# EFFECT OF EXOGENOUS PROGESTERONE ON INTERESTROUS INTERVAL AND EARLY PREGNANCY

By

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Bachelor of Science
in Agriculture

Oklahoma State University

Stillwater, Oklahoma

1985

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1987

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ON INTERESTROUS INTERVAL AND

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1290937

ACKNOWLEDGEMENTS

There are many people to whom I would like to express my sincere gratitude towards. For without their encouragement, advice and support, this thesis would never have been completed. I would like to begin by expressing my appreciation and thanks to Dr. Rodney Geisert, chairman of the committee, mentor and friend. Dr. Geisert's enthusiasm and dedication to the field of teaching and research both inspired me, at first, to continue on for a Master of Science degree and now, to hopefully always be associated with some aspect of teaching and research. I would also like to thank Susan, his wife, Ryan, his son, and Cat, their cat, for their friendship.

Appreciation is also extended to Dr. Mike Zavy, for his thought-stimulating conversations and also technical advice, to Dr. Dave Buchanan, for assisting us with the analyses of the study, and to Dr. Ulrich Melcher, whose insight from a different view brought aspects to light we had not even thought of.

Thanks is also due to Dr. Gregor Morgan for giving us technical assitance and also a taste of New Zealand humor and to Dr. Robert Wettemann for his knowledge in radioimmunoassays.

The friendship and support from LeAnn Gries, Vicki Nelson, Eugene Tinker, and Mark Richards are also greatly appreciated. The fun and fellowship with all those who played on the softball team at one time or another was also something I'll cherish for a long time. Steve Welty and all those who worked at the Nutrition and Physiology Barn deserve a big thanks as they cared for and helped in working the cows. To those people in the lab who took time to answer my "I have a question" question a hearty thanks is due and also to the work study students who kept blood tubes numbered and clean graduated cylinders in the cabinets. A big thank you is also extended to Kathy Frank and Tammy Cozzens for their friendship and secretarial assistance.

I would especially like to thank my family for their love and understanding throughout my whole graduate career. I would also like to extend my appreciation to Brad's family for their support and understanding.

A very special thanks is to my husband, Brad Williams, for his patience, love and encouragement. Without his help and being understanding, especially in these last few weeks, this thesis would not have been completed at this time.

Glory and praise also to the Lord as He has watched and cared for us through it all.

Needless to say, without the support from many people, I would not have been able to put this thesis together. And so to all of them, those mentioned and those I have not mentioned, I say, "Thank you!"

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# CHAPTER I

## INTRODUCTION

Early pregnancy of large domestic animals is a dynamic period. During this time, the uterine environment, under the influence of progesterone, develops for embryotrophic support. Progesterone-induced proteins and other select serum factors create an environment which can support and stimulate the growth of the developing embryo (Bazer, 1975). The bovine embryo is dependent on secretions from secretory glands in the uterine endometrium, as adhesion and attachment do not begin until approximately day 20 to 22 after mating (Leiser, 1975). The bovine embryo, like other mammalian embryos, may be characterized as being microlecithal. That is, the embryo has little yolk and therefore, little endogenous nutrients for development and survival.

During early pregnancy, the fertilized egg undergoes many morphological changes, developing from a one cell zygote to a multicellular conceptus. The conceptus consists of cells which have differentiated into the inner cell mass and the trophectoderm. Eventually, the inner cell mass forms the embryo proper and the trophectoderm forms the placental membranes. The conceptus must prevent corpus

luteum (CL) regression as well as block immunological recognition by the maternal system so it will not be rejected as a foreign allograft (see reviews by Thatcher et al., 1985; Bazer et al., 1986; Thatcher et al., 1986). The CL is essential for pregnancy maintenance as it provides the main source of progesterone until approximately day 210 of gestation in the bovine. If the CL undergoes regression or is removed anytime prior to this period, the conceptus will be aborted. However, if progesterone alone or a combination of estrogen and progesterone is administered exogenously, pregnancy continues (Hawk et al, 1963). Therefore, progesterone is a major steroid involved in maintenance of pregnancy of domestic farm species.

In the cyclic ewe and cow, it has been suggested that exposure to progesterone for a minimum of 8 days (Lawson and Cahill, 1983) and 10 days (Ginther, 1970), respectively, is necessary for luteolysis to occur. Administration of progesterone early in the estrous cycle of both the ewe and cow results in an earlier return to estrus (Ottobre et al, 1980; Battista et al., 1984). However, the mechanism involved with the effect of early progesterone administration has not been clearly determined for the bovine.

Studies utilizing embryo transfer have demonstrated that close synchrony (± 24 hours of the same day of the estrous cycle) between the donor and the recipient is necessary for successful establishment of pregnancy (Moor

and Rowson, 1966; Betteridge et al., 1980). Altering the endocrine system of the recipient by administering progesterone during early pregnancy can affect conceptus development. Moore (1975) reported day 4 embryos (day 0 = estrus) were able to survive and maintain pregnancy when transferred into a day 2.5 recipient ewe treated with high doses of progesterone for the first 6 days of pregnancy. Lawson and Cahill (1983) reported the survival of transferred day 10 embryos into day 6 recipient ewes which had been treated with progesterone for the first three days of the estrous cycle was comparable to the survival of embryos transferred to synchronous recipients. These data suggest that early progesterone administration may advance endometrial development allowing for the survival of older asynchronous embryos. Little is known about the effect of progesterone treatment during early pregnancy in the cow.

Therefore, the present study was designed to determine the effect of progesterone administration during the early days of the estrous cycle or pregnancy in the bovine. The literature review which follows will provide the current knowledge of the bovine estrous cycle, embryo development, maternal recognition of pregnancy, uterine environment, and embryonic mortality which is necessary for a better understanding of the mechanisms and effects of progesterone stimulation.

# CHAPTER II

# LITERATURE REVIEW

## The Estrous Cycle

### Endocrine Changes

The bovine female, like other large domestic farm animals, exhibits cyclic periods of sexual receptivity and nonreceptivity. The length of the estrous cycle in the cow ranges from 17 to 25 days, with an average of 21 days (Asdell et al., 1949). The estrous cycle is divided into four stages based on endocrine and behavioral changes. The stages of the estrous cycle are characterized as proestrus, estrus, metestrus and diestrus.

During proestrus, peripheral plasma progesterone concentrations decline. Negative feedback of progesterone on gonadotropin releasing hormone (GnRH) release from the hypothalamus is removed and the pulsatile release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are increased from the anterior pituitary. While FSH stimulates the development of follicles, inhibin, which is produced and released from the granulosa cells, allows one dominant follicle to progress to the Graafian follicle stage (Henderson and Franchimont, 1983). LH concentrations also

begin to increase in the peripheral blood (Chenault et al., 1975). That is, the pulsatile nature of LH is altered with an increase in frequency but decrease in pulse amplitude (Rahe et al., 1980). LH stimulates the theca interna cells of the follicle to synthesize androstenedione from cholesterol (Hansel and Fortune, 1978). The granulosa cells then convert androstendione to estradiol-17B (LaCroix et al., 1974).

Ayalon and Shemesh (1974) reported a proestrous surge in plasma progesterone occurred approximately 16 hours before the onset of estrus. The surge was of short duration, as indicated by low levels of progesterone four hours later, and occurred well before the LH surge. The authors suggest progesterone may act synergistically with estradiol-17B to modify or stimulate the manifestation of estrus and sexual receptivity.

The decrease in peripheral plasma progesterone is the result of a regressing corpus luteum (CL). As the CL regresses, it transforms into scar connective tissue called a corpus albican and loses its steroidogenic capabilities.

Estrus follows the stage of proestrus. In the bovine, estrus is a period usually lasting 12 to 18 hours when the female is sexually receptive to the male (Schams et al., 1977). The behavior of the cow changes as she will allow the bull to mount and copulate. Also during this period, the cow exhibits homosexual activity as she mounts and permits mounting by other cows. Mucous discharge from the

vulva of the cow during this stage is characterized by a watery, clear, and stringy appearance.

Estrus is characterized by high peripheral plasma levels of estradiol-17B, i.e. 10 pg/ml (Mellin and Erb, 1965; Henricks et al., 1971a; Hansel and Echternkamp, 1972; Wettemann et al., 1972; Glencross et al., 1973; Schams et al., 1977) and low peripheral plasma progesterone concentrations, i.e. less than 1 ng/ml (Gomes and Erb, 1965; Henricks et al., 1970; Wettemann et al., 1972; Short et al., 1973; Schams et al., 1977; Kesner et al., 1981). As plasma estrogen rises, LH secretion decreases slightly. Following this short decline, LH sharply increases with an ovulatory surge to approximately 40 ng/ml (Schams et al., 1977; Scherwood and McShane, 1977). Theoretically, the increasing estrogen first inhibits LH by decreasing the responsiveness of GnRH at the anterior pituitary. That is, there is a lack of binding of estrogen by the receptors in the preoptic anterior hypothalamic area, which stimulates the synthesis and/or secretion of GnRH (Martin et al., 1978).

However, the inhibitory effect of estradiol-17B is of short duration and is soon followed by a stimulatory or positive effect on the anterior pituitary which increases GnRH responsiveness (Martin et al., 1978).

The next stage following estrus is metestrus. Metestrus occurs on days 2 to 4 (day 0 = estrus). During this stage, the cow will no longer accept the bull, the mucous becomes thick and viscous, and some post-estrus

"bleeding" may be seen from the vulva. Post-estrus bleeding results from the rupture of estrogen-developed endometrial capillaries.

As the hypothalamus is stimulated by estrogen, a surge of GnRH is released from the preoptic, supra chiasmatic, and arcuate nuclei. Ovulation is stimulated by the large increase in LH secretion from the anterior pituitary resulting from GnRH release (Kesner and Convey, 1982). The peak of LH appears to occur in the first 8 hours of estrus in the cow (Hansel et al., 1973) with ovulation normally occurring approximately 30 hours after the plasma LH peak (Henricks et al., 1970; Hansel and Echternkamp, 1972). Ovulation therefore, occurs 10 to 20 hours after the end of estrus in the bovine.

As stated previously, the surge of LH stimulates the release of the ovum along with its cumulus oophorus cells from the Graafian follicle. Plasma estrogen levels begin to decrease shortly after ovulation (Mellin and Erb, 1965; Henricks et al., 1972; Glencross et al., 1973) and a corpus hemorrhagicum is formed at the site of the ruptured follicle. Progesterone concentrations remain less than 1 ng/ml during metestrus (Gomes and Erb, 1965; Wettemann et al., 1972; Schams et al., 1977). At the end of metestrus, plasma progesterone concentrations begin to increase as LH stimulates the theca interna and granulosa cells of the ruptured follicle to differentiate into large and small

luteal cells (Simmons and Hansel, 1964; Sherwood and McShane, 1977).

Diestrus begins with development of the functional CL. This stage lasts from approximately day 5 to 18 of the estrous cycle. During diestrus, the CL reaches its maximum size and produces the greatest levels of progesterone. Peripheral plasma progesterone concentrations rise from 2 to 3 ng/ml at the start of diestrus (day 5) to 5 to 8 ng/ml by day 10. Plasma LH concentrations remain at basal levels (0.6 ng/ml) during the luteal phase (Henricks et al., 1970).

Progesterone is the major steroidogenic product from luteal cells together with small amounts of 20B-hydroxy-4-pregnen-3-one and pregnenolone (Mason et al., 1962; Gomes and Erb, 1965; Sherwood and McShane, 1977). Functions of progesterone include preparation of the uterus for embryo attachment and development, stimulation of secretions from the uterine glands within the endometrium and possibly from the conceptus, and inhibition of uterine myometrial contraction.

Progesterone also participates in the regulation of uterine steroid receptors. Atkins et al. (1980) reported an increase in the number of binding sites per cell on the ipsilateral versus the contralateral uterine horn (in relation to the CL) on days 10 and 11. The significance of this difference is unknown. A difference in the number of binding sites of nuclear progesterone receptors in the uterine endometrium between individual cows on days 4

through 18, however, was not found. A study by Zelinski et al. (1982) indicated that the concentration of cytoplasmic progesterone receptors in the endometrium of heifers changed throughout the estrous cycle. This change appears to be dependent upon the steroids, estrogen and progesterone, as progesterone receptors increase when estrogen secretion is maximum and decrease when progesterone is maximum. It has been suggested that progesterone may antagonize the ability of estrogen to promote the synthesis and/or the replenishment of uterine estrogen and progesterone receptors.

Morphological examination of the bovine CL reveals two types of luteal cells classified as a large and small type (Priedkalns and Weber, 1968). The large luteal cells (25 to 40 um) are thought to originate from granulosa cells of the follicle (Donaldson and Hansel, 1965; Alila and Hansel, 1984) and to comprise the majority of the CL volume during this time (Parry et al., 1980). Small luteal cells, which may originate from thecal cells, are interspersed with a few fibroblasts and loose connective tissue. Alila and Hansel (1984) suggested that the small luteal cells eventually develop into large luteal cells, with the large cells originating from granulosa cells disappearing as pregnancy continues. A few contrasting reports have indicated that a distinct size difference between large and small luteal cells may not exist (Greenstein, 1958; Parry et al., 1980) in the bovine CL.

Luteal tissue in the CL is highly vascularized, allowing all the large luteal cells to be in close proximity to the vascular capillaries. Luteal cells in the bovine are morphologically similar to other steroid secreting cells and luteal cells of other mammals (Christensen and Gillim, 1969; Fawcett et al., 1969; Enders, 1973).

During the mid-luteal stage, luteal cells contain three types of granules: microperoxisomes, primary lysosomes and secretory granules. Parry et al. (1980) found the secretory granule concentration in the luteal cytoplasm correlated with the reported profile of progesterone secretion during the estrous cycle indicating some relation with progesterone release. Gulyas and Yuan (1975) suggest the microperoxisomes in the rhesus monkey may be involved in steroidogenesis in luteal cells. This theory may also hold true in the bovine.

The number of mitochondria present in luteal cells increases during mid-cycle when progesterone synthesis is increasing. The elevated number of mitochondria increases the energy potential needed during cholesterol side-chain cleavage and therefore, progesterone production. The cytoplasm also contains large nuclei along with groups of well-developed granular endoplasmic reticulum and scattered but numerous ribosomes and/or polysomes indicating protein synthesis may be another function of the luteal cells (Parry et al., 1980).

It has been suggested the large luteal cells in the bovine CL have limited steroidogenic potential (Donaldson et al., 1965), as only the small cells respond to administration of LH in vitro (Donaldson and Hansel, 1965). An in vitro study by Ursely and Leymarie (1979) indicated bovine large luteal cells synthesized and released larger amounts of progesterone compared to small luteal cells. However, when LH was added, small luteal cells synthesized and released comparable amounts of progesterone compared to large luteal cells indicating LH sensitivity or responsiveness was ten fold greater in small versus large cells. These data agree with the previous reports in the ewe. Fitz et al. (1982) indicated small ovine luteal cells contain more receptors for LH than large luteal cells. Whereas, large luteal cells have been found to contain more receptors for estradiol (Glass et al, 1984) and prostaglandin $E_2$ and $F_{2\alpha}$ compared to small luteal cells (Fitz et al., 1984).

In the ewe, the number of small and large luteal cells change throughout the estrous cycle (Farin et al., 1986; Schwall et al., 1986). Early in the cycle, small luteal cells predominate, while later in the cycle, large cells which contain $PGF_{2\alpha}$ and $PGE_2$ receptors predominate. These findings suggest the development of the CL is associated with increased numbers of large luteal cells and that luteolysis is associated with a preferential loss of small luteal cells.

Binding proteins for progesterone exist in the bovine CL (Willcox et al., 1978). These binding proteins may function in intracellular synthesis and/or transport of progesterone to the cell surface. Estrogen-binding proteins have also been found in the bovine CL (Kimball and Hansel, 1974).

The bovine CL has also been found to be a rich source of neurohypophysial peptides. Wathes et al. (1983) suggest oxytocin biosynthesis may occur within the CL. Utilizing immunocytochemical techniques, Sawyer et al. (1986) detected immunoreactive neurophysin and oxytocin in the CL as early as day 4 and until day 15 in the ovine. Regardless of the day of the estrous cycle, the large luteal cells were the only cells which stained positively. This supports previous reports in which large luteal cells have been indicated as the source of oxytocin and neurophysin in both the ewe and cow (Rodgers et al., 1983; Watkins, 1983; Guldenaar et al., 1984).

As plasma progesterone concentrations continue to increase, ovarian blood flow also increases. Ford and Chenault (1981) demonstrated a positive correlation between ovarian blood flow and systemic concentration of progesterone, but a negative correlation with systemic concentration of estradiol-17B. Uterine arterial blood flow, on the other hand, was negatively correlated with systemic progesterone concentration and positvely correlated with systemic estradiol concentration. The importance of

the direction of blood flow is unknown. Ford et al. (1979) reported blood flow to uteri in pregnant and nonpregnant cows was similar until day 14 after estrus. In nonpregnant cows, blood flow to either the uterine horn ipsilateral or contralateral to the CL did not increase, but remained constant. In pregnant cows, blood flow to the gravid uterine horn increased two to three fold between days 14 and 18. By day 19 of pregnancy, blood flow returned to approximately the same level measured on day 13. These data indicate the presence of a conceptus modifies or regulates uterine blood flow to the gravid horn during pregnancy.

If fertilization of the ovum takes place, the embryo signals the maternal system and the CL of pregnancy is maintained. Progesterone concentrations in the pregnant cow remain elevated, i.e. 5 - 10 ng/ml (Henricks et al., 1970), until parturition.

However, if a conceptus is not present, progesterone concentrations begin to decline on approximately day 17 to 18 of the cycle due to lysis of the CL (Henricks et al., 1970; Glencross et al., 1973; Chenault et al., 1976; Schams et al., 1977; Ireland et al., 1979). As stated previously, the decrease in progesterone allows one follicle to become dominant and eventually ovulate. Although follicles do continue to develop throughout the cycle, they do not ovulate but are usually destined to undergo atresia (Matton et al., 1981; Ireland and Roche, 1983; Braden et al., 1986). Only the largest follicles on day 17-18 in a cyclic cow

continue to develop to the dominant Graafian follicle which ovulates during metestrus.

Coincident with the decline in progesterone concentration is the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Nancarrow et al., 1973) by the uterine endometrium into the uterine lumen (Bartol et al., 1981) and into the utero-ovarian venous drainage (Shemesh and Hansel, 1975). Pulses of $PGF_{2\alpha}$ in the uterine-ovarian vein of the cow have been reported to occur across a two to three day period, with estrus occurring 1 to 4 days after the first increase in $PGF_{2\alpha}$ (Kindahl et al., 1976).

Prostaglandin $F_{2\alpha}$ is formed from arachidonic acid. Arachidonic acid is derived from phosphatidylethanolamine in the phospholipid bilayer of the cell membrane. Phosphatidylethanolamine, which is a major component of the cell membrane, releases arachidonic acid after being acted upon by methyl transferases and phospholipase $A_2$. Arachidonic acid is then converted to $PGF_{2\alpha}$ by cyclooxygenases, peroxidases and PG synthetases (Samuelsson et al., 1975, 1978; Wlodawer et al., 1976; Basu, 1984).

Prostaglandin $F_{2\alpha}$ is the proposed luteolysin in cattle (Kindahl et al., 1976). Hysterectomy during the mid-luteal phase extends CL function in cows, indicating the presence of a luteolytic substance originating from the uterus (Wiltbank and Casida, 1956). A number of studies have provided evidence for the involvement of $PGF_{2\alpha}$ in luteolysis. Administration of exogenous $PGF_{2\alpha}$ stimulates CL

regression in cattle (Hixon and Hansel, 1974; Louis et al., 1974; Watts and Fuquay, 1985). Intrauterine infusions of indomethacin, a PG synthetase inhibitor, given late in the estrous cycle (day 14 through 21) prolonged the lifespan of the CL and lengthened the interestrous interval of cows (Lewis and Warren, 1977). Passive (Fairclough et al., 1981) or active (Chang et al., 1987) immunization with antibody to $PGF_{2\alpha}$ results in maintenance of luteal function.

Mechanism of CL Regression

The mechanism for CL regression in the bovine is still unclear. In the ewe, McCracken et al. (1972) suggested $PGF_{2\alpha}$ from the uterus reaches the CL by a countercurrent exchange through a close association between the utero-ovarian vein and ovarian artery. A close association between the utero-ovarian vein and ovarian artery has also been reported in the bovine (Lamond and Drost, 1974; Krzymowski et al., 1981/82). Therefore, $PGF_{2\alpha}$ may reach the bovine ipsilateral artery and CL via a similar countercurrent mechanism as proposed in the ewe.

Wolfenson et al. (1985) detected a significant difference in the PGF concentration between the ovarian artery and peripheral artery in the cow which also supports a local transfer system. Hixon and Hansel (1974) presented evidence that $PGF_{2\alpha}$ is preferentially transferred to the ipsilateral ovarian artery instead of through the carotid artery and jugular vein. When the broad ligament

ipsilateral to the CL was dissected along the ovarian pedicle, estrous cycle lengths were extended. However, it should be noted that not only were vascular connections cut but lymphatic vessels were also cut.

Shemesh and Hansel (1975) have provided conflicting evidence to the McCracken theory. These authors indicate $PGF_{2\alpha}$ concentrations in the ovarian artery were not significantly different from measurements made in the peripheral circulation throughout the estrous cycle. $PGF_{2\alpha}$ levels in the utero-ovarian vein, however, were increased during days 15 to 20 of the estrous cycle.

Direct measurement of $PGF_{2\alpha}$ transversing the ovarian pedicle from the uterine vein to the ovarian artery is difficult. Surgical cannulation of the utero-ovarian vein is necessary in order to obtain an accurate measure of the uterine release of $PGF_{2\alpha}$.

Approximately 90% of the $PGF_{2\alpha}$ in the bovine is rapidly converted to 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) and other prostaglandin metabolites through one passage of the lungs (Ferreira and Vane, 1967; Granstrom, 1972; Granstrom and Kindahl, 1982; Davis et al., 1980; Maule Walker and Peaker, 1981). Therefore, peripheral measurement of $PGF_{2\alpha}$ does not accurately reflect the endogenous release of $PGF_{2\alpha}$ by the uterus.

Analysis of $PGF_{2\alpha}$ metabolites in the blood and urine have been used to measure uterine $PGF_{2\alpha}$ release. PGFM is the major metabolite of $PGF_{2\alpha}$ in cattle (Kindahl et al.,

1984) and has a considerably longer half-life (7 to 8 min) in the peripheral circulation than $PGF_{2\alpha}$ (Granstrom, 1972). Kindahl et al. (1976) reported a linear relationship between the mean increase in peripheral plasma concentrations of PGFM and concentrations of $PGF_{2\alpha}$ exogenously infused into the circulatory system. Pulses of PGFM occur during the last days of the estrous cycle in association with a decrease in plasma progesterone concentrations (Kindahl et al., 1976). In the pregnant heifer, neither a decrease in peripheral plasma progesterone nor an increase in PGFM is seen. Therefore, measurement of PGFM allows an indirect measure of the endogenous release of $PGF_{2\alpha}$ from the uterus in the bovine.

Oxytocin and estradiol-17B from the ovary may also be involved with the process of luteolysis in the ewe and cow (Ford et al., 1975; Sheldrick et al., 1980; Flint and Sheldrick, 1982; Hixon et al., 1983). Hixon et al. (1973) demonstrated an increase in plasma estrogens occurred after intraovarian administration of $PGF_{2\alpha}$ in the cow. In contrast to the ewe (Carlson et al., 1973), LH secretion did not change in the cow. In the ewe, infusion of $PGF_{2\alpha}$ into the carotid artery stimulated the release of LH. The release of LH stimulated an increase in estrogen production.

Barcikowski et al. (1974) observed that the infusion of estradiol-17B into the arterial supply of the ovine uterus had no effect on $PGF_{2\alpha}$ secretion until day 14 (the approximate time of CL regression in the ewe). At this

time, a 50 to 100 fold increase occurred in the $PGF_{2\alpha}$ release. This effect could be diminished by indomethacin, a PG synthetase inhibitor. These data suggest estradiol-17B causes the formation of $PGF_{2\alpha}$ rather than the release of stored $PGF_{2\alpha}$. Pulses of oxytocin have also been shown to occur in synchrony with pulses of $PGF_{2\alpha}$ (Flint and Sheldrick, 1983).

In both the cow (Ginther, 1974) and the ewe (Lawson and Cahill, 1983) exposure to progesterone for 10 and 8 days, respectively, has been suggested to be necessary for the initiation of luteolysis. Battista et al. (1984) conducted an experiment to determine initially if progesterone facilitated $PGF_{2\alpha}$-induced luteolysis prior to day 5 of the estrous cycle in the cow. Progesterone administration on day 0, 1, 2 and 3 (estrus=day 0) resulted in shortening estrous cycle lengths in progesterone treated cows compared to the control cows. These data agree with previous work in the cow (Woody et al., 1967; Woody and Ginther, 1968; Harms and Malven, 1969; Ginther, 1970) and ewe (Woody et al., 1967; Woody et al., 1968; Ottobre et al., 1980; Lawson and Cahill, 1983). In 1980, Hasler et al. reported that cattle exhibiting a short estrus also had a higher plasma progesterone concentration on day 3 through 7 of the estrous cycle compared to cows in estrus for a longer time. Shorter cycle lengths (17 to 19 days) also tended to be associated with higher plasma progesterone compared to cycles ranging from 19.5 to 24 days.

Ginther (1970) suggests the cycle-shortening effect of progesterone in cattle may be due to an inhibitory effect on a pituitary luteotrophic mechanism and perhaps a stimulatory effect on the local utero-ovarian luteolytic mechanism. Whereas, evidence by Ottobre et al. (1980) indicates exposure to progesterone for a certain time period is involved in the timing of the initial release of $PGF_{2\alpha}$.

McCracken et al. (1984) have suggested that progesterone, estradiol-17B and oxytocin have a combined role in $PGF_{2\alpha}$ production and release. Theoretically, progesterone initially inhibits estradiol-17B induced formation of oxytocin receptors. However, after ten days, progesterone catalyzes the destruction of its own receptor (Vu Hai et al., 1977). Therefore, the inhibition of estradiol-17B receptor replenishment is lifted and estradiol-17B interacts with its cytosolic receptors in the endometrial cells. As progesterone concentrations decrease, estrogen production from follicles increases. Estrogen aids in stimulating the formation of oxytocin receptors in the uterine endometrium within approximately 6 hours through a protein synthetic step. Oxytocin then interacts with its receptors and stimulates $PGF_{2\alpha}$ secretion through the release of arachidonic acid via a phospholipase activation. As luteolysis of the CL begins, more oxytocin is released from the CL which stimulates an increase in the release of $PGF_{2\alpha}$.

In the ewe, pulses of $PGF_{2\alpha}$ occur 6 to 12 hours apart (Thorburn et al., 1973; Barcikowski et al., 1974). Episodic

release of $PGF_{2\alpha}$ appears to be important to CL regression, since episodic administration of $PGF_{2\alpha}$ is more effective in causing luteal regression than continuous infusion (Schramm et al., 1983). The pulsatile release of $PGF_{2\alpha}$ (1 pulse/6 hours) is thought to occur as occupied oxytocin receptors are internalized and recycled to the cell surface. In a study by Sheldrick and Flint (1986), the ovine uterus appeared to undergo a transitory refractoriness in response to 2 doses of oxytocin administered 1, 2, 4, or 6 hours apart. However, no change was reported to occur in number of oxytocin receptors. Flint et al. (1986) suggest oxytocin stimulates phosphatidylinositol phosphates in the endometrium, thereby providing substrates for prostaglandin synthesis. Stimulation of uterine $PGF_{2\alpha}$ secretion by oxytocin and $PGF_{2\alpha}$-stimulated oxytocin secretion by the ovary has been proposed to form a positive feedback loop which stimulates maximal $PGF_{2\alpha}$ secretion (Flint and Sheldrick, 1983; Sheldrick and Flint, 1984; Moore et al., 1986). The 6 to 12 hour interval between $PGF_{2\alpha}$ pulses has been proposed to result from depletion of oxytocin in the CL, followed by the synthesis of more oxytocin to replenish CL content. Once sufficient oxytocin has accumulated and been released, another $PGF_{2\alpha}$ pulse is released (Sharma and Fitzpatrick, 1974; Mitchell et al., 1975; Flint and Sheldrick, 1982).

Kimball and Hansel (1974) have presented supporting evidence to the oxytocin theory in the cow. Estrogen-

binding proteins increased in the endometrium between days 15 and 21 of the estrous cycle when compared to day 2, 5 and 10. The concentration of estrogen-binding protein is also highly correlated with plasma estradiol-17B and estrone levels.

Milvae and Hansel (1980) indicated that exogenous administration of oxytocin had no effect on $PGF_{2\alpha}$ concentration in uterine venous blood or in ovarian arterial blood from oxytocin-treated or control animals. These authors proposed the presence of factor other than $PGF_{2\alpha}$ or a mechanism other than a local veno-arterial transfer of $PGF_{2\alpha}$, mediates the inhibitory effects of oxytocin on bovine CL formation.

The mechanism by which $PGF_{2\alpha}$ causes luteal regression is still basically unknown. Specific, high affinity heterogeneous $PGF_{2\alpha}$ receptors have been found in the cell membrane of bovine CL (Rao, 1975). A 200 fold increase in the binding affinity of bovine luteal cells has been demonstrated between day 13 and 20 (Rao, 1978). Rao (1975) suggests that the $PGF_{2\alpha}$-receptor interaction is one of the first steps in an array of events which must occur for luteolysis to take place. $PGF_{2\alpha}$, as a vasoconstrictor may cause a reduction in blood flow to the ovary and thus decrease the level of LH or other luteotrophic factor(s). In the ewe, Diekman et al. (1978) indicated injections of $PGF_{2\alpha}$ reduced LH receptor concentration in the CL within 23 hours. However, progesterone had already begun to decline

before LH levels were altered. Thus, it is improbable that $PGF_{2\alpha}$ causes luteal regression by depriving the CL of luteotrophic support from LH.

Umo (1975) reported ultrastructural changes within the ovine CL after administration of $PGF_{2\alpha}$ in the ewe. A decrease in the amount of smooth endoplasmic reticulum and in the number of membrane-bound granules was noted. The size and shape of the mitochondria had also changed to a more spherical shape. Carlson et al. (1982) using wide angle x-ray diffraction, observed submicroscopic structural changes in cellular membranes during luteal regression. These changes appear to be related to loss of cellular function. Utilizing fluorescence polarization and fluorescence photobleaching recovery, a decrease in the membrane fluidity of bovine luteal cells was found after *in vivo* prostaglandin treatment (Goodsaid-Zalduondo et al., 1982). Changes in the composition of the luteal cell membranes were also observed. The mechanism by which $PGF_{2\alpha}$ alters membrane composition is not known.

*In vitro* culture of large luteal cells with $PGF_{2\alpha}$ has resulted in suppression of steroidal secretion and viability of the large luteal cells (Silvia et al., 1984). It has also been suggested that $PGF_{2\alpha}$ may stimulate large luteal cells to release a cytotoxic factor that lyses small luteal cells or directly interferes with LH-stimulated progesterone secretion of small luteal cells. $PGE_2$ appears to protect

the luteal tissue from the actions of $PGF_{2\alpha}$ *in vitro* and *in vivo*.

## Early Embryo Development

The embryonic period is defined as the time extending from conception to completion of cellular and tissue differentiation, which occurs at approximately day 45 of pregnancy in the cow (Committee on Reproductive Nomenclature, 1972).

In the bovine, fertilization normally occurs during metestrous, i.e. 8 to 10 hours after ovulation. The fertilized egg or zygote is a relatively large cell (150 to 190 um) with a large cytoplasmic to nuclear ratio (Lindner and Wright, 1983). An extracellular glycoprotein matrix called the zona pellucida surrounds the zygote. In the bovine, this coating is approximately 26.9 um in thickness (Wright et al., 1977). Originating during folliculogenesis, the zona pellucida is maintained around the ovum after ovulation, fertilization and passage through the oviduct.

The initial cleavage divisions of the embryo in the bovine occur near or at the ampullary-isthmic junction of the oviduct. The first cleavage division occurs approximately 20 to 24 hours after ovulation. Basically, the zygote is cleaved vertically through the main axis of the egg from the animal pole, i.e. site of polar body extrusion, to the vegetal pole, i.e. area of yolk reserve. The cells, called blastomeres, are duplicates of each other

(Henricks et al., 1971b). The second cleavage division occurs approximately 12 hours after the first cleavage. Although cleavage of the zygote results in an increase in the number of cells, very little change is seen in the total cellular mass of the embryo (Lindner and Wright, 1983).

Early cell cleavages, along with transport of the embryo through the oviduct, are under the control of hormones, maternal factor(s) within the oviductal fluid, and perhaps even some factor(s) from the early embryo itself (Chang, 1952). The early embryo will remain at the ampullary-isthmic junction of the oviduct until approximately day 2 to 3. It is then transported towards the uterine lumen where, once again, steroids and perhaps other factor(s) have prepared an embryotrophic environment. The bovine embryo enters the uterine lumen at the 8 to 16 cell stage of development on approximately day 3 to 4 of pregnancy (Anderson, 1977; Perry, 1981).

When the embryo has developed to approximately 16 to 32 cells, it is termed a morula. Development to the morula stage in the bovine occurs around day 5 (Shea, 1981; Wright et al., 1976; Brackett et al., 1980). The cells of the morula lack well-developed intercellular junctions and are in contact only through spot and gap junctions, microvilli and other cellular projections.

Compaction of the morula occurs on day 5 to 6 (Lindner and Wright, 1983; Brackett et al., 1980) and is a calcium-dependent adhesion process. During compaction in the mouse,

the cells flatten and cell-to-cell contact is maximized. Zonular tight junctions form and maintain a permeability seal allowing fluid to be present without leaking (Blandau, 1971). Compaction functions to form two populations of cells, i.e. forming the inner cell mass and trophectoderm, and positions the trophectodermal cells so fluid can enter and expand the morula into a blastocyst.

On approximately day 7 to 8, a unilaminar blastocyst with blastocoelic cavity is formed (Hartman et al., 1942; Hamilton and Laing, 1946; Wright et al., 1976; Lindner and Wright, 1983). Fluid transport in the blastocyst is an active process involving sodium and potassium ions (Blandau, 1971). In the mouse, the sodium-potassium ATPase pumps are located only on the basal surface of the trophoblast allowing for the movement of fluid into the blastocoelic cavity. In an <u>in vitro</u> study using bovine embryos, Wright et al. (1976) noted blastocyst expansion took 12 to 15 hours. The diameter of the embryo increased by a factor of 1.32 and the zona pellucida decreased to one fifth of its original thickness.

On day 9 to 11, the blastocyst undergoes a process termed hatching in which it escapes through the zona pellucida via a small slit (Perry, 1981; Flechon and Renard, 1978; Shea, 1981). Although the exact mechanism(s) is still unknown in the bovine, proteolytic enzymes of embryonic and/or uterine origin are thought to play a role in softening the zona pellucida in the mouse (Bergstrom, 1972;

Inoue and Wolf, 1974). Blastocyst expansion and contraction may also be involved (Flechon and Renard, 1978) with extrusion of the bovine embryo. Using scanning electron microscopy, stretched fibers were observed in the angle between the edge of the slit in the "hatched" zona pellucida. Therefore, as fluid accumulates in the blastocoele and the cells undergo hyperplasia, the zona pellucida may be stretched. As the zona pellucida stretches and contracts, a slit may be created. After hatching, the bovine blastocyst remains in a spherical morphology. However, the blastocyst collapses for a short time, making identification very difficult immediately following hatching (Lindner and Wright, 1983).

At day 11 to 12 of pregnancy, the conceptus is composed of three primitive germ layers. The germ layers of the conceptus (endoderm, mesoderm and ectoderm) form the embryo and its extraembryonic membranes (Marion and Gier, 1958; Perry, 1981). The outer layer of the conceptus formed from ectoderm is termed trophectoderm. By day 15, it becomes lined with endodermal cells which originated from cells of the embryonic disc. A mesodermal layer from the inner cell mass migrates between the trophectoderm and endodermal layers forming a trilaminar trophoblast. The mesodermal layer splits into a vascular and avascular layer, with the vascular layer fusing with the endodermal layer to form a splanchnopleure yolk sac and the avascular layer fusing with the trophectoderm to form the somatopleure. As the embryo

is unattached until basically day 23 of pregnancy, the yolk sac and the glandular secretions of the uterus serve as nutritional support for the embryo (Marion and Gier, 1958; Perry, 1981). On day 19 to 20, the amnion also begins to form. The allantois first appears on day 23 from an outgrowth of the extraembryonic splachnopleure from the hindgut of the embryo (Melton et al., 1951). The allantois begins as a turgid sac but expands rapidly to 20 mm by the end of the day. On day 25, the length of the allantois has been increased to 80 mm and on day 27 measures 160 mm. As the allantois expands, the yolk sac concurrently regresses. The chorion and allantois fuse to form the chorio-allantoic placenta (Marion and Gier, 1958; Perry, 1981).

On day 15 to 16, the bovine conceptus undergoes a rapid elongation stage. The conceptus becomes filamentous with the trophoblast generally ranging from 40 to 200 mm (Chang, 1952; Greenstein et al., 1958; Betteridge et al., 1980; Geisert, 1988). The approximate height of the vesicle (2 mm) remains the same during the period of elongation, until approximately day 19 when a localized bulge appears. This bulge develops into the area which contains the embryo (Curran et al., 1986). The trophoblast comprises the majority of the conceptus surface area at the time, i.e. it is approximately 50 times longer than the embryonic disc (Northey and French, 1980). During the rapid elongation phase (days 12 through 16) in the ewe, trophoblast cell death occurs. Carnegie et al. (1985) suggest this may

indicate cellular remodelling of the blastocyst or the cells may be dying because they can not respond to the appropriate signal to differentiate. It is possible these same changes may be occurring in the bovine during its rapid elongation stage.

Apposition and adhesion of the conceptus to the uterine surface epithelium begins on approximately day 17 to 18 (King et al., 1981). Slender microvilli cover the trophoblast surface. Trophoblastic microvilli may play a role of absorbing nutrients while the conceptus is unattached within the uterine lumen. The mononuclear trophoblast epithelial cell is regular and exhibits a round, lightly stained, randomly located nucleus. The cytoplasm includes polysomes, some endoplasmic reticulum and numerous mitochondria which are located on the apical side (Wathes and Wooding, 1980).

By day 19 to 20, the conceptus has expanded and is present in both uterine horns. As attachment begins on approximately day 20 to 22, the microvilli disappear and the trophoblastic surface becomes smooth. Papillae begin to form in the area of the trophoblast opposite the openings to the uterine gland. By about day 24, the papillae encompass the area inside the glandular lumen. Guillomot and Guay (1982) suggest the conceptus inserts papillae into the glandular lumen to absorb uterine secretory products. The papillae may also serve to fix the conceptus onto the uterine epithelium so attachment can take place. Microvilli

from maternal cells are seen indenting the apical border of the embryonic cells (King et al., 1980).

Attachment of the conceptus to the uterine surface begins simultaneously in the intercaruncular and caruncular regions near the embryo (Leiser, 1975). Attachment spreads toward the end regions of the chorionic vesicle (King et al., 1981) as gestation continues.

Binucleate cells which differentiate from mononuclear cells in the trophoblast are first observed on approximately day 17. By day 21, binucleate cells comprise approximately 20% of the chorionic cells. Binucleate cells migrate into and fuse with the uterine epithelial cells to form a syncytium. Therefore, the multinucleate giant cells which result are a combination of both trophoblast, i.e. binucleate cells, and uterine epithelial cells. Multinucleate giant cells contain up to 8 nuclei and by day 24 account for approximately 50% of the uterine epithelial surface area (Wathes and Wooding, 1980). These cells also include rough endoplasmic reticulum, discrete ribosomes, mitochondria, and vesicles. As gestation continues, the number of these small, circular membrane-bound vesicles increases at the basal regions of the giant cells. It has been suggested these vesicles transport placental lactogen to the maternal vascular system.

Beginning during attachment and continuing through gestation, an increase in the accumulation of lipid material in the basal region of the trophoblast mononuclear cells has

been noted (King et al., 1980). The function of this lipid material is unknown. Carnegie et al. (1985) also noted accumulation of lipid droplets in the trophoblastic cytoplasm between days 12 and 16 of pregnancy in the ewe. These authors suggest a number of possible origins for the lipid droplets. Lipid droplets may have been absorbed from fat globules in the histotroph, released by the caruncular epithelial cells and/or synthesized by the trophoblast itself. The lipid droplets possibly serve to meet the large energy requirement needed for rapid conceptus growth and development.

On approximately day 27, attachment between the bovine conceptus and uterine epithelium is quite complex. Considerably more lipid material is present in areas of the trophoblast which are sparsely populated with giant cells. Vascularization of the trophoblast is present on day 26 (King et al., 1980) with an auditory fetal heartbeat at day 20 (Curran, 1986).

After approximately day 45, the embryo is termed a fetus. The fetal period extends from approximately day 45 until birth. This last stage is marked by rapid growth and final development of the fetus and placenta within the maternal system (for a more comprehensive review on the fetal period see Bjorkman, 1969; Eley et al., 1978).

## Maternal Recognition

Communication between the conceptus and the maternal system is necessary for pregnancy to be maintained. Without the presence of the conceptus, the CL begins to regress and the cow will return to estrus. However, if a conceptus is present, the CL is maintained. How the maternal system realizes the presence of the conceptus and therefore, maintains the CL is referred to as "maternal recognition of pregnancy." The bovine conceptus synthesizes a number of biochemically active substances during early pregnancy. These substances form an intricate communication between the conceptus and the maternal system during establishment and maintenance of pregnancy.

Several researchers have indicated the embryo may communicate with the maternal system as early as day 9 and 10. Hickey and Hansel (1987) propose a luteotrophic substance is synthesized by the bovine conceptus between days 13 and 18. These results support the findings of Henricks et al. (1971) and Lukaszewska and Hansel (1980) which indicated plasma progesterone concentrations of pregnant cows were significantly greater than those of nonpregnant cows as early as day 9 and 10 after estrus. However, these data are not conclusive since other reports find no difference in the peripheral progesterone concentration between pregnant and nonpregnant cows during this same time period (Shemesh et al., 1968; Hasler et al., 1980; Geisert et al., 1988).

Extensive research has indicated that day 16 to 17 is a critical period for pregnancy maintenance in the cow. Northey and French (1980) indicated that injection of homogenates from day 17 or 18 bovine conceptus tissue into the uterine lumen of heifers on day 14 and 15 of the estrous cycle (day 0 = estrus) prolonged the interestrous interval by 3 to 4 days through a delay in the regression of the CL. These authors also demonstrated that flushing the bovine conceptus from the uterus before day 17 resulted in cows returning to estrus around 21 days. Whereas, if the conceptus is removed on day 17, 18, or 19, the lifespan of the CL is extended. Dalla Porta and Humblot (1983) also indicated removal of conceptuses on day 9 or 14 results in no alteration in estrous cycle length or peripheral progesterone concentrations. However, when the conceptuses are removed on day 16, the interestrous interval is increased with cows returning to estrus 26 to 29 days after artificial insemination. Intrauterine infusion of day 16 conceptuses also lengthens the estrous cycle by 6 to 7 days. Whereas, neither intrauterine infusion of day 12 conceptuses nor $PGE_2$ had an antiluteolytic effect (Dalla Porta and Humblot, 1983). Betteridge et al. (1980) reported the bovine conceptus must be present in the uterus before day 16 to prevent luteolysis.

Embryo transfer studies have demonstrated that transfer of embryos after day 17 is not successful, indicating the conceptus must be present by day 16 (Sreenan, 1978).

Hysterectomy after day 16 of the estrous cycle also will not prevent luteal regression (Wiltbank and Casida, 1956; Anderson et al., 1969).

As stated previously, the bovine conceptus synthesizes and releases many biologically active substances including steroids. Shemesh et al. (1979) reported that day 13, 15 and 16 bovine conceptuses have the enzymatic capability to produce progesterone, testosterone and a small amount of estrogen. Gadsby et al. (1980) observed only a low conversion of androstenedione to phenolic steroids (estrone and estradiol-17B) by trophoblast tissue. Aromatase activity is therefore reported to be low. However, it was noted the greatest aromatase activity of the bovine conceptus was between days 16 and 22, which corresponds to the period of maternal recognition of pregnancy.

Prostaglandin $E_2$ and $PGF_{2\alpha}$ are also synthesized and released by the bovine conceptus as early as day 13 after mating, with production increasing through early pregnancy (Shemesh et al., 1979; Lewis et al., 1982). It has been suggested that prostaglandins, such as $PGE_2$, may play a role in maternal recognition of pregnancy. Prostaglandin $E_2$, which has many effects including vasodilation, may decrease endometrial vascular resistance (Thatcher et al., 1984) and thereby, increase blood flow, as has been reported by Ford et al. (1979). With an increase in blood flow, a greater amount of a luteotropic, antiluteolytic and/or luteostatic factor(s) may reach the CL and thus maintain it.

In an in vitro study by Speroff and Ramwell (1970), progesterone synthesis by luteal cells was stimulated by $PGE_2$. Prostaglandin $E_2$ and LH have the same stimulatory effect on progesterone synthesis in bovine luteal cells via increasing adenyl cyclase activity and cAMP accumulation (Kuehl et al., 1970; Marsh, 1970; Godkin et al., 1977). Reynolds et al. (1983) suggest estradiol-17B and $PGE_2$ may act synergistically to maintain luteal function during early pregnancy in the cow. Estradiol-17B is produced by the bovine conceptus by day 13 after mating (Shemesh et al., 1979; Chenault, 1980). Treatment of heifers with estradiol-17B and $PGE_2$ resulted in the maintenance of systemic progesterone levels for an extended period of time over control heifers.

The bovine blastocyst also produces a large amount of $PGF_{2\alpha}$ (Lewis et al., 1982). Geisert et al. (1988) reported a significant increase of PGF in the lumen of the ipsilateral uterine horn of pregnant cows on days 16 and 17. This period is concurrent with expansion of the bovine conceptus. The large increase of PGF content in the uterine lumen after day 16 in pregnant cows was also observed by Bartol et al. (1981) and is thought to be mainly of conceptus origin.

Lewis and Waterman (1983) indicated the bovine endometrium altered the metabolism of tritiated arachidonic acid by blastocysts. The endometrium appeared to direct blastocyst metabolism of arachidonic acid away from

synthesis of $PGF_{2\alpha}$ and towards the synthesis of $PGE_2$. The endometrium also metabolizes $PGF_{2\alpha}$ which is produced by bovine blastocysts *in vitro*. Whether or not this occurs *in vivo* has yet to be determined. If so, the amount of $PGF_{2\alpha}$ reaching the CL may be decreased, thus preventing luteolysis. Lacroix and Kann (1986) suggested the conceptus plays an active role in preventing luteolysis by influencing the amount and pattern of $PGF_{2\alpha}$ release by the uterus. Also, they suggested the sensitivity of the uterus to estradiol-17B is controlled by the conceptus as early as day 14 of pregnancy and that the embryo simultaneously exerts a protective effect at the luteal level.

Gross et al. (1987a) reported a lower secretion of $PGF_{2\alpha}$ from the endometrium of pregnant versus nonpregnant cows. Also, oxytocin treatment resulted in very little change in $PGF_{2\alpha}$ secretion in pregnancy cows. Oxytocin treatment in cyclic cows, however, resulted in increased $PGF_{2\alpha}$ secretion. Basu and Kindahl (1987) indicated pregnancy status influenced the biosynthesis of prostaglandins from arachidonic acid and the inhibitory capacity of the endometrium. Throughout the estrous cycle, nonpregnant cows transformed a greater amount of arachidonic acid into prostaglandins than pregnant cows, especially on days 17 through 19. In fact,the transformation rate was the lowest on day 18 of pregnancy. An inhibitory capability was observed in endometrial microsomes from both pregnant and nonpregnant cows. However, the inhibitory capability on day

17 during luteolysis was low compared to other days of the cycle and very high in pregnant cows during days 16 through 20.

In a study by Gross et al. (1987b), cotyledonary microsomes, which synthesize $PGF_{2\alpha}$, were co-cultured with endometrium from pregnant cows. $PGF_{2\alpha}$ synthesis by the microsomes were found to decrease. These studies suggest the presence of an intracellular endometrial inhibitor of prostaglandin synthesis which has an increased activity due to a stimulatory factor(s) from the conceptus.

In cyclic cows, the amplitude of PGF spikes is significantly higher compared to those in pregnant cows (Wolfenson et al., 1985) suggesting some biological factor from the conceptus suppresses the ability of $PGF_{2\alpha}$ to reach the ovarian circulation. Thatcher et al. (1984) reported day 17 endometrial tissue from pregnant cows synthesized less $PGF_{2\alpha}$ than tissue from cyclic cows in an in vitro study. The tissues from uterine endometrium, ovarian vein and ovarian artery of day 17 pregnant cows also accumulated less $PGF_{2\alpha}$ in vitro than tissue from day 17 cyclic cows.

The conceptus produces proteins which may function in maternal recognition of pregnancy in the bovine. Placental lactogen is a polypeptide which is synthesized and released by the bovine conceptus starting at day 17 of pregnancy (Flint et al., 1979). Although placental lactogen is produced during the time of maternal recognition of pregnancy, it is probably more of a coincidental event.

Flint et al. (1979) suggest the presence of placental lactogen at day 17 is probably related to the appearance of binucleate cells, instead of the processes of pregnancy recognition, attachment and implantation.

Knickerbocker et al. (1986) demonstrated intrauterine infusions of conceptus secretory proteins (CSP) on day 15.5 through 21 after estrus significantly extended the CL lifespan and subsequently extended the interestrous interval. Homologous serum proteins and 5B-pregnan-3a-ol-20-one, however, did not extend either CL lifespan or the interestrous interval. Endometrial PGF production was decreased in animals infused with CSP.

More specifically, the trophoblast is thought to play an important role in maintenance of pregnancy. Heyman et al. (1984) demonstrated cultured trophoblastic vesicles (minus embryonic disc) from day 14 conceptuses developed within the uterus of transfer recipients. These vesicles become threadlike and produce the pregnancy recognition signal as demonstrated by extended cycle lengths of 25 to 37 days. Martal et al. (1984a) also transferred cultured trophoblastic vesicles of day 13 bovine blastocysts and reported luteal function was extended. Cross-transfer of embryonic membranes between the ewe and the cow also resulted in longer interestrous interval in both species. Although only 20% of the transfers resulted in an extended estrous cycle length, Martal et al. (1984b) suggested the polypeptides synthesized by the ewe and the cow were similar

and perhaps the main difference between the trophoblastic vesicles was differences in histocompatibility antigens.

The trophoblast synthesizes an array of polypetides in vitro. In the ewe, ovine trophoblast protein (oTP-1) has been studied quite extensively (Godkin et al., 1984a,b; Hanson et al., 1985) and has been proposed as one of the keys in early maternal recognition of pregnancy in the ewe. This protein is synthesized and released by sheep conceptuses in vitro between days 13 and 21 of pregnancy, reaching maximum production on days 15 to 17 (Godkin et al., 1982; Hansen et al., 1985) as demonstrated by electrophoretic studies. oTP-1 is a group of polypeptide variants with isoelectric points of 5.4 to 5.7 and molecular weight of 17000 to 18000 daltons (Godkin et al., 1982).

Bartol et al. (1985a) indicated a protein similar to ovine trophoblastic protein-1 is produced in culture by the bovine conceptus. Because of its similarity to oTP-1, the protein has been named bovine trophoblast protein (bTP-1). Bovine trophoblast protein-1 is a polypeptide complex of 6 to 8 isoforms with an acidic pI of 6.5 to 6.7 and has a molecular weight group of 22000 to 24000 daltons (Bartol et al., 1985; Helmer et al., 1987; Geisert et al., 1988). Using rabbit antiserum to oTP-1 and Ouchterlony double-immunodiffusion analysis, Helmer et al. (1987) indicated bTP-1 is serologically related to oTP-1.

The major translation product of day 17 bovine conceptus mRNA, as indicated by using an antibody to oTP-1,

is a polypeptide significantly smaller (molecular weight of 18000) than the secreted protein. Helmer et al. (1986) reported that bTP-1 is probably glycosylated during post-translational modification and therefore, has a higher molecular weight than oTP-1.

The bTP-1 polypeptides are synthesized and released by the bovine conceptus between days 16 and 26 of pregnancy (Bartol et al., 1985). The presence of these polypeptides is diminished by approximately day 29 indicating a transient synthesis and release by the bovine conceptus during the critical period of pregnancy recognition (Bartol et al., 1985). Bovine trophoblast protein-1 is located in the trophectoderm of the conceptus (Murray et al., 1986). It has also been found within the glandular and surface epithelial cells of the intercaruncular endometrium and stromal cells of caruncular endometrium from pregnant cows.

In the ewe, oTP-1 will extend the cycle length of the ewe (Godkin et al., 1984). Fincher et al. (1986) suggested ovine conceptus secretory proteins (CSP) prevent luteolysis by altering the amount or pattern of $PGF_{2\alpha}$ release by the uterine endometrium. As demonstrated by these authors administration of estradiol-17B resulted in a more varied response of PGFM in nonpregnant ewes than pregnant ewes. When conceptus secretory protein versus plasma protein was administered into the uterine lumen of cyclic ewes, the mean PGFM response to oestradiol was decreased. Oxytocin elicited a greater PGFM peak response in plasma protein

treated ewes compared to ewes treated with conceptus secretory proteins. The interestrous interval of the ewes treated with CSP was also greater than in ewes treated with plasma protein. Godkin et al. (1984) suggested oTP-1 may stimulate the synthesis and release of other proteins from the uterine endometrium which in turn may provide nutrients for the developing embryo or possibly proteins which "protect" the ovary from luteolytic factors.

Bovine conceptus secretory proteins, which include bTP-1, have also been found to extend the luteal lifespan in the cow (Knickerbocker et al., 1986). However, the mechanism(s) involved with effects of these proteins, especially bTP-1, is not fully understood. Bovine conceptus secretory proteins may selectively stimulate an endometrial protein that inhibits or alters $PGF_{2\alpha}$ synthesis, and/or alters steroid-receptor interrelationships. This would correspond with the previously mentioned studies of Gross et al. (1987a,b) in which pregnant cows secreted less $PGF_{2\alpha}$ in culture than nonpregnant cows and in which the presence of an endometrial $PGF_{2\alpha}$ inhibitor had a greater effect in the presence of a conceptus.

Therefore, viability of the conceptus, i.e. the ability to synthesize and release steroids, prostaglandins and especially polypeptides may be the key to the pregnancy recognition signal in the bovine.

Another aspect of pregnancy recognition is the fact that the embryo as an allograft (tissue between two

different individuals of the same species) is not rejected from the maternal system. That is, since the embryo has both maternal and paternal genomes, it is actually a foreign tissue within the maternal system.

Several factor(s) or mechanism(s) for preventing embryo rejection by the mother have been proposed (Beer et al., 1975). One possibility is perhaps the mother produces immunoregulatory substances which prevent the embryo from being rejected. Uterine luminal proteins have been shown to have immunosuppressive activity. This activity is greatest on days 17 to 18 of pregnancy in comparison to day 10 of pregnancy or any other day of the cycle (Roberts, 1977; Segerson et al., 1984; Fisher et al., 1985). Segerson et al. (1986) demonstrated estradiol-17B and progesterone injections enhance the immunosuppressive activity of the uterine luminal proteins collected at day 17. Blocking antibodies, which are an immune response from the mother, may also block the action of effector cells on the embryonic allograft. The embryo may also produce a coating substance which protects itself from the effector cells of the mother or may "mask" itself from immunological recognition by passive acquisition or absorption of factors from the maternal system. The trophoblast may also play a role in producing specific proteins and/or steroids which may directly or indirectly block the efferent limb of the immunologic reflex, allowing the trophoblastic epithelium to

be what is termed an "immunologically privileged" tissue (Segerson et al., 1986; Roberts, 1977).

In mice, interferon which is present in the mouse placenta has been shown to prolong allograft survival (Fowler et al. 1980). Interferon is one of a group of proteins with potent intracellular antiviral properties. It also modulates a variety of lymphocyte responses to stimuli. The trophoblast has been suggested to be the main key for prevention of fetal allograft rejection. In the mouse, the trophoblast may serve to protect the embryo from the maternal system. When the trophoblast and inner cell mass cells are surgically separated and each transplanted to the kidney capsules of mice of a different genetic strain, trophoblastic cells survive while the inner cell mass cells die (Fowler et al., 1980).

Recently, Imakawa et al. (1987) indicated oTP-1 is very similar to human α-interferons. That is, about 70% of the base sequences and over 60% of the amino acid sequence show homology. Also, oTP-1 contains the highly conserved Cys-Ala-Trp-Glu sequence which is found on all α-interferon sequences. How this may also relate with bTP-1 as a maternal recognition signal and immunological protective agent in the bovine is unknown.

## Uterine Environment

Throughout the estrous cycle and early pregnancy the uterine environment is in a state of constant fluctuation.

The uterus undergoes cyclic changes to maximize the chances of the female becoming pregnant, accomodate and nurture an embryo throughout gestation, and rejuvenate and undergo uterine involution after parturition. In other words, the uterine environment is the stage for a number of biological events which occur in preparation for pregnancy or in response to such an event.

Many factors play an important role in mediating changes, both morphological and biochemical, which occur in the uterus during the estrous cycle and pregnancy. These changes may be seen through observation of the uterine epithelium and also of the biological components which are released by the uterus.

Histologically, the cells in the uterine epithelium throughout all stages of the estrous cycle are classified as pseudostratified columnar (Stinson et al., 1962). The uterine epithelial cells are mononuclear, measuring approximately 15 to 35 um in height (Wathes and Wooding, 1980) with microvilli present on the luminal surface(Stinson et al., 1962; Guillomot and Guay, 1982). The cell cytoplasm contains smooth-surfaced vesicles with a granular internal structure. The lamina propria consists mainly of fibroblasts with mast and plasma cells also being frequently found. Numerous collagenous fibrils are found in the intercellular area of the lamina propria (Stinson et al., 1962). On approximately day 18, the uterine epithelium is more regular in shape, but still columnar with cell height

measuring approximately 20 to 25 um (Wathes and Wooding, 1980).

Apical cytoplasmic protrusions are present on the uterine cell surface on day 12 to 16 of the estrous cycle (Guillomot and Guay, 1982). However, after day 16, these protrusions are no longer seen. On the other hand, the cytoplasmic protrusions are prevalent until approximately day 21 in pregnant cows. The presence of the protrusions on days 12 to 16 suggest progesterone may be involved with their formation and function. Also, their persistance to 21 days in the pregnant animal suggests the conceptus may be directly or indirectly involved in the regulation of the cytoplasmic protrusions. Guillomot and Guay (1982) suggest the cytoplasmic protrusions on the uterine cells may have a secretory and/or endocytic function.

While the early embryo is still in the oviduct, the ovarian steroids begin to prepare a favorable environment in the uterus in which the embryo may grow and develop. The bovine embryo enters the progesterone influenced uterine lumen on approximately day 3 to 4 and remains unattached until approximately day 20 to 22. Therefore, the uterine milieu must contain nutrients which are able to nourish and support the free-floating embryo.

Ovarian steroids influence the biochemical make-up of the uterine endometrial secretions. Full glandular development of the uterus appears to be dependent upon a balance between both progesterone and estrogen (Asdell et

al., 1949). Murdoch et al. (1972) reported progesterone stimulated acid phosphatase activity in the ovine endometrium. Linford and Iosson (1975) indicated the maternal endocrine status influenced bovine endometrial lysosomal presence and activity. Acid phosphatase activity, which is utilized as a marker enzyme for lysosomes, is greater in the endometrium of pregnant cows than nonpregnant cows. These authors also observed a higher content of glucose and protein in pregnant compared to nonpregnant uteri.

Wathes (1980) incubated bovine uterine tissues with progesterone and oestradiol-17B using radiolabeled amino acids to follow polypeptide production. The results indicated the addition of steroids significantly depressed protein synthesis by tissue cultured in vitro. The author suggested the addition of progesterone to culture media may inhibit the membrane transport of glucose. This, in turn, would affect the synthesis of proteins in vitro and perhaps not mimic the true effect of steroids in vivo.

Flushing of the uterine lumen with sterile saline has also been utilized to define the array of uterine luminal proteins present in cyclic cows (Bartol et al., 1981). Uterine proteins change both quantitatively and qualitatively throughout the cycle. Although, no significant correlation was found between peripheral plasma progesterone and total uterine luminal protein content, total protein did appear to increase later in the cycle,

i.e. days 14, 16 and 19. The greatest number of proteins were also present in the luminal fluid during the luteal phase, i.e. day 8 through 16. Pregnancy status of cows between day 8 and 16 does not have any effect on the presence of proteins in uterine flushings (Bartol et al., 1981). However, total protein from uterine flushings of pregnant cows appeared to be lower than that of cyclic cows on corresponding days postestrus, i.e. on days 8, 12, 14 and 16.

Four proteins (MW x $10^{-3}$ = 15.2, 306.8, 322.2 and 342.8), which were absent in flushings from any other day of the cycle or pregnancy, were identified in uterine flushings on day 19 of pregnancy. This suggests the presence of a conceptus will markedly change the content of uterine luminal protein. Geisert et al. (1988) also indicated polypeptides present in uterine flushings of nonpregnant and pregnant cows were similar. However, the number of polypeptides increased on day 17. These authors suggested that the increase in number of polypeptides may be stimulated from the uterine endometrium by the conceptus or may be from the conceptus itself.

Endometrial explants from all stages of the cycle and pregnancy (day 16, 19, 22, 24, 69 and 270) have been shown to be capable of incorporating radiolabeled amino acids into polypeptides (Bartol et al., 1985b). Analysis of fluorographs from pregnant and non-pregnant cows at varying stages revealed polypeptides in at least 4 molecular weight

($M_r$/pH) classes which are as follows: Class I, $M_r$=14 x $10^{-3}$ /pH>7.2; Class II, $M_r$=19-24 x $10^{-3}$/pH 5.4 - 6.3; Class III, $M_r$=28-31 x $10^{-3}$/pH 6.9 - 7.3; and Class IV, $M_r$>150 x $10^{-3}$/pH<5.1.

Class I polypeptides consisted of 2 polypeptides which have been identified as uterine-specific components of bovine uterine flushings (Roberts and Parker, 1976). Class II and III polypeptides are also uterine specific (Bartol et al., 1980) and found in pregnant and nonpregnant animals. Class IV polypeptides are similar to a plasma membrane-enriched fraction of porcine endometrium (Mullins et al., 1980). These polypeptides are thought to possibly play a role in apposition and attachment (Leiser and Wille, 1975) of the trophoblast to the endometrial surface.

Bartol et al. (1985b) indicated the array of polypeptides was consistent throughout the cycle and pregnancy as no qualitative changes are obvious. Geisert et al. (1988) reported an increased intensity of a group of low molecular weight polypeptides ($M_r$ 14-16 x $10^{-3}$ / pI 6.8-7.2) and a polypeptide with a $M_r$ of 35 x $10^{-3}$ and pI 7.3-8.4 in pregnant compared to nonpregnant uterine flushings on day 17. Therefore, these results indicate the changes in the secretion of luminal polypeptides may be more of a quantitative rather than a qualitative nature.

The uterine environment, as stated previously, is controlled by a number of maternal factors and hormones. However, the conceptus may also contribute to and modify the

uterine milieu. The release of prostaglandins, steroids and proteins by the conceptus into the uterine lumen has been discussed in a previous section. How these products, synthesized and released by the conceptus, influence further secretions by the uterine endometrium and thus, the uterine environment, is unknown.

## Embryo Mortality

As stated in previous sections, many factors are involved in the growth and development of the embryo throughout gestation. Even before and at fertilization, many events in both the male and female reproductive tract must occur for an embryo to be conceived. After fertilization, the proper environment, stimuli and the embryo's ability to respond to such stimuli are necessary for the pregnancy to go to term. It has been estimated that 20 to 40% of all ova shed do not result in a viable calf at term for one reason or another.

Roche (1981) indicated 20% of the loss is due to fertilization failure while approximately another 8 to 18% is caused by early embryonic mortality. Markette et al. (1985) reported pregnancy rates at day 24 were about 73.6% with 67.4% pregnant at two months of gestation. Diskin and Sreenan (1980) indicated the majority of early embryonic death occurs between day 8 and 16. However, a report by Ayalon (1973) indicates early mortality occurs by day 6 and

7. This time period corresponds to the transition of the embryo at the morula stage into a blastocyst.

Determination of early embryonic death is difficult but a general estimate can be obtained by observation of a prolonged interestrous interval, i.e. greater than 17 to 25 days. This estimate relies on the fact that a conceptus present after day 16 will synthesize and release the pregnancy recognition signal causing an extended estrous cycle length. However, this method can be inaccurate as there may be other reasons for an extended estrous cycle length. If death of the embryo occurs before day 15, the cow will exhibit a normal cycle length since the maternal recognition signal is needed on days 16 or 17 of pregnancy. Therefore, the time-slaughter method is most often used in studying embryonic mortality (see review by Ayalon, 1978).

Embryonic mortality can occur for a number of reasons. Genetic defects or aberrations have been shown to have a fatal effect on embyronic survival. A high level of inbreeding has been related to embryo death. If the embryo is inbred, death of the embryo tends to occur at an earlier stage of development. Whereas, if the dam is inbred, death of the embryo occurs later in development (Mares et al., 1961; Menge et al., 1962). Chromosome abnormalities have also been detected in day 12 to 16 blastocysts (McFeely and Rajakoski, 1968). However, death caused by genetic defects may not be detrimental to the animal producer. Bishop (1964) suggests a certain amount of embryonic mortality may

be necessary, or even beneficial, in order to rid a population of unfit or defective genotypes. Nutrition of the dam may also play a role in embryonic mortality. However, the extent of this factor is questionable, since the maternal system sacrifices much of her own needs in order to fill those of the embryo. Other factors affecting embryonic survival may include parity (Erb and Holtz, 1958) and environmental changes in ambient temperature and humidity (Biggers et al., 1987).

The major cause of embryonic mortality, however, may be related to an imbalance in the uterine environment of the maternal system. Embryo transfers have been used as a means to bypass fertilization failures and study the effect of the uterine environment on embryonic survival.

Close synchrony between the conceptus and the uterus has been demonstrated to be necessary for pregnancy to be maintained (Wilmut et al., 1985). In order for embryo transfer to be successful, the donor and recipient must be within ± 24 hours of the same day of the estrous cycle. Utilizing embryo transfer in the ewe, Lawson et al. (1983) demonstrated an interaction between the embryo and the maternal environment occurs. The objective in this study was to determine whether asynchronously transferred embryos fail to survive because of a lack of luteotrophic support or if death of the embryo was due to some other imbalance between the embryo and uterine environment. Results indicated that even though progesterone concentrations are

maintained at normal levels, embryo survival does not occur. However, the authors noted when embryos from day 4 donors were placed in a day 6 or 7 uterus, embryo development was accelerated when flushed and measured on day 12. However, after this point, the asynchronous embryos disappeared from the uterus. This suggests the uterine endometrium produces specific factor(s) which change during the estrous cycle or early pregnancy and regulate or stimulate developmental changes within the embryo. Some accommodation by the embryo to the advanced uterine environment is apparent as accelerated growth and development does occur. However, it is not complete as the embryo eventually dies.

Hawk et al. (1963) used ovariectomy and various hormonal treatments to determine the effect of the hormones on embryonic survival. Treatment after ovariectomy with progesterone and estrogen enabled an ovariectomized cow to maintain pregnancy. It has then been suggested that an imbalance in the steroids, progesterone and estrogen may lead to increased embryonic mortality. Almeida et al. (1986) studied bovine endometrial epithelium which was collected 6 and 7 days post-breeding. This is the same period Ayalon (1973) indicated as being the critical stage for embryonic survival. Results from this study indicate cows with normal pregnancy rates had a greater number of ciliated cells per square millimeter of the luminal epithelium than did repeat breeders. The significance of this finding is yet unknown. However, since ciliogenesis is

controlled by hormones, especially estrogen and progesterone, as has been shown in other species (Tachi et al., 1974; Masterson et al., 1975), an imbalance of hormones may be occurring. This imbalance of hormones may be creating an unfavorable environment even as early as day 6 or 7 resulting in death of the embryo.

Stubbing and Melton (1986) did not find a strong relationship between peripheral progesterone concentrations and establishment of pregnancy. When embryos were transferred on day 7, progesterone levels were highly variable. Boyd et al. (1969) indicated a tendency for smaller day 16 embryos to be found in cows with low plasma progesterone. Hasler et al. (1980) suggested that although progesterone levels at an extreme level may affect conceptus survival, very little embryonic mortality can be attributed to lack of luteal support through day 14 in the bovine.

The previous reports have shown that a correct balance between progesterone and estrogen is necessary for embryo survival in the uterus. However, the mechanisms and effects of these two hormones on the uterine environment and the conceptus during early pregnancy in the bovine has not been clearly shown. Therefore, the following studies were undertaken to determine the effect of progesterone on the uterine environment when administered on day 1 through 4 of the estrous cycle or of early pregnancy. Estrous cycle length, uterine secretion and conceptus development in the cow were evaluated.

# CHAPTER III

# EFFECT OF EXOGENOUS PROGESTERONE ON PROSTAGLANDIN $F_{2\alpha}$ RELEASE AND THE INTERESTROUS INTERVAL IN THE BOVINE

## Introduction

Administration of progesterone early in the estrous cycle of the cow (Woody et al., 1967; Woody and Ginther, 1968; Harms and Malven, 1969; Ginther, 1970; Battista et al., 1984) and ewe (Woody et al., 1967; Lewis et al., 1968; Ginther, 1969; Ottobre et al., 1980) shortens the interestrous interval. The mechanism by which exogenous progesterone regulates cycle length is not clearly understood. Exogenous progesterone administration has been reported to alter the pulsatile release of LH and decrease baseline LH concentration in the cow (Battista et al., 1984) and ewe (Ottobre et al., 1980),. Therefore, progesterone may shorten the interestrous interval by depriving luteal cells of luteotrophic support.

An alternative mechanism for the effect of progesterone on decreasing cycle length would be through stimulation of uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release. Woody and Ginther (1968) demonstrated that exogenous progesterone did

not shorten the length of the estrous cycle in cows after removal of the uterine horn ipsilateral to the corpus luteum (CL). Thus, early CL regression does not appear to result from luteotrophic insufficiency. Ottobre et al. (1980) suggested that progesterone stimulates an earlier release of $PGF_{2\alpha}$ from the uterine endometrium in the ewe, thus causing premature CL regression.

The effect of exogenous progesterone on CL development, secretion and the release of $PGF_{2\alpha}$ in the cow have not been clearly established. Therefore, the objective of the present study was to investigate if administration of progesterone, early in the estrous cycle of the cow, stimulated an advanced pulsatile release of $PGF_{2\alpha}$ from the uterine endometrium resulting in the decreased interestrous interval previously reported.

## Materials and Methods

General. Twenty-three cyclic beef cows maintained on pasture and fed prairie hay ad libitum were observed twice daily (AM and PM) for behavioral estrus (estrus = day 0). Only animals which exhibited an estrous cycle length of normal duration (18 to 22 days) were included in the study. Estrus was synchronized by a single injection of 25 mg of Lutalyse (Upjohn Veterinary Products, Inc., Kalamazoo, MI) administered intramuscularly to group animals prior to sampling.

Thirty-six hours after the initial observation of estrus, cows were randomly assigned to receive an intramuscular injection of either 2 ml sesame oil (vehicle) or 100 mg of progesterone (Henry Schein, Inc., Port Washington, NY) on day 1, 2, 3 and 4 of the estrous cycle.

Daily blood samples (10 ml) were collected by jugular venipuncture beginning on day 1 and continuing until the cow returned to estrus. Samples were transported to the laboratory on ice and centrifuged at 2400 x g for 15 min at 4°C. Plasma was decanted and stored at -15°C until analyzed for progesterone content.

On day 11, an intravenous vinyl catheter (Bolab Inc., Lake Havasu City, AZ) was inserted into the jugular vein toward the vena cava. Beginning on day 12 of the estrous cycle, peripheral blood samples (10 ml) were collected via the catheter at 3 h intervals until the cow returned to estrus. Blood samples were centrifuged at 2400 x g for 15 min and the plasma stored at -15°C until analyzed for the metabolite of prostaglandin $F_{2\alpha}$, 15-keto-13,14-dihydro prostaglandin $F_{2\alpha}$ (PGFM).

To determine the clearance rate of the exogenous progesterone, two cows, which had been ovario-hysterectomized two months previous, were treated with 100 mg of progesterone for 4 days as previously described. Daily blood samples (10 ml) were collected via jugular venipuncture for eleven days, beginning one day before the initial injection of progesterone. Samples were processed

for analysis of progesterone content as previously described.

Hormonal Analysis. Plasma samples were analyzed for progesterone concentration by radioimmunoassay as previously validated and described by Lusby et al. (1981). Recovery of labeled progesterone tracer after hexane extraction was 89%. The minimum sensitivity of the assay was 25 pg/ml. Intra- and interassay coefficients of variation were 7.7% and 12.2%, respectively.

Peripheral plasma PGFM concentration was quantified by a modification of the radioimmunoassay previously reported by Guilbault et al. (1984). The standard curve was generated with prostaglandin-free (PG-free) steer plasma. PG-free plasma was obtained from a steer treated with a prostaglandin synthetase inhibitor (50 mg of flunixin meglumine; Banamine, Schering Corp., Kenilworth, NJ) 16 h and 4 h prior to bleeding. Samples of PG-free plasma contained undetectable (<15 pg/ml) PGFM concentrations when assayed using standard curves in 0.05 M Tris-HCl buffer (pH 7.5). Accuracy of the assay procedure was determined by measuring known quantities of exogenous PGFM previously added to PG-free steer plasma. Plasma samples were assayed in volumes of 100, 200, and 250 ul. Recovery of added mass (0, 5, 15, 20, 40, 100, 175 pg/200 ul PG-free plasma) was described by linear regression ($Y=1.40+4.52X$; $R^2=.998$). Sensitivity of the assay was established at 50 pg/ml, as the antibody dilution of 1:10000 and an assay volume of 200 ul

permitted detection of a minimum mass of 10 pg PGFM. A 10 pg PGFM/200 ul of buffer concentration significantly displaced tritiated PGFM from PG-free plasma (96% binding; $P<.02$).

Standard solutions were made by serial dilutions in buffer of a stock solution of PGFM (Sigma Chemical Co., St. Louis, MO). Final PGFM standard concentrations were 0, 5, 10, 20, 40, 70, 100, 150 and 200 pg/100 ul 0.05 M Tris-HCl buffer. Each tube contained 200 ul of PG-free plasma and 100 ul of PGFM standard solution. Experimental plasma samples (200 ul maximum volume) were added to 100 ul of buffer. As an additional source of precipitable protein, 100 ul of 0.5% human gamma globulin (Sigma Chemical Co., St. Louis, MO) in 0.05 M Tris-HCl buffer was added to each tube. Approximately 10,500 DPM of 5,6,8,9,11,12,14(n)-$^{3}$H PGFM (184 Ci/mM; Amersham International pic, Amersham, UK) in 200 ul of buffer was added. A 100 ul aliquot of 1:10000 goat antiserum to PGFM (gift from Dr. K. Kirton, The Upjohn Co., Kalamazoo, MI) was added to give a final assay volume of 700 ul. Cross-reactivity of the antibody to PGFM with authentic $PGF_{2\alpha}$, C-16 urinary metabolites of $F_{2\alpha}$ and prostaglandins E, A and B is less than 1% (Dr. Kirton, personal communication). After a 12 h incubation at 4°C, separation of free and bound PGFM was accomplished by precipitation of proteins (plasma protein + human gamma globulin + goat anti-PGFM) with 700 ul of a cold 40% solution of polyethylene glycol-6000 (PEG; Sigma Chemical Co., St. Louis, MO) in

distilled water. Following centrifugation at 2400 x g for 30 min, the supernatant was discarded and the pellet resuspended in 0.05 M Tris-HCl buffer (700 ul) using a multi-tube vortex mixer. Forty percent PEG (700 ul) was added as before and centrifugation repeated. After centrifugation, the supernatant was discarded and the pellet resuspended with 500 ul dioxane. Scintillation cocktail (2.5 ml; 20% Triton-toluene cocktail) was added to each vial and samples were then counted using a Packard Tri-carb liquid scintillation spectrometer.

Unknown plasma samples were assayed in duplicate, while standards were run in triplicate. Sample replicates with a coefficient of variation greater than 20% were reassayed. The intra- and interassay coefficients of variation for a reference plasma sample (50 ± 16 pg/ml) run in duplicate for 28 assays were 18.8% and 28.7%, respectively.

Statistical analysis. Plasma progesterone concentration and interestrous interval data were analyzed by least square analysis of variance using the General Linear Models procedures of the Statistical Analysis System (Barr et al., 1979). The statistical model for analysis of plasma progesterone and interestrous interval included effects of treatment (control or progesterone treated), cow within treatment, day and the day by treatment interaction. Effect of treatment was tested with cow within treatment as the error. PGFM data for each cow were analyzed by the PULSAR program previously described by Merriam and Watcher (1982).

Estimated parameters included overall mean concentration, baseline concentration, pulse number, pulse amplitude, maximum amplitude, minimum amplitude, inter-pulse interval and pulse duration. The PULSAR results were analyzed by least squares to evaluate differences in the number of pulses per day between control and progesterone treated cows. The statistical model was the same as described previously for progesterone analysis.

## Results

The mean cycle length of progesterone treated cows (16±.83 days) was less ($P<0.001$) than controls (21.6±.87 days). Four cows, 2 control and 2 progesterone treated, exhibited abnormally short interestrous intervals of 7 to 10 days. The cause of the short interestrous intervals is not known. However, since equal number of cows in both control and progesterone treatment groups exhibited short cycle lengths, progesterone administration did not appear to be involved. These cows were excluded from the analysis of treatment differences.

Administration of exogenous progesterone from day 1 to 4 of the estrous cycle of the cow increased ($P<0.001$) peripheral plasma content of progesterone in treated (3.67±.14 ng/ml) compared to control (1.28±.22 ng/ml) cows on day 2 through 5 of the estrous cycle (Figure 1).

Administration of progesterone shortly after ovulation did not appear to affect development and steroidogenic

capacity of the CL as evidenced by the maintenance of peripheral plasma progesterone concentrations to at least day 9 in the progesterone treated cows. Plasma progesterone content during this period would have originated from the CL since exogenous progesterone begins to decline on day 5 and is less than 1 ng/ml on day 8 in ovario-hysterectomized cows (Figure 2).

The decline of peripheral plasma progesterone concentrations in treated cows (Figure 1) is coincident with an advanced pulsatile release of PGFM (Figure 3). A treatment by day effect ($P<0.001$) was detected for the pulse release of PGFM during diestrus. Progesterone treated cows, as represented in Figure 3, exhibited earlier episodes of PGFM release compared to control cows. Individual variation in the release of PGFM in response to progesterone treatment and normal luteolysis during the estrous cycle was observed. The majority of the control cows displayed 4 to 5 pulses of PGFM associated with CL regression (6/9). However, some displayed fewer than 4 or no pulses of PGFM associated with CL regression (3/9). Progesterone treated cows (4/9) displayed at least 3 pulses of PGFM associated with CL regression. While, others did not display any pulses (2/9) or exhibited fewer than 3 pulses of PGFM (3/9). A number of control and treated cows (7/18) exhibited very large sporadic peaks of PGFM on the first few days of the three hour sampling period. These peaks were not associated with

CL regression and may have resulted from post-cannulation stress and handling during sampling.

## Discussion

Results from the present study indicate administration of exogenous progesterone on days 1 through 4 of the estrous cycle shortens the interestrous interval by approximately 4 days. These results are in agreement with previous reports in the cow (Harms and Malven, 1969; Ginther et al., 1970; Battista et al., 1984).

Possible causes for premature luteal regression after progesterone treatment have included negative feedback on LH secretion by progesterone (Ginther, 1970) and an advanced release of uterine $PGF_{2\alpha}$ (Ottobre et al., 1985). The results of the present study support the latter theory.

Peripheral progesterone concentrations are normally low (2 to 3 ng/ml) during the first 4 to 5 days of the estrous cycle in the bovine (Henricks et al., 1970; Wettemann et al., 1972; Gomes and Erb, 1975; Schams, 1977). Therefore, the uterine endometrium is not fully under the influence of progesterone until approximately day 5 to 6 of the estrous cycle. Administration of exogenous progesterone increases the peripheral plasma content of progesterone on days 2 through 5 of the estrous cycle to a concentration comparable to that of the control estrous cycle on day 5 through 9. Consequently, the maternal system is exposed to a progesterone influence 4 to 5 days earlier. Cyclic

endocrine profiles of progesterone treated cows are similar to controls except the timing of progesterone decline is shifted by 4 days. Therefore, progesterone treated cows returned to estrus approximately 4 days earlier than control cows.

Possible causes for the abnormally short estrous cycle lengths of 8 and 10 days exhibited by 4 cows in this study are unknown. A previous study by Battista et al. (1984) indicated treatment with exogenous progesterone early in the estrous cycle results in interestrous intervals less than 13 days. However, these authors began administration of exogenous progesterone during estrus. Therefore, the presence of high concentrations of progesterone prior to or during ovulation could have interfered with ovulation resulting in an abnormally short estrous cycle length. In contrast, exogenous progesterone administration in the present experiment was begun 36 h after the initial observation of estrus to avoid interference with ovulation. Although progesterone treatment can not be ruled out, it is obviously not the only cause for the short interestrous intervals as both control and progesterone treated cows exhibited cycle lengths of 8 and 10 days.

Individual variation in the response to progesterone treatment as illustrated by the time period until return to estrus and pulsatile release of PGFM is not unexpected. Several factors may play a role, including dose response of progesterone per weight of the animal or the uterine

sensitivity to progesterone may vary in each animal. In the present study, pulses of PGFM were generally associated with decline in progesterone concentrations in both control and progesterone treated cows suggesting $PGF_{2\alpha}$ is related to regression of the corpus luteum. $PGF_{2\alpha}$ has been shown to be luteolytic in the cow (Hixon and Hansel, 1974; Louis et al., 1974; Kindahl et al., 1976). However, since measurement of the endogenous release of $PGF_{2\alpha}$ by the bovine uterine endometrium is difficult, PGFM has been utilized as an indirect measure of $PGF_{2\alpha}$ from the uterine endometrium. PGFM is the major metabolite of $PGF_{2\alpha}$ in cattle and has a half life of 7 to 8 minutes. The concentration of PGFM in the peripheral circulation is correlated with uterine $PGF_{2\alpha}$ release (Louis et al., 1977; Kindahl et al., 1976). Pulsatile release of PGFM (4 to 5 peaks) occurs during the estrous cycle in association with a decline in plasma progesterone concentration (Kindahl et al., 1976).

The advanced pulsatile release of PGFM in the estrous cycle of progesterone treated cows indicates an earlier release of $PGF_{2\alpha}$ from the uterine endometrium compared to control cows. Studies have indicated that a 10 day exposure of progesterone is necessary for initiation of luteolysis in the cow (Ginther, 1970; McCracken et al., 1984).

Results of the present study support that administration of exogenous progesterone on day 1, 2, 3 and 4 of the estrous cycle stimulates an earlier maturation of endometrial development, thereby, causing release of uterine

$PGF_{2\alpha}$ after a 10 day stimulation of progesterone. Earlier pulses of uterine $PGF_{2\alpha}$ stimulate CL regression, thus, resulting in shortened interestrous intervals.

Fig. 1. Peripheral plasma progesterone concentrations in control (solid line) and progesterone treated (dashed line) cows.

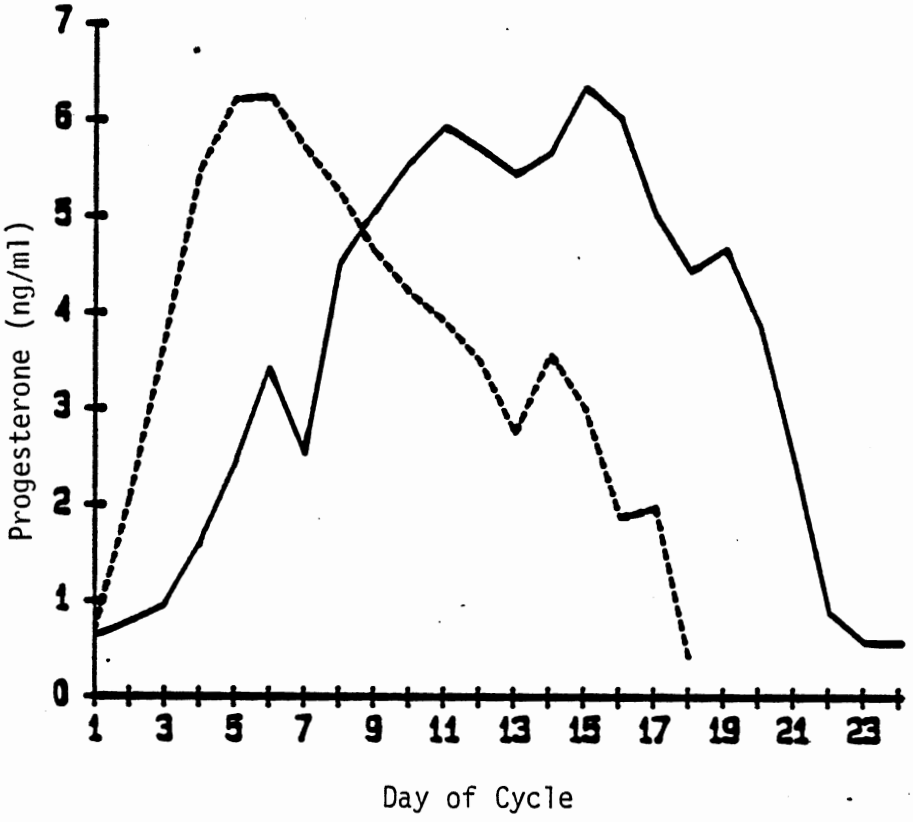

Progesterone (ng/ml)
0
1
2
3
4
5
6
7
1
3
5
7
9
11
13
15
17
19
21
23
Day of Cycle

Fig. 2. Peripheral plasma progesterone concentrations in two ovario-hysterectomized cows after progesterone administration for four consecutive days.

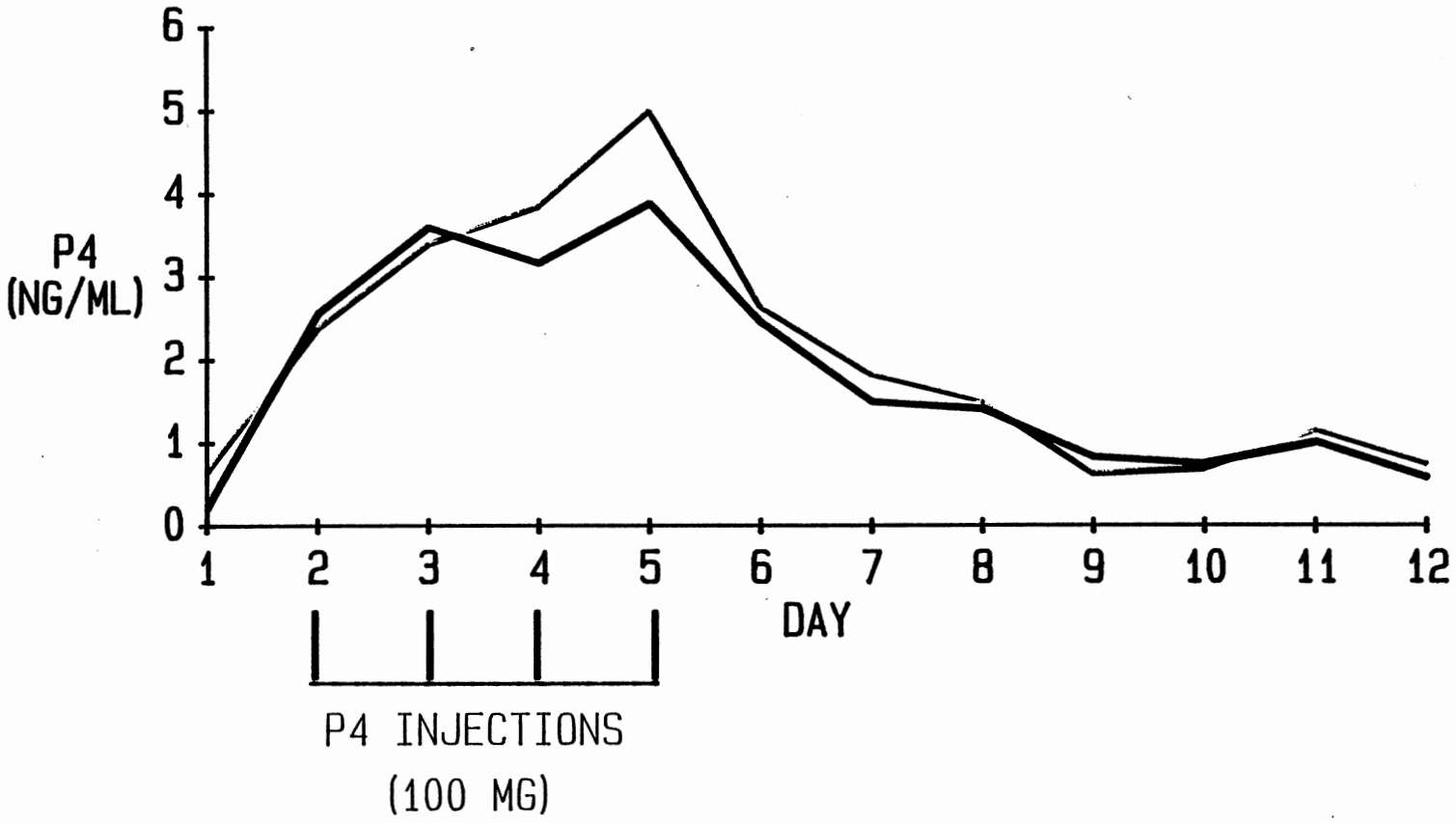
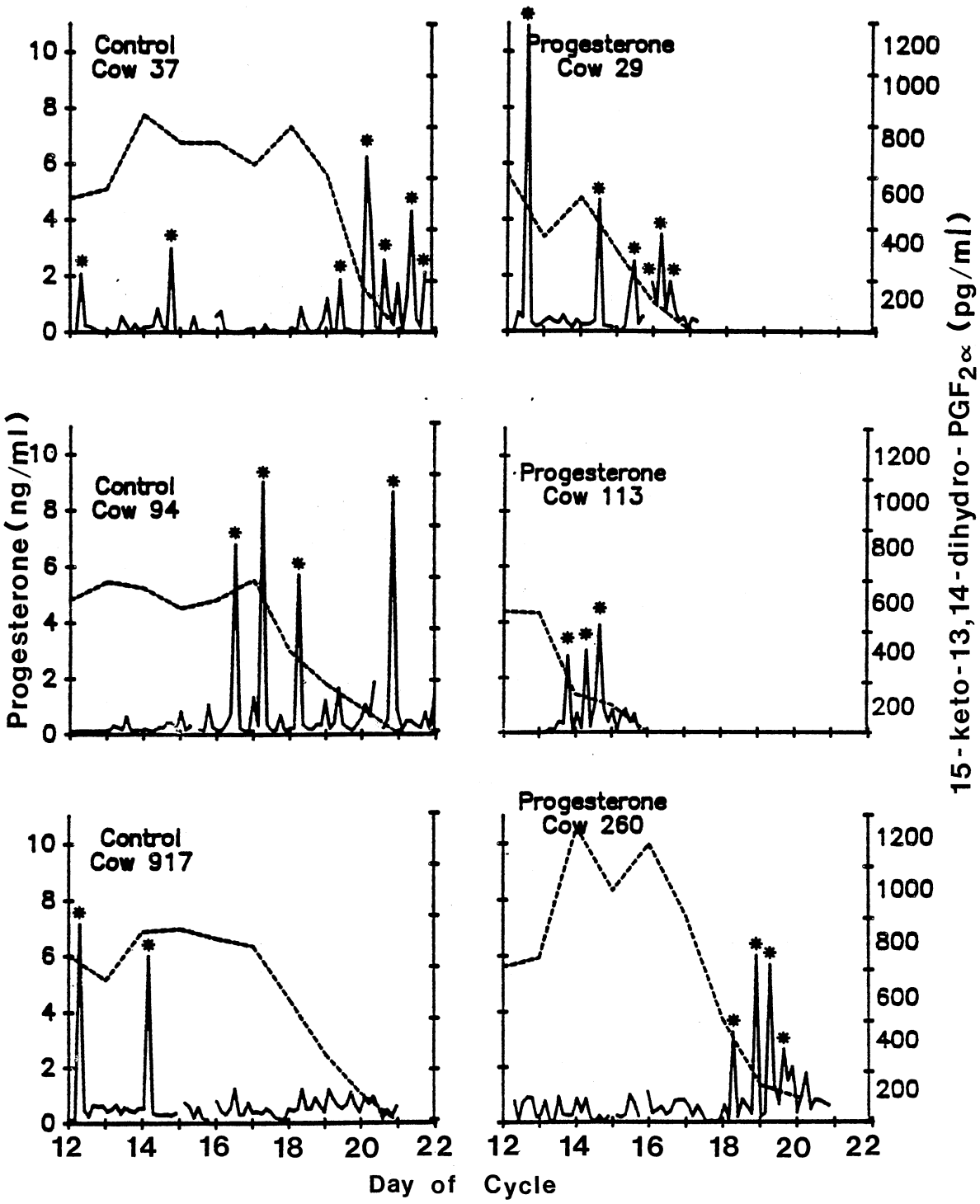

P4
(NG/ML)
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
11
12
DAY
P4 INJECTIONS
(100 MG)

Fig. 3. Peripheral concentrations of plasma progesterone (dashed line) and PGFM (solid line) in control and progesterone treated cows. Asterisks denote significant peaks.


Control
Cow 37
Progesterone
Cow 29
Control
Cow 94
Progesterone
Cow 113
Control
Cow 917
Progesterone
Cow 260
Progesterone (ng/ml)
15-keto-13,14-dihydro-PGF2α (pg/ml)
Day of Cycle
0
2
4
6
8
10
200
400
600
800
1000
1200
12
14
16
18
20
22

# CHAPTER IV

# EVIDENCE FOR MATERNAL REGULATION OF EARLY CONCEPTUS GROWTH AND DEVELOPMENT IN THE BOVINE

## Introduction

Administration of exogenous progesterone early in the oestrous cycle shortens the interestrous interval in both the ewe (Woody et al., 1967; Ottobre et al., 1980; Lawson and Cahill, 1983) and cow (Woody et al., 1967; Woody and Ginther, 1968; Harms and Malven, 1969; Ginther, 1970; Battista et al., 1984). Shortening of the oestrous cycle length is proposed to result from an earlier release of $PGF_{2\alpha}$ from the uterine endometrium (Ottobre et al., 1980; Geisert and Garrett, unpublished data). Therefore, it has been suggested that exposure to exogenous progesterone early in the oestrous cycle advances uterine secretory development.

Embryo transfer studies have emphasized the need for close synchrony (± 24 hours) between the conceptus and recipient (Moor and Rowson, 1966; Betteridge et al., 1980). Close synchrony is essential as the conceptus and maternal system form a complex communication network involving

secretions from the conceptus as well as from the uterine endometrium. These secretions stimulate and mediate changes throughout the early pregnancy period involved with maintaining pregnancy and facilitating conceptus growth and development. Ovarian steroids, especially progesterone, play an important role in regulating changes in the uterine environment conducive to attachment and survival of the conceptus.

Previous studies in the ewe have shown that progesterone administration during the first few days of the oestrous cycle allowed recipient ewes to accept and maintain older conceptuses successfully after embryo transfer (Moore, 1975; Lawson and Cahill, 1983; Vincent et al., 1985). These data suggest progesterone administration during the early oestrous cycle of the recipients results in specific changes in the uterine environment which accommodate conceptuses at a more advanced stage. The specific changes or release of specific factor(s) which mediate the development of the conceptus is unknown.

Effects of progesterone administration on conceptus development, survival and uterine endometrial secretion during early pregnancy in the cow are unknown. The objective of the present study was to determine the effects on uterine secretion and conceptus development after exogenous progesterone treatment during early pregnancy.

## Materials and Methods

Animals. Fifty-one cyclic beef cows, maintained on pasture and fed prairie hay ad libitum, were observed twice daily (AM and PM) for oestrous behaviour (oestrus = day 0). Upon detection of oestrus, cows were mated to fertile bulls.

Cows were randomly assigned to receive one of the following treatments: a)Control, intramuscular injection (2 ml) of vehicle (sesame oil) on days 1, 2, 3 and 4 of pregnancy; b)Treatment, intramuscular injection (100 mg) of progesterone (Henry Schein, inc., Port Washington, NY) on days 1, 2, 3 and 4 of pregnancy. The first injection (Day 1) was administered 36 h after the initial observation of oestrus and the remaining injections were administered at 24 h intervals thereafter. Cows within treatment groups were then randomly assigned to either be slaughtered on day 5 or 14 or rectally palpated for pregnancy on day 40.

Blood samples (10 ml) were collected daily by jugular venipuncture until slaughter or for the first 5 days after mating in animals allowed to continue pregnancy to day 40. Samples were placed on ice, transported to the laboratory and centrifuged at 2400 g for 15 min at 4°C. Plasma was decanted and stored at -15°C until analyzed for progesterone content.

Uteri from cows slaughtered on day 5 and 14 were recovered within 5 min after exsanguination, placed in a sterile beaker on ice and transported to the laboratory for processing in a sterile, laminar flow hood. The broad

ligament and ovaries were trimmed free of the uterus. Each uterine horn was clamped near the bifurcation and flushed separately with sterile 0.9% saline (20 ml) to recover uterine luminal contents. Uterine flushings were immediately examined for embryos (day 5) or conceptus tissue (day 14). Uterine flushings were centrifuged at 12000 g for 20 min at 4°C. The supernatant was decanted and stored at -15°C until analyzed for protein (quantitative and qualitative) content. After flushing, the uterine horns were opened along the antimesometrial border and endometrial explants were obtained from the horns contralateral and ipsilateral to the CL for in vitro culture.

In Vitro Culture. Endometrial tissue from the uterine horns ipsilateral and contralateral to the CL was dissected from the underlying myometrium and immediately placed in sterile modified Eagle's minimum essential media (MEM) as described by Basha et al. (1979). Tissue was cut into 2 to 3 $mm^2$ explants using sterile scalpel blades. Approximately 500 mg of wet tissue was cultured in 15 ml of sterile MEM containing one-tenth of the normal concentration of leucine. One hundred microcuries of [$^3$H]-leucine (L-leucine 4,5-$^3$H, specific activity 58.4 Ci/mmol, New England Nuclear, Boston, MA) was added to each culture dish as a tracer for de novo protein synthesis. Five day 14 conceptuses (2 control and 3 progesterone treated) recovered from uterine flushings were cultured in 3 ml MEM with 25 uCi of [$^3$H]-leucine.

Tissue cultures were rocked slowly (4.5 cycles/min) in a controlled atmospheric chamber (Bellco Biological Glassware, Vineland, NJ) which was purged with a gas mixture of 45% $O_2$/50% $N_2$/5% $CO_2$ and maintained at 37°C as described previously by Basha et al. (1979). Following a 24 h culture period, culture media from endometrial and conceptus tissue was decanted, centrifuged at 12000 g for 20 min at 4°C and stored at -87°C until analysis for polypeptide production.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Uterine flushings and culture media from conceptus and endometrial explants were dialyzed (Spec/por 3, Mr cutoff =3500, Spectrum Medical Industries, Inc., Los Angeles, CA) against several volume changes of 10 mM Tris-HCl buffer (pH 8.2) followed by one volume change of double distilled water. Following dialysis, an aliquot (100 ul) of medium from each culture was used to determine the total DPM of [$^3$H]-leucine retained as a nondialyzable product. Uterine flushings and medium from individual conceptus and endometrial cultures were lyophilized and then reconstituted in 5 mM $K_2CO_3$ containing 9.4 M urea, 2% (v/v) Nonidet P-40 and 0.5% (w/v) dithiothreitol. Two-dimensional polyacrylamide gel electrophoresis of the acidic and basic polypeptides present in uterine flushings and culture medium was performed as described by Basha et al. (1980). Three hundred micrograms of protein from each uterine flushing were applied to gels for 2D-PAGE analysis. Gels were silver

stained according to the procedure described by Wray et al. (1981).

Approximately 150,000 DPM from individual endometrial explant cultures and the 3 ml dialysate (480 to 600 x $10^{-3}$ DPM) from each conceptus culture were applied to gels. Following electrophoresis, gels were stained with Coomassie Brilliant Blue R-250, impregnated with sodium salicylate (Chamberlain, 1979), dried and fluorographs were prepared using Kodak XAR X-ray film (Eastman Kodak Co., Rochester, NY). Fluorographs were developed after an 11 week exposure period at -87°C.

Protein analysis. Protein concentration in uterine flushings were measured by the colorimetric method as described by Lowry et al. (1951) using bovine serum albumin as the standard.

Peripheral plasma progesterone analysis. Plasma samples were analyzed for progesterone concentration by radioimmunoassay as previously described and validated by Lusby et al. (1981). Recovery of labeled progesterone tracer after hexane extraction was 89%. The minimum sensitivity of the assay was 25 pg/ml. Intra- and interassay coefficients of variation were 7.7% and 12.2%, respectively.

Statistical analysis. Data were analyzed by least square analysis of variance using the General Linear Models procedures of the Statistical Analysis System (Barr et al.,

1979). The statistical model for analyses of concentration of progesterone in plasma included effects of treatment (control and progesterone treatment), cow within treatment, day and treatment by day interactions. Effect of treatment was tested with cow within treatment as the error. Conceptus length on day 14 in the control and progesterone treated groups were analyzed by Bartlett's test for homogeneity of variance (Steel and Torrie, 1960). Variances of treatment groups were different ($P<.05$). Therefore, conceptus length was analyzed for differences between treatments by student's t test for unequal variances. The statistical model for uterine protein content and incorporation of [$^3$H]-leucine by endometrial explants included effects of treatment, day and the treatment by day interaction.

## Results

A treatment by day interaction ($P<0.001$) was detected for plasma progesterone concentration. Administration of exogenous progesterone on day 1, 2, 3 and 4 of pregnancy increased ($P<0.001$) peripheral plasma progesterone concentrations on days 2 through 5 of pregnancy (3.40±.09 ng/ml) compared to control (1.22±.09 ng/ml) cows (Figure 4).

Although the recovery of day 5 embryos was low for both control (3/7) and progesterone treated (3/7) cows, progesterone treatment appeared to advance embryo development. Embryos recovered from control cows were 8 to

16 cells, while one 32 cell embryo and two empty zona pellucidae were recovered from progesterone treated cows (Figure 5). No embryos were found in the flushings containing the empty zona pellucidae.

Conceptuses recovered from day 14 uteri of progesterone treated (5/10) cows were advanced ($P<0.10$) in development (Figure 6) compared to controls (6/7). Day 14 conceptuses from progesterone treated cows averaged 37.3 ± 14.9 mm compared to 3.8 ± 1.9 mm for conceptuses from control cows (Figure 7). Progesterone treatment not only increased morphological development of day 14 conceptuses but also stimulated an advance in biosynthetic activity as indicated by fluorographs from conceptus cultures (Figure 8). The complex of polypeptides forming the bTP-1 complex ($M_r$ of 22 to 26 x $10^{-3}$, pI of 5.8 to 6.4) which are proposed to be involved with the maintenance of early CL function (Thatcher et al., 1986) were prominent in conceptus cultures from progesterone treated cows. Progesterone administration did not appear to have adverse effects upon maintenance of pregnancy as pregnancy rates determined through rectal palpation on day 40 to 60 were similar between control (6/8) and progesterone treated (7/11) cows.

Analysis of the uterine environment on day 5 and 14 indicated that progesterone administration did alter secretory activity of uterine endometrium. Total protein content in uterine flushings on day 14 was greater ($P<0.05$) in progesterone treated cows (15.2 ± 2.0 mg) compared to

controls (8.26 ± 2.4 mg). However, a day effect was not detected.

Two dimensional polyacrylamide gel electrophoresis of uterine flushings revealed several acidic polypeptides which are not present in bovine plasma (Figure 9 and 10). No obvious differences were observed between control and progesterone treatment on either day 5 or 14 of pregnancy. A day effect was noted as a group of polypeptides with a $M_r$ of 22 to 30 x $10^{-3}$; pI 4.2 to 5.5 intensified on day 5. Day 14 uterine flushings contained two groups of polypeptides ($M_r$ of 21 x $10^{-3}$, pI of 6.3 and $M_r$ of 14.4 x $10^{-3}$, pI of 6.3) which increased in intensity compared to day 5 (Figure 10).

Overall incorporation of [$^3$H]-leucine per mg of wet tissue weight was approximately 3469 ± 285 DPM for treatment and day. Evaluation of fluorographs from day 5 endometrial explant cultures from progesterone treated cows revealed an increase in the intensity of three separate groups of polypeptides compared to fluorographs from control cows (Figure 11). Group I consisted of 4 low molecular weight (16 to 20 x $10^{-3}$) polypeptides with a pI of approximately 6.3 to 7.0. These polypeptides had greater intensity on fluorographs from day 5 progesterone treated cows compared to fluorographs from day 5 control and day 14 control and progesterone treated cows. Group II consisted of polypeptides with a $M_r$ of 40 x $10^{-3}$ and pI 6.4 to 7.2 and

Group III consisted of polypeptides with a $M_r$ of 40 x $10^{-3}$ and pI 5.0 to 5.9.

Examination of fluorographs from day 14 endometrial cultures (Figure 11) indicated that a group of polypeptides with $M_r$ of 47 to 56 x $10^{-3}$ and pI of 5.2 to 6.0 was intensified in culture medium of endometrium from progesterone treated cows compared to control cows.

Evaluation of fluorographs from basic gels from day 14 endometrial explants revealed an increase in the intensity of a polypeptide with an $M_r$ of approximately 60 x $10^{-3}$ and pI of 6.7 in control cows compared to progesterone treated cows (Figure 12).

## Discussion

Results from the present study indicate administration of exogenous progesterone on day 1, 2, 3 and 4 of pregnancy increases peripheral plasma progesterone concentration on day 2 through 5 comparable to concentrations on day 5 through 9 of control cows. The results suggest that increased concentrations of progesterone during the first five days of pregnancy stimulate changes in uterine secretions which directly or indirectly stimulate conceptus growth and development.

The four day advance in the functional state of the uterus as determined by uterine secretions is consistent with shortening of the oestrous cycle by four days after a similar progesterone treatment (Woody et al., 1967; Woody

and Ginther, 1968; Harms and Malven, 1969; Ginther, 1970; Battista et al., 1984; Geisert and Garrett, unpublished data). It has been suggested that the decreased interestrous interval after treatment with exogenous progesterone is mediated through earlier maturation of the uterus as evidenced by an earlier release of $PGF_{2\alpha}$ from the uterine endometrium (Geisert and Garrett, unpublished data). The alteration in secretory activity of the endometrium in vitro support that exogenous progesterone advanced uterine endometrial secretory function.

Studies have demonstrated that administration of exogenous progesterone early in pregnancy of the ewe allows older embryos to be transferred and maintained in "younger" uteri (Moore, 1975, Lawson and Cahill, 1983; Vincent et al., 1985). These previous results suggest progesterone stimulates uterine endometrial secretions which are favorable for development and growth of older embryos. The present study indicates exposure to progesterone early in pregnancy results in quantitative secretory changes of several polypeptides from the uterine endometrium. The function of these polypeptides are presently unknown. However, it is possible that the polypeptides are involved with the development of the conceptus.

Eight to sixteen cell embryos recovered at day 5 of pregnancy in control cows are comparable to embryonic development at day 5 reported by others (Hartman et al., 1942; Hamilton and Laing, 1946; Chang, 1966; Flechon and

Renard, 1978). Although embryo recovery was low, embryonic development in progesterone treated cows appeared to be advanced. One 32 cell embryo and two empty zona pellucidae were recovered from day 5 progesterone treated cows. The empty zona pellucidae exhibited a slit on the surface indicating hatching had occurred. Blastocyst hatching normally is reported to occur on day 9 to 11 of pregnancy (Flechon and Renard, 1978; Shea, 1981).

It is evident that conceptuses from progesterone treated cows continued to develop more rapidly as conceptus length on day 14 was greater than those from control cows. Day 14 conceptuses from progesterone treated cows synthesized and released polypeptides, including bTP-1, which are associated with pregnancy recognition in the bovine (Knickerbocker et al., 1986). The early release of such polypeptides is necessary, as the pregnancy recognition signal from the conceptus is needed approximately 4 days earlier to prevent luteal regression. Similar pregnancy rates between progesterone treated and control cows on day 40 of gestation provided further evidence as to the viability of the accelerated conceptuses.

It is interesting to note, exogenous progesterone administration stimulates a marked advance in bovine conceptuses but not in ovine (Bazer, personal communication) or porcine conceptuses (Geisert, unpublished data). The cow, in contrast to the ewe and sow, exhibits a period of low concentrations of peripheral plasma progesterone during

the first five days of pregnancy. Peripheral plasma progesterone concentrations in the cow gradually increase from less than 1 ng/ml during estrus to approximately 2 ng/ml on day 5. In the ewe and sow, peripheral plasma progesterone concentrations rise more sharply after estrus (see review by Hansel et al., 1973). The uterine environment in the ewe and sow may already be under the influence of progesterone during the first few days of pregnancy. Therefore, the dramatic changes in embryo development due to exogenous progesterone administration are not seen.

Reports in other species have indicated ovarian steroids can influence uterine secretions for activation of the embryonic growth and development (for review, see Blandau, 1971). For example, in the macropodid marsupials, the embryo undergoes a period of diapause before implanting. The stimulus for activation of the embryo is an increase in progesterone production (see review by Tyndale-Briscoe, 1981).

Results of the present study indicate the bovine conceptus may undergo a similar stage of diapause during the first 5 days of pregnancy. Administration of exogenous progesterone on day 1, 2, 3 and 4 of pregnancy therefore, may stimulate an earlier activation of the bovine conceptus by advancing the release of uterine secretions necessary for conceptus development. These results suggest the maternal endocrine system, especially progesterone concentrations,

regulate early conceptus growth and development in the bovine.

Fig. 4. Peripheral plasma progesterone concentrations in control (solid line) and progesterone treated (dashed line) cows.

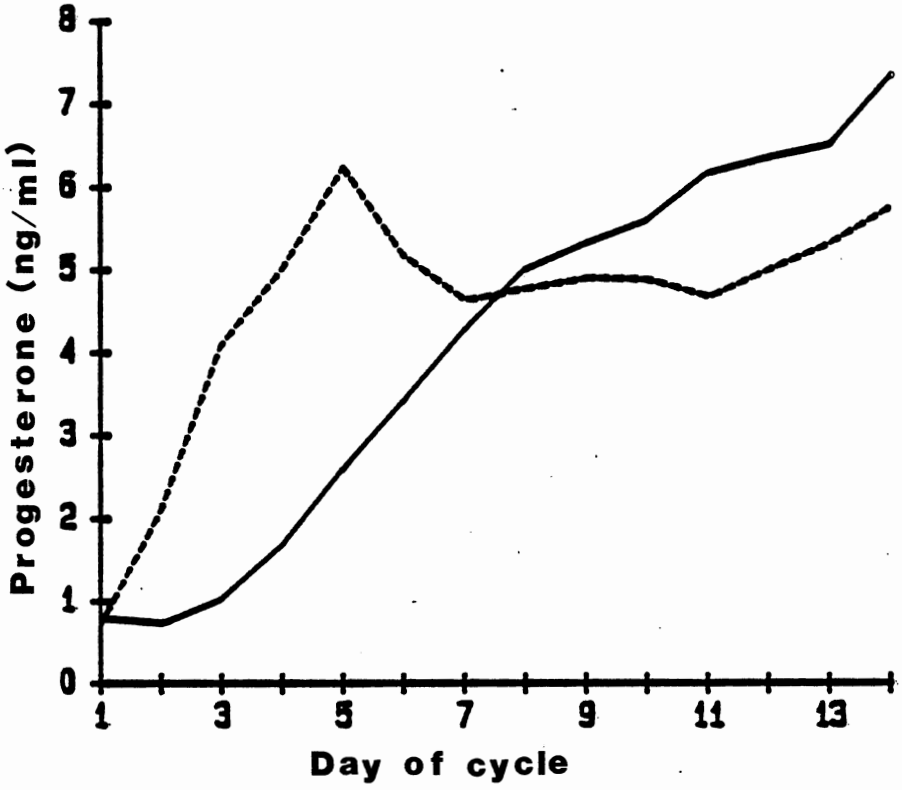
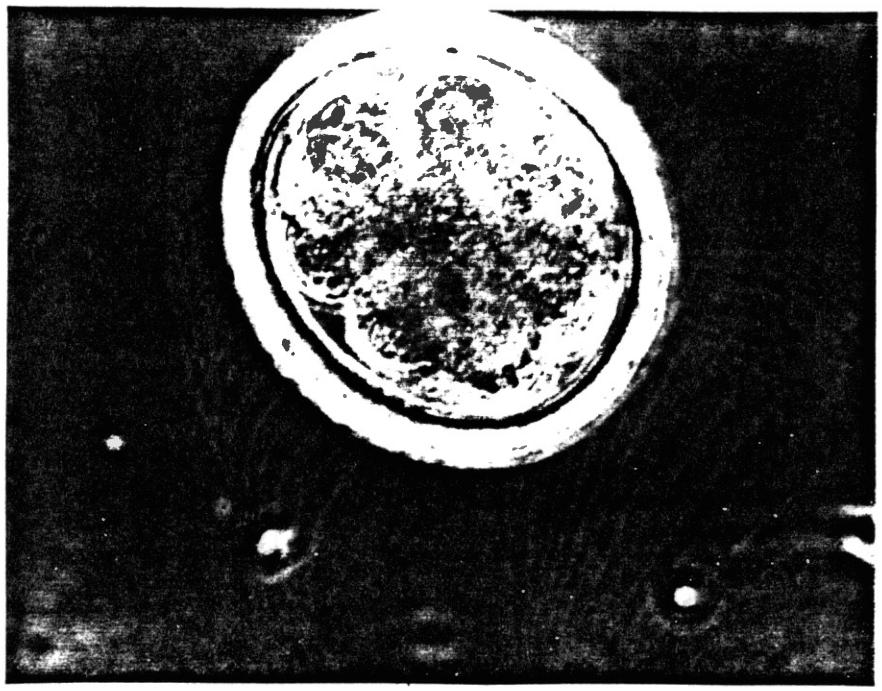

Progesterone (ng/ml)
0
1
2
3
4
5
6
7
8
1
3
5
7
9
11
13
Day of cycle

Fig. 5. Day 5 embryo recovery: (A) Sixteen cell embryo from a control cow and (B) cracked zona pellucida from a progesterone treated cow.

A.



B.

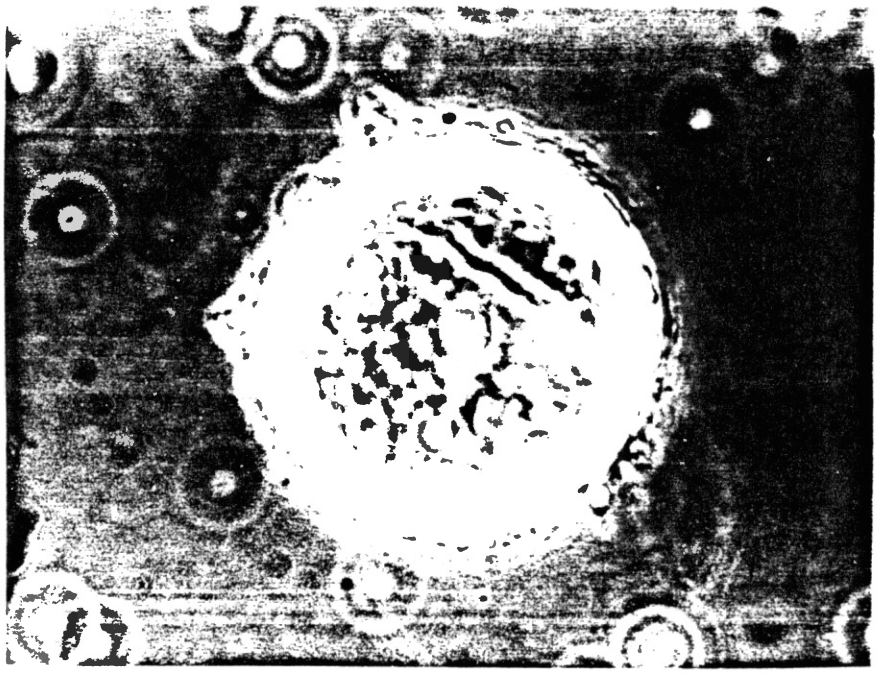
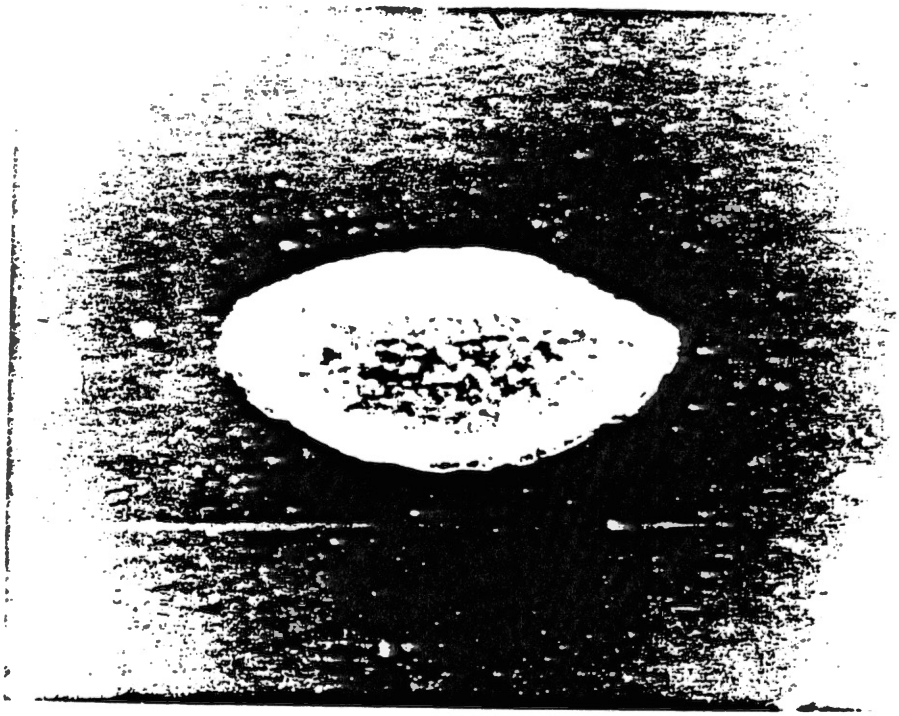


Fig. 6. Day 14 conceptus recovery: (A) 1.75 mm conceptus from a control cow and (B) 13 mm conceptus (upper) from a control cow and 47 mm conceptus (lower) from a progesterone treated cow.

A.



B.

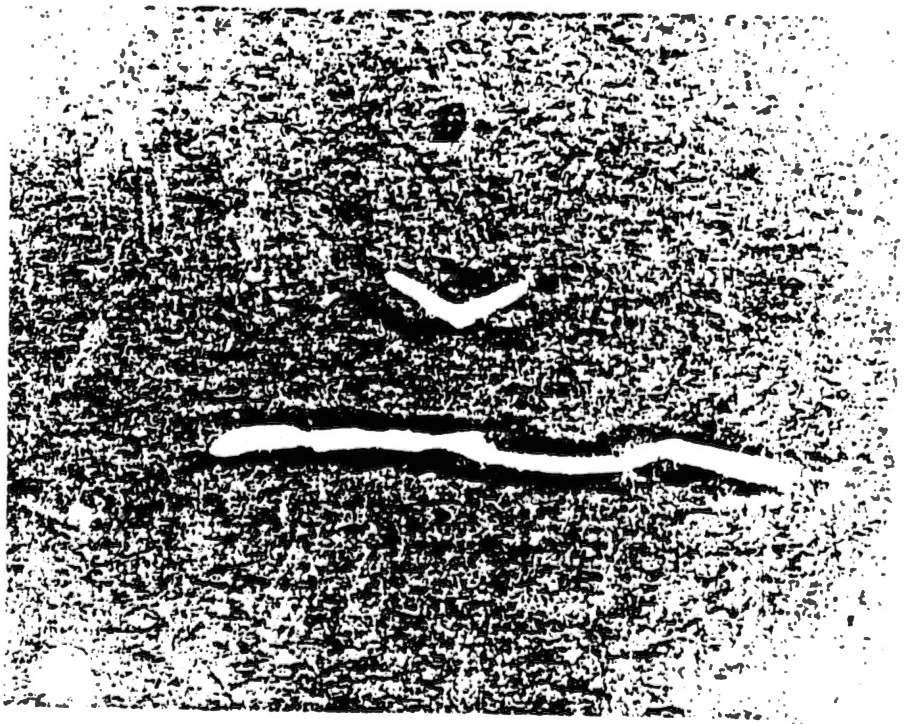


Fig. 7. Individual (closed circles) and mean lengths (bar) of conceptuses from control and progesterone treated cows on day 14.

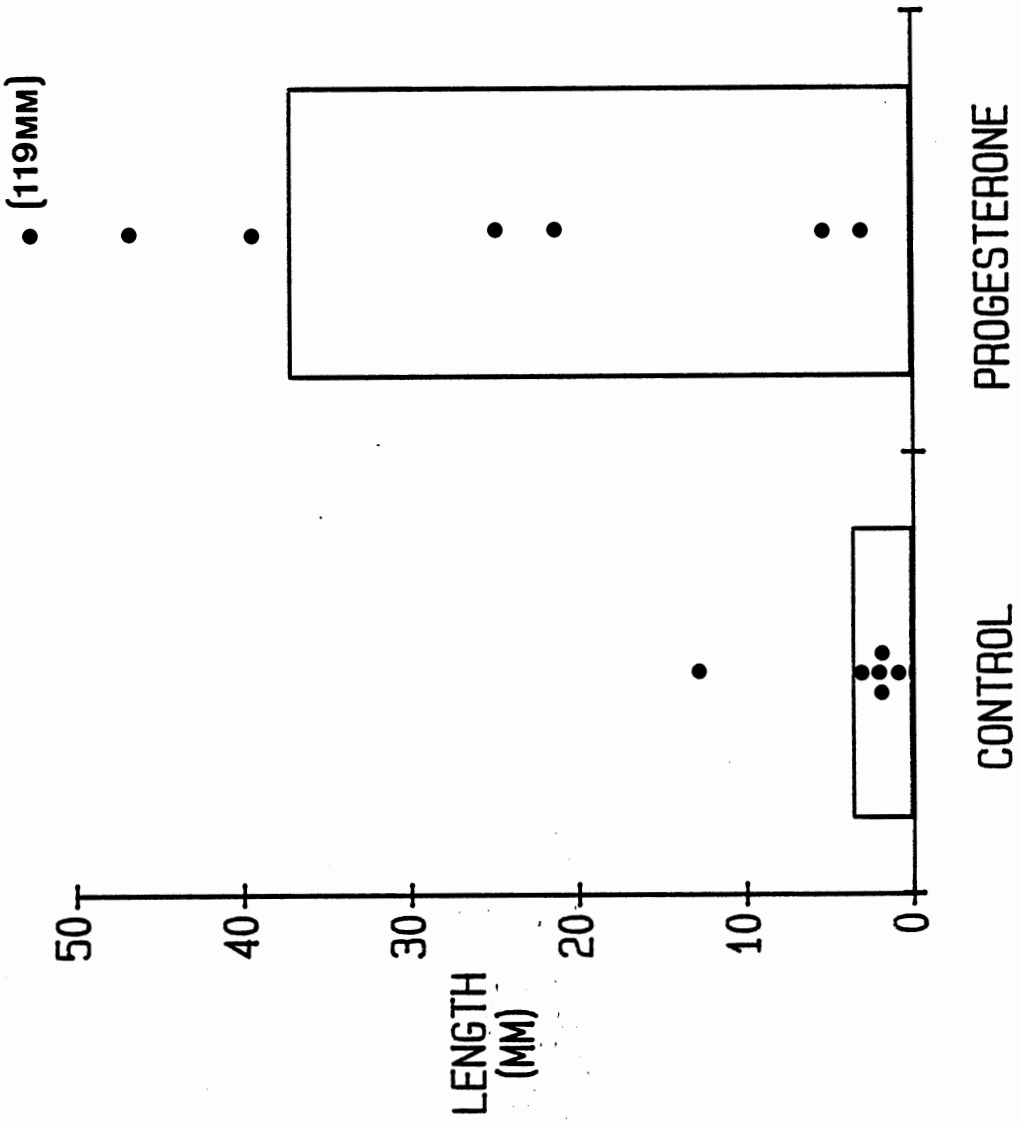

(119MM)
50
40
30
20
10
0
LENGTH (MM)
CONTROL
PROGESTERONE

Fig. 8. Fluorographs representative of 2D-PAGE acidic polypeptides in dialyzed bovine conceptus culture MEM from day 14 control (C14) and progesterone treated (P14) cows. Arrow denotes position of the bTP-1 complex.

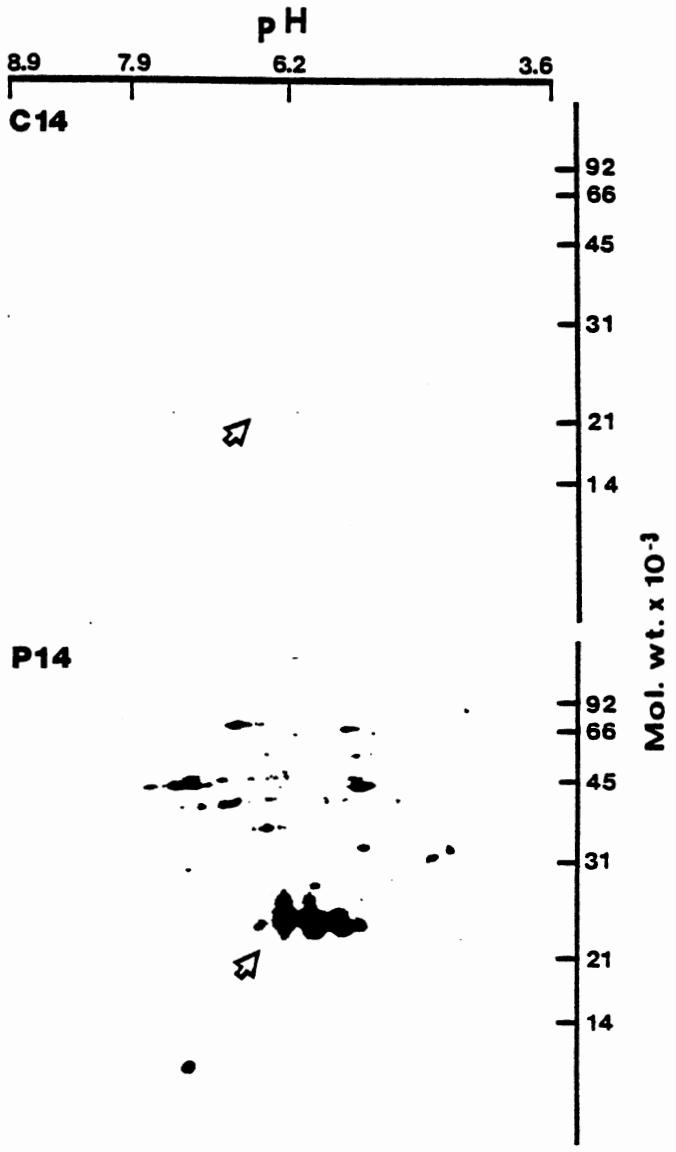

pH
8.9
7.9
6.2
3.6
C14
92
66
45
31
21
14
P14
92
66
45
31
21
14
Mol. wt. x 10⁻³

Fig. 9. Silver stained 2D-PAGE of acidic polypeptides in bovine plasma.

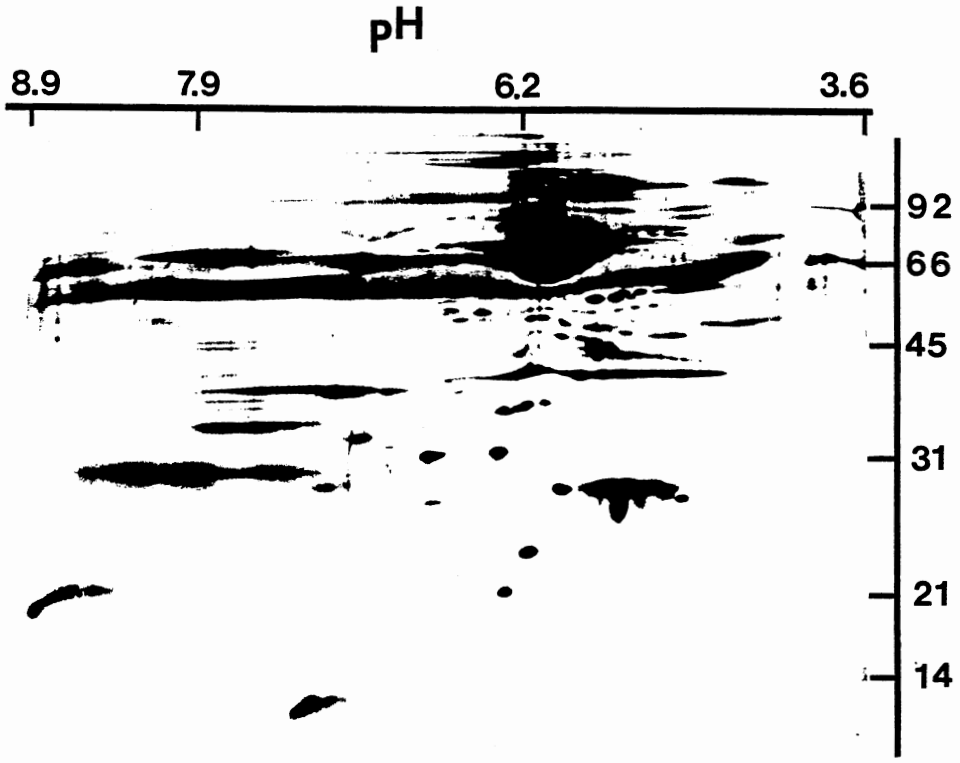

pH
8.9
7.9
6.2
3.6
92
66
45
31
21
14

Fig. 10. Silver stained 2D-PAGE of acidic polypeptides in uterine flushings obtained from day 5 control (C5) and progesterone treated (P5) and day 14 control (C14) and progesterone treated (P14) cows. Open arrow denotes group of 2 polypeptides with $M_r$ of 21 and 14.4 x $10^{-3}$, pI of 6.3. Closed arrow denotes group of polypeptides with $M_r$ of 22 to 30 x $10^{-3}$, pI of 4.2 to 5.5.

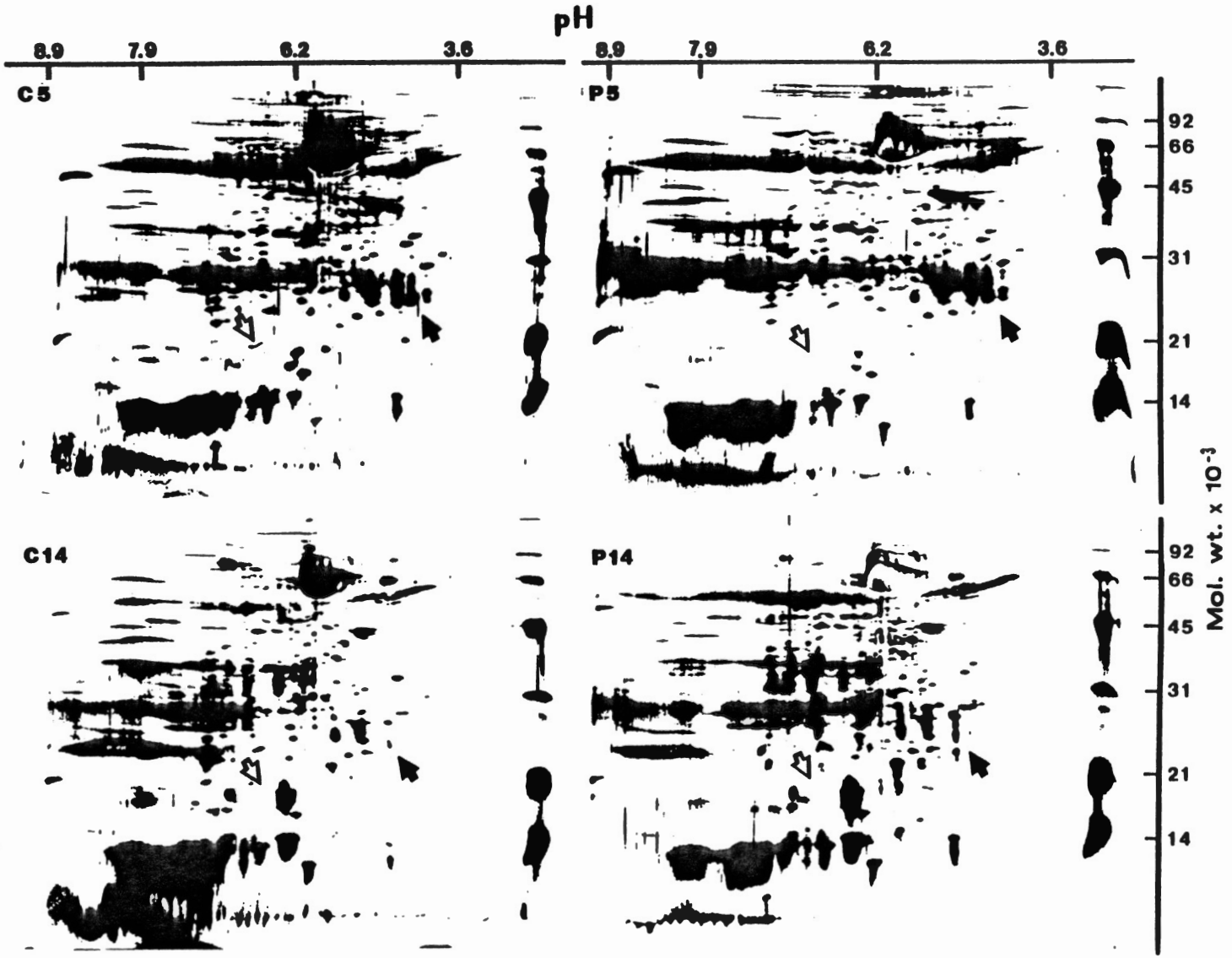

pH
8.9
7.9
6.2
3.6
C5
P5
C14
P14
92
66
45
31
21
14
Mol. wt. x 10-3

Fig. 11. Fluorographs of 2D-PAGE of acidic polypeptides in dialyzed endometrial culture MEM from day 5 control (C5) and progesterone treated (P5) and day 14 control (C14) and progesterone treated (P14) cows. Open arrow denotes group of polypeptides with $M_r$ of 16 to 20 x $10^{-3}$, pI of 6.3 to 7.0.

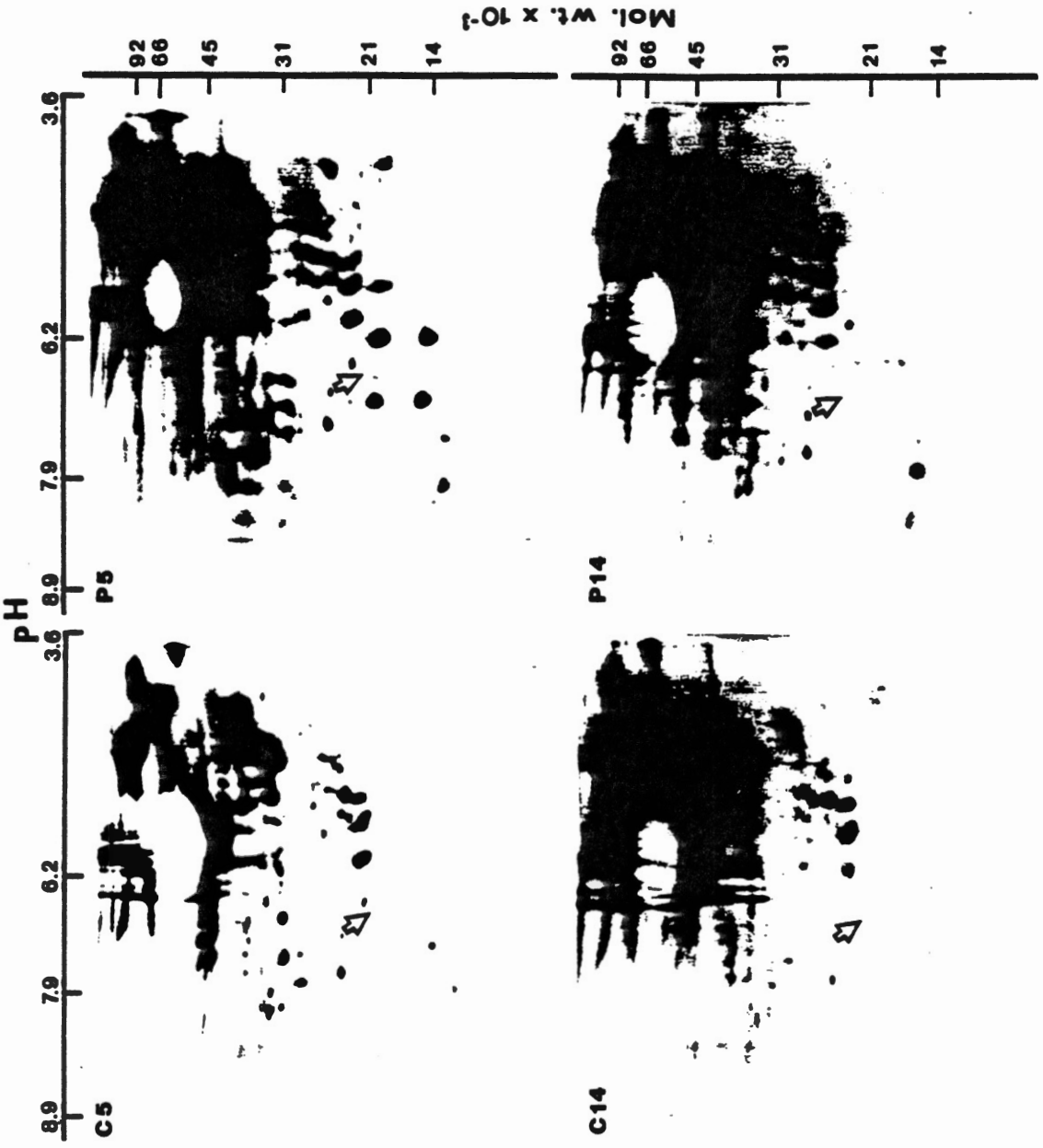
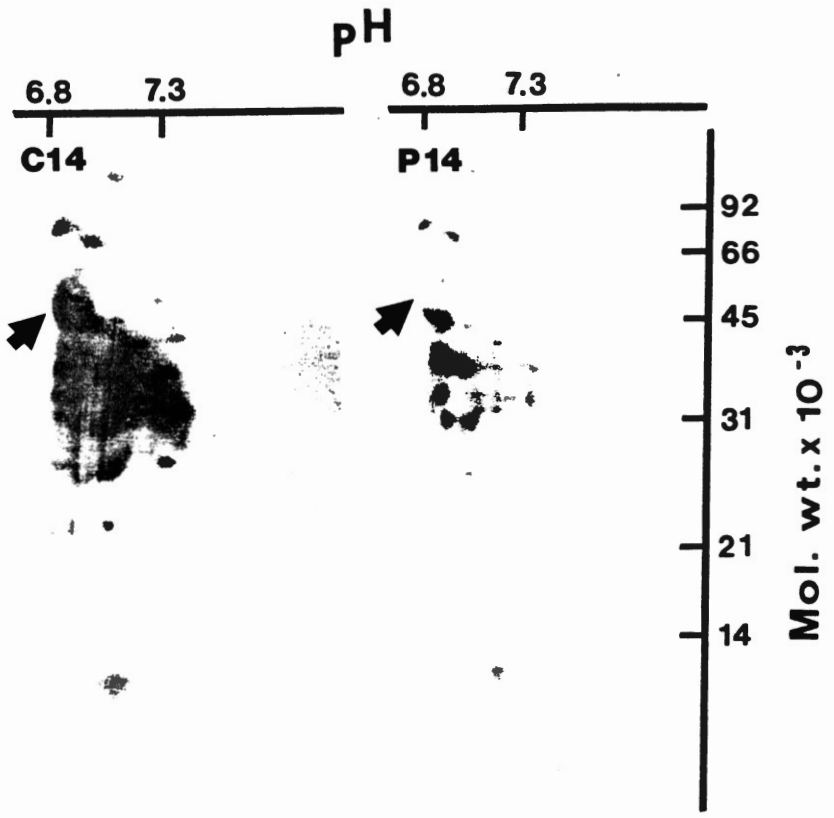

Mol. wt. x 10-3
92
66
45
31
21
14
pH
3.6
6.2
7.9
8.9
P5
P14
C5
C14

Fig. 12. Fluorographs of 2D-PAGE of basic polypeptides in dialyzed endometrial culture MEM from day 14 control (C14) and progesterone treated (P14) cows.


pH
6.8
7.3
6.8
7.3
C14
P14
92
66
45
31
21
14
Mol. wt. x 10⁻³

# CHAPTER V

## GENERAL DISCUSSION

Complex interactions between the developing conceptus and the uterine endometrium are necessary for maintenance of pregnancy in all species. The maternal endocrine system influences secretions from the endometrium and perhaps the conceptus. However, the effect of changes in uterine secretion on conceptus development and survival are not clear.

It is unknown if the conceptus develops according to an intrinsic time schedule or if it is under the extrinisic control by the maternal system. In other words, does the developing conceptus signal the maternal system and thereby, stimulate the release of secretions which are necessary for its growth and development or does the conceptus play a passive role only responding to secretions from the maternal system which regulate conceptus development within the uterine environment?

The results from the present studies suggest a combination of intrinsic and extrinsic control. Administration of exogenous progesterone on day 1, 2, 3 and 4 increase concentration of peripheral plasma progesterone on day 2 through 5 to a level comparable to day 5 through 9

in control cows. The present studies indicated that early exposure to progesterone only shifts the time period of progesterone stimulation. The maternal system is influenced by progesterone 4 days earlier and subsequently advances the synthesis and release of progesterone-induced secretions needed for conceptus growth. Earlier progesterone stimulation also results in shifting the events involved with luteal regression.

Administration of exogenous progesterone shortens the interestrous interval in the ewe (Ottobre et al., 1980) and cow (Battista et al., 1984). The results of the present thesis indicate the shortening of cycle length by progesterone is mediated through an earlier release of $PGF_{2\alpha}$ from the uterine endometrium. The mechanism by which progesterone regulates the release of $PGF_{2\alpha}$ is unknown. However, previous studies have indicated exposure to progesterone for 10 days is necessary for natural luteolysis to occur (Ginther, 1970; McCracken et al., 1984). Ten days of progesterone may be needed for maturation of the enzyme system for prostaglandin production and/or synthesis of receptors, namely oxytocin and estrogen, involved with its release.

These results open an interesting field of study in the early pregnant cow. Little is known about the effects of progesterone during early pregnancy. Much of the work has previously centered on approximately day 16 of pregnancy (Bartol et al., 1985, 1986; Geisert et al., 1988).

Therefore, the early effects of progesterone on endometrial secretions and conceptus growth and development have not been clearly defined.

Early embryo development appeared to be advanced in the progesterone treated cows as only the empty zona pellucidae from two blastocysts and one 32 cell embryo were recovered at day 5. The embryos recovered at day 5 of pregnancy are normally at the 8 to 16 cell stage. There may be some question as to whether the empty zona pellucida found were actually from the remains of hatched blastocysts or whether they were the remains of an embryo degenerating due to asynchronous uterine environment caused by the progesterone treatment. However, after examining conceptuses recovered on day 14, it is clear that development of the conceptuses has been advanced by exogenous progesterone treatment.

Conceptus lengths on day 14 were significantly greater in the progesterone treated cows compared to controls. Although there was considerable variation in conceptus length within each treatment group, it is possible this resulted from individual cow variation in response to progesterone administration. This is also evident in the variation in the pulsatile release of PGFM and return to estrus in cyclic cows after exogenous progesterone administration.

The mechanism(s) involved with progesterone stimulation of conceptus growth and development is not clearly understood. It is interesting that advancement of conceptus

development after exogenous progesterone administration is observed in the cow but not in other domestic farm species such as the ewe (Bazer, personal communication) or sow (Geisert, unpublished data). However, the cow is unique in that peripheral plasma progesterone concentrations gradually increase from less than 1 ng/ml during estrus to approximately 2 ng/ml on day 5. In the ewe and sow, peripheral plasma progesterone concentratons rise more sharply after estrus. Therefore, administeration of exogenous progesterone may not alter the release of uterine secretions by a great degree in these species.

Perhaps the gradual rise of progesterone results in advancing the initial cleavage divisions in the cow. These divisions occur 14 to 18 h after ovulation in the pig and ewe in comparison to 20 to 24 h in the cow. Entrance of the conceptus into the uterine lumen is dependent on progesterone concentration (Rider et al., 1987). The bovine conceptus enters the uterine lumen on approximately day 4 to 5 of pregnancy (Anderson, 1977; Perry, 1981). Whereas, the porcine and ovine conceptus enter the uterine lumen on approximately day 2 and 3, respectively. Progesterone administration could allow an earlier entrance into the bovine uterus. Thus, permitting an advanced stimulation by uterine secretions involved with conceptus growth and development.

Progesterone has been demonstrated to regulate the time of decidualization in the rat uterus. Psychoyos and Prapas

(1987) utilized the progesterone antagonist, RU486, to study uterine development in rat. Their results suggest progesterone plays a role in stimulating endometrial sensitivity to implantation as treatment with RU486 altered the timing of the decidualization response to oil instillation and embryo implantation. It has been suggested the uterine milieu secretes growth factors involved with regulating embryonic development (Adamson, 1987). Recently, growth factors have been identified in the uterus of the mouse (Pantazis and Howard, 1987) and pig (Brigstock et al., 1986). Isolation of growth factors from the cow uterus have not been reported. However, it is quite possible that progesterone could be involved with the synthesis and release of growth factors involved with conceptus development as observed in the present studies.

The conclusions of the present thesis are but the stepping stones on which future research will build. The mechanism by which progesterone produces its effect is yet to be determined. The function of the endometrial secretions which are released or diminished upon progesterone stimulation, and the possible direct effect of progesterone on the conceptus are of interest. Future research directed towards isolating possible growth factors involved with uterine and embryonic development are needed. Results of the present studies suggest the possibility that growth stimulating factors are present in the bovine uterus and are under progesterone regulation.

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