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Effects of prostaglandin F_2a or trilostane on pregnancy in 90 to 100 day pregnant sheep

Weems, Yoshie Suzuki, Ph.D.

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EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ OR TRILOSTANE ON PREGNANCY IN 90 TO 100 DAY PREGNANT SHEEP

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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BIOMEDICAL SCIENCES
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MAY 1994

By

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ABSTRACT

Five experiments were done to assess the effects of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) *in vivo* on pregnancy, luteal or placental function in hysterectomized (HYX), intact (INT) or ovariectomized (OVX) 90 to 100 day pregnant ewes; effect of $PGF_2\alpha$ on placental steroidogenesis *in vitro*; or the effects of trilostane on pregnancy in 90 to 100 day lutectomized pregnant ewes.

A single dose of $PGF_2\alpha$ (8 or 16 mg/58 kg/BW) decreased weights of corpora lutea and concentrations of progesterone in ovarian venous blood of INT or HYX pregnant ewes. Hysterectomy decreased progesterone in jugular venous plasma by one half and was of ovarian origin, while the other half was of placental origin. There was a significant decrease in progesterone over time in jugular or uterine venous blood in the presence or absence of the ovaries in 90 to 100 day pregnant ewes, but the profiles of progesterone were not different between vehicle and $PGF_2\alpha$-treated ewes. Uterine venous progesterone never decreased below 30 ng/ml in the presence or absence of the ovaries. There was a significant quadratic increase in uterine venous progesterone toward the end of the 72 hour sampling period indicating an increase in steroidogenic activity of the placenta. Prostaglandin $F_2\alpha$ did not cause abortion in INT or OVX pregnant ewes. Prostaglandin $F_2\alpha$ did not decrease placental secretion of progesterone *in vitro*.

Both doses of $PGF_2\alpha$ increased $PGF_2\alpha$ in ovarian venous plasma when compared with controls at 72 hour posttreatment in INT, but

not in HYX 90 to 100 day pregnant ewes. Concentrations of PGE in ovarian venous plasma averaged 7.1 ng/ml, did not differ among treatment groups and were equivalent to concentrations of PGE in uterine venous plasma. Prostaglandin E secreted *in vitro* by placental slices per ml of incubation medium/100 mg tissue was equivalent to the concentrations of PGE in uterine venous plasma. Prostaglandin E was decreased in ovarian venous plasma by $PGF_{2}\alpha$ in HYX pregnant ewes. Prostaglandin E in uterine venous plasma averaged 6 ng/ml over the 72 hour treatment period in INT and OVX pregnant ewes and was 12 fold greater than $PGF_{2}\alpha$ which averaged 500 pg/ml. Both $PGF_{2}\alpha$ and PGE increased at 64 hours in uterine venous blood of the 8 mg $PGF_{2}\alpha$-treated INT ewes. A significant quadratic increase in $PGF_{2}\alpha$ and PGE in the vehicle and both $PGF_{2}\alpha$ treatment groups of INT pregnant ewes was seen at the end of the 72 hour sampling period.

Pregnancy specific protein B (PSPB) in uterine venous blood of INT pregnant ewes averaged 51.5 ± 4.1 ng/ml and 89.8 ± 5.0 ng/ml in OVX pregnant ewes. Pregnancy specific protein B was increased by 16 mg $PGF_{2}\alpha$ in INT and decreased by 8 mg $PGF_{2}\alpha$ in OVX pregnant ewes. There was a significant increase in PSPB in the 8 mg $PGF_{2}\alpha$-treated INT pregnant ewes at 64 hours which was correlated to the increase in PGE at this time (0.76).

Eighty percent of estradiol-17ß in jugular blood was of placental origin. The 16 mg $PGF_{2}\alpha$ dose increased estradiol-17ß in jugular blood in INT and OVX pregnant ewes. Profiles of cortisol were not different in HYX, INT or OVX pregnant ewes treated with vehicle or both doses of $PGF_{2}\alpha$.

Lutectomized 90 to 100 day pregnant ewes (n = 20) were aborted with trilostane. Trilostane decreased progesterone over the 96 hour treatment period. Estradiol-17ß increased in jugular blood two hours after each treatment with trilostane and a sustained increase was seen at 36 hours after the initiation of treatments. Nineteen of the 20 ewes aborted with trilostane had a retained placenta and progesterone in uterine venous blood at 96 hours after the initiation of trilostane treatments was greater than 40 ng/ml in all treatment groups.

These data suggest that the corpus luteum at 90 to 100 day of pregnancy is functional and responsive to luteolytic actions of $PGF_2\alpha$. However, the placenta is not responsive to the doses of $PGF_2\alpha$ tested and $PGF_2\alpha$ is not abortifacient over the 72 hour treatment period. Prostaglandin E may be a placental stimulator of ovine placental secretion of progesterone or may protect placental steroidogenesis from actions of $PGF_2\alpha$. In addition, PSPB and/or estradiol-17ß may directly or indirectly through PGE regulate placental steroidogenesis. Mechanisms for abortion/parturition exist at 90 to 100 days of gestation in sheep, since trilostane terminates pregnancy.

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CHAPTER 1

REVIEW OF LITERATURE

1.1 ESTROUS CYCLE

1.1.1 INTRODUCTION

The length of the estrous cycle in sheep is 16 to 17 days (1) with a normal range between 14 to 19 days (2). In cattle, the estrous cycle averages 21 days (3) with a normal range between 19 to 23 days (4). Younger animals have a shorter interestrous interval, while older animals have a longer interestrous interestrous interval (2, 4). The major factor determining length of the estrous cycle is when secretion of progesterone by corpus luteum (CL) declines (5). With declining progesterone at the end of the estrous cycle, sheep and cattle are usually in estrus within 72 hours (5).

The estrous cycle consists of two different phases. These phases are related to changes in hypothalamic, hypophyseal, ovarian and uterine hormone secretion (5). These phases are called the follicular phase and the luteal phase (5).

The follicular phase starts with tha decline in progesterone (luteolysis) at the end of the estrous cycle. This decrease in progesterone occurs around day 14 in sheep and day 17 to 18 in cattle (6). The follicular phase includes the events of luteolysis, growth of the preovulatory follicle, estrus and ends with ovulation (8). However, these events are the terminal stage in the development of the ovulatory follicle, since theses follicles started to grow during the luteal phase (8).

The final phase of follicular development is 2 to 4 days in length in the ewe, and 3 to 6 days in the cow (6). During the follicular phase, progesterone in jugular venous blood eventually decreases to less than 1 ng/ml indicating that the CL is regressed and is followed by increases in estradiol-17ß which causes behavioral estrus. Estrus is a period of sexual receptivity by the female to male (6). This decrease in progesterone and the increase in estradiol-17ß also causes hypothalamic release of gonadotropin releaseing hormone (GnRH) which causes the hypophysis to release a preovulatory surge of luteinizing hormone (LH). This LH surge induces ovulation (8).

Removal of the CL in sheep (9) or cattle (10) during the estrous cycle causes a precipitous decline in progesterone below 1 ng/ml within 4 hours and initiates the follicular phase. Estrus and ovulation occurs within 48 to 96 hours. When the CL is removed surgically in sheep (9) or when luteolysis is induced with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in cattle (11), large (dominant) follicles on the ovary at this time do not ovulate, but become atretic. Smaller antral follicles on these ovaries grow and develop to become new dominant follicles and one or a few ovulate at the end of the follicular phase (8).

The length of estrus in sheep is 12 to 48 hours and 18 to 24 hours in cattle (1). Ovulation occurs 24 to 30 hours after the onset of estrus in sheep and 12 to 16 hours after the end of estrus in cattle (1). The time of ovulation can be regulated by hormones. When a luteolytic dose of $PGF_{2\alpha}$ is given on day 14 postestrus and GnRH is given 36 hours later, ovulation occurs 24 hours after GnRH is given in sheep (12).

Ovulation of the dominant follicle releases the oocyte for possible fertilization and marks the end of the follicular phase, but is the beginning of the luteal phase (1). The remainder of the freshly ovulated follicle develops into the CL whose main function is to secrete the steroid hormone, progesterone (1). Progesterone is necessary for implantation and maintenance of pregnancy (13). The luteal phase in sheep lasts for 13 to 14 days and for 16 to 18 days in cattle (1, 6). The end of the luteal phase during the estrous cycle occurs when the CL stops secreting progesterone (5). This occurs around day 14 in sheep (1) and days 17 to 18 in cattle (5).

Estrous cycle activity is interrupted by season of the year in sheep (2, 14) or pregnancy in sheep and cattle (1, 5, 7). Estrous cycles in sheep in the Northern Hemisphere occur from late summer through early spring. Ewes become anestrus at the end of the breeding season (14). This pattern of estrous cycle activity is reversed in sheep in the Southern Hemisphere (14). Sheep moved from the Northern to the Southern Hemisphere or from the Southern to Northern Hemisphere reverses the breeding season (15).

Onset of the ovine breeding season is associated with changes in the ratio of light:dark. Onset of the breeding season occurs with decreasing day length. The end of the breeding season is associated with increasing day length (16). Sheep located near the equator, where changes in the light:dark ratio are minimal, are affected less by season (17).

The extent of seasonality is affected by breed of sheep, altitude and temperature (18). Black-face sheep, such as Suffolk and Hampshire, are affected more by season than white-face sheep, such as the Merino and

Rambouillet (18). Moving sheep to higher altitudes, where it is cooler, advances the onset of the breeding season. This response is better in white- than in black-face sheep (18). Exposure of the ewe to the ram also advances the onset of the breeding season in sheep (19). Estrous cycles in cattle are not interrupted by season, but the degree of hypothalamo-hypophyseal activity may be altered by season (20).

1.1.2 FOLLICULOGENESIS

1.1.2.1 FOLLICULAR GROWTH AND DEVELOPMENT

Folliculogenesis is the process of growth and development of follicles until ovulation or atresia (8). All primary follicles in the ovary are formed by the time of birth in farm animals (21). Primary follicles increase in size 400 to 600 fold and hundreds of primary follicles enter the growing pool in each cycle. However, only 1 to 30 ovulate each estrous cycle in mammals, depending on the species or breed within species. The follicles that do not complete the growth process to ovulation are referred to as atretic follicles. They regress and are lost from the follicle pool of the ovary (8).

Most primary follicles are avascular, nongrowing and contain an oocyte surrounded by a single layer of simple squamous epithelium. When these follicles enter the growing pool of primary follicles, the epithelium changes from a simple squamous to a simple cuboidal epithelium. The cuboidal epithelium of growing primary follicles is mitotically active and forms 2 to 6 layers of cuboidal epithelium surrounding the oocyte. This is now called a secondary follicle (22). Regulation of movement of nongrowing

primary follicles into the pool of growing primary follicles and growth and development into the secondary follicle stage is not known, but it is not dependent on gonadotropin support, since hypophysectomy does not stop follicles from moving into the growing pool (23).

Late secondary follicles acquire a theca layer from the stroma and vascularization of the theca is initiated. The epithelial cells of the secondary follicle secrete folliclular fluid which forms a space called the antrum. Follicles with an antrum are called tertiary follicles. The tertiary follicle continues to grow. The antrum increases in size, follicular cells are mitotically active, the oocyte increases in size and the antrum accumulates follicular fluid. Components of follicular fluid come from blood plasma as well as secretions from the follicular cells. The tertiary follicle matures to become a Gräafian follicle, which is capable of undergoing preovulatory growth leading to ovulation if exposed to an ovulatory surge of LH (22). Growth of the late secondary follicle to become a Gräafian follicle is dependent on gonadotropin support from the hypophysis (23).

When secondary follicles reach the preantral stage of development, they undergo a process called recruitment. Recruitment is when follicles acquire the ability to respond to gonadotropins, which are required for continued growth of the follicle. Follicles then undergo the process of selection, where only a few of the recruited follicles are selected to escape atresia and continue to grow. From those follicles undergoing the selection process, one or a few follicles acquire dominance and the others become atretic. Dominance is the mechanism(s) whereby an ovulatory or dominant follicle uses to escape atresia, to inhibit growth of smaller follicles

and to become Gräafian follicles (8, 24). Some growing follicles undergo atresia from the early preantral through the dominance stage (8, 24).

The size of dominant follicles not undergoing preovulatory growth to ovulation ranges from 5 to 10 mm in sheep and 12 to 19 mm in cattle (25). In sheep, the time required for development of a primary follicle into a preovulatory follicle is estimated to be 6 months. Most of this time is in the preantral stage, but only 33 to 43 days are required to reach the preovulatory stage once an antrum is formed (26-28). In cattle, it takes 22 days to grow from the early antral stage to preovulatory size (29).

1.1.2.2 HORMONAL REGULATION OF FOLLICULAR DEVELOPMENT

Hypophysectomy or short term suppression of follicle stimulating hormone (FSH) inhibits development of preantral and antral follicles (30, 31). Antrum development is restored by FSH alone (8, 23). Preantral follicles acquire receptors for FSH. Recruitment occurs when the follicles become responsive to FSH. At this point, the preantral and subsequent stages of follicle growth are dependent on FSH for continued development into a dominant follicle (8, 23, 32-34). In the rat, the increase in FSH at estrus regulates the recruitment of follicles for ovulation at estrus in the next cycle. Antibodies to FSH given at proestrus-estrus results in no ovulations at estrus in the next estrous cycle. This indicates that the process from recruitment to ovulation of follicles in the rat requires 6 to 7 days (23).

All growing follicles recruited are not at the same stage of development and many become atretic during selection and dominance.

Nonatretic antral follicles produce estradiol-17ß, while estradiol-17ß and aromatase activity are absent or reduced in atretic follicles (35-39). Antral follicles produce estradiol-17ß (35-39) and inhibin (40, 41). Inhibin decreases FSH secretion by the hypophysis and causes small and medium sized follicles to become atretic (40, 41). Atresia occurs in any size of follicles in sheep (42) or cattle (39), when aromatase activity is decreased. Large nonatretic follicles continue to produce FSH receptors on granulosa cells and continue to produce estradiol-17ß.

Dominant follicles secrete growth factors that affect the FSH-dependent recruitment process. While FSH and LH are required for the final stages of follicular development, FSH, LH or estradiol-17ß do not act directly on follicles to promote mitosis of granulosa cells *in vitro* (45). Mitosis of the granulosa cells appears to be controlled by transforming growth factor-α (TGF-α) and TGF-ß from the theca (46) with assistance from activin (44). Some growth factors produced by follicles inhibit follicular growth. Dominant follicles produce inhibin and follistatin. Both inhibit growth of granulosa cells at early stages of follicular development through an inhibition of steroidogenesis (44). This may explain why FSH is more effective in causing growth of follicles before dominance is established (43). Thus, some growth factors stimulate follicular development, while others inhibit follicular development (43-45).

Follicles are the major source of estradiol-17ß. Production of estradiol-17ß involves an interaction between granulosa and theca cells (47-49). Follicular-stimulating hormone from the hypophysis binds to FSH receptors on granulosa cells and activates adenylate cyclase production of

cyclic adenosine 3',5'-monophospate (cAMP) to increase secretion of progesterone and estradiol-17ß (8, 23, 34, 49). Granulosa cells cannot produce androgens from progesterone for conversion to estradiol-17ß by granulosa cells (35, 48, 49). Androgens are produced by the theca compartment of antral follicles by LH-stimulated increases of cAMP in theca cells (35, 48, 49). Theca cells do not acquire LH receptors until an antrum is formed (8, 23). The androgens diffuse to granulosa cells and FSH stimulation of aromatase activity in granulosa cells converts the androgens to estradiol-17ß (8, 23, 34, 35, 48, 49).

Luteinizing hormone is also involved in follicular development (50-59). Secretion of LH by the hypophysis for regulation of follicular growth is increased by GnRH from the hypothalamus. Levels of LH remain low throughout the estrous cycle until progesterone secretion by the CL decreases (54). When progesterone decreases, increases in pulse frequency for GnRH and LH are seen for the preovulatory growth of follicles destined for ovulation (54). However, waves of follicular growth, which require LH, occurs during the luteal phase when LH is very low. Follicles during the luteal phase increase the number of LH receptors to respond to low levels of LH (54, 57-59).

1.1.2.3 FOLLICULAR DYNAMICS AND SECRETION OF ESTRADIOL-17ß DURING THE ESTROUS CYCLE OF SHEEP AND CATTLE

Large follicles greater than 2 mm in the ewe (58) and 8 mm in the cow (60) are present on the ovary throughout the estrous cycle. In the ewe (29) and cow (61), the major estrogen secreted throughout the estrous

cycle by dominant follicles is estradiol-17ß. When follicles are no longer dominant, secretion of estradiol-17ß declines and the follicles become atretic (23). During the estrous cycle, increases in estradiol-17ß are seen in sheep (62-67) and cattle (68). These increases occur between days 3 to 5, 8 to 11 and 16 to 17 in sheep (63, 64) and days 3 to 8, 9 to 13 and 20 to 22 in cattle (68). These increases in estradiol-17ß are associated with waves of follicular growth in sheep (58, 64) and cattle (69). Although some report only two waves of follicular growth in heifers during the estrous cycle (70, 71), most agree that there are three waves of follicular growth during the estrous cycle in cattle (72-74). A new wave of follicles occurs about every seven days in mature cycling cattle (48, 72, 73).

1.1.2.4 PREOVULATORY FOLLICULAR GROWTH

Concentrations of estradiol-17ß in jugular blood during the luteal phase ranges between 1 to 2 pg/ml, but by estrus ranges from 10 to 14 pg/ml in sheep (62) or 10 to 25 pg/ml in cattle (68). This increase in estradiol-17ß occurs prior to the preovulatory surge of LH (58, 68, 72, 73). The LH surge inhibits secretion of estradiol-17ß by the preovulatory follicle. Steroidogenesis by the granulosa cells shifts from estradiol-17ß to progesterone in preparation for oocyte maturation and ovulation (8, 23, 48, 75). Androgen production by the theca is inhibited by the LH surge (76, 77). Concentrations of estradiol-17ß secreted by the waves of growing follicles at other times of the cycle are similar, but ovulation is not induced. Progesterone from the CL inhibits ovulation. Estradiol-17ß secreted by follicular waves at midcycle, when the CL is secreting maximal levels of

progesterone, is attenuated by elevated levels of progesterone (65, 68). Mechanisms of this inhibitory action of progesterone are through inhibition of low level tonic secretion of LH (68). More follicular development occurs on the ovary with a CL than one without a CL (74). Progesterone may be increasing the recruitment and selection of follicles to the antral stage (23).

1.1.3 OVULATION

Events leading to ovulation are initiated by the decline in progesterone at the end of the estrous cycle (1, 12). Estradiol-17ß secretion by the dominant follicles destined to ovulate increases and peaks before or by the onset of behavioral estrus (8). The peak of estradiol-17ß that causes behavioral estrus also causes a hypothalamic surge of GnRH to be released. This surge of GnRH causes the preovulatory surge of LH and FSH from the hypophysis (7). The surge of LH occurs about 9 hours after the onset of estrus in sheep (65) and during the first 8 hours of estrus in cattle (3). After the LH surge and before ovulation, macrophages, leukocytes and mast cells infiltrate the dominant preovulatory follicles (12, 78-81). These cells are thought to be involved in the ovulatory process (78-81).

Ovulation involves a complex interaction among LH, progesterone, $PGF_2\alpha$ and prostaglandin E_2 (PGE_2; 78-87). Progesterone in ovulatory follicle increases 12 to 16 hours after the ovulatory surge of LH (82, 83). Inhibition of progesterone biosynthesis with isoxasol blocks ovulation. This can be reversed by giving progesterone or $PGF_2\alpha$ (84, 85). Prostaglandin $F_2\alpha$ and PGE_2 increases in the preovulatory follicle. Prostaglandin E_2 decreases by 20 hours, while progesterone and $PGF_2\alpha$

continue to increase. Indomethacin, an inhibitor of prostaglandin biosynthesis, blocks ovulation which can be reversed by $PGF_2\alpha$ or PGE_2 (84-87). Progesterone enhances follicular synthesis of $PGF_2\alpha$ from PGE_2 through activation of the enzyme, 9-keto PGE_2 reductase (88). Nine-keto PGE_2 reductase converts PGE_2 to $PGF_2\alpha$ (89, 90). Prostaglandin E_2 is produced primarily by the granulosa cells, while $PGF_2\alpha$ is produced primarily by the theca cells of the ovulatory follicle (86, 87).

Prostaglandin E_2 stimulates the dissolution of junctional complexes connecting granulosa cells and the oocyte (91). Prostaglandin $F_2\alpha$ activates collagenolysis in the theca cells (91). Luteinizing hormone causes hyperemia of the ovulatory follicle through histamine which is propagated by PGE_2, but is terminated by $PGF_2\alpha$ (91, 92). When ovulation is blocked by the inhibitor of prostaglandin synthesis, indomethacin, the follicle has a persistent hyperemia (92, 93), luteinizes with the oocyte still enclosed and secretes progesterone equivalent to that seen during the luteal phase (93). Most of these events occur only at the ovulatory stigma of the preovulatory follicle (94, 95). Thus, progesterone, PGE_2 and $PGF_2\alpha$ causes follicular rupture through a series of complex events to release the oocyte and form the corpus hemorrhagicum (25). The corpus hemorrhagicum develops into the CL (25).

Similar data for roles of LH, cAMP, $PGF_2\alpha$, PGE_2, progesterone and inflammatory cells in ovulation exists for other vertebrates as presented for the ewe (96-99). However, ovulation occurs in some animals even when nonsteroidal anti-inflammatory drugs lower prostaglandin synthesis (96). On the other hand, other inflammatory-related molecules (calcium, leukotrienes,

bradykinin, platelet activating factor) have been suggested to be involved in ovulation (96, 97). These data support the earlier theory that the surge of LH initiates an acute inflammatory reaction in ovulatory follicles (99). It has been proposed that this local accumulation of multiple inflammatory ligands results in depolarization of plasma membranes of many follicular cell types to activate proteolytic enzymes and degrade the follicular wall (96). It has also been proposed that the rise in progesterone after ovulation inhibits this inflammatory process (97).

1.1.4 FUNCTION AND DEVELOPMENT OF THE CORPUS LUTEUM

1.1.4.1 DEVELOPMENT OF THE CORPUS LUTEUM

The requirement of the CL for a successful pregnancy was first reported in rabbit. It was hypothesized that the CL was necessary for implantation of the fertilized ovum and to maintain pregnancy. When the CL of rabbits was cauterized, the embryo is resorbed or fetuses are aborted (100). This led to the identification of progesterone as the product in the CL that is necessary for maintenance of pregnancy (101).

In nonpregnant sheep, weights of CL increase from days 4 to 6 postestrus and to maximal weights by days 10 to 14. Weights of CL begin to decrease only after concentrations of progesterone decline (102-105). Weights of bovine CL increase from day 3 to maximal weights by day 12 postestrus before decreasing on day 18 after progesterone secretion starts to decrease (106, 107). Thus, functional regression of the CL occurs prior to morphological regression, since progesterone decreases prior to decreases in CL weights (6).

1.1.4.2 STEROID SECRETION BY THE CORPUS LUTEUM

Steroids secreted by the CL of sheep are progesterone, pregnenolone, 20α-dihydro-progesterone, 17α-hydroxy-progesterone and androstenedione (108). Bovine CL produce progesterone, pregnenolone and 20ß-hydroxy-4-pregn-3-one (107, 109, 110). Bovine CL do not produce androgens, since they do not have the 17,20-desmolase enzyme (110). Corpora lutea of cattle or sheep do not form estrogen, because the aromatase gene is not expressed (108-111).

In the nonpregnant ewe, a significant positive correlation exists between the content and concentration of progesterone in the CL and concentration of progesterone in the ovarian vein (102). Similar correlations exist for the concentrations of progesterone in the CL and of progesterone in jugular venous blood throughout the estrous cycle (103, 104). Progesterone content and concentration in the CL are positively correlated with CL weight (112) and to receptors for LH on luteal tissue (113). These studies indicate that concentrations of progesterone in jugular venous blood are good indicators of the degree of luteal function.

Concentrations of progesterone in jugular venous plasma of sheep during the estrous cycle are less than 1 ng/ml on days 0 (estrus) to 3 postestrus. Concentrations of progesterone increase to 1.5 to 4 ng/ml by day 8 and remains constant until day 13 postestrus. Progesterone declines after day 13 to levels less than 1 ng/ml by 12 to 24 hours prior to the onset of estrus (114-121). Similar changes in progesterone are seen in ovarian venous blood (102, 122). Similar patterns of progesterone in jugular and

ovarian venous blood are seen during the bovine estrous cycle (107). Progesterone is released in a pulsatile fashion within day by the CL (123). Weights and size of CL decrease during luteolysis, luteal cells involute and numbers of blood vessels in the CL decrease (103). This may explain the changes in color of a functional CL from pink to white in a regressed CL (corpus albicans; 99). Size, number and fragility of lysosomes in luteal tissue changes by day 15 postestrus. Lysosomes on days 11 to 14 postestrus in ovine CL are rare or small. Lipid droplets are few and small on days 11 to 14, but numbers and size of lipid droplets increase on day 15 (124).

1.1.4.3 HORMONAL REGULATION OF CORPUS LUTEUM FUNCTION

Luteal secretion of progesterone in nonpregnant sheep is stimulated by LH, although levels of LH and progesterone in jugular venous blood are negatively correlated (125). Secretion of progesterone by ovine CL is stimulated by LH *in vitro* (108, 126, 127). The negative correlation of LH and progesterone *in vivo* is probably due to the inhibition of progesterone on GnRH secretion by the hypothalamus and by a reduced sensitivity of the hypophysis to GnRH. Concentrations of LH are low when progesterone is elevated, but only low levels of LH are needed to stimulate luteal secretion of progesterone (53, 54).

Whether the hypophysis is required for regulation of luteal function, and whether only LH or both LH and prolactin are required for stimulation of luteal secretion of progesterone in sheep has been controversial for decades. Both LH and prolactin are required for luteal function in pseudopregnant rats (59). Initially, it was reported that

hypophysectomy had no effect on the formation or maintenance of CL induced in prepubertal ewe lambs (128). However, the first luteal tissue formed at puberty in sheep does not produce an estrous cycle of normal length (129). The CL fails to form in ewes hypophysectomized on day 1 postestrus. When ewes are hypophysectomized on day 5 postestrus, the partially formed CL regresses early (130). In addition, hypophysectomy of hysterectomized ewes causes early regression of the CL (131). Development of the fully functional CL requires LH after day 4 postestrus (3, 5). The uterus may also play a role in development of the CL. Corpora lutea of ewes hysterectomized early after ovulation do not develop completely and function normally based on luteal weights and progesterone secretion when compared to uterine intact ewes (132).

LH has been determined to be the only hypophyseal luteotropic factor needed for maintenance of luteal secretion of progesterone in the ewe (1, 3, 5). Initially, prolactin was thought to be the ovine luteotropin. Prolactin maintains weights of CL in ewes that are both hypophysectomized and hysterectomized, but not when the uterus is present (131, 133). Progesterone in ovarian venous plasma does not decrease throughout the estrous cycle, when a pituitary stalk section is done on days 3 or 4 postestrus (134, 135). However, concentrations of progesterone in ovarian venous plasma decrease in ewes stalk-sectioned on day 10 postestrus (135). Stalk-section of the pituitary lowers LH, but increases prolactin (53, 54). Thus, a pituitary luteotropin exists during the last third of the ovine estrous cycle (134). This was a valid conclusion at the time, since the rat was shown to require a luteotropic complex which was LH and prolactin

(59). Prolactin causes the formation of cholesterol esters by activating the enzyme cholesterol ester synthetase to form cholesterol esters, while LH activates the enzyme cholesterol esterase which frees cholesterol to be used for steroidogenesis (136). Ovine CL have cholesterol esterase and cholesterol ester synthetase (108). Others found that crude preparations of prolactin or estradiol-17ß did not maintain luteal weights in hypophysectomized ewes with an intact uterus (137, 138). In addition, prolactin does not stimulate secretion of progesterone when infused into the ovarian artery (139). Two-Br-α-ergocryptine, an inhibitor of prolactin secretion, given throughout the ovine estrous cycle lowers prolactin in blood by 95 percent. When 2-Br-α-ergocryptine, an inhibitor of prolactin release, and/or antibodies to prolactin are given daily, secretion of progesterone is not affected (140, 141). In addition, receptors for prolactin have not been detected on ovine luteal tissue (142). Crude pituitary preparations containing LH and FSH maintain luteal function in hypophysectomized, uterine intact nonpregnant and pregnant ewes (137). It was determined later that only purified preparations of LH (133), but not prolactin (144) maintains luteal function and enhances secretion of progesterone in intact ewes. Daily infusions of antibodies to LH causes premature regression of ovine CL (145). Similar data exist in cattle, LH increases concentrations of progesterone in CL of hysterectomized cows (146); LH increases secretion of progesterone by bovine luteal tissue *in vitro* (1, 3, 5); and antibodies to LH reduces luteal weights and progesterone in intact and hysterectomized cows (147).

Receptors for LH on luteal tissue are highly correlated to the growth and development, function and regression of the CL. Numbers of unoccupied or occupied receptors for LH increase from days 2 to 10, remains high from days 10 through 14, and then declines. Numbers of LH receptors on days 12 through 20 postestrus do not decrease in pregnant ewes (105, 112, 113). Numbers of unoccupied and occupied receptors for LH are highly correlated to weights of CL, progesterone in jugular blood and concentrations of progesterone in CL throughout the estrous cycle of the ewe (141, 142). Thus, receptors for LH are more positively correlated with progesterone than are circulating levels of LH (113). In summary, LH appears to be the only luteotropin needed for the development and function of the CL in nonpregnant sheep.

1.1.4.4 ORIGIN OF CELL TYPES IN THE CORPUS LUTEUM

Corpora lutea of sheep and cattle contain many cell types with distinct histological and cytological features (103, 148-151). Two types of steroidogenic luteal cells are formed in the sow CL. The larger lutein cells are derived from the granulosa cells and smaller lutein cells are derived from the theca interna (101). However, it was first reported that the CL of sheep is formed by the granulosa and theca layers of the Gräafian follicle, but the CL contains only one type of steroidogenic lutein cell. This lutein cell is derived from the stratum granulosum; connective tissue and nonstriated smooth muscle cells are derived from the theca interna; and erythrocytes, histiocytes, macrophages and leukocytes in the CL are from the blood stream. Lutein cells are reported to undergo changes in size, nuclear

structure and cytoplasmic inclusions, which can be correlated to the stage of the estrous cycle. Degenerative changes in the ovine CL occur at the end of the estrous cycle, which includes elimination of capillaries and lutein cells, degenerative changes in the nucleus and cytoplasmic inclusions, accumulation of lipids and connective tissue is left as the major constituent in the regressed CL (corpus albicantia; 150).

Later, it is reported that within 24 hours after ovulation in sheep, luteal cells are derived from the theca interna. These cells migrate into the deeper granulosa cell-derived areas and theca-derived cells are distributed throughout the CL (152, 153). Similar data for origin of two distinct steroidogenic luteal cell types and their origin exist in cattle. It has been confirmed by using monoclonal antibodies to antigenic determinants on granulosa and theca cells of Gräafian follicles that they are similar to those on two types of steroidogenically active large and small luteal cells in the bovine CL (1, 3, 164). This conclusion is further supported by studies where the granulosa cells are removed, removed and replaced, or not removed from the preovulatory follicles of cattle. When only granulosa cells are removed from the preovulatory Gräafian follicle, concentrations of progesterone are lower than in cows with only granulosa cells removed and replaced, while levels of progesterone are greater in control cows than both groups. There is no difference in the length of the estrous cycle of the three treatment groups (154).

Corpora lutea contain many cell types with distinct cytological features. These cell types include small luteal cells (SLC), large luteal cells (LLC), endothelial cells, fibroblasts, pericytes, smooth muscle cells,

macrophages, leukocytes, erythrocytes and occasionally plasma cells. The cell types are identified after enzymatic dispersion of the CL and separation of luteal cells by sedimentation on columns or by elutriation in sheep (105, 127, 155-161), cattle (162-165) and pigs (166, 167). Both SLC and LLC have been determined to be steroidogenic by biochemical and morphological techniques (152, 153, 156, 158, 168). This supports the initial hypothesis of two steroidogenic lutein cells (101, 151-153).

1.1.4.5 CELLULAR COMPOSITION OF THE CORPUS LUTEUM

While the total volume density of SLC and LLC remains constant during the ovine estrous cycle, total number of steroidogenic cells increases four fold from days 4 to 8 postestrus. This increase in total steroidogenic cells is due to an increase in the numbers of SLC, which increases four fold from days 4 to 8 postestrus. Numbers of LLC do not change during the estrous cycle, but increase two fold in size by day 8 postestrus in sheep. The size of SLC does not change during the estrous cycle (113, 159, 160). Numbers of SLC decrease at the end of the estrous cycle (105, 160) or when $PGF_2\alpha$ is given to regress the CL (169). Large luteal cells decrease in size, but not in numbers during spontaneous or a $PGF_2\alpha$-induced luteolysis (105, 160, 169). There may be conversion of SLC to LLC or LLC which are fragile may break down. Ratios of SLC to LLC increase from 1:1 on day 4 to 2:1 by day 8 and 3:1 at day 10, then remain 3:1 for the rest of the estrous cycle (105, 159, 160, 162). Total volume occupied by the LLC and SLC in the CL remains at about 55 percent throughout the estrous cycle (105, 160). Small luteal cells may be

converted to LLC, since LH or human chorionic gonadotropin (hCG) increases the number of LLC and decreases the ratio of SLC to LLC (170).

1.1.4.6 CYTOLOGICAL FEATURES OF LARGE AND SMALL STEROIDOGENIC LUTEAL CELLS

Large luteal cells occupy the greater volume of the CL, approximately 30 percent, but represents less than 10 percent of the total cells in the CL (105, 158-162). Large ovine luteal cells attain their mature size and cytological features 6 to 7 days after ovulation (158). Large luteal cells are polyhedral in shape with a diameter greater than 25 μm and contains a single, round or oval and pale nucleus with one or two nucleoli. The nucleus is centrally located in the LLC (158, 163). The nuclear to cytoplasmic ratio is 1:27 (158). The surface of the LLC is folded extensively to form microvillous or flattened projections, which extend and are parallel to the surface. These projections interdigitate with projections from the same or adjacent cells, including both LLC and SLC. At sites of interdigitation, membranes of adjacent processes are separated by cell adhesion material. The cell surface is covered by a well defined basal lamina except at areas of close contact with adjacent cells (103, 158, 163, 173-174). Electrical coupling between luteal cells during secretion has not been demonstrated (175). The cytoplasm of the LLC has large quantities of smooth endoplasmic reticulum with anastomosing networks of branching tubules and large numbers of mitochondria with tubular cristae. These are indicative of steroidogenic synthesis. Multiple, parallel and flattened cristae of isolated stacks of endoplasmic reticulum, a prominent golgi complex, and

numerous plasma membrane bound granules (0.15 to 0.3 μm in diameter) are seen. Electron dense granules are often released by exocytosis. This is indicative of polypeptide or protein synthetic and secretory function (103, 158, 163, 173-175).

Small luteal cells are stellate or elongate in shape, range in size from 12 to 22 μm (158, 163) and the nuclear to cytoplasm ratio is 1:8 (158). The nucleus is less regular in shape, stains more densely due to a coarser pattern of heterochromatin and contains one or two small nucleoli. The nuclear envelope contains many complex infoldings and nuclear inclusions with modified cytoplasmic organelles, detached from the cytoplasm. Folding of the plasma membrane and formation of basal lamina are reduced compared to LLC and surface projections are microvillous. The cytoplasm contains large amounts of tubular endoplasmic reticulum with only small groups of sparsely distributed ribosomes. Aggregates of rough endoplasmic reticulum, golgi complexes and secretory granules are lacking and small numbers of dense membrane bound granules, possibly lysosomes, are seen, but there is no evidence of exocytosis. Mitochondria contain both tubular and lamellar cristae and lipid droplets, which are less densely packed than in LLC. Mitochondria in SLC are more irregular in size than in LLC (153, 158, 163, 173-175). All of these characteristics of SLC indicate steroidogenesis, but do not indicate secretion of proteins (173-175).

Secretory granules are abundant in LLC, but not in SLC (173). Although secretory granules are similar to lysosomes, they are released at the cell surface by exocytosis (173-176), but do not contain acid

phosphatase or catalase (174). These granules in LLC contain oxytocin, vasopressin and neurophysin (176) and relaxin (177).

1.1.4.7 HORMONAL REGULATION OF STEROIDOGENIC LUTEAL CELLS

Both SLC and LLC contain adenylate cyclase, which converts adenosine triphosphate (ATP) to the second messenger cAMP. Incubation of luteal tissue *in vitro* with dibutyryl-cAMP (dbcAMP) causes dose-dependent increases in progesterone secretion (178, 179). LH also increases cAMP in a dose-dependent manner and progesterone secretion by luteal tissue *in vitro* (180, 181). However, it was reported recently that concentrations of cAMP do not change in luteal tissue in response to LH until there is a 50 percent increase in progesterone secretion. This suggests that other second messengers may be involved in the response to LH up to 50 percent maximal steroidogenesis. Other second messenger may be calcium or a leukotriene (182-184). Nordihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenase and synthesis of leukotrienes, but not indomethacin, an inhibitor of prostaglandin H synthase and thus prostaglandin synthesis, blocks the LH-induced 0 to 50 percent increase in maximal steroidogenesis (184). Thus, LH/cAMP-dependent regulation of luteal steroidogenesis may be involved only from 50 to 100 percent maximal steroidogenesis.

Basal secretion of progesterone by ovine SLC *in vitro* is 6 to 10 fold lower than that secreted by LLC (105, 157, 158, 160, 185-188). This difference appears to be related to differences in volume density of mitochondria between SLC and LLC. The greater volume density could provide more pregnenolone for 3ß-hydroxysteroid dehydrogenase (3ß-HSD)

for conversion to progesterone (186). Luteinizing hormone increases secretion of progesterone by SLC *in vitro* 6 to 20 fold (155, 185-187). Large ovine luteal cells contain LH receptors (185), but are not responsive to LH except at pharmacologic doses (175). Forskolin or cholera toxin enhances the binding of cAMP to cAMP-dependent protein kinase A, but does not increase secretion of progesterone by LLC. The steroidogenic machinery in ovine LLC appears to function at maximal capacity and cannot be stimulated further by LH (142, 188). The lack of response to LH by LLC is related to differences in gene regulation of side chain cleavage (Scc) enzyme complex activity in mitochondria. The side chain cleavage enzyme complex converts cholesterol to pregnenolone, the rate limiting step in steroidogenesis. Messenger ribonucleic acid (mRNA) for production of Scc enzyme in SLC requires stimulation by LH, which switches off rapidly after LH is withdrawn (189). Genes regulating the production of mRNA for Scc enzyme complex in LLC remain functional and do not require LH stimulation (189). It has been concluded from *in vitro* studies that 20 percent of progesterone in the ovarian vein is secreted by SLC and 80 percent is secreted by LLC, although the ratio of SLC to LLC in mature CL is 3:1 (187, 188). This probably best explains the lack of a correlation between the low levels of circulating LH and progesterone secreted during the luteal phase in ewes (116).

Interactions between ovine SLC and LLC for regulation of progesterone secretion may occur. Co-culture of SLC and LLC secrete more progesterone than the combined amount of progesterone secreted by SLC and LLC cultured separately (105, 160, 185, 188). Other tropic stimuli may

also regulate steroidogenesis by SLC or LLC. Receptors for prostaglandin E_1 (PGE_1), PGE_2, prostaglandin I_2 (PGI_2), oxytocin, norepinephrine and estradiol-17ß are also found on ovine luteal tissue (1, 187, 188). Prostaglandin E_1, PGE_2, PGI_2 and norepinephrine stimulates dose dependent increases in progesterone secretion by ovine luteal tissue *in vitro*, but $PGF_{2\alpha}$ or oxytocin do not (1, 187, 188). Norepinephrine receptors are predominantly on SLC (1, 187, 188). Receptors for PGE_1, PGE_2 and PGI_2 are 10 fold greater on ovine LLC than on SLC. Prostaglandin E_1, PGE_2 or PGI_2 stimulates secretion of progesterone by bovine SLC or LLC *in vitro* (157, 164, 187, 188, 190). Estradiol-17ß does not increase secretion by luteal tissue *in vitro*, although estradiol-17ß receptors are 8 fold greater on LLC than on SLC (191).

1.1.5 LUTEOLYSIS

1.1.5.1 UTERO-OVARIAN RELATIONSHIPS--ANATOMICAL/VASCULATURE

1.1.5.1.1 ANATOMICAL RELATIONSHIPS

Removal of both uterine horns extends luteal function in cattle (192), sheep (193-196), pigs (197), horses (198, 199), guinea pigs (200-203); and in pseudopregnant rats (204-206), rabbits (207) and mice (208). Hysterectomy does not extend luteal life span in monkeys (209), women (210), or dogs and cats (211). Naturally occurring congenital anomalies, such as an absence of one or both uterine horns, have resulted in an extension of interestrous intervals in sheep and cattle equivalent to the length of gestation (212, 213). The uterus is not only necessary for the initiation, but also for the completion of luteolysis, since bilateral

hysterectomy as late as day 15 postestrus in sheep delays the completion of luteolysis 3 to 4 days (214, 215). These data demonstrate that the uterus is the source of a uterine luteolysin to regress the CL at the end of the estrous cycle.

There is a local effect of the uterus on the life span of the CL in some mammalian species. This was first demonstrated in hemi-hysterectomized gilts (197). Corpora lutea adjacent to the remaining uterine horn of hemi-hysterectomized gilts were regressed, but CL on the side of hemi-hysterectomy were well developed and functional (197). Removal of one uterine horn results in regression of CL on ovaries adjacent to the intact uterine horn, but CL remain functional on the opposite side in ewes (194, 196, 216), cows (212, 213, 217) or guinea pigs (202-204, 208); and in the pseudopregnant rat (205, 206), hamster (208) and gerbil (218). When both the uterus and ovaries are autotransplanted to the neck in sheep, length of the estrous cycle is of normal length (219). However, when either the ovaries or uterus are transplanted alone, luteal regression is delayed (220). This demonstrates that an adjacent uterine horn is necessary to regress the CL in sheep. The amount of uterine tissue removed is directly proportional to the increase in length of the estrous cycle in sheep (195, 196), pigs (208), guinea pigs (221) and pseudopregnant rats (204-206). The pig is unique, because CL on both ovaries regress when less than one-fourth of the uterine horn is removed. When less than one-fourth of the total uterus remains, luteolysis occurs only adjacent to the retaining uterine fragment (197). Surgical separation of the ovaries and uterus in the pig delays, but does not prevent regression of the CL (222). Therefore, the uterus of pigs

controls luteolysis by both local and systemic routes. In the mare (198) and pseudopregnant rabbit (223), the uterus controls luteal regression primarily through a systemic route, since CL on both ovaries regress at the same time after unilateral hysterectomy.

1.1.5.1.2 SOURCE OF THE UTERINE LUTEOLYSIN

The source of the luteolysin in the uterus is the endometrium. Transplants of bovine endometrium but not myometrium to the cheek pouch of hysterectomized, pseudopregnant hamsters induces luteolysis as effectively as whole uterine transplants (224). This also suggests that the uterine luteolysin is similar across mammalian species. Destruction of the endometrium causes an extension of luteal function in sheep (225), pigs (226) or guinea pigs (221). Loss of the uterine luteolytic mechanism occurs when uterine infections are established at midcycle (219).

Extracts of endometrium collected around the time of onset of luteal regression in sheep (208, 224), cattle (228, 229) or pseudopregnant rats (227) are luteolytic, when injected into the ovarian artery of nonbred animals. Uterine venous plasma collected from nonpregnant ewes on days 14 to 16 postestrus causes premature regression of the CL, when infused into the ovarian artery of *in situ* or autotransplanted ovaries on day 8 postestrus. However, uterine venous plasma collected at earlier stages of the estrous cycle or jugular venous plasma collected on any day of the estrous cycle of sheep are not luteolytic (230).

1.1.5.1.3 ROUTE FOR DELIVERY OF THE UTERINE LUTEOLYSIN

The ovine uterine luteolytic pathway involves the uterine branch of the ovarian vein and the ovarian branch of the ovarian artery. Ligation of the uterine branch of the ovarian vein (231-233) or separation of ovarian and uterine vasculature (194, 231, 234) delays luteal regression. When the uterine branch of the ovarian vein or the ovarian artery adjacent to a uterine horn of bilaterally ovulating ewes is surgically anastomosed to the corresponding artery or vein on the opposite side without a uterine horn, luteal regression occurs on only the side of hemi-hysterectomy (235-238). Therefore, the uterine branch of the ovarian vein and its adjacent ovarian artery are necessary for local transfer of the uterine luteolysin. Similar data exist in cattle (239).

The ovarian artery wraps around the uterine branch of the ovarian vein many times (240-242). The ovarian artery also coils around the many collateral branches of the uterine and ovarian branches of the ovarian vein (242). Thus, a large surface area is available for transfer of the uterine luteolysin locally from uterine venous to ovarian arterial blood. Small vascular channels between the areas of contact connecting the vein and artery have been proposed for delivery of the luteolysin (243), but have not been confirmed by histological examination (244). This close anatomical association of uterine veins and ovarian arteries in the mare and rabbit where the uterine luteolysin is delivered via a systemic vascular route is reduced markedly (240). Thus, delivery of the uterine luteolysin from the

uterine vein to the adjacent ovarian artery is transferred by a countercurrent mechanism (240).

1.1.5.2 THE UTERINE LUTEOLYSIN -- $PGF_{2}\alpha$

1.1.5.2.1 INTRODUCTION

Since exogenous $PGF_{2}\alpha$ causes premature luteolysis and uterine secretion of $PGF_{2}\alpha$ occurs at the time of onset of luteolysis, the uterine luteolysin is thought to be $PGF_{2}\alpha$ (245). Prostaglandin $F_{2}\alpha$ given after day 4 postestrus in sheep advances luteolysis (245-251). Doses of $PGF_{2}\alpha$ given intramuscularly must be sufficient to overcome the catabolic activity of the lungs, if the dose is to be effective when given for delivery by systemic routes (245-251). Prostaglandin $F_{2}\alpha$ secreted endogenously is destroyed rapidly with one pass through the lungs in many mammalian species (251, 252). This is consistent with the local delivery of the uterine luteolysin in sheep or cattle (235, 240). When $PGF_{2}\alpha$ is given by different routes to sheep, smaller doses are required when given into follicles of the ovary with the CL or into the ovarian artery or intrauterine than when given intramuscularly (249-251). Four mg of $PGF_{2}\alpha$ per 58 kg body weight (BW) given intramuscularly is sufficient to cause premature luteolysis in sheep with a single CL (253). Eighty μg of $PGF_{2}\alpha$ given into the tissue space (ovarian vascular pedicle) between the ovarian vein and artery adjacent to the ovary with a single CL in sheep is sufficient to cause premature regression of the CL (254). Infusion of $PGF_{2}\alpha$ into the uterine vein of ewes at a rate of 40 μg per hour for 6 hours on day 6 or 9 postestrus in sheep causes premature luteolysis (121, 247, 248). In addition, $PGF_{2}\alpha$ infused at

2 μg per hour into the ovarian artery of sheep is luteolytic (255). These rates of infusion achieve levels of $PGF_{2}\alpha$ in the uterine vein similar to those normally found in uterine venous (10 ng/ml) or ovarian arterial blood (2 ng/ml) around the time of luteolysis in sheep (256, 257).

Prostaglandin $F_{2}\alpha$ given intramuscularly to cattle also causes luteolysis and an early return to estrus (258). Twenty-five mg of $PGF_{2}\alpha$ are required when given intramuscularly to induce premature luteolysis (259), while only 900 μg is required when given into a follicle on the ovary with the CL (260). Prostaglandin $F_{2}\alpha$ also causes luteolysis when given intramuscularly to pigs (261), mares(262), guinea pigs (263); and in the pseudopregnant hamster (263), rat (264, 265) and rabbit (264). Intrauterine infusion of $PGF_{2}\alpha$ in the mare does not give a different response from the same dose given intramuscularly (262). The uterine luteolysin in the mare is delivered primarily by a systemic route (235, 240). Only 1 mg of $PGF_{2}\alpha$ given intramuscularly in the mare is needed to cause premature luteolysis (262), but 25 mg of $PGF_{2}\alpha$ are needed when given intramuscularly in cattle (259). These data suggest that the lungs of horses are less able to catabolize $PGF_{2}\alpha$ as it passes through the lungs compared to cattle or sheep. These data further support the local utero-ovarian luteolytic pathway seen in sheep and cattle and a systemic pathway in mares (235, 240).

Additional support for local delivery of the uterine luteolysin in sheep comes from infusions of tritiated $PGF_{2}\alpha$ (^{3}H-$PGF_{2}\alpha$) into the uterine branch of the ovarian vein in sheep. Radioactive $PGF_{2}\alpha$ gradually increases in the adjacent ovarian artery and continues to rise for 30 minutes after the infusion is terminated. Only 2 to 10 percent of the infused ^{3}H-$PGF_{2}\alpha$ is

transferred locally (266). Similar data with infusion of radioactive $PGF_{2}\alpha$ and its local transfer are seen in the cow (267). The amount of $PGF_{2}\alpha$ transferred from the uterine vein to the ovarian artery is directly proportional to the log of the concentration of $PGF_{2}\alpha$ in the uterine vein. Furthermore, competitive inhibition of non-radioactive $PGF_{2}\alpha$ with ^{3}H-$PGF_{2}\alpha$ does not seem to affect this local veno-arterial transfer of $PGF_{2}\alpha$ during the estrous cycle in ewes (268). These data suggest a diffusion of $PGF_{2}\alpha$ rather than a receptor-mediated active transport system. This may explain why hysterectomy as late as day 15 postestrus delays luteal regression (213, 215). Therefore, a continual diffusion of $PGF_{2}\alpha$ over a number of days is needed to complete luteal regression, since there is a low efficiency of transfer of $PGF_{2}\alpha$ locally in sheep (266) and cattle (267). Single injections of $PGF_{2}\alpha$ given intramuscularly to synchronize estrus are given at doses that exceed the catabolic activity of the lungs (259, 265, 273).

Physiological properties of $PGF_{2}\alpha$ also supports a passive transfer system. Prostaglandin $F_{2}\alpha$ is a small molecule. It is both lipid and water soluble, and binds weakly to serum albumin (269-272).

1.1.5.2.2 UTERINE SECRETION OF $PGF_{2}\alpha$ DURING LUTEOLYSIS

Concentrations of $PGF_{2}\alpha$ increase in uterine tissue and venous plasma, ovarian arterial and venous plasma, and in ovarian tissue of sheep from the onset and throughout luteolysis (247, 256, 257, 268, 274-279). Peaks of $PGF_{2}\alpha$ in uterine venous blood may occur as early as day 11 postestrus (256) and peaks occur approximately every 4 to 6 hours from days 14 through 16 of the estrous cycle (256, 279-282). The uterus

secretes about 40 μg of $PGF_2\alpha$ per hour during luteolysis, resulting in concentrations of $PGF_2\alpha$ in uterine venous of 10 ng/ml and 2 to 4 ng/ml in ovarian arterial blood (247, 248, 256, 257). Peaks of 13,14-dihydro-15-keto-$PGF_2\alpha$ (PGFM), the major catabolite of $PGF_2\alpha$ produced in the lungs, are seen in jugular blood of sheep along with $PGF_2\alpha$ at the time progesterone is decreasing (282).

In ruminants, production of $PGF_2\alpha$ is greatest in the caruncular portion of the endometrium. Concentrations of $PGF_2\alpha$ are higher in caruncular than in intercaruncular areas of the endometrium of ewes (283) during luteolysis. Concentrations of the enzyme cyclooxygenase and $PGF_2\alpha$ reductase (89, 90), which produce $PGF_2\alpha$, are greater in caruncular than in the intercaruncular endometrium at the time of luteolysis (283). Activity of these enzymes may be regulated by progesterone, estradiol-17ß, oxytocin and an IUD. Progesterone increases cyclooxygenase activity in caruncular tissue (283) and progesterone increases $PGF_2\alpha$ production by caruncular tissue, but is amplified by estradiol-17ß (284). Also, estradiol-17ß (284, 285), progesterone (286, 287), oxytocin (288-294) and an IUD-induced (295) luteolysis are associated with advancing secretion of $PGF_2\alpha$ by the uterus in sheep. Concentrations of $PGF_2\alpha$ are increased in uterine endometrium or venous plasma at the time of onset of luteolysis in the cow (296), pig (297), mare (298), guinea pig (299); and in the pseudopregnant rat (300, 301). Furthermore, PGFM is elevated in jugular blood of heifers during luteolysis at the end of the estrous cycle (302).

Indomethacin, an inhibitor of prostaglandin biosynthesis, delays luteolysis in sheep or cattle (303) and in the pseudopregnant rabbit (304)

and rat (305). In addition, active or passive immunization against $PGF_{2\alpha}$ delays luteolysis in sheep (306) and guinea pigs (307).

1.1.5.2.3 REGULATION OF UTERINE SECRETION OF $PGF_{2\alpha}$

1.1.5.2.3.1 ROLE OF ESTROGEN IN REGULATION OF UTERINE SECRETION OF $PGF_{2\alpha}$

Regulation of uterine secretion of $PGF_{2\alpha}$ is a complex interaction between estradiol-17ß, progesterone and oxytocin. Estradiol-17ß given at midcycle in the ewe (308) or cow (309) causes premature luteolysis, but requires the presence of the uterus (309, 310). Estradiol-cyclopentyl-propionate, a long acting estrogen, given on day 10 postestrus reduces luteal progesterone concentration, but weights of the CL are maintained (311). This suggests that estrogen causes a functional luteolysis, but may delay the onset of morphological regression of the CL. Destruction of ovarian follicles, the source of estrogen, at midcycle by electrocautery or X-ray irradiation delays luteal regression in the ewe (312) and cow (313, 314). In addition, active immunization or passive immunoneutralization against estrogen in the ewe (315) or cow (316) delays luteal regression.

Estradiol-17ß causes a premature luteolysis by advancing uterine secretion of $PGF_{2\alpha}$. Time of estrogen injection, the dose and reproductive status are important factors. A single injection of estradiol-17ß given at mid-cycle in sheep causes a premature secretion of $PGF_{2\alpha}$ into uterine venous blood (284). Indomethacin blocks an estrogen-induced premature luteolysis in sheep and cattle (303). If estradiol-17ß is given on days 4 and 5 postestrus, $PGF_{2\alpha}$ does not increase prematurely in uterine

venous blood (284) and does not shorten the interestrous interval (309). Chronic intrauterine infusions of estrogen down-regulates uterine secretion of $PGF_{2\alpha}$ in sheep (285). Oxytocin increases $PGF_{2\alpha}$ in vena cava blood of anestrus sheep, but only when estrogen is also given (317, 318). Uterine response to estrogen to increase $PGF_{2\alpha}$ is related to the levels of estrogen and progesterone receptors in the endometrium. Estradiol-17ß given on days 11 and 12 postestrus increases receptors for estrogen and progesterone in the endometrium at 48 hours, but not at 24 hours. Chronic estrogen injections down-regulate estrogen receptors (318, 319). Single injections, but not chronic daily injections of estradiol-17ß with or without progesterone priming increases concentrations of $PGF_{2\alpha}$ in uterine venous plasma of chronically ovariectomized ewes (284).

1.1.5.2.3.2 ROLE OF PROGESTERONE IN REGULATION OF UTERINE SECRETION OF $PGF_{2\alpha}$

Progesterone given daily in the estrous cycle starting early after ovulation shortens the interestrous interval in sheep (320), cattle (321) and the guinea pig (320). This effect of progesterone requires the presence of the uterus (322) and is exerted through a local utero-ovarian pathway (321). Estradiol-17ß given on days 4 and 5 postestrus in sheep does not shorten the estrous cycle nor does it increase $PGF_{2\alpha}$ in uterine venous plasma unless progesterone priming is given for 5 days, starting early after estrus (284, 308, 310). There is a wave of follicular growth and an increase in estrogen secretion on days 3 to 6 (61). This could explain why progesterone priming early in the estrous cycle shortens the interestrous interval (284,

286). It has been hypothesized that progesterone alone is responsible for endometrial secretion of $PGF_2\alpha$. Progesterone given the day following estrus (323) or progesterone given at midcycle to acutely ovariectomized ewes (284, 286, 287) increases $PGF_2\alpha$ in uterine venous blood. However, in each of these studies, estrogen is elevated before progesterone was given. Thus, the uterus may require prior progesterone priming for estradiol-17ß to increase uterine secretion of $PGF_2\alpha$ in uterine venous blood.

1.1.5.2.3.3 OXYTOCIN IN REGULATION OF UTERINE SECRETION OF $PGF_2\alpha$

Oxytocin given to cattle during the first week of the estrous cycle shortens the interestrous interval (324) and is dependent on the presence of the uterus (325). A unilateral utero-ovarian mechanism is also involved, since premature luteolysis occurs only when the ovary containing the CL is adjacent to the remaining uterine horn of hemi-hysterectomized heifers (326). This luteolytic effect of oxytocin has been shown to be through premature increases of $PGF_2\alpha$ in uterine tissue and venous plasma of ewes and cows (288-294). Oxytocin increases uterine secretion of $PGF_2\alpha$ in anestrus sheep, but only when ewes are primed with estradiol-17ß (317). Oxytocin receptors in endometrium are reported to be necessary to activate endometrial prostaglandin synthesis (293). Numbers of oxytocin receptors in endometrium of sheep increase during days 13 to 16 postestrus (327). This occurs when progesterone is decreasing and estradiol-17ß is increasing (112). Estrogen increases the number of endometrial receptors for oxytocin, while progesterone inhibits estrogen-induced increases in

oxytocin receptors (328). Oxytocin increases during the period of luteolysis (292-294). This oxytocin is from LLC (329) and $PGF_2\alpha$ causes releases of oxytocin by LLC of the CL (330).

In summary, it is thought that progesterone priming of the uterus is required for estradiol-17ß secreted by midcycle dominant follicles to cause endometrial production and release of $PGF_2\alpha$ on days 12 to 13 postestrus (284). $PGF_2\alpha$ initiates the decrease in luteal secretion of progesterone (284) and numbers of progesterone receptors in the uterus (327, 330-332). This first release of $PGF_2\alpha$ causes a release of oxytocin from the CL. As progesterone and progesterone receptors decrease and estrogen and estrogen receptors increase, receptors for oxytocin increases (327). The release of oxytocin is thought to initiate the subsequent pulsatile peaks of $PGF_2\alpha$ secreted during luteolysis. This is probably facilitated by the increased preovulatory follicular secretion of estradiol-17ß. Maximum numbers of oxytocin receptors are seen at estrus , when levels of progesterone are low and levels of estradiol-17ß are greatest (327). Oxytocin receptors appear to increase after progesterone receptors are down-regulated (331, 332) and estrogen receptors are up-regulated (327). Thus, ovarian steroids appear to influence secretion of $PGF_2\alpha$ by the uterus directly as well as by regulating the levels of oxytocin receptors (327, 328, 333, 334).

1.1.5.2.4 SYNTHESIS OF PROSTAGLANDINS

Prostaglandins of the two series are synthesized from arachidonic acid, while the one series of prostaglandins are synthesized

from di-homo-γ-linolenic acid. The one or two series refers to the presence of one or two carbon-carbon double bonds on one or both of the acyl chains attached to the cyclo-pentyl ring (335). Incubation of arachidonic acid with ram seminal vesicles produces PGE_2, PGI_2 and $PGF_{2\alpha}$, while di-homo-γ-linolenic acid is converted to PGE_1 (336). Molecular oxygen is required for cyclooxygenase [also referred to as prostaglandin endoperoxide synthase or prostaglandin H synthase (PGHS)] to form PGH_2 which is the endoperoxide intermediate. The microsomal enzymes, $PGF_{2\alpha}$-reductase converts PGH_2 to $PGF_{2\alpha}$, while PGE_2-isomerase converts PGH_2 to PGE_2 (337). Intercoversion of PGE_2 and $PGF_{2\alpha}$ can also occur (89, 90). It requires the tripeptide glutathione as a cofactor and the enzyme 9-keto-PGE_2 reductase (339). Prostaglandin E_2 is similar to $PGF_{2\alpha}$, but has a keto-group on carbon 9 and an OH-group on carbon 11, while $PGF_{2\alpha}$ has an OH-group on both carbons 9 and 11 of the cyclo-pentyl ring. Arachidonic acid can also be coverted to leukotrienes by the lipoxygenase pathway (338). Arachidonic acid is stored primarily in phospholipids, triglycerides and cholesterol esters (335). Arachidonic acid from phospholipids appears to be the major source for prostaglandin biosynthesis. Arachidonic acid is made available for prostaglandin synthesis from phospholipids by the enzyme phospholipase A_2 (337).

1.1.5.2.5 CATABOLISM OF PROSTAGLANDINS

Prostaglandins are catabolized primarily by the lungs of most mammalian species. Prostaglandins are first oxidized at the 15-hydroxy group to convert it to a 15-keto moiety (251, 252). This is probably the

most important step for both biological inactivation and the formation of catabolites identified in blood or urine (337). Following the oxidation of the 15-hydroxy group to a ketone, the 13,14-trans double bond position, is reduced to form the 13,14-dihydro-15-keto-prostaglandin metabolite, which is the major inactive metabolite in the peripheral circulation (337). Prior to urinary secretion, this prostaglandin catabolite is degraded further by ß and *w* hydroxylation and oxidation and decarboxylation. Although the lungs are the primary site of catabolism of prostaglandins, other tissues also catabolize prostaglandins. A 90 to 100 percent conversion rate of biologically active prostaglandins is seen with one pass through the lungs. The enzymes have a greater specificity for prostaglandins of the E and F series than the A series (251, 252). Due to the nature of pulmonary catabolism, prostaglandins of the E and F series are quickly cleared from the peripheral circulation by the lungs (337). Thus, the E and F series of prostaglandins may function as hormones transported by local mechanisms to regulate organ and cellular functions. These catabolic data support further the local delivery mechanisms for the uterine luteolysin, $PGF_{2}\alpha$.

1.1.5.3 MECHANISM(S) OF LUTEOLYSIS CAUSED BY $PGF_{2}\alpha$

1.1.5.3.1 INTRODUCTION

Evidence exists for both an indirect and a direct effect of $PGF_{2}\alpha$ in luteolysis. Prostaglandin $F_{2}\alpha$ does not induce luteolysis through the hypothalamic/hypophyseal axis, since $PGF_{2}\alpha$ induces luteal regression in hypophysectomized pseudopregnant rats (338). In addition, levels of LH are lowest when the CL is fully functional and increases only after progesterone

decreases (121). Evidence exists for indirect actions of $PGF_2\alpha$ on the luteolytic process through the reproductive vasculature and through direct actions on the steroidogenic and nonsteroidogenic components of the CL (338). Also, $PGF_2\alpha$-induced luteolysis is not mediated through other ovarian compartments (339).

1.1.5.3.2 EVIDENCE FOR LUTEOLYSIS MEDIATED THROUGH THE VASCULATURE

1.1.5.3.2.1 REGULATION OF OVARIAN ARTERIAL BLOOD FLOW

Ovarian blood flow varies with stage of the estrous cycle in sheep (340), cows (341) or pigs (342). Blood flow to the ovary with a CL is highest during the luteal phase and lowest during the follicular phase where you have a corpus albicantia (112, 113, 141, 142, 187, 188). Blood flow and diameter of ovarian arteries differ between ovaries with or without a CL during the luteal phase. On ovaries with a CL, ovarian arterial diameter is two fold and blood flow is four fold greater than that to an ovary without a CL during the luteal phase. Within the ovary with a CL, blood flow is greater to the CL than the rest of that ovary or to an ovary without a CL (112, 113, 141, 142, 187, 188, 340).

In sheep, during spontaneous (112, 113) or $PGF_2\alpha$-induced (343) luteolysis, blood flow is reduced to the ovary with the CL and within that ovary, blood flow to the CL declines. There is a decreased volume of the capillaries (112, 113, 141, 142, 187, 188, 343) and capillary beds degenerate (168, 169, 344). Capillary blood flow decreases 90 percent during luteolysis in sheep (345-347). Blood flow to the ovary without a CL

is low and does not change during luteolysis (112, 113, 141, 142, 343). It has been suggested that $PGF_{2}\alpha$ causes luteolysis through its vasoconstrictor properties (346-348). Prostaglandin $F_{2}\alpha$ reduces blood flow only to the ovary with a CL. Reduction in luteal blood flow could cause hypoxia and which could be the cause of luteal regression. Altough blood flow decreases to luteal tissue during luteolysis, it continues to be 5 to 20 fold greater than in the surrounding ovarian tissue (112, 113, 343, 345, 349) making absolute hypoxia unlikely. It is likely that the decrease in luteal blood flow that accompanies luteal regression is due to the degeneration of capillary beds or its arterioles rather than the vasoconstrictor action of $PGF_{2}\alpha$ on the ovarian artery (112, 113, 165, 343, 349, 350).

Blood flow to the ovine luteal containing ovary is positively correlated with LH, FSH, and prolactin. Antibodies to FSH or prolactin do not reduce blood flow to the ovary with the CL. However, antisera to LH given to ewes during the estrous cycle causes a rapid decline in progesterone and blood flow to the ovary with a CL (112, 113, 141, 142, 187, 188). These data suggest that LH may have a secondary role to increase blood flow to the CL as well as to stimulate secretion of progesterone by luteal tissue (141). Since $PGF_{2}\alpha$ decreases progesterone before decreases in ovarian blood flow are seen and LH receptors on luteal tissue do not decrease until 7 hours after $PGF_{2}\alpha$ injection (112, 113), $PGF_{2}\alpha$ is probably initiating luteolysis by other mechanisms rather than through a reduction blood flow to the ovary.

Blood flow to the ovary with a CL is also positively correlated with the concentrations of progesterone in jugular blood during the luteal

phase and negatively correlated with the ratio of estradiol to progesterone during luteolysis in ewes (340), cows (341) and pigs (342). These correlations do not necessarily imply that progesterone is a vasodilator and estradiol-17ß is a vasoconstrictor of ovarian arterial vascular beds. In fact, estradiol-17ß causes dilation of the ovarian artery in sheep (351-353). Estradiol-17ß also decreases ovarian arterial contractile responses to nerve stimulation (348). The increase in ovarian blood flow during the luteal phase may not be due to progesterone, but may be due to the presence of a functional CL. The CL receives 90 percent of the total ovarian blood flow during the luteal phase (112, 113, 141, 142, 187, 188, 340, 341, 343). The decrease in ovarian blood flow at estrus is probably due to regression of the CL rather than a vasoconstrictor effect of estradiol-17ß (352, 353). Therefore, the effect of estrogen to increase ovarian blood flow may be masked by the decrease in the size of the luteal vascular beds of the ovary with a regressing CL during the follicular phase of the estrous cycle.

The effect of steroids and $PGF_2\alpha$ on contractility of ovarian arteries has been studied *in vitro*. Prostaglandin $F_2\alpha$ enhances constriction of the ovarian artery collected from the ovary with a CL, but not in ovarian arteries collected from the ovary without a CL (347). Prostaglandin $F_2\alpha$ regulates adrenergic stimulation and may decrease blood flow by increasing the synthesis and/or release of norepinephrine from the arterial blood vessels (354). However, norepinephrine causes constriction of ovarian arteries *in vitro* collected from ovaries with or without a CL (347). Ovarian arteries collected from an ovary with a CL would be exposed to higher concentrations of progesterone and estradiol-17ß. The progesterone would

be from the CL (108) and follicular fluid estrogens and numbers of antral follicles are greater on an ovary with a CL (61). Thus, blood flow to the ovary with a CL may be controlled by local concentrations of steroids or other ovarian factors.

1.1.5.3.2.2 REGULATION OF UTERINE ARTERIAL BLOOD FLOW

Uterine blood flow is low during the luteal phase and increases during the follicular phase in sheep (355), cattle (356), or pigs (357). Uterine blood flow is associated with the ratio of estrogen:progesterone. The greater the estrogen:progesterone ratio, the greater the quantity of blood that flows to the uterine vascular beds (351-356). Estrogen stimulates an increase in uterine blood flow in sheep (358), cattle (359) and pigs (360). This increase in uterine blood flow in reponse to estrogen is equally distributed among the endometrium, myometrium and caruncular vascular beds of the nonpregnant ewe (361). Uterine blood flow response to estrogen is delayed by 30 minutes and is maximal between 1 to 4 hours (358-360). This response to estrogen is blocked by protein synthesis inhibitors (357) and partially by indomethacin (362), but not actinomycin D, a transcription inhibitor (363). Thus, estrogen may be acting via translation of RNA in the cytosol.

Progesterone does not affect uterine blood flow directly, but reduces estrogen-induced increases in uterine blood flow (364). Reduction in uterine blood flow during the luteal phase is probably due to the high levels of progesterone and low levels of estrogen (104). Increases in uterine blood flow at estrus may be dependent on a previous exposure to

progesterone followed by its withdrawal and a concomitant increase in estradiol-17ß during luteolysis (364).

Since indomethacin partially blocks estrogen-induced increases in uterine blood flow, PGE_1 or PGE_2 may be mediators of estrogen to cause vasodilation. Prostaglandin E_1 and PGE_2 are vasodilators and increase uterine blood flow (346). Responses of uterine arteries collected adjacent or opposite to the ovary with the CL (348) are similar to those reported for ovarian arteries to $PGF_2\alpha$ and norepinephrine (349). This may be due to the local delivery of progesterone to the uterine horn adjacent to an ovary with the CL of sheep (365) and cattle (366). Progesterone potentiates the response of the uterine artery to the vasoconstrictiors, $PGF_2\alpha$ (346, 347) and norepinephrine (346, 347). Estrogen may counteract these vasoconstrictive effects through PGE_1 or PGE_2 (346). Uterine arteries have higher levels of α than ß adrenergic receptors (367). Alpha-adrenergic receptors mediate vasoconstriction, while ß-adrenergic receptors cause vasodilation (367).

1.1.5.3.3 EVIDENCE FOR LUTEOLYSIS MEDIATED BY DIRECT EFFECTS OF $PGF_2\alpha$ ON LUTEAL CELLS

Prostaglandin $F_2\alpha$ inhibits basal secretion or LH-stimulated secretion of progesterone by ovine luteal tissue slices *in vitro* (368), but increases progesterone secretion by bovine luteal slices *in vitro* (164). Prostaglandin $F_2\alpha$ inhibits basal or LH- or hCG-stimulated progesterone secretion by dispersed ovine (186), bovine (187), porcine (188) or rat (369, 370) luteal suspensions *in vitro* within 4 hours. These luteal suspensions

would contain not only small and large steroidogenic luteal cells, but also contain fibloblasts, leukocytes, macrophages, endothelial cells and pericytes (158, 159). This may represent the actions and interactions among different populations of luteal cells to $PGF_{2}\alpha$ (369-373). This may be important, since there is an interaction between SLC and LLC for production of progesterone prior to onset of luteolysis (160, 187, 188). Thus, it is logical to assume that interactions among SLC, LLC and other cells from CL occurs during the luteolytic process to decrease progesterone secretion.

Evidence for $PGF_{2}\alpha$ having a direct negative effect on steroidogenic cells in the CL has been obtained through the separation and characterization (155-160) and culture of purified SLC or LLC *in vitro* (371-375). Ten ng/ml of $PGF_{2}\alpha$ were needed to reduce basal secretion of progesterone by ovine LLC *in vitro* (371). This concentration of $PGF_{2}\alpha$ is seen in uterine venous blood of sheep during luteolysis (256). Prostaglandin $F_{2}\alpha$ does not decrease basal or LH-stimulated secretion of progesterone by ovine SLC *in vitro* (376). At physiological levels, LH stimulates progesterone secretion by SLC, but not by LLC in sheep. Receptors for LH on LLC are only 10 percent of those on SLC (186-188, 190). At pharmacologic levels, LH stimulates progesterone secretion by ovine LLC which can be inhibited by $PGF_{2}\alpha$ (186-188, 190). Ovine LLC have 35 fold more receptors for $PGF_{2}\alpha$ than SLC (186-188, 190). Thus, differences in luteotropic response to LH or the luteolytic response to $PGF_{2}\alpha$ by ovine SLC or LLC may be explained in part by differences in the numbers of receptors for LH or $PGF_{2}\alpha$ on the two steroidogenic cell types.

Six hours are required for $PGF_2\alpha$ to decrease progesterone secretion by ovine LLC *in vitro* (374). Also, $PGF_2\alpha$ causes degeneration and cell death of cultured LLC, but not SLC *in vitro* (371, 374). Since $PGF_2\alpha$ inhibits LH-stimulated progesterone secretion by ovine luteal slices (368) or basal progesterone secretion by ovine LLC but not by SLC, this suggests that $PGF_2\alpha$ first initiates luteolysis through stimulation of LLC to produce inhibitory factors to reduce progesterone secretion and cause death of both SLC and LLC. Alternative reasons for degeneration of SLC may be due to reduction in blood flow to the ovary with the CL (343), direct effects of $PGF_2\alpha$ on SLC not yet identified, or to immune cells which infiltrate the CL during luteolysis (375, 376).

Some studies do not see a negative effect of $PGF_2\alpha$ on progesterone secretion by bovine luteal cell suspensions *in vitro*, but see an increase in progesterone secretion (164, 377). This increase in progesterone secretion may be through increases in calcium in the SLC (377). In addition, $PGF_2\alpha$ has no effect on basal production of progesterone by bovine LLC *in vitro*, but inhibits LH, forskolin or 8-Br-cAMP stimulated progesterone secretion (164). Bovine LLC are relatively insensitive to physiologic levels of LH, but do respond to high levels of LH (164). Thus, $PGF_2\alpha$ is luteotropic in some studies with bovine luteal cells *in vitro*, but luteolytic in others (164). While $PGF_2\alpha$ may be luteotropic *in vitro*, $PGF_2\alpha$ is luteolytic *in vivo* in cattle (339).

1.1.5.4 CELLULAR MECHANISMS OF $PGF_{2\alpha}$ INHIBITION OF LUTEAL STEROIDOGENESIS

1.1.5.4.1 $PGF_{2\alpha}$ AND LH RECEPTORS

Many studies have been focused on mechanisms of $PGF_{2\alpha}$ inhibition of steroidogenesis by luteal tissue. Prostaglandin $F_{2\alpha}$ blocks the action of LH by decreasing receptors for LH *in vivo* in rats (379) or sheep (113). However, secretion of progesterone is reduced in response to $PGF_{2\alpha}$ several hours before LH receptors on luteal tissue are decreased *in vivo* (113). Thus, loss of receptors for LH after $PGF_{2\alpha}$ is an effect not the cause of luteolysis and probably ensures that regression of the CL is irreversible. Chronic infusions of LH starting early in the estrous cycle of sheep extends luteal function (137). However, a single injection of LH or prolactin stimultaneously with $PGF_{2\alpha}$ at mid-cycle in sheep does not prevent $PGF_{2\alpha}$-induced luteolysis (378). The loss of LH binding at the end of the estrous cycle may not be due only to reduced numbers of luteal LH receptors, but also to inhibitors of LH binding to receptors. Gonadotropin releasing hormone like ovarian hormone (GLOH), which has been identified to be 95 percent homologous to histone-H2A (379), inhibits binding of LH to luteal membranes (380). This GLOH reduces the luteotropic response by rat luteal cells to LH *in vitro* by 75 percent (381) and possibly inhibits activation of G-stimulatory (G_S) protein required for LH activation of adenylate cyclase (380).

These acute effects of $PGF_{2\alpha}$ *in vivo* may be through inhibition of delivery of LH to the luteal cell. $PGF_{2\alpha}$ inhibits LH uptake by rat luteal tissue *in vivo* and this parallels the decline in progesterone secretion (382-

384). In *in vitro*, $PGF_{2}\alpha$ does not affect uptake of LH by rat luteal suspensions, but progesterone secretion decreases (369, 370, 382-384). In addition, $PGF_{2}\alpha$ does not directly inhibit binding of LH to isolated luteal membranes *in vitro* (385, 386). Since $PGF_{2}\alpha$ decreases the uptake of LH and prolactin *in vivo* in rats (383, 384), but not LH binding *in vitro* (385, 386) and blood flow to the CL is not affected before (338, 387) progesterone decreases (370), $PGF_{2}\alpha$ may be inhibiting uptake of LH and prolactin *in vivo* at the capillary bed. Since $PGF_{2}\alpha$ does not affect binding of LH to receptors on luteal tissue *in vitro* (370, 384), $PGF_{2}\alpha$ may inhibit progesterone secretion by luteal tissue through an inhibition of intracellular cell signalling mechanisms that control progesterone synthesis (385-387).

1.1.5.4.2 $PGF_{2}\alpha$ AND CELLULAR SIGNALING MECHANISM

In sheep, treatment with $PGF_{2}\alpha$ decreases concentrations of cAMP and blocks LH stimulation of adenylate cyclase activity by 2 hours. Progesterone in jugular venous blood and luteal tissue does not decrease until 4 and 12 hours, respectively. Phosphodiesterase activity, which converts cAMP to AMP, is increased by 2 hours after injection of $PGF_{2}\alpha$ (388). In cattle, phosphodiesterase activity increases by 46 percent during luteolysis (389). Prostaglandin $F_{2}\alpha$ inhibits LH accumulation of cAMP induced by LH and steroidogenesis by rat luteal suspensions. This effect is independent of LH binding or cAMP degradation (370, 386, 387, 390). Luteinizing hormone increases production of progesterone in vitro through increases in cAMP (181), which stimulates protein kinase A (PKA) to phosphorylate steroidogenic enzymes (391). However, $PGF_{2}\alpha$ does not

directly inhibit LH stimulation of adenylate cyclase activity by isolated rat luteal membranes (392-396). Thus, $PGF_{2}\alpha$ must be stimulating production of an inhibitor after binding to its receptor.

Prostaglandin $F_{2}\alpha$ inhibits microaggregation of LH receptors on luteal membranes and prevents LH-induced up-regulation of LH receptors. Microaggregation of LH receptors occurs as part of LH stimulation of progesterone secretion by luteal cells (394). This inhibition on microaggregation by $PGF_{2}\alpha$ may be through a decrease in luteal membrane fluidity caused by $PGF_{2}\alpha$. Decreased luteal membrane fluidity by $PGF_{2}\alpha$ (395) also increases phospholipase A_2 activity of luteal membranes (396). Phospholipase A_2 frees arachidonic acid from phospholipids in membranes for prostaglandin biosynthesis (335-337). This could explain the secretion of $PGF_{2}\alpha$ by luteal tissue treated with $PGF_{2}\alpha$ (397). Thus, $PGF_{2}\alpha$ from the uterus may initiate luteolysis, but may also trigger luteal tissue to produce $PGF_{2}\alpha$ to complete luteolysis.

Inhibition of LH actions by $PGF_{2}\alpha$ occurs rapidly and indirectly (392). An intracellular mediator induced by $PGF_{2}\alpha$ may uncouple the activation of the enzyme adenylate cyclase from LH bound to its receptor. This rapid action could be via an inactivation of the G_s protein that links LH receptor to adenylate cyclase to produce cAMP or by the G-inhibitory (G_i) protein that inactivates adenylate cyclase. Pertussis toxin which blocks the G_i inactivation of adenylate cyclase does not prevent $PGF_{2}\alpha$ inhibition of steroidogenesis by luteal cells. Prostaglandin $F_{2}\alpha$ uncouples the G_s protein from adenylate cyclase (398).

Prostaglandin $F_{2\alpha}$ may stimulate production of inhibitors of steroidogenesis by luteal tissue. These may be reactive oxygen radicals which include hydrogen peroxide and lipid hydroperoxides. Reactive oxygen radicals damage membrane structure and function, proteins, RNA, and DNA; and cause lipid peroxidation. Biological membranes are particulary sensitive to oxidative reactions, because of the high concentrations of esterified polyunsaturated fatty acids (399). Sources of these reactive oxygen species in the ovary could be from endothelial cells, steroidogenic luteal cells, stroma cells or leukocytes, macrophages and eosinophils (400). Leukocytes, macrophages and eosinophils infiltrate luteal tissue during regression and generate superoxides in quantities sufficient to cause cell injury and death (375, 376, 401, 402). Prostaglandin $F_{2\alpha}$ stimulates early production of hydrogen peroxide and concomitant decreases in progesterone are seen (398). Hydrogen peroxide is also produced during spontaneous luteolysis (403). Also, hydrogen peroxide, at low levels, initiates luteolysis within minutes and without affecting LH binding. This is followed by a depletion of ATP in luteal cells (404). Hydrogen peroxide decreases cAMP by uncoupling of G_s protein from adenylate cyclase and blocks cAMP analogue stimulation of progesterone production by luteal tissue through PKA (405). This action of hydrogen peroxide is overcome when mitochondria permeable cholesterol analogues are used as substrates for steroidogenesis (405). Translocation of cholesterol through the mitochondrial membrane is necessary for cholesterol to be converted to pregnenolone by Scc enzyme, the rate limiting step in steroidogenesis (180).

Intracellular transport of cholesterol is dependent on protein synthesis. This is necessary for the formation of pregnenolone and is inhibited by hydrogen peroxide (405). Prostaglandin $F_{2\alpha}$ affects enzymatic sites distal to cAMP, PKA, intracellular transport of cholesterol and Scc cleavage enzyme (406). Three ß-hydroxysteroid dehydrogenase (3ß-HSD) converts pregnenolone to progesterone at smooth endoplasmic reticulum membranes (180). Prostaglandin $F_{2\alpha}$ decreases 3ß-HSD mRNA within one hour (406). Effects of hydrogen peroxide on 3ß-HSD are unknown. However, $PGF_{2\alpha}$ decreases progesterone production before effects on 3ß-HSD are seen (406). Actions of hydrogen peroxide cannot be blocked in luteal tissue by indomethacin, an inhibitor of prostaglandin synthesis (403). Hydrogen peroxide decreases luteal membrane fluidity, activates luteal membrane phospholipase A_2, and decreases the microaggregation of LH receptors on luteal cells (407-409). Hydrogen peroxide or $PGF_{2\alpha}$ inhibits progesterone secretion within minutes. Prostaglandin $F_{2\alpha}$ increases hydrogen peroxide within 10 minutes and before decreases in cAMP and progesterone are seen (407-409). It is suggested that hydrogen peroxide is a mediator of the early negative actions of $PGF_{2\alpha}$ on progesterone secretion by luteal cells. Specific effects of hydrogen peroxide on SLC or LLC or their production of hydrogen peroxide are unknown.

Since catalase injections *in vivo* inhibits a spontaneous or $PGF_{2\alpha}$-induced luteolysis but catalase would not be taken up by capillary beds, it is suggested that steroidogenic cells are not the only source of hydrogen peroxide for luteolysis (401). Endothelial cells or the invasion of luteal tissue by macrophages and eosinophils, which is seen during

luteolysis (375, 376), could also be sources for hydrogen peroxide activated by $PGF_2\alpha$. Prostaglandin $F_2\alpha$ causes an infiltration of eosinophils in luteal tissue by stimulation of a chemoattractant. This occurs before progesterone decreases (375). This could also explain why $PGF_2\alpha$ causes luteolysis in luteal suspensions (188-190) *in vitro* earlier than in pure cultures of ovine LLC (373, 374). Leukocytes, macrophages, eosinophils, lymphocytes and granulocytes generate hydrogen peroxide. This could also explain the anti-gonadotropic effects of tumor necrosis factor (TNF) and interleukin-1 (IL-1) on luteal tissue (410). Tumor necrosis factor and IL-1 are secreted by macrophages and activate neutrophils for production of hydrogen peroxide. During regression of rabbit CL, infiltration of luteal tissue by macrophages is associated with increases in TNF (411).

The antioxidants ascorbic acid (vitamin C) and tocopherol (vitamin E) may have roles in luteal function. LH decreases ascorbic acid in luteal cells, while LH increases luteal secretion of progesterone (412). Prostaglandin $F_2\alpha$ also causes an early and sustained depletion of ascorbic acid (24 hours) in luteal tissue, but decreases luteal secretion of progesterone (412). Prostaglandin $F_2\alpha$ causes lipid peroxidation, but LH does not (412). Levels of tocopherol are not affected by $PGF_2\alpha$, while LH increases tocopherols in luteal tissue by 24 hours (412). Ascorbic acid is located in the cytosol and tocopherols accumulate in the lipid bilayer of the plasma membrane. These data suggest that LH enhances accumulation of the antioxidant tocopherol to protect the plasma membrane against lipid peroxidation (412).

Other intracellular regulatory pathways, such as inositol triphosphate (IP_3), calcium, diacylglycerol, phospholipase C and protein kinase C pathways may play a role in luteolysis. In sheep (373, 374) or cattle (164, 377), $PGF_{2\alpha}$ increases intracellular concentrations of calcium 5 to 20 fold within minutes and remains elevated for 30 minutes in LLC, but not in SLC. Small luteal cells contain low levels of calcium sequestered in microsomes and mitochondria, while LLC have high levels of sequesterd calcium (374). Inositol triphosphate mediates release of intracellular calcium (160, 374). Prostaglandin $F_{2\alpha}$ stimulates turnover of phosphoinositol, the source of IP_3, in rat luteal membranes (413). Prostaglandin $F_{2\alpha}$ increases IP_3 in rat luteal cells, presumably, through the activation of phospholipase C (164, 388). In bovine luteal cells, $PGF_{2\alpha}$ stimulates release of IP_3 within 10 seconds and is followed by a sustained release of intracellular calcium that lasts for several minutes (164). However, when intracellular calcium release is blocked by thapsigargin, a blocker of calcium release, $PGF_{2\alpha}$ actions to decrease progesterone secretion by rat luteal cells *in vitro* are not affected (396). Differences may exist between intracellular regulation of luteolysis between rats and ruminants or other intracellular pathways to regulate luteolysis.

Protein kinase C (PKC) may play a role in regulation of intracellular luteolytic mechanisms to reduce progesterone secretion. Protein kinase C is activated by phosphatidyl serine, calcium and diacylglycerol. Diacylglycerol is produced from phosphatidyl inositol at the same time when phospholipase C produces IP_3 (413, 414). Protein kinase C is found in CL with levels being greater in ovine LLC than in SLC. Treatment with phorbol-

12-myristate-13-acetate (PMA), a stimulator of PKC, causes translocation of PKC to ovine luteal membranes (190, 374). In ovine (190, 374) or bovine (164), SLC secretion of progesterone induced by LH, forskolin or dbcAMP *in vitro* is inhibited by PMA. Thus, PKC activation in SLC acts at a site distal to production of cAMP. This could be either through activation of PKA by cAMP or through an inhibition of intracellular cholesterol transport used in progesterone production, since Scc enzyme is not inhibited by PKC activation (190, 374). Activation of PKC by PMA inhibits secretion of progesterone by ovine LLC and luteal cell suspensions (190, 374). However, when PKC is activated in ovine LLC, 3ß-HSD is increased (415). Activation of PKC in rat luteal cell suspensions also decreases progesterone secretion (396).

Differences for PKC in bovine luteal cells exist. Bovine SLC have high affinity, low capacity binding sites for PKC, while bovine LLC have low affinity, but high capacity binding sites for PKC. Activation of PKC by PMA does not decrease secretion of progesterone by bovine LLC *in vitro* (164). The high affinity PKC binding site on bovine SLC is puzzling, since $PGF_{2}\alpha$ increases progesterone secretion by SLC *in vitro* (164). Since $PGF_{2}\alpha$ does not decrease secretion of progesterone by purified bovine luteal cells *in vitro*, this may be due to LLC containing the low affinity binding sites for PKC (164). Thus, differences in response to $PGF_{2}\alpha$ exist between sheep, cattle and rat luteal cells.

Luteolysis starts first as a functional luteolysis where progesterone decreases (374) and is followed by morphological regression of the CL. Functional and morphological regression may involve depletion of

ATP in luteal cells. Although LH is luteotropic, LH depletes levels of ATP within 30 minutes (416) and lowers the antioxidant ascorbic acid (412). Luteinizing hormone does not decrease the antioxidant tocopherol (412). After LH, ATP and ascorbic acid levels remain depressed before returning to control levels by 24 hours. Luteinizing hormone increases levels of tocopherol by 24 hours (412). This increase in tocopherol is probably due to LH-induced release of low density lipoprotein (LDL) to deliver cholesterol to luteal cells for steroidogenesis. Tocopherol is fat soluble and transported on LDL (417). This suggests that LH causes formation of oxygen radicals in luteal cells and ascorbic acid is used to protect luteal function through enzymatic degradation of oxygen radicals by catalase and superoxide dismutase (418).

Deprivation of LH by hypophysectomy in the pseudopregnant rat also depletes ATP levels by 24 hours and results in functional and morphological regression of the CL (416, 417). Functional and morphological regression is due to a lack of metabolic production of ATP to sustain progesterone secretion (412). Loss of luteal progesterone secretion after $PGF_2\alpha$ is followed by an increase in LH (112, 113). This increase in LH after progesterone declines, probably contributes to completion of luteolysis by further decreasing ascorbic acid and ATP in luteal cells (418). During $PGF_2\alpha$-induced luteolysis *in vivo*, levels of ATP and ascorbic acid are depleted in luteal tissue by 24 hours (418). A luteolytic dose of $PGF_2\alpha$ does not deplete levels of ATP in luteal tissue of rats *in vivo* or *in vitro* within 2 hours. However, it reduces ascorbic acid (412) at the same magnitude as LH reduces ascorbic acid (412).

Prostaglandin $F_2\alpha$ induces rapid formation of hydrogen peroxide (403) and lipid peroxides by increasing malonaldehyde in rat luteal cells (412). Hydrogen peroxide decreases ascorbic acid and depletes levels of ATP in rat luteal tissue (402). Thus, $PGF_2\alpha$ probably decreases ascorbic acid while increasing hydrogen peroxide and lipid peroxides. Hydrogen peroxide decreases ATP levels. This is followed by further decreases in ATP by LH after progesterone decreases. Unlike many tissues such as muscles, luteal cells do not have phosphocreatine or creatinine phosphokinase to replenish depleted levels of ATP (419).

Morphological regression of the CL is the remodeling of the ovary by destroying destruction and removing luteal cells which is maintaining ovarian proportions. During spontaneous or $PGF_2\alpha$-induced luteal regression, lysosome activity increases. Lysosomes contain degradative enzymes which could hydrolyze luteal cell macromolecules (374). Endonucleases have been demonstrated to be involved in apoptotic programmed cell death during follicular atresia and in non-ovarian tissue. Endonucleases fragment DNA and disrupt metabolic activity of the cells. A marker of apoptosis is the formation of oligonucleosomes (420). Morphologically, CL appear to undergo apoptosis during luteolysis (421). In cattle, apoptosis occurs after spontaneous or $PGF_2\alpha$-induced luteal regression based on oligonucleosome formation, but only after progesterone decreases (421). The depletion of cellular levels of ATP may be linked to apoptosis. Reactive oxygen species not only deplete ATP levels, but are linked to DNA fragmentation and inactivation of adenosine diphosphate (ADP) ribose polymerase. Nicotinamide adenine dinucleotide (NAD), which is

utilized as a substrate for repair of this enzyme, is also depleted. NAD is involved in the formation of ATP. Thus, the ability of cells to produce ATP is reduced, but only after progesterone decreases (398).

In summary, differences in response to $PGF_2\alpha$ exist between culture of dispersed luteal cell suspensions and purified small or large steroidgenic luteal cells (SLC or LLC) cultured alone. In addition, species differences may also exist. Luteolysis is induced by $PGF_2\alpha$ secreted endogenously or given exogenously. Thus, $PGF_2\alpha$ is the uterine luteolysin. Furthermore, mechanisms of $PGF_2\alpha$-induced luteolysis occurs directly on many different cell types in luteal tissue to decrease secretion of progesterone before a decrease in blood flow to the ovary or to the CL is seen.

1.2 PREGNANCY

1.2.1 INTRODUCTION

In sheep (422) and cattle (423), fertilization in normal cycling fertile ewes and cows bred to fertile rams and bulls is close to 96 percent. Hatching of the blastocyst from the zona pellucida in preparation for implantation occurs between days 7 to 8 postbreeding (424). Sheep blastocysts are spherical (0.14 to 0.4 mm in diameter) from days 4 to 10 postbreeding. The trophoblast then undergoes intense mitosis and elongates from days 10 to 15 to the filamentous form (1 to 2 mm X 150 to 190 mm). Implantation in sheep and cattle is a gradual process, unlike in the rat. Attachment (implantation) of the sheep blastocyst begins between days 15 to 18. The cotyledons appear on the trophoblast by day 22 and gradually fuse with the caruncles on the maternal endometrium to form the placentomes. This initial phase of implantation in sheep is complete by about day 40. Formation of the tissue, organs and systems are seen by day 34 postbreeding in sheep (425). As pregnancy progresses, more cotyledons become associated with caruncles until all caruncle/cotyledon complexes (placentomes) are formed by day 90 of gestation. There are between 80 to 100 caruncles on the endometrium of sheep (426, 427).

In the cow, blastocysts are spherical (0.2 mm in diameter) on days 8 to 9, tubular (1.5 to 3.3 mm X 0.9 to 1.7 mm) on days 12 to 13, and then becomes filamentous between days 13 to 18 (1.5 mm X 160 mm). By day 24, the filamentous bovine conceptus occupies both uterine horns (425, 426). Attachment of the bovine blastocyst begins on days 28 to 32

and is completed by days 40 to 45 postbreeding, about the time of completion of embryonic differentiation (425).

Not all pregnancies are successful in producing a calf. Embryos or fetuses are lost throughout gestation, even when breeding is to healthy cows with fertile bulls (428-430). Losses occuring from conception until embryonic differentiation, approximately 34 days and 45 days in ewes and cows, respectively, are termed embryonic mortality. Losses occuring during the fetal period, which is from differentiation until parturition, are divided into abortions and premature deliveries. Abortion is the expulsion of the fetus before full term, but the fetus is incapable of independent life. A premature delivery is the expulsion of a fetus before term, but the fetus is capable of independent life (431).

When healthy cycling cows are bred by artificial insemination with semen from fertile bulls, approximately 63 calves may be expected from 100 first inseminations (428). The early losses due to embryonic mortality are manifested as repeat breeders and no physical evidence of the lost embryo may be present. Fetal abortions and premature deliveries are reported to be between 7 and 12 percent of cows identified as pregnant between 30 to 50 days postbreeding (428). In mated ewes, 20 to 40 percent of ovulations are not represented by live births. Frequency of abortion in sheep flocks runs from 1 to 5 percent in the absence of infectious disease (429). Abortions may be as high as 72 percent, when infectious diseases occur in sheep flocks (429). The greatest loss of pregnancies occurs before implantation during the first three weeks of gestation (430, 432-436). Thus, greater than 80 percent of lost pregnancies

are due to embryonic motality. Causes of lost pregnancies range from fertilization failure, genetic anomalies, heat stress, infectious agents, inadequate nutrition, toxic plants and hormonal imbalance (433, 437). If an embryo dies before days 12 to 13, the CL regresses at the normal time, the ewe returns to estrus by day 17 and most ewes have a normal conception rate when remated. Embryos that die after day 13 postbreeding extends luteal function and the conception rate at the next mating is often lower (438, 439). Most lost pregnancies occur around the time of maternal recognition of pregnancy. In sheep, this is around days 11 to 13 and days 15 to 17 in cattle (438, 439). Length of gestation in sheep and cattle is approximately 150 and 270 days, respectively, but varies with breed (438, 439).

1.2.2 MATERNAL RECOGNITION OF PREGNANCY

1.2.2.1 INTRODUCTION

Progesterone is required to maintain pregnancy throughout the 145 to 150 day gestation in sheep (4, 440, 441) or the 278 to 284 day gestation in cattle (4, 6). During early pregnancy, progesterone is from the CL. In the ewe, day 55 of pregnancy is the earliest that the ovine placenta can start to produce sufficient progesterone to maintain pregnancy independent of the ovaries (441). Thus, it is essential that luteolysis is inhibited after mating for pregnancy to be established.

The embryo either secretes or stimulates the uterus to secrete an antiluteolysin or luteotropic signal that transforms the CL of the estrous cycle to the CL of pregnancy. The CL during pregnancy may differ from the

CL of the estrous cycle by requiring a luteotropin other than LH. Response to LH stimulation of progesterone secretion *in vitro* by CL collected from midgestation cows is reduced compared to CL during the estrous cycle (442). Maternal recognition of pregnancy refers to the changes that occur starting around days 11 to 13 in sheep and days 15 to 17 in cattle, respectively, that prevents regression of the CL. These changes include embryonic signals, blood flow, and location of the embryo (443-447).

1.2.2.2 UTERO-OVARIAN RELATIONSHIPS AND ESTABLISHMENT OF PREGNANCY

1.2.2.2.1 THE CRITICAL PERIOD FOR MATERNAL RECOGNITION OF PREGNANCY

In sheep, day 12 postestrus is the last day an embryo can be transferred into a synchronous recipient nonpregnant ewes and prevent luteolysis. A higher percentage of pregnancies is seen when embryos are transferred earlier in the estrous cycle to synchronous recipients. Transfer of embryos on days 13, 14 or 15 postestrus delays luteolysis, but does not prevent luteolysis (439, 443, 448-450). Removal of embryos from pregnant sheep by day 12 postbreeding is followed by normal regression of the CL. When embryos are removed on days 13, 14 or 15 postbreeding, luteal regression is delayed for a few days (443, 448-450). Since the presence of the embryo is required by day 12 to maintain luteal function, this has been defined as the critical period for maintanance of the CL of early pregnancy in sheep (443).

In cattle, transfer of embryos to synchronous recipients as late as day 16 postbreeding results in successful pregnancies (451-453). When bovine embryos are removed on day 17 postbreeding, the interestrous interval is extended (454). In horses, the critical period for maternal recognition of pregnancy is day 16 postestrus. Transfer of embryos to synchronous recipient mares no later than day 16 maintains luteal function. Equine embryos must be removed by day 16, if the CL is to regress normally (455). In the pig, removal of embryos before day 10 postbreeding results in normal regression of the CL (456). When porcine embryos are removed on day 13 postbreeding, luteal function is extended (457).

1.2.2.2.2 RELATIONSHIP BETWEEN EMBRYOS AND THE CORPUS LUTEUM FOR ESTABLISHMENT OF PREGNANCY

Transfer of embryos to the uterine horn adjacent to or opposite the ovary with a CL results in luteal maintenance and pregnancy in sheep (443, 458, 459) or cattle (460). When an embryo is transferred to either uterine horn of ewes with CL on both ovaries, CL on both ovaries are maintained (443, 458). More CL are maintained, when embryos are transferred to the uterine horn adjacent to the ovary with the CL (443). However, when the uterine horns are separated surgically, CL are maintained only when embryos are transferred to the uterine horn adjacent to the ovary with the CL (443, 449, 458, 459). In addition, transfer of embryos to the anterior half of surgically isolated regions of the uterine horn adjacent to the ovary with the CL maintains luteal function in more ewes than when embryos are transferred to the posterior half of the uterine horn

adjacent to the ovary with a CL (443, 461). This may be due to the local delivery of factors to maintain CL or to the local delivery of progesterone from the ovary with a CL to the anterior one third of the adjacent uterine in addition to the systemic route for delivery of progesterone to the uterus of sheep (365) or cattle (367). In sheep or cattle, migration of embryos from the uterine horn adjacent to the ovary with the CL to the opposite uterine horn occurs rarely unless multiple embryos are present (462, 463).

Induction of CL on both ovaries on day 30 of pregnancy in sheep results in luteal maintenance adjacent, but not opposite to the gravid uterine horn by day 50 of pregnancy (443). This suggests that the luteolytic mechanisms exist for at least the first 50 days of pregnancy in sheep. Similarly, surgical separation of the bipartite uterine horns and induction of a unilateral pregnancy by embryo transfer in bilaterally ovulating ewes results in luteal regression only on the nongravid side (464, 465). Thus, a local relationship exists between the gravid uterine horn and the adjacent ovary to maintain luteal function only on an adjacent ovary in the ewe. Therefore, the ovine embryo appears to prevent luteolysis by a local mechanism during early pregnancy.

In the cow, the antiluteolytic influence of the bovine embryo may be passed from a uterine horn opposite to the luteal-bearing ovary to the uterine horn adjacent to the luteal-bearing ovary. When bovine embryos are transferred to the uterine horn opposite to the ovary with the CL, CL are maintained. The elongating trophoblast of bovine embryos is seen in the posterior half of the uterine horn opposite to the ovary with the CL by day 24 postbreeding (460). However, more CL are maintained when bovine

embryos are transferred to the uterine horn adjacent to the ovary with the CL (466-472). In addition, more cows appear to be pregnant when an embryo is placed in the anterior third of the uterine horn adjacent to the ovary with the CL (469). Bilateral transfer of a bovine embryo to each uterine horn adjacent and opposite to the ovary with the CL prevents luteal regression. However, embryonic death often occurs after day 24 in the uterine horn opposite to the ovary with the CL (468, 471, 472). Thus, embryos transferred to the uterine horn adjacent to the ovary with the CL has a greater chance of survival than embryos transferred to the uterine horn opposite to the ovary with a CL in the cow (467).

Local utero-ovarian mechanisms occur for maintenance of the CL during early pregnancy in pigs. In unilaterally pregnant pigs, regression of CL on both ovaries occurs and the pregnancy is lost, if seven-eighths of the nongravid horn is present. However, removal of all but the cranial one-half to one-fourth of the nongravid uterine horn causes luteolysis of the CL only on the nonpregnant side. A longitudinal incision of the nongravid uterine horn of unilaterally pregnant pigs results in regression of CL only on the nonpregnant side. The surgical incision apparently decreases, but does not eliminate the luteolytic potential of a nongravid uterine horn (473). There is a relationship between the numbers of porcine embryos and luteal maintenance. Only four porcine embryos are needed, two in each uterine horn, to delay luteolysis for a short period past the critical period. Pigs are dependent on ovarian progesterone throughout pregnancy (474). Partial hysterectomy maintains pregnancy in sows when only one embryo remains as long as all of the uterus is removed except that portion occupied by the

embryo (473). These studies support the concept of a unilateral utero-ovarian mechanism for maintenance of the CL of early pregnancy in the pig.

1.2.2.2.3 EFFECT OF EMBRYO OR PLACENTAL HOMOGENATES ON LUTEAL FUNCTION

The embryo may exert its luteotropic or antiluteolytic effect during early pregnancy through the endometrium. Removal of both the fetus and placenta of sheep prior to day 50 of gestation results in a rapid regression of the CL of pregnancy. However, when only the embryos are removed or destroyed by electrocautery, luteal function is maintained for an extended period (443). In addition, the degree of luteal maintenance in cows after embryo transfer appears to be a function of the embryo-endometrial contact on the same side as the ovary with the CL (460). Furthermore, less of a nonpregnant uterine horn is needed in unilaterally pregnant pigs to cause unilateral luteolysis in partially hysterectomized nonpregnant pigs than in unilaterally pregnant pigs (443, 473).

Chronic intrauterine infusion of homogenates of day 14 to 16 ovine embryos delays luteal regression in nonbred ewes (443, 475, 476) or prevents an IUD-induced premature luteolysis in sheep (477). Systemic infusions of homogenates of day 14 or 15 sheep embryos do not delay luteolysis (478). If homogenates of day 12, 14 or 25 sheep embryos are heated (100 ^{o}C for 5 minutes), intrauterine infusions do not delay luteolysis (443, 478). Extracts of gravid ovine uterine endometrium (479) or trophoblast tissue (476) blocks luteal regression in nonpregnant ewes. This factor produced by the preimplantation ovine embryo is heat labile (476).

The luteotropic/antiluteolytic effect of this trophoblast product disappears by day 23 postbreeding in sheep (476). This effect of ovine embryonic homogenates is not due to an LH or a prolactin-like molecule, since extracts of the embryonic homogenates do not cross-react with these hormones in a radio-receptor assay (475). In cows, a factor exists in uterine flushings which increases survival of the bovine embryo (480). When the embryonic discs are removed from day 14 bovine conceptuses and this trophoblastic tissue is transferred to the uterine horn adjacent to the ovary with the CL on day 12 postestrus, the CL is maintained until days 25 to 37 in 75 percent of the recipient heifers (481). Collectively, these data in sheep and cattle indicate that signals from the conceptus are from the trophoblast and act through the uterus.

Placental extracts of guinea pigs (482), hamsters (483) and rats (484) have luteotropic activity, since infusions of placental extracts prolong luteal function or increase weights of the CL. Increases in weights of the CL in sheep and cattle are not seen during early pregnancy (6). Thus, it appears that the embryo is overcoming the luteolytic effect of the uterus by either inhibiting the production of the uterine luteolysin or by causing the production of a luteotropin/antiluteolysin. The embryo interacts directly with the uterus to initiate maintenance of pregnancy. There are species specific differences.

1.2.2.3 EVIDENCE FOR A BLOOD BORNE LUTEOTROPIN OR ANTILUTEOLYSIN

1.2.2.3.1 INTRODUCTION

The embryo could maintain luteal function through the hypothalamic-hypophyseal axis, but is unlikely due to the local effect of the embryo to maintain luteal function (443). The local mechanism whereby the gravid uterine horn maintains luteal function is shown using surgical anastomoses of blood vessels of the reproductive tract.

1.2.2.3.2 THE LOCAL EFFECT OF THE ANTILUTEOLYSIN

When the uterine vein from a gravid horn of unilaterally pregnant, bilaterally ovulating ewe is surgically anastomosed to the uterine vein of the nongravid uterine horn only, the CL on the opposite ovary is maintained (464). Similar data are seen in cattle (485). Generally, a CL on an ovary opposite to the gravid uterine horn of a unilaterally pregnant sheep (464, 465) or cattle (485) regresses. Only a CL on an ovary adjacent to the gravid uterine horn is maintained. These data suggest that the embryo is secreting a blood borne antiluteolysin rather than acting through a suppression of the production of the uterine luteolysin ($PGF_2\alpha$). These data demonstrate that the embryonic antiluteolysin is delivered locally and has its effect at the ovarian level.

1.2.2.3.3 UTERINE AND OVARIAN BLOOD FLOW REGULATION DURING EARLY PREGNANCY

Blood flow to the uterus and ovary may be involved in maternal recognition of pregnancy or is an indicator of maternal recognition of pregnancy. Blood flow to the uterus of nonpregnant and pregnant ewes, cows and pigs is similar until maternal recognition of pregnancy. On days 13 to 15 postbreeding in ewes (486,487), days 12 to 13 in pigs (488) and days 15 to 17 in cattle (489), there is a transient 2 to 3 fold increase in uterine blood flow. Blood flow also increases to the ovary bearing the CL and to the CL directly (488, 489). Implantation is not initiated until after this transient increase in uterine blood flow in the ewe (486, 487, 490), sow (491) and cow (492). A second prolonged increase in uterine blood flow occurs after implantation in all three species (486-489). A similar increase in blood flow to the uterus occurs at the time of implantation in the guinea pig (493). The embryo either directly or indirectly, through the uterus, causes this increase in uterine blood flow at the time of maternal recognition of pregnancy, since blood flow increases only to the uterine horn containing the embryo (486-489). The second increase in uterine blood flow is highly correlated with the estradiol-17ß:progesterone ratio and blood flow increases as this ratio increases (486-493). However, the increase in uterine blood flow at the time of maternal recognition of pregnancy is not correlated to the estradiol-17ß:progesterone ratio (494-498). This suggests that the embryo either directly or indirectly through the uterus is controlling the conceptus-induced vasodilation by other mechanisms.

Contractile responses to $PGF_2\alpha$ or periarterial sympathetic nerve stimulation by uterine arteries *in vitro* from nonpregnant sheep (499) or cattle (500) is reduced by uterine flushing or the conceptus brei from pregnant uteri. The conceptus-induced increase in uterine blood flow may be due to estrogen or PGE_2 production by the embryo. Bovine or porcine conceptuses produce estradiol-17ß *in vitro* but ovine embryos produce little estradiol-17ß at the time of maternal recognition of pregnancy (499, 500). Bovine, ovine or porcine (501) embryos secrete PGE_2 *in vitro*. Both estrogen and PGE_2 are vasodilators (497). These embryonic estrogens could also be stimulating uterine prostaglandin E secretion (286, 287). Infusions of physiologic concentrations of estradiol-17ß into the lumen of one uterine horn in nonpregnant pigs increases uterine blood flow by 8 to 10 fold (498). Uterine infusions of PGE_2 increases uterine blood flow in sheep (497). While these local changes in uterine blood flow occur during maternal recognition of pregnancy, systemic blood flow and pressure do not change (494-497).

1.2.2.3.4 EFFECT OF EARLY PREGNANCY ON UTERINE SECRETION OF $PGF_2\alpha$

Although the embryo is undoubtedly responsible for maintenance of luteal function during early pregnancy through local veno-arterial pathways similar to those for delivery of the uterine luteolysin, the effect of the embryo could be exerted via suppression of $PGF_2\alpha$ production or by induction of antiluteolytic/luteotropic mechanisms which could prevent luteolysis. Many studies have measured $PGF_2\alpha$ in uterine tissue and venous blood during early pregnancy in the ewe (502-518). Only a few studies have

quantified $PGF_2\alpha$ in ovarian arterial blood or in luteal tissue during early pregnancy in sheep (257, 296, 518). Two conclusions are apparent. First, pregnancy does not turn off uterine secretion of $PGF_2\alpha$ by the uterus. Secondly, transfer of $PGF_2\alpha$ locally, accumulation of $PGF_2\alpha$ in luteal tissue, numbers of $PGF_2\alpha$ receptors on luteal membranes or binding to luteal tissue of the ovary is not reduced.

The content or concentration of $PGF_2\alpha$ in the endometrium on day 13 of pregnancy in sheep is not decreased (502). Also, $PGF_2\alpha$ is not decreased in single samples of uterine venous blood at the same time (502). Patterns of $PGF_2\alpha$ in pregnant ewes collected every 3 hours from days 11 through 15 do not appear to be different from nonpregnant ewes, although fewer peaks are detected in pregnant ewes (256). It was reported initially that there is a significant (20 percent) decrease in $PGF_2\alpha$ in uterine venous blood during early pregnancy in ewes (279). Subsequently, it was found that endometrial synthesis of $PGF_2\alpha$ *in vitro* and levels of $PGF_2\alpha$ in endometrial tissue and uterine venous blood of pregnant ewes were equal to or greater than those found in similar samples from nonpregnant sheep on days 13, 15 or 17 postestrus (503-505). No differences are seen between pregnant and nonpregnant ewes or between uterine horns for the patterns or peaks of $PGF_2\alpha$ in uterine venous blood on day 15 postbreeding (504). Later, they found greater variation in the pattern of release of $PGF_2\alpha$ in nonpregnant ewes (505). Progesterone regulation of uterine secretion of $PGF_2\alpha$ does not differ during early pregnancy when compared to nonpregnant ewes (286, 287). Other investigators report that the number of large peaks of $PGF_2\alpha$ in the uterine vein associated with luteal regression on

days 14 and 15 are reduced in pregnant ewes (279, 280, 281). Some studies monitor $PGF_2\alpha$ secretion by measuring PGFM, the major catabolite of $PGF_2\alpha$, in jugular blood, since $PGF_2\alpha$ is catabolized rapidly by the lungs. In addition, it is not easy to maintain patency of uterine venous catheters These studies (280, 281) consistently show a reduction in PGFM in jugular blood during early pregnancy. This may not be a good approach to assess uterine secretion of $PGF_2\alpha$. Measurement of PGFM would reflect the secretion of $PGF_2\alpha$ by all tissues, not just the uterus. Although jugular PGFM has been positively correlated to $PGF_2\alpha$ in uterine venous blood in one laboratory (280, 281), other laboratories have not confirmed this relationship. Concentrations of $PGF_2\alpha$ or PGE_2 in uterine venous blood at parturition in cows is not significantly correlated with their catabolites in jugular blood (506). Both ovine (507) and bovine (508) placental tissue metabolize $PGF_2\alpha$. In addition, concentration of 15-hydroxy-prostaglandin dehydrogenase in rat placental, lung and kidney tissues vary markedly with the day of pregnancy (509, 510). Prostaglandin $F_2\alpha$ could also be metabolized to some other catabolite during pregnancy in ewes. For all of these reasons, direct measurement of $PGF_2\alpha$ is a more reliable estimator of uterine secretion of $PGF_2\alpha$.

Some of the variations in data may be due to differences in caruncular secretion and catabolism of $PGF_2\alpha$ or to secretion or catabolism of $PGF_2\alpha$ by the conceptus. Production of $PGF_2\alpha$ and protein production are altered *in vitro* by ovine caruncular endometrium on day 15 of pregnancy (511-514). Caruncular tissue has a great concentration and basal release of $PGF_2\alpha$ even in the presence of indomethacin (514), a prostaglandin

synthesis inhibitor, but a lower net production rate for $PGF_2\alpha$ by caruncular endometrium of pregnant than in nonpregnant ewes. The synthesis of $PGF_2\alpha$ *in vitro* or levels of PGFM in uterine flushings is highly variable (511-514). Evidence for inhibitors of prostaglandin synthesis exist in pregnant endometrium. Extracts of endometrium from pregnant sheep (515), cattle (516) and horses (517) reduces secretion of $PGF_2\alpha$ by endometrium from nonpregnant animals *in vitro*. The differences reported between many measurements of $PGF_2\alpha$ or its metabolite *in vitro* and *in vivo* may be due to the increase in protein synthesis in caruncles; catabolism of $PGF_2\alpha$ by endometrium of pregnant animals; secretion of PGE_2 and/or changes in blood flow to the uterus seen at the time of maternal recognition of pregnancy.

Peaks of $PGF_2\alpha$ or PGFM variable magnitude occur every 6 hours from days 14 through 16 postestrus in nonpregnant and early pregnant ewes (256, 280, 281). In pregnant ewes on days 14 through 16 postbreeding, the number of peaks of PGFM is reduced. Basal secretion of PGFM is increased in these pregnant ewes (283). Since uterine blood flow increases only in the uterine horn containing a conceptus (487, 498), some of the reduction in PGFM measured during early pregnancy compared to nonpregnant ewes could be due to a dilution effect as a result of increased uterine blood flow (497) unless there is an increase in uterine production of $PGF_2\alpha$.

Although the uterine luteolysin is transferred through local veno-arterial (240, 241) pathways, less than 5 percent of $PGF_2\alpha$ infused into the uterine vein is transferred (267, 268). Only one study has measured

levels of $PGF_2\alpha$ in ovarian arterial blood and in luteal tissue of pregnant ewes during the period of maternal recognition of pregnancy. In sheep, levels of $PGF_2\alpha$ in uterine venous, ovarian venous or ovarian arterial blood, or in luteal tissue or the remainder of the ovary are not reduced on day 15 of pregnancy when compared to nonpregnant ewes (257). When corrected for differences in ovarian arterial blood flow between pregnant and nonpregnant ewes, the quantity of $PGF_2\alpha$ transported to the ovaries of pregnant ewes is 4 fold greater than in nonpregnant ewes (257). Levels of $PGF_2\alpha$ in the uterine vein or ovarian arterial tissue are similar between pregnant and nonpregnant ewes on day 16 (504, 505). In cows, levels of $PGF_2\alpha$ in the uterine vein are lower in day 18 pregnant than in nonpregnant cows. However, the levels of $PGF_2\alpha$ in ovarian venous or arterial blood and in the CL are similar on day 18 of gestation (518). Moreover, binding of radioactive $PGF_2\alpha$ (^3H-$PGF_2\alpha$) by luteal membranes or the numbers of receptors for $PGF_2\alpha$ are not reduced by pregnancy in sheep on days 13 through 16 (519).

Deciduomal tissue is the artificially induced maternal contribution to the placenta in laboratory animals. Deciduomal tissue in pseudopregnant rats extends luteal function for approximately the same length as pregnancy, but no embryos/fetuses are present (303). Deciduomal tissue does not extend luteal function in pseudopregnant rats by reducing concentrations of $PGF_2\alpha$ in uterine venous, ovarian venous or ovarian arterial blood or in luteal tissue or binding of ^3H-$PGF_2\alpha$ to luteal membranes (301). Therefore, it is concluded that secretion or local transfer of $PGF_2\alpha$; accumulation of $PGF_2\alpha$ in luteal tissue; or binding of $PGF_2\alpha$ by luteal tissue

are not affected to explain maintenance of luteal function during early pregnancy.

1.2.2.3.5 EFFECT OF $PGF_{2}\alpha$ ON EARLY PREGNANCY

Although the endogenous luteolysin is still delivered to the CL, pregnant ewes are more resistant to an exogenous luteolytic dose of $PGF_{2}\alpha$ or estradiol-17ß (253, 520-523). Estradiol-17ß is luteolytic through increasing $PGF_{2}\alpha$ in uterine venous blood of nonpregnant ewes (284), but less so in early pregnant ewes (520). Injection of $PGF_{2}\alpha$ into the ovarian artery of the luteal-bearing ovary on day 13 causes luteolysis in 86 percent of hysterectomized, but only 25 percent of pregnant ewes (255, 261). Only 4 mg of $PGF_{2}\alpha$ *per* 58 kg body weight (BW) given intramuscularly are necessary to regress the CL on day 13 postestrus in nonpregnant ewes (253, 522). However, at least 6 mg of $PGF_{2}\alpha$/58 kg/BW are needed to regress the CL on day 13 of pregnancy in the ewe. This resistance is lost by day 23 post breeding (522). In superovulated pregnant ewes with multiple embryos, the ability of cloprostenol, a synthetic $PGF_{2}\alpha$ analogue, to cause luteolysis is inversely related to the number of embryos in the uterus (523). This further supports the observation that higher levels of $PGF_{2}\alpha$ are required to induce luteolysis in ewes during early pregnancy. Collectively, these data suggest that the embryo exerts a local antiluteolytic effect to counteract the response of the CL to the uterine luteolysin. The important issue is how the conceptus protects the CL from the actions of $PGF_{2}\alpha$ and what is the blood borne antiluteolysin of pregnancy in sheep.

1.2.2.4 POTENTIAL ANTILUTEOLYSINS OF PREGNANCY

Since the antiluteolytic action of the embryo is *via* a local veno-arterial route similar to $PGF_2\alpha$, it is possible that the antiluteolysin is a molecule similar to $PGF_2\alpha$, but with opposite actions.

Antiluteolytic activity of homogenates of day 14-16 conceptuses is abolished by heat treatment (80 ^{o}C for 30 minutes) or treatment with the proteolytic enzyme, pronase (476). Heat treatment or dialysis of days 14 to 15 ovine conceptus homogenates destroys their ability to stimulate progesterone secretion by ovine luteal tissue in vitro (524). These studies suggest that the antilutelysin is a protein. Similar early reports that the uterine luteolysin was a protein were incorrect (525). However, the luteolytic activity in the endometrium of cows was found in the lipid extracts of the protein fraction (526). This suggests that the luteolysin is bound to the protein fraction. The antiluteolytic activity of the conceptus homogenates may also be contained in a lipid fraction bound to protein. This has not been tested. Prostaglandins similar to $PGF_2\alpha$, but with actions opposite to $PGF_2\alpha$ are PGI_2, PGE_1 and PGE_2 (335-337, 344). Prostaglandin I_2, PGE_1 or PGE_2 are unstable even at room temperature (270, 271) and heat treatment could destroy their activity. Since methoxy-inulin, a small molecular weight substance, does not cross the veno-arterial pathway of the rat pampiniform plexus (235). The pampiniform plexus and utero-ovarian vasculature arterial/venous channel relationships are similar (235). A protein antiluteolysin would have to be delivered via a systemic vascular route. This is not consistent with transfer of the blood borne antilyteolysin through a local veno-arterial pathway.

Other local pathways for delivery of other hormones exist. Progesterone, a small molecular weight lipid, is transferred locally from the uterine vein to the ovarian artery in sheep (527). Estrogen, also acts locally to increase uterine blood flow in sheep, cattle and pigs (497, 498). Estrogen is luteotropic in pigs (528). It appeares to block the release of $PGF_{2}\alpha$ by the porcine uterus to maintain pregnancy (348). Estradiol-17ß is produced by pig or cow blastocysts (501). Intrauterine infusion of estradiol-17ß extends luteal function in pigs (529). However, sheep conceptus on days 12 to 18 produce no estradiol-17ß from radiolabelled precursors (501). In addition, estradiol-17ß is luteolytic in nonpregnant sheep (286) or cattle (309).

The antiluteolysin substance produced by the gravid uterus should be a small molecular weight molecule, which could be transferred by a local veno-arterial pathway and is probably heat labile. Although prostaglandins A_1 or A_2 (PGA_1; PGA_2) are vasodilators (344), they are not rapidly catabolized by the lungs (337-339).

Prostaglandin E_1 and PGE_2 are structually similar to $PGF_{2}\alpha$, they are rapidly catabolized by one pass through the lungs and are heat labile (335-337). In addition, PGE1 and PGE_2 are vasodilators (346) and increase uterine blood flow, while $PGF_{2}\alpha$ is a vasoconstrictor (346) and decreases uterine blood flow.

1.2.2.5 EFFECTS OF PGE_1, PGE_2 OR PGI_2 *IN VITRO*

Separate receptors for $PGF_{2}\alpha$, PGE_1 or PGE_2 are seen on luteal membranes (187, 188, 374, 519). Prostaglandin $F_{2}\alpha$ decreases secretion of progesterone *in vitro* (370), while PGE_1 or PGE_2 increases secretion of

progesterone by luteal tissue *in vitro* (187, 188, 530). Luteinizing hormone stimulates secretion of progesterone by luteal tissue *in vitro* (109) through increases in cAMP (110, 160). Prostaglandin E_1, PGE_2 or PGI_2 increases levels of cAMP in luteal tissue and stimulates secretion of progesterone (160, 530). Prostaglandin E_1 or PGE_2 is between 0.5 to 1 percent as effective as LH on a per molar basis in some studies (531) and PGE_2 is 50 percent as potent as LH on a per molar basis in another study (532). Luteinizing hormone and PGE2 have an additive effect on stimulation of progesterone secretion by luteal tissue (530), but PGE_1 or PGE_2 are not intermediates in LH stimulation of progesterone secetion (532). In addition, neonatal rat ovaries acquire the ability to respond to PGE_1 or PGE_2 increases in cAMP before the ability to respond to LH (532).

Prostaglandin $F_{2\alpha}$ increases levels of cyclic guanosine monophosphate (cGMP) and causes constriction of vasculature, while PGE_2 increases cAMP and relaxes smooth muscle of the vasculature and increases blood flow (533, 534). Increases in the cGMP:cAMP ratio in uterine tissue are associated with an increase in the ratio of $PGF_{2\alpha}$ to PGE_2 and myometrial contractions (534).

1.2.2.6 EFFECT OF ESTRADIOL-17ß, PGE_1, PGE_2 OR PGI_2 *IN VIVO* ON PREGNANCY

1.2.2.6.1 INTRODUCTION

Infusions of estradiol-17ß starting early in the estrous cycle of sheep extends luteal function (529). This was thought to be due to estradiol-17ß increases in secretion of LH (535). Since estradiol-17ß alters

the levels of cyclic nucleotides and $PGF_{2}\alpha$ and PGE_2 in the uterus (534), it could alter the synthesis of prostaglandins in favor of PGE_2. Estradiol-17ß rapidly increases uterine secretion of PGE_2 in ovariectomized rats. This effect is independent of progesterone priming (534).

Prostaglandin E_2 terminates pregnancy or pseudopregnancy in rats (536-538). However, PGE_2 is much less potent than $PGF_{2}\alpha$ in terminating pregnancy (536-538). Higher doses of PGE_1 are required to terminate pregnancy in rats (537, 538), but does not regress the CL (538, 539). Prostaglandin E_1 does not induce CL regression in pseudopregnant rats, while PGE_2 will at high doses when given into the uterus (539). Twenty-five mg of PGE_1 or PGE_2 given on days 9 and 10 postestrus does not alter cycle length or profiles of progesterone in nonpregnant ewes (444). A single injection of 25 mg of PGE_1 given on days 11 to 13 postestrus to cows increases progesterone in jugular blood for 24 hours, but does not alter cycle length (540). In addition, a single injection of 200 mg of PGE_2 given into the largest follicle on the ovary with the CL on day 9 postestrus increases progesterone in jugular blood above control ewes at 4 and 24 hours in nonpregnant ewes (521). Collectively, these in vitro and in vivo data indicate that PGE_1 or PGE_2 have actions opposite to $PGF_{2}\alpha$ on luteal tissue. Furthermore, PGE_1 or PGE_2 are not luteolytic at comparable doses or higher than those required by $PGF_{2}\alpha$ to induce luteolysis. Since PGE_1 or PGE_2 are less effective than $PGF_{2}\alpha$ as an abortifacient, PGE_1 or PGE_2 are good candidates as the embryonic antiluteolysin of eary pregnancy in sheep.

1.2.2.6.2 SECRETION OF PGE_1 OR PGE_2 DURING EARLY PREGNANCY

Three questions must be answered to conclude that PGE_1 or PGE_2 are candidates as an embryonic antiluteolysin: 1. Does the uterus secrete PGE_1 and/or PGE_2 at the time of maternal recognition of pregnancy?; 2. Will acute exposure to PGE_1 and/or PGE_2 *in vitro* or *in vivo* prevent a $PGF_2\alpha$-induced premature luteolysis?; and 3. Will chronic intrauterine infusion of PGE_1 and/or PGE_2 prevent spontaneous luteolysis?. If the PGE series is the antiluteolysin of early pregnancy in sheep, levels should be increased in uterine endometrium and venous plasma during the critical period of maternal recognition of pregnancy.

Ovine or bovine blastocysts collected around the time of maternal recognition of pregnancy synthesize PGE_2 *in vitro* from radioactive arachidonic acid (508). In addition, concentrations of PGE_1 or PGE_2 are increased 2 fold in uterine endometrium of pregnant ewes on day 13 compared to nonpregnant ewes (502). The PGE/PGA fraction from homogenates of ovine embryos and endometrium collected on days 13 to 15 increases weights of CL and progesterone in jugular blood when injected into the perivascular space adjacent to the ovary with the CL. This response increases as the number of embryo equivalents is increased (541). Intrauterine infusions of homogenates of days 14 to 15 embryos (3 embryo equivalents per 24 hours) from days 12 to 18 postestrus extends luteal life span and peripheral progesterone for 2 days in nonpregnant ewes (475). In pseudopregnant rats, synthesis and release of PGE2 by the uterus is higher in those with artificially induced contributions to the maternal placenta than in control pseudopregnant rats (542, 543). Indomethacin, a prostaglandin

synthesis inhibitor, prevents formation of the maternal contributions to the placenta in pseudopregnant rats (543).

Concentrations of PGE_2 in uterine endometrium and venous plasma on day 13 postbreeding is higher in pregnant ewes than in nonbred ewes (502). Concentrations of PGE_2 in uterine endometrium or venous blood of pregnant ewes are not different compared to nonbred ewes on days 15 to 16 post estrus. However, levels of PGE_2 are elevated in ovarian arterial and uterine venous tissue (504, 505). Secretion of PGE_2 *in vitro* by endometrium from pregnant ewes on days 12 and 15 is greater than in nonpregnant ewes (503). Synthesis of PGE_2 by endometrial tissue *in vitro* and the concentration of PGE_2 in endometrium or utero-ovarian venous serum are higher on days 15 and 17 in pregnant than in nonpregnant ewes (503). Synthesis of prostaglandins by blood samples collected in this experiment was not inhibited. Although PGE_2 values from pregnant ewes tended to be higher on day 13, these effects may have been caused by synthesis of prostaglandins by platelets after collection of the blood sample until serum was collected (503). Other studies show that secretion of PGE_2 *in vitro* by endometrium collected from pregnant ewes is greater than endometrium from nonbred ewes (511, 513, 514). In addition, stroma of the ovine endometrium secretes more PGE_2 than does the uterine luminal epithelium (544).

When utero-ovarian venous plasma is collected every 30 minutes for 2 hours twice daily from days 10 through 14 postbreeding, a 3 fold increase in PGE_2 is seen on day 13 compared to nonbred ewes from days 10 through 13. Prostaglandin E_2 and PGE_1 are increased 2 fold in

ovine endometrium on day 13 postbreeding (502). The PGE:$PGF_2\alpha$ ratio increases from 0.1 to 1.0 in uterine venous plasma on day 13 (286, 287, 545). Furthermore, changes in $PGF_2\alpha$ and PGE_2 in uterine tissue, embryonic tissue, ovarian arterial blood, uterine vein and ovarian vein from days 10 to 15 postbreeding support these data (546). Prostaglandin $F_2\alpha$ increases from days 10 through 15 in uterine caruncular and intercauncular tissue and in uterine venous, ovarian venous or ovarian arterial plasma (546). Prostaglandin $F_2\alpha$ increases 5 fold in intercaruncular and 3 fold in caruncular endometrium, 4 fold in ovarian arterial plasma, 2 fold in ovarian venous plasma and 5 fold in uterine venous plasma. Increases in PGE_2 are also seen from days 10 to 15 (546). Prostaglandin E_2 increases 3 fold in intercaruncular and 2 fold in caruncular endometrium, 9 fold in ovarian arterial plasma, and 3 fold in uterine venous plasma, but no change in ovarian venous levels of PGE_2 is seen. Levels of PGE_2 in caruncular endometrium from days 10 to 45 post breeding are similar. However, PGE_2 increase 2 fold on days 50 and 55 postbreeding (546). This is interesting, since the ovaries or pituitary can first be removed in sheep around day 55 without interrupting pregnancy (441).

When day 10 embryos are transferred to day 6 recipient ewes, none of the recipients maintain luteal function. However, when day 10 embryos are transferred to day 10 recipients, 70 percent of the CL are maintained in the recipient ewes. If day 6 recipients are primed with progesterone after ovulation to advance the age of uterus, day 10 embryos transferred to day 6 recipients maintain 70 percent of the CL (547). When

day 6 ewes are primed with progestererone after ovulation and receive a day 10 embryo, secretion of PGE_2 is advanced (548).

In cattle, content of PGFM in uterine flushings is not different between cycling and pregnant cows on day 17 postestrus (549, 550). Bovine conceptuses collected on days 16 or 19 of pregnancy produce more PGE_2 and PGFM than endometrial tissue and endometrial tissue metabolizes more $PGF_{2}\alpha$ than the conceptus (447, 508). Thus, production of PGE_2 and PGFM increases with day of early pregnancy (551, 552). In addition, PGE_2 in uterine flushings of pregnant cattle are greater than those from nonpregnant cows (553). Although endometrial production of prostaglandins *in vitro* is less than that produced by the conceptus, co-incubation of these tissues changes metabolism of arachidonic acid by the conceptus from $PGF_{2}\alpha$ to PGE_2 (553). This suggests that endometrial tissue determines the amount and type of prostaglandins secreted by the conceptus. Concentrations of PGFM in jugular blood is higher in pregnant than in nonpregnant cows at the time of maternal recognition of pregnancy (447). Other metabolites of arachidonic acid may also be involved in luteolysis or antiluteolysis in cattle. Nordihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenase conversion of arachidonic acid to leukotrienes, delays luteolysis (554). Further studies on roles of leukotrienes and luteal function need to be explored.

1.2.2.6.3 EFFECTS OF PGE_1 OR PGE_2 ON LUTEAL FUNCTION IN NONPREGNANT EWES AND COWS

Prostaglandin E_1 or PGE_2 (333 μg) given into the ovarian vascular pedicle sheath adjacent to the ovary with a CL prevents luteolysis by a luteolytic dose of $PGF_2\alpha$ (80 μg) given via the same route (555). Progesterone in jugular blood of $PGF_2\alpha$-treated ewes decreases to less than 1 ng/ml by 48 hours. At 24 hours in $PGE_1 + PGF_2\alpha$- or $PGE_2 + PGF_2\alpha$-treated ewes, progesterone decreases by 50 percent and by 48 hours progesterone in the $PGE_1 + PGF_2\alpha$-treated ewes returned to levels that were not different from vehicle-treated controls. Weights of the CL of $PGF_2\alpha$-treated ewes are decreased at 48 hours, but weights of the CL in $PGE_1 + PGF_2\alpha$- or $PGE_2 + PGF_2\alpha$-treated ewes are not reduced at 48 hours. Unoccupied receptors for LH are reduced by 24 hours in all treatment groups receiving $PGF_2\alpha$ and decreases further in ewes treated only with $PGF_2\alpha$. Unoccupied receptors for LH at 48 hours in the $PGE_2 + PGF_2\alpha$-treated ewes remain at levels seen at 24 hours in this same group, while unoccupied receptors for LH in the $PGE_1 + PGF_2\alpha$-treated ewes are 3 fold greater than controls at 48 hours (555). In a separate experiment, PGE_1 or PGE_2 increase progesterone in jugular blood of sheep at 24 hours (556). By 48 hours, progesterone returns to levels not different from controls in PGE_2-treated ewes, while progesterone increases further in PGE_1-treated ewes. Occupied receptors for LH double at 48 hours by treatment with PGE_1 but PGE_2 does not affect the amount of LH bound to luteal membranes (555). When three doses of PGE_2 (180-290 μg, 305-345 μg, 345-505 μg) plus 80 μg of $PGF_2\alpha$ are given into the sheath of the ovarian vascular pedicle

adjacent to the ovary with a CL on days 8, 9 or 10 postestrus, the intermediate dose range prevents $PGF_{2}\alpha$ decreases in CL weights at 48 hours, while the high dose range acts synergistically with $PGF_{2}\alpha$ and decreases luteal weights. The lowest dose range is ineffective (254). When PGE_2 or $PGF_{2}\alpha$ alone or both PGE_2 and $PGF_{2}\alpha$ are infused into the ovarian artery of sheep at a rate of 10 μg/hr for 4 hours, $PGF_{2}\alpha$ alone but not PGE_2 reduces ovarian venous progesterone. When PGE_2 and $PGF_{2}\alpha$ are infused stimultaneously, progesterone secretion is maintained for 5.5 hours, but decreases after infusions of PGE_2 are terminated (557). Thus, acute injections of PGE_1 or PGE_2 prevents a $PGF_{2}\alpha$-induced premature luteolysis. Although PGE_1 and PGE_2 may share common mechanisms only PGE_1 increases unoccupied receptors for LH when the CL is threatened with luteolysis induced by $PGF_{2}\alpha$ (555) and only PGE1 increases the amount of LH bound to receptors at the times analyzed (556).

Chronic intrauterine infusions of PGE_2 (300, 600 or 900 μg) every 6 hours from day 12 postestrus until ewes return to estrus increases plasma progesterone for 2 days and delays the return to estrus by 3 to 5 days posttreatment (558). Chronic intrauterine infusions of PGE_2 (37.5 μg/hr) with an osmotic minipump inserted on day 11 delays estrus for 2 days in 2 out of 7 bilaterally ovulating ewes with surgically separated uterine horns. Infusions of PGE_2 (75 μg/hr) in unilaterally ovulating ewes does not alter interestrous intervals, but does increase progesterone in jugular blood for an average of 2 days (558). Prostaglandin E_1 infused (500 μg every 4 hours) into the uterine lumen via an intrauterine cannula adjacent to the ovary with a CL from day 10 until day 25 postestrus extends luteal

function to an average of 37 days compared to 16 days for control infusions (444, 445). Intrauterine infusions of PGE_2 (300 μg every 4 hours) into the uterine lumen via an intrauterine cannula from day 9 to day 22 adjacent to the ovary with CL also delay return to estrus from 30 to 48 days postestrus (444, 445). Intrauterine infusions of PGE_1 (559) or PGE_2 (560) every 4 hours (500 μg) via an intrauterine cannula adjacent to the ovary with the CL prevent an estradiol-17ß-induced premature luteolysis. Also, chronic intrauterine infusions of PGE_1 (561) or PGE_2 (562) every 4 hours (500 μg) via an intrauterine cannula adjacent to the ovary with a CL prevents an IUD-induced premature luteolysis. Moreover, PGE_1 or PGE_2 (500 μg) given every 4 hours extends luteal function only when infused into the uterine lumen adjacent to the ovary with a CL, but not when infused into the uterine lumen opposite to the ovary with a CL (563, 564).

Evidence exists for a direct effect of PGE1, PGE2 or PGI_2 on ovine luteal cells *in vitro* and for PGE_2 inhibition of $PGF_{2\alpha}$ actions *in vitro*. Prostaglandin E_1, PGE_2 or PGI_2 increases progesterone secretion 16 fold by ovine LLC *in vitro*. Prostaglandin I_2 increases progesterone secretion by ovine SLC *in vitro* at 4 hours, some 3 hours later than increases stimulated by LH. Prostaglandin E_1 or PGE_2 does not increase progesterone secretion by SLC after 4 hours of culture (565). Prostaglandin E_2 increases secretion of progesterone by ovine LLC *in vitro* at doses of 1, 10 or 100 ng/ml (565). These data are consistent with location of receptors for PGE_1 or PGE_2, since numbers of PGE_1 or PGE_2 receptors are greater on LLC than SLC in sheep (160). Prostaglandin $F_{2\alpha}$ decreases progesterone secretion by ovine LLC *in vitro* (372). Furthermore, PGE_2 inhibits $PGF_{2\alpha}$-induced declines in

progesterone by ovine LLC *in vitro* (373, 374). Moreover, PGE_2 attenuates the $PGF_2\alpha$-induced increases in free intracellular calcium in ovine LLC (566). The mechanism of luteolysis in LLC has been reported to be through increases in intracellular calcium (160, 190).

Prostaglandin E_2, estradiol-17ß or PGE_2 + estradiol-17ß were infused intrauterine every 6 hours from day 13 to day 21 in cows. Cows receiving estradiol-17ß, PGE2 or control are in estrus by day 21. None of the cows receiving PGE_2 + estradiol-17ß are in estrus at autopsy on day 21. Luteal weights and concentration of progesterone are higher in PGE_2 + estradiol-17ß-treated cows than in controls or cows receiving estradiol-17ß or PGE_2. Concentrations of progesterone in jugular blood of control, estradiol-17ß or PGE_2-treated cows from days 16 to 18 decreases to less than 1 ng/ml by day 21. In cows treated with PGE_2 + estradiol-17ß, progesterone decreases from 8 ng/ml on day 19 to 3 ng/ml on day 21 post estrus, but none of these cows were in estrus on day 21 (567). Thus, PGE_2 + estradiol-17ß in cattle delays the decline in progesterone.

Intrauterine infusion of PGE_2 adjacent to the ovary with the CL of heifers every 4 hours from days 9 to 21 (568) or day 14 through days 25 to 28 or when heifers are observed in estrus, luteal function is maintained. Prostaglandin E_2 does not increase secretion of progesterone, but the decline in progesterone is delayed. Forty percent of the heifers treated with PGE_2 have regressed CL during the infusion period, but have estrous cycles extended to 24 to 27 days. When the infusions of PGE_2 are terminated in heifers with maintained luteal function, progesterone decreases and heifers

are in estrus within 3 days (569). A single intramuscular injection of PGE_1 (25 mg) in cattle increases progesterone in jugular blood for 48 hour (540).

Direct effects of PGE_2 and PGI_2 on bovine luteal cells are seen. Prostaglandin I_2 increases basal secretion of progesterone by bovine SLC *in vitro* and causes marked increases in progesterone secretion by LLC *in vitro* (164). Differences in distribution of receptors for PGE_1, PGE_2 or PGI_2 on bovine SLC and LLC as seen in sheep have not been determined.

In summary, PGE_1 or PGE_2 prevents luteolysis in sheep and only when infused intrauterine adjacent to the ovary with the CL. This fits with the local veno-arterial pathway established for delivery of the uterine luteolysin ($PGF_2\alpha$) and the embryonic antiluteolysin. Concentrations of PGE_1 and PGE_2 are increased in uterine endometrium during the period of maternal recognition of pregnancy. Prostaglandin E_2 is secreted into uterine venous blood of the gravid uterine horn at the time of maternal recognition of pregnancy in sheep. Also, the ovine conceptus collected around the time of maternal recognition of pregnancy secretes PGE_1 and PGE_2 *in vitro*.

1.2.2.7 PREGNANCY PROTEINS AND/OR THEIR POSSIBLE ACTIONS FOR MATERNAL RECOGNITION OF PREGNANCY

A number of proteins or their interaction may play a role in maternal recognition of pregnancy in sheep, cattle, pigs and horses. These may include but are probably not limited to ovine trophoblastic protein-1 (oTP-1; interferon α), bovine trophoblastic protein-1 (bTP-1; interferon α), oxytocin, neurophysin, ovine conceptus secretory proteins (oCSP), bovine conceptus secretory proteins (bCSP), porcine conceptus secretory protein

(pCSP), pregnant mare serum gonadotropin (PMSG) and equine conceptus secretory proteins (eCSP; 438).

Ovarian oxytocin is involved in releasing $PGF_{2}\alpha$ from the uterus to cause regression of the CL (570). Oxytocin increases are seen in blood at the time of luteolysis and are released in a pulsatile fashion similar to that seen for $PGF_{2}\alpha$ (570, 571). During early pregnancy, receptors for oxytocin in uterine endometrium (572) and oxytocin in blood (573) decrease suggesting that the luteolytic mechanism is suppressed. Since estradiol-17ß stimulates uterine production of oxytocin receptors (292-294), it is difficult to interpret these data when estradiol-17ß in jugular blood is not increasing during days 14 to 18 of early pregnancy compared to nonpregnant ewes during luteolysis (6). Progesterone is decreasing during luteolysis in nonpregnant but not in pregnant sheep (277, 278) and progesterone inhibits estradiol-17ß induction of its own receptors (318). Moreover, conception rates of ewes actively immunized against oxytocin are only 28 percent compared to 91 percent for ewes immunized against the carrier protein only (574). In fact, evidence exists for a need for oxytocin for establishment of pregnancy, since depletion of endogenous oxytocin in the previous cycle reduces numbers of pregnancies by 80 percent (575). This is further supported by the presence of oxytocin receptors on luteal tissue (576) and oxytocin stimulation of progesterone release by luteal tissue *in vitro* (577). Oxytocin and progesterone are not released stimultaneously and only oxytocin is released from the granular secretory vesicles in the LLC of the CL (578, 579). Thus, oxytocin may be involved not only in luteolysis, but also in luteal maintenance.

Ovine trophoblast protein-1 and bTP-1 are secreted by the conceptus between days 10 to 21 (580) and days 25 to 45 (581) in sheep and days 16 to 19 postbreeding in cattle (582). Thus, oTP-1 and bTP-1 are produced by the conceptus at the time of maternal recognition of pregnancy. Infusion of oTP-1 (583) or bTP-1 (584) into the uterus extends luteal function in sheep and cattle. Ovine trophoblastic protein-1 and bTP-1 are thought to interfere with the luteolytic mechanism by reducing the number of oxytocin receptors, by decreasing the affinity of the oxytocin and estrogen receptors to inhibit uterine secretion of $PGF_2\alpha$ and thus reduce the luteolytic process (332-334, 585, 586). Ovine trophoblastic protein-1 or bTP-1 (580) are not found in uterine venous blood nor do they stimulate PGE secretion by the uterus (438). It is difficult to support oTP-1 or bTP-1 as molecules responsible for maternal recognition of pregnancy, since they do not appear in uterine venous blood. Thus, they do not fit with the route of delivery for the embryonic antiluteolysin shown by vascular anastomoses experiments (464, 465).

Since oTP-1 and bTP-1 are interferons and interferon-α (IFN-α) given systemically extends luteal function, oTP-1 and bTP-1 may be suppressing the immune system (332-334, 585, 586). Bovine trophoblastic protein-1 and bovine IFN-α_1 inhibits stimulation of lymphocyte proliferation induced by phytohemagglutinin, concanavalin-A and pokeweed mitogen (587). Thus, oTP-1 and bTP-1 may be involved in protecting the allogenic conceptus from rejection.

Ovine trophoblastic protein-1 does not inhibit $PGF_2\alpha$ decreases in progesterone secretion by ovine LLC *in vitro*. However, one oCSP does

inhibit luteolytic actions of $PGF_2\alpha$ on ovine LLC (588). Porcine CSP infused intrauterine extends luteal function (590) by increasing uterine secretion of PGE_2 *in vivo* (589) or stimulating progesterone secretion *in vitro* (590). In pigs, the endometrial stroma produces PGE_2 during early pregnancy (591). Porcine endometrial stroma binds epidermal growth factor (EGF) more than uterine luminal epithelium and stimulates PGE_2 secretion by porcine endometrial stroma cells (592). Thus, stimulation of growth factors may regulate endometrial production of prostaglandins.

1.2.3 PLACENTATION IN THE RUMINANT

The sheep endometrium is composed of uterine caruncular and intercaruncular areas through which the uterine glands open. Caruncles are nonglandular structures that interact with the trophectoderm to form the fetal placenta called a placentome. The external form of the placenta is cotyledonary (593, 594). The bovine caruncle forms a convex knob and the chorionic tissue covers it, while the ovine caruncle is concave and forms a cup and the chorionic tissue fills it. At the interface of the cotyledon of the chorion, villi from the cotyledon portion of the trophectoderm and fills depressions in the caruncle called crypts (594). Thus, the placentome is a complex of interlocking fetal and maternal elements, which may also fuse (594, 595). Electron dense material is seen between the villi and crypts of the caruncle and is assumed to be adhesive material (595). Manual separation of the cotyledon and caruncle cannot be done without chorionic villi being left in the crypts. Chorionic villi also break off and remain in the caruncules at parturition (593).

Numbers of caruncles range from 64 to 115 in Merino and Merino-cross bred ewes, but numbers vary within breed. Numbers of caruncles are also distributed evenly between the two uterine horns (596). Numbers of cotyledons formed range from 88 to 96 (490). Thus, most of the caruncles are associated with cotyledons during pregnancy. Birth weights are positively correlated with placental and cotyledon weight. Reduction of the number of caruncles surgically reduces placental, cotyledon and fetal weights (597). All of the caruncle/cotyledon complexes (placentomes) are formed by day 70 to 80 in sheep. Maximum cotyledon size and placental weight is seen by day 85 of gestation. More placentomes are formed as pregnancy progresses (598). The bulb shape of many cotyledons changes to a flat button shape late in pregnancy as the fetal tissue envelops the maternal tissue (599). While sheep placenta weight is maximal by day 85, bovine placental weight increases exponentially from days 100 to 250 of pregnancy. This increase in weight is caused by hyperplasia in the cotyledon, but only hypertrophy is seen in the caruncle (600). Progesterone receptors in nuclei of caruncular endometrium increase 3 fold from breeding to day 28 of pregnancy in sheep. Nuclear progesterone receptors increase in intercaruncular endometrium between days 84 to 112 in sheep. Estrogen receptors are low from days 56 to 112 in ovine cotyledon, caruncles and intercaruncular endometrium. Concentrations of protein in intercaruncular endometrium increases 25 fold between days 0 and 112 of gestation in sheep, but does not change in caruncular endometrium (601). Thus, receptors for estrogen or progesterone are not significantly correlated with ovine placental growth.

Although ovine placental weight is maximal by day 85 in sheep (490, 598), development of the vasculature within the placentome increases throughout the remainder of gestation. By the end of gestation, the placenta consists primarily of vascular tissue (602, 603). Eighty to 90 percent of the total uterine and umbilical blood flow is distributed to the maternal and fetal portions of the placenta (604-606). Heat stress reduces blood flow to the uterus (607) and may be responsible for reduced birth weights, reduced placental weights and reduced neonatal survival (599). Reduced placental weight is associated with reduced birth weight and neonatal survival (599).

The ovine and bovine placenta is a syndesmochorial/cotyledonary placenta. All six of the fetal and maternal layers (fetal endothelium, fetal connective tissue, trophoblast epithelium, uterine luminal epitheium, stromal connective tissue and maternal endothelium) contributing to placental formation are retained (594). Placentation in sheep begins on days 15 to 18 when contact by the blastocyst and uterus occurs (608, 609). Attachment of the chorion to the uterine epithelium begins in the middle of the uterine horn near the embryonic disc and spreads toward the tip of the uterine horn (610). The trophectoderm has villi which invade the uterine glands from day 15 to the end of gestation. These villi may not only anchor the conceptus during the adhesion stage but may also provide a route for the exchange of uterine histotrophic secretions. At the point of the contact between trophectoderm and caruncular crypt epithelium, most microvilli dissapear (611, 612). There is no phagocytosis of the uterine caruncular epithelium, which forms a syncytium by the break down of its lateral cell boundaries (611, 613).

Three cell types are seen at the interface of the chorion and caruncule; mononucleate, binucleate and trinucleate cells. The mononucleate cells of the trophectoderm undergo karyokinesis, but not cytokinesis to form the binucleate cells (614). These binucleate cells are seen as early as day 16 and migrate from the trophectoderm to the adhesion site (613). The trinucleate cells, which are seen occasionally, appear to come from fusion of binucleate cells with the caruncular epithelial cells (615). These binucleate and trinucleate cells are numerous at the tip of the trophectoderm villus in the caruncular crypts (616, 617). Binucleate cells account for 15 to 20 percent of the cells of the trophectodermal epithelium (618) throughout gestation (619). The binucleate cells migrate through chorionic tight junctions to fuse with uterine epithelial cells. Thus, binucleate cells modify the uterine epithelium and the placenta is actually synepitheliochorial rather than epitheliochorial or syndesmochorial placenta (619).

Numbers of binucleate cells decrease just before parturition in the trophoblastic ectoderm (619, 620). Hypophysectomy of the fetus, but not treatment with an anti-progesterone antisera or inhibitors of prolactin prevents the decrease in numbers of binucleate cells before parturition. A synthetic analogue of adrenocorticotropic hormone (ACTH) causes an early decrease in binucleate cell numbers (620). The binucleate cells are not destroyed at parturition (621). Although the binucleate cells do not move beyond the uterine luminal epithelium, microvilli penetrate the syncytium and appear to release secretory products (622). Granules in the binucleate cells contain ovine placental lactogen and appear to accumulate in these microvilli

(619, 620). The binucleate cell contains large amounts of Golgi apparatus and endoplasmic reticulum and may secrete other proteins (614). The binucleate cells are also thought to secrete estrogen, progesterone and prostaglandins in sheep and cattle (623-628). When glucocorticoids are used to induce parturition, binucleate cells may be stabilized and not decrease leading to retention of fetal membranes. Prostaglandin $F_{2}\alpha$ reduces numbers of binucleate cells in the placenta *in vitro* (630).

1.2.4 PROGESTERONE DURING GESTATION IN RUMINANTS

Progesterone appears to be the only steroid required for implantation and maintenance of gestation through day 55 of pregnancy in sheep (440). However, progesterone, estradiol-17ß and other placental products may be required to maintain pregnancy after day 55 of gestation in the absence of the ovaries in sheep.

When ewes are ovariectomized for months, then primed with progesterone and estradiol-17ß to induce behavioral estrus, embryos transferred into these ewes implant and survive (630). Implants of progesterone (113) or weekly intramuscular injections of medroxy-progesterone acetate (632), a synthetic long lasting progestin, given to ewes ovariectomized on day 20 postbreeding maintains pregnancy until day 120 (631) or to term although progesterone implants are removed between days 89 to 94 of gestation (117). Ovariectomy of ewes at day 84 of pregnancy without giving exogenous progesterone does not interrupt gestation (632). When ewes are ovariectomized at different stages of gestation, the ovaries are needed only to days 55 to 60 of gestation in

sheep (441, 633). This suggests that the placenta is able to secrete sufficient progesterone and/or other placental products to maintain pregnancy after day 55 of gestation in sheep.

Additional studies in sheep indicate that neither the maternal pituitary nor ovaries are needed after day 55 postbreeding to maintain pregnancy (633-638). If ewes are hypophysectomized on day 10 post breeding and pregnancy is maintained with daily injections of progesterone, the CL regresses by day 20. The pituitary appears to convert the CL of the estrous cycle to the CL of pregnancy (635, 636). If ewes are hypophysectomized on day 30 of pregnancy and after implantation, the CL regresses slowly, but the fetus eventually aborts (635, 636). If ewes are hypophysectomized on day 60 of pregnancy, the fetus is not aborted although the weight of the CL is decreased by 25 percent (635-638). The placenta may secrete a pituitary luteotropin, at this time as this coincides with an increase in peripheral progesterone (639, 640) or the placenta secretes enough progesterone to maintain pregnancy (113, 631, 632). Thus, the embryo/placenta appears to be only antiluteolytic for the first 30 days of pregnancy, but may become both antiluteolytic and increasingly luteotropic from days 30 to 60 of pregnancy in sheep (633-640).

Sheep placenta appear to increase progesterone secretion when the ovaries are removed (641). Concentrations of progesterone in placental tissue of day 100 intact and ovariectomized pregnant ewes are 4 μg/kg and 9 μg/kg, respectively (641). Concentrations of progesterone in jugular blood during pregnancy in sheep are similar; and then increase gradually from days 50 to 85; and increase rapidly from days 85 to 130 of pregnancy (117,

640, 642). Concentrations of progesterone in jugular blood between days 80 to 130 in ewes depends on the number of fetuses in the uterus (643). Profiles of progesterone in amniotic and allantoic fluid throughout gestation (644) are similar to jugular blood (113, 640, 642). Placental secretion of progesterone may be affected by exogenous estrogens. Ewes grazing Yarloop clover, an estrogenic pasture, have progesterone levels 50 percent lower from day 90 until parturition than ewes grazing rye grass, a non-estrogenic pasture (645).

Regulation of ovine placental secretion of progesterone is unknown. It was reported that ovine LH stimulates conversion of pregnenolone to progesterone *in vitro* by minced fetal cotyledons collected on days 45 or 55 of pregnancy in sheep (646). In another study, hCG given on day 80 to ewes ovariectomized on day 75 or hCG incubated with 100 mg of fetal cotyledons did not increase progesterone in jugular blood or in culture medium after 2 hours of incubation. In addition, dbcAMP did not increase progesterone by fetal cotyledons *in vitro*. Progesterone in ewes ovariectomized on day 75 increases 5 fold from day 80 to day 115 (647). In another study, 8-Br-cAMP, LH, forskolin, phorbol esters, GnRH, calcium ionophore or a GnRH antagonist did not stimulate secretion of progesterone *in vitro* after 3 hours by minced ovine fetal cotyledons collected on day 35, 52, 68 or 118 of pregnancy. Addition of pregnenolone increased progesterone secretion in a dose dependent manner, while 25-hydroxy cholesterol does not (648). When enzymatically dispersed ovine fetal cotyledons collected on days 100 to 120 are incubated with a calmodulin antagonist, pimozide, a calcium ionophore or phorbol esters *in vitro*,

progesterone secretion decreases. Ethylene glycol tetra-acetate (EGTA), a calcium chelator, increases progesterone secretion and blocks the calcium ionophore decrease in progesterone secretion (649). Luteinizing hormone does not increase progesterone secretion by minced placentome slices from intact or ovariectomized 90 to 100 day pregnant sheep *in vitro* (C. Weems, personal communication, unpublished data). Placentome slices would contain both fetal and maternal contributions to the placenta. The binucleate cells of the fetal placenta appear to be the source of progesterone (619-629). In human placental slices, cAMP increases steroidogenic enzymes *in vitro*, but not necessarily progesterone secretion (650).

Some investigators conclude that progesterone is derived predominately from the placenta and that luteal secretion of progesterone decreases markedly after day 60 of pregnancy in sheep (639, 651). Corpora lutea of pregnancy in sheep may be functional through at least days 120 to 130. Luteal weights and progesterone content do not decrease and structural evidence of luteal regression is not seen until day 142 which is several days before parturition (652). In addition, cellular composition, numbers of SLC and LLC, cell volume and the cytoplasmic:nuclear ratio are similar until day 142 of gestation to that seen at mid-cycle in nonpregnant sheep (652). At day 133 of gestation, ovine CL contain only 20 and 12 percent of cytochrome p450 Scc enzyme and adrenodoxin, respectively, when compared to CL collected at mid-cycle from nonpregnant ewes (653). This is about the time when progesterone declines in ovarian venous blood of pregnant ewes (639, 654, 655), weights of CL decrease (655, 656) and progesterone content in CL decreases (654, 655).

Hysterectomy early after ovulation in nonpregnant ewes extends luteal function for the same length of time as pregnancy; undergoes similar changes in CL weight , progesterone content and ultrastructure (657) to those seen during late gestation in sheep (655, 656). Corpora lutea from late pregnant sheep (654) or mid- to late pregnant cows (658, 659) produce less progesterone *in vitro* in response to LH than luteal tissue collected from mid-cycle nonpregnant ewes or cows. Ovine CL collected at 90 to 100 days of pregnancy are not responsive to LH, but PGE_2 increse secretion of progesterone by luteal slices *in vitro* (C. Weems, personal communication, unpublished data). This reduced response to LH is not due to numbers of receptors on luteal tissue for LH or prolactin, since numbers of these receptors do not change throughout gestation and are similar to ovine luteal tissue collected at mid-cycle from nonpregnant ewes (660). When ewes are given GnRH at different stages of gestation, release of LH is less at day 118 than earlier stages of pregnancy in sheep (661). Although numbers of LH receptors on luteal tissue do not change throughout pregnancy in sheep (660), luteal secretion of progesterone in sheep may be regulated by other factors during pregnancy. This stage of pregnancy is when progesterone peaks in jugular blood (631, 632, 639, 640, 642, 643). Progesterone decreases GnRH secretion by the hypophysis and the sensitivity of the hypophysis to GnRH (44, 53, 54).

These negative effects of high progesterone and estradiol-17ß during late gestation in sheep and cattle reduce the content of LH and mRNA for both α- and ß- subunits of LH in the hypophysis. Messenger RNA for the α-subunit of LH increases 3 fold by day 2 postpartum and levels of

mRNA for the ß-subunit of LH does not increase until day 13 postpartum in sheep (662). Gonadotropin releasing hormone increases progesterone secretion *in vivo* in late pregnant sows, but not in ewes (660). This difference may be due to a lack of placental secretion of estradiol-17ß and progesterone during gestation in pigs. Levels of progesterone and estradiol-17ß in pigs is no higher during gestation than those seen on day 15 post breeding (663, 664). Pigs require ovaries for progesterone secretion throughout gestation (665), unlike sheep where the ovaries are required only through day 55 of pregnancy (441, 643).

Collectively, these data suggests in sheep that CL of pregnancy are functional until days 130 to 140 of gestation. In addition, secretion of progesterone may require some luteotropin other than LH to maintain luteal secretion of progesterone after day 55 of pregnancy.

Concentrations of progesterone in jugular blood increase to an average of 6 ng/ml by day 12 post breeding (666, 667) and increases further to 10 ng/ml at the time of maternal recognition of pregnancy in cows (666). Some report that levels of jugular progesterone are similar from day 20 throughout the remainder of gestation in cows where samples were collected every 10 days (667). Others report that jugular progesterone increases through day 85 of pregnancy in cows, but do not increase for the remainder of gestation (666). Weights of CL are similar throughout gestation in cows (668, 669). In another study (670), progesterone in jugular blood between days 140 to 200 of pregnancy is about half that in cows between days 10 to 20 of pregnancy. In large numbers of cows sampled throughout gestation, progesterone content and concentration in CL peak between 37

to 81 days, decline through day 185, increase 100 percent by day 204 and then decrease 10 days prior to parturition (668, 669). Progesterone in ovarian venous blood of cattle is similar through day 120 and decline through day 230 before increasing on day 240 (668). However, progesterone in jugular blood is similar throughout gestation in cattle (668, 671). Levels of the 20α- or 20ß- metabolites of progesterone do not change during gestation (668).

In Brahman cattle with twin fetuses, progesterone is higher in jugular blood through 8 weeks of gestation than cows with a single fetus, but thereafter, levels of progesterone are similar throughout the remainder of gestation (672). Secretion of progesterone by enzymatically dispersed maternal caruncles *in vitro* increases 4 fold between days 90 to 135 of gestation and decreases on days 182 to 244 before increasing on days 275 to 285 (626, 673-676). However, arterio-venous concentrations of progesterone indicate that the gravid uterus does not secrete significant quantities of progesterone (677, 678). Lutectomy of cows at 250 to 270 days of gestation reduces progesterone in jugular blood to 1 ng/ml within 16 hours and decreases slowly to less than 0.5 ng/ml by 72 hours. Concentrations of progesterone in the uterine vein decrease to 1.5 ng/ml and 0.5 ng/ml by 16 and 24 hours, respectively, after lutectomy on days 250 to 270. Progesterone remains at these levels in samples collected for 72 hours post lutectomy and none of the cows abort (679). Ovariectomy between days 190 to 251 of gestation in cows decreases progesterone in jugular blood by 50 percent and levels of progesterone are similar throughout gestation (671). Corpora lutea are required for the first half of

gestation (680-682), but not the last half of gestation in cattle. However, removal of ovaries during the second half of gestation increases calving difficulties (671, 683-687).

Both fetal and maternal contributions to the placenta in cattle produce progesterone from pregnenolone *in vivo* and *in vitro* (688). Fetal cotyledons produce more progesterone than maternal contributions to the placenta when cultured alone, but fetal cotyledon production of progesterone declines when co-cultured with maternal tissue. If CL are regressed *in vivo* with $PGF_2\alpha$, secretion of progesterone increases by the fetal cotyledon *in vitro* but not by the maternal contribution to the placenta (688). This suggests that the placenta secretes progesterone, but that a functional CL inhibits placental secretion of progesterone in cattle. Thus, it is difficult to understand how pregnancy is maintained when progesterone is less than 1 ng/ml in jugular blood for 72 hours (679), unless jugular progesterone does not reflect adequately placental secretion and distribution of progesterone.

The adrenal may play a role in maintenance of pregnancy in cattle. When cattle are ovariectomized and adrenalectomized at 215 day of gestation, abortion occurs within 30 days. However, cows left intact, ovariectomized or adrenalectomized, pregnancy will go on to deliver calves between days 270 to 278 of gestation (689). In this study, cows ovariectomized and adrenalectomized received exogenous cortisol, but the other three treatment groups did not. Progesterone is 10 to 100 times greater in the adrenal vein than in the adrenal artery at 240 day of gestation in cows (690).

Regulation of bovine placental secretion of progesterone is also controversial. Cyclic AMP (679) or inhibitors of cAMP degradation (673, 674) increase bovine placental secretion of progesterone. However, 8-Br-cAMP or LH does not (673, 674). Calcium ionophores increase bovine placental secretion through protein kinase C activation and activation of cholesterol Scc enzyme (673, 674, 691-693). These actions on progesterone secretion occurs in fetal cotyledons throughout pregnancy, but only after 100 to 120 days of gestation in the maternal caruncle (694).

Fetal cotyledon secretion of progesterone is by the binucleate cells (625-629). Binucleate cells from fetal cotyledons of cows also secrete Progesterone E_2 and PGI_2. Twice as much PGE_2 is secreted than PGI_2 (625). Prostaglandin E_2 or PGI_2 could be the regulators of bovine placental secretion of progesterone.

Regression of the LLC has been reported to occur before SLC at parturition in cattle (658, 695). Regulation of progesterone secretion by the CL of the cow may be by LH throughout pregnancy, since progesterone secretion in response to LH does not differ between early and late pregnancy (696). However, other studies suggest that the CL response to LH decreases during mid- to late pregnancy in cattle (674). Evidence exists for both a pregnancy protein gonadotropin (697) and a lipophilic gonadotropic substance (698) secreted by conceptuses of cows. This lipophilic substance could be PGE_2, since luteal tissue from pregnant cows contains PGE_1, PGE_2 and PGI_2 receptors (699). Prostaglandin $F_{2\alpha}$, relaxin and oxytocin (700, 701) also increase progesterone secretion by pregnant bovine luteal tissue in a dose-dependent manner. Effects of PGE_1, PGE_2 or

PGI_2 on secretion of progesterone by pregnant bovine luteal or placental tissues have not been tested. Insulin-like growth factor-1 (IGF_1) may also play a role in progesterone secretion by luteal tissue from pregnant cows. Secretion of IGF_1 by luteal tissue from nonpregnant cows increase during the estrous cycle and decrease at luteolysis. Secretion of IGF_1 by pregnant bovine CL is high during the first months of pregnancy and decreases progressively during 3 to 5 and 6 to 9 months of gestation (702). Direct effects of IGF_1 on luteal secretion of progesterone has not been tested.

1.2.5 ESTROGENS DURING PREGNANCY IN RUMINANTS

Concentrations of estradiol-17ß in uterine venous plasma are similar from day 80 through day 115 of gestation and increases by day 125 in sheep. Similar levels of estrone are seen, but without an increase by day 125. Estradiol-17ß increases progressively from days 70 to 125 in fetal plasma (703). In another study, estradiol-17ß in maternal jugular and in fetal blood were similar throughout pregnancy in sheep. Concentrations of estradiol-17ß are similar through day 130, but increases thereafter (704). In a later and more complete study, estradiol-17α, estradiol-17ß, estrone and their sulfur conjugates were measured in amniotic fluid, allantoic fluid or in ovarian, uterine, or jugular venous blood of sheep (705). Three stages of estrogen production appear to occur between days 0 to 55, days 55 to 140 and days 140 to 150 (705). Concentrations of estradiol-17ß and estrone in amniotic fluid; allantoic fluid; or in ovarian, uterine or jugular venous blood of sheep is one-tenth that seen for estrone sulfate or estradiol sulfate (705).

During the last trimester, total estrogens are highly correlated to the number of fetuses (706) and total birth weight (707). In addition, total estrogens are highly correlated with total placental weight (708). The principal estrogen based on mass is estrone sulfate, which appears to be coming from the fetus as early as day 30 of gestation in sheep. Levels of estrogen are consistently higher in fetal than in maternal fluids. However, the placenta may modify the form seen in maternal blood (709). Follicles are sources of estrogen also, but follicles less than 2 mm in diameter produce little estrogen (705). Although estrone and its sulfur conjugate levels are greater than estradiol and its sulfur conjugate, the patterns and variability suggest sulfated forms are reserve pools (705). Estrone is easily converted to estradiol-17ß and sulfatases convert estrone sulfate and estradiol sulfate to free estrone and free estradiol (650).

Metabolic clearance rate of estrone secreted at days 24, 32, 82, 110, 116, 139, 142-144 does not appear to vary during pregnancy (709). Estrogen binding by erythrocytes from day 15 pregnant sheep is 15 fold greater than nonpregnant ewes (709). In addition, binding of estradiol-17ß to ovine serum albumin is 6 fold greater than binding of estrone (710). The ovine placenta has steroidogenic enzymes to produce all of the precursors to produce estradiol-17ß, unlike primates which cannot produce androstenedione (650). Binucleate cells appear to be the source of placental estrogen in sheep (619, 620, 623-629).

Estrogens in jugular blood of goats during pregnancy increase progressively from day 40 to peak at the time of parturition. This concentration of estrogen is greater in those with twin fetuses than a single

fetus in goats (711-714). In addition, fetal death in utero is related to low levels of estrogen in goats (714). The goat placenta produces little or no progesterone or estrogen and ovariectomy at any stage of gestation results in abortion (714). These data in goats indicate estrogen may be a marker of fetal growth and viability during gestation.

In cows, secretion of estrone in jugular blood or urinary excretion of estrone increases progressively from day 100 to peak at parturition by 7 fold (668, 715). Similar changes are seen for total estrogen, estradiol-17ß and estradiol-17α, but not estriol (716). Similar changes in estrogen concentration in allantoic and amniotic fluid occur as those seen in sheep, but occur at day 135 in cattle (717). Ovariectomy or ovariectomy and adrenalectomy at day 215 of gestation in cows prevents the increase in estradiol-17ß seen at parturition (689). However, levels of total estrogens secreted in uterine venous blood are not different between 1 to 74 days after ovariectomy (671). Samples before 1 hour were not collected. The ovary may inhibit placental secretion of estrogens as seen for placental secretion of progesterone in cows during gestation (688).

1.2.6 PROSTAGLANDINS DURING GESTATION IN RUMINANTS

Prostaglandin $F_2\alpha$, PGFM, PGE_2, PGI_2 or its metabolite, 6-keto $PGF_1\alpha$, and TXB_2 (TXA_2 metabolite) have been quantified in maternal or fetal blood, placental, endometrial or myometrial tissues, or in amnionic or allantoic fluid at different stages of gestation in sheep (718-725). Production of $PGF_2\alpha$, PGE_2, and 6-keto $PGF_1\alpha$ by cotyledons, allantoic and

trophectoderm cells at different stages of gestation *in vitro* has been measured (725-744).

Concentrations of $PGF_2\alpha$ in fetal blood are one-tenth of those seen in utero-ovarian venous blood (1.1 ng/ml) in pregnant sheep on days 116 to 141 (718, 719, 722). Concentrations of PGFM, the metabolite of $PGF_2\alpha$, are 10 fold greater than $PGF_2\alpha$ in fetal blood and 3 fold greater than $PGF_2\alpha$ in utero-ovarian venous blood from days 116 to 141 of pregnancy in sheep (718, 719, 722). Concentrations of $PGF_2\alpha$ in the uterine lumen range from 3 to 8 fold greater than $PGF_2\alpha$ in utero-ovarian venous blood from days 90 to 140 of gestation in sheep (723, 724). Concentrations of $PGF_2\alpha$ in tissue from the fetal cotyledon, chorioallantois and amnion are 500 fold greater than in endometrial tissue from days 50 to 145 of gestation in sheep (725). Concentrations of $PGF_2\alpha$ in allantoic and amnionic fluid are similar to those seen for $PGF_2\alpha$ in utero-ovarian venous blood during gestation in sheep. The lower levels of $PGF_2\alpha$, but higher levels of PGFM in fetal and utero-ovarian venous blood and in amnionic and allantoic fluid (725) seem to be related to maternal and fetal placental catabolism of $PGF_2\alpha$ to PGFM (720). Ninety-seven and 77 percent of tritiated $PGF_2\alpha$ incubated *in vitro* with ovine maternal or fetal contributions to the placenta is converted to PGFM (720). Catabolism of $PGF_2\alpha$ to PGFM by these tissues at parturition is decreased (720). Concentrations of $PGF_2\alpha$ increase many fold in utero-ovarian venous and fetal blood; and in placental tissues; and amnionic fluid at parturition in sheep (718-725). These increases in $PGF_2\alpha$ at parturition in sheep are thought to initiate labor in sheep (718-725).

Other eicosanoids also change throughout gestation in sheep. Concentrations of PGE_2 in fetal and utero-ovarian venous blood; and in amnionic and allantoic fluid have been reported to be similar to $PGF_2\alpha$ throughout gestation in sheep (718-725), but are 2 to 4 fold greater than $PGF_2\alpha$ from days 120 to 140 of gestation in sheep (721). Concentrations of PGE_2 and 6-keto $PGF_1\alpha$ are 100 to 1000 fold greater than $PGF_2\alpha$ in endometrial, fetal cotyledonary, allantoic and amnionic tissues throughout gestation (725). Concentrations of PGE_2, 6-keto $PGF_1\alpha$ and TXB_2 increase many fold greater than $PGF_2\alpha$ in fetal and utero-ovarian blood; placental tissue; and amnionic fluid at parturition in sheep (718-725).

Concentrations of eicosanoids in blood and in uterine/placental tissues may also be affected by rate of synthesis of eicosanoids. Synthesis of PGE_2 and $PGF_2\alpha$ by dispersed trophectoderm cells (726) or microsomes from cotyledons (727) is low from day 25 until day 110 of pregnancy in sheep and is not stimulated by the addition of arachidonic acid. These cultures would contain many types of cotyledonary cells. Synthesis of $PGF_2\alpha$ and PGE_2 increases from day 110 to parturition (726-732). Prostaglandin production by the placenta *in vitro* is inhibited by extracts of endometrial tissue (516, 517), allantoic fluid (733) and myometrial cytosolic (515) products during gestation. The inhibitor in allantoic fluid increases during gestation in sheep and decreases just prior to parturition (733, 734). If the fetus is hypophysectomized, PGE_2 in the maternal carotid artery only increases 3 fold from days 75 to 130 of pregnancy in sheep (722). Thorburn and coworkers conclude that the increase in PGE_2 during late gestation initiates parturition (734, 735).

The rate of synthesis of eicosanoids may be affected by the location and concentration of the rate limiting enzyme, PGH synthase (335). Recently, immunoreactive PGH synthase in the maternal caruncular stroma, the fetal cotyledon, the maternal luminal epithelium and the chorionic vascular endothelial cells has been determined throughout gestation in sheep. Immunoreactive PGH synthase is undetectable from day 30 in the fetal cotyledon until day 110 and then increases. In the caruncular stroma, immunoreactive PGH synthase stains intensly from days 30 to 90 and decreases by day 110, but increase again on day 140. Immunoreactive PGH synthase in caruncular luminal epithelium is undetectable from days 30 to 60, then increases 3 fold from day 70 to peak on day 100 and does not change after that. Immunoreactive PGH synthase in chorionic vascular endothelial cells is undetectable on day 30, but increases 3 fold by day 50 and does not change for the remainder of gestation (736). This study does not differentiate between the two types of PGH synthase descovered in reproductive tissue, recently.

One form of PGH synthase (PGHS-1 and PGHS-2) is constitutively expressed, while the other form is mitogen-induced (737-743). Recently, the form in ovine fetal cotyledons during late gestation has been reported by immunoreactive antibodies to be the mitogen inducible form of PGH synthase-2 (PGHS-2). Maternal myometrium, caruncle and endometrium have PGH synthase-1 (PGHS-1) at 79 and 144 days of gestation. PGHS-1 also increases at parturition in the fetal cotyledon (744).

1.2.7 REGULATION OF UTERO-PLACENTAL BLOOD FLOW DURING PREGNANCY

During pregnancy, uterine blood flow increases progressively to the gravid uterus (745). Presumably, PGE_1, PGE_2 or PGI_2, estradiol-17ß, and endothelial derived relaxing factor (EDRF) reduce the potentiation of vascular smooth muscle contraction caused by progesterone. This potentiation of increased vascular smooth muscle tone is through α-adrenergic receptors on uterine arterial vascular smooth muscle (746). During gestation, α-adrenergic receptors are greater than ß-adrenergic receptors on uterine arteries (746, 747). Epinephrine given to late pregnant sheep reduces blood flow to the uterus (748). In addition, stimulation of α-adrenergic receptors increase contractions in uterine arterial smooth muscle and reduces blood flow (749-752). This appears to be mediated through protein kinase C (753), but PKC is decreased during late pregnancy by estradiol-17ß (754). Arterial vasculature at the fetal-maternal placental interface does not respond to α-agonist stimulation (749-752).

Caruncular arteries of ovine placenta may contract in response to endothelin and adenosine. Although adenosine is a vasodilator of uterine arteries, it reduces blood flow through caruncular arteries (755). In addition, endothelin causes vascular smooth muscle contraction and a reduction in blood flow at the fetal-maternal placental interface through a release of the vasoconstrictor TXB_2 (756).

Vasoconstrictor response to angiotensin I is reduced pregnancy in sheep (751). Uterine blood flow increases are through the local production of prostacyclin (PGI_2) and endothelial-derived relaxin factor

(EDRF). Prostaglandin I_2 and EDRF are produced by the uterine arterial endothelium and increase blood flow by relaxing vascular smooth muscle through increases of cAMP and cGMP in the uterine arterial smooth muscle. Prostaglandin I_2 increases cAMP and EDRF increases cGMP in the vascular smooth muscle (757, 758).

1.2.8 CORTICOSTEROIDS AND CATECHOLAMINES DURING GESTATION

Weight of the fetal adrenals in sheep increases from day 50 of pregnancy to day 130 and increases further by the time of parturition (759, 760). Hypophysectomy of the fetus at day 120 of gestation results in lower adrenal weight increases by the time of anticipated parturition. Inhibition of fetal adrenal growth by hypophysectomy occurs only in the fetal adrenal cortex (761, 762). This indicates that fetal adrenal cortex development is partially independent of maternal control. Development of catecholamine biosynthetic enzymes and the ratio of epinephrine to norepinephrine is reduced by fetal hypophysectomy (762). Catecholamines increase in fetal blood at the end of gestation, which is blocked by fetal adrenalectomy (763). This increase in catecholamines in fetal adrenal tissue at the end of gestation is thought by some to release ACTH by the fetal pituitary as well as directly stimulate steroidogenesis by the fetal adrenal to facilitate parturition (762, 763). Injection of clenbuterol, a synthetic ß-adrenergic agonist, on day 143 of gestation in sheep is associated with an earlier decline in progesterone (764).

Concentrations of cortisol in jugular blood of pregnant ewes are similar throughout gestation and increases at parturition. In fetal blood,

cortisol increases gradually from day 140 to 145 and increases rapidly by parturition (765). This increase in cortisol in ovine fetal blood at parturition is preceeded by an increase in ACTH in fetal blood and the increase in cortisol in fetal blood occurs before increases in cortisol in maternal blood (766-769).

Receptors for ACTH and adenylate cyclase in fetal adrenal cortical tissue increases 4 fold during the last 5 days of gestation (767-770). The fetal hypophysis is active throughout gestation, since incubation of the fetal pituitary at any stage of pregnancy with maternal adrenal tissue increases cortisol secretion by the maternal adrenal. Ovine fetal adrenals respond to ACTH-stimulated increases in cortisol secretion on day 50 or at term, but not on days 100 or 130 of gestation in sheep (767-770). This inhibition of ACTH action on days 100 and 130 by fetal adrenal tisue may be a function of angiotensin II and transforming growth factor ß (769). This change in response to ACTH by fetal adrenal tissue at the end of gestation may be through stimulatory or inhibitory elements of guanine nucleotide binding components (767-770). Although the fetal adrenal cortex does not mature until the last 5 days of gestation, receptors for cortisol on sheep placenta increase 5 fold from days 30 to 60; 4 fold more by days 80 to 100; and then decrease by 50 percent from days 120 to 145 (771).

1.2.9 RELAXIN, OXYTOCIN, PREGNANCY SPECIFIC PROTEIN B (PSPB), ENDORPHIN AND PLACENTAL LACTOGEN DURING GESTATION OF RUMINANTS

Concentrations of oxytocin in maternal jugular blood are less than 5 pg/ml from days 80 to 143 of pregnancy in sheep. Similar levels of oxytocin are also seen in fetal blood (772, 773). Oxytocin usually does not increase prior to initiation of labor, but increase as the fetus enters the cervix and vagina (773). However, an oxytocin receptor antagonist delays expulsion of the fetus (773). Moreover, oxytocin stimulation of myometrial contractions is similar to those seen at parturition (772-774).

Relaxin concentrations in placentomes of sheep are similar on days 21 to 40, 41 to 60, 61 to 80, 81 to 100 and 101 to 120, but decrease in placentomes from days 120 to parturition (775, 776). However, evidence of biological activity was not demonstrated (777). In the pig, relaxin promotes growth and relaxation of the uterine horns (778). In addition, porcine relaxin given late in gestation causes cervical dilation and parturition in cattle and reduces the incidence of dystocia (779).

Beta-endorphin in fetal lamb plasma increases from day 120 to 140 of gestation (780). In cattle, ß-endorphin increases from the third month of gestation to the sixth month and enkephalin increases 3 fold at parturition (781).

Pregnancy specific protein B (PSPB) is first detected in peripheral blood of cows on day 20 of gestation (782, 783). Concentrations of PSPB increase to 1 ng/ml from days 20 to 30, increases gradually to 9 ng/ml by 3 months, increases to 39 ng/ml by 6 months, increases to 150

ng/ml by 9 months and increases further to 542 ng/ml two days prior to parturition. In addition, PSPB is detectable in sera up to 3 weeks postpartum in cattle (784). The accuracy of diagnosis of pregnancy in cattle with PSPB as early as day 20 postbreeding exceeds 90 percent. When embryos/fetuses die in utero, levels of PSPB decrease in jugular blood of cows (785). PSPB is detectable by day 20 postbreeding in jugular blood of ewes and increases to 10 ng/ml by day 30 postbreeding, increases from 31 ng/ml 3 weeks before parturition to 42 ng/ml by parturition. Pregnancy specific protein B is detectable up to 3 weeks postpartum in sheep (786). Accuracy of pregnancy diagnosis in sheep at 60, 90 and 120 days of pregnancy by PSPB radioimmunoassay is 90 percent (787). Secretion of PSPB is by the binucleate cells of the bovine trophoblast (785). Secretion of PGE_2 is increased by PSPB by day 16 bovine intercaruncular endometrium *in vitro* alone or in the presence of oxytocin (788). In addition, PSPB increases secretion of PGE_2 by day 16 bovine caruncular endometrium in vitro (C. Weems, personal communication, unpublished data).

Ovine placental lactogen is produced by both uninucleate and binucleate cells of the placenta (789, 790). Ovine placental lactogen is detectable by day 14 in the fetal cotyledon and by day 18 in the maternal caruncle (791). Ovine placental lactogen is first detected in maternal and fetal blood by days 50 and 40, respectively (792). Ovine placental lactogen increases progressively in maternal and fetal blood by days 41 to 50 to a maximum by days 121 to 130, before decreasing to low levels by parturition (791, 792). Ovine placental lactogen is not correlated with progesterone secretion, but is highly correlated to placental weight (793). In

addition, ovine placental lactogen is higher in ewes with twin than with single fetuses (794). Fetal weight is highly correlated with ovine placental lactogen (790, 791, 794, 795). Secretion of ovine placental lactogen is stimulated by arachidonic acid (796), the precursor of prostaglandins (335).

Placental lactogen may be controlling growth of the fetus and placenta through somatomedin C (insulin-like growth factor 1; IGF_1). Concentrations of somatomedin C do not vary with stage of gestation in the ewe, but increases 7 fold in fetal blood from day 100 to parturition (797). Hypophysectomy of fetal lambs on day 100 decreases IGF_1, but not IGF_2 (798). Insulin-like growth factor 1 does not stimulate secretion of progesterone by 90 to 100 day ovine placenta from intact or ovariectomized ewes (C. Weems, personal communication, unpublished data).

In summary, PSPB can be used to diagnose pregnancy and a viable fetus and may have a role in regulation of uteine/placental secretion of prostaglandins (782-788). While ovine placental lactogen may be used to diagnose pregnancy and placental lactogen and somatomedin C may regulate placental and fetal growth, little evidence exists for regulation of placental steroidogenesis (789-797). No evidence exists for a role of endorphin, relaxin, or oxytocin during gestation except may be at parturition (772-781).

1.2.10 MYOMETRIAL ACTIVITY DURING GESTATION

Myometrial activity occurs throughout gestation in sheep, either as contractures or contractions. Contractions last approximately one minute and occur during parturition. Contractures last for several minutes and are

associated with smaller amplitude changes in intrauterine pressure and occur throughout gestation (799). The amplitude of contractions is probably suppressed during gestation by relaxin to cause contractures (800). The switch at parturition from contractures to contractions by the myometrium is probably related to changes in progesterone, estrogen, cortisol, oxytocin and prostaglandins (799-802). This may be PGE_2, since PGE_2 increases progressively during the last 30 days of pregnancy (722). This probably involves the number, size and permeability of gap junctions between myometrial cells. Gap junctions appear between myometrial smooth muscle cells just before the parturition (803-806).

Gap junctions are low resistance cell-to-cell channels, which allow the passage of electrolytes and small molecules between cells, thereby coupling cells together both electrically and metabolically (803). Increased gap junctions formed in the myometrium during labor improve electrical interaction between smooth muscle fibers. Electrical activity generated in one region of the myometrium would easily propagate throughout the uterus and promote electrical and contractile synchrony of the myometrium. This would result in coordinated and forceful contractions (804-806).

Cyclic AMP causes a rapid and reversible decrease in the coupling of myometrial gap junctions (807, 808). Beta-adrenergic receptor stimulation inhibits spontaneous contractile activity of the myometrium through increased levels of cAMP in the myometrium (805). Formation of these gap junctions occurs just prior to parturition and are through the connexin 43 protein (806).

Since estrogen, prostaglandins and oxytocin causes myometrial contractions and increase at parturition (799), it is suggested that they cause the appearance of these gap junctions (806-808). However, appearance of these gap junctions may just be coincident with the increases in prostaglandins, estrogen, cortisol and oxytocin (799, 809-812). This may explain why pregnant ovine myometrium has inhibitors of phospholipase A2 during gestation (813). This may be to prevent release of arachidonic acid for prostaglandin synthesis and calcium release (811, 812), thus maintaining the uterus in a relative quiescent state to facilitate fetal development (799). Relaxin may also play a role in reducing myometrial activity during gestation (814).

1.3 PARTURITION IN RUMINANTS

1.3.1 THE CERVIX DURING PARTURITION

The cervix consists of smooth muscle, collagen and connective tissue ground substance. The connective tissue ground substance contains the glycosaminoglycans (GAGS), dermatan sulfate, chondroitin sulfate and hyaluronic acid. Maturation or ripening of the cervix for parturition is associated with the connective tissue ground substance becoming more prominent and a loss of collagen fibrils (816, 817). During maturation of the cervix, cervical distensibility increases and protein and collagen concentrations decrease (816-818). Collagen is made of tropocollagen, which is a helix consisting of three collagen chains of 100,000 molecular weight each (820-822). Breakdown of the cervical collagen at parturition (819) is thought to be by P-Z peptidase, which increases in cervical tissue (820).

Concentrations of GAGS remain unchanged during gestation, however, cervical hyaluronic acid increases by the time of parturition (821-823). Cervical proteins decrease by parturition. Hyaluronic acid is greater and chondroitin sulfate and dermatan sulfate in the cervix decreases by two-thirds at parturition (824-827). The decreases in chondroitin sulfate and dermatan sulfate probably occur, because they are associated with collagen (828). The increase in hyaluronic acid at parturition increases water retention in the cervix and causes a softening of the cervix (829).

Hormonal regulation of cervical ripening is probably related to the increase in the estrogen:progesterone ratio (821, 822, 827-830), relaxin and PGE_2 seen at parturition (722). Progesterone given at parturition

decreases collagen breakdown (831). Intraaortic infusion of PGE_2 or intracervical administration of $PGF_{2\alpha}$ or PGE_2 causes ovine cervical ripening at doses that do not promote myometrial contractions (832). The softening of the cervix is stimulated by PGE_2 *in vitro* indicating a direct action on the cervix (824-833). In addition, cervical softening following progesterone withdrawal is prevented by inhibitors of prostaglandin synthesis (834-836). Relaxin given into the cervix also causes cervical maturation and ripening (837). It is suggested that relaxin, released at parturition, stimulates cervical production of prostaglandins (832-837). There is a progressive increase in uterine secretion of PGE_2 during the last 30 days of gestation (722).

1.3.2 HORMONAL REGULATION OF PARTURITION

Hormonal initiation of parturition involves the fetal hypothalamic/hypophyseal/adrenal axis. Evidence to first suggest this relationship was in the human where it was observed that parturition is delayed or does not occur in women with an anencephalic fetus (838-840). In sheep, consumption of *Veratrum californicum*, a type of weed, during gestation causes a cyclops anomaly with a displacement or absence of the fetal hypophysis and prolongs gestation. Adrenals from these lambs have a marked reduction in the ability to convert prognenolone to cortisol (841, 842). A similar malfunction is seen in cyclops human fetuses (839, 840). Ingestion of *Salsola tuberculata*, a type of weed, by sheep also prolongs gestation by causing adrenal and hypophyseal atrophy in the fetus (843). Genetic influences also exist to prolong gestation in Guernsey and Holstein cattle as their fetuses are often hypoadrenal (844, 845) due to a lack of

fetal hypophyseal ACTH (846, 847). In contrast, shortened gestation can be associated with hyperplasia of the fetal adrenal in the human (847) and habitual aborter goats (848, 849).

Destruction of the ovine fetal hypophysis prior to 134 days of gestation prolongs gestation, if at least 70 percent of the hypophysis is destroyed. Parturition still occurs at term, when the hypophysis is removed from only one lamb of ewes with multiple fetuses (850-852). This prolongation of gestation after hypophysectomy of the fetal lamb may last as long as 40 days past the normal length of gestation (853-855). Bilateral adrenalectomy of the lamb fetus also prolongs gestation (856), which can be overcome by infusions of cortisol acetate (852) or ACTH (857-860) into the adrenalectomized fetus.

Increases in cortisol are seen in fetal blood before increases in cortisol occur in maternal blood during the last 20 days of gestation in sheep. There are marked increases in cortisol during the last 2 to 3 days. Maternal cortisol does not increase until the last 1 to 2 days before parturition (861, 862). Pulsatile increases in fetal ACTH are seen at this time (863, 864). Infusion of ACTH (865-868) or corticotropin releasing hormone (CRH) into fetal blood during the last 20 days of gestation induces premature parturition. This is the time when the fetal adrenal cortical tissue develops adenylate cyclase activity and are steroidogenic (869). In addition, concentrations of CRH, ACTH and cortisol increase in fetal blood during the last 20 days of pregnancy in sheep (860). Moreover, dexamethasone, a synthetic glucocorticoid, given in a dose dependent manner the last 10 days of pregnancy to ewes advances parturition (771, 870-872). Receptors for

glucocorticoids increase in the placenta (771) and in the hypophysis, hypothalamus and lungs of fetal sheep during the last few days of pregnancy (872).

Concentrations of progesterone in ewes decrease in a variable fashion over the last 15 days of pregnancy (873). Premature parturition induced by ACTH or cortisol given to the fetal lamb is preceded by a decrease in progesterone in maternal blood (860). Progesterone maintains uterine quiesence through suppression of the spontaneous generation and propagation of action potentials. This suppression is lost when progesterone decreases (874), when receptors to progesterone decrease in the myometrium (875) or when receptors for progesterone are blocked by RU-486 (876). Myoelectrical activity, intrauterine pressures and myometrial contractility increases at the end of gestation when progesterone decreases (877). These waves of contraction increase in frequency about 12 hours before parturition and delivery of a single lamb fetus lasts 20 to 30 minutes. When twin lambs are born this delivery lasts about 40 to 60 minutes. If the fetus is not expelled in 8 hours, the ewe has dystocia (665). The placenta is expelled 2 to 4 hours after delivery of the fetus (878, 879). If the ovine placenta is not expelled within 8 hours, the placenta is judged to be retaoned (665).

Myometrial contractions are probably facilitated by increases in oxytocin. Secretion of oxytocin, estradiol-17ß, $PGF_{2}\alpha$ and PGE_{2}; and their receptors on the myometrium increase at parturition (880-887). Cortisol increases production of placental 17α-hydroxylase (650, 880-882) and 17,20-desmolase (650) to produce androgens for estrogen synthesis.

Concentrations of unconjugated estrogens increase during the last 24 hours of gestation in sheep by the placenta (883, 884). These increases in estrogen occur even when lambs are adrenalectomized, indicating that the fetal adrenal is not necessary for the production of precursors to estrogens (884, 885). Estrogen increases contractility of the myometrium (799), probably through increases in myometrial receptors for oxytocin (886, 887) and prostaglandins (888), both of which increase myometrial contractions (799). Receptors for estradiol-17ß increase in myometrial tissue at parturition (875). In addition, numbers of receptors for oxytocin (886, 887) and prostaglandins (888) increase in myometrial tissue and oxytocin secretion increase at parturition (772). Also, oxytocin increases secretion of prostaglandins by the uterus of the pregnant ewe (799).

Estrogen increases $PGF_{2}\alpha$ secretion by uterus of the pregnant ewe at parturition (853) even when the fetus has been adrenalectomized (858). Progesterone can block estrogen release of $PGF_{2}\alpha$ by the uterus (860) and oxytocic actions of $PGF_{2}\alpha$ even when concentration of $PGF_{2}\alpha$ have increased in the maternal caruncle and myometrium (859). Estrogen lowers the dose of oxyocin needed to stimulate myometrial contractions, presumably by increasing the number of oxytocin receptors and release of $PGF_{2}\alpha$ (860). If progesterone synthesis is reduced by an inhibitor of 3ß-HSD during late pregnancy, secretion of progesterone decreases followed by increases in PGFM and premature delivery occurs in most animals. Also, a 3ß-HSD inhibitor increases estrogen (889, 890). This suggests that $PGF_{2}\alpha$ decreases secretion of progesterone by luteal tissue to lower progesterone. The increasing estrogen:progesterone ratio seen a few days before

parturition is probably responsible for the initial release of $PGF_2\alpha$ at the end of gestation (860). The increases in estrogen at parturition by the placenta may increase contractility of the myometrium by stimulating myometrial synthesis of proteins, especially contractile proteins (799). However, myometrial actin and myosin are doubled by day 100 of pregnancy in sheep and do not change for the remainder of gestation (C.R. Rosenfeld, personal communication, unpublished data).

The increase in placental secretion of $PGF_2\alpha$ and PGE_2 seen at parturition appears to be essential for parturition to occur (860). Initially, increases of PGE_2 are greater than $PGF_2\alpha$ in amniotic fluid at parturition. With the progression of labor, this ratio changes in favor of $PGF_2\alpha$ (891). In uterine venous blood, increases in $PGF_2\alpha$ are greater than PGE_2, but are reversed in fetal blood at parturition (892). When prostaglandins increase in amnionic fluid, increases in arachidonic acid, the precursor for prostaglandin synthesis, are also seen (893). The amnion may be a major site of prostaglandin synthesis at parturition (894).

Some believe that the increases in PGE_2 seen in late pregnancy is the trigger for initiation of parturition (722). However, others believe that prostaglandin secretion at parturition is not involved with parturition and increases occur only because of microbial contamination of the uterus as a result of cervical dilation (895). This group believes that the increase in oxytocin seen at parturition is all that is needed for labor (895, 896). Oxytocin antagonists will delay parturition (897). Onapristone, a progesterone receptor antagonist, increases the effectiveness of oxytocin-induced premature parturition without changes in myometrial oxytocin

receptors (898). In addition, estrogen, oxytocin or cortisol (722) increases prostaglandins in pregnant uterine tissue. Estrogen (899, 900), oxytocin (901), cortisol (902), $PGF_2\alpha$ (903-905) or PGE_2 (722) given the last few days of gestation advances parturition. Prostaglandins increase in uterine tissue at parturition before increases in uterine mechanical activity implying that prostaglandins mediate parturition and are not a result of parturition (722).

Infusions of PGE_2 into fetal lambs does not change intrauterine pressure at any stage of gestation (906) and PGE_2 does not enhance fetal secretion of cortisol in response to ACTH (907-909). However, there is a progressive increase in uterine secretion of PGE_2 during the last 30 days of gestation. In addition, PGE_2 given into the amnion of late pregnant sheep induces premature parturition. These same investigators also conclude that placental secretion of PGE_2 is inhibited until the last 30 days of gestation in sheep (722).

1.4 ABORTION/PRE-TERM LABOR IN RUMINANTS

Many plant toxins, infections, estrogen, oxytocin, corticosteroids are associated with abortion in livestock. In addition, abortion with prostaglandins is dependent on the stage of gestation. Microbial-induced abortions are thought by some to be by increasing endogenous secretion of prostaglandins. Likewise, pre-eclampsia and pre-term labor is thought to be due to an increase in endogenous uterine secretion of prostaglandins (910-986).

Ingestion of ponderosa pine needles may cause abortion in bison or cattle (910-913), but not in sheep or goats (913) at any stage of gestation. The incidence of abortion is greater, the further along in gestation that cows ingest the pine needles (910-913). Pine needles cause abortion through disturbing hormonal control of uterine arterial blood flow to the gravid uterine horn (914, 915), by causing a marked vasoconstriction of the caruncular artery (916). Fescue toxicosis contains endophyte, which produce an ergot alkaloid, and causes abortion in cattle (917) and sheep (918) through its vasoconstrictive activity. In addition, *Veratrum*, lupines, loco weed, poison hemlock, snake weed and broom weed cause abortion in cattle and sheep, but these mechanisms of abortion are unknown (919-921). Also, mycotoxins produced by various fungi contain estrogens, aflatoxin and ergot alkaloids which cause abortion (922), presumably by increasing endogenous uterine secretion of prostaglandins.

Abortions induced by plant products may range from 5 to 100 percent. Some of these abortions may be due to a malformed placenta, which runs as high as 15 percent in some herds (923). Trichosanthin, an

18,000 molecular weight protein from a curcubit root tuber, induces abortion in rats, monkeys and women. Trichosanthin causes necrosis of placental villi and a decline in hCG in women (924-926). Trichosanthin presumably causes abortion through increases in secretion of estrogen (926), $PGF_2\alpha$ and PGE_2 (927-929). Abortion occurs in women an average of 100 hours after trichosanthin is injected intramuscularly (924-928).

Microbial-induced abortions in sheep and cattle are caused by *Toxoplasma gondii* (929, 930), *Trypanosoma vivax* (931), *Escherechia coli* (932), *Chlamydia psittaci* (933), *Salmonella abortus ovis* (934), bovine *Herpes virus-1* or bovine *Rhinotracheitis virus* (935), *Anaplasma marginale* (936), *Brucella abortus* (937), *Vibrio fetus* (938), *Salmonella dublin* (939) and *Pasteurella spp* (940). Abortion induced by microbial infections may be associated with culture-positive and culture-negative fetuses and placenta. In culture-positive cases, bacteremia occurs in the mother and leads to an infection of the placenta and/or fetus, causes fetal death, and expulsion. In culture-negative cases, endotoxemia associated with diarrhea and damaged gut mucosa produces abortion in 2 to 3 days (941).

The uterus is resistant to infections during an estrogenic phase, but highly susceptible to infections in a progesterone dominated uterus, presumably by decreased activation of leukocytes (942). Lipopolysaccharide endotoxins from gram-negative bacteria cause abortion either through colonization in the fetal/placental unit or systemically through the gastro-intestinal tract. Abortions caused by microbial colonization of the fetal/placental unit or by endotoxins seems to be through stimulation of $PGF_2\alpha$ secretion by the placental unit (929, 930, 932, 933, 942-944).

However, examination of these data from microbial-induced abortions indicate that increases in $PGF_2\alpha$ secretion usually do not occur until the fetus dies (944).

In cows given *Escherichia coli* (*E. Coli*) endotoxin during the first trimester of pregnancy, increases in PGFM, TXB_2, 6-keto-$PGF_1\alpha$ and cortisol, but not PGE_2, are seen within 3 hours and 60 percent of the cows abort within 60 to 72 hours. Progesterone is decreased by 18 hours, but not to less than 1 ng/ml in jugular blood until 48 hours after endotoxin treatment. Treatment with flunixin meglumine, an inhibitor of prostaglandin synthesis, prior to giving endotoxin prevents abortion. Fetal death occurs within a few hours after endotoxin is given to the mother. Endotoxin does not cause death of the fetus, when given directly into the fetus. However, endotoxin given to the mother causes fetal death, even if pregnancy has been maintained by exogenous progesterone (932). One common feature of endotoxin-induced abortion is the formation of clots in the placental and fetal vasculature. This may be through interleukin-1 and interleukin-8, which increase $PGF_2\alpha$ secretion (945-949). In brain tissue, endotoxin increases PGE_2 and causes clots in this tissue, but can be blocked by dexamethasone, a synthetic corticosteroid (950).

In pregnant ewes infected on day 90 of pregnancy with *Chlamydia psittaci*, abortion occurs by 135 days of gestation in infected ewes and parturition occurs normally in control ewes. In control ewes, progesterone decreases rapidly 2 to 3 days before parturition. In infected ewes progesterone declines slowly over 15 days. Increases in estradiol were

seen in all infected and control ewes at day 130, but PGE_2 increased in amnionic fluid in only 4 out of 12 ewes at day 130 (943, 944).

Abortion and increases in PGFM do not occur in bacterial-induced abortions until after the fetus dies, which is weeks later after infection. Thus, the decline in progesterone, the increase in PGFM and abortion are probably due to deteriorating placental and fetal tissues, and represents an effect rather than a cause. This effect is probably through the formation of clots in the placenta and fetus (945, 946). When fetuses die in utero and are not aborted, $PGF_2\alpha$ in placental compartments is increased (943-951).

Corticosteroids increase $PGF_2\alpha$ in uterine venous plasma, when given the last 3 weeks of pregnancy and advance parturition (860-869). Dexamethasone, a synthetic glucocorticid, given intramuscularly every 6 hours (2.5 mg) for 5 days starting at day 88 of gestation in sheep induces abortion in 3 of 5 ewes within 4 days (952). Dexamethasone given alone, or along with estradiol or oxytocin to cows ranging between 5 to 8.5 months of gestation induces abortion in 14 of 15 cows. The interval to abortion is greater in those cows receiving estradiol or oxytocin with dexamethasone (953). Dexamethasone (20 to 40 mg) induces abortion in 81 percent of cows with dropsy, when given between the seventh and ninth month of gestation (954).Cows appear to be more responsive to corticosteroids as abortifacients than sheep (952, 954).

Some growth factors are abortifacient during gestation in sheep. Murine epidermal growth factor (EGF) given to pregnant ewes at days 25, 50 or 75 of pregnancy induces abortion in some ewes. In ewes at

day 25 or day 50 of gestation, 15 to 20 percent of ewes abort and progesterone decreases to less than 1 ng/ml in jugular blood 8 to 48 hours after initiation of treatment. None of the ewes treated at day 75 with EGF abort (955). Whether EGF-induced abortion is mediated through prostaglandins is unknown.

In ewes at days 119 and 142 postbreeding, oxytocin increases secretion of $PGF_2\alpha$, but does not affect concentrations of progesterone or advance parturition. At day 135 of gestation, dexamethasone advances parturition (956). In one, day 100 pregnant cow, fenprostalene, a synthetic $PGF_2\alpha$ analogue, decreased progesterone by 75 percent in 5 hours, which decreases further to less than 1 ng/ml at 12 hours and the cow aborted at 66 hours (957). Prostaglandin $F_2\alpha$ (25 or 30 mg) in a total of two cows at 5 and 7 months of gestation with hydroallantois decreased progesterone within 24 hours to 0.8 ng/ml and calves were born at 40 and 72 hours (958).

In a number of well designed experiments, $PGF_2\alpha$ given up to 125 days of gestation in cattle causes abortion, but the closer these cows are to day 130 of gestation, effectiveness of $PGF_2\alpha$ decreases (959-970). When $PGF_2\alpha$ or PGE_2 is given to intact or ovariectomized cattle between days 180 to 200, abortion does not occur or occurs in only a few cases with single or multiple injections of $PGF_2\alpha$ (959, 965-970). Similar data are seen for comparable stages of gestation in the guinea pig (971). Prostaglandin E_2 given into the vaginal canal does not induce abortion in humans (972).

Prostaglandin $F_2\alpha$ (8 mg/58 kg/ body weight) given on day 85 of pregnancy in hysterectomized ewes did not decrease progesterone in jugular blood until 40 hours and then increased by 64 hours. In addition, progesterone did not decrease below 2 ng/ml in jugular blood of intact day 85 pregnant ewes throughout the 64 hours after $PGF_2\alpha$ and no ewes aborted (973).

Pre-eclampsia, abortions and infection driven pre-term labor are associated with increases in intrauterine prostaglandins (974, 975). Infection-induced abortions may be due to phospholipases in the bacteria, which hydrolyze phospholipids to release arachidonic acid for prostaglandin biosynthesis (976). However, 15 to 25 percent of pregnancies end in abortion and anti-phospholipid antibodies are associated with some of these spontaneous abortions (977-981). This suggests that some prostanoid may be necessary for maintenance of gestation as well as being involved in pre-term labor.

RU-486, a progesterone receptor antagonist, alone or along with $PGF_2\alpha$ terminates pregnancy in the human at any stage of gestation (982) or when given with oxytocin on day 135 of pregnancy in sheep (983). This supports the necessity of progesterone or progesterone action in the maintenance of gestation. A single intrajugular injection of trilostane, a 3ß-HSD inhibitor, that blocks the conversion of pregnenolone to progesterone or dehydro-epiandrosterone to androstenedione for estrogen synthesis, given on day 136 (984) or day 146 (985) of pregnancy in sheep decreases jugular progesterone by 75 percent and advances parturition in most ewes. However, a single intrajugular injection of trilostane given on days 118 to

130 at doses of 25 to 200 mg caused abortion in only 2 out of 15 ewes, although levels of progesterone decrease by 75 percent for 12 hours after injection (986).

CHAPTER 2

STATEMENT OF THE PROBLEM

Induction of abortion/premature parturition in sheep during the last 15 days of gestation is easy with injections of corticosteroids (771, 870-872), $PGF_{2}\alpha$ and PGE_2 (722), estradiol-17ß (853) or trilostane (984, 985). Thorburn's group states that: " In most mammalian species, parturition may be induced at any stage of gestation by increasing intrauterine prostaglandin concentrations. This may be achieved by exogenous administration of prostaglandins, induction of prostaglandin synthesizing enzymes, or by bacterial-induced stimulation of prostaglandin synthesis" (731). No data or references to data are given to support these statements (731).

Single injections of trilostane given before day 135 of gestation in sheep is not an effective abortifacient (986). In addition, 8 mg $PGF_{2}\alpha$/58 kg/BW given on day 85 of pregnancy in sheep decreased progesterone, but not below 1 ng/ml in jugular blood within 64 hours in hysterectomised ewes, and $PGF_{2}\alpha$ did not cause abortion in intact pregnant ewes by 64 hours in a preliminary experiment (973). However, different doses of $PGF_{2}\alpha$ or frequency of trilostane injections have not been tested to determine their effects on different sources of progesterone or on pregnancy status between 55 to 130 days of gestation in sheep.

Concentrations of PGE_2 and $PGF_{2}\alpha$ reported in uterine venous plasma throughout pregnancy in sheep differs between studies (718-725). Some studies report that both $PGF_{2}\alpha$ and PGE_2 in uterine venous plasma

are low and increase at parturition, while other studies indicate that concentrations of PGE_2 in uterine venous plasma during gestation are greater than $PGF_2\alpha$ (718-744). Changes in concentrations of $PGF_2\alpha$ relative to PGE_2 may determine whether abortion does or does not occur. In microbial-induced abortion, $PGF_2\alpha$ increases in uterine venous blood, while PGE_2 decreases in uterine venous blood (988).

Since progesterone rises rapidly by day 85 and peaks by day 125 of pregnancy in sheep, this would probably be the most resistant stage of gestation to assess potential abortifacient agents (631, 632, 642, 655). Progesterone decreases a few days before parturition and exogenous progesterone given at the onset of parturition delays delivery of the fetus even when $PGF_2\alpha$ is increased in the placenta of sheep (720-733, 860, 865).

The objective of these experiments was to determine the effect of different doses of $PGF_2\alpha$ on pregnancy status and sources of progesterone in 90 to 100 day pregnant sheep. In addition, determine whether trilostane given more frequently is abortifacient in 90 to 100 day pregnant sheep. These data will serve to establish a model system for determining the regulation of progesterone secretion by the placenta of sheep and maintenance of pregnancy in sheep or for use in the development of abortifacient agents. In addition, these data may serve to develop strategies to prevent microbial-induced "abortion storms".

CHAPTER 3

MATERIALS AND METHODS

3.1 GENERAL

3.1.1 ANIMALS

Mature crossbred (Merino x Romadale) ewes were penned with brisket-painted vasectomized rams for detection of estrus. Paint-marked ewes were removed from the flock twice daily, morning and late afternoon, and marked ewes were mated with each of two intact fertile rams in the morning or late afternoon until they were out of estrus. Estrus is designated as day 0 of gestation. Bred ewes were returned to the flock on day 4 postbreeding. Between 90 to 100 days of gestation, pregnant ewes were weighed, caged and transported (45 miles) to the Magoon Campus Small Animal Facility. Ewes were housed in digestion crates and fed alfalfa cubes and water *ad libitum* throughout the experiments except that feed and water were withdrawn 12 to 18 hours before surgery.

3.1.2 SURGERY

Ewes were given intramuscularly, 1 cc (15 mg) of atropine sulfate (Phoenix Pharmaceutical Inc., St. Joseph, MO) 30 minutes prior to induction of anesthesia. A 1.02 mm bore polyvinyl catheter (Cole Parmer, Chicago, IL) was installed in a jugular vein and anesthesia was induced to effect with pentobarbital sodium (64.5 mg/ml; Anthony Products Co., Arcadia, CA). A midventral incision was made under sterile conditions and the gravid uterus was exposed for collection of samples or installation of a

1.02 mm bore polyvinyl catheter in a prominent uterine vein at the uterine body. The uterine venous catheter was exteriorized through a flank incision and secured under the wool for subsequent collection of samples.

Ewes were either hysterectomized (uterine arteries were ligated and the uterus/fetus/placental unit anterior to the cervix was removed), ovariectomized (only the ovaries were removed but the uterus/fetus/placental unit was left *in situ*), lutectomized (only the corpus luteum was removed), left intact (sham operation), or intact ewes were used for tissue collection for in vitro studies. Ewes left intact, ovariectomized, hysterectomized, or lutectomized received subcutaneously 10 cc Di Pen (200,000 units procaine penicillin G and 250 mg dihydrostreptomycin/1 cc; G.C. Hanford Co., Syracuse, NY) after closure of the incision and were permitted to recover for collection of samples. These ewes were relaparotomized either 72 or 168 hours later under pentobarbital sodium anesthesia for collection of the last samples, euthanized by an overdose of pentobarbital sodium, and the carcass was incinerated.

3.2 EXPERIMENT 1

The null hypothesis tested in experiment 1 was that corpora lutea of hysterectomized 90 to 100 day pregnant ewes are not responsive to a dose of $PGF_2\alpha$ that is luteolytic in nonpregnant ewes at midcycle. The alternative hypothesis tested was that corpora lutea of hysterectomized 90 to 100 day pregnant ewes are responsive to a dose of $PGF_2\alpha$ that is luteolytic in nonpregnant ewes at midcycle.

Hysterectomized 90 to 100 day pregnant ewes received intramuscularly injections of vehicle [Tromethamine (Tham) buffer; Sigma Chemical Co., St. Louis, MO] or 8 or 16 mg of $PGF_2\alpha$ (Lutalyse, the Upjohn Co Inc., Kalamazoo, MI) per 58 kg body weight (BW). The minimum effective dose of $PGF_2\alpha$ given on day 13 of pregnancy in ewes to cause a significant decrease in jugular progesterone is 6 mg/58 kg/BW while 4 mg $PGF_2\alpha$/58 kg/BW is effective in decreasing jugular progesterone on day 13 of nonpregnant ewes (253). These data determined the doses of $PGF_2\alpha$ used in the present study. There were 5 ewes in each of the three treatment groups.

Ovarian venous blood was collected at 0 and 72 hours for analysis for progesterone, $PGF_2\alpha$ and PGE. Jugular venous blood was collected at 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56, 64 and 72 hours for analysis for progesterone, estradiol-17ß and cortisol by radioimmunoassay (RIA). Blood samples were collected via a heparinized syringe and needle. Plasma was collected following centrifugation and stored at -20 ^{o}C until assayed. Catheters were filled with 3 cc of heparin sodium (250 I.U./ml; Sigma Chemical Co., St. Louis, MO) in 0.9 % saline between samples and plugged. Samples collected for analysis for PGFα and PGE received 0.1 ml of 0.1 N HCL (Sigma Chemical Co., St. Louis, MO) per ml of blood collected to inhibit platelet prostaglandin biosynthesis (256, 289). The visibly 10 largest placentomes ipsilateral to the CL were collected and weighed at 0 hours. Corpora lutea were collected at 72 hours and weighed. Numbers and size of surface antral follicles on both ovaries were determined.

3.3 EXPERIMENT 2

The null hypothesis tested in experiment 2 was that corpora lutea of 90 to 100 day intact pregnant ewes are not responsive to a dose of $PGF_2\alpha$ that is luteolytic in nonpregnant ewes at midcycle and that $PGF_2\alpha$ is not abortifacient in 90 to 100 day intact pregnant ewes. The alternative hypothesis tested was that corpora lutea of intact 90 to 100 pregnant ewes are responsive to a dose of $PGF_2\alpha$ that is luteolytic in nonpregnant ewes at midcycle and that $PGF_2\alpha$ is abortifacient in 90 to 100 day intact pregnant ewes.

Intact 90 to 100 day pregnant ewes had jugular and uterine venous catheters surgically placed as described earlier and received intramuscularly injections of vehicle (Tham buffer) or 8 or 16 mg $PGF_2\alpha$/58 kg/BW. Ten ewes were treated with vehicle, 10 ewes were treated with 8 mg $PGF_2\alpha$/58 kg/BW, and 11 ewes were treated with 16 mg $PGF_2\alpha$/58 kg/BW. However, only 6, 6, and 5 ewes in the vehicle, 8 mg $PGF_2\alpha$, and 16 mg $PGF_2\alpha$ treatment groups, respectively, had patent uterine venous catheters throughout the 72 hour sampling period.

Ovarian venous blood was collected at 0 and 72 hours for analysis for progesterone, $PGF_2\alpha$, and PGE; jugular venous blood was collected at 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56, 64, and 72 hours for analysis for progesterone, estradiol-17ß and cortisol; and uterine venous blood was collected at 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56, 64, and 72 hours for analysis for progesterone, $PGF_2\alpha$, PGE, and PSPB by RIA. Samples for plasma were collected, processed, and stored until assayed as in experiment 1. The 10 visibly largest placentomes ipsilateral to the CL and

CL were collected at 72 hours and weighed. Numbers and size of surface antral follicles on both ovaries were determined.

3.4 EXPERIMENT 3

The null hypothesis tested in experiment 3 was that placental secretion of progesterone is not responsive to the potential negative effect of $PGF_2\alpha$ on placental secretion of progesterone *in vivo* in ovariectomized 90 to 100 day pregnant ewes and that $PGF_2\alpha$ is not abortifacient in 90 to 100 day pregnant ewes in the absence of the ovaries. The alternative hypothesis tested was that placental secretion of progesterone is responsive to the potential negative effect of $PGF_2\alpha$ on placental secretion of progesterone *in vivo* in ovariectomized 90 to 100 day pregnant ewes and that $PGF_2\alpha$ is abortifacient in 90 to 100 day pregnant ewes in the absence of the ovaries.

Ovariectomized 90 to 100 day pregnant ewes received jugular and uterine venous catheters and received intramuscularly injections of vehicle (Tham buffer) or 8 or 16 mg $PGF_2\alpha$/58 kg/BW. Eleven ewes received vehicle, 8 ewes received 8 mg $PGF_2\alpha$/58 kg/BW, and 14 ewes received 16 mg $PGF_2\alpha$/58 kg/BW. However, only 5, 6, and 7 ewes in the vehicle, 8 mg $PGF_2\alpha$, and 16 mg $PGF_2\alpha$ treatment groups, respectively, had patent uterine catheters throughout the 72 hour sampling period.

Jugular venous blood samples were collected at 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56, 64, and 72 hours for analysis for progesterone, estradiol-17ß and cortisol and uterine venous blood was collected at 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56, 64, and 72 hours for analysis for

progesterone, $PGF_2\alpha$, PGE, and PSPB by RIA. Samples for plasma were collected, processed, and stored until assayed as in experiment 1. The 10 visibly largest placentomes ipsilateral to the CL were collected at 72 hours and weighed.

3.5 EXPERIMENT 4

The null hypothesis tested in experiment 4 was that $PGF_2\alpha$ does not directly affect secretion of progesterone, PGE or estradiol-17ß by placentome slices from 90 to 100 day intact pregnant ewes *in vitro*. The alternative hypothesis tested was that $PGF_2\alpha$ does directly affect secretion of progesterone, PGE or estradiol-17ß by placentome slices from 90 to 100 day intact pregnant ewes *in vitro*.

Placentome slices were collected from 90 to 100 day intact pregnant sheep under pentobarbital anesthesia. A thin slice of placentome was weighed, minced and incubated in 5 ml of medium 199 containing 25 mM Hepes buffer and Earle's salts (Gibco, Gland Island, NY), 0.1 % bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO), 100 IU/ml penicillin (Sigma Chemical Co., St. Louis, MO), 0.2 mg/ml streptomycin sulfate (Sigma Chemical Co., St. Louis, MO), and 20 μg/ml 25-hydroxycholesterol (Sigma Chemical Co., St. Louis, MO). Minced placentome slices were preincubated for 30 minutes followed by a 4 hour incubation in fresh medium in the presence of $PGF_2\alpha$ (0, 1, 10, or 100 ng/ml; Cayman Chemical, Ann Arbor, MI), Indomethacin (0 or 10 μg/ml; Sigma Chemical Co, St. Louis, MO), and/or androstenedione (0 or 10 ng/ml; Steraloids, Wilton, NH) at 37 ^{o}C at 95% O_2/5% CO_2. Media were

separated from tissue at the end of the 4 hour incubation and kept frozen at -20 ^{o}C until assay for progesterone, PGE, $PGF_{2}\alpha$, and estradiol-17ß by RIA. There were seven replicates (animals) per treatment group. Only one slice per placentome was used and different placentomes were used for each treatment group from each of seven ewes. Treatment groups were randomized within each ewe.

3.6 EXPERIMENT 5

The null hypothesis tested in experiment 5 was that trilostane does not affect pregnancy status or placental secretion of progesterone or estradiol-17ß *in vivo* in 90 to 100 day lutectomized pregnant sheep. The alternative hypothesis tested was that trilostane does affect pregnancy status or placental secretion of progesterone or estradiol-17ß *in vivo* in 90 to 100 day lutectomized pregnant sheep.

Ewes between 90 to 100 days of gestation received a jugular venous catheter and were lutectomized through a midventral incision. Treatment groups were as follows: 1. vehicle-1 (Safflower oil) + vehicle-2 [Dimethyl Sulfoxide (DMSO); Sigma Chemical Co., St. Louis, MO] + vehicle-3 (Tham buffer); 2. vehicle-1 + vehicle-2 + $PGF_{2}\alpha$; 3. Cortisol (Sigma Chemical Co., St. Louis, MO) + vehicle-2 + vehicle-3; 4. Cortisol + vehicle-2 + $PGF_{2}\alpha$; 5. vehicle-1 + Trilostane (Sterling Winthrop, Rensselaer, NY) + vehicle-3; 6. Cortisol + Trilostane + vehicle-3; 7. vehicle-1 + Trilostane + $PGF_{2}\alpha$; and 8. Cortisol + Trilostane + $PGF_{2}\alpha$.

Cortisol was dissolved in Safflower oil and Cortisol or Safflower oil was given intramuscularly. Trilostane was dissolved in one ml DMSO and

Trilostane or DMSO was given into the jugular vein. Prostaglandin $F_2\alpha$ was dissolved in Tham buffer and $PGF_2\alpha$ or Tham buffer was given intramuscularly. The first dose of Trilostane or vehicle-2 given was 100 mg starting at 72 hours postlutectomy followed by 50 mg every 12 hours (84, 96, 108, 120, 132, and 144 hours), and the last dose was 25 mg of Trilostane at 156 hours postlutectomy. Cortisol (25 mg in 4 ml Safflower oil) or vehicle-1 was given at 24 hour intervals from 72 hours postlutectomy (72, 96, 120, and 144 hours) with the last injection given at 144 hours postlutectomy. Prostaglandin $F_2\alpha$ (16 mg/58 kg/BW) or vehicle-3 was given at 84 hours postlutectomy only, which is 12 hours after the initiation of the Trilostane treatments. Five ewes were used in each treatment group.

Jugular venous blood was collected at 0, 24, 48 and 72 hours before initiation of treatments at 72 hours and at 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 108, 120, 132, 144, 156, and 168 hours after lutectomy for analysis for progesterone and estradiol-17ß by RIA. Blood samples were collected prior to treatments. Uterine venous plasma was collected at 168 hours postlutectomy for analysis for progesterone by RIA. Blood samples for plasma were collected, processed, and stored until assayed as in experiment 1.

The 10 visibly largest placentomes ipsilateral to the side of lutectomy were collected at 168 hours postlutectomy and weighed. Ewes were observed for abortion, time to abortion, lactation, dystocia, and retained placenta during the experimental period.

3.7 RADIOIMMUNOASSAYS

Procedures and reagents for the radioimmunoassay are given for progesterone (Appendix A), $PGF_{2}\alpha$ (Appendix B), PGE (Appendix B), estradiol-17ß (Appendix C), pregnancy specific protein B (Appendix D), and cortisol (Appendix E). Samples for PSPB were analyzed in the laboratory of Dr. Garth Sasser (783).

3.8 STATISTICAL ANALYSIS

3.8.1 GENERAL

All data sets were analyzed for homogeneity of variance by Bartlett's Box F test (987-990). When a data set was not homogeneous, data were transformed before being analyzed statistically. Where percentage data were collected, data were transformed by Arc Sine before being analyzed by a completely randomized design for an analysis of variance (ANOVA; 987-990). Only animals with complete blood samples over time for the experimental protocols were included in the statistical analysis for hormones and are indicated in the figure legends and tables. When significant treatment differences were found, differences between treatment means were tested by a Least Significant Difference test (987-990).

3.8.2 ABORTION

Percent data on numbers of abortions in experiments 2, 3, and 5 were analyzed by a one way completely randomized design for an ANOVA (987, 989, 990).

3.8.3 WEIGHTS OF CORPORA LUTEA AND PLACENTOMES

Weights of corpora lutea in experiments 1 and 2 were analyzed by a one way completely randomized design for an ANOVA (987, 989). Data for weights of placentomes in experiments 1, 2, 3, and 5 were analyzed by a one way completely randomized design for an ANOVA (987, 989).

3.8.4 NUMBERS OF SURFACE ANTRAL FOLLICLES

Numbers of visible surface antral follicles on ovaries with or without a CL of hysterectomized or intact 90 to 100 day pregnant ewes were analyzed by a 2x3 factorial completely randomized design for an ANOVA (987-989).

3.8.5 OVARIAN VENOUS PROGESTERONE, $PGF_2\alpha$ AND PGE

Data for ovarian venous progesterone in experiments 1 and 2 were transformed ($\log(x+1)$) to meet the assumptions of homogeneity of variance before being analyzed by a 2x3 factorial completely randomized design for an ANOVA (987-990). Data for PGE in ovarian venous blood in experiments 1 and 2 and $PGF_2\alpha$ in experiment 2 were transformed (square root) before analysis by a 2x3 factorial completely randomized design for an ANOVA (987-990). Data for $PGF_2\alpha$ in ovarian venous blood for experiment 1 were homogeneous and analysed by a 2x3 factorial completely randomized design for an ANOVA (987-990).

3.8.6 TIME TO ABORTION, LACTATION AND RETAINED PLACENTA

In experiment 5, data on time to abortion, percent data on lactation, and percent data on retained placenta were analyzed by a one way completely randomized design for an ANOVA (987-990).

3.8.7 SINGLE SAMPLES OF UTERINE VENOUS PROGESTERONE

In experiment 5, uterine venous progesterone at 168 hours was analyzed by a one way completely randomized design for an ANOVA (987-990).

3.8.8 PLACENTAL SECRETION OF PROGESTERONE, ESTRADIOL-17ß, $PGF_2\alpha$ AND PGE *IN VITRO*

Data on placental secretion of progesterone, estradiol-17ß, $PGF_2\alpha$, and PGE *in vitro* in experiment 4 were analyzed by a 4x2x2 factorial completely randomized design for an ANOVA (987-990). All data sets were transformed ($\log(x \div 2 + 10)$) prior to analysis (987-990).

3.8.9 PROFILES OF PROGESTERONE, ESTRADIOL-17ß, CORTISOL, $PGF_2\alpha$, PGE OR PSPB IN JUGULAR OR UTERINE VENOUS PLASMA

Profiles of progesterone and estradiol-17ß in jugular plasma in experiments 1, 2, 3, and 5; profiles of cortisol in jugular plasma in experiments 1, 2, and 3; and profiles of progesterone, $PGF_2\alpha$, PGE, and PSPB in uterine venous plasma in experiments 2 and 3 were analyzed using a general linear model for ANOVA for repeated measures using a split-plot design. Treatments served as the main plots and time within treatment

served as the subplots (990, 991). Assumption of independent variances of subplots were tested (990, 991). Animal within treatment was included in the model and the type III mean square for animal within treatment was used as the error term for detection of treatment effects. When profiles of hormones were significant for time or treatment x time effects, data were analyzed by a one way completely randomized design for an ANOVA or a factorial completely randomized design for an ANOVA, respectively (987-990). In addition, regression analysis was used to determine linear and quadratic components and in some cases comparison of regression lines (990, 991).

Data for progesterone in jugular plasma were transformed (square root$(x+0.5)\div 2+10$) and data for estradiol-17ß in jugular plasma were transformed (log$(x+1)$) in experiment 1 before analysis. Data for cortisol in jugular plasma in experiment 1 were transformed (log$(x\div 2+1)$) before analysis (987-990).

Data for jugular venous cortisol in experiment 2 were transformed (square root$(x\div 2+10)$) prior to analysis. Data for jugular venous estradiol-17ß in experiment 3 were transformed (square root) prior to analysis (987-990).

Data for progesterone in uterine venous plasma were transformed (square root$(x+1)$), data for PSPB in uterine venous plasma were transformed (square root$(x\div 2+10)$) and data for uterine venous PGE were transformed (log$x$) in experiment 2 prior to analysis (987-990).

Data for uterine venous progesterone and PSPB were transformed (square root$(x\div 2+10)$) and data for uterine venous $PGF_{2\alpha}$ and

PGE were transformed ($\log(x+1)$) in experiment 3 prior to analysis (987-990).

Data for progesterone and estradiol-17ß in jugular plasma in experiment 5 were transformed ($\log(x+2)\times10$) prior to analysis (987-990).

CHAPTER 4

RESULTS

4.1 ABORTION, TIME TO ABORTION, LACTATION AND RETENTION OF PLACENTA

In experiment 2, one intact ewe in each of the vehicle, 8, or 16 mg $PGF_2\alpha$/58 kg/BW-treated 90 to 100 day pregnant ewes aborted and thus were not different among treated groups ($P \geq 0.05$, Table 1). One ovariectomized 90 to 100 day pregnant ewe in the 16 mg $PGF_2\alpha$/58 kg/BW-treated group in experiment 3 aborted ($P \geq 0.05$, Table 1). All pregnant ewes in experiments 2 and 3 treated with $PGF_2\alpha$ were observed to have abdominal contractions and exhibited alternating recumbent and standing positions between 32 and 48 hours after treatment, which are characteristic of maternal delivery behavior in sheep. This behavior was not observed in control ewes in the presence or absence of the ovaries. At laparotomy 72 hours after treatment, the uterus of all $PGF_2\alpha$-treated intact or ovariectomized 90 to 100 day pregnant ewes in experiments 2 and 3 was tight, while the uterus of vehicle control ewes was flaccid.

In experiment 5, abortion was induced in all 90 to 100 day lutectomized pregnant ewes treated with trilostane, trilostane + $PGF_2\alpha$, trilostane + cortisol, or trilostane + $PGF_2\alpha$ + cortisol ($P \leq 0.05$, Table 2). One ewe in the trilostane + cortisol treatment group had dystocia. No abortions occurred in control, cortisol- or $PGF_2\alpha$ + cortisol-treated ewes ($P \geq 0.05$, Table 2) and only one 90 to 100 day lutectomized pregnant ewe treated with $PGF_2\alpha$ alone aborted ($P \geq 0.05$, Table 2). Time to abortion in

TABLE 1: NUMBER OF 90-100 DAY PREGNANT SHEEP ABORTED BY PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$)

	INTACT		OVARIECTOMIZED	
Treatment*	No. of ewes	No. of abortions#	No. of ewes	No.of abortions#
Vehicle	10	1^a	11	0^a
$PGF_{2\alpha}$-8mg	10	1^a	8	0^a
$PGF_{2\alpha}$-16mg	11	1^a	14	1^a

* Dose of $PGF_{2\alpha}$ was per 58 kg body weight.
\# Mean $\pm$ S.E.M. with similar superscripts do not differ at $P \geq 0.05$ within experiment.

TABLE 2: INCIDENCE OF ABORTION, TIME TO ABORTION, RETAINED PLACENTA, AND LACTATION 96 HOURS AFTER TREATMENT WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), CORTISOL OR TRILOSTANE IN 90-100 DAY LUTECTOMIZED PREGNANT EWES

Treatment	No. of ewes	No. of ewes* aborted	Time to abortion*,# (minutes)	Retained placenta(%)	No. of ewes* lactating
Vehicle	5	0^a	5760 ± 0000^a	0 ± 0^a	0^a
$PGF_{2\alpha}$	5	1^a	4973 ± 0162^a	0 ± 0^a	1^a
Cortisol	5	0^b	5760 ± 0000^a	0 ± 0^a	0^a
Trilostane	5	5^b	3342 ± 0129^b	100 ± 0^b	5^b
Cortisol + $PGF_{2\alpha}$	5	0^a	5760 ± 0000^a	0 ± 0^a	0^a
Trilostane + $PGF_{2\alpha}$	5	5^b	3080 ± 0282^b	80 ± 20^b	5^b
Trilostane + Cortisol	5	5^b	2784 ± 0195^b	100 ± 0^b	5^b
Trilostane + Cortisol + $PGF_{2\alpha}$	5	5^b	2988 ± 0313^b	100 ± 0^b	5^b

* Mean ± S.E.M. within columns with different superscripts differ at $P \leq 0.05$.
\# Time to abortion or necropsy at 96 hours after initiation of treatments.

those ewes that aborted compared to ewes that did not abort by the time of necropsy at 96 hours after the initiation of treatments was shortened ($P \leq 0.05$, Table 2), but time to abortion did not differ among the four different treatment groups receiving trilostane ($P \geq 0.05$, Table 2). Of the 44 pregnant ewes lutectomized for experiment 5, four ewes aborted before the initiation of treatments at 72 hours post lutectomy.

All ewes that aborted in the four trilostane treatment groups and the one $PGF_2\alpha$ alone-treated ewe that aborted were lactating at laparotomy, 96 hours after the initiation of treatments ($P \leq 0.05$, Table 2). All of the trilostane, trilostane + cortisol, and trilostane + $PGF_2\alpha$ + cortisol-treated ewes and 80 percent of the trilostane + $PGF_2\alpha$-treated ewes which aborted had retained placentas at necropsy, 96 hours after initiation of treatments ($P \leq 0.05$, Table 2).

4.2 WEIGHTS OF CORPORA LUTEA AND PLACENTOMES

Weights of corpora lutea at 0 hour in ovariectomized 90 to 100 day pregnant ewes in experiment 3 for the vehicle, 8 or 16 mg $PGF_2\alpha$/58 kg/BW treatment groups did not differ ($P \geq 0.05$) and averaged 571 ± 25, 576 ± 39 and 539 ± 35 mg, respectively. Weights of corpora lutea at the time of lutectomy (0 hours) in the vehicle, $PGF_2\alpha$, cortisol, cortisol + $PGF_2\alpha$, trilostane, trilostane + $PGF_2\alpha$, trilostane + cortisol, and trilostane + cortisol + $PGF_2\alpha$ treatment groups in experiment 5 did not differ ($P \geq 0.05$) and averaged 511 ± 32, 542 ± 27, 519 ± 33, 564 ± 24, 546 ± 38, 551 ± 42, 543 ± 34, and 526 ± 37 mg, respectively.

Weights of corpora lutea were reduced at 72 hours post treatment by either the 8 or 16 mg $PGF_2\alpha$/58 kg/BW dose when compared to controls in both the intact (Experiment 2) or in hysterectomized (Experiment 1) 90 to 100 day pregnant ewes ($P \leq 0.05$, Table 3). Weights of corpora lutea between the 8 or 16 mg $PGF_2\alpha$/58 kg/BW treatment groups in either the intact or hysterectomized 90 to 100 day pregnant ewes did not differ ($P \geq 0.05$, Table 3).

Weights of the ten visibly largest placentomes ipsilateral to the ovary with the CL of the fifteen 90 to 100 day pregnant ewes at the time (0 hour) of hysterectomy averaged 6.8 ± 0.2 g. Treatment of intact 90 to 100 day pregnant ewes with 8 mg $PGF_2\alpha$/58 kg/BW increased placentome weights in experiment 2 at 72 hours when compared to vehicle-treated ewes ($P \leq 0.05$, Table 4). Weights of placentomes in ovariectomized 90 to 100 day pregnant ewes (Experiment 3) at 72 hours post treatment did not differ among vehicle, 8, or 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes ($P \geq 0.05$, Table 4). Weights of placentomes in 90 to 100 day lutectomized pregnant ewes in experiment 5 at 96 hours after the initiation of treatments tended to be decreased only in the trilostane + $PGF_2\alpha$ treatment group ($P \leq 0.10$, Table 5).

4.3 NUMBERS AND SIZE OF SURFACE ANTRAL FOLLICLES

Numbers of visible surface antral follicles on ovaries with or without a CL of vehicle, 8 and 16 mg $PGF_2\alpha$-treated hysterectomized 90 to 100 day pregnant ewes at 72 hours post treatment in experiment 1 were 4.3 ± 0.2, 3.9 ± 0.5; 4.6 ± 0.4, 4.1 ± 0.4; and 4.8 ± 0.6, 4.2 ± 0.5;

TABLE 3: EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) ON WEIGHT OF THE CORPUS LUTEUM OF INTACT OR HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP 72 HOUR AFTER $PGF_{2\alpha}$

	INTACT		HYSTERECTOMIZED	
Treatment*	No. of ewes	Corpus Luteum# (mg)	No. of ewes	Corpus Luteum# (mg)
Vehicle	6	520 ± 33^a	5	536 ± 61^a
$PGF_{2\alpha}$-8mg	6	301 ± 55^b	5	212 ± 30^b
$PGF_{2\alpha}$-16mg	5	202 ± 29^b	5	324 ± 71^b

* Dose of $PGF_{2\alpha}$ was per 58 kg body weight.
\# Mean $\pm$ S.E.M. in columns within experiment with different superscripts differ at $P \leq 0.05$.

TABLE 4: WEIGHTS OF PLACENTOMES FROM 90-100 DAY PREGNANT SHEEP AFTER TREATMENT WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$)

	INTACT@		OVARIECTOMIZED@	
Treatment&	No. of ewes	Placentome*,# (gm)	No. of ewes	Placentome*,# (gm)
Vehicle	6	6.7 ± 0.2^{a}	5	7.2 ± 0.3^{a}
$PGF_{2\alpha}$-8mg	6	7.9 ± 0.3^{b}	6	7.1 ± 0.4^{a}
$PGF_{2\alpha}$-16mg	5	$7.2 \pm 0.2^{a,b}$	7	7.1 ± 0.3^{a}

& Dose of $PGF_{2\alpha}$ was per 58 kg body weight.
* Mean $\pm$ S.E.M of the ten visibly largest placentomes per ewe.
# Mean $\pm$ S.E.M. within columns within experiment with different superscripts differ at $P \leq 0.05$.
@ Average weight of 10 placentomes from each of 15 hysterectomized 90-100 day pregnant ewes was 6.8 ± 0.2 gm.

TABLE 5: WEIGHTS OF PLACENTOMES IPSILATERAL TO THE OVARY WITH THE CORPUS LUTEUM OF 90-100 DAY LUTECTOMIZED PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), CORTISOL OR TRILOSTANE

Treatment	No. of ewes	Placentome[*,#] (gm)
Vehicle	5	6.2 ± 0.6^{a}
$PGF_{2\alpha}$	5	6.2 ± 0.6^{a}
Cortisol	5	5.7 ± 0.5^{a}
Trilostane	5	$5.5 \pm 1.2^{a,b}$
Cortisol + $PGF_{2\alpha}$	5	$5.1 \pm 0.9^{a,b}$
Trilostane + $PGF_{2\alpha}$	5	4.1 ± 0.6^{b}
Trilostane + Cortisol	5	6.7 ± 0.7^{a}
Trilostane + $PGF_{2\alpha}$ + Cortisol	5	6.1 ± 1.4^{a}

[*] Mean ± S.E.M. with different superscripts differ at $P \leq 0.10$.
[#] Mean ± S.E.M of the ten visibly largest placentome.

respectively, and did not differ within ovaries ($P \geq 0.05$) or among treatment groups ($P \geq 0.05$). Numbers of visible surface antral follicles on ovaries with or without a CL of vehicle, 8 and 16 mg $PGF_2\alpha$-treated intact 90 to 100 day pregnant ewes at 72 hours post treatment in experiment 2 were 4.4 ± 0.3, 4.0 ± 0.4; 4.6 ± 0.6, 3.8 ± 0.8; and 4.7 ± 0.5, 4.1 ± 0.5; respectively, and did not differ within ovaries ($P \geq 0.05$) or among treatment groups ($P \geq 0.05$). Size of visible surface antral follicles did not exceed 2 mm in any treatment group of hysterectomized or intact 90 to 100 day pregnant ewes at 72 hours post treatment.

4.4 PROGESTERONE

Concentrations of progesterone in ovarian venous plasma at 0 hours in the vehicle, 8, or 16 mg $PGF_2\alpha$/58 kg/BW treatment groups did not differ within the hysterectomized (Experiment 1) or intact (Experiment 2) 90 to 100 day pregnant ewes ($P \geq 0.05$, Table 6). Concentrations of progesterone in ovarian venous plasma were reduced at 72 hours after hysterectomy when compared to the 0 hour sample in vehicle-treated ewes ($P \leq 0.05$, Table 6). Both doses of $PGF_2\alpha$ reduced the concentration of progesterone at 72 hours when compared to either the 0 hour sample within the two $PGF_2\alpha$ treatment groups of hysterectomized or intact pregnant ewes or when compared to the 72 hour sample of control ewes within the hysterectomized or intact pregnant ewe experiments ($P \leq 0.05$, Table 6). There was no dose dependent effect of $PGF_2\alpha$ on ovarian venous progesterone in either the hysterectomized or intact 90 to 100 day pregnant ewes ($P \geq 0.05$, Table 6).

TABLE 6: EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) ON OVARIAN VENOUS PROGESTERONE OF INTACT OR HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP 0 OR 72 HOUR AFTER $PGF_{2\alpha}$ TREATMENT

Treatment*	INTACT			HYSTERECTOMIZED		
	No.of ewes	Progesterone# (ng/ml)		No.of ewes	Progesterone# (ng/ml)	
		0 hr	72 hr		0 hr	72 hr
Vehicle	6	1256 ± 454^{a}	1627 ± 457^{a}	5	3004 ± 1166^{a}	1112 ± 548^{b}
$PGF_{2\alpha}$-8mg	6	1282 ± 382^{a}	119 ± 035^{b}	5	1901 ± 0483^{a}	39 ± 001^{c}
$PGF_{2\alpha}$-16mg	5	798 ± 267^{a}	62 ± 021^{b}	5	1758 ± 0260^{a}	13 ± 006^{c}

* Dose of $PGF_{2\alpha}$ was per 58 kg body weight.
\# Means $\pm$ S.E.M. in columns or rows within experiment with different superscripts differ at $P \leq 0.05$.

Profiles of progesterone in jugular venous blood of vehicle, 8, and 16 mg $PGF_2\alpha$/58 kg/BW-treated hysterectomized 90 to 100 day pregnant ewes differed ($P \leq 0.05$, Figure 1). There was a treatment x time decrease ($P \leq 0.05$) in concentrations of progesterone in jugular plasma in the vehicle, 8, and 16 mg $PGF_2\alpha$/58 kg/BW-treated hysterectomized (Experiment 1) 90 to 100 day pregnant ewes (Fig.1). Concentrations of progesterone in jugular venous blood of vehicle-treated hysterectomized ewes were reduced by 4 hours post hysterectomy ($P \leq 0.05$, Fig.1) and remained between 4 to 5 ng/ml but did not differ ($P \geq 0.05$) throughout the remainder of the 72 hour sampling period (Fig.1). Concentrations of progesterone in jugular venous blood of hysterectomized 90 to 100 day pregnant ewes were reduced by 4 hours ($P \leq 0.05$) in the 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes and declined further by 16 hours ($P \leq 0.05$) when compared to hysterectomized control ewes over the 72 hour sampling period (Fig.1). Profiles of progesterone were not different between the 8 or 16 mg $PGF_2\alpha$-treated hysterectomized pregnant ewes ($P \geq 0.05$, Fig.1).

Concentrations of progesterone in jugular venous blood of vehicle, 8, and 16 mg $PGF_2\alpha$-treated intact (Experiment 2, Fig.2) or ovariectomized (Experiment 3, Fig.3) 90 to 100 day pregnant ewes decreased over time ($P \leq 0.05$) but never fell below 5 ng/ml. However, profiles of jugular venous progesterone were not different ($P \geq 0.05$) among the vehicle, 8, and 16 mg $PGF_2\alpha$/58 kg/BW intact (Fig.2) or ovariectomized (Fig.3) pregnant ewes. Concentrations of progesterone in jugular venous plasma decreased ($P \leq 0.05$) by 12 hours in $PGF_2\alpha$-treated intact and by 48 hours in vehicle-treated pregnant ewes (Fig.2). Concentrations of

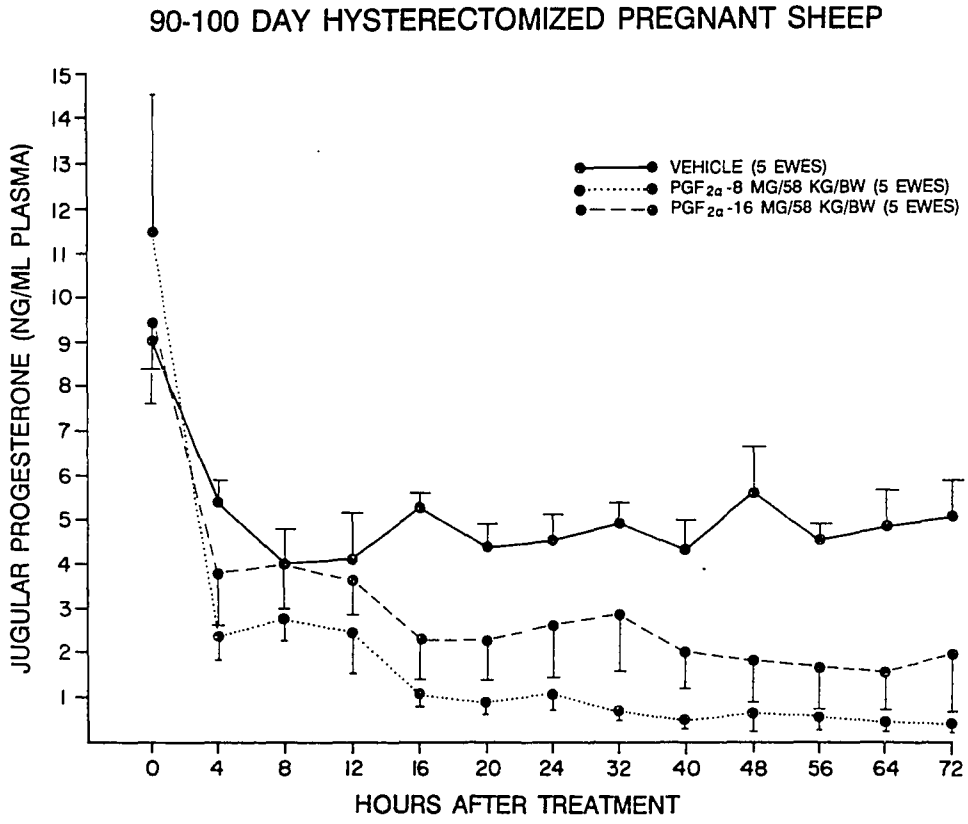

FIGURE 1. PROFILES OF PROGESTERONE (LEAST SQUARE MEASNS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TREATMENT, TIME, AND TREATMENT x TIME ($P \leq 0.05$).

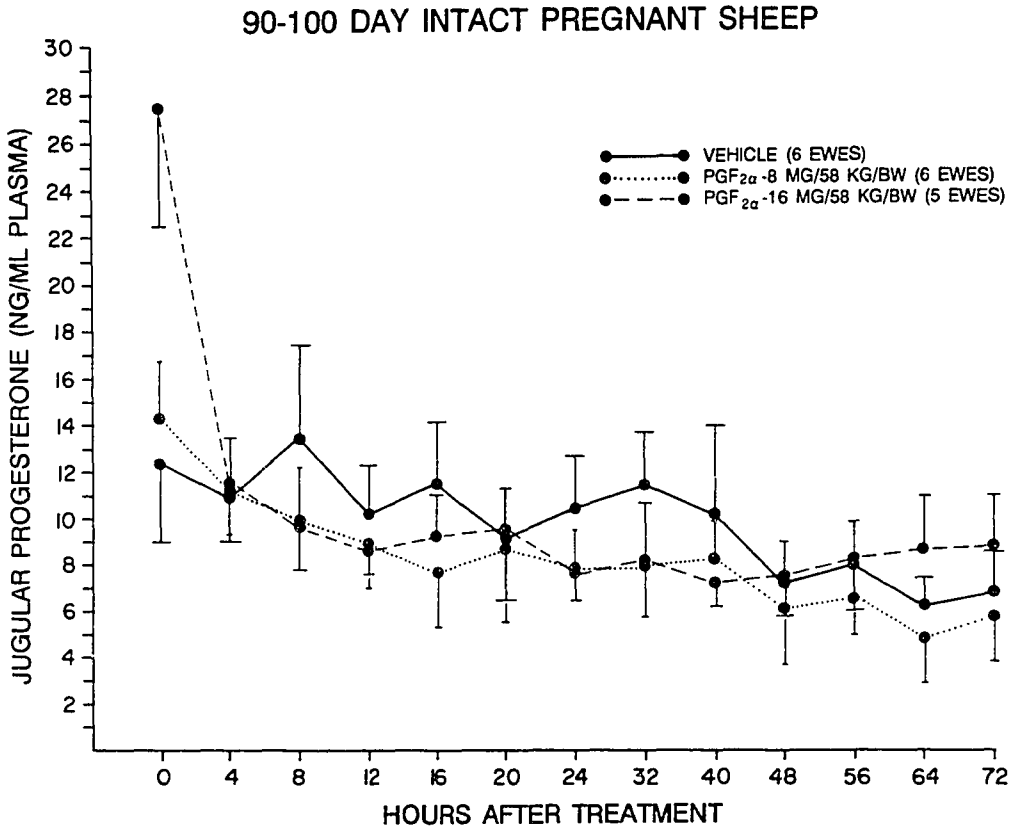

FIGURE 2. PROFILES PROGESTERONE (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF INTACT 90-TOO DAY PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

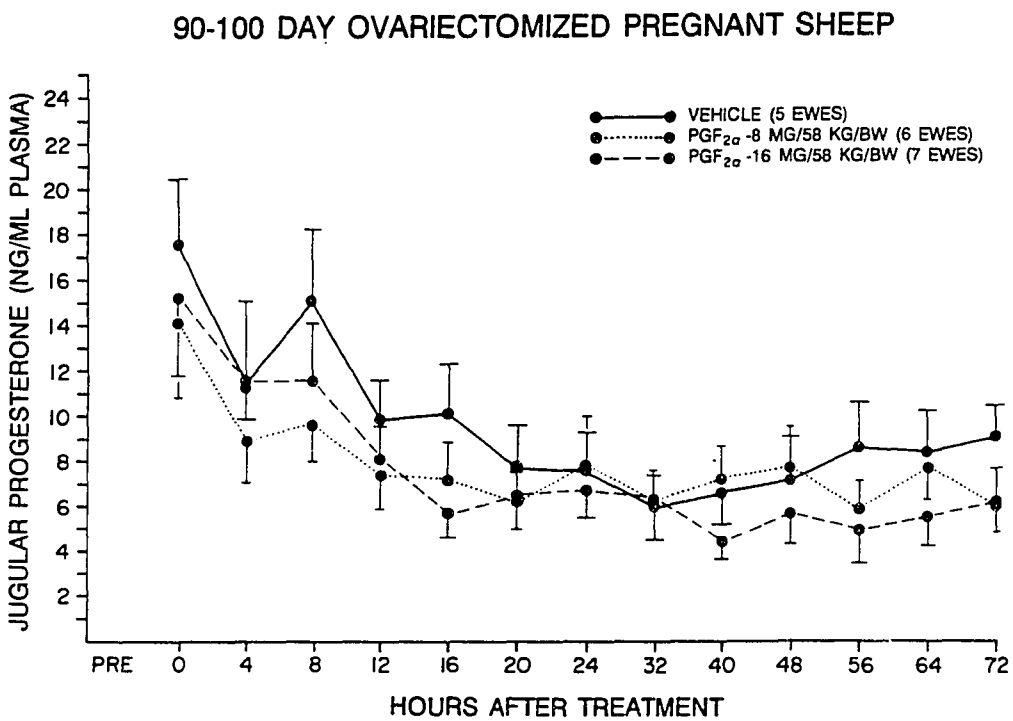

FIGURE 3. PROFILES OF PROGESTERONE (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

progesterone in jugular venous blood decreased ($P \leq 0.05$) by 12 hours in vehicle, 8 or 16 mg $PGF_2\alpha$/58 kg/BW- treated ovariectomized 90 to 100 day pregnant ewes (Fig.3).

Concentrations of progesterone in uterine venous blood decreased over time ($P \leq 0.05$), but never fell below 30 ng/ml over the 72 hour sampling period in vehicle, 8, or 16 mg $PGF_2\alpha$-treated intact (Experiment 2, Fig.4) or ovariectomized (Experiment 3, Fig.5) 90 to 100 day pregnant ewes. However, profiles of uterine venous progesterone did not differ among vehicle, 8, or 16 mg $PGF_2\alpha$-treated intact ($P \geq 0.05$, Fig.4) or ovariectomized ($P \geq 0.05$, Fig.5) 90 to 100 day pregnent ewes. There was a quadratic increase in uterine venous progesterone in the vehicle, 8, or 16 mg $PGF_2\alpha$-treated intact ($P \leq 0.05$, Fig.4) or vehicle and 16 mg $PGF_2\alpha$-treated ovariectomized ($P \leq 0.05$, Fig.5) 90 to 100 day pregnant ewes toward the end of the 72 hour sampling period.

In experiment 4, secretion of progesterone *in vitro* by placentome slices from 90 to 100 day intact pregnant ewes in a four hour incubation averaged 1.9 ± 0.5 ng/ml/100 mg of tissue and ranged from 1 to 3.4 ng/ml/100 mg of tissue by the 16 treatment groups (Fig.6). Secretion of progesterone by placentome slices *in vitro* from 90 to 100 day intact pregnant ewes was not affected ($P \geq 0.05$) by treatment with $PGF_2\alpha$, indomethacin, androstenedione or their interactions during the 4 hours of culture (Fig.6).

In experiment 5, concentrations of progesterone in jugular venous blood decreased ($P \leq 0.07$) over the first 72 hours in all 8 treatment groups, but did not differ ($P \geq 0.05$) among the 8 treatment groups for the

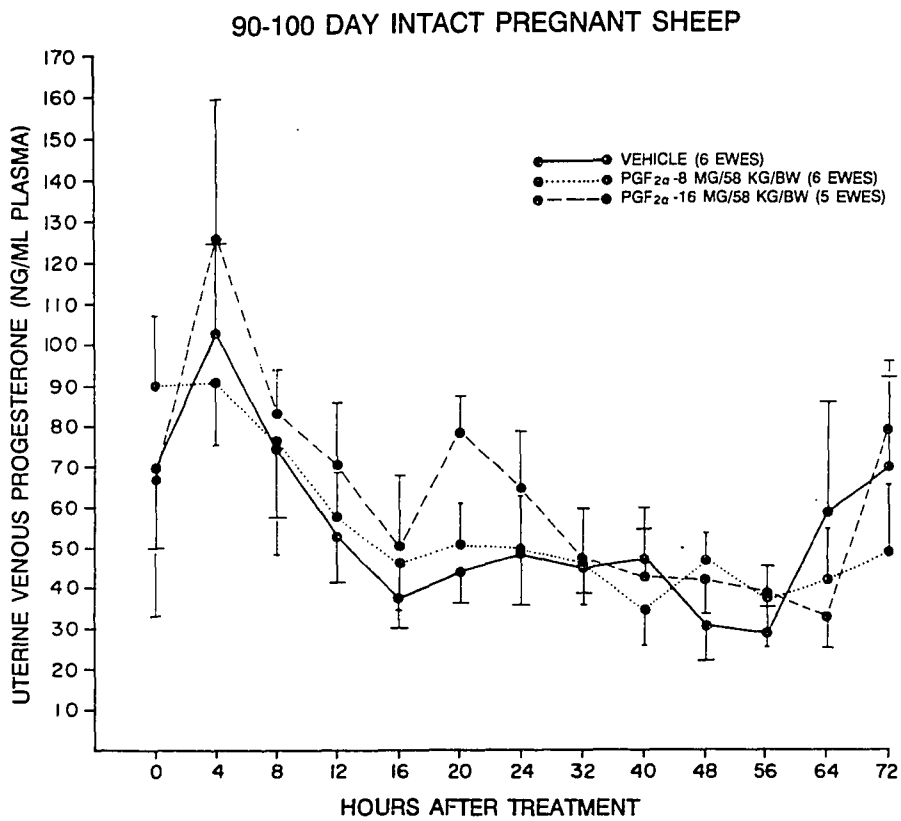

FIGURE 4. PROFILES OF PROGESTERONE (LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE VENOUS PLASMA OF 90-100 DAY INTACT PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). LINEAR AND QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.05$).

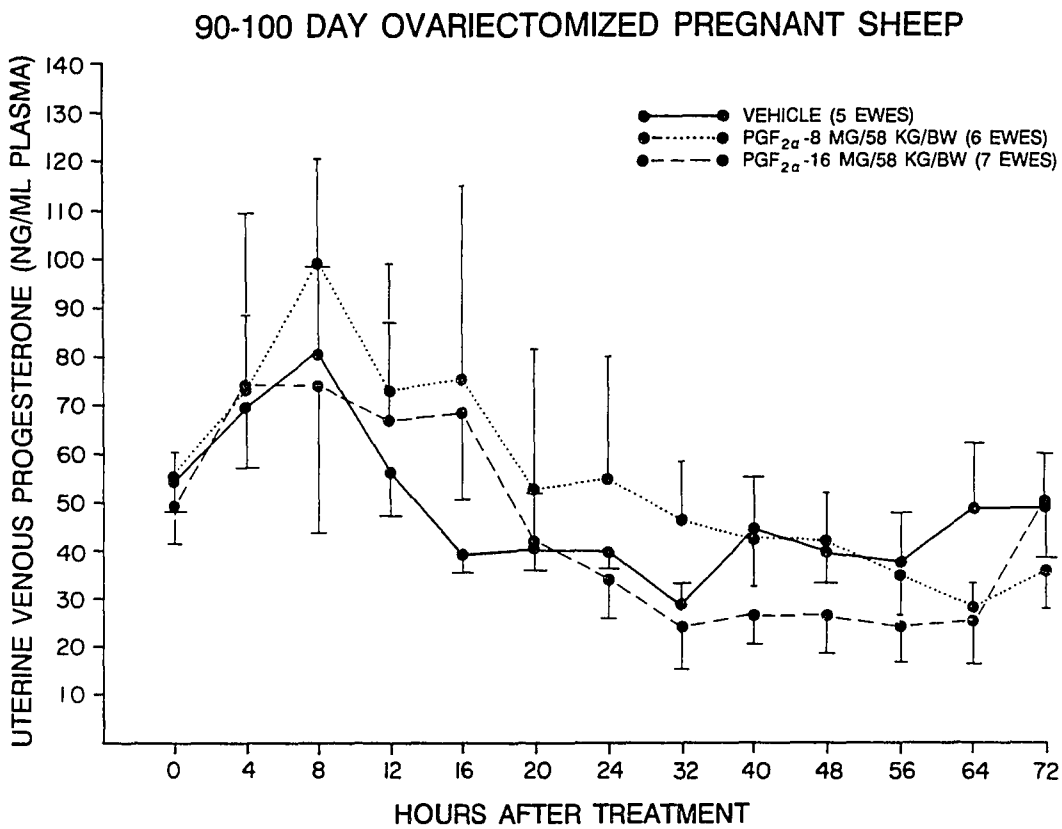

FIGURE 5. PROFILES OF PROGESTERONE (LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE VENOUS PLASMA OF 90-100 DAY OVARIECTOMIZED PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). LINEAR AND QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.05$).

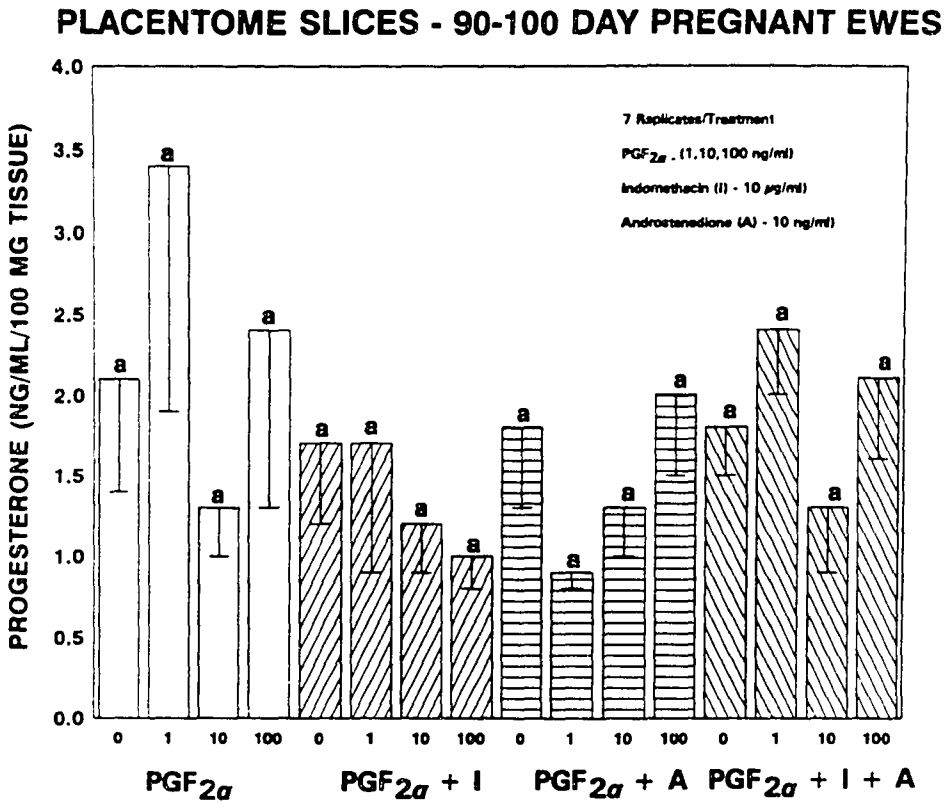

FIGURE 6. MEANS ± S.E.M. FOR PROGESTERONE *IN VITRO* BY PLACENTOME SLICES FROM 90-100 DAY INTACT PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), INDOMETHACIN (I) AND/OR ANDROSTENEDIONE (A). TREATMENT DIFFERENCES WERE NOT SEEN ($P \geq 0.05$).

first 72 hours (Figs. 7 and 8). There was a significant ($P \leq 0.05$) treatment x time difference in progesterone from 72 to 168 hours in jugular venous blood of lutectomized 90 to 100 day pregnant ewes (Figs. 7 and 8). Profiles of jugular venous progesterone from 72 through 168 hours differed ($P \leq 0.05$) in trilostane + $PGF_2\alpha$ and trilostane + $PGF_2\alpha$ + cortisol, and trilostane + cortisol ($P \leq 0.08$) treatment groups when compared to vehicle-treated lutectomized 90 to 100 day pregnant ewes (Fig. 8). Profiles of jugular venous progesterone from 72 through 168 hours did not differ ($P \geq 0.05$) among vehicle, $PGF_2\alpha$, cortisol, $PGF_2\alpha$ + cortisol, or trilostane-treated lutectomized 90 to 100 day pregnant ewes (Fig.7 and 8). Concentrations of progesterone in uterine venous plasma of lutectomized 90 to 100 day pregnant ewes at 168 hours tended to be lower ($P \leq 0.06$, Table 7) in trilostane and trilostane + $PGF_2\alpha$-treated ewes than in control ewes.

4.5 PROSTAGLANDIN $F_2\alpha$

Concentrations of $PGF_2\alpha$ in ovarian venous plasma at 0 hour in all hysterectomized (Experiment 1) and intact (Experiment 2) pregnant ewes averaged 93 and 39 pg/ml, respectively. Concentrations of $PGF_2\alpha$ in ovarian venous blood at 72 hours post treatment in the 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated 90 to 100 day hysterectomized pregnant ewes were not different ($P \geq 0.05$) when compared to vehicle-treated ewes nor when compared with the 0 hour ovarian venous samples collected from 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes (Table 8). There was a treatment x time ($P \leq 0.05$) effect of $PGF_2\alpha$ on concentrations of $PGF_2\alpha$ in ovarian venous blood of 90 to 100 day intact pregnant ewes. Concentrations of $PGF_2\alpha$ in

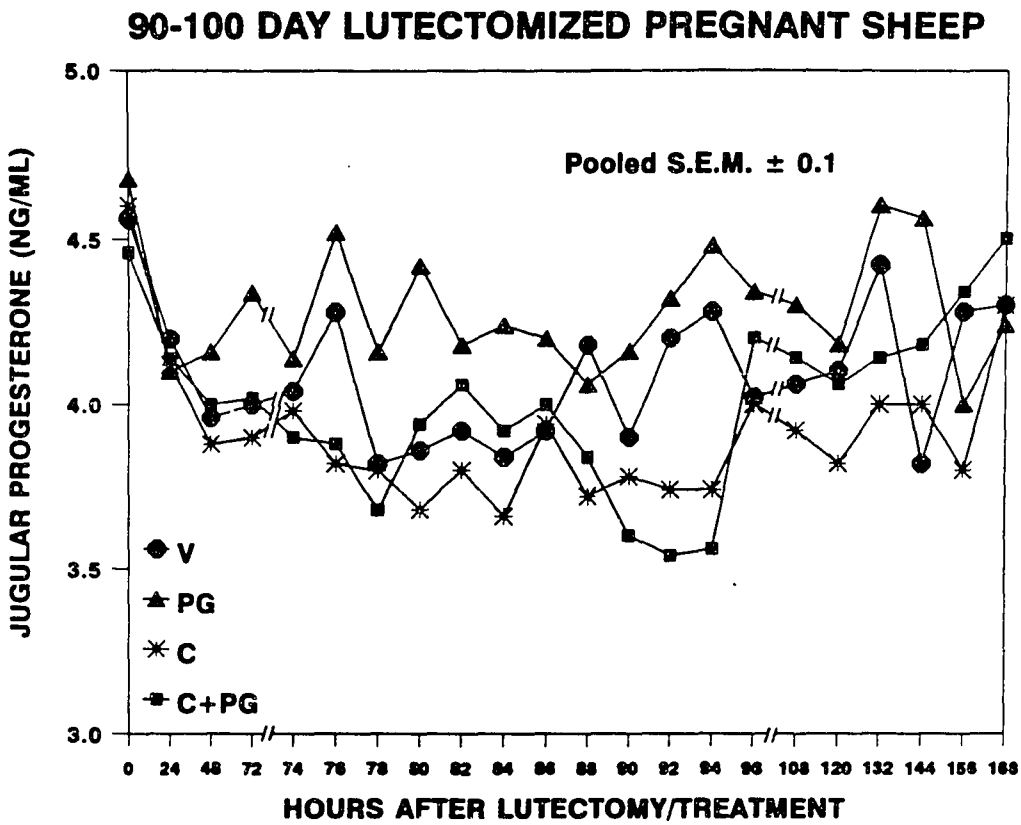

FIGURE 7. PROFILES OF PROGESTERONE (LEAST SQUARE MEANS $\pm$ POOLED S.E.M.) IN JUGULAR VENOUS PLASMA OF LUTECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH VEHICLE (V), CORTISOL (C), PROSTAGLANDIN $F_{2\alpha}$ (PG; 16 MG/58 KG/B.W.) OR CORTISOL + PROSTAGLANDIN $F_{2\alpha}$ (C+PG). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

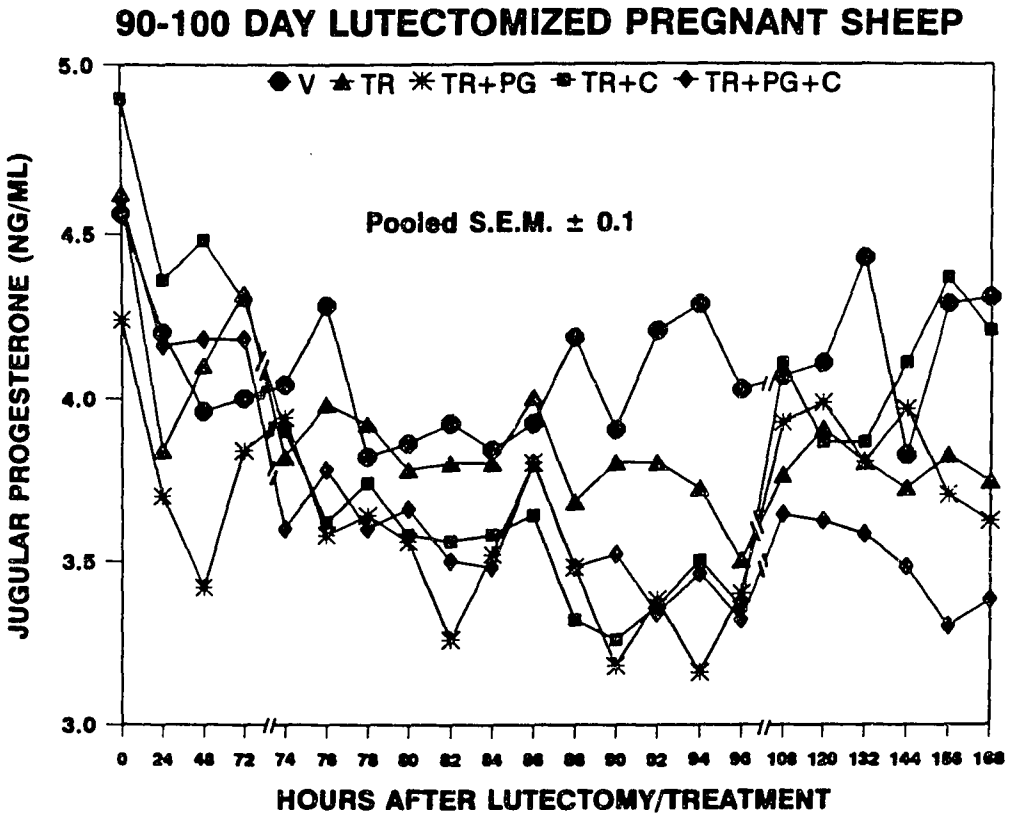

FIGURE 8. PROFILES OF PROGESTERONE (LEAST SQUARE MEANS ± POOLED S.E.M.) IN JUGULAR VENOUS PLASMA OF LUTECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH VEHICLE (V), TRILOSTANE (TR), TRILOSTANE + PROSTAGLANDIN $F_{2\alpha}$ (TR+PG), TRILOSTANE + CORTISOL (TR+C) OR TRILOSTANE +PROSTAGLANDIN $F_{2\alpha}$ + CORTISOL (TR+PG+C). DIFFERENCES WERE SEEN FOR TREATMENT, TIME, AND TREATMENT x TIME ($P \leq 0.05$).

TABLE 7: UTERINE VENOUS PROGESTERONE 96 HOURS AFTER TREATMENT WITH TRILOSTANE, PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), OR CORTISOL IN 90 - 100 DAY LUTECTOMIZED PREGNANT EWES

Treatment	No. of ewes	Progesterone* (ng/ml)
Vehicle	5	69.3 ± 14.1^{a}
$PGF_{2\alpha}$	5	$58.1 \pm 17.9^{a,b}$
Cortisol	5	81.4 ± 24.4^{a}
Trilostane	5	39.8 ± 11.3^{b}
Cortisol + $PGF_{2\alpha}$	5	73.6 ± 14.3^{a}
Trilostane + $PGF_{2\alpha}$	5	34.2 ± 10.9^{b}
Trilostane + Cortisol	5	$55.6 \pm 09.9^{a,b}$
Trilostane + Cortisol + $PGF_{2\alpha}$	5	$45.2 \pm 13.4^{a,b}$

* Mean ± S.E.M. with different superscripts differ at $P \leq 0.06$.

TABLE 8. EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) ON $PGF_{2\alpha}$ IN THE OVARIAN VEIN OF INTACT OR HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP

Treatment*	Intact			Hysterectomized		
	Number of ewes	PROSTAGLANDIN $F_{2\alpha}$# (pg/ml) 0 hr	72 hr	Number of ewes	PROSTAGLANDIN $F_{2\alpha}$# (pg/ml) 0 hr	72 hr
Vehicle	6	40 ± 013^{a}	38 ± 006^{a}	5	42 ± 12^{a}	39 ± 09^{a}
$PGF_{2\alpha}$ - 8 mg	6	48 ± 1.7^{a}	1392 ± 881^{b}	5	309 ± 08^{a}	44 ± 10^{a}
$PGF_{2\alpha}$ - 16 mg	5	$192 \pm 107^{a,b}$	431 ± 135^{b}	5	44 ± 13^{a}	39 ± 08^{a}

*Dose of $PGF_{2\alpha}$ was per 58 kg body weight.

#Mean $\pm$ S.E.M. in rows or columns within either the intact or hysterectomized treatment groups with different superscripts differ at $P \leq 0.05$

ovarian venous plasma were increased ($P \leq 0.05$) at 72 hours post treatment in the 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated 90 to 100 day intact pregnant ewes when compared to vehicle-treated ewes at 72 hours or to the 0 hour ovarian venous sample within the 8 mg $PGF_2\alpha$/58 kg/BW-treated intact ewes (Table 8).

Concentrations of $PGF_2\alpha$ in uterine venous plasma of intact (Experiment 2) and ovariectomized (Experiment 3) 90 to 100 day pregnant ewes over the 72 hour sampling period averaged 500 pg/ml and levels of $PGF_2\alpha$ at 0 hour were 211 pg/ml (Figures 9 and 10). There was a significant ($P \leq 0.05$) treatment and treatment x time interaction for concentrations of $PGF_2\alpha$ in uterine venous plasma of intact 90 to 100 day pregnant ewes after treatment with $PGF_2\alpha$ (Fig.9). Profiles of $PGF_2\alpha$ in uterine venous plasma collected from the 8 mg $PGF_2\alpha$/58 kg/BW-treated intact 90 to 100 day pregnant ewes were different ($P \leq 0.05$) from vehicle-treated ewes (Fig.9). Concentrations of uterine venous $PGF_2\alpha$ were increased ($P \leq 0.05$) at 64 hours post treatment in the 8 mg $PGF_2\alpha$/58 kg/BW-treated ewes (Fig.9). There was a quadratic increase ($P \leq 0.05$) in uterine venous $PGF_2\alpha$ in all of the intact 90 to 100 day pregnant ewe treatment groups toward the end of the 72 hour sampling period (Fig.9).

There was a trend for a treatment x time effect ($P \leq 0.10$) on profiles of $PGF_2\alpha$ in uterine venous plasma of ovariectomized 90 to 100 day pregnant ewes (Fig.10). Prostaglandin $F_2\alpha$ in uterine venous plasma of the 16 mg $PGF_2\alpha$/58 kg/BW-treated ovariectomized 90 to 100 day pregnant ewes tended to increase ($P \leq 0.10$) at 32 hours post treatment (Fig.10). A trend for a quadratic increase in uterine venous $PGF_2\alpha$ was detected in the

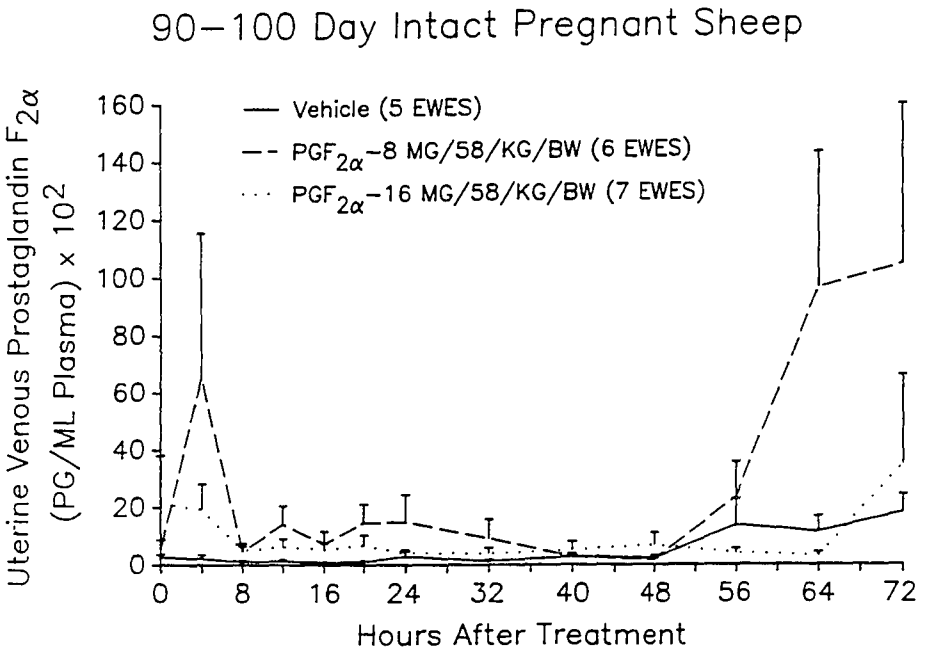

FIGURE 9. PROFILES OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$; LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE VENOUS PLASMA OF INTACT 90-100 DAY PREGNANT EWES TREATED WITH $PGF_{2\alpha}$. DIFFERENCES WERE SEEN FOR TREATMENT AND TREATMENT x TIME ($P \leq 0.05$), AND QUADRATIC EFFECTS FOR TIME ($P \leq 0.05$).

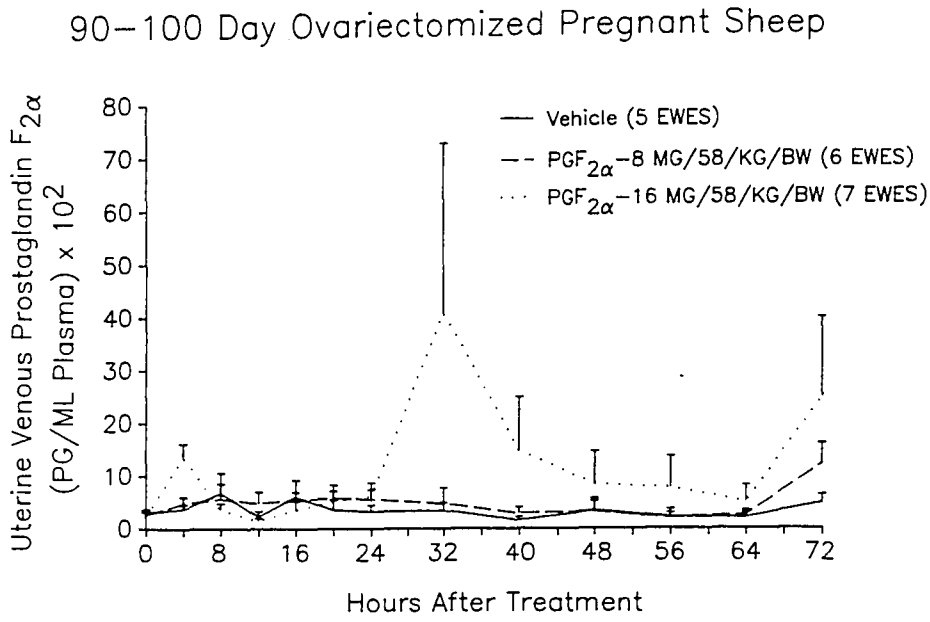

FIGURE 10. PROFILES OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$; LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH $PGF_{2\alpha}$. DIFFERENCES WERE SEEN FOR TREATMENT x TIME ($P \leq 0.10$) AND QUADRATIC EFFECTS ON TIME ($P \leq 0.10$).

8 mg $PGF_2\alpha$/58 kg/BW treatment group toward the end of the 72 hour sampling period ($P \leq 0.10$, Fig.10).

In experiment 4, secretion of $PGF_2\alpha$ in medium from placentome slices *in vitro* of 90 to 100 day intact pregnant ewes averaged 0.3 ± 0.1 ng/ml/100 mg tissue and ranged between 0.1 to 53.7 ng/ml/100 mg tissue across all treatment groups (Fig.11). Secretion of $PGF_2\alpha$ *in vitro* by placentome slices was decreased by indomethacin ($P \leq 0.05$, Fig.11) and tended to be increased by androstenedione ($P \leq 0.10$, Fig.11). Concentrations of $PGF_2\alpha$ in medium were increased by the treatment dose of $PGF_2\alpha$ used ($P \leq 0.05$, Fig.11).

4.6 PROSTAGLANDIN E

Concentrations of PGE in ovarian venous plasma at 0 hour in all 90 to 100 day hysterectomized (Experiment 1) and intact (Experiment 2) pregnant ewes averaged 6.9 and 7.2 ng/ml, respectively (Table 9). There was a treatment x time effect ($P \leq 0.07$) of $PGF_2\alpha$ on concentrations of PGE in ovarian venous plasma of 90 to 100 day hysterectomized pregnant ewes. Concentrations of PGE in ovarian venous plasma of hysterectomized 90 to 100 day pregnant ewes at 72 hour post treatment were not different among controls and the 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes ($P \geq 0.05$). Concentrations of PGE in ovarian venous plasma were lower at 72 hours in the 8 or 16 mg $PGF_2\alpha$/58 kg/BW-treated 90 to 100 day hysterectomized pregnant ewes when compared with their 0 hour samples ($P \leq 0.07$, Table 9). Concentrations of PGE in ovarian venous plasma of 90 to 100 day intact pregnant ewes did not differ ($P \geq 0.05$) at 0 or 72 hours (Table 9).

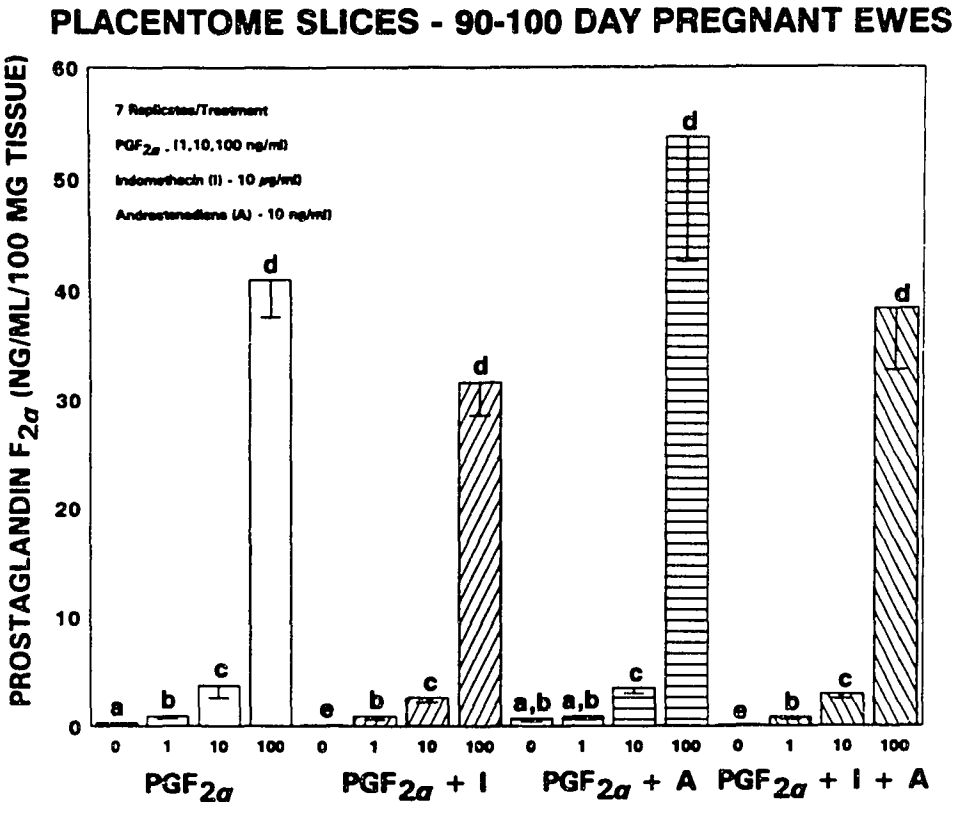

FIGURE 11. MEANS ± S.E.M. FOR SECRETION OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) *IN VITRO* BY PLACENTOME SLICES FROM 90-100 DAY INTACT PREGNANT EWES TREATED WITH $PGF_{2\alpha}$, INDOMETHACIN (I) AND/OR ANDROSTENEDIONE (A). DIFFERENCES WERE SEEN FOR TREATMENT AND DOSE ($P \leq 0.05$).

TABLE 9. EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) ON PROSTAGLANDIN E (PGE) IN THE OVARIAN VEIN OF INTACT OR HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP

Treatment*	Intact			Hysterectomized		
	Number of ewes	PROSTAGLANDIN E# (ng/ml) 0 hr	72 hr	Number of ewes	PROSTAGLANDIN E# (ng/ml) 0 hr	72 hr
Vehicle	6	6.8 ± 1.8^{a}	6.4 ± 1.8^{a}	5	4.2 ± 0.7^{a}	$4.7 \pm 0.7^{a,b}$
$PGF_{2\alpha}$ - 8 mg	6	5.7 ± 1.7^{a}	7.6 ± 2.2^{a}	5	7.9 ± 3.1^{a}	3.5 ± 0.7^{b}
$PGF_{2\alpha}$ - 16 mg	5	9.0 ± 2.1^{a}	10.5 ± 2.1^{a}	5	8.7 ± 4.4^{a}	$3.9 \pm 0.9^{b,c}$

*Dose of $PGF_{2\alpha}$ was per 58 kg body weight.

#Mean $\pm$ S.E.M. in rows or columns within either the intact or hysterectomized treatment groups with different superscripts differ at $P \leq 0.07$.

Concentrations of PGE in uterine venous plasma of intact (Experiment 2) and ovariectomized (Experiment 3) 90 to 100 day pregnant ewes averaged 6 ng/ml over the 72 hour treatment period (Figs. 12 and 13). Profiles of PGE in uterine venous plasma did not differ ($P \geq 0.05$) among intact treatment groups, but differed ($P \leq 0.05$) over time within treatment groups (Fig.12). Concentrations of PGE in uterine venous plasma increased ($P \leq 0.05$) by 64 hours in the 8 mg $PGF_2\alpha$/58 kg/BW intact treatment group (Fig.12). There was a quadratic increase ($P \leq 0.05$) in uterine venous PGE in vehicle, 8, and 16 mg $PGF_2\alpha$/58 kg/BW intact 90 to 100 day pregnant ewe treatment groups toward the end of the 72 hour sampling period (Fig.12).

Profiles of PGE in uterine venous plasma of ovariectomized 90 to 100 day pregnant ewes did not differ ($P \geq 0.05$) among the vehicle and 8 or 16 mg $PGF_2\alpha$ treatment groups (Fig.13). There was a quadratic effect ($P \leq 0.05$) for time on PGE in uterine venous plasma toward the end of the 72 hour sampling period in the vehicle and 8 mg $PGF_2\alpha$/58 kg/BW treatment groups of ovariectomized 90 to 100 day pregnant ewes (Fig.13).

In experiment 4, secretion of PGE by placentome slices *in vitro* from 90 to 100 day intact pregnant ewes during the 4 hour incubation was 10.6 ng/ml/100 mg tissue and ranged from 4.7 to 12.6 ng/ml/100 mg tissue across all treatment groups (Fig.14). Secretion of PGE by placentome slices *in vitro* was decreased ($P \leq 0.05$) by indomethacin (Fig.14).

The overall ratio of PGE:$PGF_2\alpha$ in ovarian venous and uterine venous plasma exceeded 100:1 and 12:1, respectively.

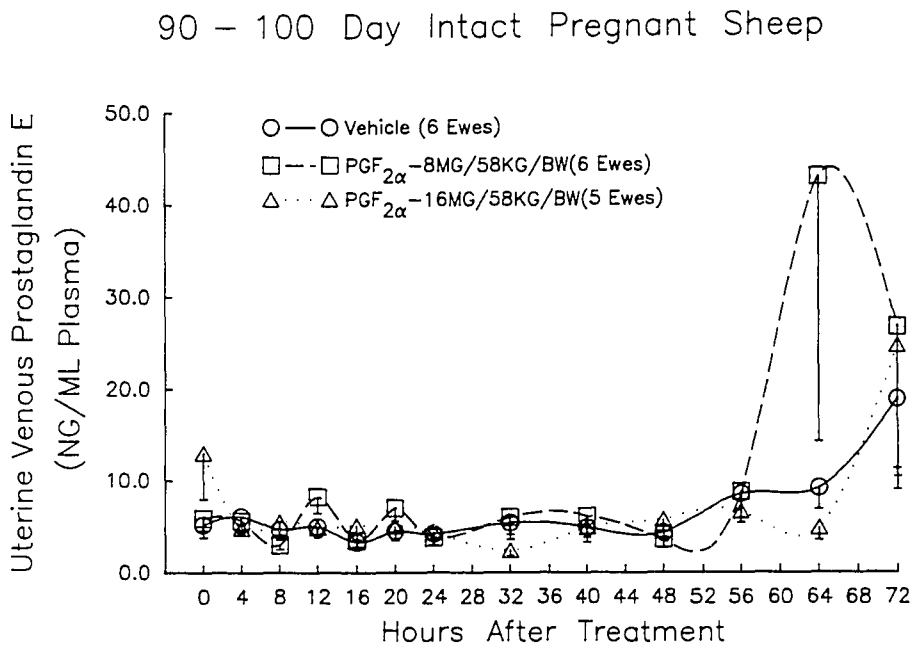

FIGURE 12. PROFILES OF PROSTAGLANDIN E (PGE; LEAST SQUARE MEANS ± S.E.M.) IN UTERINE VENOUS PLASMA OF INTACT 90-100 DAY PREGNMANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.05$).

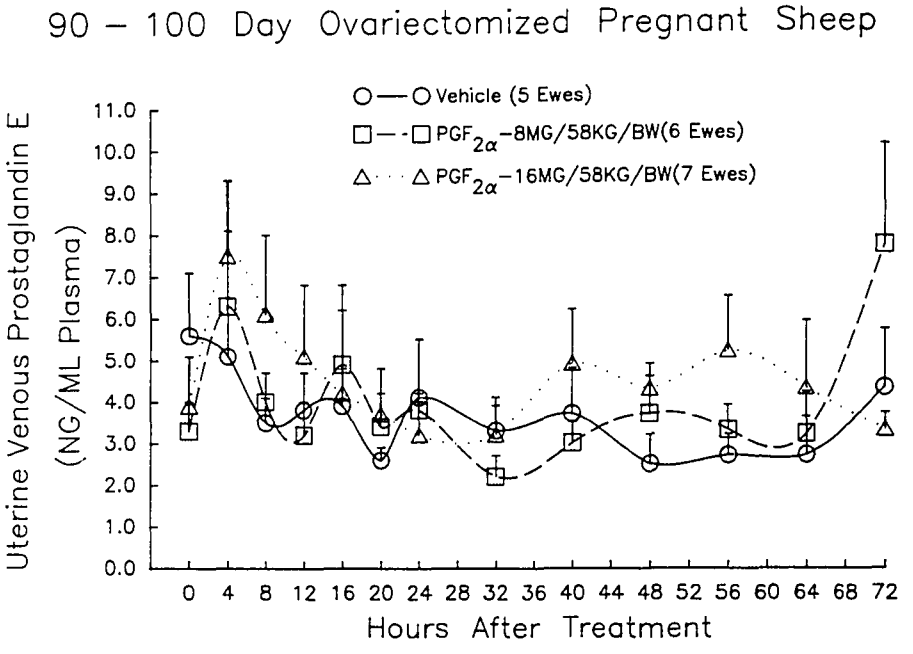

FIGURE 13. PROFILES OF PROSTAGLANDIN E (PGE; LEAST SQUARE MEANS ± S.E.M.) IN UTERINE VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.05$).

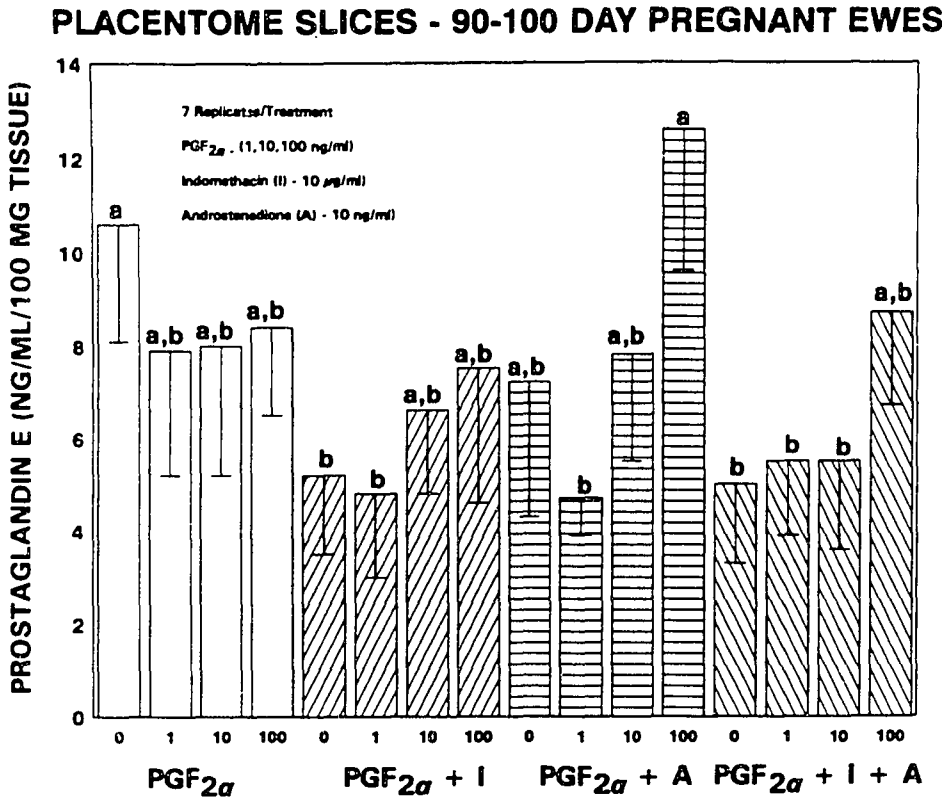

FIGURE 14. MEANS ± S.E.M. FOR SECRETION OF PROSTAGLANDIN E (PGE) *IN VITRO* BY PLACENTOME SLICES FROM 90-100 DAY INTACT PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), INDOMETHACINE (I) AND/OR ANDROSTENEDIONE (A). DIFFERENCES WERE SEEN FOR TREATMENT x DOSE ($P \leq 0.05$).

4.7 ESTRADIOL-17ß

Profiles of estradiol-17ß in jugular blood of hysterectomized 90 to 100 day pregnant ewes (Experiment 1) did not differ ($P \geq 0.05$) among vehicle, 8, or 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes, but did differ over time ($P \leq 0.05$; Fig.15). Hysterectomy reduced ($P \leq 0.05$) jugular venous estradiol-17ß within 16 hours by 80 percent in vehicle or $PGF_2\alpha$-treated 90 to 100 day pregnant ewes (Fig.15).

There were treatment ($P \leq 0.10$) and time ($P \leq 0.05$) effects on profiles of estradiol-17ß in jugular venous blood of intact 90 to 100 day pregnant ewes (Experiment 2, Fig.16). Profiles of estradiol-17ß in jugular venous blood of 90 to 100 day intact pregnant ewes treated with 16 mg $PGF_2\alpha$/58 kg/BW were different ($P \leq 0.05$) when compared to control pregnant ewes (Fig.16). Comparison of regression lines revealed a treatment effect ($P \leq 0.05$) with both the 8 and 16 mg $PGF_2\alpha$/58 kg/BW treatment groups being different when compared to control pregnant ewes. Estradiol-17ß in jugular venous blood of vehicle (4 Hours)- and 8 mg $PGF_2\alpha$/58 kg/BW (40 hours)-treated intact 90 to 100 day pregnant ewes declined with time ($P \leq 0.05$) with a trend ($P \leq 0.10$) for a quadratic increase at the end of the 72 hour sampling period in control pregnant ewes (Fig.16). There was a quadratic increase ($P \leq 0.05$) in estradiol-17ß in jugular venous blood of 90 to 100 day intact pregnant ewes treated with 16 mg $PGF_2\alpha$/58 kg/BW toward the end of the 72 hour sampling period (Fig.16).

There were treatment ($P \leq 0.10$) and time ($P \leq 0.05$) effects on profiles of estradiol-17ß in jugular venous blood of ovariectomized 90 to 100 day pregnant ewes (Experiment 3, Fig.17). Profiles of estradiol-17ß in

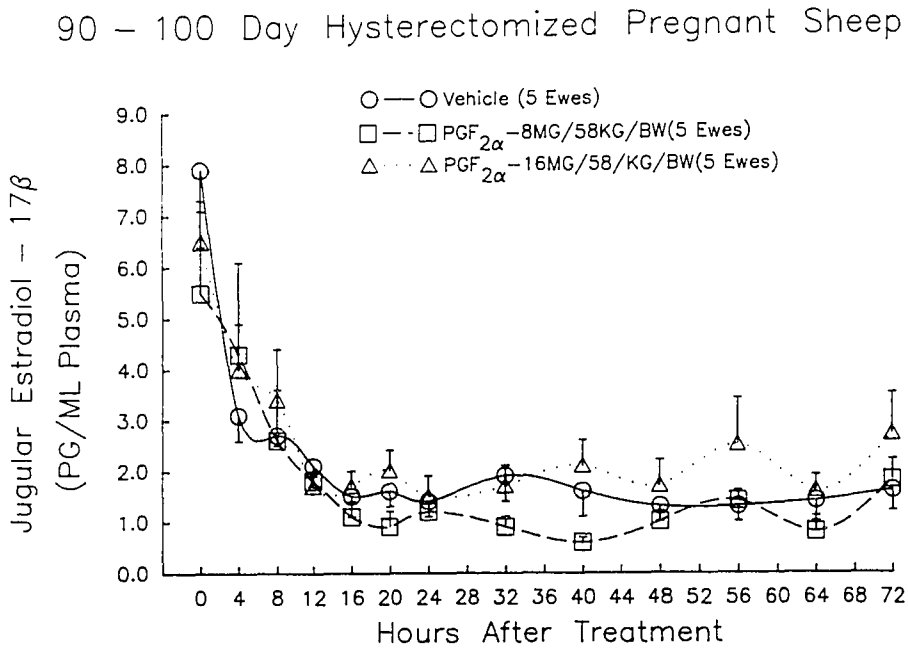

FIGURE 15. PROFILES OF ESTRADIOL-17ß (LEAST SQUARE MEANS ± S.E.M.) IN JUGULAR VENOUS PLASMA OF HYSTERECTOMIZED 90-100 DAY PREGNANT SHEEP TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

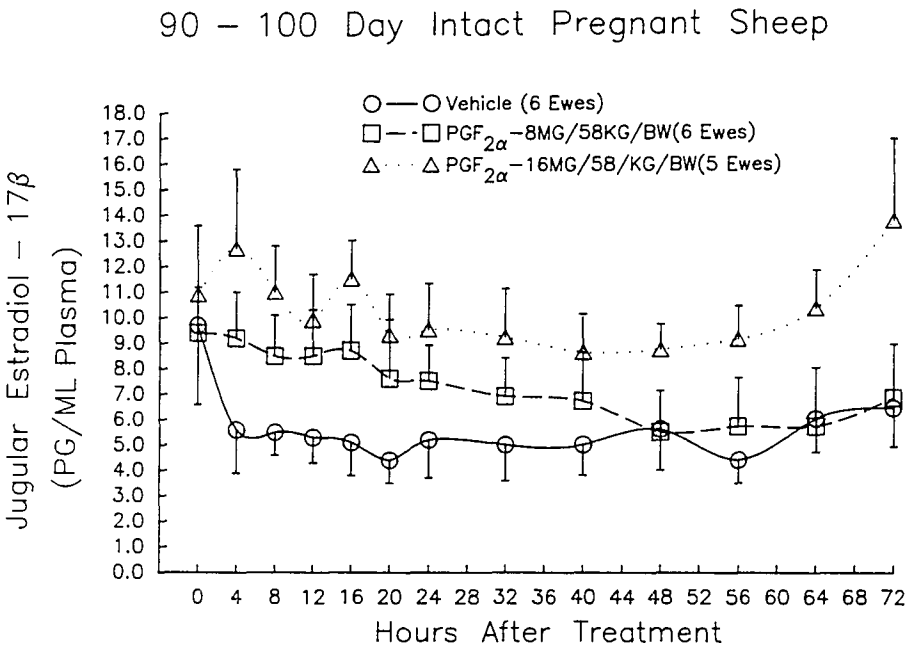

FIGURE 16. PROFILES OF ESTRADIOL-17ß (LEAST SQUARE MEANS ± S.E.M.) IN JUGULAR VENOUS PLASMA OF INTACT 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TREATMENT ($P \leq 0.10$), AND LINEAR ($P \leq 0.05$) AND QUADRATIC ($P \leq 0.10$) EFFECTS FOR TIME.

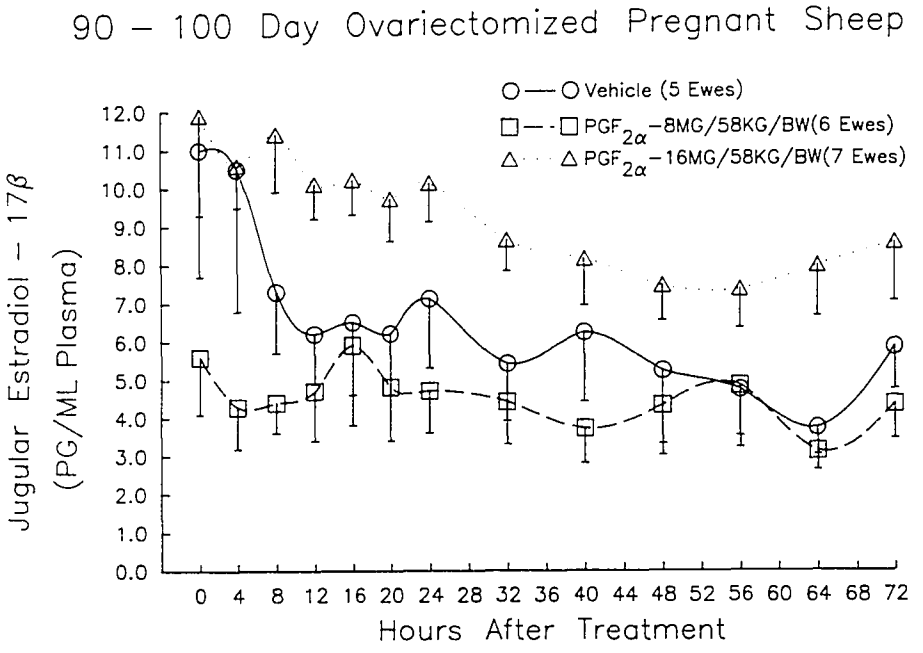

FIGURE 17. PROFILES OF ESTRADIOL-17ß (LEAST SQUARE MEANS ± S.E.M.) IN JUGULAR VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TREATMENT ($P \leq 0.10$), AND LINEAR ($P \leq 0.05$) AND QUADRATIC ($P \leq 0.10$) EFFECTS FOR TIME.

jugular venous blood of 16 mg $PGF_2\alpha$/58 kg/BW-treated ovariectomized 90 to 100 day pregnant ewes differed ($P \leq 0.10$) from control pregnant ewes or those treated with 8 mg $PGF_2\alpha$/58 kg/BW ($P \leq 0.05$; Fig.17). There was a decline ($P \leq 0.05$) in estradiol-17ß with time in control and 16 mg $PGF_2\alpha$/58 kg/BW-treated ewes and a trend ($P \leq 0.10$) for a quadratic increase toward the end of the 72 hour treatment period.

In experiment 4, secretion of estradiol-17ß by placentome slices *in vitro* from 90 to 100 day intact pregnant ewes averaged 3.2 pg/ml/100 mg tissue during the 4 hour culture and ranged from 1.9 to 7.6 pg/ml/100 mg tissue across all treatment groups (Fig.18). Addition of androstenedione to the culture medium increased ($P \leq 0.05$) secretion of estradiol-17ß by placentome slices, but was not affected by indomethacin, $PGF_2\alpha$ or their interactions during the 4 hour incubation period ($P \geq 0.05$, Fig.18).

In experiment 5, profiles of estradiol-17ß in jugular venous blood did not differ ($P \geq 0.05$) among treatment groups from 0 to 72 hours. There was a treatment ($P \leq 0.08$) and time and treatment x time effect ($P \leq 0.05$) on profiles of estradiol-17ß in jugular venous blood of lutectomized 90 to 100 day pregnant ewes over the 72 to 168 hour treatment period (Figs. 19 and 20). Profiles of estradiol-17ß in jugular venous blood did not differ ($P \geq 0.05$) over the 72 to 168 hour treatment period of lutectomized 90 to 100 day pregnant ewes treated with vehicle, cortisol or cortisol + $PGF_2\alpha$ (Figs. 19 and 20). Profiles of estradiol-17ß in jugular venous blood were different ($P \leq 0.05$) in trilostane, trilostane + cortisol, and trilostane + cortisol + $PGF_2\alpha$; and in trilostane + $PGF_2\alpha$

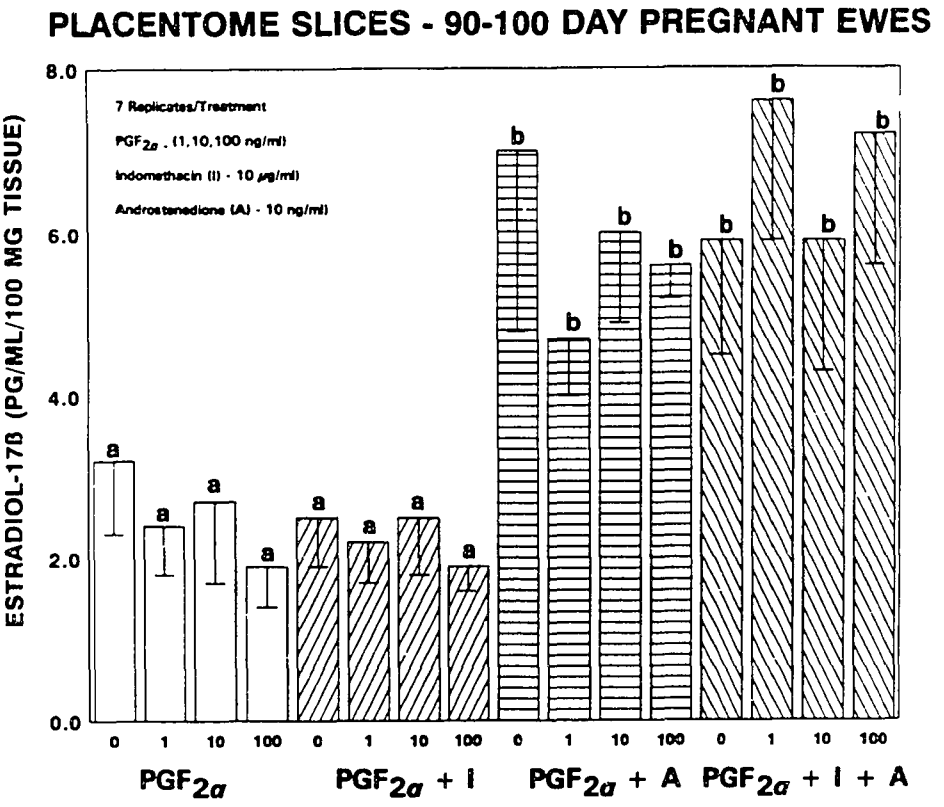

FIGURE 18. MEANS ± S.E.M. FOR SECRETION OF ESTRADIOL-17ß *IN VITRO* BY PLACENTOME SLICES FROM INTACT 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$), INDOMETHACIN (I) AND/OR ANDROSTENEDIONE (A). DIFFERENCES WERE SEEN FOR TREATMENT ($P \leq 0.05$).

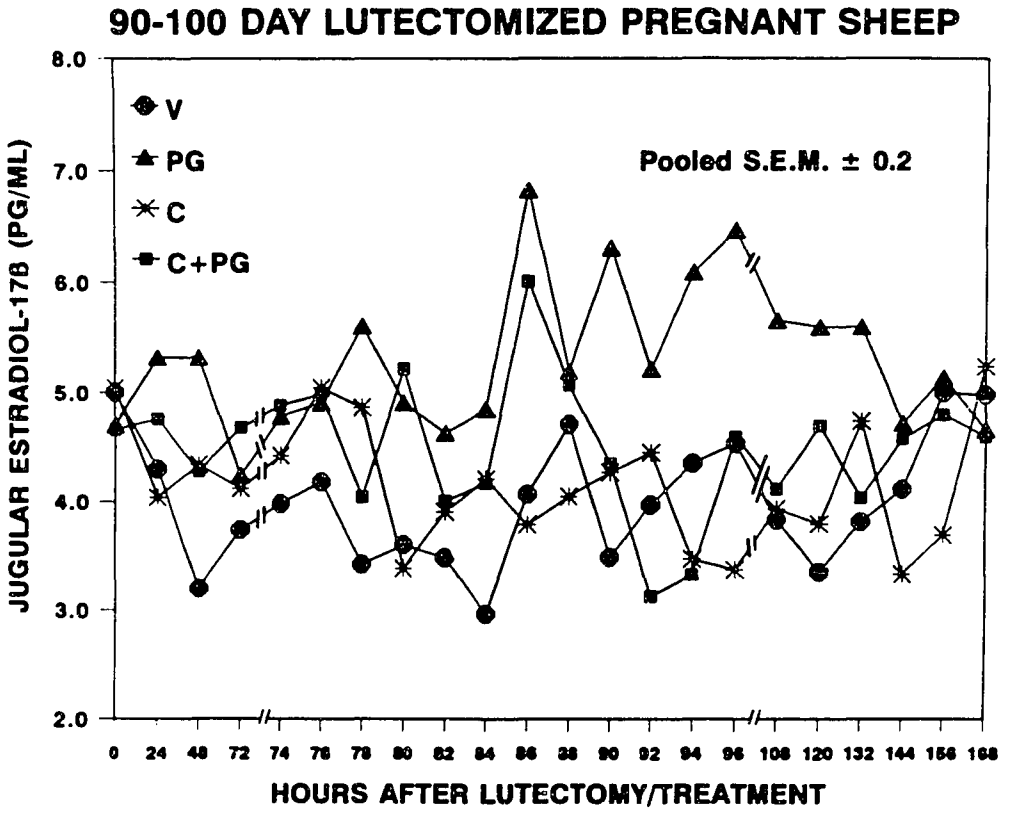

FIGURE 19. PROFILES OF ESTRADIOL-17ß (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF LUTECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH VEHICLE (V), CORTISOL (C), PROSTAGLANDIN $F_{2\alpha}$ (PG; 16 MG/58 KG/B.W.), OR CORTISOL + PROSTAGLANDIN $F_{2\alpha}$ (C+PG). DIFFERENCES WERE SEEN FOR TREATMENT AND TIME ($P \leq 0.05$).

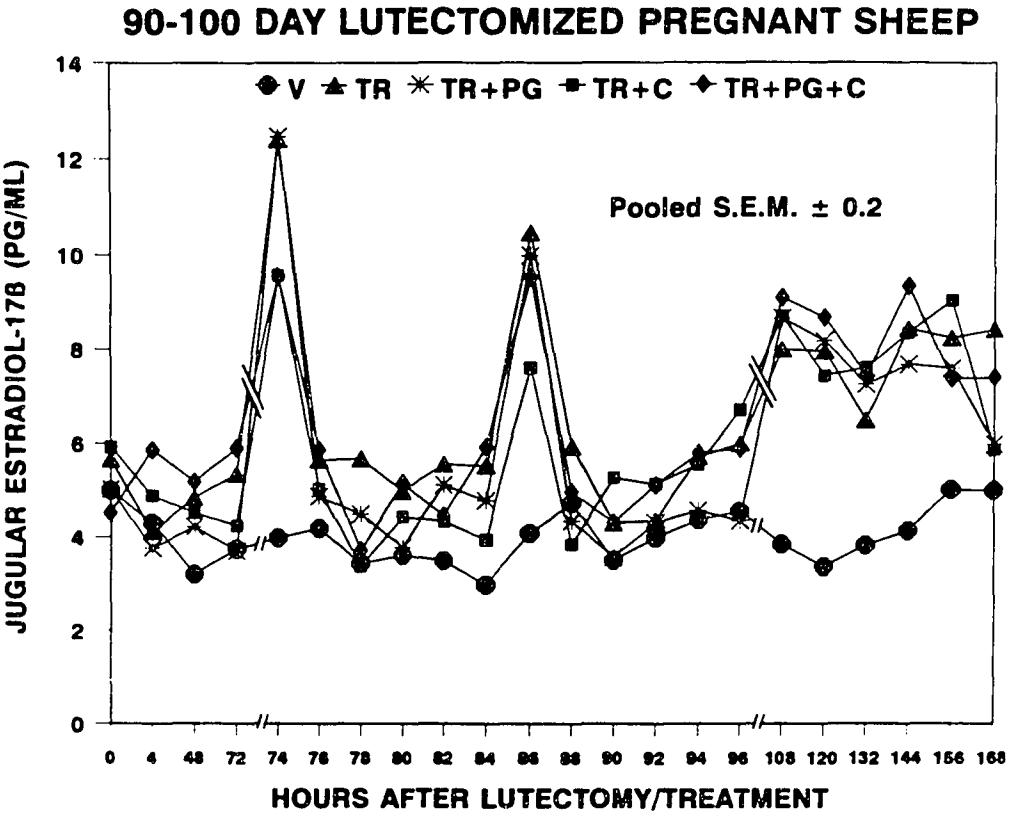

FIGURE 20. PROFILES OF ESTRADIOL-17ß (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF LUTECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH VEHICLE (V), TRILOSTANE (TR), TRILOSTANE + PROSTAGLANDIN $F_{2\alpha}$ (TR+PG), TRILOSTANE + CORTISOL (TR+C), OR TRILOSTANE + PROSTAGLANDIN $F_{2\alpha}$ + CORTISOL (TR+PG+C). DIFFERENCES WERE SEEN FOR TREATMENT, TIME, AND TREATMENT x TIME ($P \leq 0.05$).

($P \leq 0.08$)-treated ewes when compared to control pregnant ewes over the 72 to 168 hour treatment period. Concentrations of estradiol-17ß in jugular venous blood of lutectomized 90 to 100 day pregnant ewes were increased ($P \leq 0.05$) at 74 and 86 hours, two hours after infusion of trilostane, trilostane + $PGF_2\alpha$, trilostane + cortisol and trilostane + cortisol + $PGF_2\alpha$ treatments and returned to levels not different ($P \geq 0.05$) from controls 2 hours later at 76 and 88 hours (Fig.20). This was followed by a sustained increase ($P \leq 0.05$) in concentrations of estradiol-17ß in jugular venous blood starting at 108 hours in trilostane, trilostane + PGFα, trilostane + cortisol and trilostane + cortisol + $PGF_2\alpha$-treated lutectomized 90 to 100 day pregnant ewes (Fig.20).

4.8 PREGNANCY SPECIFIC PROTEIN B (PSPB)

Concentrations of PSPB in uterine venous plasma of control 90 to 100 day intact pregnant ewes over the 72 hour treatment period averaged 51 ng/ml. There were treatment ($P \leq 0.05$) and time ($P \leq 0.05$) effects on profiles of PSPB in uterine venous plasma of intact 90 to 100 day pregnant ewes (Fig.21). Profiles of PSPB in uterine venous blood of 90 to 100 day intact pregnant ewes treated with 16 mg $PGF_2\alpha$/58 kg/BW averaged 78 ng/ml and differed ($P \leq 0.05$) when compared to vehicle-treated intact 90 to 100 day pregnant ewes (Fig.21). Concentrations of PSPB in uterine venous blood of intact 90 to 100 day pregnant ewes were increased ($P \leq 0.05$) at 64 hours after treatment with 8 mg $PGF_2\alpha$/58 kg/BW (Fig.21). There was a quadratic increase ($P \leq 0.09$) in PSPB in uterine venous plasma

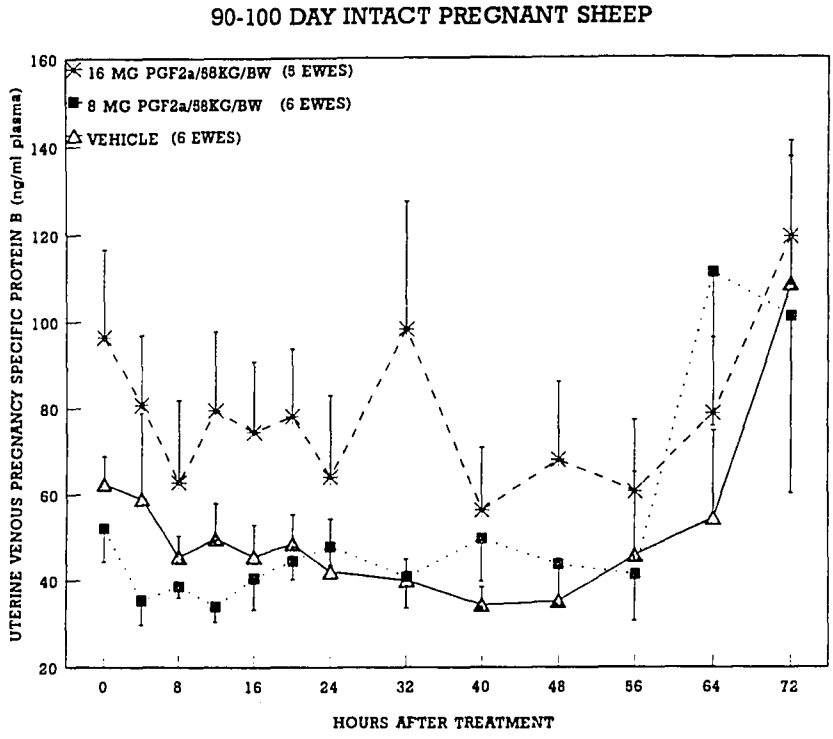

FIGURE 21. PROFILES OF PREGNANCY SPECIFIC PROTEIN B (PSPB; LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE PLASMA OF INTACT 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TREATMENT AND TIME ($P \leq 0.05$), AND QUADRATIC EFFECTS FOR TIME ($P \leq 0.09$).

of control, 8 and 16 mg $PGF_2\alpha$/58 kg/BW-treated intact 90 to 100 day pregnant ewes toward the end of the 72 hour sampling period.

Concentrations of PSPB in uterine venous plasma of control 90 to 100 day ovariectomized pregnant ewes over the 72 hour treatment period averaged 90 ng/ml (Fig.22). There was a trend for a quadratic increase ($P \leq 0.10$) in PSPB in uterine venous plasma of the 8 and 16 mg $PGF_2\alpha$/58 kg/BW-treated ovariectomized 90 to 100 day pregnant ewes toward the end of the 72 hour sampling period (Fig.22).

4.9 CORTISOL

Concentrations of cortisol in jugular venous blood of hysterectomized (Experiment 1, Fig.23), intact (Experiment 2, Fig.24) and ovariectomized (Experiment 3, Fig.25) 90 to 100 day pregnant ewes declined over time ($P \leq 0.05$), but did not differ ($P \geq 0.05$) among treatment groups within experiments. Concentrations of cortisol in jugular venous plasma declined ($P \leq 0.05$) from 65 μg/dl at 0 hour to a low of 10 μg/dl by 24 hours in all three treatment groups of hysterectomized 90 to 100 day pregnant ewes (Fig.23). Concentrations of cortisol in jugular venous blood decreased ($P \leq 0.05$) from 58 μg/dl at 0 hour to 18 μg/dl by 12 hours in intact 90 to 100 day pregnant ewes (Fig.24). There was a quadratic increase ($P \leq 0.05$) in cortisol in jugular venous plasma of intact 90 to 100 day pregnant ewes treated with 8 mg $PGF_2\alpha$/58 kg/BW (Fig.24). Concentrations of cortisol in jugular venous plasma decreased ($P \leq 0.05$) from 57 μg/dl at 0 hour to 12 μg/dl by 20 hours in all three treatment groups of ovariectomized 90 to 100 day pregnant ewes and did not change

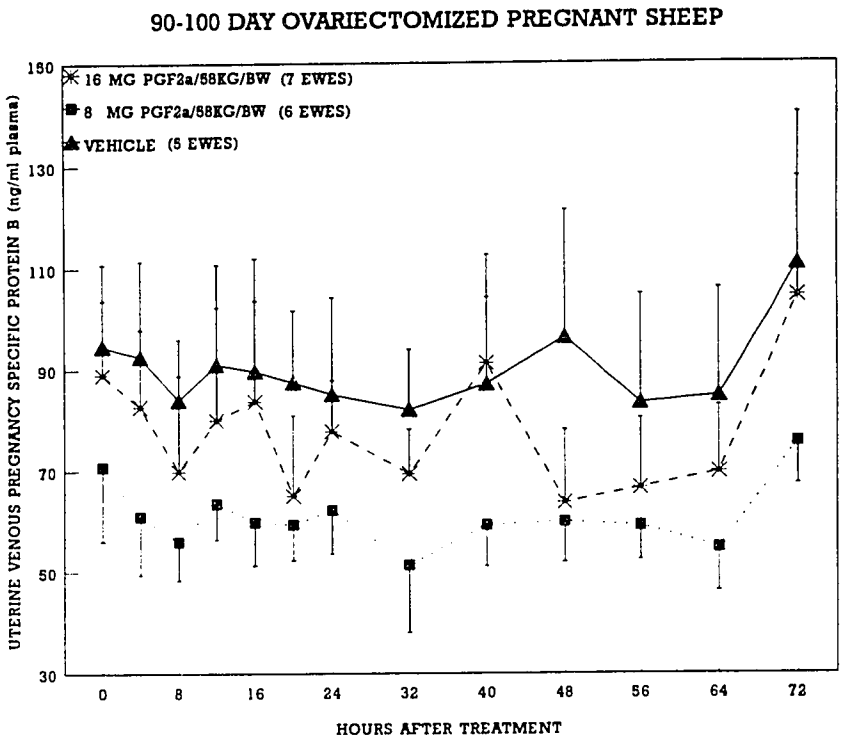

FIGURE 22. PROFILES OF PREGNANCY SPECIFIC PROTEIN B (PSPB; LEAST SQUARE MEANS $\pm$ S.E.M.) IN UTERINE VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.10$).

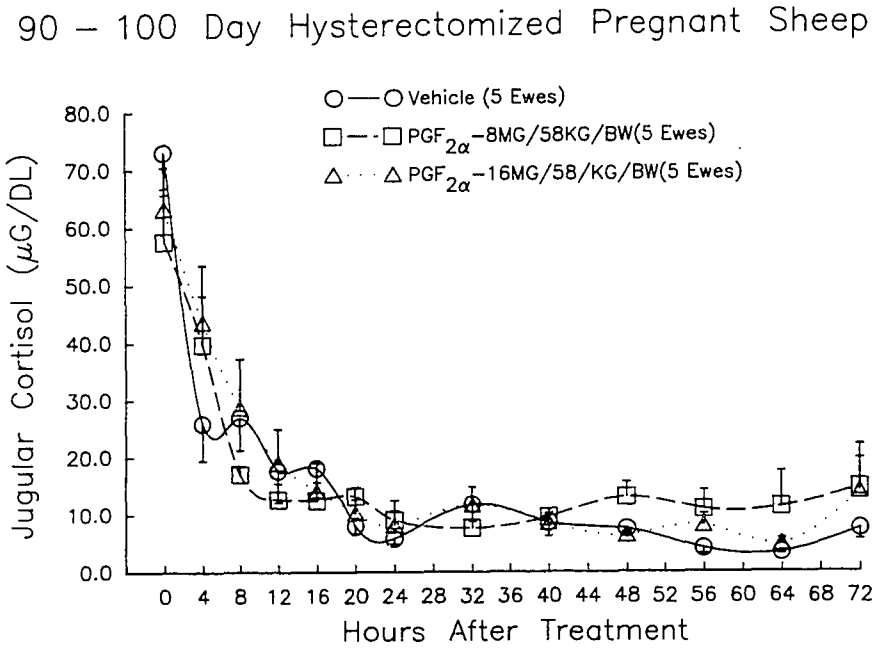

FIGURE 23. PROFILES OF CORTISOL (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF HYSTERECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

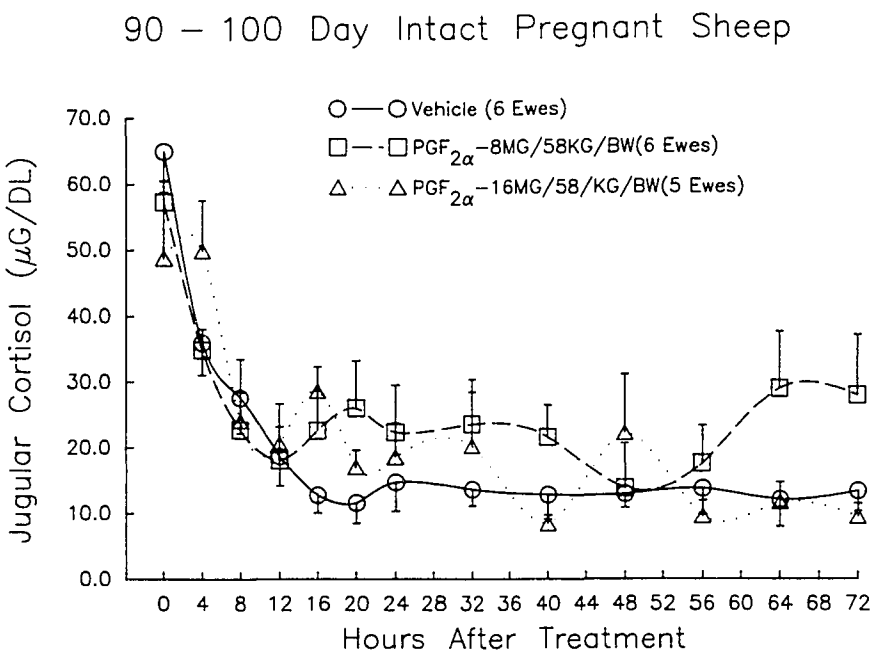

FIGURE 24. PROFILES OF CORTISOL (LEAST SQUARE MEANS ± S.E.M.) IN JUGULAR VENOUS PLASMA OF INTACT 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$). LINEAR AND QUADRATIC EFFECTS WERE SEEN FOR TIME ($P \leq 0.05$).

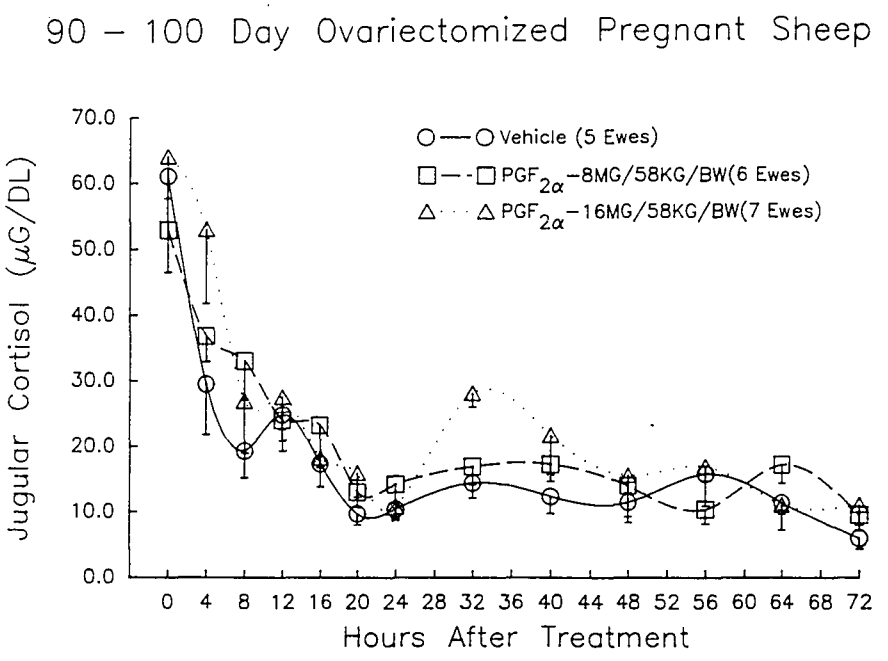

FIGURE 25. PROFILES OF CORTISOL (LEAST SQUARE MEANS $\pm$ S.E.M.) IN JUGULAR VENOUS PLASMA OF OVARIECTOMIZED 90-100 DAY PREGNANT EWES TREATED WITH PROSTAGLANDIN $F_{2}\alpha$ ($PGF_{2}\alpha$). DIFFERENCES WERE SEEN FOR TIME ($P \leq 0.05$).

($P \geq 0.05$) thereafter in the vehicle or 8 mg $PGF_2\alpha$/58 kg/BW treatment groups, but tended to increase ($P \leq 0.10$) at 32 hours in the 16 mg $PGF_2\alpha$/58 kg/BW treatment group (Fig.25).

CHAPTER 5

DISCUSSION

Prostaglandin $F_{2\alpha}$ did not induce abortion in 90 to 100 day pregnant ewes at a dose of 8 or 16 mg/58 kg/BW in the presence or absence of the ovaries within the 72 hour experimental period or 16 mg $PGF_{2\alpha}$/58 kg/BW in the 96 hour experimental period of lutectomized pregnant ewes. These data are supported by others where abortion was not induced with a dose of 8.12 mg $PGF_{2\alpha}$/58 kg/BW given on day 74 to intact pregnant ewes. These ewes had a normal gestation length (992). In addition, 8 mg $PGF_{2\alpha}$/58 kg/BW given on day 85 of gestation in sheep did not induce abortion within 64 hours (973). When $PGF_{2\alpha}$ is given before day 60 of gestation, ewes abort but not when $PGF_{2\alpha}$ is given between days 60 to 120 of gestation (Miguel Garcia-Winder, personal communication, unpublished data). When $PGF_{2\alpha}$ is given on day 135, premature parturition is induced in sheep (854, 859-861; Miguel Garcia-Winder, personal communication, unpublished data). These data suggest that the uterus changes its responsiveness to $PGF_{2\alpha}$ around day 135 in preparation for parturition. The data reported herein are in agreement with some studies in the pregnant cow. In intact (686, 959, 969) or ovariectomized cows (686, 965, 969), pregnancy is not terminated by $PGF_{2\alpha}$ between 150 and 250 days of gestation. In addition, oxytocin given to pregnant cows on day 150 of pregnancy increases PGFM in jugular venous blood and regresses the CL in 67 percent of the cows. All fetuses, however, are carried to term. Oxytocin induces abortion in cows when given on day 60 of pregnancy

(993). The data in my studies conflict with others who state that sheep are responsive to the abortifacient actions of any prostaglandin at any stage of gestation (731). However, these authors offer no data or references to data to substantiate their statement. Abortions are induced in some studies with $PGF_2\alpha$ in cattle, but these reports are either on one cow (957), cows with different pathological conditions (962-964) or occur when treatments are given before day 130 of pregnancy (959, 965, 968).

Abortion was induced with trilostane given at 12 hour intervals by 60 hours in all 90 to 100 day pregnant ewes lutectomized 72 hours before the initiation of the treatments. Trilostane is an inhibitor of 3ß-HSD and blocks the conversion of pregnenolone to progesterone (890, 891). A single intrajugular injection of trilostane given to intact day 136 (890) or 146 (891) pregnant sheep decreases progesterone in jugular venous blood and advances parturition in most ewes. However, a single intrajugular injection of trilostane given on days 118 to 130 at doses of 25 to 200 mg caused abortion in only 2 of 15 ewes even though concentrations of progesterone in jugular venous plasma were decreased by 70 percent for 12 hours (984). Blockage of the progesterone receptor with RU-486, advances parturition when it is given at 135 to 140 days of pregnancy in sheep (983). Progesterone in jugular venous blood of sheep is not decreased with RU-486 (983). Pregnancy in women is terminated by RU-486 at any stage of gestation (982). The interval to abortion is decreased and the number of abortions is increased when $PGF_2\alpha$ is given along with RU-486 in women (982) or when oxytocin is given with RU-486 in sheep (983). The effect on pregnancy of RU-486 alone in these sheep was not tested (983). It is

concluded that sheep are resistant to abortifacient actions of $PGF_2\alpha$ between days 60 to 120 of gestation.

It is suggested that multiple injections of trilostane are required to terminate pregnancy in sheep prior to day 135 of pregnancy. This may be due to the effect of a single injection of trilostane lasting for only a short period. Estradiol-17ß was increased 2 hours after trilostane was given, but estradiol-17ß decreased by 4 hours after trilostane. It is suggested that the change in the ratio of estradiol-17ß:progesterone by trilostane increased oxytocin receptors to result in abortion (876, 887, 888). Trilostane effects on pregnancy prior to day 90 in sheep have not tested. Whether or not $PGF_2\alpha$ or trilostane terminates pregnancy may be due to differences in the species or animal model (intact, ovariectomized, lutectomized) used, frequency of injections, dose, site(s) of action (ovary, placenta, adrenal, hypothalamo-hypophyseal axis) or other changes that may occur within the CL or placenta.

Although $PGF_2\alpha$ did not terminate pregnancy in intact or ovariectomized 90 to 100 day pregnant ewes, both the 8 and 16 mg $PGF_2\alpha$/58 kg/BW treatment doses decreased weights of CL and progesterone in ovarian venous plasma in intact and hysterectomized 90 to 100 day pregnant ewes. This indicates that corpora lutea from these 90 to 100 day pregnant ewes are responsive to the luteolytic actions of $PGF_2\alpha$. One ewe in the 16 mg $PGF_2\alpha$/58 kg/BW hysterectomized treatment group did not respond to $PGF_2\alpha$. This is seen in the variance in jugular and ovarian venous progesterone and mean luteal weights when compared to the 8 mg $PGF_2\alpha$/58 kg/BW-treated hysterectomized ewes. Treatment with 8.12 mg

$PGF_2\alpha$/58 kg/BW on day 74 of pregnancy reduces progesterone in jugular venous blood of ewes, but pregnancy is maintained. The reduction in progesterone was presumed to be through a loss of luteal function (992). At day 85 of gestation, 8 mg $PGF_2\alpha$/58 kg/BW did not decrease jugular venous progesterone below 1 ng/ml in intact or hysterectomized ewes within 64 hours (973). The minimum effective dose of $PGF_2\alpha$ to reduce luteal weights and to cause a reduction in jugular venous progesterone has not been determined at 90 to 100 days of gestation. The minimum effective dose of $PGF_2\alpha$ to cause a reduction in jugular venous progesterone on day 13 of pregnancy in ewes with a single CL is 6 mg/58 kg/BW (253, 372). This resistance to $PGF_2\alpha$ (522) or its synthetic analogue (523) is lost between days 16 and 26 of pregnancy in sheep and could be due to changes in numbers of receptors to $PGF_2\alpha$ on CL. However, numbers of receptors for $PGF_2\alpha$ on luteal membranes through day 40 of pregnancy in ewes are similar to those seen on luteal membranes from nonpregnant ewes at midcycle (519). Thus, ovine corpora lutea presumably retain receptors throughout gestation since they are responsive to $PGF_2\alpha$ at early (253), middle (992) and late (731) stages of gestation. Bovine CL appear to be responsive through day 250 of pregnancy in cattle (688, 968).

Corpora lutea of 90 to 100 day pregnant ewes were functional at the time of treatment since both the 8 or 16 mg $PGF_2\alpha$/58 kg/BW treatment doses reduced concentrations of progesterone in ovarian venous plasma of intact and hysterectomized ewes. Further support that these CL were functional is seen in the vehicle-treated hysterectomized 90 to 100 day pregnant ewes where jugular venous progesterone remained above 4

ng/ml throughout the 72 hour sampling period. These concentrations of progesterone in the vehicle-treated 90 to 100 day hysterectomized pregnant ewes are similar to concentrations of jugular venous progesterone at midcycle in nonpregnant ewes when the CL secretes maximal amounts of progesterone (102, 115). Corpora lutea appear to contribute 40 to 50 percent of the circulating progesterone at 90 to 100 days of gestation based on the relative decreases in progesterone in jugular venous blood of vehicle-treated hysterectomized and ovariectomized pregnant ewes after hysterectomy or ovariectomy of 90 to 100 day pregnant ewes presented herein. However, a functional CL is not necessary to maintain pregnancy after day 55 in sheep (441, 634, 636). A single CL at 90 to 100 days of gestation averages around 550 mg and secretes more progesterone per unit mass than the approximately 100 placentomes (596, 597) which average in excess of 6 g each. Corpora lutea of pregnant sheep may be functional through day 120 since luteal weights, content and concentration of progesterone in CL, luteal volume density and luteal cell numbers are not different from midcycle nonpregnant ovine CL (652). Corpora lutea of pregnant sheep appear to undergo regression between days 133 to 142 since cytochrome p450 Scc enzyme and adrenodoxin in CL are 20 and 12 percent, respectively, at day 133 when compared to CL of nonpregnant ewes at midcycle (653). Structural evidence of luteal regression is seen by day 142 (653). Collectively, data in the present experiments indicate that ovine CL are functional at 90 to 100 days of gestation and through day 120 of pregnancy based on other data (652, 653). The data presented within these studies and the data cited above conflict with suggestions by other

investigators (639, 654) that secretion of progesterone by ovine CL declines after day 60. This conflict is probably due to the use of the autotransplanted ovarian model with only three ewes where vascular anastomosis of a smaller artery and vein (ovarian) to a larger artery and vein (carotid, jugular) is difficult and to the use of a different experimental model and limited animals and samples (639).

Progesterone did not decrease below 5 ng/ml in jugular or 30 ng/ml in uterine venous blood of vehicle, 8 or 16 mg PGFα-treated intact or ovariectomized 90-100 day pregnant ewes. This indicates that the placenta is functional. Further support that the placenta is functional is seen from the data that progesterone decreases earlier in jugular than in uterine venous blood in $PGF_2\alpha$-treated intact or in vehicle or $PGF_2\alpha$-treated ovariectomized 90 to 100 day pregnant ewes, but never below 30 ng/ml in uterine venous plasma. Also, further support that the placenta is functional comes from the in vitro data where placentome slices secreted significant concentrations of progesterone. The placenta appears to produce approximately one half of the circulating progesterone at 90 to 100 days of gestation in the ewe based on the changes in concentrations of progesterone in jugular and uterine venous blood of the hysterectomized and ovariectomized 90 to 100 day pregnant ewes. Bovine placenta at 200 days of gestation converts radiolabelled pregnenolone to progesterone *in vitro* only when regression of CL has been induced *in vivo* (688). This suggests that the bovine placenta does not secrete significant quantities of progesterone *in vivo* in the presence of a functional CL, which differs from the in vivo data on 90 to 100 day pregnant ewe presented herein.

Ovariectomy of ewes without progesterone replacement therapy prior to day 55 of pregnancy results in abortion or resorption of the fetus, while ovariectomy after day 55 of pregnancy permits gestation to proceed to term in most ewes (441, 634, 636). In ewes ovariectomized between days 25 to 34 of pregnancy can be maintained by the synthetic progestin, medroxyprogesterone acetate (631). In these ewes, progesterone remains less than 1 pmol/ml in jugular venous blood, increases to 2 to 3 pmol/ml by days 60 to 90 and then increases rapidly from 3 pmol/ml on day 90 to a peak greater than 8 pmol/ml by day 120 (631, 632, 640, 642, 643). Removal of silastic implants containing progesterone in days 89 to 94 postbreeding does not terminate gestation in pregnant ewes ovariectomized between days 7 to 67 of gestation (117). Patterns and concentrations of progesterone after day 60 in jugular venous blood of intact pregnant sheep are similar to those seen in jugular venous blood of ewes ovariectomized on day 60 of pregnancy during the last 90 days of gestation (631, 632, 640, 642, 643) except in Barbary sheep (1033). The placenta appears to compensate for the loss of luteal progesterone. However, the placenta of some sheep may not produce sufficient quantities of progesterone or other products to maintain pregnancy in the absence of a functional CL since 4 of 44 ewes lutectomized for the trilostane experiment aborted within 72 hours and before the initiation of the trilostane treatments. Progesterone in jugular venous blood of Barbary sheep does not increase after day 60 of pregnancy (1033). In addition, the placenta of the one ewe each in the intact vehicle, 8 or 16 mg $PGF_2\alpha$/58 kg/BW and the one ewe in the ovariectomized 16 mg $PGF_2\alpha$/58 kg/BW 90 to 100 day pregnant ewe treatment groups that

aborted was apparently unable to secrete sufficient progesterone or other placental products to maintain pregnancy. This is further supported by the lack of an effect of $PGF_2\alpha$ to decrease placental secretion of progesterone below 30 ng/ml in uterine venous plasma in the intact or ovariectomized pregnant ewes or to inhibit secretion of progesterone by placental slices *in vitro* in experiments 2 to 4. Receptors for $PGF_2\alpha$ on placental tissue of sheep are low during gestation in sheep, but contain many receptors for PGE_2 (727, 728, 730-732, 735, 802, 803, 811, 812). Receptors for oxytocin on caruncular and cotyledonary tissue of cows is low and increases at parturition (887, 888). Since the uterus of $PGF_2\alpha$-treated ewes was tight rather than flaccid, the myometrium but not the placenta appears to respond to $PGF_2\alpha$.

Placental secretion of progesterone is resistant to the luteolytic actions of $PGF_2\alpha$ at the doses tested *in vivo* and *in vitro*. Profiles of progesterone in jugular or uterine venous plasma were not different in vehicle or $PGF_2\alpha$-treated intact or ovariectomized 90 to 100 day pregnant ewes over the 72 hour sampling period. In addition, $PGF_2\alpha$ did not inhibit secretion of progesterone by placental slices *in vitro* and uterine venous progesterone did not decrease below 30 ng/ml in uterine venous or below 5 ng/ml in jugular venous plasma of intact or ovariectomized pregnant ewes over the 72 hour sampling period. The decrease in progesterone in jugular or uterine venous plasma over the first 20 to 48 hours in vehicle and $PGF_2\alpha$-treated ewes may be due to the elevated levels of cortisol as a result of surgical stress (768). Basal concentrations of cortisol throughout pregnancy in sheep are around 20 μg/dl (763-765) and increase at parturition (766-

769). Cortisol in jugular venous blood of intact, ovariectomized or hysterectomized 90 to 100 day pregnant ewes in all three treatment groups was approximately 80 μg/dl at 0 hour and declined to concentrations around 20 μg/dl a few hours after surgery. These elevated levels of cortisol may have potentiated the vasoconstrictive effects of catecholamines on the uterine arteries and reduced blood flow to the uterus (745, 748, 752, 755). Catecholamines cause vasoconstriction of uterine arteries *in vivo* in unanesthesized pregnant sheep and decreases blood flow to the uterus (347, 348, 352, 359, 362). This may explain why multiple injections of large doses of dexamethasone alone (25 mg) every 6 hours for 5 days during midgestation in unanesthesized sheep terminates pregnancy in 40 percent of the ewes (871, 952). In addition, corticosteroids inhibit placental secretion of progesterone *in vitro* and *in vivo* (969, 970) and have been reported to increase placental estrogen secretion *in vivo* (994, 995).

The increase in uterine venous progesterone toward the end of the 72 hour sampling period of intact or ovariectomized 90 to 100 day pregnant ewes suggests a factor(s) is blocking the actions of $PGF_2\alpha$ and/or stimulating secretion of progesterone by the ovine placenta. These factors may be PGE_1 and/or PGE_2. Prostaglandin E_1 or PGE_2 given during the estrous cycle of sheep are antiluteolytic and extends luteal function (555-564). In addition, embryos produce PGE_2 *in vitro* and concentrations of PGE_2 increase in uterine venous plasma during early pregnancy (286, 287, 505, 508, 545, 546, 548, 549). Further support is seen from data on hypophysectomy or ovariectomy at different stages of gestation. Removal of the maternal hypophysis of sheep on day 60 of gestation results in

maintenance of CL weights at 75 percent of controls and pregnancy is not terminated. However, hypophysectomy earlier than day 60 causes luteal regression and abortion, but the time required for luteal regression to occur is prolonged as the day when hypophysectomy is performed is closer to day 60 of pregnancy in ewes (131, 133-135, 634-638). In addition, ovariectomy after day 55 of pregnancy does not terminate pregnancy in ewes, while ovariectomy before day 55 results in abortion (4, 441). These data on hypophysectomy and ovariectomy during pregnancy in ewes suggests that the fetus and/or placenta secretes a luteotropin to sustain luteal and placental secretion of progesterone independent of the maternal hypophysis. The ratio of PGE to $PGF_{2}\alpha$ reported herein supports this hypothesis.

Since $PGF_{2}\alpha$ did not inhibit placental secretion of progesterone *in vitro* or *in vivo* at the doses tested, other factors may be necessary for $PGF_{2}\alpha$ to inhibit placental secretion of progesterone or PGE stimulates secretion of factor(s) that block the actions of $PGF_{2}\alpha$ on the placenta. Factor(s) from the placenta of pregnant ewes have been demonstrated to inhibit secretion of $PGF_{2}\alpha$ by nonpregnant ovine endometrium (580, 731). Alternatively, other factors may be necessary for $PGF_{2}\alpha$ to reduce placental secretion of progesterone such as cortisol, estradiol-17ß, relaxin, or oxytocin. Increases in cortisol, relaxin, estradiol-17ß, $PGF_{2}\alpha$, PGE_{2}, and oxytocin in jugular or uterine venous blood are seen prior to the decline in progesterone for the initiation of parturition (859-869, 874). However, concentrations of estradiol-17ß were increased *in vivo* or *in vitro* and $PGF_{2}\alpha$ did not decrease placental secretion of progesterone. Since progesterone in uterine venous plasma did not decrease below 30 ng/ml, this probably

prevented $PGF_2\alpha$ from inducing abortion in intact and ovariectomized 90 to 100 day pregnant ewes. Exogenous progesterone given to ewes with parturition initiated 2 weeks prior to anticipated lambing blocks parturition, although increases in $PGF_2\alpha$ were seen in the maternal contributions to the placenta and in the myometrium (860, 885). Thus, successful induction of abortion occurs only when secretion of progesterone and the mechanism(s) through which progesterone works to inhibit fetal expulsion to overcome the progesterone block proposed originally in the pregnant rat (996).

Trilostane given intrajugular every 12 hours starting 72 hours after lutectomy induced abortions in all 90 to 100 day lutectomized pregnant ewes and decreased progesterone in jugular venous plasma. Trilostane inhibits the conversion of pregnenolone to progesterone by 3ß-HSD (891). Progesterone of luteal or placental origin is necessary throughout gestation to maintain pregnancy (440, 441). A single injection of trilostane given on days 136 (984) or 146 (890, 891) to intact pregnant ewes decreases progesterone in jugular venous plasma by 75 percent and advances parturition in most ewes. However, a single intrajugular injection of trilostane given on days 118 to 130 of pregnancy at doses of 25 to 200 mg caused abortion in only 2 of 15 ewes, although progesterone in jugular venous blood was decreased within 2 hours by 70 percent and for 12 hours after the treatment (984). These two experiments in intact pregnant ewes had a limited number of animals per treatment dose and did not include control injections of the vehicle, DMSO (890, 891, 984). In my study, progesterone in jugular blood of 90 to 100 day lutectomized ewes given trilostane 72 hours after lutectomy was not decreased as much as that seen

for the 70 percent reduction by single injections of trilostane in intact pregnant ewes between day 118 to 146 of gestation (890, 891, 984). This suggests either that the 3ß-HSD in luteal tissue is more sensitive to trilostane than 3ß-HSD in the placenta or that inhibition of placental production of progesterone by trilostane results in a diffusion gradient of progesterone from placental tissue to the blood compartment in the absence of a luteal source of progesterone to cause abortion. There is evidence that 3ß-HSD for conversion of pregnenolone to progesterone is different from 3ß-HSD for the conversion of dehydro-epiandrosterone to androstenedione (Ian Mason, personal communoication, unpublished data). This is further supported by the increase in estradiol-17ß but decrease in progesterone seen after trilostane in my studies, presumably by pregnenolone being diverted to the "delta-five" pathway. Whether 3ß-HSD for conversion of pregnenolone to progesterone differs in the CL and placenta is unknown. Since trilostane was given 12 hours apart in the present study, this may explain the reduced success of abortion in ewes treated with a single injection of trilostane between days 118 to 130 of pregnancy. The greater success of abortion in ewes treated with a single injection of trilostane at 136 to 146 days of pregnancy in the presence of the ovaries (890, 891) than at earlier stages of pregnancy (984) may be facilitated by the increased size of the fetus, placenta and fetal fluid content in the uterus at the end of pregnancy to serve as a mechanical stimulus when progesterone is decreased. The uterus appears to be prepared for parturition by day 100 of pregnancy in sheep. The content of actin and myosin in uterine smooth muscle doubles by day 100 of gestation in sheep and does not change for

the duration of gestation (Charles Rosenfeld, personal communication, unpublished data). Myosin light chain kinase which phosphorylates actin and myosin to cause smooth muscle contractions (803-812) changes from predominantly a myosin light chain kinase-I to myosin light chain kinase-II in uterine smooth muscle of sheep during pregnancy and is not reversed until the postpartum period (Charles Rosenfeld, personal communication, unpublished data). However, differences in the ability of myosin light chain kinase-I and -II to phosphorylate myosin in uterine smooth muscle and cause uterine contraction are not known.

The failure of $PGF_2\alpha$ and the success of trilostane to cause abortion in 90 to 100 day pregnant ewes may be related to the lack of $PGF_2\alpha$ to reduce progesterone and to alter gap junctions in the myometrium. Gap junctions are regions on cell membranes where channels form between adjacent cells permitting the exchange of small ions and metabolites (803). Gap junctions appear to be formed by the protein, connexin-43, in each membrane of adjacent cells (806). Gap junctions are low resistance pathways between cells, thereby increasing electical coupling between two adjacent cells (803, 807). During pregnancy, gap junctions are either undetectable or present in low numbers in the uterine myometrium (804-806, 809). At parturition, numbers and the size of gap junctions increase and are associated with increased electrical conductivity of the myometrium and the development of spontaneous but coordinated contractions of the pregnant uterus (809). Regulation of production of the gap junction protein, connexin-43, is increased by estrogen but decreased by progesterone (803, 809). Progesterone did not decrease below 30 ng/ml

in uterine venous plasma of intact or ovariectomized 90 to 100 day pregnant ewes and may have prevented the development of well coordinated myometrial contractions. However, $PGF_2\alpha$ did increase estradiol-17ß. The uterus of $PGF_2\alpha$-treated intact or ovariectomized 90 to 100 day pregnant ewes at 72 hours after treatment was tight, while the uterus of controls was flaccid. The success of trilostane to induce abortion in 90 to 100 day lutectomized pregnant ewes may be related to the decrease in jugular venous progesterone and the increase in estradiol-17ß seen at 2 hours after the first two trilostane treatments. The two increases in estradiol-17ß for the first 24 hours after the initiation of trilostane treatments occurred only at 2 hours after trilostane and then decreased. This suggests that the inhibition of placental steroidogenesis by trilostane is short lived. There was a sustained increase in estradiol-17ß in jugular venous plasma starting at 36 hours after the initiation of trilostane treatments and before abortions occurred. These sustained increases in estradiol-17ß in trilostane-treated pregnant ewes may have caused increases in gap junctions in the uterine myometrium leading to the development of well coordinated uterine contractions to expel the fetus when progesterone was decreased. Secretion of estradiol-17ß in previous experiments with trilostane has not been determined (890, 984). Although estradiol-17ß was increased in the 16 mg $PGF_2\alpha$/58 kg/BW-treated intact and ovariectomized 90 to 100 day pregnant ewes and only for a short period in the 8 mg $PGF_2\alpha$/58 kg/BW-treated ewes, abortions did not occur. In addition, progesterone never fell below 30 ng/ml in uterine venous plasma and may

have blocked estrogen-induced (803, 809) gap junction formation in the uterine myometrium.

Failure of $PGF_2\alpha$ to terminate pregnancy in 90 to 100 day pregnant ewes could also be related to changes in uterine secretion of $PGF_2\alpha$. Concentrations of $PGF_2\alpha$ in ovarian venous plasma of intact and hysterectomized 90 to 100 day pregnant ewes were approximately one tenth of those for $PGF_2\alpha$ in uterine venous plasma of intact or ovariectomized 90 to 100 day pregnant ewes and were near the lower limit of the sensitivity of the RIA for $PGF_2\alpha$. This is consistent with previous data where less than 5 percent of $PGF_2\alpha$ is transferred locally from the uterine venous to the adjacent ovarian arterial vasculature (266). These data further suggest that uterine secretion of $PGF_2\alpha$ *in vivo* and *in vitro* by 90 to 100 day pregnant ewes is inhibited since concentrations of $PGF_2\alpha$ in uterine venous plasma or $PGF_2\alpha$ secreted by uterine tissue *in vitro* are lower when compared to those reported for nonpregnant ewes during luteolysis (135, 247, 256, 257, 268, 273-279). Previous reports indicate that placental extracts suppress secretion of $PGF_2\alpha$ by nonpregnant endometrium *in vitro* (580, 734). This suggests that secretion of progesterone by luteal and placental tissues may be protected partially through an inhibition of $PGF_2\alpha$ production. Secretion of $PGF_2\alpha$ into uterine venous plasma during early pregnancy has been reported to decrease in some studies, especially, a reduction in the number of peaks of $PGF_2\alpha$. However, basal concentration of $PGF_2\alpha$ in uterine venous plasma increase (280, 281). These data have been offered as an explanation for maintenance of the CL which is essential during pregnancy in ewes until at least day 55 (441). Others offer

alternative explanations for maintenance of CL of early pregnancy in ewes where uterine secretion (256, 257, 286, 287, 444, 445), local transfer of $PGF_2\alpha$ to the adjacent ovarian arterial vasculature or the accumulation of $PGF_2\alpha$ in the adjacent CL (257), numbers of receptors for $PGF_2\alpha$ on luteal membranes (519), or binding of $PGF_2\alpha$ to luteal membranes (519) are not reduced but the luteolytic response to $PGF_2\alpha$ is suppressed by early pregnancy factors (521). In addition, the minimum effective dose of $PGF_2\alpha$ given intramuscularly on day 13 postbreeding to cause a decrease in jugular venous progesterone is 6 mg/58 kg/BW, while only 4 mg/58 kg/BW is needed on day 13 in nonpregnant ewes (255, 522). While uterine secretion of $PGF_2\alpha$ into ovine uterine venous drainage during early pregnancy is controversial, data reported herein suggests that uterine secretion of $PGF_2\alpha$ at 90 to 100 days of gestation is suppressed but the uterus can secrete large amounts of $PGF_2\alpha$ since increases in uterine venous $PGF_2\alpha$ were seen at 32 and 64 hours in the 16 mg $PGF_2\alpha$/58 kg/BW ovariectomized and 8 mg $PGF_2\alpha$/58 kg/BW intact treatment groups, respectively. These increases in $PGF_2\alpha$ in ovariectomized and intact 90 to 100 day pregnant ewes may be due to the oxytocic effect of $PGF_2\alpha$ on the uterus that was injected (292, 294, 300, 570, 571) rather than quantification of the injected $PGF_2\alpha$. Oxytocin increases postpartum uterine secretion of $PGF_2\alpha$ (997). In addition, oxytocin given to day 150 pregnant cows increases the PGFM in jugular blood and CL were regressed in two thirds of the cows but all pregnancies were carried to term (993). Treatment of late pregnant ewes with $PGF_2\alpha$ also increases uterine secretion of $PGF_2\alpha$ *in vivo*. At 72 hours posttreatment with $PGF_2\alpha$, ovarian venous $PGF_2\alpha$ was increased in intact

but not hysterectomized 90 to 100 day pregnant ewes. This indicates that the source of this increase of $PGF_2\alpha$ in ovarian venous plasma at 72 hours in intact 90 to 100 day pregnant ewes was of uterine origin. Although luteal secretion in addition to uterine secretion of $PGF_2\alpha$ has been reported in the pseudopregnant and pregnant rat (397), the CL does not appear to be the source for the increases in $PGF_2\alpha$ seen in ovarian venous plasma at 90 to 100 days of gestation in sheep. Secretion of $PGF_2\alpha$ by placentome slices *in vitro* from 90 to 100 day pregnant ewes was not increased by treatment with $PGF_2\alpha$ since the associated myometrium was not present. However, these tissues were incubated for only 4 hours. A sustained pulsatile secretion of $PGF_2\alpha$ by the uterus may be necessary to cause abortion or parturition. A sustained pulsatile secretion of $PGF_2\alpha$ into uterine venous plasma is seen at parturition (722, 731) or during abortion induced by microbial agents (998).

Failure of $PGF_2\alpha$ to cause abortion in 90 to 100 day pregnant ewes may be related to placental secretion of PGE to protect secretion of progesterone by the placenta from $PGF_2\alpha$ inhibition of steroidogenesis. Concentrations of PGE in uterine and ovarian venous plasma were similar. Concentrations of PGE in ovarian venous plasma at 0 hour averaged 7.1 ng/ml in intact and hysterectomized 90 to 100 day pregnant ewes and were not affected by treatment with $PGF_2\alpha$ in intact 90 to 100 day pregnant ewes. Concentrations of PGE were reduced at 72 hours compared to the 0 hour sample in the two $PGF_2\alpha$ hysterectomized treatment groups. This also suggests that the placenta contributes PGE to the ovarian vein in 90 to 100 day pregnant ewes. Concentrations of PGE in uterine venous plasma of

intact and ovariectomized ewes averaged 6 ng/ml. Ratios of PGE to $PGF_2\alpha$ in ovarian and uterine venous plasma were 100:1 and 12:1, respectively. Since concentrations of PGE in ovarian venous plasma were not affected by hysterectomy and were lowered by 50 percent by $PGF_2\alpha$ within the hysterectomized, $PGF_2\alpha$ treated groups, half of the PGE in ovarian venous plasma was of luteal origin and the other half was from the remainder of the ovary. This is supported by data in pregnant rats where secretion of PGE_2 by day 19 pregnant rat CL is 5 to 10 fold greater than $PGF_2\alpha$ (397). Prostaglandin E_2 can be converted to $PGF_2\alpha$ by the enzyme 9-keto-PGE_2-reductase (89, 90, 999). During early pregnancy in sheep, the ratio of PGE_2:$PGF_2\alpha$ in uterine venous plasma changes from 0.1 in early postbreeding to 1.0 at the time of maternal recognition of pregnancy (288, 345). This change may be explained by changes in the activity of 9-keto-PGE_2-reductase which is decreased in the endometrium but not in luteal tissue at the time of maternal recognition of pregnancy (999). Decreased activity of 9-keto-PGE_2-reductase could also explain the high ratios of PGE to $PGF_2\alpha$ in ovarian and uterine venous plasma of 90 to 100 day pregnant ewes to sustain luteal and placental secretion of progesterone and to protect placental secretion of progesterone from inhibitory actions of $PGF_2\alpha$. Chronic intrauterine infusions of PGE_1 (444, 445, 539, 559-564) or PGE_2 (444, 445, 539, 559-564) prevents a spontaneous luteolysis and acute challenges of PGE_1 or PGE_2 given with a luteolytic dose of $PGF_2\alpha$ prevents $PGF_2\alpha$-induced premature luteolysis in nonpregnant ewes (555, 556). Eighty μg of $PGF_2\alpha$ given at the ovarian vascular pedicle adjacent to the ovary with a CL of nonpregnant ewes is luteolytic and a five fold greater amount of

PGE_2 is required to prevent $PGF_2\alpha$-induced premature luteolysis (254). In addition, PGE_1 and PGE_2 are luteotropic *in vitro* (188-190, 530). Thus, it is suggested that PGE secreted by luteal tissue is luteotropic in 90 to 100 day pregnant ewes. This could explain the reduced *in vitro* response to LH as a luteotropin by bovine CL during midpregnancy (659) and in 90 to 100 day pregnant sheep (Charles Weems, personal communication, unpublished data). In addition, PGE_2 stimulates secretion of progesterone by CL from 90 to 100 day pregnant ewes *in vitro* (Charles Weems, personal communication, unpublished data). Furthermore, PGE_2 blocks $PGF_2\alpha$-induced increases in cytosolic free calcium, which appears to be the mechanism of $PGF_2\alpha$-induced luteolysis (1000).

The placenta of 90 to 100 day pregnant ewes secretes large quantities of PGE *in vivo* in the presence or absence of the ovaries or when placental slices are incubated *in vitro*. In addition, increases in PGE and $PGF_2\alpha$ were seen at 64 hours post treatment in intact 90 to 100 day pregnant ewes with 8 mg $PGF_2\alpha$/58 kg/BW. It is suggested that PGE prevents $PGF_2\alpha$ inhibition of secretion of progesterone by the ovine placenta. Furthermore, it is suggested that PGE stimulates secretion of progesterone by the ovine placenta. This may explain why most of the receptors on placental tissue are PGE rather than $PGF_2\alpha$ (727, 728, 730-732, 735, 802, 803, 811, 812). Microbial induced abortion in sheep is associated with increases in $PGF_2\alpha$ and decreases in PGE_2 in uterine venous plasma (998). This suggestion is further supported by the observation that ovine placental binucleate cells secretes progesterone and PGE_2. These binucleate cells comprise one fifth of the trophectodermal layer at the ovine

feto-maternal interface (619, 620, 623-629). In addition, PGE_2 stimulates conversion of pregnenolone to progesterone by binucleate cells from the ovine placenta and indomethacin, an inhibitor of prostaglandin synthesis, decreases this conversion of pregnenolone to progesterone *in vitro* (623-629). However, PGE_1 or PGE_2 at dose of 1, 10 or 100 ng/ml did not increase secretion of progesterone by 90 to 100 day placentome slices *in vitro* where 25-OH-cholesterol was used as a precursor for steroidogenesis (Charles Weems, personal communication, unpublished data). However, these tissues secrete ng amounts of PGE_2 based on the *in vitro* data in experiment 4. Differences between these two studies may also be due to the type of tissue preparation used (dispersed binucleate cells vs. placentome slices) or the type of precursor added to the medium (pregnenolone vs. 25-OH-cholesterol). However, addition of pregnenolone alone increases progesterone secretion by minced ovine placental tissue *in vitro* (648, 649). What is most important are events at the plasma membrane and the transport of cholesterol to the mitochondria for conversion to pregnenolone and then to progesterone (369, 398, 405). Addition of ovine LH, FSH, prolactin or cAMP analogues does not stimulate secretion of progesterone by ovine binucleate cells (648, 649) or placentome slices *in vitro* (Charles Weems, personal communication, unpublised data). However, inhibition of phosphodiesterase, which inactivates cAMP, increases progesterone secretion by binucleate cells (623-629) or minced placental tissue *in vitro* (648, 649). Overall analysis of data on hCG indicates that hCG does not stimulate secretion of progesterone in the human, but cAMP increases steroidogenic enzymes in the human

placenta (650). Thus, it remains to be determined whether PGE stimulates placental secretion of progesterone.

Previous reports indicate that secretion of $PGF_{2}\alpha$ and PGE_2 and activity of PGH-synthase are very low until day 120 of gestation in sheep when cotyledons are incubated *in vitro* with arachidonic acid (722, 728, 729). This was followed by a progressive increase in secretion of $PGF_{2}\alpha$, PGE_2 and increased PGH-synthase activity for the remainder of gestation to reach a peak by the time of parturition to initiate the birth process (722, 728, 729). These authors concluded that the levels of PGE_2 at the end of gestation in sheep reached levels that were greater than the catabolic activity of the lungs. Thus, PGE_2 spills over into arterial blood and initiates parturition (722). However, these studies were only at a few selected times during pregnancy and from only a few sheep (722). Immunoreactive PGH-synthase has been reported to be located in ovine maternal contributions to the placenta for the first half of gestation and then decreases to undetectable levels followed by progressive increases in PGH-synthase in the fetal cotyledon from day 114 to term (236, 1002). These data are not supported by the basal and $PGF_{2}\alpha$-stimulated concentrations of PGE in uterine venous plasma of intact 90 to 100 day pregnant ewes reported here. Furthermore, human placental and amnion homogenates at different stages of gestation do not convert radiolabelled PGH_2 to TXA_2, PGH_2 to $PGF_{2}\alpha$ but shifts PGH_2 placental microsome metabolism to PGE_2 *via* a heat stable factor (1001). Since basal secretion of PGE and $PGF_{2}\alpha$ by 90 to 100 day pregnant ewes was relatively constant but peaks of PGE and $PGF_{2}\alpha$ were seen, it is suggested that basal concentrations of PGE and $PGF_{2}\alpha$ are

formed by the constitutively expressed PGH-synthase, while the peak increases in PGE and $PGF_2\alpha$ at 64 hours after 8 mg $PGF_2\alpha$/58 kg/BW treatment intact 90 to 100 ewes is from the inducible form of PGH-synthase (737-743, 1003-1011). Indomethacin decreased PGE secretion by placentome slices from 90 to 100 day pregnant ewes by only half *in vitro* but did not affect secretion of progesterone. Indomethacin inhibits the constitutively expressed form of PGH-synthase, but not the mitogen-inducible form of PGH-synthase (1003). Sources of PGE_2 secretion by ovine placentome slices *in vitro* may be the endometrial stroma, since the stroma of pregnant or pseudopregnant rats (1012, 1013) secretes PGE_2 and epidermal growth factor stimulates secretion of PGE_2 by the stroma of pig endometrium (593). Evidence exists for PGE_2 stimulation of ovine binucleate cell secretion of progesterone (1014), although calcium may also be involved (1015). Since these regulators (1014, 1015) may also negatively affect steroidogenesis (1000), this may explain the rapid turnover of binucleate cells (1016-1020). These binucleate cells (1020) are necessary for placental development, maintenance, and secretion of progesterone (1021).

An additional role for placental secretion of PGE during gestation may be for the progressive increase in uterine blood flow that occurs during gestation to facilitate growth of the fetus (486-493). Prostaglandin E_1 and PGE_2 are vasodilators (497). Progesterone potentiates vasoconstriction of uterine arteries and progesterone increases progressively during the last half of pregnancy in sheep (347-350, 352, 497, 499). Heat stress decreases uterine blood flow to the gravid uterus (607) and is

associated with a decrease in PGE_2 secretion, but has no effect on secretion of $PGF_2\alpha$ by sheep (1022). Another role for PGE may be immunosuppression of the maternal immune system during gestation to prevent rejection of the allogenic trophoblast or fetus (1023, 1024). Fifteen to 25 percent of pregnancies in women end in spontaneous abortion and 50 percent of those are of unexplained etiology (977). Antibodies to the negatively charged phospholipids which contain the precursor for prostaglandin synthesis (arachidonic acid), have been associated with spontaneous abortion, stillbirths, intrauterine fetal growth retardation and preterm birth in the human (978). Antiphospholipid antibodies decrease between pregnancies in these patients only to reappear with increased activity in subsequent pregnancies and again result in abortion (979-981). Caution should be used when using these data as many of these patients have more than one autoimmune antibodies (981). Whether antibodies are produced against phospholipase A_2, which frees arachidonic acid from phospholipids for prostaglandin synthesis, or to either of the two forms of PGH-synthase, which converts arachidonic acid to the prostaglandin endoperoxide intermediates, is not known. Spontaneous abortion or fetal resorption are also associated with increases in the cytokines, transforming growth factor-α, interferon-τ and interleukin-2, which increase $PGF_2\alpha$ and may either increase or decrease PGE secretion by the placenta (1025-1029).

Pregnancy specific protein B and/or estradiol-17ß may play a role in regulating placental steroidogenesis in ruminants. Concentrations of PSPB secreted by the binucleate cells (784) and progesterone in uterine or jugular venous plasma are greater in ewes with twin lambs than in ewes

with a single lamb (1030). Secretion of PSPB is seen by the ovine embryo at the time of maternal recognition of pregnancy (1031) and concentrations of PSPB continue to increase in jugular venous blood throughout gestation to peak just before parturition (782-785, 788), but do not return to undetectable levels until about 3 weeks postpartum (1032). It is suggested that PSPB regulates placental secretion of PGE to control ovine placental secretion of progesterone. An increase in PSPB, PGE, and $PGF_2\alpha$ was seen at 64 hours in the 8 mg $PGF_2\alpha$/58 kg/BW-treated intact 90 to 100 day pregnant ewes. In addition, concentrations of PSPB were greater in ovariectomized than in intact 90 to 100 day pregnant ewes. Also, the 8 mg $PGF_2\alpha$/58 kg/BW dose decreased PSPB in ovariectomized 90 to 100 day pregnant ewes. The 16 mg $PGF_2\alpha$/58 kg/BW dose increased PSPB in intact but did not decrease PSPB in ovariectomized 90 to 100 day pregnant ewes. Whether PSPB directly stimulates secretion of PGE or progesterone by the ovine placenta is unknown. Pregnancy specific protein B increases secretion of PGE by postpartum bovine endometrium in the presence or absence of oxytocin (788). Preliminary data in 90 to 100 day pregnant ewes ovariectomized for 7 days indicates that secretion of PGE and progesterone *in vitro* is greater than secretion by placentome slices from intact 90 to 100 day pregnant ewes (Charles Weems, personal communication, unpublished data). Whether the concentration of PGE is greater in ewes with twins than in ewes with single lambs is unknown.

The 16 mg $PGF_2\alpha$/58 kg/BW dose increased estradiol-17ß in intact or ovariectomized 90 to 100 day pregnant ewes and for a brief period in the 8 mg $PGF_2\alpha$/58 kg/BW-treated intact 90 to 100 day pregnant ewes.

Estradiol-17ß was lower in vehicle-treated intact or vehicle and 8 mg $PGF_2\alpha$/58 kg/BW-treated ovariectomized 90 to 100 day pregnant ewes. This decrease was probably due to the elevated cortisol in all ewes at 0 hour as discussed previously for progesterone. Most of the circulating estradiol-17ß was from the placenta, since estradiol-17ß was decreased by 80 percent in the vehicle and both $PGF_2\alpha$ treatment groups within 12 hours after hysterectomy in 90 to 100 day pregnant ewes. Also, addition of androstenedione to cultures of placentome slices from 90 to 100 day pregnant ewes increased estradiol-17ß secretion. Estradiol-17ß is increased 100 fold, while estrone sulfates is increased 10 fold after day 60 of gestation in Barbary sheep compared to early pregnancy (1033). Patterns of estradiol-17ß are similar to those seen for progesterone which is increased 4 fold (1033). Estradiol-17ß has been associated with placental development (1035) and maintenance of pregnancy in primates (1036-1039). Reduced concentrations of estradiol-17ß during pregnancy in monkeys are associated with decreased placental and fetal weights and an increase in abortion (1036-1039). Estradiol-17ß in primates facilitates the utilization of lipoprotein to deliver cholesterol for placental steroidogenesis (1040). The placenta of primates is dependent on fetal adrenal androgens for conversion to estrogen (1035-1039), but not in sheep (722). Estradiol-17ß may also have adverse effects on pregnancy. Trilostane decreased progesterone, but increased estradiol-17ß within two hours following the first two injections for the first 24 hours in 90 to 100 day lutectomized pregnant ewes. This was followed by a sustained increase in estradiol-17ß starting at 108 hours in all trilostane-treated 90 to 100 day pregnant ewes and occurred prior to

abortion. Estradiol-17ß and $PGF_{2}\alpha$ increase prior to parturition in sheep (722). Estradiol-17ß is a stimulator of uterine secretion of $PGF_{2}\alpha$ (284). However, estradiol-17ß in the 16 mg $PGF_{2}\alpha$/58 kg/BW-treated intact, ovariectomized or lutectomized 90 to 100 day pregnant ewes was different, but did not cause abortion. It is suggested that a sustained increase in estradiol-17ß when progesterone is decreasing may initiate parturition through the pulsatile release of $PGF_{2}\alpha$ and activation of steroid sulfatases (1041). In microbial-induced abortion, the microbial infection causes a pulsatile release of $PGF_{2}\alpha$ and PGE decreases in uterine venous blood (998).

Both doses of $PGF_{2}\alpha$ regressed CL and decreased progesterone below 1 ng/ml in jugular venous blood of hysterectomized 90 to 100 day pregnant ewes, but estradiol-17ß did not increase and numbers of surface antral follicles or size of the follicles did not increase at 72 hours. In addition, size of the surface antral follicles was less than 2 mm in diameter. Numbers and the size of antral follicles in previous studies on day 120 of gestation in sheep were reported to be reduced and follicles were no larger than 1.7 mm in diameter (1042-1044). When ewes were hysterectomized on day 30 of pregnancy and ovaries were examined on day 120, some follicles on the ovary ranged from 2 to 4 mm in diameter (1044). These data suggest that the pregnant ovine uterus inhibits the growth of the follicle beyond a diameter of 2 mm, since lutectomy at day 75 does not result in follicles greater than 2 mm on day 140 (1043, 1044). This inhibition is released between day 140 of pregnancy and day 5 postpartum when follicles between 2 to 4 mm in diameter are seen (1045-1047). Although the follicles on the ovary at day 120 do not grow past 2 mm in size, numbers of

preantral growing follicles are greater on the ovary with a CL (1042). This suggests that luteal products enhance follicular growth to the preantral stage but atresia of follicles occurs before growth past 2 mm in size (1044). In these antral follicles, concentrations of estradiol-17ß are reduced , while progesterone is increased (1045-1047). This may even occur during early pregnancy (1048-1051). This could explain the greater follicular atresia reported (1042-1047). High estradiol-17ß secretion from the placenta as seen in the 90 to 100 day pregnant ewes may be responsible for these changes. Estradiol-17ß decreases 9-keto-reductase in ovarian tissue and directs prostaglandin synthesis to PGE_2 (1052, 1053). Prostaglandin E_2 was secreted in ng amounts by the ovary of 90 to 100 day hysterectomized or intact pregnant ewes and could shift follicular steroidogenesis to progesterone rather than to estradiol-17ß. At ovulation, LH shifts steroidogenesis of the preovulatory follicle from estradiol-17ß to progesterone as well as causing secretion of PGE_2 by the preovulatory follicle (96-99). Atretic follicles secrete progesterone, but not estradiol-17ß (8). In addition, PGE_2 stimulates production of inhibin by antral follicles (1053). Failure of follicles to grow past 2 mm in size during pregnancy may also be related to alterations in secretion of FSH and LH. Concentrations of FSH and LH are decreased in the hypophysis and blood (1055-1060). Growth of antral follicles requires both FSH and LH (11, 34, 35, 43-57).

It is interesting that induction of abortion in trilostane-treated 90 to 100 day lutectomized ewes resulted in unexpected retained placentae in 19 of 20 ewes. A placenta is considered to be retained in sheep when the placenta is not expelled within 8 hours after expulsion of the fetus (665).

Retention of the placenta is thought to occur when concentrations of estrogen are low prior to parturition (1061). However, estradiol-17ß was elevated in all trilostane-aborted 90 to 100 day pregnant ewes. However, uterine venous progesterone at 168 hours was greater than 40 ng/ml in all treatment groups in experiment 5. Progesterone in uterine venous plasma of 90 to 100 day pregnant ewes treated with $PGF_2\alpha$ never fell below 30 ng/ml in experiments 2 and 3. Thus, it is suggested that the altered progesterone : estradiol-17ß ratio probably resulted in the high incidence of retained placenta. Others suggest that retention of the placenta and delayed uterine involution in the cow and the delayed interval to ovulation, estrus and decreased profitability postpartum is impeded by PGE_2 (1062-1065). However, secretion of PGE_2 and TXB_2 are lower by caruncular tissue but allantochorion tissue secretes more PGE, leukotriene B_4, 6-keto-$PGF_1\alpha$ but less TXB_2 and $PGF_2\alpha$ (1062). Some suggest that any interference (injections of hormones, ovariectomy) with normal synchronized multiple hormonal changes such as those seen at parturition can increase the incidence of retained placenta (1064). However, induction of parturition 6 days before anticipated lambing with dexamethasone + cloprostenol, $PGF_2\alpha$ analogue, does not increase the incidence of retained placenta (1066) but does in other studies (64 %) where parturition was induced with dexamethasone + cloprostenol on days 276-278 in cows (1067). A retained placenta has more type III collagen and collagenase, but not hyaluronidase. Hyaluronidase causes cotyledon-caruncle separation (1068, 1069). Thus, it is suggested that the high incidence of retained placenta seen in trilostane-treated lutectomized 90 to 100 day pregnant ewes was

due to progesterone secretion by the placentome not being turned off, even after abortion.

In summary, 1. the ovine corpus luteum at 90 to 100 days of gestation is functional and responsive to luteolytic actions of $PGF_2\alpha$. 2. Placentomes and CL contribute equally to the concentrations of circulating progesterone at this stage of gestation. 3. Single injections of $PGF_2\alpha$ do not terminate pregnancy at 90 to 100 day of gestation in the presence or absence of the ovaries. 4. Placentomes do not appear to be responsive to $PGF_2\alpha$ inhibition of progesterone secretion *in vivo* or *in vitro* to the doses of $PGF_2\alpha$ tested over the experimental period. Failure of $PGF_2\alpha$ to inhibit placental secretion of progesterone may be due to the substantial quantities of PGE secreted by the ovine placentome *in vivo* or *in vitro* or to a lack of receptors for $PGF_2\alpha$. Regulation of placental secretion of progesterone, directly or indirectly, may protect placental steroidogenesis from inhibitory actions of $PGF_2\alpha$ may be mediated by PGE. Regulation of PGE and progesterone secretion may be through PSPB and/or estradiol-17ß. It is suggested that estradiol-17ß in the presence of progesterone in pregnant sheep regulates secretion of PSPB; PSPB regulates secretion of PGE; and PGE regulates secretion of progesterone by the placenta. 5. Trilostane aborts 90 to 100 day lutectomized pregnant ewes but causes a high incidence of retained placenta. This high incidence of retained placenta may be due to the failure of placentome secretion of progesterone to be inhibited. Although trilostane decreases placental secretion of progesterone, estradiol-17ß is increased. The retained placenta does not appear to be due to a lack of sufficient estradiol-17ß. It is suggested that the inhibition of

growth or atresia of follicles during gestation in sheep is caused by estradiol-17ß, secreted by the placenta, and/or PGE, secreted by the placenta and ovaries.

APPENDIX A

RADIOIMMUNOASSAY FOR PROGESTERONE

This assay was validated in our laboratory and was modified from Berardinelli et al (1069) which has been reported previously (1070). Jugular (50 μl), uterine (2 μl) or ovarian (0.2 μl) venous or their respective 90 to 100 day pregnant sheep pooled plasma samples in duplicate were diluted to 200 μl with 0.1 % gelatin in phosphate buffered saline (PBS). Medium 199 from in vitro incubations (50 μl) were diluted in duplicate to 100 μl with medium 199 and diluted further to 200 μl with PBS. Media samples from the in vitro experiment were assayed without extraction. Plasma samples were extracted twice in 13x100 mm borosilicate glass culture tubes with 3 ml of petroleum ether (Fisher Scientific, Fairlawn, NJ). Tubes were placed in a dry ice/methanol bath to freeze the aqueous phase, the organic phase was decanted into assay tubes (12x75 mm glass tubes), and petroleum ether from the extract was evaporated under a stream of prepurified nitrogen gas in a water bath at 37 ^{o}C. Tritiated progesterone (0.01 μci) was added before extraction to separate pooled plasma samples to estimate recovery of progesterone.

Progesterone standards (Steroids, Wilton, NH) in methanol (Burdick and Jackson, Muskegon, MI) used in the assay were 0, 10, 20, 50, 100, 200 and 400 pg/tube and were in duplicate. Progesterone standards were incubated in a water bath at 37 ^{o}C under prepurified nitrogen to evaporate the methanol. All assay tubes received 100 μl each of PBS, tritiated progesterone (0.01 μCi; 1,2,6,7-^{3}H CN-progesterone, New England

Nuclear, Boston, MA) and progesterone antisera (1:2000 dilution; R.L.Butcher, West Virginia University, Ewe 123, 10-21-74). The total and nonspecific binding tubes received another 100 μl of PBS instead of antisera to maintain a constant volume. Contents of the tubes were mixed and incubated in a water bath at 37 ^{o}C for 5 minutes followed by incubation at 4 ^{o}C overnight.

Bound from free radiolabelled steroid was separated by adding 0.5 ml dextran (Kodak Chemical Co., Rochester, NY) -coated charcoal (Norit A, Fisher Scientific Inc., Fairlawn, NJ) suspension to individual tubes at 4 ^{o}C within 2 minutes of a 15 minute incubation period, except the total count tubes which received 0.5 ml distilled water instead. These tubes were mixed and incubated for the remainder of the 15 minute incubation period at 4 ^{o}C followed by centrifugation at 3000 r.p.m. (Sorvall RC5C, Newtown, CT) for 10 minutes at 4 ^{o}C. The supernatant fluid was decanted into 4 ml scintillation vials (Omnivials, Wheaton Scientific, Millville, NJ) containing 2.5 ml of scintillation fluid (Universol, ICN Radiochemicals, Irvine, CA) and radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4550, Downers Grove, IL) for 2 minutes per sample.

Average binding by the antisera in all assays was 35$\pm$0.1 % and sensitivity of the assay was 0.05 ng/ml. Parallelism of the assay was determined by extraction twice of pooled jugular, uterine and ovarian venous plasma of 90 to 100 day pregnant ewes with petroleum ether. Extracts of the three different pooled plasma volume or unextracted media were parallel to the standard curve.

Crossreactivity of the progesterone antisera with other steroids was as follows: 18-hydroxyprogesterone, 11 %; 17α-hydroxyprogesterone, and testosterone, 2 % each; 11α-hydroxyprogesterone, allopregnenolone, and 6-dihydrotestosterone, 1 % each; and less than 0.05 % each with corticosterone, 4-androsten-3ß,17ß-diol, 4-pregnen-20α-ol-3-one, dihydrotestosterone, 19-nortestosterone, androstenediol, androstenedione, dehydroepiandrostene, androstanedione, estradiol-17ß, 6-keto-estradiol-17ß, 5α-androstan-3ß,17ß-diol, estrone, cholesterol, estradiol-17α, and estriol.

The range of concentrations for jugular progesterone of 39 randomly chosen samples were also run in duplicate using a Coat-A-Count progesterone radioimmunoassay kit (Diagnostic Products Co., Los Angeles, CA) to verify the assay values. The results for concentrations of progesterone in each samples determined by Coat-A-Count were not different from the results obtained by the RIA for progesterone.

Concentrations of progesterone in samples were adjusted individually for procedural losses, estimated by recovery of radiolabelled progesterone. Percent recoveries for jugular (n=97 assays), uterine (n=30 assays), and ovarian (n=7 assays) venous plasma in experiments 1, 2, 3, and 5 were 96±3, 84±4, and 82±7 percent, respectively.

Coefficients of variation within and between assays for jugular, uterine and ovarian venous plasma were estimated using the pooled plasma samples. Intraassay coefficients of variation for jugular, uterine, and ovarian venous plasma samples in in vivo experiments were 7, 15, and 10 percent, respectively. Interassay coefficient of variation for jugular, uterine, and ovarian venous plasma were 15, 22, and 16 percent, respectively. In in vitro

samples from incubation of placentome slices, intraassay and interassay coefficients of variation were 13 and 12 percent, respectively.

Reagents for the progesterone RIA are as follows:

1. Gelatin Phosphate Buffered Saline (PBS)

Two stock solutions A and B were made in a 2 liter volumetric flask and brought to volume with double distilled water.

Stock Solution A for PBS

Sodium Phosphate Dibasic ($Na_2HPO_4.7H_2O$)--5.36 g

Sodium Chloride (NaCl)--16.36 g

Stock Solution B for PBS

Sodium Phosphate Monobasic ($NaH_2PO_4.H_2O$)--2.76 g

Sodium Chloride (NaCl)--16.36 g

Stock solution A was poured into a 4,000 ml beaker and 1,400 to 1,450 ml of stock solution B was added and pH was adjusted to 7.0 by adding varying volumes of stock solution B. Knox gelatin (0.1 percent weight/volume) was added and the solution was warmed on a stirring hot plate. The solution was permitted to cool and thimerosol (0.01 percent) was added, mixed, and transferred to two 2,000 ml Erlenmeyer flasks with caps and stored at 4 ^{o}C. Chemicals used were from Sigma Chemical Co. (St. Louis, MO). This buffer is 0.01 M phosphate/0.14 M NaCl and was adjusted to pH 7.0.

2. Dilution of Progesterone Antisera

For a "1:2000 dilution of the antiserum", 100 μl of progesterone antisera (R.L.Butcher, West Virginia University, Sheep 123, 10-21-74) were transferred to a 200 ml volumetric flask and was brought to volume with PBS. The solution was gently mixed well and stored at 4 ^{o}C.

3. Radiolabelled Progesterone

Twenty-five μl (0.02 mCi) of tritiated progesterone (1,2,6,7-^{3}H(Ω); New England Nuclear, Boston, MA) were transferred to a 200 ml volumetric flask and brought to volume with PBS. The solution was gently mixed well and stored at 4 ^{o}C. Final concentration was 0.01 μCi/100 μl. Percent binding to progesterone antisera should be between 35 to 40 percent.

4. Progesterone Standards

Two stock solutions were used (1 ng/ml and 100 pg/ml). Two hundred μl of a "1 μg/ml stock solution of progesterone" were transferred to a 200 ml volumetric flask and brought to volume with freshly opened methanol (Burdick and Jackson, Muskegon, MI) to make the "1 ng/ml stock solution". For the "100 pg/ml stock solution", 10 ml of the 1 ng/ml progesterone stock solution were transferred to a 100 ml volumetric flask and brought to volume with methanol. A ground glass stopper was placed in the flask, the stopper was wrapped with parafilm, and refrigerated (4 ^{o}C) between use.

5. Dextran-Coated Charcoal Suspension

Dextran (0.25 g, Kodak Chemical, Rochester, NY) and 2.5 g Norit A Charcoal (Carbon decolorizing alkaline, Fisher Scientific Co., Fairlawn, NJ) was placed in an Erlenmeyer flask and 1000 ml of doubled distilled water were added. The solution was mixed on a magnetic stirrer and stored at 4 ^{o}C.

APPENDIX B

RADIOIMMUNOASSAY FOR PROSTAGLANDIN $F_{2\alpha}$ AND PROSTAGLANDIN E

Validation of the assays for $PGF_{2\alpha}$ and PGE has been reported previously (1070) and modified from Pexton et al. (295) as modified by Lewis et al. (504, 505), Vincent et al. (548) and Christensen and Leyssac (1072). Uterine venous or ovarian venous plasma in Experiments 1, 2 and 3 or their respective 90 to 100 day pregnant pooled plasma samples in duplicate were extracted twice for $PGF_{2\alpha}$ (400 μl) or PGE (200 μl) with 100 μl 0.1 N HCl and 4 ml of freshly opened ethyl acetate (Burdick and Jackson Laboratories, Muskegon, MI) in 13x100 mm borosilicate glass tubes. Tritiated $PGF_{2\alpha}$ or PGE_2 was added to separate pooled plasma samples to estimate recovery. Tubes were vortexed for 30 seconds, centrifuged at 3000 r.p.m. for 10 minutes at 4 ^{o}C, the supernatant was decanted into 12x75 mm tubes, and solvent was evaporated under a stream of prepurified nitrogen gas in a water bath at 37 ^{o}C. This extraction process was repeated once.

Prostaglandin $F_{2\alpha}$ and PGE were separated on Sephadex LH-20 columns (Sigma Chemical Co., St.Louis, MO or Pharmacia Fine Chemicals, Piscataway, NJ) with a 98:2 mixture of methylene chloride : methanol (Burdick and Jackson Laboratories, Muskegon, MI) as described previously (504, 505, 548) and modified later (1072). This column elute must be made at least 30 minutes before building the column in order to expand the mixture. A 5 cc disposable glass pipet was used as the column and was

placed on the column rack. A small glass bead (4 mm) was placed inside each pipet and presoaked Sephadex LH-20 (500 mg/column in 5 ml of the column elute soaked at least 2 hours) was added and followed with several ml of the column elute. The column was permitted to drip until it ran dry and a small disk of Whatman filter paper was gently placed on the top of each Sephadex column. One hundred and twenty-five μl of glacial acetic acid in 25 ml of the column elute mixture were used as the sample elute to transfer extracted samples to the column. Fifty μl of the sample elute were added to a sample tube, vortexed, and the sample was transferred to the filter paper disk on top of the column. This process was repeated twice followed by 1 ml of column elute added to the sample tube, mixed, and then added to the column.

The amount of column elute used for $PGF_{2\alpha}$ and PGE separation is dependent on the lot number and/or source of the Sephadex. Elution patterns were determined using tritiated $PGF_{2\alpha}$ or PGE before using the columns for prostaglandin separation. Fractions of column elute collected for assays were as follows: for uterine $PGF_{2\alpha}$ (lot# 75F-0067, Sigma), fractions 10-20 ml; for ovarian $PGF_{2\alpha}$ (lot# 18F-0061, Sigma), fractions 12-22 ml; for uterine PGE (lot# PL-10694, Pharmacia), fractions 6-20 ml; and for ovarian PGE (lot# 18F-0061), fractions 6-16 ml. Media samples from experiment 4 were assayed directly without extraction or column chromatography.

Antisera for $PGF_{2\alpha}$ (RA 546 C-15; L.Levine, Brandeis University, Waltham, MA) and PGE (Mason 001475; N.Mason, Lily Research Laboratories, Indianapolis, IN) were used at a dilution of 1:2000 and

1:15,000, respectively. Standards for $PGF_{2}\alpha$ (Cayman Chemical Co., Ann Arbor, MI) used in the assay were 0, 50, 100, 250, 500, 1000, and 5000 pg/tube and PGE_2 standards (Cayman Chemical Co., Ann Arbor, MI) used in the assay were 0, 10, 50, 100, 250, 500, and 1000 pg/tube. The column elute collected was evaporated under prepurified nitrogen gas and then reconstituted in 1 ml of methanol. Reconstitued fractions for $PGF_{2}\alpha$ (1 ml) or PGE (250 μl) were transferred to 12x75 borosilicate glass tubes (assay tubes) and evaporated under prepurified nitrogen gas in a water bath at 37 ^{o}C. Media samples from Experiment 4 for $PGF_{2}\alpha$ (50 μl + 50 μl medium 199) or PGE (10 μl + 90 μl medium 199) were placed directly into the assay tubes. All assay tubes received 100 μl each of prostaglandin phosphate assay buffer (Buffer A), tritiated $PGF_{2}\alpha$ (0.01 μCi/tube) or PGE (0.0083 μCi/tube), and $PGF_{2}\alpha$ or PGE antisera. The total and nonspecific binding tubes received another 100 μl of Buffer A instead of antisera to maintain a constant total volume of 300 μl. Tubes other than samples for assays for Experiment 4 received an additional 100 μl of medium 199 to maintain constant volume of 400 μl. Tubes were mixed and incubated overnight at 4 ^{o}C.

Bound from free $PGF_{2}\alpha$ or PGE was separated by adding 1.0 ml dextran (Kodak Chemicals, Rochester, NY) -coated charcoal (Norit-A, Fisher scientific, Fairlawn, NJ) suspension to tubes at 4 ^{o}C within 2 minutes of a 10 minute incubation period. Tubes were mixed, incubated the rest of the 10 minute incubation period at 4 ^{o}C, and then centrifuged at 3000 r.p.m. for 10 minutes at 4 ^{o}C. The supernatant fluid was then decanted into 4 ml scintillation vials (Omnivials, Wheaton Scientific, Millville, NJ) with 2 ml of

liquid scintillation cocktail (Universol, ICN Radiochemicals, Irvine, CA) and radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4550, Downers Grove, IL) for two minutes. Average binding by antisera for $PGF_2\alpha$ and PGE in all assays averaged 35 and 37 percent, respectively. Sensitivity for $PGF_2\alpha$ and PGE was 30 pg/ml and 10 pg/ml, respectively.

Crossreactivity of the $PGF_2\alpha$ antiserum with other prostaglandins was as follows: 6-keto-$PGF_1\alpha$, 3.5 %; and less than 0.5 % each with 13,14-dihydro-15-keto-$PGF_2\alpha$; 15-keto-$PGF_1\alpha$; 11-deoxy-13,14-dihydro-14-keto-11ß,16ϵ-cycloprostaglandin E_1; PGA_1; PGA_2; PGE_1; PGE_2; 13,14-dihydr-15-keto-PGE_2; 13,14-dihydro-15-keto-$PGF_1\alpha$; and 13,14-dihydro-15-keto-PGE_1. Crossreactivity of the PGE_2 antisera with other prostaglandins was as follows: PGE_1, 55 %; PGA_1, 35 %; PGA_2, 27 %; and less than 1 % each with 11-deoxy-13,14-dihydro-14-keto-11ß,16ϵ-cycloprostaglandin E_1; 15-keto-$PGF_1\alpha$; $PGF_2\alpha$; 13,14-dihydro-15-keto-$PGF_2\alpha$; 13,14-dihydro-15-keto-PGE_2; 6-keto-$PGF_1\alpha$; 13,14-dihydro-15-keto-$PGF_1\alpha$; 13,14-dihydro-15-keto-PGE_1. Crossreacting prostaglandins were separated by partition chromatography on Sephadex LH-20 columns as described above except PGE_1 and PGE_2 could not be separated and values are reported as PGE.

Parallelism of the assays was determined by extraction twice of pooled uterine or ovarian venous plasma from 90 to 100 day pregnant ewe with ethyl acetate and separation of $PGF_2\alpha$ or PGE on Sephadex LH-20 columns. Extracts of the different pooled plasma volumes were parallel to the standard curve.

Concentrations of $PGF_2\alpha$ and PGE in samples were adjusted individually for procedural losses estimated by recovery of radiolabelled $PGF_2\alpha$ or PGE_2. Percent recoveries for uterine venous $PGF_2\alpha$ (n=46 assays), uterine venous PGE (n=38 assays), ovarian venous $PGF_2\alpha$ (n=7 assays), ovarian venous PGE (n=8 assays) in Experiments 1, 2 and 3 and $PGF_2\alpha$ (n=6 assays) and PGE (n=4 assays) secretion *in vitro* in Experiment 4 were 66±1, 44±3, 29±6, 41±2, 98±2, and 82±2 percent, respectively.

Intraassay coefficients of variation for uterine venous $PGF_2\alpha$, uterine PGE, ovarian venous $PGF_2\alpha$, ovarian venous PGE in experiments 1, 2 and 3 and $PGF_2\alpha$ and PGE *in vitro* in Experiment 4 were 12.7, 18.9, 34.7, 33.4, 14.8, and 10.1 percent, respectively. Interassay coefficients of variation for uterine venous $PGF_2\alpha$, uterine venous PGE, ovarian venous $PGF_2\alpha$, ovarian venous PGE in Experiments 1, 2, and 3 and $PGF_2\alpha$ and PGE *in vitro* in experiment 4 were 24.3, 27.4, 27.3, 28.3, 16.7, and 22.5 percent, respectively.

Reagents for the $PGF_2\alpha$ and PGE radioimmunoassays were as follows:

1. <u>Prostaglandin Phosphate Assay Buffer (Buffer A)</u>

   Sodium Phosphate Dibasic ($Na_2HPO_4.7H_2O$)--8.66 g

   Sodium Phosphate Monobasic ($NaH_2PO_4.H_2O$)--5.38 g

   Sodium Chloride (NaCl)--58.44 g

   Sodium Azide (NaN_3)--1.00 g

   Bovine Gamma Globulin--2.00 g

(Sigma Chemical Co., St.Louis, MO)

Reagents were placed in an one liter volumetric flask, brought to volume with double distilled water, mixed gently, adjusted to pH 7.0, and stored at 4 ^{o}C.

2. Dilution of $PGF_2\alpha$ and PGE Antisera

One ml of 1:10 dilution of $PGF_2\alpha$ antiserum (RA 546-C, L.Levine, Brandeis University, Waltham, MA) was transferred to a 200 ml volumetric flask and brought to volume with Buffer A. The solution was mixed gently and stored at 4 ^{o}C. Final dilution was 1:2000.

One ml of 1:10 dilution of PGE_2 antiserum was transferred to a 10 ml volumetric flask, brought to voiume with Buffer A, and gently mixed well. One ml of this dilution was transferred to an Erlenmyer flask and 149 ml of Buffer A were added. The solution was mixed gently and stored at 4 ^{o}C. Final dilution was 1:15,000.

3. Radiolabelled $PGF_2\alpha$ and PGE_2

One hundred μl of ^{3}H-$PGF_2\alpha$ (5,6,8,9,11,12,14,15-^{3}H(N); 10 μCi) were transferred to a 100 ml volumetric flask and brought to volume with Buffer A. The solution was mixed gently and stored at 4 ^{o}C. The final concentration was 0.01 μCi/100 μl.

One hundred and twenty-five μl of tritiated PGE_2 (5,6,8,11,12,14,15-^{3}H(N); 12.5 μCi) were transferred to a flask and 150 ml of freshly opened methanol were added. The solution was mixed well and stored at 4 ^{o}C. The

flask was sealed well with a glass stopper and parafilm between use. The final concentration was 0.0083 μCi/100 μl.

Tritiated $PGF_{2}\alpha$ and PGE_2 were from E.I. Du Pont de Nemours, Wilmington, Delaware.

4. $PGF_{2}\alpha$ Standard Solutions

Powdered $PGF_{2}\alpha$ tromethamine (10 μg) in the original vial (Cayman Chemical, Ann Arbor, MI) was dissolved with 1 ml Buffer A and mixed gently to make a "stock solution" of 10 μg/ml. One hundred μl of the "stock solution" were transferred to a 10 ml volumetric flask, brought to volume with Buffer A, and mixed gently, to make a "working solution-I" of 10 ng/100 μl. Two hundred and fifty μl of the "stock solution" were transferred to a 10 ml volumetric flask, brought to volume with Buffer A, and mixed gently to make a "working solution-II" of 25 ng/100 μl.

Two ml of "working solution-II" were transferred to a scintillation vial and 8 ml of Buffer A were added and mixed well to make the "5000 pg/100 μl $PGF_{2}\alpha$ standard". One ml of "working solution-I" was transferred to a scintillation vial and 9 ml of Buffer A were added and mixed well to make the "1000 pg/100 μl $PGF_{2}\alpha$ standard". One ml of the "5000 pg/100 μl standard" was transferred to a vial and 9 ml of Buffer A were added and mixed well to make the "500 pg/100 μl $PGF_{2}\alpha$ standard". Five hundred μl of the "5000 pg/100 μl standard" were transferred to a vial and 9.5 ml of Buffer A were added and mixed well to make the "250 pg/100 μl $PGF_{2}\alpha$ standard". One ml of the "1000 pg/100 μl standard" was transferred to a vial and 9 ml of Buffer A were added and mixed well to make the "100

pg/100 μl $PGF_{2}\alpha$ standard". Five hundred μl of the "1000 pg/100 μl standard" were transferred to a vial and 9.5 ml of Buffer A were added and mixed well to make the "50 pg/100 μl $PGF_{2}\alpha$ standard". All the standard solutions were stored at 4 ^{o}C between use, except "stock solution", "working solution-I" and "working solution-II" which were stored at -20 ^{o}C.

5. <u>PGE_2 Standard Solutions</u>

Prostaglandin E_2 (Cayman Chemical, Ann arbor, MI) was received as 1 mg powder in 100 μl methanol. Ten μl of this original PGE_2 were transferred to a 10 ml volumetric flask, brought to volume with freshly opened methanol, and mixed well to make a "stock solution" of 1000 ng/100 μl. One ml of "stock solution" was transferred to a 10 ml volumetric flask, brought to volume with methanol, and mixed well to make a "working solution-I" of 100 ng/100 μl. Two hundred and fifty μl of "working solution-I" were transferred to a 10 ml volumetric flask, brought to volume with methanol, and mixed well to make a "working solution-II" of 2500 pg/100 μl.

One hundred μl of "working solution-I" were transferred to a graduated glass tube with a cap, 9.9 ml of methanol were added, capped, and mixed well to make the "1000 pg/100 μl PGE_2 standard". Fifty μl of "working solution-I" were transferred to a graduated glass tube, 9.95 ml of methanol were added, capped, and mixed well to make the "500 pg/100 μl PGE_2 standard". One ml of "working solution-II" was transferred to a graduated glass tube, 9 ml of methanol were added, capped, and mixed well to make the "250 pg/100 μl PGE_2 standard". One ml of the "1000 pg/100

μl standard" was transferred to a graduated glass tube, 9 ml of methanol were added, capped, and mixed well to make the "100 pg/100 μl PGE_2 standard". One ml of the "500 pg/100 μl standard" was transferred to a graduated glass tube, 9 ml of methanol were added, capped, and mixed well to make the "50 pg/100 μl PGE_2 standard". One ml of the "100 pg/100 μl standard" was transferred to a graduated glass tube, 9 ml of methanol were added, capped, and mixed well to make the "10 pg/100 μl PGE_2 standard". All PGE_2 standard solutions were sealed well with parafilm and stored at -20 ^{o}C between use.

6. Dextran-Coated Charcoal Suspension for $PGF_2\alpha$ and PGE_2 RIA

Dextran (Kodak Chemicals, Rochester, NY) 250 mg and 2.5 g Carbon decolorizing alkaline Norit-A Charcoal (Fisher Scientific, Fairlawn, NJ) were placed in a flask and 1000 ml of double distilled water were added. The solution was mixed with a magnetic stirrer and stored at 4 ^{o}C.

APPENDIX C

RADIOIMMUNOASSAY FOR ESTRADIOL-17ß

Estradiol-17ß was quantified using the procedures described by Butcher (1073) and validated in our laboratory (1074). Five hundred μl of jugular venous plasma or pooled jugular venous plasma samples in duplicate in 13x100 mm borosilicate glass tubes were extracted twice by vortexing for a one minute period each with 4 ml freshly opened anesthesia grade diethylether (Mallinkrodt, Paris, KY). Iodinated estradiol-17ß (3-iodo-estradiol-17ß, ICN Biomedical, Costa Mesa, CA) was added to separate pooled plasma samples to determine percent recoveries. Tubes were placed in a dry ice/methanol bath to freeze the aqueous phase and the organic phase was decanted into 12x75 mm assay tubes. The solvent was evaporated under prepurified nitrogen gas in a 37 ^{o}C water bath. Media samples collected from Experiment 4 were assayed directly without extraction. Four hundred μl of medium samples were assayed in duplicate, while other tubes received 400 μl of medium 199 instead to maintain total of 700 μl volume per assay tube.

Estradiol-17ß standards (Steraloids, Wilton, NH) used were 0, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 pg/tube in duplicate in methanol and the solvent was dried under prepurified nitrogen in water bath. All assay tubes received 100 μl of 1 % bovine serum albumin (Fraction V, Sigma Chemical Co., St.Louis, MO) and 100 μl of estradiol-17ß antiserum (1:100,000 dilution; R.L.Butcher, West Verginia University, Ewe 12, 1-17-72). The total and nonspecific binding tubes received 100 μl of serum assay buffer instead

of antiserum. Tubes were vortexed, incubated for five minutes at 37 ^{o}C, and then incubated for an hour at 4 ^{o}C. Tubes received 100 μl of ^{125}I-estradiol-17ß (0.01 μCi), tubes were vortexed, and then incubated for a minimum of 15 hours at 4 ^{o}C.

Bound from free radiolabelled hormone was separated by adding 0.8 ml dextran-coated charcoal suspension to all tubes within the first 2 minutes for a 10 minute incubation period, except total tubes which received double distilled water instead. Tubes were vortexed, incubated for the rest of the 10 minutes at 4 ^{o}C, and then centrifuged for 10 minutes at 3000 r.p.m. (Sorvall RC5C, Newton, CT) at 4 ^{o}C. The supernatant fluid was decanted into 4 ml scintillation vials (Omnivials, Wheaton Scientific, Millville, NJ) containing scintillation fluid (Universol, ICN Radiochemicals, Irvine, CA) and radioactivity was counted for two minutes in a gamma counter (Autogamma 5000 series, Packard Instruments, Downers Grove, IL).

Parallelism of the assay was confirmed by measuring estradiol-17ß in 3 different volumes and comparing it to the standard curve. Sensitivity of the assay was 1 pg/ml. Crossreactivity of other steroids tested with the estradiol-17ß antisera was as follows: estriol, 1.4 %; estradiol-17α, 1 %; 19-nortestosterone, 0.8 %; and less than 0.01 % each with 18-hydroxyprogesterone, dihydrotestosterone, 6-dehydrotestosterone, testosterone, 4-androsten-3ß,17ß-diol, dehydroepiandrostene, androstenedione, corticosterone, 4-pregn-20α-ol-3-one, androstenediol, allopregnenolone, 17α-hydroxyprogesterone, 11α-hydroxyprogesterone, androstanedione, 5α-androstan-3ß,17ß-diol, cholesterol, 6-keto-17ß-estradiol, estrone sulfate, and estradiol sulfate.

Percent recoveries for jugular venous (n=21) and in vitro media (n=5) samples averaged 86±1 and 97±3 percent, respectively. Intrasaay coefficient of variation for jugular venous and in vitro medium samples were 14.2 and 17.3 percent, respectively. Interassay coefficient of variation for jugular venous and in vitro media samples were 23 and 19 percent, respectively.

Reagents for the estradiol-17ß RIA were as follows:

1. 2.5 M Sodium Phosphate Stock Solution

Sodium Phosphate Dibasic ($Na_2HPO_4.7H_2O$)--335.09 g

Sodium Phosphate Monobasic ($NaH_2PO_4.H_2O$)--172.49 g

Sodium phosphate dibasic was placed into beaker, 500 ml of double distilled water were added, and dissolved on a magnetic stirrer by gentle heat. Sodium phosphate monobasic was placed into another beaker, 500 ml of double distilled water were added, and dissolved on a magnetic stirrer by gentle heat. The two solutions were mixed in 1000 ml Erlenmeyer flask with a magnetic stirrer at a ratio of approximately 300 ml of dibasic to 275 ml monobasic solutions. The pH was adjusted to 6.8, capped, cooled and stored at 4 ^{o}C between use.

2. Serum Assay Buffer (SAB)

Sodium Chloride (NaCl)--9 g

2.5 M Sodium Phosphate Stock Solution--4 ml

Gelatin (Knox Inc., Englewood Cliffs, NJ)--1 g

Thimerosol--0.1 g

The first two reagents were placed in a 1000 ml volumetric flask, brought to volume with double distilled water, and then transferred to an Erlenmeyer flask. The solution was heated gently on a magnetic stirrer and gelatin was added to dissolve. The mixture was cooled before thimerosol was added. The solution was mixed well, pH was adjusted to 7.0-7.2, and stored at 4 ^{o}C between use.

3. Antiserum to Estradiol-17ß

Reconstituted original antiserum (anti-estradiol-17ß-3-hemisuccinyl-human serum albumin, R.L. Butcher, West Virginia University, ewe 12, 1-17-72) was added (11.7 μl) to a 100 ml volumetric flask and brought to volume with SAB. The solution was mixed gently and stored at -20 ^{o}C between use. This is the "anti-estradiol-17ß stock solution" at a 1:10,000 dilution.

Twenty ml of "stock solution" were transferred to a 200 ml volumetric flask and brought to volume with SAB. The solution was mixed well and stored at 4 ^{o}C between use. This is the "antiserum to estradiol-17ß" used in the assays at a dilution of 1:100,000.

4. Iodinated Estradiol-17ß

Refer to shipping information to determine total concentration of isotope in ^{125}I-estradiol-17ß (ICN Biomedical, Costa Mesa, CA) and prepare

a final concentration of 0.01 μCi/100 μl dilution with SAB. Mix well and store at 4 ^{o}C.

5. <u>1 % Bovine Serum Albumin Solution (1 % BSA)</u>

Bovine Serum Albumin (BSA)--1 g

Sodium Chloride (NaCl)--0.9 g

2.5 M Sodium Phosphate Stock Solution--400 μl

Bovine serum albumin and NaCl were transferred to a 100 ml erlenmeyer flask, 100 ml of double distilled water were added, and then phosphate stock solution was added. Mix well and store at 4 ^{o}C. This solution needs to be replaced about every week to keep it fresh.

6. <u>Estradiol-17ß Standard Solutions</u>

Fifty μl of "original stock solution (1 mg/ml)" were transferred to a 50 ml volumetric flask, brought to volume with freshly opened methanol, and mixed well to make a "1 μg/ml standard". Fifty μl of the "1 μg/ml standard" were transferred to a 50 ml volumetric flask, brought to volume with methanol, and mixed well to make a "1 ng/ml standard". Both standards along with the stock solution were sealed well and kept at -20 ^{o}C.

Five ml of the "1 ng/ml standard" were transferred to a 100 ml volumetric flask, brought to volume with methanol, and mixed well to make the "50 pg/ml estradiol-17ß standard solution" used in the assays. Five hundred μl of "1 ng/ml standard" were transferred to a 100 ml volumetric flask, brought to volume with methanol, and mixed well to make the "5

pg/ml estradiol-17ß standard solution" used in the assays. Both solutions were sealed well and stored at 4 ^{o}C between use.

7. Dextran-Coated Charcoal Suspension

Carbon decolorizing alkaline (2.5 g; Norit-A, Fisher Scientific, Fairlawn, NJ) and dextran (10 g; Kodak Chemicals, Rochester, NY) were placed into a 1000 ml Erlenmyer flask and 1000 ml of double distilled water were added. The solution was mixed well on a magnetic stirrer and stored at 4 ^{o}C.

APPENDIX D

RADIOIMMUNOASSAY FOR PREGNANCY SPECIFIC PROTEIN B

Pregnancy specific protein B (PSPB) was assayed in uterine venous plasma of 90 to 100 day pregnant ewes by Dr. Garth Sasser, Department of Animal Science, University of Idaho according to the procedures of Sasser et al. (783).

The samples and standards were run in triplicate. Uterine venous plasma samples and assay buffer were kept on ice. Samples (200 μl) were pipetted into 12x75 polypropylene tubes and adjusted to a total volume of 500 μl with 1 % bovine serum albumin-phosphate buffered saline (BSA-PBS) mixture. Standards of PSPB used in the assay were 39.065, 78.15, 156.25, 312.5 or 625 pg and 1.25, 2.5 and 5 ng/tube. Two hundred μl of first antibody (rabbit anti-PSPB) were added to all tubes except total and nonspecific binding tubes. Tubes were vortexed briefly, covered, and incubated for 8 hours at room temperature. One hundred μl of ^{125}I-PSPB were added to all tubes (30,000 cpm/tube) and total tubes were capped. Tubes were vortexed briefly, covered, and incubated overnight (minimum of 8 hours) at room temperature. Two hundred μl of second antibody (sheep anti-rabbit gamma globulin) were added to all tubes except total tubes to separate bound from free hormone. Tubes were vortexed briefly, covered, and incubated for 4 hours at room temperature. One ml of 4 % polyethylene glycol (PEG)-PBS at 4 ^{o}C was added to all tubes, except for total tubes which were not centrifuged or decanted. Tubes were centrifuged at 3000 r.p.m. for 30 minutes at 4 ^{o}C. The supernatant was

discarded. Radioactivity in the tubes was counted in a gamma counter (Packard Instruments, Downers Grove, IL). Sensitivity of the assay was 135 pg. Intraassay and interassay coefficients of variation for 17 assays were 7 and 12 percent, respectively.

Reagents for the PSPB RIA are as follows:

1. Phosphate Buffered Saline (PBS)

Sodium Chloride (NaCl)--16.34 g

Sodium Phosphate Monobasic (NaH_2PO_4)--1.02 g

Sodium Phosphate Dibasic (Na_2HPO_4)--1.64 g

Thimerosol--0.2 g

All chemicals were dissolved in 1800 ml of deionized water and adjusted to pH 7.0 with NaOH. This solution was transferred to a 2 liter volumetric flask, brought to volume with deionized water, and stored at 4 ^{o}C.

2. PBS-ethylenediaminetetraacetic acid (EDTA)

Ethylenediaminetetraacetic acid (1.86 g) was dissolved in 80 ml of PBS and pH was adjusted to 7.0. This solution was transferred to a 100 ml volumetric flask, brought to volume with PBS, and stored at 4 ^{o}C.

3. 1 % Bovine Serum Albumin (BSA)-PBS

Five grams of BSA were dissolved in 450 ml of PBS and pH was adjusted to 7.0. This solution was transferred to a 500 ml volumetric flask, brought to volume with PBS, and stored at 4 ^{o}C.

4. 4 % Polyethylene Glycol (PEG)-PBS

Twenty grams of PEG were dissolved in 450 ml of PBS and pH was adjusted to 7.0. This solution was transferred to a 500 ml volumetric flask, brought to volume with PBS, and stored at 4 ^{o}C.

5. Normal Rabbit Serum (NRS)

A 1:400 dilution of NRS was made with EDTA-PBS.

6. First Antibody

Rabbit anti-PSPB serum (RGS 38-1) was diluted to 1:90,000 in NRS.

7. Second Antibody

Sheep anti-rabbit gamma globulin was diluted to 1:8 with EDTA-PBS.

8. Iodinated PSPB

Iodinated PSPB was diluted to approximately 30,000 cpm/100 μl in PBS-BSA.

9. PSPB Standard Solutions

One ml of PSPB (preparation R-37; 50 ng/ml PBS) was diluted with 1 ml of virgin heifer serum to make a "5 ng/200 μl PSPB standard". One ml of the "5 ng/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "2.5 ng/200 μl PSPB standard". One ml of the "2.5 ng/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "1.25 ng/200 μl PSPB standard". One ml of the "1.25 ng/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "625 pg/200 μl PSPB standard". One ml of the "625 pg/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "312.5 pg/200 μl PSPB standard". One ml of the "312.5 pg/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "156.25 pg/200 μl PSPB standard". One ml of the "156.25 pg/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "78.125 pg/200 μl PSPB standard". One ml of the "78.125 pg/200 μl standard" was diluted with 1 ml of virgin heifer serum to make a "39.065 pg/200 μl PSPB standard". These standard solutions were prepared fresh for each assay.

APPENDIX E

RADIOIMMUNOASSAY FOR CORTISOL

Cortisol was quantified in jugular venous samples from Experiments 1, 2 and 3 by a Coat-A-Count solid phase radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). Polypropylene tubes (12x75 mm) coated with cortisol antiserum received 100 μl of plasma or standards (0, 1, 5, 10, 20, 50 μg/dl) and 1.0 ml ^{125}I-cortisol, and were incubated for 45 minutes at 37 ^{o}C. The supernatant fluid was decanted and the tubes were counted for 1 minute in a gamma scintillation counter (Packard Instruments, Downers Grove, IL).

Different volumes of plasma were used to verify parallelism with the standard curve. Percent binding averaged 65 % and sensitivity of the assay was 0.2 μg/dl. Crossreactivity of the other steroids with the antisera were as follows: aldosterone, 0.01 %; corticosterone, 1.4 %; cortisone, 0.6 %; 11-deoxycorticosterone, 1.5 %; 11-deoxycortisol, 0.25 %; 21-deoxycortisone, 0.04 %; estriol, 0.0005 %; estrone, 0.025 %; 17α-hydroxyprogesterone, 0 %; and progesterone, 0.15 %. Intraassay and interassay coefficients of variation determined by use of pooled plasma averaged 8 and 8.03 percent, respectively.

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