintained through days 16 to 18 when luteolysis ensues, progesterone in blood then declines to basal levels observed throughout estrus.

Estradiol: In cows, estradiol in the blood is difficult to quantify during most stages of an estrous cycle. For this reason changes in concentrations of estradiol during diestrus are variable and difficult to assimilate. Wetteman et al. (1972) quantified estradiol in single serum samples taken from heifers on days 2, 4, 7 and 11 postestrus and reported values between 3.0 pg/ml and 3.9 pg/ml with no appreciable variation over time. Significant increases in concentrations of estradiol occurred during proestrus and estrus. Shemesh et al. (1972) measured estradiol in blood of nine cows throughout their estrous cycles and observed three periods of increased concentrations of estradiol. The greatest increase was associated with estrus and smaller increases were noted on days 4 and 11 postestrus. Dobson and Dean (1974) reported a small rise in estradiol

around day 4 postestrus but did not observe an increase at midcycle. Hansel and Ecternkamp (1972) and Glencross et al. (973) observed high concentrations of estradiol at estrus and another increase during metestrus, but very low concentrations at midcycle in postpartum cows. However, concentrations were noted to increase on day 12 postestrus and remained slightly elevated until proestrus when concentrations of estradiol increased markedly concomitant with the decline in progesterone. In summary, researchers agree that the greatest concentrations of estradiol occur at proestrus and estrus with a less dramatic increase during metestrus. There is equivocal evidence that concentrations of estradiol increase during mid to late diestrus. However, frequency of sampling, preparation of blood samples, site of sampling and limitations of assays in reports cited above, may account for variable tests of this concept.

Luteinizing hormone (LH): Relatively low (baseline) concentrations of LH are observed throughout most of a bovine estrous cycle. Mean concentrations of LH in blood range between 1 and 2 ng/ml during diestrus (Wetteman et al, 1972). Rahe et al. (1980), aware that LH is characteristicly released in a pulsatile fashion, quantified LH in blood of four heifers on days 3, 10, and 19 postestrus. Observations were that concentrations of LH in blood fluctuate in a pulsatile fashion that varies with stage of an estrous cycle. During metestrus (day 3 postestrus), pulses of LH were of low amplitude (ΔLH = .3 to 1.8 ng/ml) and high frequency (20 to 30 pulses/day). The mean concentrations of LH varied among animals (1.3 ng/ml to 2.2 ng/ml). At mid-diestrus (day 10 postestrus) pulses of LH were characterized at high amplitude (ΔLH = 1.2 to 7.0 ng/ml) and low frequency (6 to 8 pulses/day). Again, mean concentrations of LH differed among animals on day 10. Mean concentration of LH on day 10

postestrus did not differ from mean on day 3. Thus, there is no change in basal concentrations of LH between day 3 and day 10, however the pattern of secretion of LH did differ. Sources of variation and potential biological effects of differences in secretory patterns of LH between days 3 and 10 postestrus are not known.

Frequency of pulses of LH increase as the preovulatory surge of LH approaches. The preovulatory surge of LH lasts from 5 to 10 hours and is composed of numerous episodic peaks high in magnitude (Rahe et al., 1980; Chenault et al., 1975).

Follicle stimulating hormone (FSH): A definitive study aimed to adequately characterize the pattern of circulating concentrations of FSH in cows has not been done. Increased concentrations of FSH occur in blood at the time of the LH surge during proestrus (Roche and Ireland, 1981; Dobson, 1978). Decreased concentrations of FSH were found in pituitary glands during this same interval (days 18 through 20) (Hackett and Hafs, 1969). However, a depletion of FSH in pituitaries was also noted to occur at midcycle (Desjardens and Hafs, 1968). Although this observation supports the possibility that secretion of FSH into blood increases at this time, no direct evidence exists. Although the LH and FSH surges associated with proestrus occur concomitantly, the secretory patterns of FSH do not always parallel patterns observed for LH (Dobson, 1978). Concentrations of FSH in blood do vary during metestrus and diestrus, however discrete patterns are not yet readily discernable (Akbar et al., 1974). With the advent of better assays and knowledge that acute changes in secretion of pituitary hormones contribute to the overall hormonal patterns, the nature of secretion of FSH in cattle will become known.

Corpora Lutea of the Estrous Cycle

Role of the CL in cyclicity: The primary role of the corpus luteum is to produce progesterone. Significant variations in concentrations of progesterone in blood affects estrous cyclicity. Early regression of corpora lutea results in shortened cycle length. Prolongation of luteal function results in longer than normal estrous cycles.

In cows and ewes, concentrations of progesterone in blood rise during metestrus as the CL develops. Concentrations of progesterone remain elevated throughout diestrus and decrease in late diestrus-early proestrus (Wetteman et al., 1972). Estrus and ovulation occur spontaneously only after luteal regression and production of progesterone ceases. Continuous administration of progesterone prevents estrus and ovulation in cows (Christian and Casida, 1948) and ewes (Dutt and Casida, 1948) thus prolonging estrous cycles. Withdrawal of exogenous progesterone is followed by estrus and ovulation in cattle (Roche, 1976) and sheep (Dutt and Casida, 1948). Enucleation of corpora lutea at midcycle in cattle results in premature estrus and ovulation (Anderson et al., 1965; Snook et al., 1969; Hobson and Hansel, 1972). Therefore, relative consistency of normal estrous cycle length is in part dependent on interval of luteal function.

There have been numerous studies attempting to elucidate mechanisms by which corpora lutea and/or progesterone influence estrous cycles. In cycling cows and ewes regression of corpora lutea is followed by rapid development of follicles and preovulatory surge of LH (Chenault et al., 1975). This sequence was also noticed following enucleation of CL in cattle (Hobson and Hansel, 1972) and in sheep (Karsch et al., 1979).

Ovariectomy during the luteal phase of an estrous cycle results in increased concentrations of LH in sera of cattle (Hobson and Hansel, 1972) and sheep (Butler et al., 1971). This is termed the "post castrational rise in LH" and does not achieve the magnitude of the preovulatory LH surge. Replacement of endogenous progesterone following ovariectomy with progesterone implants reduces magnitude of the post castrational rise in LH (Beck et al., 1976). The ability of progesterone to prevent a surge of LH and ovulation following removal of corpora lutea in sheep was demonstrated by Karsch et al. (1979). Exogenous progesterone blocked estradiol induced surges of LH in ovariectomized ewes (Howland et al., 1978). Kesner (1981) showed that progesterone is an effective blocker of LH and FSH surges in cattle. Therefore, the LH surge does not occur during a period of luteal function.

Karsch et al. (1979) suggested that the slight increase in concentration of LH following luteal regression might stimulate increased secretion of estradiol. In the absence of progesterone, exogenous estradiol is capable of eliciting a LH surge in ewes (Hobson and Hansel, 1972) and in cattle (Beck and Convey, 1977; Short et al., 1979). However, Fogwell et al (1978) observed increased concentrations of estradiol to occur in blood of heifers following removal of CL whether or not increased secretion of LH was blocked by anesthesia. Thus, the small increase in concentration of LH observed just following luteal regression may not be important to increased secretion of estradiol thought to stimulate the LH surge. Clearly, corpora lutea do influence events associated with cyclicity primarily through production of progesterone. In order for estrus and ovulation to occur and cyclicity to resume, the corpus luteum must regress.

Although timing of luteal regression influences lengths of estrous cycles, additional evidence indicates timing of formation of corpora lutea is also important. Administration of progesterone from estrus through metestrus shortened estrous cycle lengths in cattle and sheep (Woody et al., 1967).

Formation of the CL: Following ovulation, granulosa and theca cells of an ovulated follicle differentiate into luteal cells. Luteal cells derived from the theca interna dominate the population of luteal cells observed by day 7 postestrus. Luteal cells derived from granulosal cells are prominent and dividing up to day 4 postestrus. Luteal cells derived from the theca interna appeared to retain ability to respond to LH by proliferating after day 4 when granulosa luteal cells have ceased mitotic activity. Mitosis of luteal cells is complete by day 8 postestrus but hypertrophy of existing luteal cells and proliferation of connective tissue may continue for 3 more days (Rajakoski, 1960). This period (days 8 through 11) was characterized by increased ability to bind LH, increased secretion of progesterone, increased adenylate cyclase activity and increased luteal weight (Fitz et al., 1980; Erb et al., 1971).

Regulation of luteal function: Hypophysectomy of ewes during the luteal phase of an estrous cycle results in loss of luteal function (Kaltenbach et al., 1968; Hixon and Clegg, 1969). Continuous infusion of pituitary extract prevents loss of luteal function following hypophysectomy (Kaltenbach et al., 1968). However, purified pituitary extract high in LH activity required concurrent administration of prolactin in order to maintain luteal function in hypophysectomized ewes (Denamur et al., 1973). Administration of antisera to bovine LH during the period of luteal development decreased luteal weights and concentration and

content of progesterone of corpora lutea. However, the same treatment during late diestrus resulted in lengthening of the estrous cycle. While LH is critical for luteal development it may also be involved in luteal regression due to support of follicular development (Snook et al., 1969). In vitro, LH has been demonstrated to be the primary luteotropin of ewes (Kaltenbach et al., 1967).

Concentrations of progesterone in blood have been shown to fluctuate during diestrus yet no alteration in concentrations of LH have been associated with these changes (Rahe et al., 1980; Roche and Ireland, 1981). However, techniques in handling, storing and processing blood samples can alter assayable concentrations of progesterone (Vahdat et al., 1981) and account for some variation.

Changes in numbers of receptors or binding characteristics for LH on luteal cells may account for variation in secretion of progesterone. However, Diekman et al. (1978a) reported no change in affinity of receptors for LH on luteal cells during diestrus in ewes. No significant changes in numbers of receptors occurred during mid-diestrus. However, comparisons were of data pooled within day and may have masked transient changes. Suter et al. (1980) demonstrated that large doses of LH increased total numbers of receptors for LH in luteal tissue associated with increased production of progesterone in ewes.

Receptors for LH associated with lysosomes were demonstrated to have similarities to those on plasma membranes. However, because some characteristics were different, it was suggested that these receptors were of different origins (Rao et al., 1981). The possibility that differences in characterisitics were due to the dynamic process of receptor degradation by lysosomes was not tested. Characterization of

receptors for the luteolytic substance prostaglandin $F_{2}\alpha$ ($PGF_{2}\alpha$) in luteal tissue of cows have been reported. Compared to other subcellular fractions, there were greater numbers of receptors for PGE_{1}, $PGF_{2}\alpha$ and LH in both lysosomes and plasma membranes of luteal cells (Mitra and Rao, 1978). Binding of $PGF_{2}\alpha$ to lysosomal and plasma membranes increased from days 3 to 20 postestrus then declined by days 21 to 24 in cows. However, competitive binding characteristics of receptors for hCG were not different on days 13 and 20. Concentrations of progesterone in plasma were higher on day 13 postestrus than day 20. Thus, although a temporal association existed between secretion of progesterone and binding of $PGF_{2}\alpha$ to luteal cells, acute or chronic cause effect relationships have not been tested (Rao et al., 1979).

Exogenous estradiol causes luteal regression in cows (Kaltenbach et al., 1964; Greenstein et al., 1958) and ewes (Warren et al., 1973; Stormshak et al., 1969). Variations in circulating concentrations of estradiol have been recorded in cows during diestrus (Shemesh et al., 1972; Hansel and Ecternkamp, 1972; Dobson and Dean, 1974) and ewes (Scaramuzzi et al., 1970; Hauger et al., 1977). However, a direct relationship between changes in concentrations of estradiol and progesterone has not been demonstrated. Sheridan et al. (1975) did demonstrate variations in uptake of estradiol into luteal tissue during the luteal phase of the ovine estrous cycle. To date, there is no evidence that acute or transient changes in receptor characteristics of trophic or lytic substances occur during diestrus in cycling cows or ewes. It is possible that changes in concentrations of gonadotropins and luteolysins in blood interact with transient changes in their receptors to influence luteal function. This theory would be hard to test because it is likely

that each animal differs slightly in those parameters such that pooling data from different animals would result in no significant change in pattern.

The mechanism by which progesterone is secreted from corpora lutea may influence fluctuations in circulating concentrations of progesterone in blood. Progesterone was shown to be characteristically packaged into distinct secretory granules within luteal cells and released by exocytosis (Gemmell et al., 1974; Willcox and Alison, 1982; Quirk et al., 1979). Sawyer et al. (1979) reported a relationship between formation and release of secretory granules and the secretory pattern of progesterone during the ovine estrous cycle. Furthermore, formation and release of secretory granules in vitro was enhanced by the addition of LH. Willcox and Alison (1982) reported the existence of a specific binding protein for progesterone within luteal cells. Condon and Pate (1981) found addition of serum to potentiate release of progesterone, independant of synthesis, from bovine luteal cells in vitro. Thus, a factor contained in serum may influence secretion of progesterone. Adenosine has been shown to potentiate LH induced secretion of progesterone by rat luteal cells in vitro. Adenosine also antagonized the depressant effect of $PGF_{2}\alpha$ on production of progesterone (Behrman et al., 1982).

In summary, luteal function is dependant upon gonadotropic support, however, variations in circulating concentrations of progesterone do not appear to be due to changes in secretion of pituitary gonadotropins. Changes in packaging and release of progesterone as well as influences of luteolytic factors or balance of luteolysins and luteotropins may account, at least in part, for variations in secretion of progesterone. Additionally, collection, handling and storage of blood samples may

account for some variation of results of hormonal assays.

Regression of Corpora Lutea

Luteal regression involves a complete loss of production of progesterone (functional regression) and degeneration of luteal tissue (structural regression). Regression of corpora lutea begins around day 16 postestrus in cattle and around day 14 postestrus in ewes (Hansel et al., 1973). Donaldson and Hansel (1965) reported early aspects of luteal regression were associated with loss of cytoplasmic stippling and rounding of cell outline. Vacuolation was observed at the cell periphery followed by cytoplasmic condensation and pyknosis. Gemmell et al. (1974, 1976) reported decreased numbers of densely staining granules associated with decreased secretion of progesterone and appearance of autophagocytic bodies within luteal cells as the first morphological signs of luteolysis. These changes were observed as early as day 12 of an ovine estrous cycle. By day 15 postestrus degradation progressed to a point were cellular organization was lost. Lysosomal activity appeared increased and accumulation of lipid droplets were observed. The dramatic increased degenerative changes was temporally associated with final decline in concentrations of progesterone in blood of cycling ewes (Thorburn and Mattner, 1971). McClellan et al. (1977) reported lysosomal activity to increase in luteal tissue by day 15 postestrus in ewes. Dingle et al. (1968) reported total lysosomal enzyme content in corpora lutea of ewes not to change throughout diestrus, but fragility and enzyme activity of lysosomes increased during late diestrus. Rao et al. (1979) reported reduced binding of gonadotropin to bovine luteal membranes to occur after concentrations of progesterone

had declined in blood. However, Diekman et al. (1978a) and Spicer et al. (1981) reported a loss of luteal receptors for LH occurred concomitantly with luteal regression in ewes and cattle. In bovine corpora lutea, binding of $PGF_2\alpha$ increased during late diestrus (Rao et al., 1979).

Role of the Uterus in Luteal Regression

Hysterectomy during midcycle prolongs lifespan of corpora lutea in cattle (Wiltbank and Casida, 1956; Brunner et al., 1969; Anderson et al., 1962), in ewes (Wiltbank and Casida, 1956) and in many other mammalian species (Melampy and Anderson, 1968). In most studies the luteolytic effect of the uterus has been shown to be generated from the uterine horn adjacent to the ovary bearing a CL in both cows and ewes (Woody and Ginther, 1968; Ginther et al., 1967; Ginther, 1967; Mapletoft et al., 1975).

Integrity of vasculature between ovaries and uteri was necessary for luteal regression (Dobrowolski et al., 1970). Vascular anatomy of sheep and cattle, species in which the uterine luteolytic effect is unilateral, had ovarian arteries in close apposition to ovarian veins. This arrangement was not evident in mares, an animal in which the luteolytic effect of the uterus is not local (DelCampo and Ginther, 1973; Ginther and DelCampo, 1974). Blood taken from uterine veins of ewes on day 15 postestrus caused premature luteolysis when infused into arterial supply of autotransplanted ovaries (McCracken et al., 1972).

Although the uterus is important for luteal regression, it may not be the only component or variable. In primates, luteal regression is independent of the uterus and is controlled principally within the ovary

(Karsch and Sutton, 1976; Auletta et al., 1978). In addiiton, hysterectomy does not consistently prolong CL in cows (Ward et al., 1976; Brunner et al., 1969; Anderson et al., 1962; Hansel and Seifart, 1967). Therefore, control of luteal regression in cows may be influenced by ovarian as well as uterine factors.

Prostaglandins and Luteal Regression

Prostaglandin $F_2\alpha$ induced luteal regression in pseudopregnant rats (Phariss and Wyngarden, 1969) and in most other mammals tested (see Horton and Poyser, 1976). Concentrations of $PGF_2\alpha$ have been observed to increase in utero-ovarian venous plasma of ewes during the period of luteal regression (Bland et al., 1971; Thorburn et al., 1972). Concentrations of prostaglandin forming cyclooxygenase in ovine endometrial tissue increased during the period of luteal regression (Huslig et al., 1979). Increased content of $PGF_2\alpha$ in uterine endometrium and increased concentration of $PGF_2\alpha$ in ovarian venous blood occurred during the period of luteal regression in heifers (Shemesh and Hansel, 1975a). McCracken et al. (1972) reported infusion of $PGF_2\alpha$ into an ovarian vein of a ewe resulted in preferential transfer to the adjacent ovarian artery. Interestingly, first detection of veno-arterial transfer of $PGF_2\alpha$ was delayed relative to onset of infusion but transfer was sustained for 90 minutes following cessation of infusion. Hixon and Hansel (1974) reported peak concentrations of $PGF_2\alpha$ in ovarian arterial blood of heifers to occur 40 minutes after infusion into a uterine lumen.

Episodes of increased concentrations of $PGF_2\alpha$ in blood from both uterine and utero-ovarian veins of ewes were not always temporally correlated with each other or with progesterone and thus implicating a

potential ovarian source of $PGF_2\alpha$ (Nett et al., 1976). Hansel et al., (1976) demonstrated that bovine luteal and follicular tissues can synthesize PGF in vitro. Injection of $PGF_2\alpha$ into the largest follicle on the ovary bearing a CL caused premature luteal regression in ewes (Inskeep et al., 1975) and heifers (Fogwell et al., 1978). The mechanism of transfer of $PGF_2\alpha$ from follicle to CL involved a local (unilateral) yet extraovarian pathway (Fogwell et al., 1977). These observations raise the possibility that in addition to the uterus, ovarian factors may contribute to luteal regression.

Oxytocin and Luteal Regression

Administration of oxytocin early in an estrous cycle results in premature luteal regression (Hansel and Wagner, 1960; Anderson et al., 1965; Black and Duby, 1965). This luteolytic effect of oxytocin is dependant upon presence of the uterus (Ginther et al., 1967; Anderson et al., 1965). Administration of atropine or epinephrine inhibited the luteolytic effect of oxytocin but did not affect spontaneous luteal regression (Black and Duby, 1965). However, ewes immunized against oxytocin exhibited prolonged estrous cycles (Sheldrick et al., 1980). Newcomb et al. (1977) showed that injection of oxytocin early in the bovine estrous cycle resulted in increased concentrations of PGF in the posterior vena cava. This effect was not observed when oxytocin was administered after day 4 postestrus. In ewes, oxytocin injected on days 4 and 14 postestrus caused increased uterine secretion of $PGF_2\alpha$ but not on day 8 postestrus (Roberts and McCracken, 1976). Circulating concentrations of estrogen have been reported to be increased on days 3 and 14 but not day 8

of the ovine estrous cycle (Hauger et al., 1977). In anestrous ewes, oxytocin augmented estradiol induced increased release of PGF but in the absence of estradiol, oxytocin was without effect (Sharma and Fitzpatrick, 1974). Although inconclusive, these results are consistent with the concept proposed by Roberts and McCracken (1976) that estrogen and oxytocin interact to cause increased release of $PGF_2\alpha$ for the luteolytic process.

Estrogens and Luteal Regression

Administration of estrogen to cattle after midcycle causes premature luteal regression (Wiltbank et al., 1961; Niswender et al., 1965; Brunner et al., 1969). However, estrogen administered early in the estrous cycle has variable effects on luteal regression (Greenstein et al., 1958; Loy et al., 1960; Kaltenbach et al., 1964). Administration of estradiol on days 1 through 3 did not alter lengths of estrous cycles (Ginther, 1970). Piper and Foots (1965) reported estrogen given daily starting on day 4 postestrus extended luteal life span in ewes. Administration of estradiol on days 4 through 7 of the ovine estrous cycle lengthened cycles. Howland et al. (1971) reported surges of LH to occur in blood of ewes given a single injection of estradiol on day 4 but not day 11 postestrus. Thus, lengthening of estrous cycles following administration of estradiol early in estrous cycles may be due to increased luteotropic support. Moreover, estradiol may not provide any luteolytic influence without prior progestational "exposure". Administration of progesterone during metestrus facilitated estradiol-induced luteolysis on days 5 and 6 of the ovine estrous cycle (Warren et al., 1973).

Hysterectomy prevents the luteolytic effect of estrogen in ewes

Bolt and Hawk, 1972; Stormshak et al., 1969; Chakraborty and Stormshak, 1976). Although, Kaltenbach et al. (1964) and Brunner et al. (1969) reported reduced luteal weights following treatment of hysterectomized heifers with estrogen, the uterus was necessary for luteal regression (Brunner et al., 1969). Estradiol did however, facilitate luteolysis induced with $PGF_2\alpha$ in ewes (Gengenbach et al., 1977). Thus, both uterine and extra-uterine factors may influence luteal regression induced with estrogen in cattle and sheep.

Duration of treatment with estrogen may be more important to luteolysis than dose of estrogen administered. Luteal weights were reduced further by infusion of low amounts (10 ug/hr) of estradiol for 24 hours than by higher doses (41 ug/hr) for 12 hours. However, treatment for 48 hours was no more efficacious than 24 hour treatment (Bolt, 1974).

Infusion of estradiol into uterine arteries of ewes for 6 hours on day 14 postestrus resulted in increased secretion of $PGF_2\alpha$ into uterine veins but not days 6 or 10 postestrus. However, Ford et al. (1975) detected increased secretion of PGF into uterine venous blood on day 11 postestrus following injections of estradiol on days 9 and 10. This effect may have been due to two consecutive days of treatment with estrogen rather than only on day 10. Inhibition of prostaglandin synthesis with indomethacin prevented luteolysis induced with estrogen in cows and ewes (Lewis and Warren, 1974, 1977). Increased uterine content of prostaglandin specific cyclooxygenase occurred during late diestrus (Huslig et al., 1979) and inhibition of synthesis of RNA with actinomycin D prevented induction of luteolysis with estradiol (French and Casidy, 1973). Therefore, induction of luteolysis with estradiol is

mediated at least in part by increased uterine synthesis of PGF.

Progesterone and Luteolysis

Administration of progesterone during metestrus generally shortens estrous cycles in cows and ewes (Woody and Ginther, 1968). Hysterectomy prevents this effect of progesterone (Woody et al., 1967). Administration of progesterone during metestrus also facilitates estradiol-induced luteal regression on days 4 or 5 postestrus (Warren et al., 1973).

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

The concept that prostaglandins are involved in luteal regression is based primarily on:

1. Exogenous $PGF_{2}\alpha$ induces luteal regression in a manner similar to that observed histologically in spontaneous regression (McClellan et al., 1977).
2. Inhibition of synthesis of prostaglandin prohibits spontaneous luteal regressing (Lewis and Warren, 1976, 1977).
3. Elevated concentrations of $PGF_{2}\alpha$ in utero-ovarian venous blood are temporally associated with luteal regression (Land et al., 1976; Thorburn et al., 1972; Shemesh and Hansel, 1975a).

The mechanism by which $PGF_{2}\alpha$ exerts its luteolytic effect is still being investigated. There is no evidence that reduced concentrations of gonadotropins in blood are involved in spontaneous or $PGF_{2}\alpha$ induced luteal regression. Mechanisms still being investigated include reduction

in blood flow to CL, interference with binding and/or actions of gonadotrophin, activation of lytic enzymes and/or direct effect on luteal metabolism.

Blood Flow

Pharriss and Wyngarden (1969), aware of the venoconstrictive properties of prostaglandin $F_2\alpha$ and its abundance in uterine secretions, demonstrated exogenous $PGF_2\alpha$ to be luteolytic. Further studies have not been able to provide direct evidence that luteal regression is a result of diminished blood flow to luteal tissue.

The ovary bearing a CL has greater blood flow than the contralateral ovary. Moreover, the CL receives the major portion of ovarian blood flow (Niswender et al., 1975; Brown et al., 1980; Bruce and Moor, 1975). Einer-Jensen and McCracken (1976) reported that secretion of progesterone declined prior to reduced capillary blood flow through the CL. Rates of flow for blood through an ovary bearing a corpus luteum decreased only after production of progesterone declined in serum of ewes during luteolysis induced with $PGF_2\alpha$. However, when $PGF_2\alpha$ was infused in larger amounts into ovarian arteries, rates of flow decreased immediately by 50 percent (McCracken et al., 1972). Brown et al. (1980) reported a linear relationship between blood velocity in the artery supplying an ovary with a corpus luteum, plasma concentrations of progesterone and luteal weights. Velocity of blood in arteries supplying ovaries without corpora lutea did not change throughout ovine estrous cycles. In pseudopregnant rats, luteal blood flow did not change during 12 hour test period following administration of $PGF_2\alpha$. Flow of blood to ovarian interstitium increased

during this period and did not return to basal flow until after concentrations of progesterone in serum declined fully (Pang and Behrman, 1981). Redistribution of blood flow from luteal tissue to ovarian interstitium and follicles was also observed in rabbits following treatment with $PGF_{2\alpha}$ (Novy and Cook, 1973). Redistribution of blood flow could create an anoxic environment for luteal tissue and thus account for early morphological signs of luteal regression (Deane et al., 1966). Therefore diminished blood flow to luteal tissue has not been established as a cause or effect of luteal regression.

Role of Lysosomes in Luteal Regression

Dingle et al. (1968) observed increased lysosomal activity in luteal tissue taken from ewes at the time of luteal regression. McClellan et al. (1977) reported lysosomes increased in enzyme activity and packaging within luteal cells at the onset of spontaneous and $PGF_{2\alpha}$ induced luteal regression in ewes. Discrete receptors for $PGF_{2\alpha}$ have been detected in lysosomal membranes within bovine luteal cells (Rao et al., 1978). Receptors for LH, PGE, and $PGF_{2\alpha}$ were detected on lysosomal membranes and plasma membranes of bovine corpora luteal (Rao et al., 1978; Mitra and Rao, 1978). Affinity of bovine luteal receptors for $PGF_{2\alpha}$ increased at the time of luteal regression prior to alterations in binding of LH (Rao et al., 1979). Although lysosomes may play a role in degradation of cellular components during luteolysis, it is not known if lysosomes alter steroidogenesis during initial phases of luteal regression.

Effects of $PGF_{2\alpha}$ on Metabolism in Luteal Cells

Secretion of progesterone from luteal tissue incubated in vitro is reduced after 4 hours of exposure to $PGF_{2\alpha}$ (Demers et al., 1973; Henderson

and McNatty, 1975; Evrard-Herouard et al., 1981). Marsh (1971) observed an additive effect of PGE_2 and LH on luteal synthesis of cAMP and progesterone in vitro. Henderson and McNatty (1975) suggested that PGE_2 increased synthesis of progesterone through increased production of cAMP. Conversely, $PGF_2\alpha$ acted on the coupling component of the LH induced adenylate cyclase system, reducing cAMP and thus decreased synthesis of progesterone. Fitz et al. (1980) reported decreased luteal adenylate cyclase activity associated with decreased concentrations of progesterone in serum.

Diekman et al. (1978b) quantified receptors for LH in luteal preparations of ewes following treatment in vivo with $PGF_2\alpha$. No change in binding sites for LH were observed until after concentrations of progesterone in serum had declined. Similarly, characteristics of receptors for LH in bovine luteal tissue were not altered until after spontaneous luteal regression was near completion (Rao et al., 1979).

In summary, alterations in binding of gonadotropic to luteal tissue are not involved in initiation of luteolysis. However, $PGF_2\alpha$ may exert its effect in part by disrupting the adenylate cyclase system without affecting binding of LH. Although lysosomal activity increases during luteal regression and receptors for $PGF_2\alpha$ are associated with lysosomal membranes, a causative role of lysosomes in luteolysis has not been established.

Role of Development of Follicles in Luteal Regression

Two major roles of follicles are the production of oocytes and secretion of steroids (Richards, 1978). In view of the effects of estrogen on luteal function, characterization of follicular function throughout the

estrous cycle is warranted. During estrous cycles of heifers the proportion of atretic (degenerating) follicles to normal follicles remained constant within size category. Most (2/3) follicles were less than 3 mm in diameter in all stages of an estrous cycle. Follicles greater than 4 mm diameter comprised less than 11 percent of the total population of follicles. The number of non-atretic follicles varied greatly among heifers and no cyclic pattern was observed. However, the pattern of growth and atresia of the largest follicle demonstrated a tendency toward two waves of follicular growth during bovine estrous cycles (Rajakowski, 1960). Waves or peaks in follicular activity have also been described in ewes (Brand and deJong, 1973). Matton et al. (1981) reported enhanced ability of bovine ovaries to develop large follicles after day 15 postestrus compared to metestrus or early to mid-diestrus. Numbers of follicles less than 3 mm decreased from days 3 through day 13 postestrus whereas numbers of medium follicles (3 to 6 mm) increased on day 13 and 18 postestrus relative to days 3 or 8. Numbers of large follicles (>6 mm) did not vary among days tested (days 3, 8, 13 and 18 postestrus).

Choudary et al. (1968) reported no pattern of growth or atresia to occur during bovine estrous cycles and concluded that growth of follicles from one size class to another is continuous and independent of stage of cycle. Richards (1980) proposed a continuum of follicular growth and atresia occurs between surge of gonadotrophic at proestrus. Marion et al. (1968) theorized that all follicles that pass through a "competent" stage without estrous stimulation become atretic and are replaced by smaller developing follicles. Follicles can become atretic at any stage of growth.

The follicle destined to ovulate does not emerge as the largest

follicle until day 18 of the bovine estrous cycle (Dufour et al., 1973). Moor et al. (1978) reported the greatest concentration of estrogen in follicular fluid and greatest secretion of estrogen was associated with large non-atretic follicles. England et al. (1973) reported the largest follicle on the ovary bearing a CL contained more total estrogen and greater concentration of estrogen than the largest follicle from non-CL ovaries on day 14 of the bovine estrous cycle. However, the size of the largest follicle from ovary bearing CL and non-CL ovary do not differ at this time of the bovine estrous cycle (Matton et al., 1981). In ewes, presence of a CL has been shown to enhance follicular development (Dufour et al., 1971; Fogwell et al., 1977).

Luteal Function in the Absence of Ovarian Follicles

Karsch et al. (1970) first demonstrated that arresting follicular development by irradiation lengthened luteal function in ewes. Other studies in ewes confirmed these results (Hixon et al., 1975; Gengenbach et al., 1977). Additionally, Hixon et al. (1975) reported exogenous $PGF_2\alpha$ to be less effective in causing luteal regression in ewes with follices destroyed. Administration of estradiol benzoate or estradiol benzoate plus $PGF_2\alpha$ resulted in luteal regression in ewes with or without ovarian follicles. In a follow up experiment a larger dose of $PGF_2\alpha$ was effective in inducing luteolysis in ewes with or without follicles present. However, in hysterectomized ewes, estradiol benzoate with $PGF_2\alpha$ caused luteal regression with follicles present and reduction of progesterone in serum in the absence of follicles (Gengenbach et al., 1977). This was the first evidence in ewes that estrogen could facilitate luteal

regression in the absence of the uterus. Importantly, estradiol may affect luteal regression from a site in addition to the uterus.

Luteal life span has been reported to be increased in cows with visible follicles destroyed by cautery during late diestrus (Chupin and Saumande, 1979). Administration of $PGF_2\alpha$ or estradiol valerate reduced luteal lifespan and interval to estrus in cows with follicles destroyed by cautery (Chupin and Saumande, 1981). Definitive studies addressing the role of ovarian follicles in luteal regression in cows have not been conducted. However, based primarily on studies in sheep, it would appear that ovarian follicles contribute to the process of luteal regression.

Effects of X-irradiation on Ovarian Tissue

Numerous studies utilizing selective X-irradiation of ovaries have been conducted. Sublethal doses of X-rays cause selective changes in ovarian functions. Cells undergoing division are very sensitive to X-rays during metaphase and during the part of interphase involving synthesis of DNA. While large doses of X-rays can kill a cell, lower doses can affect nuclear chromatin sufficiently to "kill the nucleus" yet cytoplasmic metabolism continues (Baker and Neal, 1977).

The inherent lifespan of cells contributes to the metabolic manifestations of sublethal doses of X-rays on a tissue. Thus, the effects of disruption of cellular division might not detectably alter function of a tissue for a long period (Lacassagne et al., 1962). X-irradiation of adrenal tissue resulted in disrupted steroidogenesis over a ten week period (Berliner et al., 1964).

Corpora lutea were able to develop after X-irradiation of graafian

follicles in rabbits (Lacassagne et al., 1962). Whole ovary X-irradiation of ewes resulted in occasional incidence of follicles becoming luteinized and/or cystic (Gengenbach et al., 1977; Hixon et al., 1975). In ewes (Hixon et al., 1975; Karsch et al., 1970), in monkeys (Hobson and Baker, 1979) and in hamsters (Norman and Greenwald, 1971) whole ovary X-irradiation did not impair secretion of progesterone.

Whole ovary irradiation at midcycle in ewes resulted in nearly complete absence of follicles, complete impairment of follicular development and no histological evidence of altered corpora lutea (Karsch et al., 1970; Hixon et al., 1975; Gengenbach et al., 1977). However, a low incidence of follicular cysts which had luteinized was reported (Hixon et al., 1975; Gengenbach et al., 1977). In summary, based on the studies utilizing whole ovary X-irradiation, sublethal irradiation of ovaries containing fully functional corpora lutea effectively inhibits follicular development without impariing luteal development. Electrocautery of macroscopically visible follicles removes larger antral follicles but does not appear to inhibit further follicular development.

Summary of Review of Literature

1. Corpora lutea influence estrous cycle length.
2. Luteal lifespan is determined by variation in luteolytic factors, not by luteotropic factors.
3. The uterus is an important component in luteal regression.
4. Ovarian follicles and estrogens apparently contribute to luteal regression in part via the uterus.
5. Follicles and presumably estrogens are temporally associated with luteal regression.

This study is intended to address the following question. Is the presence of ovarian follicles after midcycle important to the luteolytic process in cows? Furthermore, by monitoring secretory patterns of LH, progesterone, estradiol and $PGF_{2\alpha}$ elucidation of the roles of these hormones in the luteolytic process is possible.

MATERIALS AND METHODS

General

This study was conducted from Fall 1978 through Winter 1979. At midcycle (days 10 or 11 postestrus), cows underwent surgery marking the beginning of the experimental period which ended on day 22 postestrus. In treated cows (X-irradiated, n = 6) follicles were destroyed by X-irradiation and cautery. Controls (sham-irradiated, n = 6) were manipulated similarly, but follicles were not destroyed. All cows received indwelling catheters into a jugular vein and both utero-ovarian veins in order to monitor hormonal patterns during the experimental period. Ovaries were removed on day 22 postestrus and returned to the laboratory for inspection and to weigh corpora lutea.

Animals

Non-lactating, parous Jersey and Guernsey cows were observed for estrus 3 times daily. After two or more estrous cycles of 19 to 24 days, cows underwent surgery at midcycle (day 10 postestrus n = 9, day 11 postestrus n = 2).

Surgery

Feed and water were withheld for 36 hours prior to surgery. Polyvinyl chloride (PVC) cannulae were installed and secured into a jugular vein to facilitate sampling of blood prior to and throughout the experimental period.

Anesthesia was induced with rapid intravenous administration of a 5% solution of glyceryl guaiacolate (Glycodex, Burns-Bioted Lab, Chromalloy Pharmaceuticals, Oakland, CA) and 0.2% thiamylal (Surital, Parke-Davis, Detroit, MI) to effect. Upon intubation, anesthesia was maintained with halothane (Fluothane, Ayerst Lab, New York, NY) by inhalation.

Cows were transferred to a custom built surgical cradle modeled after that described by Anderson et al. (1962) and maintained in dorsal recumbency. Following routine preparation of the abdomen, a midventral celiotomy was performed. The incision began at the pubis and extended 25 to 30 cm anteriorly. The reproductive tract was exteriorized and a uterine branch of both utero-ovarian veins was identified within the mesometrium. A 1 to 2 cm incision was made in mesometrium and tissue dissected down to the vein. After adequate isolation of the vein, it was grasped with vascular forceps and incised transversely. Sterilized PVC cannulae (Ico Rally, Palo Alto, CA) coated with 0.7 TDMAC-heparan comlex (Tri-docecylmethyl ammonium chloride, Polysciences, Inc. Warrington, PA) was placed into each vein and positioned to collect blood from the utero-ovarian vein prior to the vena cava. Cannulae were secured to the mesometrium, marked to identify left and right, filled with heparinized saline (400 u/ml, Ha-heparin US Biochemical Corp., Cleveland, OH) and exteriorized through the paralumbar fossa.

Treatment

Cows in the X-irradiated group had all follicles at the surface of each ovary destroyed by electro-cautery. For radiation, each ovary was isolated above a 1/8 inch thick lead shield to protect surrounding tissues. Ovaries were irradiated with a General Electric Maxitron - 300 X-ray machine

operated at 300 peak kilovolts (300 KvP) and 20 millamperes (20 Ma). Quality of the X-ray beam was 2.0 mm Cu half-value layer. Each ovary received a total dose of 1500 rads over a ten minute interval with a target distance of 50 cm. Ovaries of sham-irradiated (control) cows were exteriorized and held above the lead shield for 10 to 12 minutes and no follicles were cauterized.

Following routine surgical closure, cows were moved to tie stalls for the remainder of each cow's experimental period. Each cow received 6,000,000 units of procaine penicillin-G (300,000 u/ml, Pfizer, Inc., New York, NY) initially then 3,000,000 units twice daily for three days.

To preserve patency of utero-ovarian venous cannulae, sterilized saline (0.9% NaCl) with heparian (100 u/ml) and penicillin-G (Na-penicillin-G, Sigma Chemical Corp, St. Louis, MO, 1,000 u/ml) was infused continuously through each cannula at the rate of 10 ml/hour with a constant infusion apparatus (Harvard Apparatus Co., Cambridge, MA) for the duration of the experiment or until patency of cannulae was lost.

Sampling of Blood

Five jugular venous samples were taken 15 minutes apart to characterize patterns of secretion of LH one hour prior to anesthesia and three times daily for three days after treatment. These samples were to provide control data on LH within and between groups of cows after treatment. Collectively, the five samples will be referred to as LH windows. LH windows were resumed three times daily on days 16 to 19 postestrus in order to determine potential differences in the secretion of LH between treatment groups during the period of anticipated luteal regression.

Jugular venous and utero-ovarian venous samples were taken every four hours for the duration of the experiment. Additional samples were

taken every hour for eight hours, once daily, on days 15 to 19 postestrus in order to characterize acute changes in the secretion of estradiol-17β and $PGF_2\alpha$ during the period of luteal regression. Each sample from each utero-ovarian vein was divided between two tubes. Blood in the tube marked for determination of $PGF_2\alpha$ was acidified with 0.1 ml of 0.1N HCl per ml blood to prevent synthesis of prostaglandins by platelets (Pexton et al., 1975b) Blood in the other tube was reserved for the determination of estradiol-17β. All samples were heparinized upon collection and stored in a refrigerator until centrifugation. Following separation by centrifugation, plasma was transferred to a new tube, labelled and stored frozen until assayed.

Quantification of Hormones

Plasma collected via jugular cannulae was reserved primarily for determination by radioimmunoassay of concentrations of LH (Convey et al., 1976) and progesterone (Louis et al., 1973). Concentrations of estradiol-17β were determined in plasma from blood samples taken from utero-ovarian veins throughout the experimental period and from jugular venous blood sampled on day 22 postestrus. Assay for estradiol was a modified version of that described by Oxender et al. (1977). Steroids were extracted from plasma with ether and the extract chromatographed with Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, NJ) as described by Butcher et al. (1974). To account for procedural losses, 5,000 cpm of ^{3}H-1,2,5,7-estradiol-17β were added to four additional plasma samples in each assay. These samples were extracted and chromatographed with unknowns and recovery was calculated as percentage of total radioactivity recovered. The interassay coefficient of variation was 10 percent. Plasma samples determined to

contain more than 25 pg/ml estradiol were reassayed for verification. Because of the specificity of the antibody and chromatographic procedure, results are expressed as pg of estradiol-17β per ml of plasma.

$PGF_{2}\alpha$

Concentrations of $PGF_{2}\alpha$ were determined in utero-ovarian venous plasma using the extraction procedure described by Pexton et al. (1975) and chromatography described by Lewis et al. (1978). This procedure effectively separates $PGF_{1}\alpha$ from $PGF_{2}\alpha$ thus making the assay specific for $PGF_{2}\alpha$ (Cornette et al., 1972; Stellflug et al., 1975). Results are expressed as ng $PGF_{2}\alpha$ per ml plasma. Percent recovery was determined in a manner similar to that for estradiol. Ability of the assay to measure 5 ng/ml, 10 ng/ml and 25 ng/ml was demonstrated. However, the coefficient of variation at 0.4 ng/ml was 32 percent. Therefore, surges in secretion of $PGF_{2}\alpha$ (values greater than 1 ng/ml) can be characterized but sensitivity was not sufficient to measure differences in basal concentrations of $PGF_{2}\alpha$ (values less than 1 ng/ml). Samples determined to contain more than 1 ng/ml of $PGF_{2}\alpha$ were reassayed to increase confidence in values above basal concentrations.

Ovariectomy

At the end of the experimental period (day 22 postestrus n = 10, day 20 postestrus n = 2) ovaries were removed from cows through a supra-vaginal incision. Corpora lutea were enucleated from ovaries and following removal of extraneous tissue, weighed to the nearest 0.01 g. Visible follicles were counted and their diameters measured. Ovaries from the X-irradiated group were sliced every 2 mm and inspected visually for

antral follicles. Presence of any visible follicles in ovaries which were cauterized and X-irradiated excluded that animal from the experiment.

Statistical Analysis

Differences in weights of corpora lutea between treatment groups were evaluated by students t test. Profiles of concentrations of progesterone were examined for parallelism between groups by least squares regression analysis using time as split plot. Differences between treatment groups in mean concentrations of estradiol-17β in utero-ovarian venous plasma on days 10 and 15 and in jugular venous plasma on day 22 postestrus were evaluated by students t test. Orthogonal contrasts were utilized to determine variations in mean concentrations of estradiol-17β in utero-ovarian venous plasma among days in control animals. Students t test was utilized to determine differences in mean concentrations of LH between treatment groups on each day.

RESULTS

Efficacy of Treatment

1) No evidence of follicles in ovaries of treated cow at the time of ovariectomy.

2) Cows were verified to be in luteal phase of estrous cycle at time of treatment. This assesment based on inspection of luteal tissue at time of surgery and concentrations of progesterone in blood. Criteria for inclusion of animals in results included: Of the six cows treated at midcycle with cautery of visible follicles and whole ovary X-irradiation, five had no visible follicles at the time of ovariectomy. One cow had two follicles (14 mm and 8 mm) present in one ovary. Data from this animal were not included in the results. Another treated cow had experienced luteal regression prior to treatment. This assessment was based on concentrations of progesterone in blood samples taken prior to surgery. Therefore these results are based on four treated and six control cows.

Ovaries of four cows which were X-irradiated following cautery of visible follicles at midcycle had no visible follicles (< 2 mm) on day 22 postestrus. Prior to cautery of follicles and X-irradiation of ovaries concentrations of estradiol-17β in utero-ovarian venous plasma (Table 1) did not differ between groups. However, on day 15 postestrus cows in the X-irradiated group had lower concentrations of estradiol-17β in utero-ovarian venous plasma compared to the sham-irradiated group ($P<.025$) or compared to pretreatment levels ($P<.005$) (Table 1). Figure 1 illustrates

TABLE 1. CONCENTRATIONS OF ESTRADIOL-17β IN PLASMA AND INVENTORY OF FOLLICLES PRIOR TO AND FOLLOWING X-IRRADIATION OR SHAM-IRRADIATION.[a]

Treatment	n	Estradiol-17β (pg/ml)			Number of follicles ≥ 8 mm per ovary	
		Utero-ovarian		Jugular		
		Pre-treat[b]	Day 15	Day 22	Day 10	Day 22
Sham-irrad.	6	12.9±2.6	16.3±5.8	5.7±1.5	0.5±.2	1.5±.4
X-irrad.	4	11.1±1.5	5.6±0.7*	2.6±0.3**	0.6±.2	0.0

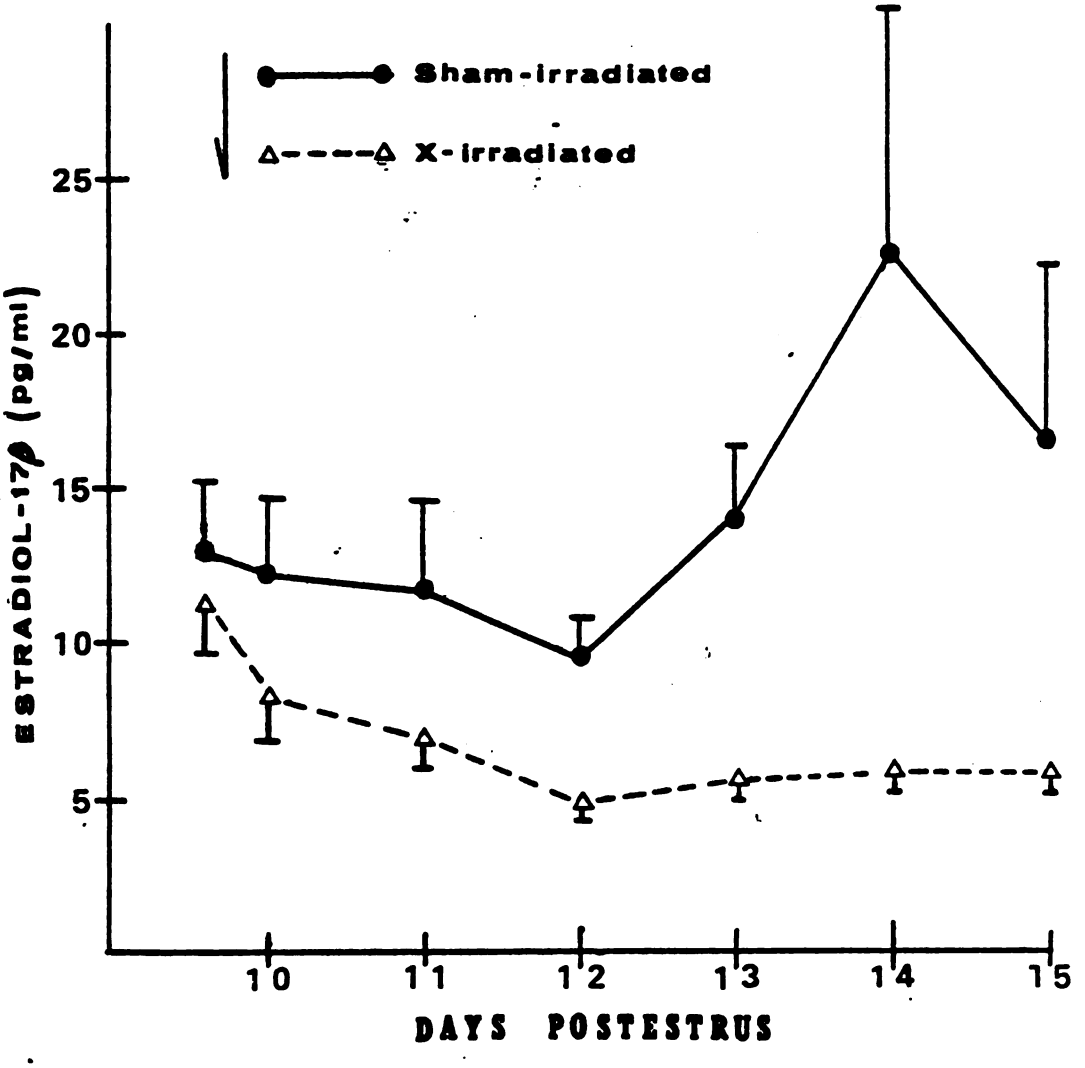
[a]Values given as mean ± standard error.

[b]Blood samples taken prior to electro-cautery of follicles or sham-cautery.

*Less than respective control value ($P<0.025$).

**Less than respective control value ($P<0.05$).

Figure 1. Effect of sham-irradiation or X-irradiation of ovaries on concentrations of estradiol-17β in plasma from utero-ovarian veins draining both ovaries. Values represent daily mean concentrations ± s.e. of all animals within treatment group. ✓ signifies time of treatment.


Sham-irradiated
X-irradiated
ESTRADIOL-17β (pg/ml)
25
20
15
10
5
10
11
12
13
14
15
DAYS POSTESTRUS

the daily mean concentrations of estradiol-17β in utero-ovarian venous plasma draining both ovaries of treated and control cows. Following destruction of follicles, lowest-concentrations of estradiol-17β were observed after two days following treatment ($P<.05$). Concentrations of estradiol-17β also declined in plasma from control cows over the same interval ($P<.005$) however, this trend reversed markedly after day 12. Days 12 to 15 postestrus, concentrations of estradiol-17β in X-irradiated cows remained low with very little variation, while concentrations of estradiol-17β in sham-irradiated cows increased.

Concentrations of estradiol-17β in jugular venous plasma on day 22 postestrus (Table 1) were lower in treated cows than in controls ($P<.05$). Estradiol-17β measured in serum from an ovariectomized cow was $2.2 \pm .38$ pg/ml which did not differ (Students t test, $P>.40$) from concentrations of estradiol-17β in jugular venous plasma in cows with no visible ovarian follicles on day 22 postestrus.

Based on these parameters, (reduction in secretion of estradiol-17β and absence of follicles) it is concluded that the combination of electrocautery and X-irradiation of ovarian follicles was effective in reducing follicular function and preventing follicular development.

Effects of Treatment on Concentrations of LH in Plasma

Mean concentrations of LH (Figure 2) in plasma did not differ between groups prior to treatment, within or between treatment groups on any day after treatment. The schedule for sampling was not adequate to examine secretory patterns of LH. It is therefore possible that differences in the pulsatile patterns of LH existed even though basal secretion of LH was not affected by treatment or stages of estrous cycle tested.

Figure 2. Effect of cautery of follicles and X-irradiation or sham-irradiation on concentrations of LH in jugular venous plasma. Values represent daily mean concentrations ± s.e. ↓ signifies time of treatment.

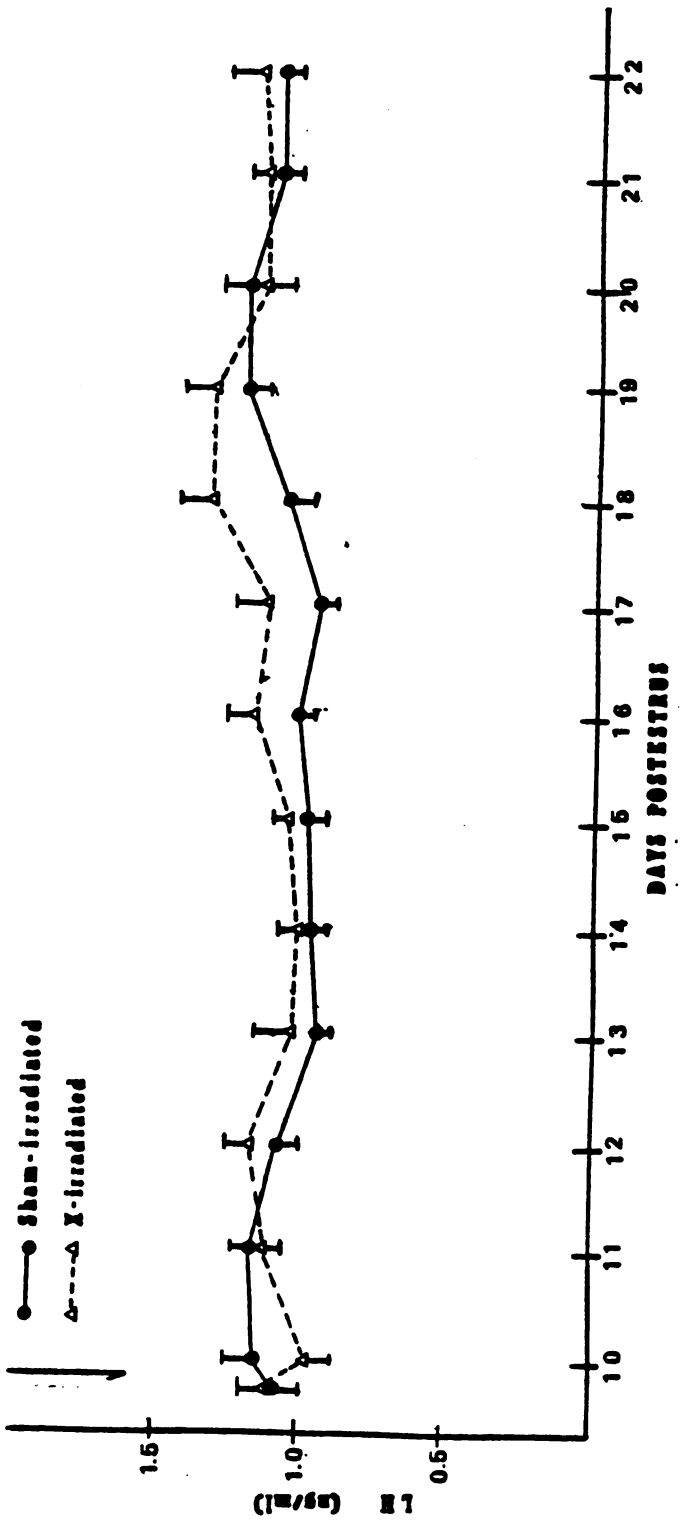

Sham-irradiated
X-irradiated
1.5
1.0
0.5
10
11
12
13
14
15
16
17
18
19
20
21
22
DAYS POSTSTRESS

TABLE 2. PLASMA PROGESTERONE AND WEIGHTS OF CORPORA LUTEA OF COWS ON DAY 22 POSTESTRUS, FOLLOWING X-IRRADIATION ON DAY 10.

Treatment	n	Progesterone (ng/ml)	Luteal weight (g)
Sham	5	$2.0 \pm .5^{a}$	$3.8 \pm .6^{c}$
X-irradiation	3	4.7 ± 1.0^{b}	8.3 ± 1.7^{d}

Values presented are mean $\pm$ standard error.

Values within a column with different superscripts are significantly different (ab, $P<.01$; cd, $P<.001$).

Effect of Treatment on Luteal Function

Based on comparisons of concentrations of progesterone in plasma of control and treated cows, there was no effect of X-irradiation on luteal function through day 20 postestrus. However, by day 22 postestrus mean concentrations of progesterone in plasma (Table 2) of sham-irradiated cows were lower than in X-irradiated cows ($P<.01$). In addition the profile (Figure 3) of concentrations of progesterone in plasma was maintained in treated cows but declined in control cows ($P<.001$). Luteal regression had not begun by day 22 postestrus in one control cow. All cows in the treated group had sustained luteal function through day 22 postestrus. Weights of corpora lutea removed from treated cows on day 22 postestrus were greater ($P<.001$) than those from control cows (Table 2). Based on these results, it is concluded that destruction of follicles prolonged luteal function.

Relationship of secretion of estradiol-17β to progesterone

Concentrations of estradiol-17β were determined in utero-ovarian venous plasma of five cows from day 10 postestrus through day 19 to determine if increased secretion of estradiol is associated with luteal regression. Profiles of estradiol-17β and progesterone are depicted in Figure 4. Based on analysis of variance, secretion of estradiol-17β increases after midcycle, prior to decline in progesterone ($P<.001$). The increase in estradiol-17β secretion is transient since by day 19 postestrus concentrations of estradiol-17β were not different from those observed at midcycle. Since estradiol-17β increased prior to decline in progesterone, these observations support the notion that estradiol, a product of follicular steroidogenesis, may mediate the effect of follicles in luteal regression.

Figure 3. Concentrations of progesterone in jugular venous plasma of cows following electrocautery of follicles, X-irradiation of ovaries or sham-irradiation.

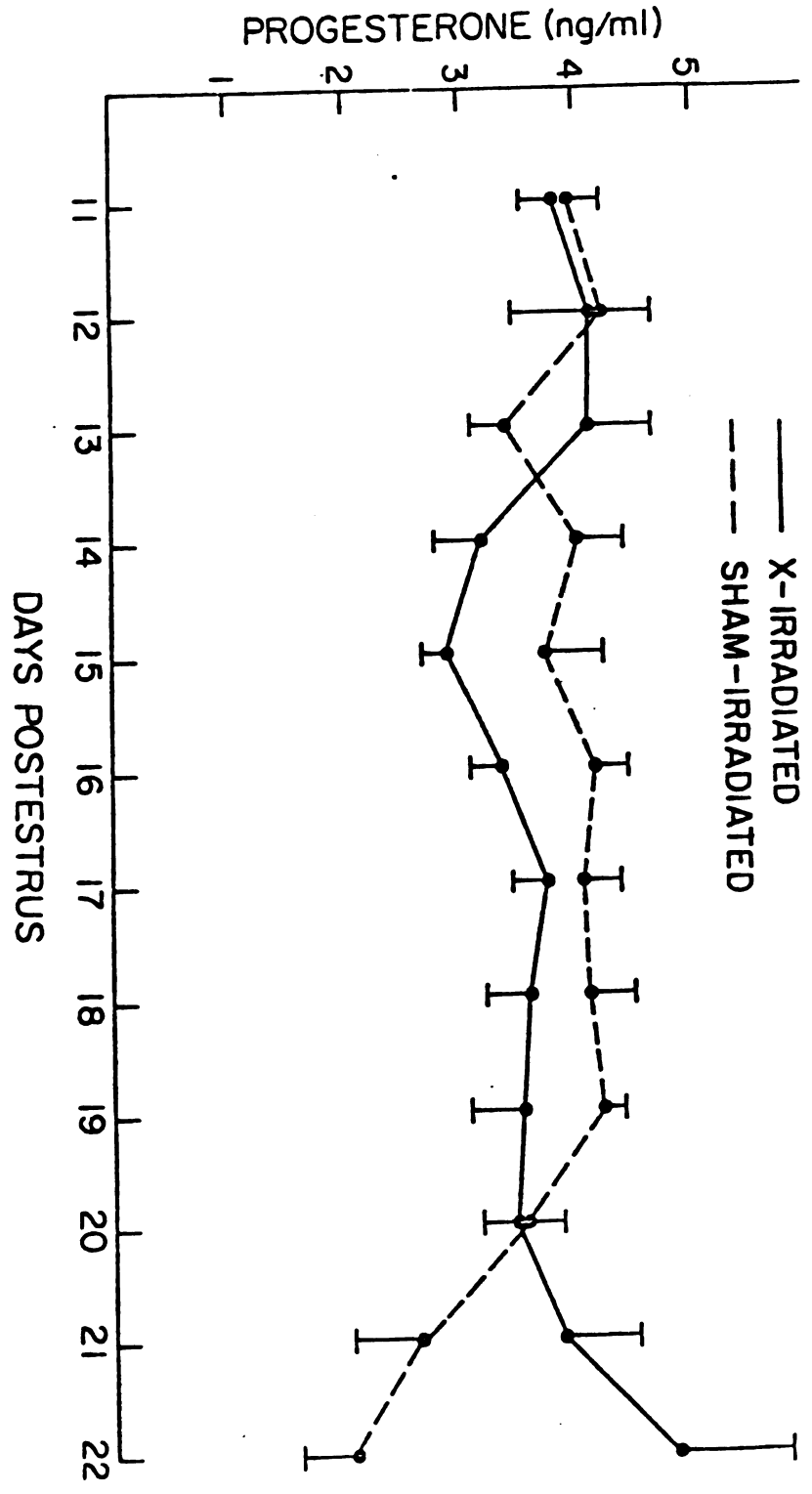
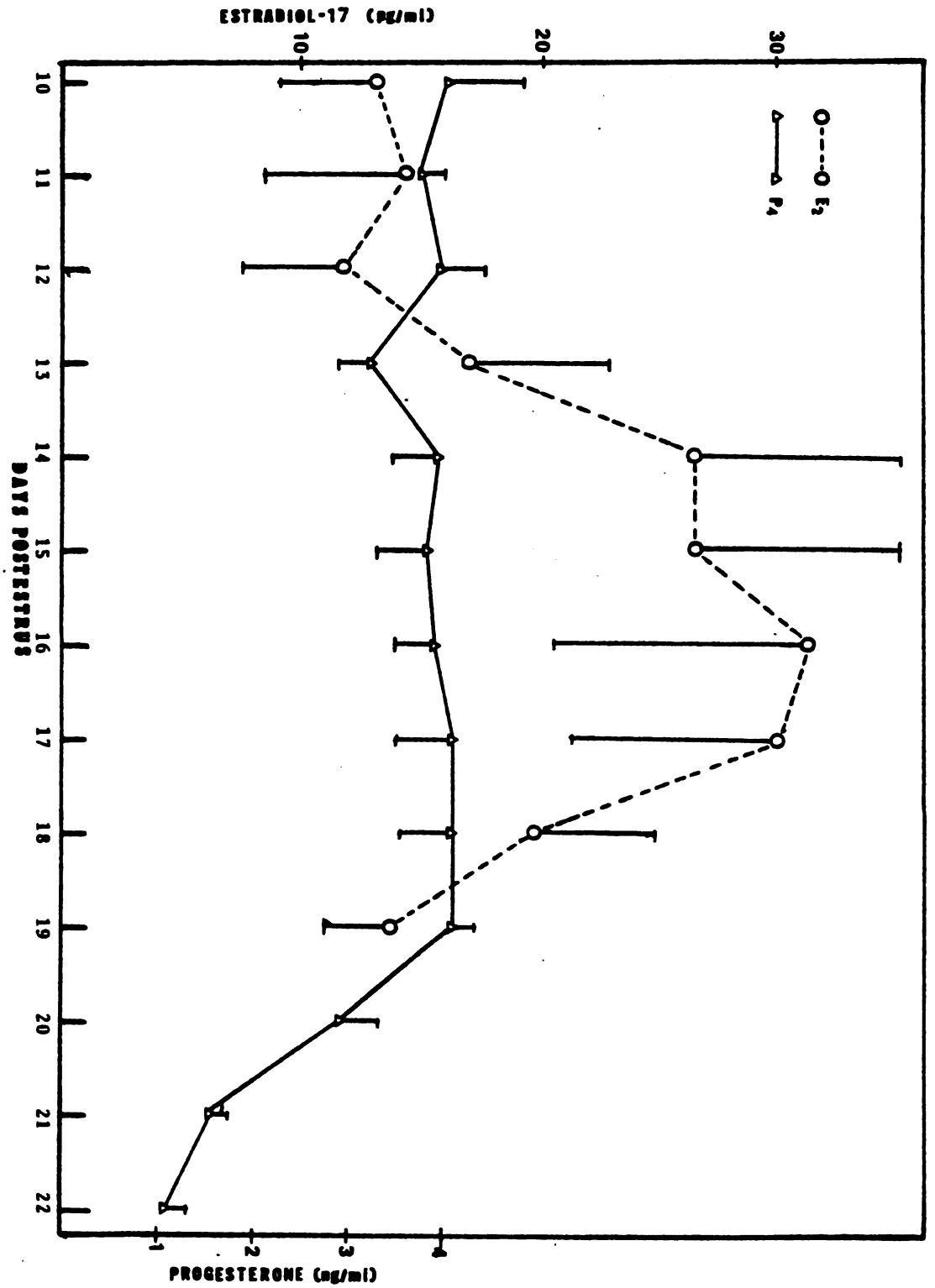

PROGESTERONE (ng/ml)
1
2
3
4
5
11
12
13
14
15
16
17
18
19
20
21
22
DAYS POSTESTRUS
X-IRRADIATED
SHAM-IRRADIATED

Figure 4. Concentrations of estradiol-17β in plasma from the utero-ovarian vein draining the ovary bearing a corpus luteum and progesterone from the jugular vein in five sham-irradiated cows which underwent luteal regression.


ESTRADIOL-17 (pg/ml)
10
20
30
DAYS POSTESTRUS
10
11
12
13
14
15
16
17
18
19
20
21
22
PROGESTERONE (ng/ml)
1
2
3
4
P4
E2

Relationships of Estradiol-17β, $PGF_{2}\alpha$ and Progesterone Characterized in Individual Cows

In three sham-irradiated cows most planned blood samples from the utero-ovarian vein draining the ovary with a CL were obtained. Estradiol-17β was quantified in all samples and $PGF_{2}\alpha$ was quantified in most samples. Luteal regression occurred in a normal fashion in two cows (Figures 5 and 6). One cow (Figure 7) did not undergo luteolysis as evidenced by high concentrations of progesterone in jugular venous plasma taken on day 22 postestrus. When luteolysis occurred (Figure 5 and 6), increases in concentrations of utero-ovarian venous estradiol-17β and $PGF_{2}\alpha$ were observed prior to and then concomitant with decline in progesterone (Figure 5 and 6). However, the cow in which no decline in plasma concentrations of progesterone occurred (Figure 7), utero-ovarian venous concentrations of estradiol-17β and $PGF_{2}\alpha$ did not increase. These observations are consistent with the hypothesis that follicles influence luteal regression via increased secretion of estrogen. In addition, increased secretion of $PGF_{2}\alpha$ occurred only after increases in concentrations of estradiol-17β.

During periods of frequent sampling (every hour), increased concentrations of estradiol-17β were not observed for more than two consecutive samples. Similarly, increased concentrations of $PGF_{2}\alpha$ were not observed in more than three consecutive samples. Thus, increased secretion of both estradiol-17β and $PGF_{2}\alpha$ occurs over very short intervals. These observations illustrate the importance of frequency of sampling blood to accurately characterize secretory patterns of estradiol or $PGF_{2}\alpha$.

Figure 5. Concentrations of estradiol-17β and $PGF_{2\alpha}$ in plasma from the utero-ovarian vein draining the ovary bearing a corpus luteum and progesterone from a jugular vein in cow number 6493.

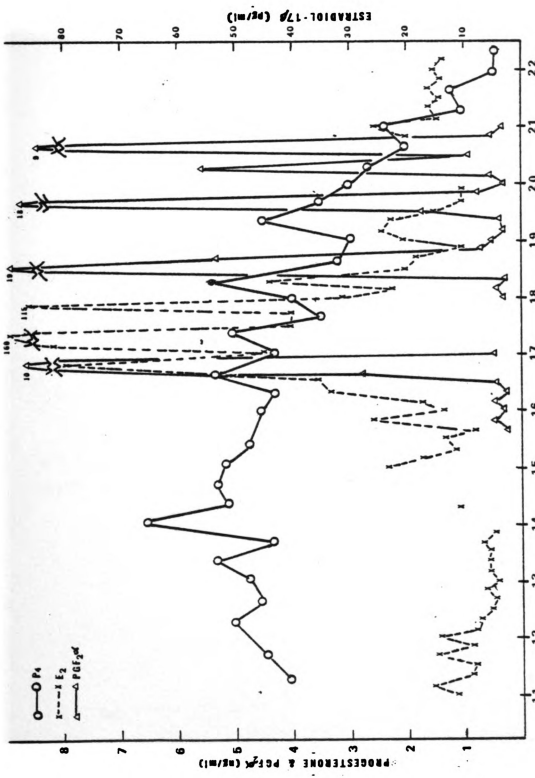

PROGESTERONE & PGF2α (ng/ml)
ESTRADIOL-17β (pg/ml)
DAYS POSTESTRUS
P4
E2
PGF2α

Figure 6. Concentrations of estradiol-17β and $PGF_{2}\alpha$ in plasma from the utero-ovarian vein draining the ovary bearing a corpus luteum and progesterone from a jugular vein in cow number 6592.

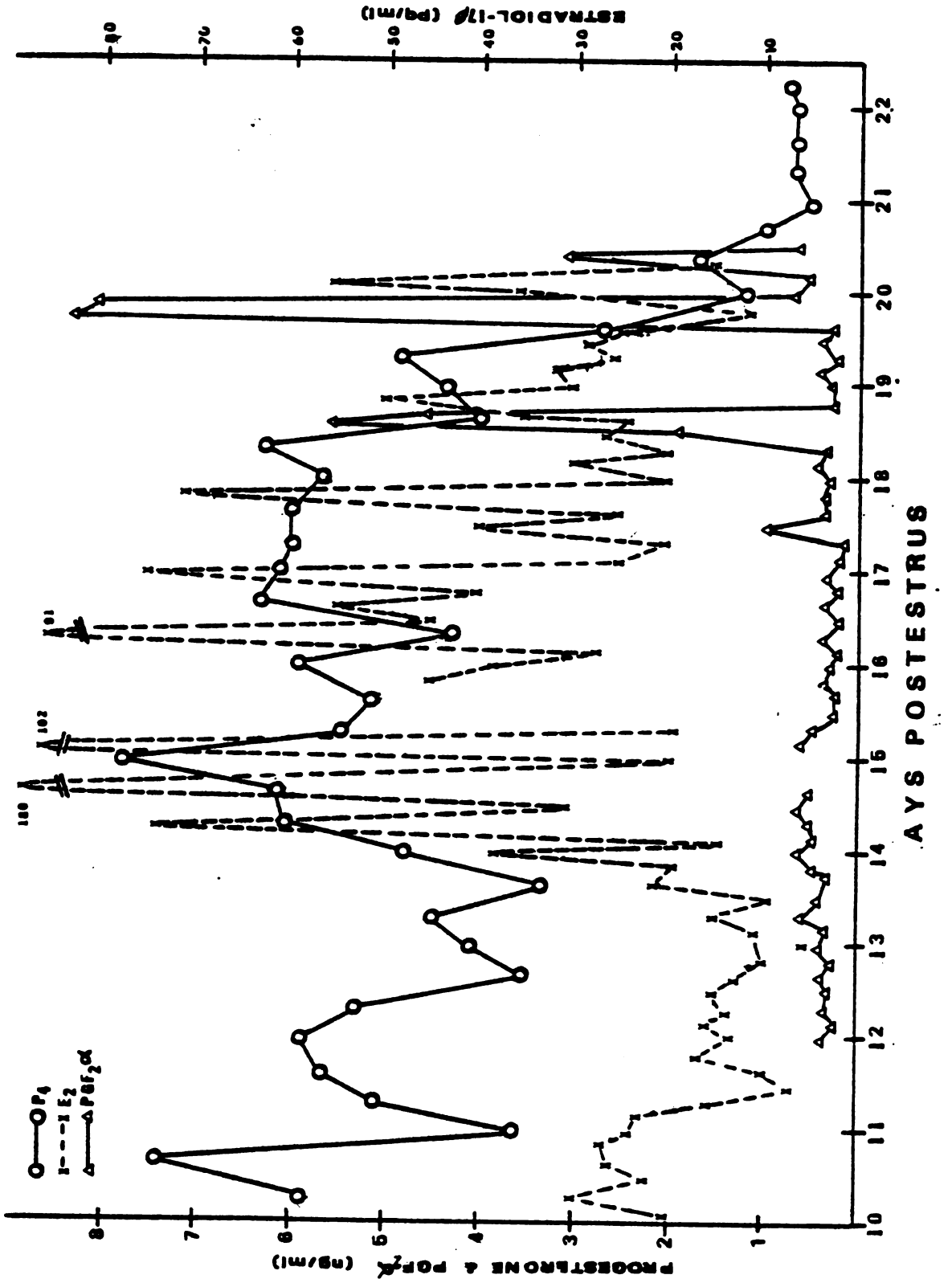
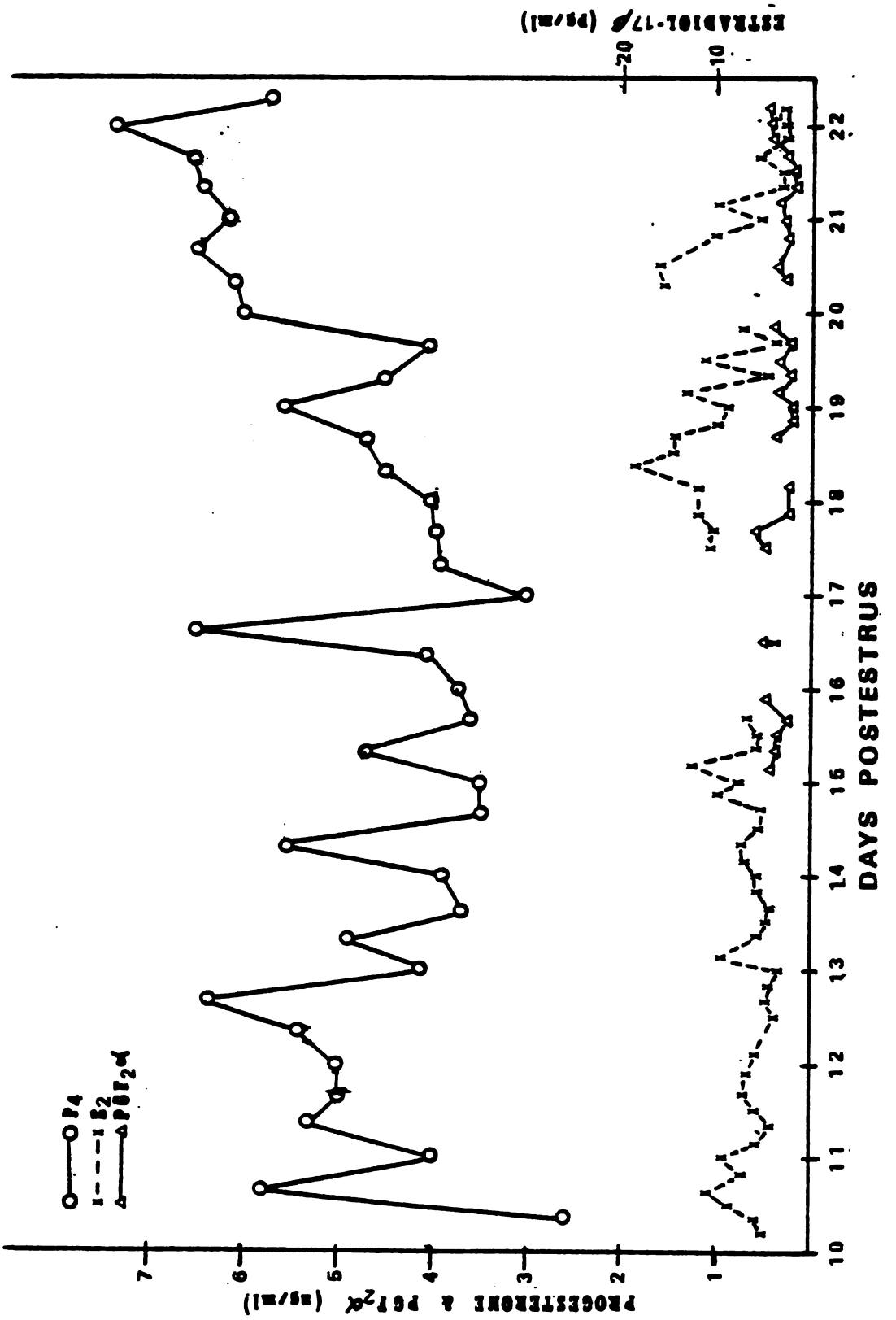

ESTRADIOL-17β (pg/ml)
PROGESTERONE & PGF2α (ng/ml)
DAYS POSTESTRUS
P4
E2
PGF2α

Figure 7. Concentrations of estradiol-17β and $PGF_{2}\alpha$ in plasma from the utero-ovarian vein draining the ovary bearing a corpus luteum and progesterone from a jugular vein in cow number 6585.


P4
E2
PGF2α
PROGESTERONE & PGF2α (ng/ml)
ESTRADIOL-17β (pg/ml)
DAYS POSTESTRUS

DISCUSSION

Electro-cautery of surface follicles and whole ovary X-irradiation was intended to destroy visible follicles and prevent further follicular development. Based on lower concentrations of estradiol-17β on days 15 and 22 postestrus and absence of visible follicles at ovariectomy on day 22 postestrus this basic goal was achieved. Studies utilizing isolated whole ovary X-irradiation in cattle have not been reported. Studies utilizing follicular cautery in cattle have been reported by Staigmiller et al. (1980) and Chupin and Saumande (1979). In ewes (Dufour et al., 1971) and in cows (Chupin and Saumande, 1979), electrocautery of follicles delayed but did not prevent onset of estrus. Hixon et al. (1975) and Gengenbach et al. (1977) reported inhibition of follicular development with whole ovary X-irradiation but luteinization of follicles occurred in some ewes. Lacassagne et al. (1962) reported luteinization of follicles following X-irradiation of rabbit ovaries. Luteinization was thought to have occurred in follicles present on the surface of ovaries at the time of irradiation. During development of techniques for this study, luteinized tissue was observed at a site where a follicle existed at the time of cautery and X-irradiation six days previous. It was concluded that cautery of follicles had to be complete and care was taken that minimal follicular tissue remained following electro-cautery. In the present study, luteinization of follicular tissue was not observed on any ovary removed on day 22 postestrus following electro-cautery and X-irradiation on day 10 postestrus. Apparently extensive electro-cautery of surface follicles

prevented luteinization of follicular tissue.

Because large non-atretic follicles are thought to be the primary sources of estradiol (Moor et al., 1978), it was expected that concentrations of estradiol-17β in utero-ovarian venous plasma would decline rapidly following electro-cautery and X-irradiation of ovaries, however the decline occurred over two days. Choudary et al. (1968) reported all follicles greater than 5 mm diameter (medium and large follicles) present during the luteal phase of cattle are atretic. Rajakowski (1960) reported most large follicles present at midcycle in heifers are atretic. In the present study there was no apparent relationship between presence of large (>6 mm) follicles on individual ovaries at the time of surgery and concentrations of estradiol-17β at midcycle (days 10 to 12 postestrus) were lower than between days 14 and 18 postestrus. Therefore, a likely source of estradiol secreted at midcycle would be small follicles (<2 mm) which might not have been cauterized, yet retained steroidogenic capacity for a period following X-irradiation. Even by day 15 postestrus, five days after treatment, concentrations of estradiol-17β in utero-ovarian venous plasma of treated cows were still greater than concentrations observed in jugular venous plasma on day 22 postestrus. Therefore, it appears that treatment effectively reduces but does not prevent secretion of estradiol. This observation is consistent with the goal of disrupting follicular development without cellular destruction.

Critical to understanding the role of follicles in luteal regression was determining whether or not secretion of the primary luteotropin, LH, was affected by treatment. Following initiation of this experiment, Rahe et al. (1980) reported high magnitude pulses of LH occurring every three to four hours during diestrus. Therefore, the one hour windows of frequent

sampling utilized in the present experiment would not suffice to characterize secretory patterns of LH. However, in this study mean concentrations of LH in jugular venous plasma did not differ between treatment groups on any day. Therefore, continued luteal function in treated cows was not due to increased basal secretion of LH.

Staigmiller et al. (1980) reported increased concentrations of LH in cows following cautery of follicles during proestrus. Beck et al. (1976) reported progesterone and estradiol were both necessary to suppress post-castrational increases in concentrations of LH in heifers. However, in those studies the magnitude of change in concentration of LH due to treatment was large enough to be detected with infrequent sampling of blood. Therefore, in the present study, the failure of secretion of LH to increase following follicular destruction may be due to continued presence of estradiol.

Comparison of concentrations of progesterone in blood of X-irradiated and sham-irradiated cows showed no detectable acute effects of irradiation on luteal function. This is in agreement with studies in ewes utilizing similar doses of X-irradiation (Hixon et al., 1975; Gengenbach et al., 1977). Ichikawa et al. (1968) used a higher dose of X-irradiation (2000 rads) on ovaries of ewes at midcycle without altering secretion of progesterone. Thus, based on unaltered profiles of progesterone after X-irradiation in the present study, an additional goal of irradiating ovaries without directly affecting luteal function was realized.

The primary objective of this study was to determine whether or not ovarian follicles, present after mid-cycle, affect luteal regression in cows. Based on greater luteal weights and greater concentrations of progesterone in plasma of cows with follicles destroyed than in controls, it is concluded the ovarian follicles are involved in the normal luteolytic

process. This effect was also observed in ewes (Karsch et al., 1970; Hixon et al., 1975; Gengenbach et al., 1977) in which follicles were destroyed by X-irradiation. Administration of LH during diestrus can prolong luteal function (Donaldson and Hansel, 1975a). However, Karsch et al. (1970) reported a six fold increase in circulating concentration of LH was required to extend luteal lifespan in ewes. Thus, in order for increased secretion of LH to prevent luteal regression large (easily detectable) increases may be required. The absence of obvious changes in secretion of LH in the present study precludes the pituitary as being involved in this process.

A potential role of follicles in luteal regression is increased secretion of estradiol during a period of luteal and/or uterine sensitivity to the luteolytic effects of estradiol. Secretion of estradiol-17β in control cows as relatively stable at midcycle but significant increases in secretion were observed after day 13 postestrus. This is the first conclusive evidence that secretion of estradiol increases prior to the onset of luteal regression in cattle.

Concentrations of estradiol-17β were determined in plasma from both utero-ovarian veins in each cow from days 10 through 15 postestrus. Interestingly, with cows in which comparisons could be made, secretory peaks of estradiol-17β (>25 pg/ml) were observed only in samples taken from veins draining ovaries containing a CL. These comparisons, although not definitive, are consistent with observations by England et al. (1973) that greater concentrations of estradiol are found in fluid from large follicles on ovaries bearing CL on day 14 postestrus in heifers than from contralateral ovaries. Additionally, Dufour et al. (1971) proposed that corpora lutea promote follicular development via a local (unilateral) mechanism

in ewes.

Karsch et al (1970) demonstrated a potential local effect of follicles on luteal function in ewes and Fogwell et al. (1978) observed a local, yet extraovarian pathway for the transfer of a luteolytic substance ($PGF_{2\alpha}$) from follicles to corpora lutea in ewes. Evidence in the present study that secretion of estradiol-17β from the ovary bearing a CL increases after midcycle, but prior to luteal regression, illustrates a temporal and local association between follicular steroidogenesis and luteal regression.

The duration and magnitude of observed peaks of estradiol-17β in utero-ovarian venous plasma may be masked in peripheral blood. Most studies have failed to detect increased concentrations of estradiol in jugular venous blood just prior to luteal regression. Thus, it would appear that if enhanced secretion of estradiol is involved in luteal regression, it does so via a local pathway in order to achieve higher concentrations of estradiol in responsive ovarian or uterine tissues. This pathway may be intraovarian or utero-ovarian. Responsive tissues include ovary and uterus. Cook et al. (1974) demonstrated that intraluteal injection of estrogen induced luteal regression in ewes. This effect could not be reversed with administration of LH systemically thus further implicating estrogen as being luteolytic locally.

The duration of increased secretion of estradiol prior to luteal regression may be important to luteolysis. Bolt et al. (1978) reported smaller doses of estradiol were luteolytic if administered over 24 hours versus larger doses over 12 hours. Although in the present study observed peaks in secretion of estradiol-17β were transient, lasting less than two hours, the interval in which peaks occurred lasted more than 24 hours.

Thus the average concentration of estradiol-17β in utero-ovarian venous plasma was in fact elevated for a period greater than 24 hours.

Interestingly, increased secretion of estradiol observed just prior to luteal regression was probably not associated with follicles destined to ovulate. In cattle, follicles destined to ovulate are not thought to emerge as the largest follicle until day 18 postestrus, 3 to 4 days prior to estrus (Dufour et al., 1973). In the present study, four of five cows in the control group ovariectomized on day 22 postestrus had the largest follicle present on the ovary not bearing a CL. Whereas, increased secretion of estradiol-17β was observed in utero-ovarian venous plasma associated with ovaries bearing corpora lutea. Additionally, in cows in which a corpus luteum regressed, concentrations of estradiol-17β in utero-ovarian venous plasma were increased only transiently, having returned to basal concentrations by day 22 postestrus. These observations do not preclude the possibility that follicles influencing luteal regression are from a pool of follicles from which the ovulatory follicle emerges (Brand and deJong, 1973). Yet a discrete increase in secretion of estradiol occurs prior to luteal regression and does not contribute directly to proestrous secretory patterns of estradiol-17β. Importantly, this increase in secretion of estradiol may be crucial and unique to initiation of luteal regression.

Characterization of acute changes in secretion and temporal associations of $PGF_2\alpha$, estradiol-17β and progesterone in individual control cows during diestrus may help pinpoint potential roles of these hormones in the luteolytic process. Secretory patterns of estradiol-17β and $PGF_2\alpha$ were characterized in two cows prior to and during luteal regression and in another cow which had not initiated luteal regression by day 22

postestrus. In the cows undergoing luteolysis, increased secretion of estradiol-17β was followed by increased secretion of $PGF_2\alpha$ prior to decreases in concentrations of progesterone. In the cow with prolonged luteal function no increases in estradiol-17β or $PGF_2\alpha$ were observed in utero-ovarian venous plasma nor were any follicles greater than 5 mm observed on ovaries removed on day 22 postestrus. These observations are consistent with the theory that ovarian follicles influence luteal regression by increased secretion of estradiol which directly and/or indirectly by increased synthesis and release of $PGF_2\alpha$ cause luteal regression. Lewis and Warren (1977, 1974) implicated synthesis of prostaglandin by the uterus as a mediator of estrogen induced luteolysis in cattle and ewes.

While most studies implicate the uterus as necessary for estrogen induced or spontaneous luteolysis, other studies indicate an intra-ovarian element of luteal regression may also be involved. Shemesh and Hansel (1975b) demonstrated synthesis of PGF from follicles and luteal tissue *in vitro*. In monkeys, exogenous estradiol induces luteolysis, at least in part through increased ovarian synthesis of prostaglandins (Auletta *et al.*, 1978). In ewes, uptake of estradiol into CL changes during diestrus (Sheridan *et al.*, 1975) and intra-luteal injections of estrogen caused luteolysis (Cook *et al.*, 1974). Binding proteins for estrogen are reported to exist in bovine luteal tissue (Kimball and Hansel, 1974). In hysterectomized ewes, administration of estradiol benzoate reduced the dose of $PGF_2\alpha$ necessary to induce luteal regression (Gengenbach *et al.*, 1977).

Although one should consider both ovarian and uterine contributions of $PGF_2\alpha$ in the luteolytic process, other studies gave evidence for luteal regression induced by estradiol independant of prostaglandin $F_2\alpha$.

Gengenbach et al. (1977) reported 3.5 mg $PGF_2\alpha$ to be luteolytic if given with estradiol benzoate to hysterectomized ewes with or without follicles present. Seven mg $PGF_2\alpha$ was luteolytic in four of four ewes with follicles present but luteolytic in only two of four ewes with follicles destroyed by X-irradiation. In cows with large (>4 mm) follicles destroyed by electrocautery, time from cautery to luteolysis was shortest if $PGF_2\alpha$ was given at the time of cautery or if given two days after treatment of cautery plus estradiol valerate. These observations lead one to consider estrogen and $PGF_2\alpha$ act synergistically in the luteolytic process. However, one should not rule out the possibility that the effect is merely additive with estrogen's contribution being stimulation of further synthesis of prostaglandin.

Although peaks in secretion of estradiol-17β and $PGF_2\alpha$ were observed in utero-ovarian venous plasma prior to decreased production of progesterone, no direct temporal association was apparent among these hormones. Robinson et al. (1976) found removal of endometrial tissue, a primary source of uterine $PGF_2\alpha$ resulted in greater concentrations of progesterone in blood of ewes during diestrus. In the present study, concentrations of progesterone in plasma were not different between control and treated cows until luteal regression began in controls. Moreover, variation in concentrations of progesterone in plasma was observed on all days in all cows independent of treatment. In the present study, concentrations of $PGF_2\alpha$ in utero-ovarian venous plasma of sham-irradiated cows did not vary from basal levels until late diestrus when significant elevations were observed. However, during this period variations in concentration of progesterone in jugular venous plasma occurred. Thus variations in concentrations of progesterone observed during diestrus are not always associated

with increased release of $PGF_2\alpha$ into utero-ovarian venous blood. Additionally, absence of significant changes in secretion of estradiol-17β between days 10 and 13 postestrus preclude estradiol-17β as a source of variation in luteal function during this period. Although not tested in the present study, it would be of interest to examine the temporal association between secretory patterns of LH and progesterone. Interpretation of data involving concentrations of progesterone in blood determined by radioimmunoassay is potentially confounded by the possibility that variations are in part due to storage and processing of blood samples (Vahdat et al., 1981).

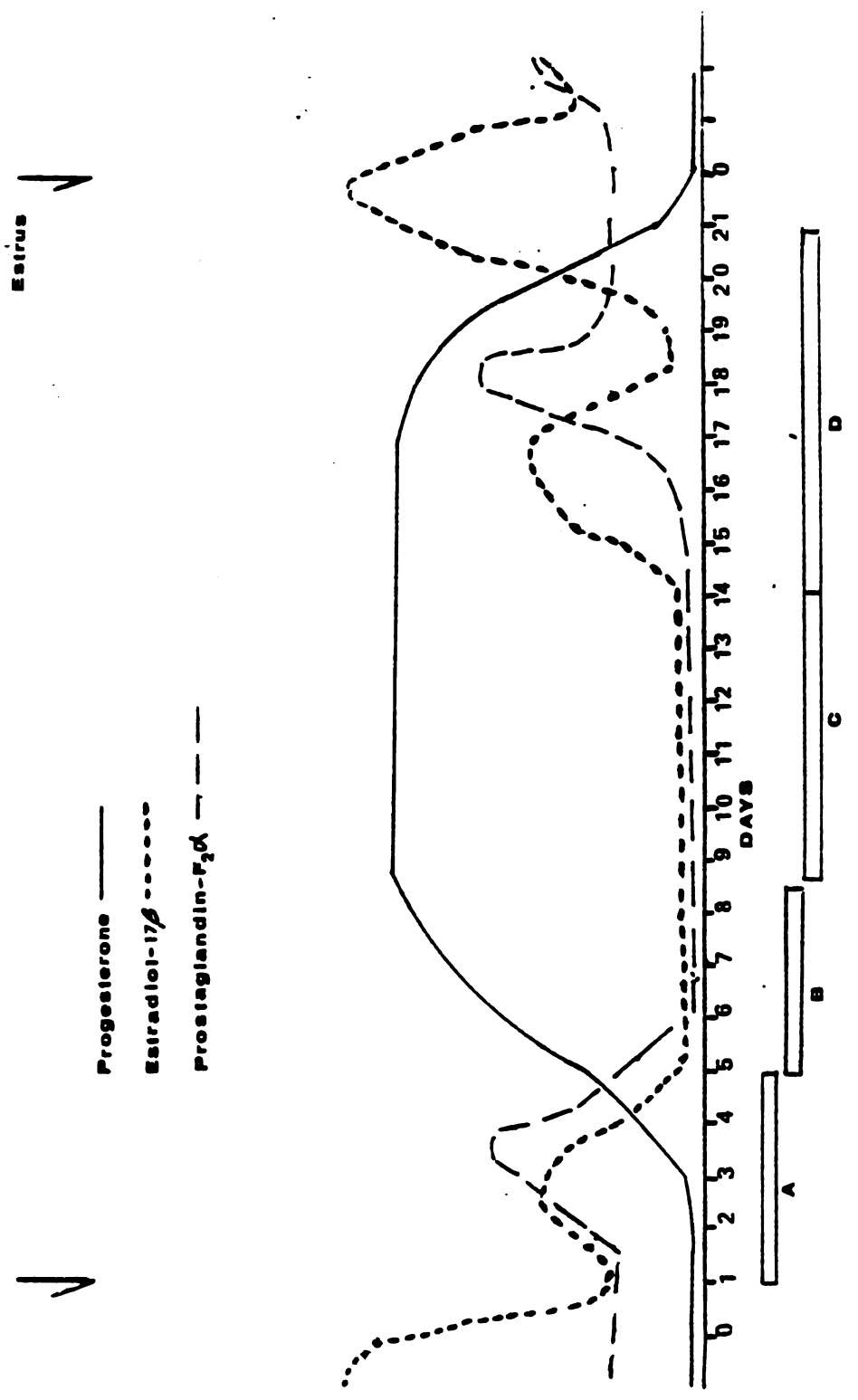
One cow in the control group failed to undergo luteal regression by day 22 postestrus. At ovariectomy (day 22 postestrus) no follicle greater than 5 mm in diameter were present on either ovary. No significant changes in secretion of estradiol-17β or $PGF_2\alpha$ were detected in utero-ovarian venous plasma from this cow. It would appear that failure of luteolysis was due to absence of follicles, no increase in secretion of estradiol and no increase in secretion of $PGF_2\alpha$.

The information learned from this study along with that reviewed can be synthesized into a model for the timing of luteal regression (Figure 8). Because the antiluteolytic effect of an embryo is not evident until mid to late diestrus, a period of luteal function, independent of conception is necessary for the reproductive process. Although secretion of estrogen and $PGF_2\alpha$ may be increased during metestrus, during early development (Period A) corpora lutea are resistant to the luteolytic influences of $PGF_2\alpha$ and estradiol. During days 5 to 8 postestrus (B) estradiol is not luteolytic due to its inability to stimulate synthesis of $PGF_2\alpha$. Exogenous $PGF_2\alpha$ is luteolytic during this period. Estradiol attains its luteolytic ability after day 8 postestrus (B and C) due to a period of progestational

influence. However days 8 to 14 (C) is a period of follicular estrogenic quiescence. After day 14 (D) postestrus, follicular steroidogenesis increases and secretion of estradiol is increased for 3 to 4 days. This extended (>24 hr) period of estrogenic influence is necessary for luteolysis. The luteolytic effect of estradiol is mediated at least in part through increased synthesis and release of prostaglandin. Significant increases in concentrations of $PGF_{2\alpha}$ in utero-ovarian venous plasma follow a period of increased secretion of estradiol.

In summary, luteal regression in cattle is initiated by increased steroidogenesis of ovarian follicles present after midcycle. Increased secretion of $PGF_{2\alpha}$ into utero-ovarian venous blood occurs after the increased secretion of estradiol and is followed by a decline in secretion of progesterone (luteolysis). The question remains as to what initiated follicular steroidogenesis observed to increase during late diestrus.

Figure 8. Hormones that influence luteal regression in cattle: A model of the bovine estrous cycle.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