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Title: **In Vivo** and **In Vitro** Effects of a Cyclopropenoid Fatty Acid on Progesterone Synthesis by the Ovine Corpus Luteum

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Two experiments were conducted to examine the effect of a cyclopropenoid fatty acid on luteal cell function. In Exp. 1, 12 mature ewes were mated to a fertile ram, assigned to two groups (n = 6/group) and laparotomized on day 18 of gestation. Ewes with corpora lutea (CL) in both ovaries were unilaterally ovariectomized while ewes with a CL in one ovary only were allowed to remain intact. An extract of Sterculia foetida seeds (1.09 mg), consisting of a mixture of fatty acid methyl esters including 750 ug of sterculic acid (SA), or 1.09 mg oleic acid methyl ester (OA) was injected into the artery supplying the ovary bearing CL. Jugular blood was collected on day 18 before surgery and daily thereafter until day 30 of gestation or until detected estrus, whichever occurred first. Sera were assayed for progesterone (P_4) by radioimmunoassay. In Exp. 2, 12 mature ewes were laparotomized on day 10 of the

estrous cycle and CL were removed, weighed and sliced for incubation. Corpora lutea from two ewes were pooled for each incubation. Slices of CL were preincubated in medium containing 145 ng/ml of S. foetida extract (100 ng/ml sterculic acid methyl ester) or 145 ng/ml oleic acid methyl ester (control) for 90 min. Then, slices of tissue were washed and reincubated in fresh medium containing 25 ug 22(R)-hydroxycholesterol/ml (0.079 nM final concentration) or 25 ug 5-pregnen-3ßol-20-one/ml (0.084 nM final concentration) for 120 min. Tissue plus medium were analyzed for P_4. Injection of SA or OA on day 18 of gestation caused a reduction in serum concentrations of P_4 within 24 h, after which concentrations of steroid remained low and relatively constant in control and those SA-treated ewes that remained pregnant until day 30 of gestation. Three of six ewes that were injected with SA exhibited estrus within 3 to 5 days after treatment. Serum concentrations of P_4 of SA-treated ewes differed from those of OA-injected control ewes ($P<0.01$). Luteal tissue subjected to SA or OA in vitro did not differ in ability to synthesize P_4 during subsequent incubation in the absence of precursor substrate (incubated controls). Relative to respective incubated controls, P_4 synthesis by tissue previously exposed to SA or OA was not altered by incubation in the presence of 22(R)-hydroxycholesterol. Presence of 5-pregnen-3ßol-20-one (pregnenolone) in the medium significantly increased P_4 synthesis by luteal tissue preincubated with SA or OA compared with that of controls. However, response of SA-treated tissue

was markedly less than that of tissue exposed to OA ($P<0.05$).

Results of this study suggest that SA can cause regression of CL in 50% of pregnant ewes. Apparently, the luteolytic effect of SA may be caused by its ability to interfere in the conversion of pregnenolone to P_4 by 3ß-hydroxysteroid dehydrogenase.

In Vivo and *In Vitro* Effects of a Cyclopropenoid Fatty Acid on Progesterone Synthesis by the Ovine Corpus Luteum

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INTRODUCTION

Development of a functional corpus luteum (CL) is required for the maintenance of pregnancy and normal menstrual or estrual cycles. Removal of the CL or administration of any agent that impairs luteal function during early gestation results in abortion. Results of a preliminary study indicated that cyclopropenoid fatty acids (CPFA), which are naturally occurring plant lipids, can inhibit luteal function in ewes. Sterculic acid (SA) is believed to be the most biologically active of these CPFA and has been shown to be carcinogenic and adversely affect growth and reproductive processes in various animal models. Because of the previously demonstrated action of the SA on the CL, treatment with this fatty acid provides a novel way to gain further understanding about the regulation of luteal function. These studies are important in the investigation of luteal insufficiency and reproductive success in domestic animal populations. Therefore, the aim of this research was to determine whether sterculic acid, one of the two CPFA extracted from *Sterculia foetida* seed oil, alters the synthesis of progesterone by CL of nonpregnant and pregnant ewes.

Factors that regulate corpus luteum function during the estrous cycle and early pregnancy, as well the source, structure, biological effects, and metabolism of CPFA will be reviewed.

FORMATION AND MORPHOLOGY OF THE CORPUS LUTEUM

The corpus luteum arises from the proliferation and hypertrophy of theca and granulosa cells of the follicle after ovulation. Prior to ovulation under the influence of luteinizing hormone (LH), a series of morphological and biochemical changes begin to occur in the theca interna and membrana granulosa cells. This process termed luteinization, is characterized by hypertrophy of granulosa cells accompanied by dispersion of nuclear chromatin, and formation of a nucleolus (McClellan et al., 1975). Thirty to forty hours after the ovulatory surge of LH, the amount of smooth endoplasmic reticulum, which is necessary for normal biosynthesis of steroids, increases, and concomitantly there occurs an increase in the number of mitochondria (Hay and Moor, 1975). As a result, these luteal cells are capable of synthesizing progesterone.

Based on the size of luteal cells, the corpus luteum of sows (Corner, 1919), rats (Pederson, 1951), cows (Donaldson and Hansel, 1965a), women (Guraya, 1971), and ewes (O'Shea et al., 1980) consist of two different cell types: large luteal cells with diameters of 22 to 40 um (depending upon species) and small luteal cells of less than 22 um in diameter (Koos and Hansel, 1981). Both large and small luteal cells contain the elements of steroid secreting cells. Large luteal cells have a distinct nucleus, numerous mitochondria, smooth

endoplasmic reticulum, Golgi apparatus, abundant membrane bound secretory granules, and highly convoluted cell surfaces. Small luteal cells have an irregularly-shaped nucleus, Golgi apparatus, and no secretory granules, but they do contain numerous lipid droplets. Like large luteal cells, small luteal cells possess abundant mitochondria and a smooth endoplasmic reticulum.

Donaldson and Hansel (1965a) reported that bovine luteal cells are derived both from granulosa and theca interna cells of the follicle, and that theca-derived cells can become large cells. Using highly specific labelled monoclonal antibodies to theca and granulosa cell surface antigens, Alila and Hansel (1984) reported that 77% of the large luteal cells bound the granulosa antibody (GrAb) on days 4 to 6 and thereafter declined as the age of the CL advanced. Only 14% of small luteal cells bound GrAb on days 4 to 6 of the cycle and none were labelled during subsequent stages. In contrast, using thecal antibody (TAb), the proportions of large cells that were labelled increased from 10% between days 4 to 6 to 46% on days 10 to 12. About 70% of the small cells were labelled between days 4 and 12 of the estrous cycle and decreased so that about 60% were bound between days 16 and 18. These data confirmed the results of the experiment by Donaldson and Hansel (1965a). Luteinizing hormone has been reported to promote the conversion of small luteal cells into large luteal cells (Cran, 1983; Farin et al., 1986). Moreover, the number

of granulosa-luteal cells remain constant throughout the estrous cycle because these cells do not undergo secondary mitosis following ovulation (Donaldson and Hansel, 1965a; McClellan et al., 1975).

The plasma membrane of the luteal cell has been recognized as a signal transducer in hormone-receptor interaction (Powell et al., 1974a; Hoyer and Niswender, 1985). Primary constituents of plasma membranes are glycoproteins, cholesterol and phospholipid. Zelinski and Stormshak (1983) and Zelinski et al. (1988) characterized the plasma membrane lipid of ovine CL. Phospholipid composition of luteal plasma membranes was found to be 50% phosphatidylcholine, 4 to 6% phosphatidylserine, 33% phosphatidylethanolamine, 4 to 6% phosphatidylinositol, 6 to 10% sphingomyelin and less than 4% cardiolipin.

Natural and induced luteolysis in cows (Carlson et al., 1982) and rats (Carlson et al., 1984) is associated with a reduction in membrane fluidity. It seems likely that lipids play a critical role in regulating membrane fluidity that is essential for ensuring coupling of the hormone and its receptors, internalization of the hormone-receptor complexes and exocytosis of secretory products. Principal fatty acids present in phospholipids of cow (Scott et al., 1968) and ewe (Waterman, 1980,1988) luteal tissue are palmitic (16:0), stearic (18:0), oleic (18:0), linoleic (18:2) and arachidonic (20:4) acids, with a few unsaturated fatty acids of chain

length greater than 20 carbons.

HORMONES SYNTHESIZED BY THE CORPUS LUTEUM

The primary hormone secreted by the corpus luteum (CL) in most mammals is progesterone. The CL has been reported to synthesize relaxin in women (Weiss, 1981), nonhuman primates (Walsh et al., 1974), sows (Sherwood and O'Byrne, 1974) and rats (Anderson et al., 1973a). Oxytocin is also produced in the CL of ewes (Wathes and Swann, 1982), cows (Wathes et al., 1983), women (Wathes et al., 1982) and monkeys (Khan-Dawood et al., 1984). Prostaglandins, hormones of uterine origin in some species, have been reported to be synthesized by the CL of women (Shutt et al., 1975) and nonhuman primates (Balmaceda et al., 1979).

PROGESTERONE

The main function of progesterone is to prepare the reproductive system for support of pregnancy. Progesterone can function successfully if cells of the reproductive organ have been exposed to estradiol, which induces the formation of progesterone receptors (Muldoon, 1980). Together with estrogen and anterior pituitary hormones, progesterone acts to promote lobulo-alveolar development of mammary glands during pregnancy (Tooper and Freeman, 1980), and transforms the uterine endometrium from a proliferative to a secretory state. More importantly, during the luteal phase of the cycle, a high level of serum progesterone has a direct

influence on the gonadotropin-releasing hormone pulse generator in the hypothalamus. As a result, pulses of luteinizing hormone (LH) are infrequent but are of higher amplitude than those observed during the follicular phase of the cycle (Yen et al., 1972; Santen and Barden, 1973; Foster et al., 1975; Baird, 1978). By this action progesterone regulates the occurrence of estrus in domestic and laboratory animals.

Progesterone is synthesized and secreted from both small and large luteal cells. Luteinizing hormone has been identified as the major luteotropin in the cow (Simmon et al., 1964; Donaldson and Hansel, 1965b), ewe (Kaltenbach et al., 1968; Fuller et al., 1970), women (Vande Weile et al., 1970), and monkeys (Hutchison and Zeleznik, 1984, 1985). Injection of luteinizing hormone (LH) into the jugular vein of cows on days 5, 10, 15 and 20 of the estrous cycle (Schomberg et al., 1967) or infusion of LH into the ovarian artery of ewes on day 9 of the estrous cycle (Niswender et al., 1976) increased the synthesis and secretion of progesterone. Moreover, increased <u>in vitro</u> progesterone production occurs in response to LH by bovine (Armstrong and Black, 1966; William and Marsh, 1978), porcine (Cook et al., 1967), and ovine (Kaltenbach et al., 1967) luteal slices, or by ovine luteal cell suspensions (Simmons et al., 1976). Luteinizing hormone stimulates progesterone synthesis by the small luteal cells, while large luteal cells spontaneously secrete progesterone at a high rate

(Niswender et al., 1985).

Progesterone is synthesized primarily from cholesterol derived from low or high density lipoproteins or via de novo synthesis of cholesterol from acetate in luteal cells. Depending upon the species, cholesterol from the liver is incorporated into low density lipoprotein (LDL) or into high density lipoprotein (HDL), and is transported to the luteal cells. The cholesterol of lipoproteins is liberated upon endocytosis and the action of lysosomal enzymes. Free cholesterol leaves the lysosome and is either esterified and stored as lipid droplets, used for steroid biosynthesis or used for synthesis of cell membrane constituents. Cholesterol is transported to the mitochondria where it is converted to pregnenolone by the cytochrome P-450 side chain cleavage complex (Ichii et al., 1963; Roberts et al., 1967). Pregnenolone is then converted to progesterone by 3ß-hydroxysteroid dehydrogenase in the smooth endoplasmic reticulum (Miller and Turner, 1963; Rubin et al., 1963; Niswender and Nett, 1988) and this latter steroid is secreted into the blood.

RELAXIN

Relaxin is a peptide hormone of approximately 6,000 molecular weight. Relaxin, like insulin, consists of dissimilar A and B chains linked by two disulfide linkages (Schwabe and McDonald, 1977; Bedarkar et al., 1977). However,

amino acid homology is less than 25% between relaxin and insulin (Schwabe et al., 1982).

Relaxin has been shown to be localized in the CL. Using the avidin-biotin immunoperoxidase method and an antiserum to porcine relaxin, this peptide hormone was detected in porcine CL from days 3 to 18 of the cycle (Ali et al., 1986), and during gestation (Anderson et al., 1973b). Relaxin has also been found in CL of cows during the third trimester of gestation (Fields et al., 1980, 1985), and in pregnant rats (Anderson et al., 1975; Anderson and Long, 1978), primates (Walsh et al., 1974), and women (Weiss et al., 1976; O' Byrne et al., 1978a,b; Mathieu et al., 1981).

Belt et al. (1970) employed electron microscopy to examine the fine structure of porcine luteal cells throughout gestation and after hysterectomy. Granulosa cells contained a larger Golgi apparatus, more granular and agranular endoplasmic reticulum and an immense number of membrane-limited dense bodies compared to the fine structure of granulosa cells during the estrous cycle. Electron microscopy of CL from rats on days 14 to 20 of gestation revealed an increased population of electron-dense, and membrane-bound granules that did not contain lysosomal enzymes (Long, 1973). In addition, the appearance and accumulation of dense granules in the cytoplasm of porcine luteal cells during early and midgestation, and the last day of gestation paralleled closely the rise and fall of ovarian vein relaxin concentrations (Belt

et al., 1971). These data suggest that the observed dense bodies or granules most likely represented stored relaxin in the CL.

Weiss et al. (1976) collected simultaneous peripheral blood and ovarian vein blood samples at term from women having cesarean sections. Relaxin concentrations were found to be four times higher in the ovarian vein draining the ovary, which contained the CL of pregnancy, than in either the peripheral vein or the contralateral ovarian vein. Relaxin was not detected in the sera of three ovariectomized pregnant monkeys (Weiss et al., 1981) or in sera from intact male or cycling female monkeys (Weiss et al., 1981) and women (O'Byrne et al., 1978a). Collectively, these data indicate that relaxin is a hormone of pregnancy, which in a majority of species, appears to be synthesized by the CL. The function of ovarian relaxin is not clear. Hisaw, (1926) was the first to report that relaxin caused relaxation of the pelvic ligaments in estrogen-primed guinea pigs. In primates, relaxin may act synergistically with estrogen and progesterone to promote the growth of the uterus, cervix and pubic symphysis (Hisaw and Hisaw, 1964). Intramuscular injection of relaxin into intact and luteectomized pigs (Kertiles and Anderson, 1979), and intracervical administration of relaxin to beef heifers (Musah et al., 1986) during late gestation induced cervical dilatation and relaxation of pelvic ligaments. Maximal opening of the pelvic canal of the cow

occurred between 12 to 36 hours after relaxin treatment. These data indicated that relaxin plays an important physiological role in preparing for the process of parturition.

An in vivo study was conducted to examine the induction of relaxin secretion in nonpregnant women by human chorionic gonadotropin (hCG). Injection of hCG on alternate days beginning on day 8 of the luteal phase of the menstrual cycle resulted in prompt rescue of the corpus luteum and an abrupt rise in progesterone secretion. However, relaxin was detectable only after 2 to 6 days of treatment (Quagliarello et al., 1980). Moreover, intramuscular injection of increasing doses of hCG into female monkeys twice daily for 10 days beginning during the early, mid- or late-luteal phase of the menstrual cycle stimulated relaxin synthesis and secretion (Ottobre et al., 1984). Addition of hCG to a monolayer culture of luteal cells obtained during early gestation and term pregnancy extended relaxin synthesis for an additional 2 days (Nixon, 1982). These data suggest that the secretion of relaxin could be induced by hCG.

OXYTOCIN

Oxytocin is a nonapeptide hormone containing internal disulfide bonds linking cysteine residues at positions 1 and 6, forming a ring structure that is necessary for biological activity. Oxytocin is known to be synthesized in the

hypothalamus and stored in the neurohypophysis. Several lines of evidence point to the corpus luteum as a second source of oxytocin in ewes (Wathes and Swann, 1982; Flint and Sheldrick, 1983a; Sawyer et al., 1986), cows (Wathes et al., 1983; Fields et al., 1983; Abdelgadir et al., 1987), women (Wathes et al., 1982; Khan-Dawood and Dawood, 1983), and monkeys (Khan-Dawood et al., 1984).

Ivell and Richter (1984) determined that the oxytocin gene is highly transcribed in the bovine CL during the early luteal phase of the estrous cycle. Luteal cDNA sequence analysis as well as cell-free translation studies have shown that luteal oxytocin mRNA is essentially similar to that in the hypothalamus, except that in the bovine CL the poly (A) tail of mRNA is shorter. Comparing the relative amounts of oxytocin per organ, the active CL produces 250 times more oxytocin mRNA than does the hypothalamus. Swann et al. (1984) demonstrated that ovine and bovine luteal cells synthesize oxytocin via a precursor protein similar to that found in the hypothalamus. Immunoprecipitation of cell extracts with anti-rat oxytocin-neurophysin followed by SDS-PAGE yielded two radioactive bands of 14kDa and 11-12kDa. Immunoprecipitation with anti-oxytocin yielded one band at 14kDa. Using SDS-PAGE the 14kDa band had a similar mobility to that of rat-hypothalamic oxytocin precursor. Thus, the synthesis of both hypothalamic and luteal oxytocin requires an approximate 14kDa precursor.

Immunoreactive oxytocin was demonstrated in large luteal cells of nonpregnant ewes, but not in CL of pregnant ewes (Guldenaar et al., 1984; Sawyer et al., 1986). Small luteal cells were not found to contain oxytocin at any stage of the cycle or gestation (Guldenaar et al., 1984). In addition, Rodgers et al. (1983) reported that ovine large luteal cells contained 30 times more oxytocin than small luteal cells. Oxytocin was reported to be localized in granules in large luteal cells of the ovine corpus luteum (Theodosis et al., 1986). These data indicate that large luteal cells are the source of oxytocin synthesis and storage. Oxytocin concentration in both ovine (Sheldrick and Flint, 1983a) and bovine (Abdelgadir et al., 1987) luteal tissue is maximal during the early luteal phase with peak levels occurring around day 8 of the cycle. Oxytocin secretion is stimulated by intramuscular injection of cloprostenol ($PGF_2\alpha$ analog) in ewes (Walters et al., 1983; Flint and Sheldrick, 1983b) and cows on day 12 of estrous cycle (Schallenberger et al., 1984). Injection of cows with $PGF_2\alpha$ can increase degranulation of luteal cells (Heath et al., 1983).

Apart from establishment of its role in lactation and labor, oxytocin is thought to be an important regulator of the estrous cycle. Injection of oxytocin into heifers on days 3 to 6 of the estrous cycle caused a reduction in the duration of the cycle (Armstrong and Hansel, 1959; Hansel and Wagner, 1960). This observation has been confirmed in the cow (Harms

et al., 1969) and goat (Cooke and Knifton 1981; Cooke and Homeida, 1982). Active and passive immunization against oxytocin extended the length of the cycle in ewes (Sheldrick et al., 1980; Schams et al., 1983) and goats (Cooke and Homeida, 1985).

Oxytocin concentration was measured in CL tissue obtained from six women at laparotomy (Wathes et al., 1982) and from monkeys (Khan-Dawood et al., 1984). The concentration of oxytocin in monkey CL was found to be higher than in human CL, and it was found to be highest during the midluteal phase of the cycle. However, oxytocin concentration in the human CL is four orders of magnitude greater than that in the peripheral circulation of nonpregnant women (Amico et al., 1981). There is some evidence suggesting that in primate CL oxytocin may have a local effect on steroidogenesis. Four milliunits/ml of oxytocin incubated with dispersed human luteal cells obtained from nonpregnant women produced a slight increase in basal progesterone production. However, 400-800 mU/ml oxytocin markedly inhibited both basal and hCG-induced progesterone production (Tan et al., 1982). Thus, these data are equivocal and need confirmation to determine whether oxytocin can serve as a paracrine regulator of steroidogeneis in human and lower primate CL. However, in sheep and cattle, oxytocin appears to be essential for regression of CL.

PROGTAGLANDINS

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and prostaglandin E_2 (PGE_2) have been reported to be synthesized by the human CL the during early and mid-luteal phase of the menstrual cycle (Challis, 1976; Shutt et al., 1975; Pathwardan and Lanthier, 1985).

Prostaglandin E_2 concentrations are constant throughout the menstrual cycle, and a positive correlation exists between the CL concentration of progesterone and PGE_2 during the luteal phase (Vijayakumar and Walters, 1983; Balmaceda et al., 1979). Moreover, PGE_2, like hCG, stimulates steroidogenesis in vitro in human CL slices (Marsh and LeMaire, 1974). These data suggest that PGE_2 may exert a tropic influence on luteal steroidogenesis.

In several animal species, there is strong evidence that uterine $PGF_2\alpha$ is the essential luteolytic agent. However, in the human, a luteolytic role of $PGF_2\alpha$ remains equivocal. Nevertheless, in the primate, ovarian $PGF_2\alpha$ may act as a luteolytic agent. Highest concentrations of $PGF_2\alpha$ in CL of the human (Vijayakumar and Walters, 1983) and other primates (Balmaceda et al., 1979) are found during the late luteal phase, coincident with the lower CL concentrations of progesterone.

Intravenous infusion of $PGF_2\alpha$ into women on days 4 to 10 postovulation did not shorten the length of the menstrual cycle (LeMaire and Shapiro, 1972; Jewelewicz et al., 1972;

Jones and Wentz, 1972). However, administration of $PGF_2\alpha$ every 2 hours for 10 hours on day 23 of the menstrual cycle depressed plasma concentrations of progesterone (Hillier et al., 1972). Moreover, direct intraluteal administration of $PGF_2\alpha$ produced only a transient suppression of progesterone secretion (Korda et al., 1975). In addition, Hamberger et al. (1979) and Denefors et al. (1982) demonstrated that LH- or hCG-stimulated cAMP and progesterone production by CL slices from the mid-luteal phase of the menstrual cycle could be counteracted by $PGF_2\alpha$. Morover, administration of ibuprofen ($PGF_2\alpha$ inhibitor) to cycling women at 2 to 5 days before menses had little effect on the duration of luteal phase (Gibson and Auletta, 1986). Although receptors for $PGF_2\alpha$ have been found in the human CL (Powell et al., 1974b), substantial evidence exists that $PGF_2\alpha$ may not be a relevant luteolysin in humans.

In nonhuman primates, subcutaneous injection of $PGF_2\alpha$ twice daily for 5 days beginning on days 11, 12 or 13 of gestation significantly depressed progestin levels (Kirton et al., 1970). Administration of $PGF_2\alpha$ into the ovarian artery of monkeys on day 22 of the menstrual cycle caused luteolysis (Auletta et al., 1973). Treatment of monkeys with indomethacin, a prostaglandin inhibitor, after diethylstilbestrol treatment during the early and mid-luteal phase, extended the luteal phase of the cycle (Auletta et al., 1976). Later, Auletta et al. (1984) administered chronic

infusions of $PGF_2\alpha$ at 10 ng/ml/hour directly into the CL on days 5 to 7 postovulation and produced premature functional luteolysis. Peripheral progesterone and estradiol concentrations were decreased, but serum LH concentrations were not altered. Therefore, Auletta et al. (1984) concluded that $PGF_2\alpha$ is luteolytic in the primate only when delivered locally to the CL and decreased secretion of LH is not a prerequisite for $PGF_2\alpha$-induced luteolysis. Nevertheless, how $PGF_2\alpha$ acts to bring about the regression of the CL is unknown.

In conclusion, it appears that $PGF_2\alpha$ is luteolytic in several domestic and laboratory animals as well as in nonhuman primates. The evidence for a luteolytic role of $PGF_2\alpha$ in the human is equivocal.

MAINTENANCE OF CORPUS LUTEUM FUNCTION

Hormones, usually of extrinsic origin, that prolong the life span of the CL and(or) stimulate luteal synthesis and secretion of progesterone *in vivo* and *in vitro* are called luteotropins. In most mammalian females the primary luteotropins that regulate luteal life span during the estrual or menstrual cycle are produced by the pituitary gland or the gonad. In some species luteal maintenance during gestation is ensured by a luteotropin produced by the conceptus. The luteotropic functions of two gonadotropins (luteinizing hormone and prolactin), estrogen and conceptus secretory proteins will be discussed in this section of the thesis.

LUTEINIZING HORMONE

Luteinizing hormone (LH) is the major endocrine factor responsible for regulation of the CL. This peptide hormone stimulates progesterone synthesis and at the same time maintains the structural integrity of the CL in most mammalian species (Hansel et al., 1973; Rothchild, 1981; Niswender et al., 1985). Continuous infusion of crude pituitary extract containing LH and follicle-stimulating hormone (FSH) into hypophysectomized pregnant (Kaltenbach et al., 1968) and nonpregnant ewes (Karsch et al., 1971) maintained luteal function. Donaldson and Hansel (1965b) reported that the life span of the bovine CL during the estrous cycle was prolonged

by exogenous LH. In addition, LH has been shown to prevent the luteolytic effect of oxytocin administered to heifers early in the cycle (Donaldson et al., 1965). Furthermore, luteinizing hormone increased ovine luteal synthesis of progesterone in vivo (Niswender et al., 1976) or when incubated with luteal cells in vitro (Kaltenbach et al., 1967; Simmons et al., 1976). Treatment of ewes with anti-LH serum decreased progesterone secretion and caused regression of the CL (Reimers and Niswender, 1975). Luteal function during the menstrual cycle in women (Vande Weile et al., 1970; Mais et al., 1986) and monkeys (Moudgal et al., 1971; Groff et al., 1984) is also dependent upon LH. Hutchison and Zeleznik (1984) reported that in the nonhuman primate the primary role of LH is to maintain steroidogenesis, while the structural integrity of the CL may be dependent upon other factors.

Due to their structural (Pierce and Parson, 1981) and functional similarities, LH and hCG bind to the same receptor site in gonadal tissue. Receptors for LH are localized in the plasma membrane of target cells (Rajaniemi and Vanha-Pertulla, 1972; Han et al., 1974; Anderson et al., 1979). There is controversy about the structure of the LH/CG receptor. Dattatreyamurty et al. (1983), Rapoport et al. (1984) and Ascoli and Segaloff (1986) reported that the LH/CG receptor is a glycoprotein and a noncovalently bonded oligomer probably consisting of two different chains joined by disulfide bonds.

Ascoli and Segaloff (1989) recently provided evidence indicating that the LH/CG receptor is composed of a single polypeptide rather than a more complex structure composed of several subunits. Anderson et al. (1979) reported that in rat luteal cells the majority of hCG binding sites are localized along the region of the cell surface facing capillaries, which is characterized by microvillus folds, whereas the basolateral surface of the luteal cells is characterized by junction complexes and contain few binding sites. Thus LH receptors appear to be concentrated in a specific region of the plasma membrane and not distributed uniformly throughout the entire cell membrane.

Once the corpus luteum begins to develop, the secretion of progesterone by the CL in women (Lee et al., 1973), rats (Hacik and Kolena, 1975) and cows (Spicer et al., 1981) appears to be highly correlated with the number of LH receptors. Diekman et al. (1978a) reported that the total number of LH receptors in ovine CL increases 40-fold between days 2 and 14 of the cycle. There was a sixfold increase in both number of receptors occupied by endogenous hormone and weight of the CL during the interval of the cycle studied. Further, a tenfold increase in serum concentration of progesterone occurred during the same period. However, less than 2.5% of the total number of receptors was occupied by endogenous hormone. On day 16 (late luteal phase) both total number of receptors and number occupied by endogenous LH had

decreased by 75%. During early pregnancy the number of total and occupied receptors were very similar to those observed during the midluteal phase of the cycle.

A variety of factors may influence the concentration of LH receptors in the ovary (Richards, 1979), but the primary factor appears to be LH itself. Exposure of luteal cells to high concentrations of LH or hCG invariably results in a dramatic loss (up to 90% in some cases) of LH receptors, a condition known as "down-regulation" (Conti et al., 1976, 1977; Harwood et al., 1978; Suter et al., 1980; Rodger and Stormshak, 1986). The loss induced by homologous hormone is time and dose dependent, and is accompanied by a concomitant loss in hCG-stimulated adenylate cyclase activity and (or) steroid production (Harwood et al., 1978; Catt et al., 1978).

The LH-receptor complex is internalized (Chen et al., 1977) via endocytotic vesicles (Chen et al., 1977; Anderson et al., 1979; Conn et al., 1987), and the hormone is subsequently degraded by lysosomal enzymes (Ascoli and Puett, 1978; Ahmed et al., 1981). Ascoli and Puett (1978) reported that chloroquine, a lysosomal enzyme inhibitor, blocks degradation of the hormone by target cells but does not reduce steroid secretion. This indicates that internalization of the hormone is a degradatory process rather than a mechanism of hormone action. The current working hypothesis is that the functional life of LH receptors in ovine luteal cells involves a single binding of hormone followed by internalization and

degradation of the hormone. The receptors appear to be subsequently recycled to the plasma membrane (Suter et al., 1984).

Prostaglandin $F_2\alpha$ also appears to be involved in regulating the number of luteal LH receptors in rats (Behrman et al., 1979). The number of LH receptors in rats administered a luteolytic dose of $PGF_2\alpha$ decreased dramatically. However, $PGF_2\alpha$ does not appear to have the same effect in ewes (Diekman et al., 1978b).

EFFECT OF LH ON STEROIDOGENESIS

The mechanism of action of LH on luteal cells has been shown to conform to the "second messenger" model. The second messenger hypothesis of hormone action was proposed in the early 1960s when adenosine 3',5' monophosphate (cyclic AMP) was found to activate glycogen phosphorylase by epinephrine and glucagon. Briefly, LH binds to its receptor in the luteal plasma membrane. The LH-receptor complex via coupling with a G_s protein (Gilman, 1984) activates adenylate cyclase (Condon and Black, 1976; Jordan et al., 1978). In turn, adenylate cyclase catalyzes the conversion of ATP to cAMP (Flockart and Corbin, 1982). The binding of cAMP to a protein kinase promotes activation of the catalytic subunit of the enzyme, which then phosphorylates endogenous proteins that are necessary for the synthesis and secretion of progesterone. Recent research indicated that LH incubated with a purified

preparation of bovine small luteal cells stimulated both cAMP production and activation of an inositol 1,4,5 trisphosphate-diacylglycerol (IP_3-DAG) transmembrane signalling system (Davis et al., 1989). Moreover, Hoyer and Kong (1989) reported that measurement of ^{32}P incorporation into proteins was indicative of stimulation of protein kinase C (calcium, phospholipid-dependent) activity in the soluble fraction of ovine luteal cells. These data suggest a possible role for C kinase in regulating steroidogenesis in ovine small luteal cells.

The LH-induced stimulation of steroidogenesis in luteal cells seems to occur via several mechanisms. The exact sites in the steroidogenic pathway where protein synthesis or phosphorylation is required have not been elucidated, but several have been suggested. Cholesterol is the initial substrate for luteal steroidogenesis. The release of cholesterol from pools of fatty acid ester is a possible target for the acute action of LH (Behrman and Armstrong, 1969). Results of <u>in vitro</u> studies suggest that cholesterol esterase is activated by cAMP-dependent protein kinase (Trzeciak and Boyd, 1974; Caffrey et al., 1979a). Therefore, this enzyme appears to be one important point for the regulation of steroidogenesis by LH. Activation of the mitochondrial cholesterol side chain cleavage complex by cAMP-dependent protein kinase has also been reported, and it has been suggested that the phosphorylated moiety is cytochrome

P-450 (Caron et al., 1975). This enzyme is crucial for steroidogenesis and is thought to be the rate limiting step in the steroidogenic pathway. The enzyme 3ß-hydroxysteroid dehydrogenase appears to be present in considerable excess in luteal tissue (Caffrey et al., 1979b) and is responsible for catalyzing the conversion of pregnenolone to progesterone. However, there is no evidence for acute regulation of 3ß hydroxysteroid dehydrogenase by LH.

PROLACTIN

Prolactin (PRL) is a 23,000 molecular weight protein hormone that is secreted by lactotropic cells in the pars distalis of all vertebrates. Alone or in combination with other pituitary gonadotropins, PRL is critical in promoting CL function in rats, mice and hamsters. However, PRL appears to be without effect in stimulating steroidogenesis by luteal tissue and plays no role in regulating luteal function during the estrous cycle in cows (Smith et al., 1957; Mason et al., 1962), ewes (Karsh et al., 1971; Niswender et al., 1976), and sows (Duncan et al., 1961). Prolactin has also been reported to have no role in regulating steroidogenesis by luteal tissue during the menstrual cycle in humans (Rice et al., 1964) and monkeys (Stouffer et al., 1980).

Rats, mice and hamsters are characterized by a short estrous cycle. Luteal function in these species is not dependent upon hypophyseal gonadotropins during early stages

of gestation or pseudopregnancy. Hypophysectomy of the rat (Smith, 1930) or hamster (Greenwald and Rothchild, 1968) shortly after ovulation, or hypophyseal stalk-section of the guinea pig (Illingworth and Perry, 1971) shortly after ovulation does not alter the normal life span of the CL.

Cervical stimulation by mating or similar artificial stimulation to induce pseudopregnancy in rats (Smith and Neill, 1976) stimulates the secretion of PRL. Prolactin supports luteal maintenance up to day 8 or 9 of pregnancy or pseudopregnancy. Sinha et al. (1978) reported that in the mouse, concentrations of PRL in the serum increased threefold within 60 min after cervical stimulation, which was sufficient to induce pseudopregnancy. Serum concentrations of prolactin also have been reported to be increased during early gestation in mice (Barkley et al., 1978). Moreover, injection of the PRL inhibitor 2-bromo-α-ergocriptine after cervical stimulation in the mouse reduced the incidence of pseudopregnancy (Sinha et al., 1978). These data suggest that PRL is the primary hormone involved in the induction and maintenance of pseudopregnancy and pregnancy in rats and mice.

Prolactin in hamsters apparently does not act alone to maintain pregnancy. Injection of PRL into pregnant hamsters that were hypophysectomized on day 4 of gestation for four days did not maintain the pregnancy. Pregnancy could be maintained, however, by concurrent injection of 100 ug ovine PRL and 10-200 ug ovine follicle-stimulating hormone (FSH;

Greenwald, 1967, 1973). Thus in the hamster the maintenance of CL function is dependent upon a minimal luteotropic complex of FSH and PRL. In addition, Illingworth and Perry (1971) reported that daily administration of PRL to hypophysectomized guinea pigs during the cycle promoted luteal maintenance, indicating that PRL is luteotropic in the nonpregnant guinea pig.

Evidence has been reported that PRL is not responsible for CL maintenance throughout gestation. Luteal function in the pregnant hamster (Mukku and Moudgal, 1975; Terranova and Greenwald, 1979) and mouse (Choudary and Greenwald, 1969; Mednick et al., 1980) becomes dependent upon LH at or near the time of implantation. However, in the rat, LH dependency occurs at or after day 8 of implantation (Morishige and Rothchild, 1974).

Information about the mechanism by which PRL can affect luteal function is very limited. Armstrong et al. (1970) reported that PRL may serve to prevent reduction of progesterone to 20α-hydroxy-4-pregnen-3-one in rat luteal cells, and PRL appears to be essential for maintaining the concentration of LH receptors in rat CL (Grinwich et al., 1976; Gibory and Richards, 1978).

ESTROGEN

Estrogen has been reported to be luteotropic in rats (Merckel and Nelson 1940) , pigs (Kidder et al., 1955; Gardner

et al., 1963) and rabbits (Robson, 1937, 1939).

RATS

Estrogen administration by daily injection or solid implant in rats at the beginning of the estrous cycle stimulated CL function (Everett, 1964). Daily subcutaneous injection of 10 ug estrone into hypophysectomized and hysterectomized rats on days 12 to 15 of gestation maintained both plasma and luteal progesterone at concentrations similar to those of intact pregnant rats. A single injection of 25 ug estradiol into hypophysectomized and hysterectomized rats on day 12 of gestation significantly increased plasma and luteal concentrations of progesterone but failed to restore plasma progesterone concentrations to the levels of intact pregnant rats (Takayama and Greenwald, 1973). In this latter study, rats were hypophysectomized and hysterectomized on day 12 of gestation. Administration of 100 ug estradiol/day or a high level of testosterone given via a silastic capsule to hypophysectomized and hysterectomized rats on days 12 to 14 of gestation increased serum estradiol concentration and the nuclear content of estradiol receptors in the CL (Gibory and Keyes, 1978). Serum progesterone from days 12 to 15 was maintained at a concentration similar to that in pregnant rats. However, administration of dihydrotestosterone (DHT), a nonaromatizable androgen, did not prevent the decline in serum progesterone after hysterectomy and hypophysectomy.

Testosterone treatment had no effect in hypophysectomized, hysterectomized and ovariectomized rats on day 12. These results suggest that estrogen formed by aromatization of androgen within the CL may play an important role in the regulation of luteal function during pregnancy in the rat.

Androgen concentrations in plasma of the mouse increased from 50 to 72 pg/ml on day 1 after mating and remained, relatively unchanged throughout day 8 of gestation. A dramatic increase in plasma androgen concentration occurred on day 9 of gestation, then declined to a lower concentration, which was still greater than that present prior to day 9 of gestation. A second rise in plasma androgen occurred on day 14 of gestation which remained unchanged until day 18, followed by a marked decline on the day of parturition (Barkley et al., 1977). In rats, serum concentrations of androgen increased at the beginning of mid-gestation, attained peak levels on days 17 and 18, and then declined significantly by day 19. However, the high serum concentration of androgen on days 17 and 18 was not associated with a similar increase in ovarian concentration of androgen, suggesting that the serum androgen was from an extra-ovarian source (Gibory et al., 1979). Subsequently, Gibory and Sridaran (1981) suggested that during the second one-half of gestation the placenta produces androgen and that the ovaries and fetuses produce estradiol. Thus, during mid-gestation in the rat, estrogen derived from aromatization of placental androgen by

the ovary and fetuses may play an important role in the regulation of the CL.

In addition, estrogen receptors have been identified in the rat CL (Richards, 1974).

PIGS

Kidder et al. (1955) and Gardner et al. (1963) reported that daily intramuscular injection of estrogen into sows starting on day 11 of the estrous cycle prolonged CL life span. Estrogen also stimulated synthesis of progesterone by cultured porcine granulosa cells (Goldenberg et al., 1972). Estradiol protected the CL from the luteolytic action of $PGF_2\alpha$ when both estradiol benzoate and $PGF_2\alpha$ were administered in hysterectomized gilts on days 8 to 10 of the estrous cycle (Kraeling et al., 1975). These data suggest that estrogen plays a role in regulating luteal function in pigs; however, the mechanism involved in the CL response to estrogen in this species is not clear.

In pregnant sows, estrogen is synthesized by blastocysts as early as day 12 of gestation (Perry et al., 1976). Estrogen synthesis decreases between days 13 to 14 and then increases after day 15 (Pope et al., 1982; Bazer et al., 1986). Daily infusion of an extract of day 20 to 25 embryos into unilaterally pregnant gilts, initiated on day 9 of gestation, maintained CL function (Longenecker and Day, 1972; Ball et al., 1982a,b). However, daily infusion of a charcoal

absorbed extract of day 16-25 embryos into unilaterally pregnant gilts with bilateral corpora lutea, beginning on day 12 of gestation, did not maintain pregnancy up to day 19 (Ball and Day, 1982a). Compared to untreated embryonic extract, the total estrogen content of charcoal absorbed embryonic extract was reduced 89.5%. Ball and Day (1982b) implanted charcoal absorbed embryonic extract and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) into CL on days 30 to 35 of gestation. The charcoal absorbed embryonic extract failed to protect the CL from the local luteolytic effect of exogenous $PGF_2\alpha$. These data suggest that estrogen was responsible for protection of CL from $PGF_2\alpha$ action.

Prostaglandin $F_2\alpha$ is luteolytic in hysterectomized gilts when given on day 14 after detected estrus (Moeljono et al., 1976) and in pregnant pigs when given during early gestation (Diehl and Day, 1974; Kraeling and Rampacek, 1977). Concentrations of utero-ovarian vein (UOV) plasma $PGF_2\alpha$ of nonpregnant gilts were consistently increased between days 12 to 17 of the estrous cycle. However, such increases in $PGF_2\alpha$ did not occur in pregnant pigs on the same days of gestation. Moreover, UOV estradiol concentrations were increased on days 12 to 17 of gestation compared to those of nonpregnant gilts on the same days of the estrous cycle (Moeljono et al., 1977). Administration of estradiol to gilts from days 11 to 15 of the estrous cycle significantly increased concentrations of $PGF_2\alpha$ in the uterine lumen (Bazer and Thatcher, 1977; Frank et al.,

1978). These results indicate that estradiol does not reduce the synthesis and secretion of $PGF_2\alpha$, but affects the direction of uterine secretion of $PGF_2\alpha$.

Collective data have shown that estrogen synthesized by the blastocyst can convert the porcine uterus from an endocrine to exocrine organ, with respect to its secretion of $PGF_2\alpha$ (Bazer and Thatcher, 1977; Frank et al., 1978; Bazer et al., 1986). Therefore, in the sow, estrogen has an indirect luteotropic effect on the CL.

RABBITS

The role of estrogen as an essential luteotropin in pseudopregnant and pregnant rabbits has been known since the early reports of Robson (1937, 1939), who observed the maintenance of CL in hypophysectomized rabbits given injections of estrogen. Ovarian follicles are the primary source of estrogen in rabbits. Estrogen concentrations were increased in cultured follicle cells obtained 2 hours post-coitus (Mills and Sarvard, 1973). Experimental evidence indicated that if follicles were destroyed by cautery (Rennie, 1968) or X-irradiation (Keyes and Nalbandov, 1967; Keyes and Armstrong, 1968), or were luteinized in response to exogenous gonadotropin (Stormshak and Casida, 1965; Keyes and Nalbandov, 1968) rapid regression of the CL occurred.

The rabbit is among those species in which the placenta secretes physiologically insignificant quantities of

progesterone (Thau and Lanman, 1974). Therefore, CL must continue to secrete progesterone throughout gestation for successful maintenance of pregnancy (Hilliard, 1973). During gestation of the rabbit, two luteotropins are required to maintain CL function: estrogen from the follicle and a placental luteotropin.

Spies et al. (1968) reported that injection of 2 ug of estradiol into the hypophysectomized rabbit during the second week of gestation sustained the CL and progesterone secretion and maintained the pregnancy. Pregnant rabbits with one X-irradiated ovary containing only normal appearing CL and interstitial tissue aborted 27 to 60 hours after the removal of the contralateral normal ovary. However, injection of 2-4 ug/day of estradiol into pregnant rabbits with only one X-irradiated ovary containing just CL and interstitial tissue maintained the pregnancy. The CL and ovarian plasma from these rabbits contained significant quantities of progesterone. Injection of LH or whole pituitary extract into rabbits with only one X-irradiated ovary containing only CL and interstitial tissue did not maintain pregnancy (Keyes and Nalbandov, 1967). Silastic implants containing estradiol-17ß were implanted into hypophysectomized rabbits on day 1 after sterile matings. Removal of the implant on day 10 after implantation caused a decrease in serum concentration of progesterone within 12 hours (Bill and Keyes, 1983). These data suggest that estrogen from the follicle is the primary

luteotropin in rabbits. This is supported by the observation that CL of rabbits contain significant quantities of estrogen receptors (Lee et al., 1971).

Hysterectomy of pregnant rabbits during the first half of gestation shortens the life of the corpus luteum (Greep, 1941; Chu et al., 1946). However, implantation of placenta into the abdominal cavity in the hysterectomized-pregnant rabbit at about mid-term of gestation prolongs the survival of the corpora lutea (Chu et al., 1946). Holt and Ewing (1974) removed the fetuses of 21 day pregnant rabbits, leaving the placenta ***in situ***. Plasma progesterone concentration and ovarian secretion of progesterone remained at normal pregnancy levels after 24 hours. However, when the placenta also were removed, plasma progesterone and ovarian secretion of progesterone declined after 24 hours. These data suggest that placenta plays an important role in the functional regulation of the CL during gestation. Moreover, progesterone production in the pregnant rabbit is acutely dependent upon tropic influences emanating from the placentas (Holt and Ewing, 1974; Lanman and Thau, 1979).

Gadsby et al. (1983) investigated the role of a placental luteotropic factor in maintaining CL function in the pregnant rabbit in the absence of estrogen. Follicular estrogen was withdrawn on day 21 of gestation by injecting 10 IU human chorionic gonadotropin (hCG) into pregnant rabbits to ovulate the follicles. On the next day laparotomy was performed and

the newly ovulated follicles(CL) were carefully dissected out, leaving the original CL of pregnancy intact. Viability of fetuses were ensured by injecting the rabbit with medroxyprogesterone acetate (MPA) on day 20 of gestation. Absence of estrogen on day 21 of gestation caused a dramatic decline in serum progesterone after 24 hours in both MPA-treated and control animals. Subsequently, CL regressed and abortion occurred on days 24 to 27 in control animals. In contrast, estrogen replacement on day 22 of gestation, after ovulation of follicles on day 21 of gestation by injection of 10 IU of hCG, was fully capable of restoring serum progesterone concentrations in MPA-treated and control rabbits. These data indicate that during the third week of gestation, in the absence of estrogen, the rabbit placental factor maintained by the progestagen MPA has no direct luteotropic effect on the CL. In addition, hysterectomy on day 21 of gestation rapidly reduced serum progesterone concentrations within 24 hours. Estradiol implanted 24 hours before or after hysterectomy on day 21 of gestation did not prevent luteal regression. Luteal estradiol-17ß concentration 24 hours after hysterectomy and sham hysterectomy of rabbits did not differ (Gadsby and Keyes, 1984). These data suggested that in the absence of placental luteotropin, estradiol has no luteotropic action. Further these investigators suggested that placental luteotropin may regulate the responsiveness of CL to estrogen. It is clear that during gestation estradiol

and the response of the conceptus can stimulate and restore progesterone synthesis. However, neither estrogen nor any factor of placental origin have demonstrated luteotropic effects when acting alone.

During the second one-half of gestation, luteal progesterone synthesis is acutely dependent upon estradiol and a placental factor. In pseudopregnant rabbits, the CL are maintained and secrete normal quantities of progesterone through the action of estradiol even in the absence of the pituitary (Bill and Keyes, 1983). Thus the rabbit is the only species in which estrogen has been shown to be the primary luteotropin (Hilliard, 1973; Bill and Keyes, 1983).

EMBRYONIC LUTEOTROPIN

MATERNAL RECOGNITION OF PREGNANCY

During early pregnancy in mammals an endocrinological and immunological adjustment due to the interaction between the developing conceptus and maternal tissues must occur to ensure continued maintenance of pregnancy. The phenomenon by which the conceptus signals the mother (dam) of its presence is known as maternal recognition of pregnancy (Short, 1969). Presence of a viable conceptus in the uterus prevents regression of the corpus luteum (CL), but precisely how this is accomplished is unknown.

Removal of embryos from ewes up to and including day 12

of gestation allowed regression of CL to occur at the normal time (Moor and Rowson, 1966a), whereas removal of embryos on day 13 or 15 resulted in a marked extension of luteal function. Similarly, embryos transferred to nonpregnant ewes with intact uteri up to day 12 of the cycle were capable of maintaining CL irrespective of whether the embryos were transferred to the uterine horn ipsilateral or contralateral to ovary bearing the CL (Moor and Rowson, 1966b; Moor, 1968). Because embryos were not isolated in each uterine horn, it is possible that extra embryonic membranes or secretory products of the conceptus were able to migrate to the opposite horn. Therefore, in the case of the experimental group in which an embryo was transferred to the horn contralateral to the ovary bearing the CL, this movement of secretory product could have resulted in maintenance of the CL. However, transfer of embryos after this time did not inhibit regression of CL in ewes (Moor and Rowson, 1966c). Infusion of homogenates of sheep embryos collected on days 14 and 15 of gestation into nonpregnant ewes increased the length of the estrous cycle, but infusion of homogenates of embryos removed on day 25 of gestation was without effect on the function of CL (Rowson and Moor, 1967; Martal et al., 1979; Ellinwood et al., 1979a). When one uterine horn in ewes that had ovulated unilaterally was transected at the cervical end, so that access from the lumen of one uterine horn to the lumen of the other was prevented, only embryos transferred into the horn ipsilateral

to the ovary bearing the CL could maintain the CL (Moor and Rowson, 1966b). These data suggest that the signal from the conceptus does not act systemically but rather acts locally in the uterus. Thus, there appears to be a critical period during which maternal recognition of pregnancy occurs. Ford (1985) defined this critical period as a brief 24 -48 hours during early gestation of domestic species when the maternal organism first responds to the presence of an embryo within the uterus. This period occurs on days 12-13 in ewes (Moor and Rowson, 1966c), and days 15-17 in cows (Northey and French, 1980).

CONCEPTUS SECRETORY PROTEIN

Considerable research has been conducted with sheep and cattle to identify and characterize a conceptus secretory protein that plays a role in maternal recognition of pregnancy (Godkin et al., 1984a,b; Knickerbocker et al., 1984). The active protein has been determined to be heat labile and protease sensitive (Martal et al., 1979; Ellinwood et al., 1979b), and has been given the name trophoblastin (Martal et al., 1979).

Using two-dimensional polyacrylamide gel electrophoresis two major proteins produced *in vitro* by the sheep conceptus between days 13 and 23 of pregnancy were purified (Godkin et al., 1982). One of these proteins has a low molecular weight (17,000 - 21,000) with acidic properties (pI 5.5), and is

produced as a major product between days 13 and 21 of gestation (Godkin et al., 1982; Hansen et al., 1985). This protein was named ovine trophoblast protein-one (oTP-1; Godkin et al., 1982). Trophectoderm has been reported to be the site of synthesis of oTP-1 based upon detection of the protein by immunocytochemical methods (Godkin et al., 1984a) and in situ hybridization of oTP-1 mRNA (Farin et al., 1989) in trophectoderm cells of the day 12 or 13 blastocyst. Further, oTP-1 mRNA is produced between days 11 and 23 of gestation with peak quantities produced per cell at approximately day 13 of gestation (Farin et al., 1989). In addition, daily intrauterine infusion of purified oTP-1 (0.2 mg) into ewes on days 12 to 21 of the cycle prolonged CL function (Godkin et al., 1984b). Therefore, these investigators suggested that oTP-1 may play a vital role in maternal recognition of pregnancy. Another protein, bovine trophoblast protein one (bTP-1), presumed to have a similar function as oTP-1, has been identified in cows. It is an acidic protein with a molecular weight of 22,000 - 26,000, and is secreted between days 16 and 24 of gestation (Bartol et al., 1985). Although bTP-1 is of higher molecular weight than oTP-1, the two proteins crossreact immunologically (Helmer et al., 1987). In addition, using Western blots of oTP-1, this secretory protein was not detected in flushings of nongravid uterine horns of pregnant ewes in which the conceptus had been confined to one uterine horn (Kazemi et al., 1988). These

data confirmed that the signal from the conceptus acts locally in the uterus.

The presence of oTP-1 and bTP-1 appears to exert an antiluteolytic effect. Lafrance and Goff (1985) have reported that the presence of bTP-1 inhibits oxytocin-induced release of $PGF_2\alpha$ on days 17, 18 or 19 of gestation in pregnant cows. Similarly, intrauterine injection of total conceptus protein obtained from cultured day 16 conceptuses, given twice daily on days 12, 13 and 14 of the cycle suppressed the total quantity, amplitude and frequency of pulsatile $PGF_2\alpha$ release from uteri of nonpregnant ewes treated either with estradiol or oxytocin (Fincher et al., 1986). Lacroix and Kann (1986) concluded that the conceptus controls the amount and pattern of $PGF_2\alpha$ released by the uterus as early as day 14 of gestation. Nevertheless, the mechanism by which conceptus-secretory proteins act to reduce $PGF_2\alpha$ synthesis is unknown. Roberts and McCracken (1976) reported that secretion of $PGF_2\alpha$ from uterine endometrium in ewes was stimulated by oxytocin treatment. Oxytocin may stimulate the uterine phosphotidylinositol cycle to affect prostaglandin production (Flint et al., 1986a; Mirando et al., 1990). Hooper et al. (1986) have indicated that pulsatile release of oxytocin from the CL and posterior pituitary is similar for pregnant and cyclic ewes. However, the episodic release of $PGF_2\alpha$ from the uterus that normally occurs during luteolysis is reduced to only a single episode on similar days of gestation in ewes

(McCracken et al., 1984; Zacro et al., 1984,1988) and cows (Kindhal et al., 1976; Thatcher et al., 1985). Thus, it seems likely that the decrease in pulsatile release of $PGF_2\alpha$ in early gestation probably results from decreased uterine responsiveness to oxytocin.

Uterine concentrations of oxytocin receptors during early gestation are lower than during luteolysis (McCracken et al., 1984; Sheldrick and Flint, 1985). Ovine trophoblast protein-1 alone can block oxytocin-stimulated production of $PGF_2\alpha$ (Vallet et al., 1988). In addition, Mirando et al. (1990) reported that oxytocin administered to ewes on day 16 of gestation or to ewes on day 15 of the cycle that had been treated with ovine conceptus secretory protein did not increase uterine phosphotidylinositol turnover in vivo. However, oTP-1 is unable to inhibit oxytocin stimulation of endometrial inositol turnover once the oxytocin receptors have formed (Vallet and Bazer, 1989). Because oTP-1 does not appear to bind directly to oxytocin receptors (Bazer et al., 1986), these data suggest that oTP-1 may inhibit the synthesis of oxytocin receptors.

The antiluteolytic effect of oTP-1 may also be due to its ability to shift endometrial production of $PGF_2\alpha$ to prostaglandin E_2 (PGE_2). The ovine conceptus can synthesize PGE_2 (Lacroix and Kann, 1982), which is structurally similar to $PGF_2\alpha$ and can pass from the uterine vein into the ovarian artery (Mapletoft et al., 1976). Ellinwood et al. (1979b)

reported that uterine synthesis and secretion of PGE_2, were greater on days 15 and 17 of gestation than during the same days of the estrous cycle in ewes. Similarly, concentrations of PGE_2 in utero-ovarian venous plasma of ewes from days 10 to 14 of gestation were higher than in nonpregnant ewes on the same days of the cycle (Silvia et al., 1984). Therefore, the increase in PGE_2 secretion observed in pregnant ewes during the critical period for maternal recognition of pregnancy may be contributed by the uterus and conceptus. In addition, intrauterine infusions of PGE_2 at 8 hour intervals into ewes beginning on day 12 of cycle and continuing until next estrus or until day 20 of cycle prevented luteal regression (Pratt et al., 1977). Thus, PGE_2 has been shown to exert an antiluteolytic effect in ewes. However, the mechanism by which PGE_2 maintains the function of the CL is not known. Evidence has been reported that PGE_2 may act directly to stimulate luteal cells (Fletcher and Niswender, 1982) or indirectly by antagonizing the luteolytic action of $PGF_2\alpha$ (Fitz et al., 1984)

Infusion of $PGF_2\alpha$ into the ovarian artery during early gestation in seven ewes resulted in luteolysis in only two animals (Mapletoft et al., 1976), and injection of 125 ug of cloprostenol (an analog of $PGF_2\alpha$) into ewes on day 20 to 23 of gestation caused only 63% abortions (Reid and Crothers, 1980). These data indicate that the presence of conceptuses affords protection against the luteolytic action of $PGF_2\alpha$.

Estradiol has been reported to be luteolytic when injected into intact cyclic ewes for two consecutive days after day 8 of the estrous cycle (Stormshak et al., 1969; Hawk and Bolt, 1971), but not in hysterectomized ewes (Stormshak et al., 1969; Bolt and Hawk, 1975). Uterine venous plasma $PGF_2\alpha$ increased in ewes treated with estradiol on days 9 and 10 of the estrous cycle (Ford et al., 1975). These latter data are supported by the observation that uterine vein concentrations of $PGF_2\alpha$ in ewes on days 13 and 14 of the estrous cycle are associated with elevated concentrations of endogenous estradiol (Barcikowski et al., 1974). Therefore, it seems likely that estrogen-induced luteolysis is probably mediated through stimulation of uterine prostaglandin secretion. However, intramuscular injection of 250 ug of estradiol-17ß (E_2) in oil on days 11 and 12 or days 12 and 13 of gestation resulted in luteolysis in only six of twelve pregnant ewes (Kittok and Britt, 1977). Lacroix and Kann (1986) reported that twice daily intramuscular injections of 125 ug E_2 on days 14, 19 and 33 of gestation failed to induce pulsatile release of $PGF_2\alpha$ and termination of pregnancy. Collectively these data suggest that conceptus secretory proteins exert a luteotropic effect; a function of these proteins first proposed by McCracken and Schramm (1983). However, Godkin et al. (1984a) concluded that oTP-1 does not act at the level of the CL to extend its life span. These investigators reported that no significant ^{125}I-oTP-1

accumulated in the CL and ovarian tissue tested, and oTP-1 failed to compete with human chorionic gonadotropin (hCG) or bovine luteinizing hormone (bLH) for binding to LH receptors in ovine luteal cells. Further, oTP-1 does not stimulate progesterone production by dispersed luteal cells obtained from day 12 cycling ewes (Ellinwood et al., 1979a; Godkin et al., 1984a).

Godkin et al. (1984a) reported that receptors for oTP-1 were uniformly distributed throughout the endometrium, and immunocytochemically, oTP-1 appeared to bind specifically in the upper glandular and superficial epithelium. Numbers of receptors for oTP-1 were maximal on day 4 of the estrous cycle and declined progressively to day 12 in both cyclic and pregnant ewes. After day 12, the quantity of unoccupied receptors for oTP-1 increased gradually to day 16 in cyclic ewes, but declined further in endometrium of pregnant ewes (Knickerbocker and Niswender, 1989). Moreover, Knickerbocker and Niswender (1989) indicated that weight and progesterone content of corpora lutea were negatively correlated with the concentration of unoccupied receptors of oTP-1. These data suggested a role of ovarian steroids in regulating the number of endometrial receptors of oTP1. Thus, conceptus secretory proteins (oTP-1 and bTP-1) appear to have an antiluteolytic effect, but not by direct luteotropic action on the CL.

INTERFERONS

Three major groups of interferons (α, β, and γ) are produced by vertebrate cells in response to viral infection in order to inhibit viral growth. In addition, interferons have the ability to inhibit the growth of a wide range of animal tumors and have been reported to play an important role in regulation of the immune response (Taylor-Papadimitriou, 1984). Interferons are synthesized by leukocytes, fibroblasts and other somatic cells (Stewart, 1979). Fowler et al. (1985) reported that interferon was detected in murine placenta, and that concentrations of interferon increased significantly between days 10 to 13 of gestation and continued to increase until day 17 of gestation. Subsequently, on day 17 of gestation the interferon concentration plateaued until term. Interferon was also detected in human placenta at term (Bocci et al., 1985). Moreover, transient appearance of interferon in murine uteri on days 10 to 15 of gestation developed abruptly and was highest at day 13 of gestation (Fowler et al., 1985).

Recently, oTP-1 and bTP-1 have been classified as interferons (IFN). The amino acid sequence of oTP-1 (Imakawa et al., 1987; Stewart et al., 1987) is 70.3% homologous to IFN-α_{II}. Bovine trophoblast protein-1 has also shown to be an IFN-α_{II} (Imakawa et al., 1989). Purified oTP-1 can inhibit binding of radiolabelled human interferon-α (hIFN-α) to

membrane receptors in endometrium of cyclic ewes (Stewart et al., 1987; Hansen et al., 1988), and binding of [^{125}I]oTP-1 can be blocked by hIFN and bovine recombinant interferon (brIFN; Flint et al., 1988) . Ovine trophoblast protein-1 has been shown to possess antiviral activity (Pontzer et al., 1988) comparable to that of α interferon. Similarly, total bovine conceptus secretory proteins (Betteridge et al., 1988) and purified bTP-1 (Godkin et al., 1988) also have been shown to possess antiviral activity. Moreover, Roberts (1989) reported that oTP-1 exerts an antiproliferative characteristic. Similar to recombinant human interferon, oTP-1 inhibits the growth of bovine kidney epithelial cells in culture. Therefore, these data have served to establish that oTP-1 and bTP-1 belong to the family of interferons. Recently, results of some studies have shown that recombinant interferon can mimic the biological effects of oTP-1 and bTP-1. Infusion of brIFN into the uteri of cyclic ewes (Lamming et al., 1988; Stewart et al, 1989) or infusion (Plante et al., 1988) of brIFNα_I into uteri of cows during the period of maternal recognition of pregnancy extended luteal life span. In addition, secretion of $PGF_2\alpha$ was reduced in ewes subjected to intrauterine infusions of brIFNα_I (Lamming et al., 1988). Human interferon (hIFN) incubated with endometrial cells obtained from day 12 cyclic ewes attenuated PGE_2 and $PGF_2\alpha$ release (Salamonsen et al., 1988). Human interferon also mimicked the stimulatory effect of oTP-1 on protein synthesis

by endometrial cells (Salamonsen et al., 1988; Silcox et al., 1988). The proteins synthesized were similar to proteins synthesized by cultures of endometrial cells obtained from ewes on day 13 of gestation or by cultures of epithelial endometrial cells from ewes on day 13 of the estrous cycle that were incubated in the presence of concentrated culture medium of day 15 sheep blastocysts (Salamonsen, 1986).

Thus, conceptus secretory proteins as functional interferons may participate in establishing immunoprotection to the fetal allograft, possess antiviral characteristics, and exhibit antiproliferative roles during maternal recognition of pregnancy.

In summary, it seems likely that oTP-1 and bTP-1 are pregnancy specific proteins that are secreted into the uterine lumen, where they act as antiluteolytic and immunoregulatory agents. Moreover, the two proteins seem to be biologically active in both species because reciprocal interspecies transfer of bovine and ovine trophoblast into uteri of cyclic recipients modified CL life span (Martal et al., 1984).

LUTEOLYSIS

Luteolysis is defined as a sustained decrease in the ability of the corpus luteum (CL) to secrete progesterone. There are two aspects of this phenomenon: functional luteolysis, which involves a sustained loss in progesterone synthesis and secretion, and structural luteolysis, which entails regression and degeneration of the CL.

The uterus has been reported to be involved in luteolysis in some domestic and laboratory animals. Prostaglandin $F_2\alpha$ secreted by the uterus in these species has been reported to be the luteolytic agent.

UTERINE INVOLVEMENT IN LUTEAL REGRESSION

Loeb (1923) reported the first evidence that removal of the uterus (hysterectomy) in guinea pigs prolonged CL life span, indicating a uterine involvement in the luteolytic process. A similar effect of hysterectomy was subsequently demonstrated for the pseudopregnant rat (Bradbury, 1937; Bradbury et al., 1950), mouse (Critser et al., 1980), hamster (Cadwell et al., 1967; Duby et al., 1969a,b), and rabbit (Asdell and Hammond, 1933; Chu et al., 1946). Maintenance of CL also occurred after hysterectomy of the cow, ewe (Wiltbank and Casida, 1956), sow (Spies et al., 1960) and mare (Ginther and First, 1971; Stabenfeldt et al., 1974). The structural and functional integrity of the CL is retained for a period

equivalent to the normal duration of gestation in hysterectomized guinea pigs (Rowlands and Short, 1959; Poser and Horton, 1975), ewes (Kiracofe and Spies, 1966; Moor et al., 1970; Sheldrick and Flint, 1983b), cows (Malven and Hansel, 1964; Anderson et al., 1965), sows (Spies et al., 1960; Anderson et al., 1961; Moeljono et al., 1976) and rats (Pepe and Rotchild, 1974). However, in pseudopregnant rabbits (Miller and Keyes, 1976) and in mares (Stabenfeldt et al., 1974; Squires et al., 1975) CL function is maintained for a shorter time than during gestation. Removal of the uterus of monkeys (Neill et al., 1969; Castracane et al., 1979) and women (Beling et al., 1970; Doyle et al., 1971) has no effect on CL maintenance during the menstrual cycle.

Unilateral hysterectomy of ewes (Moor and Rowson, 1966d; Inskeep and Butcher, 1966), cows (Ginther et al., 1967), sows (du Mesnil du Buisson, 1961), guinea pigs (Fisher, 1965; Butcher et al., 1969), pseudopregnant hamsters (Duby et al., 1969a) and pseudopregnant rats (Barley et al., 1966) resulted in luteal regression in the ovary adjacent to the intact uterine horn but prevented regression in the opposite ovary. However, CL of pseudopregnant rabbits (Hunter and Casida, 1967) and mares (Ginther and First, 1971) undergo regression despite unilateral removal of the adjacent uterine horn. It appears that the luteolytic factor emanating from the uterus in most species exerts its effect only on the adjacent ovary, and thus the effect of the uterus on luteal function is

exerted locally. It appears unlikely that the luteolytic agent is transported from the uterus to the ovary through the systemic circulation.

Ginther et al. (1973) reported that anastomosis of the main uterine vein from the uterine-intact side to the contralateral utero-ovarian vein in unilaterally hysterectomized ewes caused regression of the CL in the contralateral ovary. Anastomosis of the ovarian artery from the uterine-intact side to the ovarian artery on the contralateral side in these unilaterally hysterectomized ewes also caused regression of CL in the contralateral ovary. These data suggest that the luteolytic effect of the uterus is exerted through a local veno-arterial pathway between a uterine horn and its adjacent ovary.

Anatomically there are no vascular connections between the uterine vein and ovarian artery. However, there are regions of extensive contact between these two vessels throughout the broad ligament up to the point of their respective junctions with the vena cava and aorta (Del Campo and Ginther, 1973a, b). Ginther and Del Campo (1973) examined histological preparations of areas of apposition of the utero-ovarian vein and the ovarian artery in ewes. The ovarian artery is extremely tortuous, and collateral channels and venules arising from the uterine vein wrap around the artery and thus increase the area of contact between these vessels. These vessels share a common tunica adventia and have thinner

walls in regions where they make contact (Del Campo and Ginther, 1974). Similar gross morphological veno-arterial relationships have been observed in the guinea pig, rat, hamster (Del Campo and Ginther, 1973c), sow (Del Campo and Ginther, 1973a) and cow (Ginther and Del Campo, 1974). Interestingly there is very little contact between the uterine vein and ovarian artery in the rabbit (Del Campo and Ginther, 1973b) and mare (Ginther et al., 1972); two species in which the uterine luteolytic effect is not exerted locally.

PROSTAGLANDIN $F_2\alpha$ AS THE UTERINE LUTEOLYTIC FACTOR

Considerable evidence has been accumulated to suggest that $PGF_2\alpha$ is the uterine factor responsible for luteolysis in species of large animals and rodents. Prostaglandin $F_2\alpha$ has been found to be luteolytic in pseudopregnant rats (Pharriss and Wyngarden, 1969), mice (Bartke et al., 1972), hamsters (Harris and Murphy, 1981), rabbits (Scott and Rennie, 1970; Gutknecht et al., 1972) and in cows (Lauderdale, 1972; Rowson et al., 1972) ewes (McCracken et al., 1970; Barret et al., 1971; Thorburn and Nicol, 1971), guinea pigs (Chaichareon et al., 1974), does (Ott et al., 1980), mares (Douglas and Ginther, 1972; Noden et al., 1972; Allen and Rowson, 1973), and to a limited extent in sows (Gleeson, 1974; Hallford et al., 1975).

Injection of 20 mg indomethacin ($PGF_2\alpha$ inhibitor) into the uterine horn adjacent to the ovary with CL twice daily on

days 12 - 17 of the estrous cycle prevented normal luteal regression in ewes (Lewis and Warren, 1977). The same effect occurred in heifers when 40 mg indomethacin was injected into the uterine horn adjacent to the ovary bearing CL twice daily on days 14 - 21 of the estrous cycle (Lewis and Warren, 1977). Moreover, treatment with indomethacin has been reported to prolong the length of pseudopregnancy of rabbits (O'Grady et al., 1972) rats, hamsters (Lau et al., 1975) and mice (Critser et al., 1981). These data indicate that synthesis of $PGF_2\alpha$ appears to be required for normal luteolysis.

Active and passive immunization of ewes (Scaramuzzi and Baird, 1976; Fairclough et al., 1976), cows (Fairclough et al., 1981) and guinea pigs (Poser and Horton, 1975) against $PGF_2\alpha$ prevented spontaneous luteal regression. In addition, $PGF_2\alpha$ has been isolated from endometrial tissue and its concentration is maximal during the period of luteal regression in cows (Shemesh and Hansel, 1975), ewes (Wilson et al., 1972), guinea pigs (Poyser, 1972), mares (Vernon et al., 1981), sows (Guthrie and Rexroad, 1980), pseudopregnant rats (Doebler et al., 1981) and pseudopregnant rabbits (Lytton and Poyser, 1982a). The concentration of $PGF_2\alpha$ in uterine venous blood increases as spontaneous luteolysis commences in sows (Gleeson et al., 1974; Moeljono et al., 1977), mares (Douglas and Ginther, 1976) and pseudopregnant rabbits (Lytton and Poyser, 1982b) and rats (Castracane et al., 1976). Collectively, these data suggest that $PGF_2\alpha$ is the factor of

uterine origin responsible for luteolysis in large domestic species and pseudopregnant rodents.

Luteal development and steroidogenesis in a number of species have been shown to be dependent upon LH-induced accumulation of cAMP. Therefore, Henderson and McNatty (1975) proposed that $PGF_2\alpha$ initiates functional luteolysis by interfering with LH activation of adenylate cyclase. In support of this hypothesis, exposure of rat (Lahav et al., 1976; Thomas et al., 1978; Jordan, 1981), ovine (Ervard et al., 1978; Fletcher and Niswender, 1982) and bovine (Marsh, 1971) CL to $PGF_2\alpha$ in vitro was shown to inhibit gonadotropin stimulation of adenylate cyclase activity. Natural luteolysis in cows (Garverick et al., 1985) and sows (Ritzhaup et al., 1986), and $PGF_2\alpha$ -induced luteolysis in ewes (Agudo et al., 1984) and pseudopregnant rats (Khan and Rosberg, 1979) was accompanied by decrease in basal and(or) LH-stimulated adenylate cyclase activity. A direct action of $PGF_2\alpha$ on the luteal cell is supported by research demonstrating the existence of specific receptors for this agent located within the plasma membrane of ovine (Powell et al., 1974a), rat (Luborsky-Moore et al., 1979) and bovine (Powell et al., 1976; Lin and Rao, 1977) CL. The majority of receptors for LH are in small luteal cells, whereas those for $PGF_2\alpha$ are in large luteal cells (Fitz et al., 1982).

The precise mechanism(s) by which $PGF_2\alpha$ causes luteolysis is unknown. However, this compound may ultimately interfere

with several cellular functions. Results of some research suggest that $PGF_2\alpha$ interferes with signal transduction evoked by LH. Ordinarily, the LH receptor complex couples with a G_s protein which in turn activates adenylate cyclase. The G protein consists of α, β and γ subunit. The $G\alpha_s$ subunit possesses intrinsic guanosine trophosphatase (GTPase) activity and in the inactive state is characterized by the binding of a molecule of guanosine diphosphate (GDP). Binding of LH to its receptors triggers the dissociation of GDP from the $G\alpha_s$ subunit and its replacement by a molecule of GTP which then causes activation of the G_s protein brought about by dissociation of the α from the $\beta\gamma$ subunits. The $G\alpha$-GTP complex then binds to the catalytic subunit of adenylate cyclase to promote the conversion of ATP to cAMP. The intrinsic GTPase activity then converts GTP to GDP and the $G\alpha$-GDP complex reassociates with a $\beta\gamma$ subunits to return to its inactive state. Agudo et al. (1984) reported that basal LH-activated , guanylylimidodiphosphate [Gpp(NH)p]-activated and LH plus [Gpp(NH)p]-activated adenylate cyclase in homogenates of ovine CL collected on day 9 of the estrous cycle decreased by 2 hours after $PGF_2\alpha$ injection. In contrast, phosphodiesterase activity was increased at 2 and 4 hours post-$PGF_2\alpha$ injection. However, activation of adenylate cyclase by Mg^{2+} or the non-hydrolyzable guanosine triphosphate (GTP) analogue guanosine 5'-(β,γ -imido)-triphosphate [GMp-p(NH)p] was not altered in homogenates of ovaries from

cloprostenol-treated rats (Torjesen and Aakvaag, 1986). Thus, whether $PGF_2\alpha$ acts at the level of the cell membrane to interfere with activation of adenylate cyclase remains enigmatic at this time and requires further research.

Another process by which $PGF_2\alpha$ blocks the action of LH is by inducing a loss of LH receptors in the CL (Hichens et al., 1974; Grinwich et al., 1976; Behrman et al., 1978). Changes in the membrane arrangement of the LH receptor appear to be necessary for the expression of LH action in luteal cells. By high resolution ultrastructural analysis, Luborsky et al. (1984b) found that LH-receptor complexes were rapidly organized into small aggregates that increased in size as ferritin-LH binding and progesterone secretion increased. Acute exposure of rat luteal cells to $PGF_2\alpha$ prevented this ferritin-LH aggregation (Luborsky et al., 1979). In addition, $PGF_2\alpha$ prevented LH-induced up-regulation of LH receptors by a process that involves unmasking of cryptic membrane receptors (Luborsky et al., 1984a). Therefore, it seems likely that the initial action of $PGF_2\alpha$ results in a decrease in membrane fluidity, which reduces the ability of the LH receptor to aggregate and activate adenylate cyclase. Reduced membrane fluidity during $PGF_2\alpha$-induced luteal regression has been confirmed by fluorescent probe analysis (Carlson et al., 1981). In addition, progesterone secretion is reduced 22 hours before a decrease in LH receptor content occurs in rats (Grinwich et al.,1976). Moreover, there was no decrease in

the number of receptors for LH in ewes administered a luteolytic dose of $PGF_2\alpha$ until after a dramatic decrease in serum progesterone occurred (Diekman et al., 1974b). Attenuation of adenylate cyclase activity was noted by 15 and 120 min after administration of $PGF_2\alpha$ to rats (Lahav et al., 1976) and ewes (Agudo et al., 1984), respectively. Therefore, down regulation of LH receptors is eventually induced by $PGF_2\alpha$ in vivo, but it is not the initial cause of luteolysis. However, after induced loss of LH receptors occurs, exposure of the CL to $PGF_2\alpha$ for an additional 24 to 48 hours results in irreversible structural and enzymatic changes that ultimately complete luteal regression.

Thomas et al. (1978) reported that $PGF_2\alpha$ stimulated in vitro basal progesterone production by dispersed rat luteal cells. However, incubation of luteal cells with $PGF_2\alpha$ plus LH resulted in a dose dependent inhibition of progesterone secretion. Luteinizing hormone-stimulated adenylate cyclase activity and cAMP accumulation was inhibited by $PGF_2\alpha$. The block in progesterone secretion was reversed by addition of dbcAMP but not by theophylline. These data suggest that a physiological concentration of $PGF_2\alpha$ acutely inhibits the cAMP and steroidogenic responses of isolated luteal cells to LH, independent of an effect of LH receptor binding activity or cAMP degradation. Thus, $PGF_2\alpha$ does not directly inhibit LH-sensitive adenylate cyclase activity in rat luteal membranes. Therefore, it seems likely that some agent must

mediate the intracellular action of $PGF_2\alpha$ following its binding to specific membrane receptors (Wright et al., 1980).

The cellular mechanism whereby functional luteolysis is initiated by $PGF_2\alpha$ has only recently been demonstrated to involve phosphoinositide metabolism in rat (Leung et al., 1986) and bovine (West et al., 1986) luteal cells in vitro. Hormone-induced hydrolysis of plasma membrane phosphotidylinositol 4,5, bisphosphate (PIP_2), a metabolite of phosphotidylinositol (PI) results in the generation of inositol 1,4,5, triphosphate (IP_3) and diacylglycerol (DAG). As a second messenger in target cells, IP_3 elicits calcium mobilization from the endoplasmic reticulum. This ion along with DAG promotes an activation of protein kinase C (Berridge and Irvine, 1984; Nishizuka et al., 1984). Within seconds of exposure to $PGF_2\alpha$, rat and bovine luteal cells exhibited a rapid decrease in PIP_2 and a concomitant increase in IP_3, which presumably led to increased intracellular calcium. Thus, increased intracellular calcium levels in luteal cells as a result of $PGF_2\alpha$-mediated PIP_2 hydrolysis may provide a central mechanism whereby functional luteolysis ensues in many species. In addition, Rothchild (1981) proposed that $PGF_2\alpha$, whether of uterine or ovarian origin, could stimulate its own production in luteal tissue of all species, thus contributing to luteolysis in a paracrine fashion.

In summary, $PGF_2\alpha$ blocks the action of LH by an indirect mechanism. An intracellular mediator of $PGF_2\alpha$ must,

therefore, be involved which somehow prevents the activation of adenylate cyclase by the occupied LH receptors. The acute antigonadotropic response to $PGF_2\alpha$ also appears to be linked to inhibition of the lateral movement of occupied LH receptor into microaggregates within the plasma membrane, which is necessary for the action of LH to be expressed.

Research has shown that the interaction between LH and its receptor, in addition to stimulating adenylate cyclase, was able to trigger a negative regulatory signal at a step beyond cAMP synthesis (Benhaim et al., 1987a). Benhaim et al., 1987b) reported that an activation of protein kinase C (PKC) by phorbol 12-myristate-13-acetate (PMA) or diacylglycerol inhibited the progesterone production induced by high doses of LH. Recently, phospholipase C has been shown to mimic the post-cAMP negative regulating signal induced in vitro by high doses of LH, in the presence of an activation of protein kinase C in bovine small luteal cells. Whereas, with lower doses of LH, phospholipase C only slightly stimulated steroidogenesis (Benhaim et al., 1990). These data suggest that both the luteolytic action of $PGF_2\alpha$ and the inhibitory effect of high doses of LH induced the phospholipase C-generated second messengers which antagonized the adenylate cyclase-generated second messenger system.

REGRESSION OF THE OVINE CORPUS LUTEUM

Control of uterine luteolysin secretion in ewes is

regulated by estrogen (Barcikowski et al., 1974) of follicle origin and oxytocin (OT; Amstrong and Hansel, 1959) of luteal origin. Estrogen induces differentiation of the uterus, increases the capacity of the uterus for synthesis of $PGF_2\alpha$ and induces the appearance of uterine oxytocin receptors (Soloff, 1982). Pulsatile surges of OT originating from the CL occurs during luteolysis in the ewe (Flint and Sheldrick, 1986b). Increased utero-ovarian venous concentrations of $PGF_2\alpha$ were observed prior to oxytocin secretory pulses on day 15 of the estrous cycle of ewes (Moore et al., 1986), suggesting that uterine $PGF_2\alpha$ initiates OT release from the CL during the late stages of luteolysis.

McCracken et al. (1984) postulated the following sequence regulating luteolysis in the ewe: because the uterotropic action of progesterone appears to decline as the luteal phase progresses, endogenous estradiol is able to stimulate OT receptor synthesis in the endometrium. If a sufficient number of OT receptors are induced, occupancy of these receptors by OT acutely stimulates the arachidonic cascade, which results in $PGF_2\alpha$ secretion (Roberts et al., 1976). Subsequent to binding of OT to its receptor, desensitization of OT receptors occurs. Luteal regression is initiated as a result of the countercurrent transfer of $PGF_2\alpha$ from the uterine vein to the ovarian artery. Further release of OT from the CL caused by $PGF_2\alpha$, and OT binding to its receptors in endometrium further reinforces $PGF_2\alpha$ release in a positive manner. This latter

release appears to cause the pulsatile secretion of $PGF_2\alpha$ on days 14-15 of the estrous cycle. Prostaglandin $F_2\alpha$ is released every 6 hour because this is the time necessary for estradiol to induce synthesis of new OT receptors. Permanent luteal regression occurred when five pulses of $PGF_2\alpha$ were administered to ewes over a 24 hour period (McCracken and Schramm, 1983). However, Hooper et al. (1986) recently demonstrated that uterine $PGF_2\alpha$ release during luteolysis in the ewe was not associated with luteal OT release. These latter data suggest that secretion of OT is not always dependent upon stimulation by uterine $PGF_2\alpha$.

CYCLOPROPENOID FATTY ACIDS

Cyclopropenoid fatty acids (CPFA) occur naturally in plants of the order Malvales (Carter and Frampton, 1964). The function of these fatty acids in plants is not well understood, however, it has been suggested that CPFA are produced by plants for self-protection against parasites and predators (Chan et al., 1978).

Some plant species in the order Malvales are important in the human and animal food chain. The source and structure, biological effects, and the metabolism of cyclopropenoid fatty acids will be discussed.

SOURCE AND STRUCTURE OF CYCLOPROPENOID FATTY ACIDS

Cyclopropenoid fatty acids were discovered to be significant components of oil from seed and other tissue of plants in the order Malvales. There are four families in this order: Malvaleceae, Sterculiaceae, Bombacaceae and Tiliaceae. Some species from these families consumed by humans and animals include cotton and kapok seed, okra, limes, durian and China chestnut (Carter and Frampton, 1964).

Relative to consumption by humans and farm animals there are two plants that are important. Cotton (Gossypium hirsutum), which yields an oil or flour used in many western food preparations, and the kapok plant (Eridendran anafractuosum), whose seed oil is important in Asiatic diets.

In Brazil and the West Indies, people extensively consume the nut Pachira aquatica that has 50% lipid, 26% of which consists of cyclopropenoid fatty acids (Bohannon and Kleiman, 1978). Sterculia foetida seeds, which are a rich source of CPFA, are occasionally consumed in India and tropical countries. The lipid content of these seeds has been found to consist of 65% CPFA.

During the 1950's, two cyclopropenoid fatty acids were identified. These CPFA consisted of a highly strained and reactive unsaturated three membered ring (a cyclopropene ring) in the center of a 15 and 16 carbon chain. The eighteen carbon fatty acid was termed malvalic acid (2-octyl-1-cyclopropene-1-heptanoate; Macfarlene et al., 1957) and the nineteen carbon fatty acid was termed sterculic acid (2-octyl-1-cyclopropene-1-octanoate ; Nunn, 1952). The presence of these fatty acids in the plant can be detected by using the Halphen test. Briefly, into an oil or fat sample, one volume of carbon disulfide containing 1% dissolved sulfur and one volume of pentanol (amyl alcohol) are added. Then, the solution is heated slowly to 110° C in an open tube and the carbon disulfide is slowly distilled. Appearance of a pink or red color is indicative of the presence of CPFA (Phelps et al., 1965).

In general, the concentration of malvalic acid predominates over that of sterculic acid in most plant oils. However, there are two species of plants that are exceptions.

The sterculic acid concentration is four times greater than that of malvalic acid in seeds of Bombax olegineum and eight times greater than that of malvalic acid in seed oil of Sterculia foetida. It should be noted that oil of Sterculia foetida is a rich source of cyclopropenoid fatty acids. Compared to crude cottonseed oil, S. foetida oil contains 20 - 140 times more CPFA, although with a different ratio of sterculic acid : malvalic acid. Therefore, Sterculia foetida is a good oil source of cyclopropenoid fatty acids for research purposes (Phelps et al., 1965).

Purification of sterculic acid from the Sterculia foetida seed oil has been accomplished via crystallization of urea inclusion complexes (Nunn, 1952; Kircher, 1964). However, this technique cannot provide a total separation of sterculic acid from malvalic acid. Pawlowski et al. (1981), using high vacuum spinning band distillation, completely separated methyl sterculate from methyl malvalate. However, this is a laborious procedure and the yields of each acid are low.

BIOLOGICAL EFFECTS

Much research has been conducted to examine the biological effect of CPFA in animals. This has generally been accomplished by feeding animals diets containing cottonseed oil, Sterculia foetida oil, or the semi-pure fatty acids.

The biological effects of CPFA appears to depend upon their structure. As early as 1959, Shenstone and Vickery

first noticed that the activity of malvalic acid was less than that of sterculic acid in regard to the ability of these CPFA to cause pink discolorization of avian eggs. In addition, Pawlowski et al. (1985) reported that sterculic acid was more active than malvalic acid in depressing the activity of cytochrome P_{450}.

Nixon et al. (1977b) reported that dietary CPFA were detectable in animal tissue. Subsequently, Einerson (1982) found CPFA to be incorporated into phospholipids of microsomal and plasma membranes. Thus, it is possible that the biological effects of CPFA occur as a result of their incorporation into lipid constituents of membranes.

EFFECTS ON REPRODUCTION

Sherwood (1931) reported that the white of eggs of hens fed cottonseed meal developed a pink color after storage. The pink color was subsequently shown to be an iron chelate of choralbumin in the egg white caused by increased ability of iron to diffuse from the yolk (Schaible and Bandemer, 1946). Cyclopropenoid fatty acids were subsequently reported to increase the permeability of the vitelline membrane (Doberenz et al., 1960). In addition, retardation of ovary and oviduct development and consequent inhibition of egg production occurred in pullets fed a diet containing 200 mg/day of Sterculia foetida oil (Schneider et al., 1962).

Long term feeding of female rats from the weanling stage

with diets containing up to 3% of S. foetida oil delayed sexual maturity and thereafter prolonged estrous cycles (Sheehan and Vavich, 1964). These investigators also found that uteri of rats fed diets containing CPFA were significantly smaller than those of control rats.

Cyclopropenoid fatty acids in the diet of lactating rats (Nixon et al., 1977a) and cows (Hawkins et al., 1985) resulted in detectable concentrations of CPFA in milk fat.

EFFECTS ON LIPID METABOLISM

Cyclopropenoid fatty acids have been reported to be responsible for altering the fatty acid composition of body lipids. An increased level of stearic acid and decreased level of oleic acid in lipids of plasma, liver and ovaries were reported in hens fed with crude cottonseed oil and S. foetida oil (Evans et al., 1962). Johnson et al. (1967) also reported that CPFA altered the egg yolk lipid composition. The metabolic effects of CPFA could not be eliminated by supplying oleic acid in addition to those fatty acids normally synthesized by the animal (Evans et al., 1963). In vivo studies by Reiser and Raju (1964) indicated that the increased concentration of stearic acid was due to the inhibition of fatty acid delta^{9}-desaturase activity. Inhibition was attributed to the ability of sterculic acid to react with crucial sulfhydryl groups in this enzyme (Holloway et al., 1963). Increased tissue concentrations of stearic acid at the

expense of oleic acid result in pasty egg yolk, hard lard and sticky butter.

In CPFA-fed mice, the percentage of saturated fatty acid residues increased at the expense of monounsaturates in the cholesterol ester, triglycerides and phosphatidylcholine fraction of serum lipids (Matlock et al., 1985). Feeding white leghorn cockerels with Sterculia foetida and cottonseed oil caused increased plasma cholesterol, aortic atherosclerosis, liver weight, volume of bile in the gall bladder, bile acids in the bile and bile acid excretion (Goodnight and Kemmerer, 1967). In rabbits, dietary CPFA induced aortic atherosceloris (Ferguson et al., 1976).

EFFECTS ON PROTEIN SYNTHESIS

Dietary cyclopropenes were reported to significantly decrease the activity of mixed-function-oxidase enzymes, such as benzopyrene, cytochrome P_{450}, and NADPH cytochrome P_{450} reductase in trout (Eisele et al., 1978, 1983). An alteration in these particular enzyme activities by CPFA resulted in a change in the metabolic product derived from aflatoxin B1; therefore, CPFA could increase the biological half-life of this carcinogen (Bailey et al., 1982).

Feeding CPFA for 10 weeks to fish decreased acetyl-CoA carboxylase, which is a critical enzyme in the synthesis of malonyl CoA essential for fatty acid synthesis (Perdew et al., 1986). Impairment of fatty acid synthesis disrupted general

lipid synthesis, and affected energy storage capabilities, membrane synthesis and hormonal balance .

COCARCINOGENIC AND CARCINOGENIC EFFECTS

Sinnhuber et al. (1976) demonstrated that the incidence of hepatoma in trout was increased sixfold when 220 ppm CPFA was fed together with 4 ppb aflatoxin. Aflatoxin is a toxin produced by the mold Aspergillus flavus and is a potent hepatocarcinogen in rainbow trout (Ayres et al., 1971). Typical histological damage caused by active cyclopropenes includes: pale, fibrous liver with bile duct hyperplasia, large cells with parallel arrays of endoplasmic reticulum resembling striation, necrotic cells and a high mitotic index (Lee et al., 1968; Struthers et al., 1975). Cyclopropenoid fatty acids had no effect on tumor incidence in female rats intubated with aflatoxin B1 (Nixon et al., 1974), but the response of rabbits to dietary CPFAs was similar to the response in rainbow trout although not as pronounced (Eisele et al., 1982).

The carcinogenic and synergistic activities of CPFA have been examined in trout, rats and mice. In trout, CPFA's have apparent hepatocarcinogenic activity (Hendrick at al., 1980) which may be due to their extremely powerful promotional ability acting upon an otherwise undetectable low rate of spontaneous tumor formation. A significant promotional activity of mouse mammary tumors by cottonseed oil with 0.5%

CPFA was observed as compared with twelve other lipid sources (Tinsley et al., 1981, 1982). A promotional effect of CPFA in rat livers was observed by Lee et al. (1969) and Wells et al. (1974), but Nixon et al. (1974) failed to observe a statistically valid effect of CPFA in rats due to a very small sample size.

METABOLISM OF CYCLOPROPENOID FATTY ACIDS

Metabolism of ^{14}C-sterculic acid has been investigated in rats (Nixon at al., 1977b; Eisele et al., 1977) and trout (Eisele et al., 1979,1983). Apparently, metabolism of CPFA occurs primarily in the liver, and microsomal and mitochondrial subcellular fractions of the liver may be involved in the metabolism of sterculic acid (Nixon at al., 1977b).

The only identified steps in the metabolism of ^{14}C sterculic acid are ring saturation which leads to total inactivity of CPFA (Pawlowski et al., 1985), and alpha, beta, and omega oxidation (Eisele et al., 1977; Nixon et al., 1977b). Metabolites of CPFA retain the three-membered ring in the form of cyclopropane derivatives that are eliminated in urine and feces (Eisele et al., 1977).

Finally, although there exist numerous pathways for transfer of CPFA to man through commercial food, the potential dangers are small because the dietary level of CPFA is very

low and cooking probably destroys the activity of CPFA (Scarpelli, 1968).

EXPERIMENTS 1 AND 2: IN VIVO AND IN VITRO EFFECTS OF A CYCLOPROPENOID FATTY ACID ON PROGESTERONE SYNTHESIS BY THE OVINE CORPUS LUTEUM

INTRODUCTION

Cyclopropenoid fatty acids (CPFA) are found in the seeds and tissues of numerous plants in the order Malvales (Carter and Frampton, 1964). Two plant oils of this order are particularly important in diets of humans and animals. Kapok seed oil (Eridendran anafractuosum) is important in Asiatic diets, while cottonseed oil (Gossypium hirsutum) is present in many Western food preparations and livestock feeds (Matlock et al., 1985).

A CPFA is an unsaturated fatty acid having a highly strained and reactive cyclopropene ring. Two naturally occurring fatty acids with this cyclopropene ring have been isolated. Sterculic acid (2-octyl-1-cyclopropene-1-octanoate) was isolated from Sterculia foetida seeds (Nunn, 1952) and malvalic acid (2-octyl-1-cyclopropene-1-heptanoate) was isolated from Malva parviflora (Macfarlane et al., 1957).

Effects of CPFA on mammalian reproduction have not been extensively examined. However, it has been reported that long-term feeding of weanling female rats diets containing up to 3% of S. foetida seed oil delayed sexual maturity and thereafter prolonged the duration of the estrous cycle (Sheehan and Vavich, 1964). Uteri of rats fed CPFA were

significantly smaller than those of control rats. These data suggest that CPFA may have altered ovarian function of rats. Ability of a CPFA to impair ovarian function has recently been demonstrated utilizing ewes. Injection of 500 or 750 ug of sterculic acid (SA) into the ovarian artery supplying the ovary bearing corpora lutea (CL) on day 2 of the estrous cycle suppressed serum concentrations of progesterone and shortened the estrous cycle in the majority of ewes (Slayden and Stormshak, 1988). In addition, injection of 750 ug SA into the ovarian artery of ewes on day 10 of the cycle suppressed serum progesterone concentrations within 12 hour of treatment (Slayden et al., 1989).

The present experiments were conducted to determine the in vivo effect of SA on luteal function in ewes during early gestation and the in vitro effect of SA on the ability of luteal tissue to synthesize progesterone from pregnenolone or 22(R)-hydroxycholesterol.

MATERIALS AND METHODS

EXPERIMENT 1

Twelve mature ewes were observed twice daily (morning and evening) for behavioral estrus (day of detected estrus = day 0 of the cycle) using vasectomized rams. After exhibiting at least two estrous cycles of normal duration ($\bar{X}$ ± SE, 17.2 ± 0.2 days), ewes were assigned randomly to two groups of six

animals each, and were bred to a fertile ram at the time of detected estrus and again the subsequent evening or morning. On day 18 postmating, a 10 ml sample of blood was collected from each animal via jugular venipuncture. Animals were then anaesthetized by intravenous injection of sodium thiamylal (2.5%), and anaesthesia was maintained by inhalation of halothane. A midventral laparotomy was performed to expose the uterine horns and ovaries. Pregnancy status was subjectively assessed by visually evaluating the size and the tone of the uterine horns and the size and color of the CL. Ewes with CL in both ovaries were unilaterally ovariectomized while those with CL in one ovary only were allowed to remain intact. Treatment consisted of injection of a mixture of fatty acids extracted from Sterculia foetida seeds, which consisted of the methyl esters of SA 69%, malvalic, oleic, stearic, palmitic acids 31%. Thus ewes in group 1 were treated with 1.09 mg of the fatty acid mixture containing 750 ug of SA methyl ester which was injected into the ovarian artery supplying the ovary bearing the CL. Control ewes (group 2) were similarly injected with 1.09 mg of oleic acid methyl ester. After injection, the reproductive organs were returned to the abdominal cavity. The peritoneum was closed with # 2 chromic catgut and the skin sutured with surgical silk. All ewes were given an intramuscular injection of 6 ml of combiotic (dihydrostreptomycin/penicillin) to prevent any infection. Ewes were allowed to recover from surgery for 24

hour and then checked twice daily for estrous behavior. A jugular vein sample of blood was taken daily until ewes exhibited behavioral estrus or until day 30 postmating, whichever occurred first. Blood samples (10 ml) were stored at 4° C for 24 hour and then centrifuged at 500 X g at 4° C for 10 min. The resulting sera were stored frozen (-20°C) until analyzed for progesterone by use of radioimmunoassay. On day 30 postmating, those ewes that had not exhibited behavioral estrus were relaparotomized as described above. The uterine horns were palpated to locate the fetus and a small incision was made in the uterine horn through which the fetus was expressed. The fetus was considered viable if a heart beat was observed. The incision was closed with # 2-O catgut. Corpora lutea in the remaining ovary were enucleated and weighed.

EXPERIMENT 2

Twelve mature crossbred ewes were checked for behavioral estrus twice daily using vasectomized rams. All ewes were allowed to complete at least one estrous cycle of normal duration ($\bar{X}$ ± SE, 16.8 ± 0.3 days) before being utilized for the experiment. Corpora lutea were removed from ewes via laparotomy on day 10 of a subsequent estrous cycle as described for Exp.1. The CL were placed into cold Ham's F-12 medium containing 24 nM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2 ethanesulfonic acid)], 100 U/ml penicillin, 100 ug/ml

streptomycin, 0.25 ug/ml amphotericin B, 5 ug/ml insulin, 5 ug/ml transferrin, and 5 ng/ml selenium (Pate and Codon, 1978), and transported to the laboratory. Corpora lutea were trimmed of excess connective tissues, weighed and cut in half. Each half was sliced (0.3 mm thickness) using a Harvard tissue slicer maintained at 4° C. Luteal slices were then washed with 30 ml of cold Ham's F-12 medium containing 24 nM HEPES, and 100 U/ml penicillin, 100 ug/ml streptomycin, 0.25 ug/ml amphotericin B. The interval between collection of CL to initiation of incubation was less than 2 hour.

Tissue Incubation

Aliquots of luteal slices (90 - 150 mg, $\bar{X}$ ± SE, 115 ± 9.6 mg) were placed into two sets of four flasks with each flask containing 2.97 ml of Ham's F-12 medium (pH 7.2, 4° C). Incubation of luteal slices consisted of two phases. In phase I, oleic acid methyl ester (435 ng dissolved in 30 ul dimethyl sulfoxide; DMSO) was added to each of four flasks (controls), and 435 ng of an extract of S. foetida seeds in 30 ul DMSO (100 ng/ml SA methyl ester) were added to each of the remaining four flasks. Flasks were incubated under 95% O_2 - 5% CO_2 for 90 min at 37° C using a Dubnoff metabolic incubator after which incubation was terminated by placing flasks into an ice bath. Tissue in each flask was then washed with fresh medium, and resuspended in 2.97 ml of fresh Ham's F-12 medium. Each set of four flasks received the following treatments:

Flask 1, 30 ul absolute ethanol (unincubated control), Flask 2, 30 ul absolute ethanol (incubated control), Flask 3, 75 ug 5-pregnen-3ßol-20-one (pregnenolone) dissolved in 30 ul absolute ethanol (final concentration 0.079nM), and Flask 4, 75 ug 22(R)-hydroxycholesterol dissolved in 30 ul absolute ethanol (final concentration 0.084nM). Flasks 2, 3, and 4 were placed back into the Dubnoff metabolic incubator for another 120 min incubation under 95% O_2 - 5% CO_2, 37° C. Six milliliters of cold distilled absolute ethanol were added to the unincubated control flasks to terminate further synthesis of progesterone. A similar quantity of distilled absolute ethanol was added to each of the remaining flasks at the end of incubation. The contents of each flask (tissue, medium and ethanol) were then transferred to vials, and stored at -20° C until analyzed for progesterone.

Extraction of Tissue

Each tissue sample was warmed to room temperature after which 41,000 dpm of [^{3}H]-progesterone (115.0 Ci/mmol, New England Nuclear) was added and the sample allowed to equilibrate for 15 - 30 min before homogenization. Each sample was homogenized using a ground-glass homogenizer, filtered, and the filtrate dried under vacuum at 45° C. The dried residue was resuspended by addition of 3 ml 0.1% gel-PBS and then extracted for 2 min using 20 ml of hexane:benzene (1:2 vol/vol). Samples were stored at -20° C for 2 hour to

freeze the aqueous phase, and the organic solvent containing the steroid was quickly decanted into 20 ml screw-cap vials. The hexane: benzene was dried under a stream of air and the sample was then redissolved in 20 ml absolute ethanol (Koligian and Stormshak, 1976). Mean extraction efficiency for 54 samples was 94.3 ± 0.4%. Samples were stored at -20 °C until assayed for progesterone.

RADIOIMMUNOASSAY FOR PROGESTERONE

In Exp. 1, sera were analyzed for progesterone as described by Koligian and Stormshak (1977). The antibody (1 : 1000 dilution using 0.1% gelatin in phosphate-buffered saline) and [^{3}H]-progesterone (41,000 dpm, 115.0 Ci/mmol, New England Nuclear) were each added to assay tubes in a volume of 100 ul. Separation of free and antibody-bound progesterone was accomplished by the addition of 1.0 ml dextran-charcoal suspension (2.5 g neutralized norit, and 0.25 g dextran T-70 in 1 liter phosphate-buffered saline).

In Exp. 2, samples were analyzed for progesterone by radioimmunoassay as described for Exp 1. However, samples were diluted with distilled absolute ethanol to yield 1 : 100 dilution, and 100 ul was assayed.

The intra- and interassay coefficients of variation for the assay of progesterone for Exp.1, were 8.6% and 16.5%, respectively, and for Exp. 2, were 7.1 % and 11.4 %, respectively. The sensitivity of the assay was 10

pg/tube.

STATISTICAL ANALYSIS

Because three SA-treated ewes in Exp.1 exhibited behavioral estrus within 5 days of treatment, data for serum concentrations of progesterone for 6 control and 6 treated ewes on days 18, 19 and 20 were analyzed separately from data of three treated and six control ewes for day 22 to 30 of gestation. Each data set was analyzed by split-plot analysis of variance. Weights of CL collected from three treated and six control ewes on day 30 were analyzed by unpaired Student's t-test (Devore and Peck, 1986).

Data on *in vitro* progesterone synthesis (after substraction of appropriate unincubated controls) for Exp.2 were analyzed by split-plot analysis of variance and differences among means were tested for significance by use of Fisher's Protected LSD analysis (Steel and Torrie, 1980).

RESULTS

Serum concentrations of progesterone in ewes injected with OA and SA were lower 24 hour after treatment than presurgery systemic concentrations of steroid and remained low until day 30 of gestation. The reduction in serum concentrations of progesterone during the 48 hour after treatment was greater in SA-treated ewes than in control animals (Figure 1, $P<0.01$).

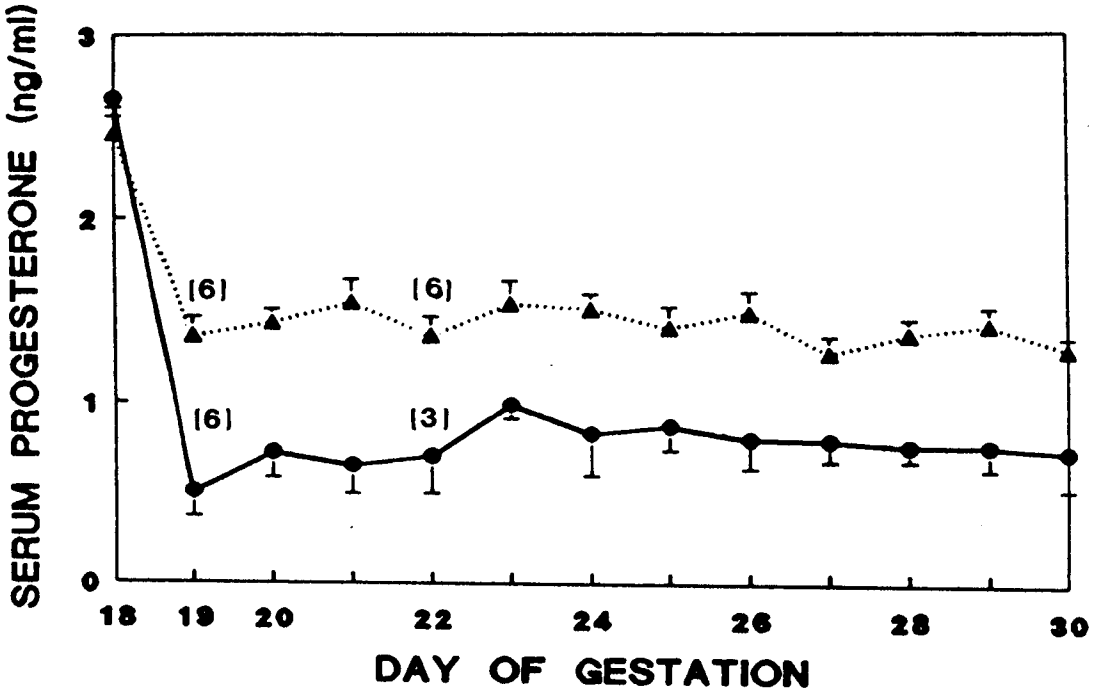

Figure 1. Mean serum concentrations of progesterone (mean ± SE) in ewes injected with either 1.09 mg extract of Sterculia foetida seeds (750 ug SA methyl ester; ●—●) or 1.09 mg oleic acid methyl ester (▲--▲) on day 18 of gestation. Because three SA-treated ewes exhibited behavioral estrus within 5 days of treatment, data for days 18 - 20 were analyzed separately from those for days 22 - 30. Values in parentheses represent the number of SA-treated ewes that had not exhibited estrus (treatment x day interaction; $P<0.01$).

Three SA-treated ewes exhibited behavioral estrus between days 20 and 22 of gestation suggesting that luteal regression had occurred in these animals. Mean serum concentrations of progesterone in the remaining three SA-treated ewes continued to be lower than those of control ewes during the interval from day 22 to 30 of gestation ($P<0.09$). Laparotomy of ewes on day 30 revealed that all six control ewes were pregnant as verified by the presence of a viable fetus in each ewe. Daily mean serum concentrations of progesterone in the remaining three SA-treated ewes, ranging from 0.7 to 0.9 ng/ml between days 18 to 30 (approximately 0.6 ng/ml less than in control ewes), were apparently sufficient to maintain pregnancy for a few days but not until day 30. All three of these ewes had nonviable fetuses on day 30. Corpora lutea of SA-treated ewes removed at laparotomy on day 30 of gestation did not differ in weight from those of control ewes ($\bar{X}$ ± SE, SA-treated 369.5 ± 89.2 vs controls, 421.1 ± 36.3 mg).

In Exp. 2 exposure of luteal tissue to OA and SA for 90 min did not affect progesterone production (unincubated controls, $\bar{X}$ ± SE; SA, 63.9 ± 19.6 vs OA, 38.7 ± 11.7 ng/mg tissues). Similarly, during a subsequent 90 min incubation, in the presence or absence of 22(R)-hydroxycholesterol <u>de novo</u> progesterone synthesis by luteal tissue preincubated with OA and SA did not differ ($P>0.05$). Presence of pregnenolone in the incubation medium stimulated an increase in progesterone synthesis by luteal tissue preincubated with OA or SA compared

with progesterone production by respective incubated control tissue (Figure 2, $P<0.05$). However, the conversion of pregnenolone to progesterone by luteal tissue exposed to SA was markedly less than by tissue preincubated with OA ($P<0.01$).

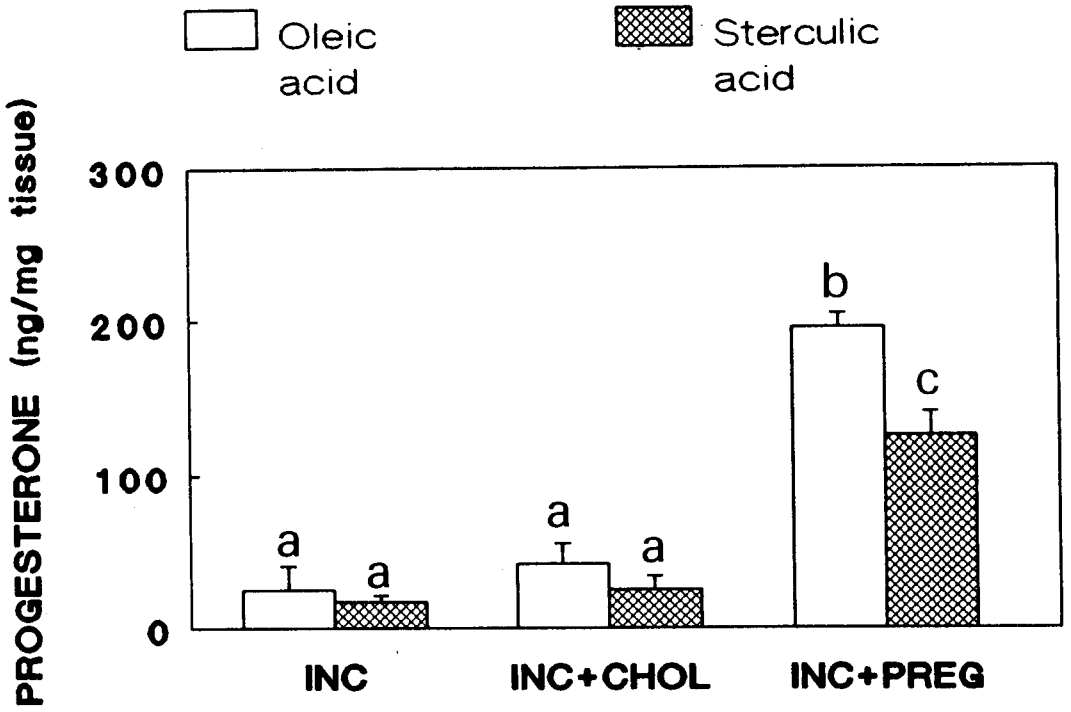

Figure 2. Mean tissue plus medium concentrations of progesterone (mean ± SE) resulting from incubation of luteal slices for 120 min with 25 ug 22(R)-hydroxycholesterol (chol), 25 ug 5-pregnen-3ßol-20-one (preg) or vehicle (inc.control) after preincubation for 90 min with 100 ng/ml SA methyl ester or OA methyl ester (fatty acid x substrate interaction; $P<0.05$). Means with different superscripts differ ($P<0.01$).

DISCUSSION

Results of the present study indicate that injection of a mixture of plant fatty acids, consisting predominantly of SA, into the ovarian artery of ewes on day 18 of gestation impaired luteal function. Serum concentrations of progesterone in treated ewes were significantly lower than in control ewes. These data are in agreement with those of Slayden and Stormshak (1988) who found that injection of SA into ewes on days 2 or 10 of the estrous cycle (Slayden et al., 1989) suppressed luteal function and caused premature luteal regression. It is noteworthy that presurgery serum concentrations of progesterone in control and treated ewes were greater than the concentrations of steroid detected 24 h after treatment, with the reduction in treated ewes being greater than in controls. The reasons for this decrease in serum concentrations of progesterone may be attributed in part to unilateral ovariectomy of those ewes with CL in both ovaries. However, the number of CL removed in treated ewes was similar to the number removed in control ewes (1 CL/two ewes/group). It is also conceivable that the stress of surgery including anesthesia (Lewis et al., 1985) may have contributed to the reduction in luteal progesterone secretion. This latter possibility is supported by the data of Mirando et al. (1990) who found that plasma concentrations of progesterone were reduced 24 h after surgery of ewes.

The CL is the primary source of progesterone in ewes during the first 50 days of gestation (Casida and Warwick, 1945). It has been established that a conceptus must be present by days 12 to 13 postmating to ensure maintenance of the CL in ewes (Moor and Rowson, 1966a). The ovine conceptus produces a polypeptide referred to as ovine trophoblastic protein one (oTP-1) which has been found to prevent luteal regression (Martal et al., 1979; Godkin et al., 1984a,b). This protein is produced by the conceptus from days 13 to 21 of gestation (Godkin et al., 1982). Presumably this protein acts to inhibit the uterine secretion of prostaglandin $F_2\alpha$ which is the natural luteolysin in ewes (Goding, 1974), but whether this protein also acts in some other manner to protect the CL has not been determined. It is obvious from the present study that treatment with SA was able to override the luteotropic effects of the conceptus in 50% of the ewes within 5 days after treatment as determined by detection of behavioral estrus. However, the fact that the remaining ewes had fetuses present, although nonviable, on day 30 of gestation suggest that the presence of conceptuses in these ewes prevented complete luteolysis. By contrast, injection of SA into ewes on day 10 of the cycle, a stage when the CL has attained maximal size and function, caused luteal regression in five of six ewes (Slayden et al., 1989). It is unlikely that the observed effect of treatment on luteal function was due to either the mass of lipid injection or the

other fatty acids present in the administered plant extract. Administration of a 1.09 mg of a mixture of fatty acids consisting of oleic, stearic, palmitic and linoleic acids into the ovarian artery of ewes on day 10 of the cycle did not interfere with luteal function (Slayden, unpublished data). Thus it appears that the SA present in the mixture was responsible for altering the function of the CL in pregnant ewes. It is not known precisely which luteal cell organelles or systems are affected by this fatty acid. Injection of SA into ewes on day 10 of the cycle has been shown to reduce luteal concentrations of unoccupied LH receptors (Cortell et al., 1990). In vitro preincubation of luteal tissue collected from ewes on day 10 of the cycle with SA reduced progesterone synthesis in response to LH during subsequent incubation (Slayden et al., 1989) but did not interfere with the ability of dbcAMP to stimulate steroidogenesis (Cortell, unpublished data). Results of this study indicate that in vitro exposure of luteal tissue of ewes on day 10 of the cycle to SA interferes with the conversion of pregnenolone to progesterone. Conversion of pregnenolone to progesterone occurs in the smooth endoplasmic reticulum and is catalyzed by the enzyme 3ß-hydroxysteroid dehydrogenase. These data suggest that SA somehow impaired the activity of this enzyme. Injection of rats with SA resulted in an accumulation of this fatty acid in hepatic microsomal and mitochondrial membranes (Nixon et al., 1977b). Further, inhibition of stearic fatty

acid desaturase occurred in livers of trout fed SA thus increasing the concentration of hepatic saturated fatty acids. Thus, it is possible that CPFA either directly or indirectly alter the composition of membrane phospholipids and hence membrane fluidity. A reduction in membrane fluidity may account for suppressed synthesis of progesterone from pregnenolone.

The rate limiting step in the synthesis of progesterone is the conversion of cholesterol to pregnenolone by the cytochrome P_{450} cholesterol side chain cleavage complex in mitochondria. Toaff et al. (1982) found that incubation of dispersed rat luteal cells with 22(R)-hydroxycholesterol (25 ug/ml of medium) stimulated an increase in progesterone synthesis. Progesterone synthesis in the presence of 22(R)-hydroxycholesterol did not differ from that of incubated controls regardless of whether luteal tissue was preincubated with OA or SA. Synthesis of progesterone resulting from conversion of 22(R)-hydroxycholesterol in SA-treated slices tended to be slightly lower than that of slices exposed to OA but the difference was not significant statistically. Apparently a greater concentration of 22(R)-hydroxycholesterol was required and(or) duration of incubation was not sufficient to permit adequate amounts of this steroid to gain entry into the cells.

In summary, injection of SA into the ovarian artery of ewes on day 18 of gestation suppressed luteal progesterone

secretion to a level that ultimately resulted in termination of pregnancy. Although the data are equivocal, it appears that the presence of the conceptus exerted a luteotropic effect sufficient to maintain limited luteal function for 12 days post-treatment in 50% of the ewes. Results of an *in vitro* experiment suggest that SA-induced reduction in luteal progesterone synthesis is caused by the ability of this unique fatty acid to interfere with the conversion of pregnenolone to progesterone in the endoplasmic reticulum.

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