University of Tennessee, Knoxville

TRACE: Tennessee Research and Creative Exchange

Masters Theses

Graduate School

8-1996

Effects of elevated concentrations of prostaglandin $F_2\alpha$ on early embryonic survival in cows supplemented with exogenous progestogen

Richard C. Seals

Follow this and additional works at: https://trace.tennessee.edu/utk_gradthes

Recommended Citation

Seals, Richard C., "Effects of elevated concentrations of prostaglandin $F_2\alpha$ on early embryonic survival in cows supplemented with exogenous progestogen. " Master's Thesis, University of Tennessee, 1996.
https://trace.tennessee.edu/utk_gradthes/6800

This Thesis is brought to you for free and open access by the Graduate School at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Masters Theses by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.

To the Graduate Council:

I am submitting herewith a thesis written by Richard C. Seals entitled "Effects of elevated concentrations of prostaglandin $F_2\alpha$ on early embryonic survival in cows supplemented with exogenous progestogen." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

F. Neal Schrick, Major Professor

We have read this thesis and recommend its acceptance:

Jim Godkin, Fred Hopkins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Richard C. Seals entitled "Effects of Elevated Concentrations of Prostaglandin $F_{2\alpha}$ on Embryonic Survival in Beef Cattle Supplemented with Exogenous Progestogen." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

F. Neal Schrick, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

EFFECTS OF ELEVATED CONCENTRATIONS OF PROSTAGLANDIN $F_{2\alpha}$ ON EARLY EMBRYONIC SURVIVAL IN COWS SUPPLEMENTED WITH EXOGENOUS PROGESTOGEN

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Richard C. Seals

August 1996

AG-VET-MED.
Thesis
96
.S4

Acknowledgments

I would like to express my gratitude to Dr. Neal Schrick for allowing me to further my graduate education at the University of Tennessee. I would like to thank Dr. Schrick for his patience, guidance and friendship during my graduate career. Thanks are extended to Dr. Fred Hopkins for his candid "Case of the Day" and for critically reviewing this thesis. I would also like to thank Dr. Jim Godkin for keeping me on my toes in journal club and for critically reviewing this thesis.

A special thanks goes out to Jackie Martin, Dale Rose and Kevin Thompson for procurement and care of the animals used in this experiment. I would also like to thank the work-study students (Mark Davis, Jason Vickers, Elizabeth Doane and Bryan Moore) for their help and assistance. I would also like to thank Nancy Rohrbach for conducting the radioimmunoassays performed in this study as well as countless others.

A special thanks goes to my fellow graduate students (Mike Buuck, Willard Lemaster, Mark Franklin and Chad Robbins) for their help and friendship. I wish each of you

the best of luck in all that you do and your friendship will never be forgotten.

I would also like to thank my wife, Laurie, for her love, encouragement and support during my graduate study at the University of Tennessee. I could not have completed this accomplishment without you. I would also like to thank my parents for their love and encouragement.

ABSTRACT

An experiment was performed to determine the stage of embryonic development detrimentally affected by elevated prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the presence or absence of luteal oxytocin. Ninety-one beef cows had their estrous cycles synchronized and were bred by natural service and artificial insemination (Day 0). Cows were randomly assigned to receive either 3 ml saline (CON), 15 mg $PGF_{2\alpha}$ (PGF) or 15 mg $PGF_{2\alpha}$ + lutectomy (P+L) administered i.m. at 8 h intervals on either Days 5-8, 10-13 or 15-18 (3 X 3 factorial). Lutectomies were performed by transrectal digital pressure before initiation of treatment on Day 5, 10 or 15 for Days 5-8, 10-13 and 15-18, respectively. All cows were fed 4 mg/d of melengesterol acetate (MGA) from two days prior to initiation of treatment until Day 30. Lutectomies were performed on nine CON cows to verify the ability of MGA to sustain pregnancy. Seven of these nine animals were diagnosed pregnant by transrectal ultrasonography at Day 30. All cows were bled by jugular venipuncture at 0600 and 2200 h of their respective treatments for determination of progesterone (P_4), estradiol-17β (E_2), and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM). Additionally, cows were bled at 30 min

following initiation of treatment for determination of oxytocin (OT) concentrations. Concentrations of P_4 were reduced ($p < 0.05$) in all cows administered PGF and P+L by 2200 h on the first day of treatment. Mean concentrations of PGFM were increased in cows administered PGF and P+L treatments (456 ± 35 and 398 ± 35 pg/ml, respectively; $p < 0.01$) compared to CON cows (84 ± 58 pg/ml) regardless of daygroup. Mean concentrations of OT were significantly increased in cows administered PGF when compared to CON and P+L in the Day 5-8 ($P < .07$) and in the Day 10-13 and 15-18 groups ($P = .0001$). Pregnancy rates were reduced ($p = 0.03$) in the PGF(5-8) group (3/13, 23%) compared to CON (5/7, 72%). Lutectomy tended to improve pregnancy rate in P+L(5-8; 6/11, 55%) compared to PGF(5-8; $p = 0.1$). Pregnancy rates tended ($p = 0.07$) to increase across daygroups in the PGF treatment (3/13, 23%; 5/10, 50%; and 6/10, 60% for Days 5-8, 10-13 and 15-18, respectively). Pregnancy rates did not differ between CON, PGF and P+L groups for Days 10-13 and 15-18. In conclusion, the most susceptible period of embryonic growth to the negative effects of $PGF_{2\alpha}$ appears to be during morula to blastocyst development. Removal of the luteal source of oxytocin diminishes the negative effects of

$PGF_{2\alpha}$ administration on the bovine embryo during early development, possibly through interruption of the luteal oxytocin-uterine $PGF_{2\alpha}$ feedback loop.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Pregnancy failure in beef cows is a vital concern to producers and consumers. For a cow to be profitable, she must produce and wean a calf annually. Embryonic loss results in the animal either not producing an offspring or causing a delay in the time of a "maintained" pregnancy resulting in the failure of weaning a calf every 365 days. In the United States, the net calf crop has been estimated to be between 65 to 81% (Bellows et al., 1979). Increasing the calf crop to 85% has been projected to produce an annual savings of $558 million in the United States (Gerrits et al., 1979). Furthermore, Inskeep and Peters (1982) estimated the cost of wintering nonpregnant cows to be $1.1 billion per year. Several factors influence conception rate including breed, age, season, length of postpartum anestrus, nutrition, management, lactation, and disease.

Although results have been variable in establishing the exact time of embryonic death in beef cattle, several studies have reported the majority of loss occurs prior to Day 18 after mating. According to Ayalon (1978), the majority of the loss occurs between Days 6-7 postestrus (the time period in which the embryo is developing from a morula

to a blastocyst). Maurer and Chenault (1983) observed that 67% of the mortality had occurred or was occurring by Day 8 of gestation. However, Roche et al. (1981) suggested a gradual loss occurs between Day 8 to 18 after mating.

For pregnancy to be established in cattle, the corpus luteum (CL) must be maintained beyond the time of normal luteolysis (Days 15-19). The presence of a live embryo in the horn ipsilateral to the CL capable of secreting conceptus proteins during the time of normal luteolysis in a nonpregnant animal is a necessity (for review see Bazer et al., 1986; Thatcher et al., 1986). Possible reasons for embryonic loss include failure of normal embryonic development (d 1-16), failure of embryo-derived signals initiating maternal recognition of pregnancy (d 16-24), or luteal dysfunction (d 5-parturition). Embryonic mortality in either of these periods may be associated with toxic factors in the uterine environment which destroy the embryo and/or the CL.

Following estrus, concentrations of $PGF_{2\alpha}$ decrease to basal levels with slight increases on Day 5 (as determined by PGFM) which are associated with metestrus bleeding in cattle (Kindahl et al., 1976). Elevated concentrations of

$PGF_{2\alpha}$ have been observed in association with thermal stress (Malayer et al., 1990; Dunlap and Vincent, 1971), uterine manipulation and irritation (typically encountered with artificial insemination and embryo transfer techniques; (Seguin et al., 1974; Roberts et al., 1975), and administration of oxytocin for milk letdown (Armstrong and Hansel, 1959; Farin and Estill, 1993).

Administration of $PGF_{2\alpha}$ will result in the release of oxytocin (OT) from the regressing CL (Flint and Sheldrick, 1982). This luteal oxytocin is believed to stimulate further release of uterine $PGF_{2\alpha}$, which then feeds back to stimulate further release of luteal OT, thus setting up a "positive-feedback loop" (for reviews see Flint et al., 1990; Silvia et al., 1991).

The first ovulation in the postpartum cow is associated with a decrease in the length of the luteal phase due to a premature release of $PGF_{2\alpha}$ (Ramirez-Godinez et al., 1981, 1982; Pratt et al., 1982; Cooper et al., 1991). In cows which were bred or had "good" embryos transferred to their uteri at their first postpartum ovulation, pregnancies were not maintained even with progesterone supplementation to replace the short-lived CL (Breuel et al., 1993b; Butcher

et al., 1992). Schrick et al. (1993) observed elevated luminal uterine concentrations of $PGF_{2\alpha}$ in cows with short luteal phases which was negatively correlated with embryo quality ($r=-.42$; $P < .07$). A rapid deterioration of embryo quality between Day 3 (Breuel et al., 1993b) and Day 6 (Schrick et al., 1993) in cows with short luteal phases suggests that the problem must be occurring after the embryo enters the uterus. From these findings in cows with short luteal phases, $PGF_{2\alpha}$ may be having a direct negative effect on embryonic survival in cows as suggested in other species: rabbit (Maurier and Beier, 1976), mice (Harper and Skarnes, 1972) and rats (Breuel et al., 1993a).

Elevated concentrations of $PGF_{2\alpha}$ have been observed in heifers up to Day 6 after mating (Schallenberger et al., 1989) and during maternal recognition of pregnancy (Days 16 and 19; Bartol et al., 1981). Buford et al. (1996) reported that administration of $PGF_{2\alpha}$ to normal, cycling beef cows from Days 4-7 or 5-8 after mating decreased pregnancy rates even with exogenous progestogen supplemented. Furthermore, removal of the regressing CL improved pregnancy rates to control values. Inhibition of $PGF_{2\alpha}$ with flunixin meglumine, a prostaglandin

endoperoxidase synthase inhibitor, was ineffective in increasing pregnancy rates in postpartum cows (Buford et al., 1996). However, lutectomy in combination with flunixin meglumine treatment increased pregnancy rates in postpartum cows (Buford et al., 1996). Therefore, the objective of this study was to determine the effect of elevated prostaglandin $F_{2\alpha}$ at different stages of embryonic development in the presence or absence of luteal oxytocin on establishment of pregnancy.

REVIEW OF THE LITERATURE

Endocrine Events of the Bovine Estrous Cycle

The bovine estrous cycle consists of four distinct phases: estrus, metestrus, diestrus and proestrus. Estrus is the result of increased estradiol-17β (E_2) which is produced by the ovulatory follicle and induces estrous behavior and the luteinizing hormone (LH) surge. During metestrus (d 1-5), the developing corpus luteum (CL) increases progesterone (P_4) concentrations with a slight increase in uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Kindahl et al., 1976). Diestrus (d 5-18) is associated with increased P_4 along with a maturing CL. Decreasing concentrations of P_4 and increasing concentrations of E_2 and $PGF_{2\alpha}$ are associated with proestrus (d 18 until subsequent estrus; as reviewed by Hansel and Convey, 1983).

Luteal Development and Progesterone

After ovulation of the dominant follicle caused by a surge of luteinizing hormone (LH), theca and granulosal cells luteinize to become small and large luteal cells, respectively (Niswender and Nett, 1988). Weight and size of the CL increase during metestrus and the first portion of diestrus and attains its mature size by Day 7 (Henricks et

al., 1972). A mature CL will reach 20-25 mm diameter in size and will produce P_4 concentrations of ≈6.0 ng/ml in serum. Luteal secretion of P_4 is essential throughout gestation for maintenance of pregnancy in cattle or compared to som other species, such as sheep, that do not require luteal function after Day 50 (Lauderdale, 1986; Niswender and Nett, 1988). In each case, E_2 must prime the reproductive tract, by inducing formation of P_4 receptors (Muldoon, 1980), to support the environment required for normal embryonic growth and development (Niswender and Nett, 1988). Three days prior to estrus, P_4 decreases exponentially with subsequent increases in E_2 (Wettemann et al., 1972) and a new estrous cycle begins.

Estrogen and Follicular Development

Estradiol exhibits a biphasic pattern during the estrous cycle. The first rise in serum E_2 (about d 8-10), from the dominant follicle of the first follicular wave (Ireland and Roche, 1983), reaches concentrations of 10 pg/ml. After atresia of the dominant follicle, E_2 decreases to basal levels (2-5 pg/ml). The second rise in E_2 occurs about 2-3 days prior to estrus (Hansel and Convey, 1983). This rise in E_2 serves to induce the formation of oxytocin

receptors in the uterine endometrium such that a "positive feedback loop" can occur between $PGF_{2\alpha}$ and oxytocin. For ovulation to occur, a decrease in P_4 and a decrease in E_2 (i. e., less than 1.0 ng/ml in serum) must occur, which induces a LH surge.

Biosynthesis and Secretion of Prostaglandins

Milvae (1986) reviewed several pathways in which prostaglandins are synthesized from arachidonic acid. These pathways include the cyclooxygenase pathway, which produces prostaglandins and thromboxanes, and the lipooxygenase pathway, which produces hydroxyacids and leukotrienes. Several pathways exist which can lead to the production of $PGF_{2\alpha}$, but the most energy efficient pathway is through cyclooxygenase pathway.

The primary precursor of all prostaglandins are fatty acids obtained from the diet or membrane lipids, mainly arachidonic acid which is converted from linoleic acid by desaturase enzymes (Moore, 1985). Prostaglandins are often increased during uterine manipulation and irritation, typically encountered with artificial insemination and embryo transfer techniques (Schallenberger et al., 1989; Seguin et al., 1974), heat stress (Malayer et al., 1990),

and in the short luteal phase cow (Ramirez-Godinez et al., 1981; Pratt et al., 1982; Cooper et al., 1991). New reports indicate cows with gram negative mastitis have elevated concentrations of milk $PGF_{2\alpha}$ and shortened interestrus interval (reviewed by Cullor, 1990).

Luteal Regression

Prostaglandin $F_{2\alpha}$ has been implicated as the primary luteolysin in cattle as well as many other ruminant animals (Goding, 1974). Prostaglandin $F_{2\alpha}$ must be released in a series of five to eight pulses to initiate luteolysis in cattle (Kindahl et al., 1976). These pulses appear just prior to the initiation of luteolysis (i.e., significant decreases in serum P_4). Transfer of uterine $PGF_{2\alpha}$ to the ovary has been proposed to occur by a countercurrent mechanism between the uteroovarin vein and ovarian artery (McCracken et al., 1972). McCracken et al. (1971) provided the first evidence of a countercurrent mechanism by observing that tritium-labeled $PGF_{2\alpha}$ infused into the ovarian pedicle was transferred from the uterine vein to the ovarian artery.

In order for spontaneous luteolysis to occur, the uterus requires a period of P_4 exposure (approximately 10 d;

Homanics and Silvia, 1988; Lafrance and Goff, 1988). This period is needed to allow for accumulation of lipid droplets in uterine epithelial cells and upregulation of the cyclooxygenase enzyme, stimulation of IP_3 turnover and pulsatile release of $PGF_{2\alpha}$ during spontaneous luteolysis. Furthermore, P_4 inhibits the ability of E_2 to stimulate OT receptors (as reviewed by Silvia et al., 1991). McCracken et al. (1984) suggested that luteolysis involves the formation of endometrial OT receptors induced by a pulse of E_2 such that a positive feedback loop can occur. Following P_4 exposure, the receptor for P_4 down regulates itself causing the formation of OT receptors and allowing for OT to enhance a pulsatile release of $PGF_{2\alpha}$.

The positive feedback between OT and $PGF_{2\alpha}$ causes the decline in P_4, growth of the ovulatory follicle and E_2 to increase. This increase in E_2 helps to drive luteolysis to completion by inducing the formation of additional OT receptors, stimulation of arachidonic acid turnover in phospholipid and triglyceride pools, stimulation of cyclooxygenase enzyme production and further release of $PGF_{2\alpha}$ (Raw et al., 1988; as reviewed by Silvia et al., 1991).

Elevated concentrations of $PGF_{2\alpha}$ may cause embryonic death through the actions of estrogen. Continued infusion or twice daily injections of $PGF_{2\alpha}$ increased size and number of large follicles in postpartum cows and ewes (Villeneuve et al., 1988, 1989). Villeneuve (1990) observed increased concentrations of E_2 following $PGF_{2\alpha}$ treatment of early postpartum cows. Therefore, caution must be used in determining the cause of embryonic loss associated with $PGF_{2\alpha}$ since elevated E_2 has also been reported to cause embryonic mortality in rats (Butcher, 1977), rabbits (Stormshak and Casida, 1965) and cows (Butcher et al., 1992).

A direct relationship between concentrations of 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM), a metabolite of $PGF_{2\alpha}$, and $PGF_{2\alpha}$ has been documented in cattle (Kindahl et al., 1976). However, Carver (1989) observed that concentrations of PGFM measured in jugular or the posterior vena cava did not accurately reflect the production of $PGF_{2\alpha}$ by the uterus or reproductive tract in cattle. However, measurement of PGFM is still considered the best indicator of peripheral $PGF_{2\alpha}$ concentrations.

Following estrus, concentrations of $PGF_{2\alpha}$ decrease to

basal levels with slight increases on Day 5 (as determined by PGFM concentrations) which is associated with metestrus bleeding in cattle (Kindahl et al., 1976). Schallenberger et al. (1989) also observed an increase in $PGF_{2\alpha}$ until Day 6 after estrus following artificial insemination compared to non-inseminated cyclic heifers.

Cellular Mechanisms of $PGF_{2\alpha}$

The receptor for $PGF_{2\alpha}$ has been cloned and its presence has been characterized throughout the estrous cycle in sheep and cattle (Rao et al., 1979; Graves et al., 1995; Wiltbank et al., 1995). The $PGF_{2\alpha}$ receptor is a G-protein coupled receptor containing seven hydrophobic transmembrane spanning regions which represent α-helices (Ostrowski et al., 1992). The $PGF_{2\alpha}$ receptor has been primarily localized to the large luteal cells of the CL (Balapure et al., 1989). Receptors for $PGF_{2\alpha}$ are present as early as Day 3 after estrus (Rao et al., 1979; Wiltbank et al., 1995) and increase in concentration and binding affinity throughtout the estrous cycle. However, the CL is non-responsive to administration of $PGF_{2\alpha}$ until Day 5 postestrus (Rawson et al., 1972). Furthermore, this effect does not appear to be due to a lack of high-affinity receptors for $PGF_{2\alpha}$ on the CL (Wiltbank et

al., 1995).

When $PGF_{2\alpha}$ binds to its receptor, phospholipase C (PLC) is activated and the receptor-$PGF_{2\alpha}$ complex is translocated to the plasma membrane (Wiltbank et al., 1990). $PGF_{2\alpha}$ increases phosphotidylinositol hydrolysis, specifically hydrolysis of phosphoinositol bisphosphate to inositol phosphate (IP_3) and diacylglycerol (DAG), within the luteal cell (Jacobs et al., 1991). DAG can act as a second messenger through activation of protein kinase C (PKC; Nishizuka, 1984). PKC may phosphorylate (and thereby activate) enzymes involved in liberating free arachidonic acid from intracellular storage pools to serve as a substrate for prostaglandin synthesis. Cytosolic free calcium is increased from the intracellular pool in the large luteal cells in response to $PGF_{2\alpha}$ (Wegner et al., 1990) and luteal oxytocin is released. Sustained intracellular concentrations of free calcium are cytotoxic in numerous cell systems (Rasmussen and Barrett, 1984) suggesting that the luteolytic effects of $PGF_{2\alpha}$ are mediated in this manner.

$PGF_{2\alpha}$ also exhibits antisteroidogenic activity in luteal tissue. $PGF_{2\alpha}$ blocks LH induced stimulation of

adenylate cyclase, thus reducing cyclic AMP (cAMP) concentrations in luteal tissues (Lahav et al., 1976). Henderson and McNatty (1977) suggested that this reduction in cAMP concentrations causes the cholesterol esterase enzyme to be converted from its active phosphorylated form to an inactive dephosphorylated form. The net effect of this step is a reduction of free cholesterol for P_4 biosynthesis by luteal cell mitochondria. Furthermore, mRNA for the LH receptor on the rat CL is decreased with the process of luteolysis (Bjurulf and Selstam, 1996). LH is the major luteotrophic hormone in the cow and ewe (Hansel et al., 1973; Niswender et al., 1981). LH is known to stimulate P_4 production by luteal tissues in vitro (Kaltenbach et al., 1967) and frequent injection of LH at mid-cycle prolonged the functional lifespan of the CL and lengthened the estrous cycle.

$PGF_{2\alpha}$ and Involvement with Oxytocin

Following a pulse of $PGF_{2\alpha}$, oxytocin (OT) is released from the secretory granules of large luteal cells of the CL (Fuchs, 1987). It is believed that OT (either from the CL and/or posterior pituitary) stimulates uterine release of $PGF_{2\alpha}$ which feeds back to stimulate release of luteal

oxytocin, which then stimulates the uterus to release additional $PGF_{2\alpha}$, thus a "positive-feedback loop" is established (for reviews see Flint et al., 1990; Silvia et al., 1991).

Oxytocin is considered to only play a passive role in luteolysis. Kotwica and Skarzynski (1993) reported heifers infused with noreadreneline (induces release of OT from the CL) on Days 11 and 12 and on Days 15 and 16 of their estrous cycle had increased release of OT with no effects on spontaneous luteolysis or estrous cycle duration. Therefore, ovarian OT may have a passive role rather than a direct action on luteolysis.

Intrauterine infusion of ovine trophoblast interferon-τ (IFN-τ; the major anti-luteolytic protein produced by the trophoblast during maternal recognition of pregnancy) to ewes between Days 11 and 15 of the estrous cycle reduced estrogen receptor mRNA, OT receptors and well as affinity of OT receptors for OT (reviewed by Bazer et al., 1994). Furthermore, endometrial OT receptors are low in pregnant versus cyclic cows during the luteolytic period (Jenner et al., 1991). This trophoblast protein likely inhibits synthesis of endometrial estrogen and OT receptors to slow

uterine production of luteolytic pulses of $PGF_{2\alpha}$.

Utilizing a frequent bleeding regimen in ewes, an endogenous pulse of $PGF_{2\alpha}$ occurred at an average of 17 minutes prior to a pulse of OT during luteolysis (Moore et al., 1986). Furthermore, OT concentrations in ovarian, utero-ovarian and jugular blood in sheep was increased following an injection of cloprostenol (a $PGF_{2\alpha}$ analogue; Flint and Sheldrick, 1983). Therefore, both endogenous and exogenous sources of $PGF_{2\alpha}$ will result in OT release from the regressing CL.

Cellular Mechanisms of Oxytocin

Oxytocin is synthesized and stored in the large luteal cells as well as the posterior pituitary of several ruminant animals (reviewed by Fuchs, 1988). When OT binds with its receptor in the uterine endometrium, activation of phosholipase C (PLC) causes metabolism of phosphotidylinositol phosphate to IP_3 and DAG. These second messengers enhance $PGF_{2\alpha}$ secretion by enhancing phospholipase A_2 (PLA_2) activity (which increases intracellular calcium concentrations) and providing an additional source of arachidonic acid by metabolism of DAG by DAG lipases, or through the action of PLC (Lafrance and

Goff, 1990).

Silvia and co-workers have dissociated the responsiveness of the PLC signal transduction system from OT-stimulated $PGF_{2\alpha}$ release. Silvia and Raw (1993) observed no rise in PLC activity from endometrial tissue from cycling sheep from Days 12, 14 and 16; however, a clear rise in the OT-stimulated release of $PGF_{2\alpha}$ occurred. Futhermore, Silvia et al. (1994) observed the dose of OT required to increase PLC activity was ten times more than needed to stimulate $PGF_{2\alpha}$ release. Therefore, OT-stimulated $PGF_{2\alpha}$ release must act through a mechanism other than phosphotidylinositol-specific PLC. Lee and Silvia (1994) demonstrated that a stimulator of PLA_2 (melittin) enhanced $PGF_{2\alpha}$ release comparable to that of OT. Aristocholic acid (a PLA_2 inhibitor) inhibited the rise in $PGF_{2\alpha}$ secretion by OT and PLA_2; however, blocking of PLA_2 only blocked OT-induced release of arachidonic acid by 22% (Lee and Silvia, 1994). Therefore, both PLA_2 and PLC appear to regulate $PGF_{2\alpha}$ release.

Early Embryonic Development

Ovulation and Oocyte Maturation

Following the LH surge and ovulation of the dominant follicle, the oocyte resumes meiosis and continues maturation so that fertilization can occur. The developing oocyte enters the oviduct where sperm capacitation, oocyte maturation, transport of gametes, fertilization and early cleavage of the embryo takes place (Ellington, 1991). The oviduct consists of four distinct regions: infundibulum, ampulla, isthmus and the ampullary-isthmic junction.

The oocyte undergoes the final stages of maturation in the ampulla before fertilization. After capacitation of the sperm in the isthmus, sperm penetrate the oocyte to form the zygote (i.e., less than two cell divisions). The zygote undergoes cleavage and development while traveling through the isthmus until the 8- to 16-cell stage, at which time the embryo is transported to the uterus (reviewed by Bazer et al., 1993).

The proper uterine environment is necessary for correct development of the embryo. Pregnancy rates in cattle that had embryos placed into their uteri with embryos -3, -2, -1, 0, +1, +2 and +3 days out of synchrony with the uterus were

0, 30, 52.2, 91.1, 56.5 40 and 20%, respectively (Rawson et al., 1972). Furthermore, Newcomb and Rawson (1975) reported that Day 3 embryos were too immature to survive in the uterus. Elevated concentations of E_2, $PGF_{2\alpha}$, and OT are possible reasons for accelerated rate of movement of embryos through the oviduct (Chang, 1966; Booth et al., 1975). Therefore, accurate synchrony between the embryo and uterus is necessary for survival and development of the early embryo.

Morula to Blastocyst Development

The 8- to 16-cell embryo enters the uterus approximately 96 hours after ovulation (Hamilton and Laing, 1946; Chang, 1952). During the first week of development, the embryo increases in cell numbers, a switch occurs from the maternal genome to the embryonic genome, and development of the three germ layers (endoderm, mesoderm, and ectoderm) is established. During morula development, the daughter cells (blastomeres) flatten on each other to form a rounded embryo and internal cellular components. The combined flattening and polarization are referred to as "compaction". Tight junctions form within the trophoblast when the blastomeres are in close contact during compaction. This

formation provides a permeability seal to allow fluids to move from the outside to the inside of the embryo as the result of solute gradient established by active ion transport (i.e. Na^+/K^+ ATPase pump). The accumulation of fluid induces the formation of the blastocoele and allows development of the blastocyst to form cells (inner cell mass) which give rise to the embryo proper and the trophectoderm and the trophoblast which forms the extraembryonic membranes (for review see Bazer et al., 1993).

Hatching of the Embryo

The developing blastocyst will be released (hatched) from the zona pellucida (ZP) in the uterus at about 8 to 11 days postovulation in cattle (Chang, 1952). Expansion and contraction of the blastocyst appears to play a major role in embryo hatching in cattle. Expansion of the blastocyst involves hyperplasia and fluid acculmulation in the blastocoele. The ZP becomes distended by the blastocyst and breaks in a equitorial plane; however, enzymes may also play an important role in ZP hatching. After the ZP ruptures, the blastocyst will squeeze between the two edges of the opening in the ZP (reviewed by Bazer et al., 1993). Biggers

et al. (1978) suggested that prostaglandins (in particular, the E series) are involved in the hatching process in mice.

Elongation Phase

The developing bovine blastocyst transforms from a 3 mm sphere on Day 13 to a 25 cm filamentous shape on Day 17. By Day 18, the embryo has entered both the ipsilateral and the contralateral horns of the uterus. This elongation has been suggested to be due to a cellular remodeling rather than a change in cell numbers (Geisert et al., 1982).

Maternal Recognition of Pregnancy

The next major feat the embryo must complete is to suppress uterine secretion of $PGF_{2\alpha}$, thus extending CL lifespan. The pulsatile secretion of $PGF_{2\alpha}$ around the time of expected luteolysis (Days 14-19) in pregnant versus cyclic cows has been examined in great detail (Thatcher et al., 1986, 1995).

To suppress uterine secretion of $PGF_{2\alpha}$, the conceptus must produce a luteotrophic factor to alert the mother of the presence of a viable embryo and extend luteal lifespan, thus termed "maternal recognition of pregnancy". This subject has received worldwide attention and has been the subject of many reviews (Bazer et al., 1986; Thatcher et

al., 1986; Roberts et al., 1992; Bazer et al., 1995). In ruminants, the trophoblast produces a protein called Interferon-τ (IFN-τ; also termed bovine and ovine trophoblast protein-1, bTP-1 and oTP-1, respectively). Interferon-τ is classified as a Type I interferon due to its similarity in structure and anti-viral activity of other interferons (Roberts et al., 1992). Interferon-τ has been characterized (Godkin et al., 1982) and is synthesized specifically by the trophectoderm from Days 16-24 and 13-21 of pregnancy in cattle and sheep, respectively (Godkin et al., 1984; Roberts et al., 1992). Godkin et al. (1982) reported the presence of high-affinity receptors for IFN-τ on sheep endometrial tissue, specifically on the luminal epithelial cells and on the outer glandular epithelial cells (the two major sources of $PGF_{2\alpha}$).

IFN-τ reduces prostaglandin secretion by inducing an intracellular endometrial inhibitor which inhibits the cyclooxygenase enzyme complex (Gross et al., 1988) such that the luteolytic pulses are not expressed during expected luteolysis. Concentrations of $PGF_{2\alpha}$ in the uterine lumen are high on Days 16 and 19 of pregnancy (Bartol et al., 1981). Thus, it seems unlikely that $PGF_{2\alpha}$ is detrimental to

embryos as old as 14 days of age.

Early Embryonic Mortality

The Committee on Reproductive Nomenclature (1972) defines embryonic mortality as any loss which occurs during the first 42 days of pregnancy (i.e., period from conception to completion of differentiation). Possible reasons for embryonic loss include failure of normal embryonic development (d 1-16), embryo-derived signals initiating maternal recognition of pregnancy (d 16-24) or luteal dysfunction (d 5-parturition). Embryonic loss in either of these time periods may involve toxic factors in the uterine environment which destroy the embryos and/or the CL.

Fertilization rates in cattle are accepted to be close to 90 to 95%, but diagnosed pregnancy rates are between 50 and 60% (Sreenan and Diskin, 1983). The majority of the studies completed on embryonic or fetal loss have shown the majority of the loss occur during the embryonic period. Boyd et al. (1969) reported only a 5-8% fetal loss from Day 42 until parturition.

Several studies have been performed to establish the exact timing in which embryonic loss actually occurs. Embryonic loss has been estimated to be between 20 (Ayalon,

1978) and 42% (Diskin and Sreenan, 1980). However, results concerning the timing of embryonic loss have been variable and very inconsistent. For example, Boyd et al. (1969) reported an 8% loss before 25 days after artificial insemination, whereas Roche et al. (1981) reported an embryonic loss of 24% over the same time period. Maurer and Chenault (1983) observed that 67% of the mortality has occurred or was occurring by Day 8 of gestation. Furthermore, Ayalon (1978) reported that the majority of embryonic loss occurred before Day 8 postestrus, with Day 7 being the period with the majority of the loss. However, Diskin and Sreenan (1980) reported the majority of the loss occurs between Day 16 and 18 of pregnancy. To reconcile these differences, Roche et al. (1981) suggested that a gradual loss occurs between Day 8 and 16 of pregnancy.

Prostaglandin Effects on the Embryo

Prostaglandins have been implicated in both growth of the embryo and the implantation process in rabbits (El-Banna et al., 1976; Hoffman et al., 1978). Rabbits treated with indomethacin, a cyclooxygenase inhibitor, did not maintain pregnancy which suggests the importance of prostaglandins for this species (Hoffman et al., 1978). Furthermore,

treatment of hamsters, mice and rats with indomethacin disrupted implantation (Evans and Kennedy, 1978; Oettel et al., 1979).

However, Harper and Skarnes (1972) suggested that elevated concentrations of $PGF_{2\alpha}$ increased the number of fetal deaths and treatment with P_4 prevented abortion, but not fetal death in mice. Furthermore, treatment of rat embryos with .1-10 ng/ml of $PGF_{2\alpha}$ decreased blastocyst formation and hatching (Breuel et al., 1993a). Likewise, Stormshak and Casida (1965) reported that treatment with P_4 prevented abortion but not fetal death when E_2 was used to rescue the CL from LH-induced luteolysis in rabbits.

As documented by Lewis (1989), the blastocyst of several species (porcine, ovine, bovine and laboratory animals) secretes $PGF_{2\alpha}$ when incubated with arachidonic acid in vitro. $PGF_{2\alpha}$ from the conceptus is involved in intrauterine migration, ZP hatching, ion transport across the trophectoderm, fluid accumulation in the blastocoele, increase endometrial permeability and glucose metabolism by the blastocyst.

The Postpartum Cow as a Model

The first ovulation in the postpartum cow is associated

with a short lived CL due to a premature release of $PGF_{2\alpha}$ (Ramirez-Godinez et al., 1981, 1982; Pratt et al., 1982; Cooper et al., 1991). Cows bred at their first postpartum ovulation were not able to maintain pregnancy even when supplemented with exogenous progestogen (Breuel et al., 1993b). Furthermore, Breuel et al. (1993b) reported the occurrence of ovulation, fertilization and normal embryonic development to Day 3 postmating did not differ in cows with normal or short estrous cycles. Butcher et al. (1992) reported that when two "good" embryos were transferred into uteri of cows with short luteal phases supplemented with exogenous progestogen, pregnancy rates were 50% lower when compared to cows with normal luteal phases. Furthermore, Schrick et al. (1993) reported that Day 6 embryos from cows with short luteal phases were capable of producing pregnancies when transferred to cows with normal luteal phases.

Uterine luminal concentrations of $PGF_{2\alpha}$ were higher in cows with short luteal phases when compared to cows with normal luteal phases (Schrick et al., 1993). Those cows with higher concentrations of $PGF_{2\alpha}$ had lower quality embryos ($r=-.42$; $P = .07$). The significant deterioration in

embryo quality between Day 3 (Breuel et al., 1993b) and Day 6 (Schrick et al., 1993) in cows with short luteal phases suggests that the problem is likely after the embryo enters the uterus. As stated previously, culture of rat embryos with 0.1-10 ng/ml $PGF_{2\alpha}$ prevented development to the expanded or hatched blastocyst (Breuel et al., 1993a).

Buford et al. (1996) reported that postpartum cows (≈28 days) supplemented with exogenous progestogen and administered saline, flunixin megulmine (FM; a cyclooxygenase inhibitor), or FM + lutectomy (lutectomy performed on Day 6) from Days 4 to 9 after mating had pregnancy rates of 21, 27 and 53%, respectively ($P<.05$ for saline and FM vs FM+lutectomy). The surpise finding that lutectomy increased pregnancy rate suggested that the regressing CL was required for the embryotoxic effect of $PGF_{2\alpha}$.

The Cyclic Cow

Cattle supplemented with exogenous progestogen and administered either saline, 15 mg of $PGF_{2\alpha}$, or 15 mg $PGF_{2\alpha}$+lutectomy (lutectomy was performed on Day 5) every 8 hours from Days 5-8 had pregnancy rates of 67, 22 and 80%, respectively (Buford et al., 1996). Lutectomy prevented the

negative effects of $PGF_{2\alpha}$ in both early postpartum and normally cycling cows. Concentrations of PGFM did not differ between cows administered $PGF_{2\alpha}$ and $PGF_{2\alpha}$ + lutectomy. Furthermore, lowered embryo survival during luteolysis were not due to increased $PGF_{2\alpha}$ *per se*, but appears to be manifested through the regressing CL. From these data, even subluteolytic concentrations of $PGF_{2\alpha}$ (Schramm et al., 1983) could play a role in embryonic loss during the early period of embryonic development via release of an embryotoxin (possibly luteal OT) from the CL. Therefore, the removal of the luteal source of OT diminishes the negative effects of $PGF_{2\alpha}$ on the developing embryo in cattle.

STATEMENT OF THE PROBLEM

Reports concerning the timing of embryonic loss have been variable and very inconsistent. Ayalon (1978) reported the majortiy of embryonic loss had occurred or was occurring by Day 8. In contrast, Diskin and Sreenan (1980) reported the majority of embryonic loss occurred from Days 16 to 18. To reconcile these differences, Roche et al. (1981) suggested that a gradual loss occurs between Days 8 and 16.

The first ovulation in the postpartum cow is associated with a short luteal phase due to a premature release of $PGF_{2\alpha}$ (Ramirez-Godinez et al., 1981, 1982; Pratt et al., 1982; Cooper et al., 1991). Previous experiments suggest the presence of a regressing CL is required to manifest the negative effects of increased concentrations of $PGF_{2\alpha}$ (Butcher et al., 1992; Breuel et al., 1993b; Schrick et al., 1993; Buford et al., 1996) in the postpartum cow as well as normally cycling cattle. Furthermore, treatment of rat embryos with 0.1-10 ng/ml $PGF_{2\alpha}$ prevented formation of expanded or hatched blastocysts as well as formation of the morula stage (Breuel et al., 1993a). Therefore, increased concentrations of $PGF_{2\alpha}$ during early embryonic development, possibly mediated through OT, may reduce pregnancy rates by

a direct action of $PGF_{2\alpha}$ (retarded embryonic development) or an embryotoxic factor from the regressing CL (luteal OT).

Few reports have been found on the effects of $PGF_{2\alpha}$ on older embryos (Day 10 until maternal recognition of pregnancy) and if lutectomy would alleviate the effects (if any) of elevated concentrations of $PGF_{2\alpha}$. To test this hypothesis, an experiment was conducted to examine the role of luteal regression on embryonic survival during three periods of embryonic development in cattle supplemented with exogenous progestogen. The objective was to determine if embryo survival in the presence of luteolytic concentrations of $PGF_{2\alpha}$ differs with developmental age of the embryo and if presence of the CL is required to manifest the effects of $PGF_{2\alpha}$ on embryo survival.

MATERIALS AND METHODS

General Methods

Ninety-one primiparous and multiparous cycling, nonlactating cows (3 replicates) were randomly allotted to receive saline (CON), 15 mg of $PGF_{2\alpha}$ (PGF), or 15 mg of $PGF_{2\alpha}$ + lutectomy (P+L). Cows were further assigned to one of three periods of embryonic development; Days 5 through 8, Days 10 through 13 or Days 15 through 18 after mating using a 3 X 3 factorial treatment arrangement (Figure 1).

All cows were in moderate body condition (Spitzer et al., 1986) and estrus was synchronized with two injections of 25 mg of $PGF_{2\alpha}$ ten days apart (im, Lutalyse, The Upjohn Company, Kalamazoo, MI). Cows were observed for estrus twice daily and two Polled Hereford bulls of known fertility were rotated twice daily for maximal detection of estrus. Cows were artificially inseminated with semen from a single Holstein bull of high fertility at estrus (Day 0) and bred by natural service to Polled Hereford bulls. Furthermore, cows were artificially inseminated again twelve hours after first observed signs of estrus to remove any "male" effect from the current study. All cows were fed 4 mg/day of melengestrol acetate (MGA) in the a.m. beginning 2 days

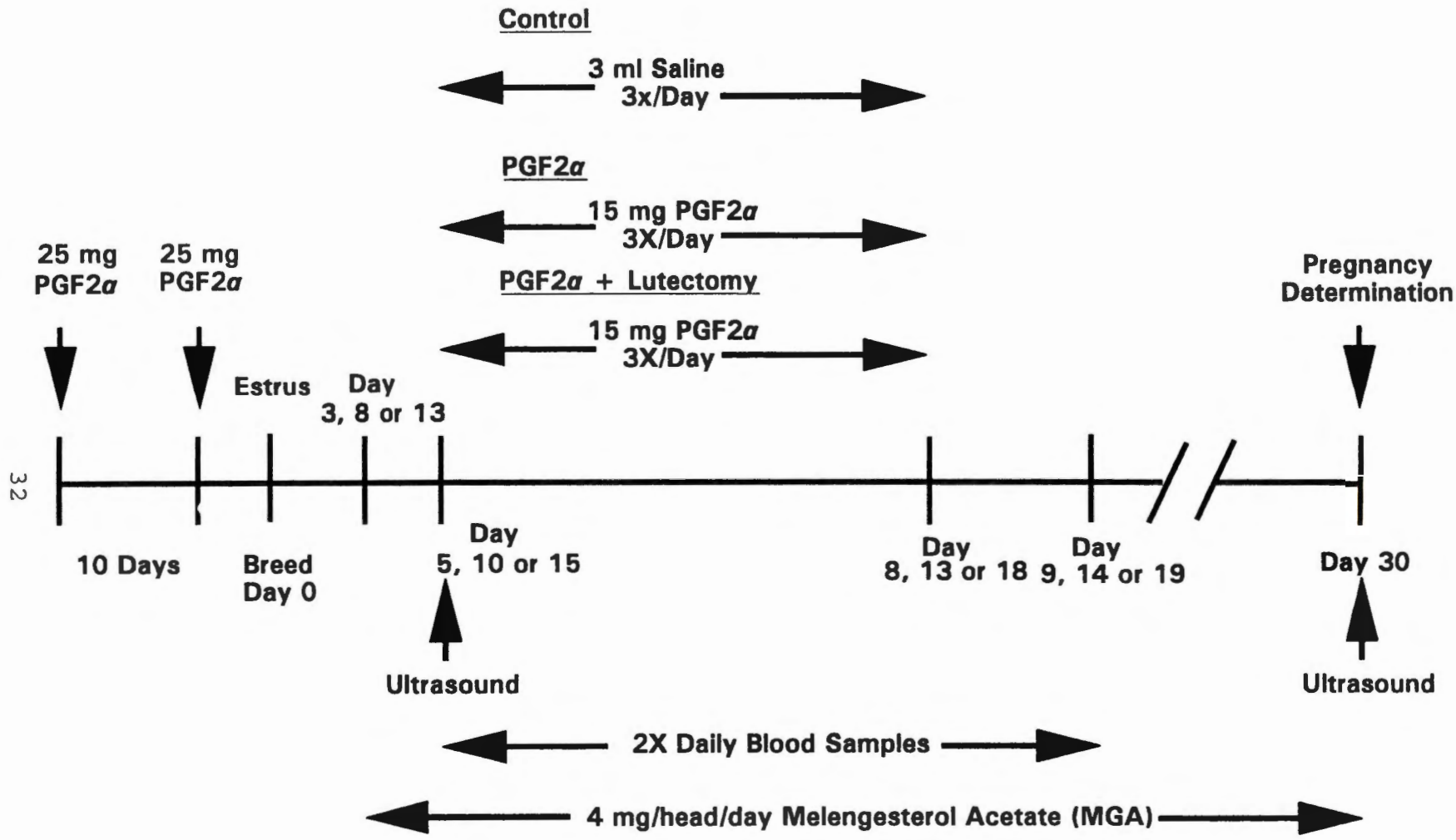

Figure 1: Design of the Experiment

before initiation of their respective treatments (ie, Day 3, 8 and 13 for days 5-8, 10-13, and 15-18, respectively) and continued until Day 30 after mating. Cows were allowed ad libitum access to bermuda grass pasture, orchardgrass hay and water. Furthermore, non-treated feed was also fed in the p.m. to provide supplemental energy and to stimulate appetite. The non-treated and the MGA-treated feed (1 mg MGA/lb feed) was in pelleted form and was purchased from Tennessee Farmers Coop (see appendix 1 for exact composition of feed).

Cows in the CON group received injections of 3 ml sterile saline each day at 8 hour intervals (0600, 1400, and 2200 h) during their respective treatment periods. Cows in PGF and P+L received intramuscular injections of 15 mg $PGF_{2\alpha}$ every 8 hours during their respective treatment periods. Lutectomies were performed during rectal palpation in 9/22 CON cows(4, 3 and 2 in Days 5-8, 10-13 and 15-18, respectively) and all cows in the P+L group at the initiation of treatment. Lutectomies were performed in these CON cows to verify that 4 mg/cow/d of MGA could sustain a pregnancy. Lutectomies were performed transrectally at 0600 on the first day of treatment prior to

injection. The CL was removed by using digital pressure with the thumb and forefinger and dropped into the abdominal cavity. Verification of CL loss was determined by ultrasonography at the time of lutectomy, progesterone and oxytocin assays. All animals were intensively observed for blood loss by monitoring changes in respiratory rate, evidence of depression, feed refusal, abdominal pain and mucous membrane color of the vulva every 8 hours for 24 hours following lutectomy. If evidence of possible blood loss was apparent, then blood was drawn for determination of packed-cell volume (PCV) count. No cows were removed due to symptoms of blood loss. Pregnancy rates were determined by ultrasonography 30 days after mating.

In a secondary study, eleven (11) cycling, nonlactating cows which had exhibited at least two normal estrous cycles were randomly allotted to one of three groups. The objective of the second experiment was to determine concentrations of uterine $PGF_{2\alpha}$ by flushing the uteri of progestogen-supplemented cows. Treatments included saline (CON; n=3), 15 mg of $PGF_{2\alpha}$ (PGF; n=5), and 15 mg of $PGF_{2\alpha}$ + Lutectomy (P+L; n=3). All cows were treated as previously described from Days 5 through 7 after mating (Figure 2),

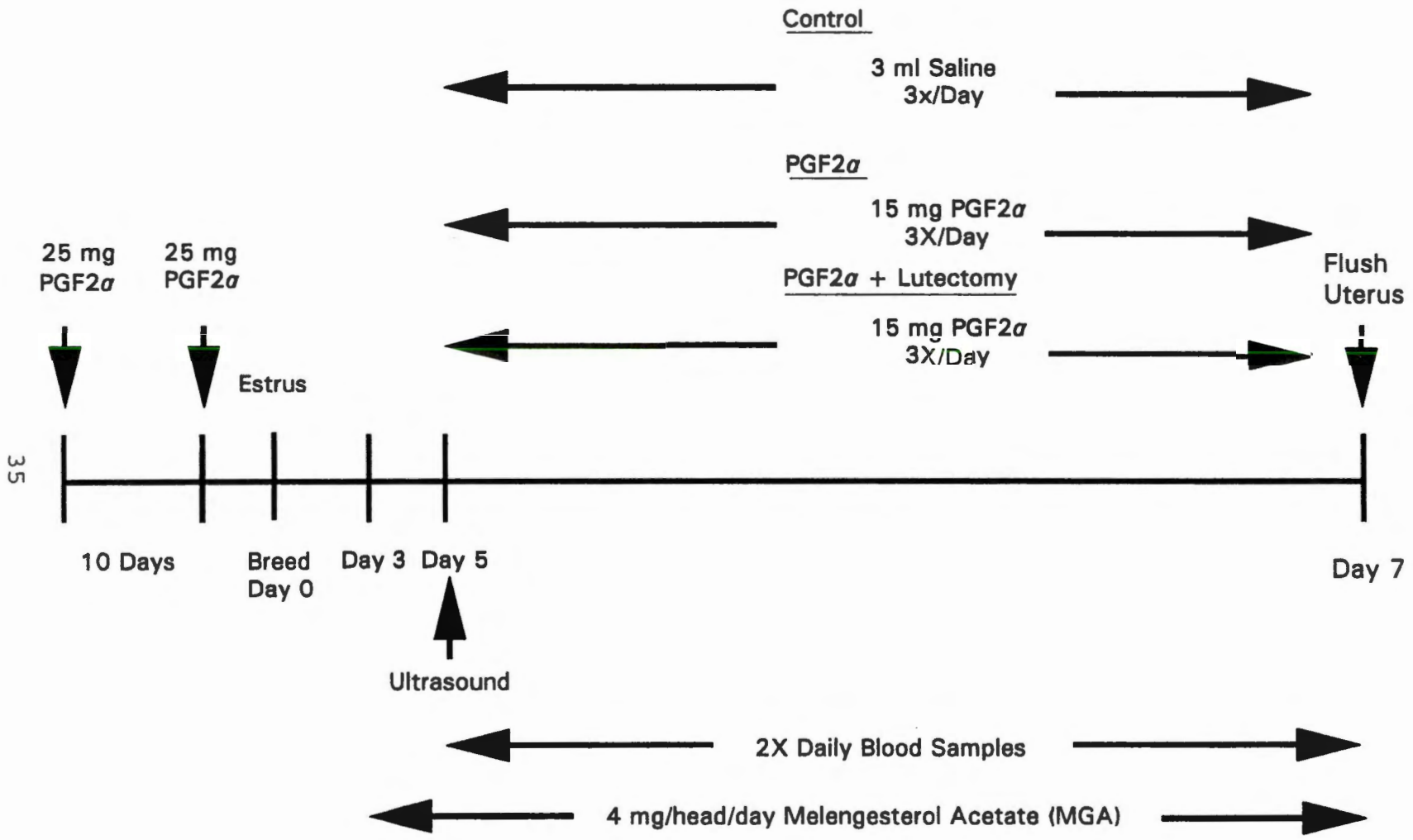

Figure 2: Design of Secondary Experiment

which corresponds to the stage when the embryo is developing from a morula to a hatched blastocyst.

Synchronization and detection of estrus, breeding and feeding of all cows was performed as in the previous experiment. All cows were fed 4 mg/day of melengestrol acetate (MGA) in a.m. beginning on Day 3 after mating and continued until Day 7 after mating.

On Day 7 at 1000, a jugular blood sample was collected and the uterine horn ipsilateral to the CL of all cows was flushed with 500 ml of Dulbecco's phosphate buffered saline (Gibco, Inc., Grand Island , NY) as described by Elsden and Seidel (1982). Fifteen ml of flush medium was collected from each cow and were centrifuged at 2500 X g to remove debris present in the flush media. In analyses of concentrations of uterine $PGF_{2\alpha}$, only data from cows with recovery scores of 1 or 2 were utilized (Schrick et al., 1993). Recovery score refers to the ease of insertion of the Foley catheter into the uterus and the degree of difficulty the uterus had to evacuated of the media at the time of flushing.

Ultrasonography

Ultrasonograms were performed with an Aloka 500

ultrasound unit equipped with a 7.5 MHz rectal probe (Corometrics Medical Systems., Wallingford, CT; Model UST-556I-7.5). Ultrasonography was used to verify loss of the CL at the time of lutectomy and establishment of pregnancy (presence of an embryo with a visible heartbeat) in all cows 10 days after mating.

Blood Preparation and Radioimmunoassay

Approximately 12 ml of jugular blood was collected at 0600 and 2200 prior to injection and at 30 min and 1 hour after the initial injection of $PGF_{2\alpha}$/saline and placed in chilled tubes containing 200 μl of sodium citrate solution (25 mg/ml; Baxter Scientific Products). Blood and uterine flush samples were immediately placed on ice until arrival at the Endocrine Laboratory. Blood samples were centrifuged (2500 X g), plasma was harvested and both uterine flush and plasma samples were stored at -20°C until assays were performed. Plasma samples were assayed for determination of concentrations of progesterone (P_4), estradiol-17β (E_2; 2 reps of experiment one),and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM). Furthermore, plasma from samples collected at time 0 (initial injection), 30 min and 1 hour were assayed to determine concentrations of oxytocin and P_4 (verification of

complete lutectomy). Additionally, uterine flush samples were assayed for $PGF_{2\alpha}$.

Progesterone

Concentrations of P_4 in plasma were determined as described by Plata et al. (1990). Sensitivity of the assay was .05 ng/tube (100 μl), with intra- and inter-assay coefficients of variation of 8.6 and 13.6%, respectively.

Estradiol-17ß

Concentrations of E_2 in plasma were determined by the procedure by Howard et al. (1990). Sensitivity of the assay was .15 pg/tube (150 μl), with an intra-assay coefficient of variation of 6.5%.

13,14-dihydro-15-keto-$PGF_{2\alpha}$

Concentrations of PGFM in plasma were determined as described by Silvia and Taylor (1988) with modifications described by Homanics and Silvia (1989). Sensitivity of the assay was 15 pg/tube (200 μl), with intra- and inter-assay coefficients of variation of 10 and 12%, respectively.

Prostaglandin $F_{2\alpha}$

Concentrations of $PGF_{2\alpha}$ in the uterine flush samples were determined by the procedure of Cooper et al. (1991). Sensitivity of the assay was 25 pg/tube (200 μl), with an

intra-assay coefficient of variation of 3%.

Oxytocin

Concentrations of OT in plasma samples were determined by the procedure of Gorewit (1979) with modifications described by Cooper et al. (1991). Sensitivity of the assay was 3 pg/tube (100 μl), with intra- -assay coefficient of variation of 3%.

Statistical Analysis

Pregnancy data were analyzed using Chi-square test. Changes in concentrations of plasma P_4, E_2, OT and PGFM were analyzed by analysis of variance for a RBD split-plot design with treatment as the main plot and time as the subplot variable using the MIXED procedure of SAS (1989). Effects of treatment were considered significant when $P < .05$.

RESULTS

Pregnancy Rates

Pregnancy rates did not differ between CON and C+L, therefore, these animals were combined into one CON group per daygroup. Pregnancy rates were significantly reduced in the PGF(5-8) groups when compared to CON (3/13, 23% and 5/7, 72%, respectively; $P < .03$). Lutectomy tended to increase pregnancy rates in P+L(5-8) group (6/11, 55%) compared to PGF(5-8) animals (3/13, 23%; $P = .1$). Furthermore, pregnancy rates tended to increase across daygroups in the PGF treatment groups (3/13, 23%; 5/10, 50%; and 6/10, 60% for Days 5-8, 10-13 and 15-18, respectively; $P = .07$; Figure 3). Pregnancy rates did not differ between any other treatment*daygroup interactions.

Concentrations of E_2, P_4, PGFM and OT

Differences in E_2, P_4 and PGFM did not occur between CON and C+L except for concentrations of P_4, therefore, these animals were combined into one CON group. The initial P_4 value collected for each daygroup was significantly higher when compared across daygroups ($1.2 \pm .4$, $3.9 \pm .3$ and $5.5 \pm .4$ for Days 5-8, 10-13 and 15-18, respectively; $P < .05$). Concentrations of P_4 were significantly reduced by

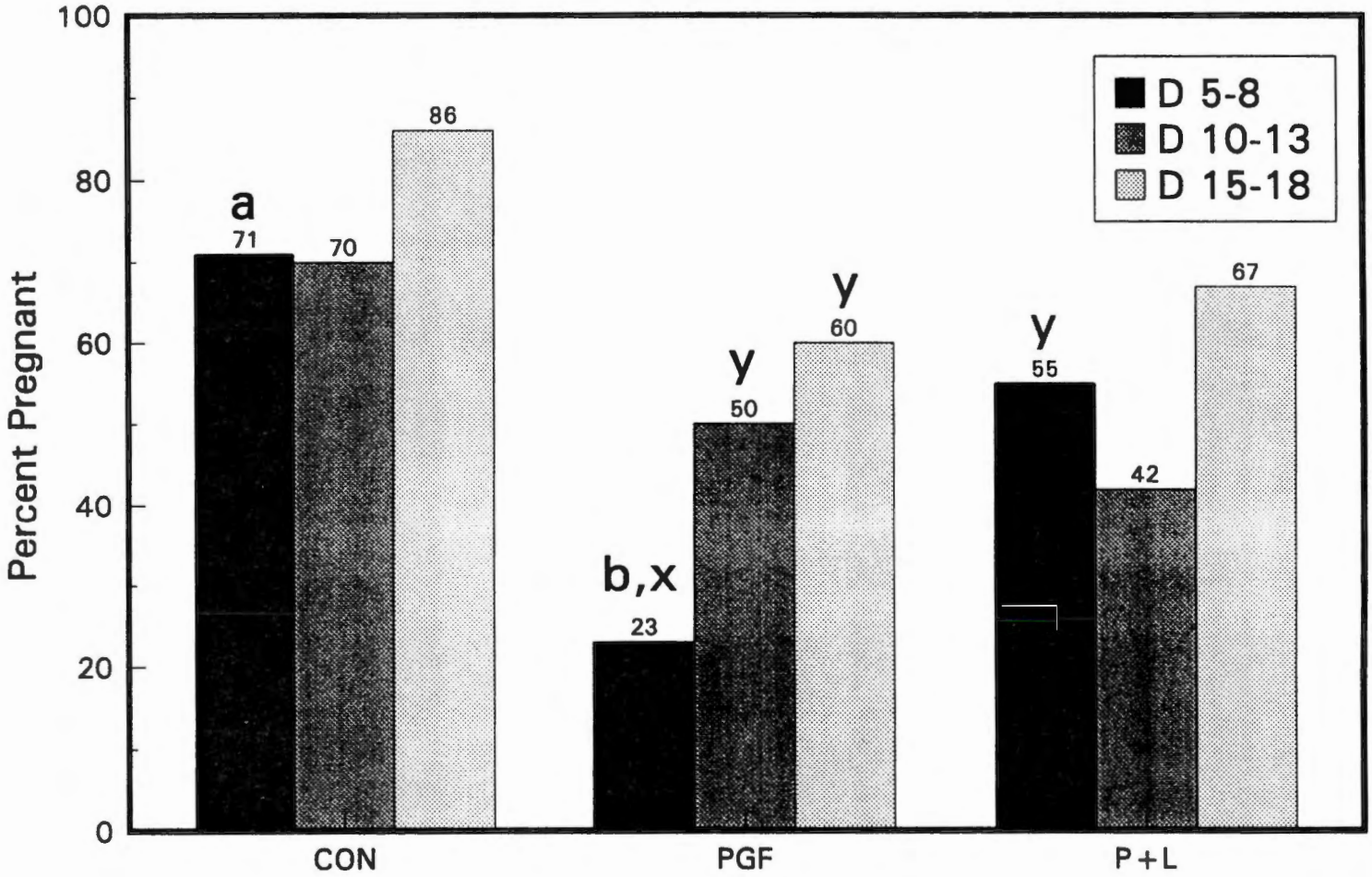

Figure 3: Pregnancy rates in Experiment 1 at 30 d postestrus. CON=Control, PGF=$PGF_{2\alpha}$, P+L=$PGF_{2\alpha}$ + Lutectomy. ^{a,b}Treatments differ ($P < .05$) when letters above the bars are different. ^{x,y}Treatments tend to differ ($P < .1$) when letters above the bars are different.

2200 of the first day of treatment in cows administered C+L, PGF and P+L when compared to the 0600 sample. No differences were observed in concentrations of E_2 between any treatment, daygroup or any of treatment*daygroup interactions except for Days 10-13 (Figure 4). Concentrations of E_2 for CON, PGF and P+L in the Day 10-13 daygroup were 2.8 ± .4, 3.7 ± .4 and 3.3 ± .4 pg/ml, respectively ($P < .05$).

Mean concentrations of PGFM were significantly elevated in animals receiving PGF and P+L treatments (398 ± 23 and 413 ± 22 pg/ml, respectively) when compared to CON animals (80 ± 29 pg/ml; $P < .01$; Figure 5). No differences were observed between PGF and P+L across daygroups. Furthermore, PGFM concentrations did not different between the first sample collected between the PGF and P+L across all daygroups (79 ± 43 and 92 ± 43 pg/ml for PGF and P+L, respectively). Also, no differences in PGFM concentrations were observed 24 hours following the last administration of $PGF_{2\alpha}$ (94 ± 42 and 119 ± 42 pg/ml for PGF and P+L, respectively). Therefore, endogenous $PGF_{2\alpha}$ did not appear to be released before the first injection and after the last injection.

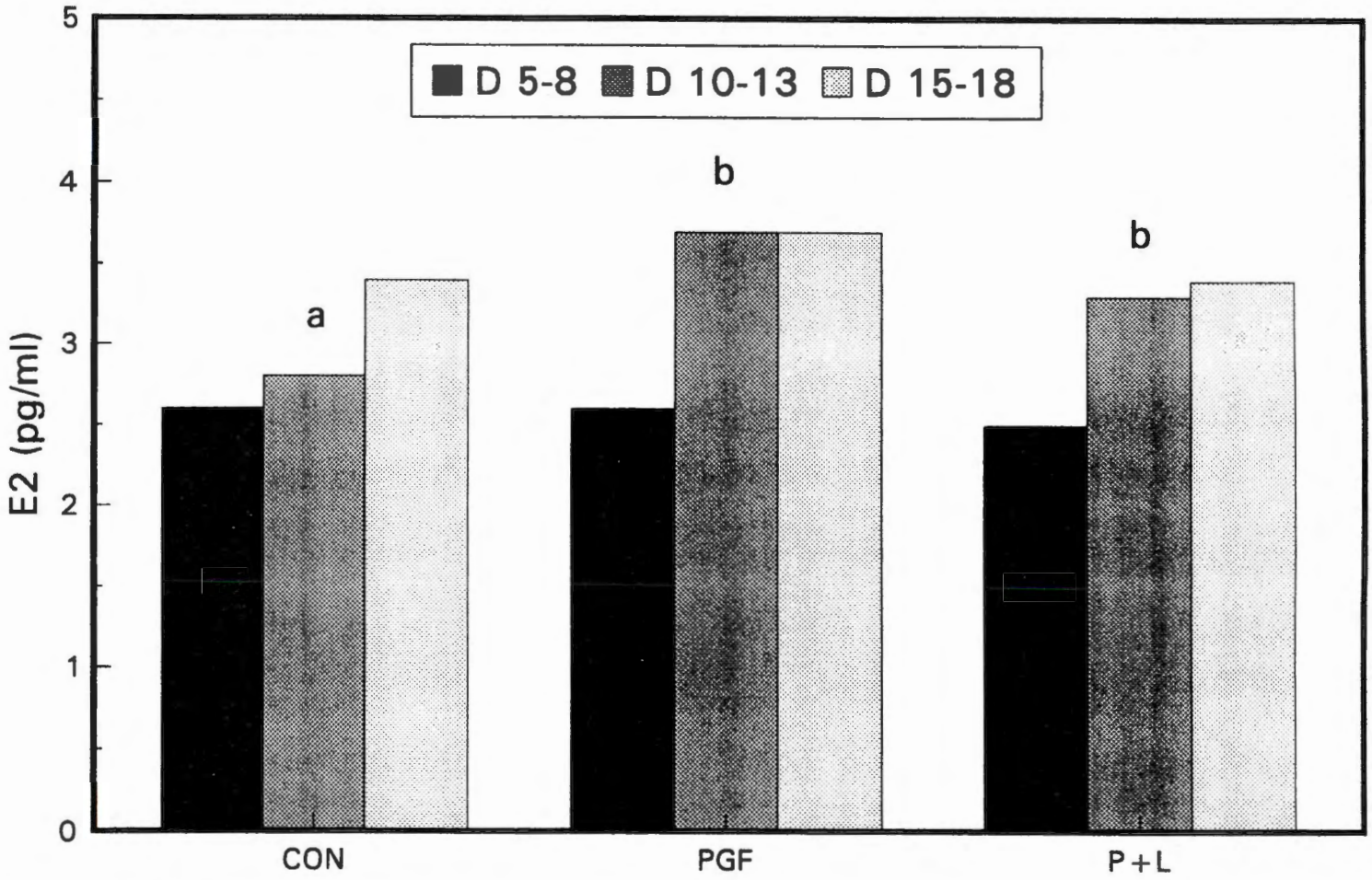

Figure 4: Mean Estradiol concentrations in Experiment 1 over the treatment periods. [a,b]Treatments differ (P < .05) when letters above the bars are different.

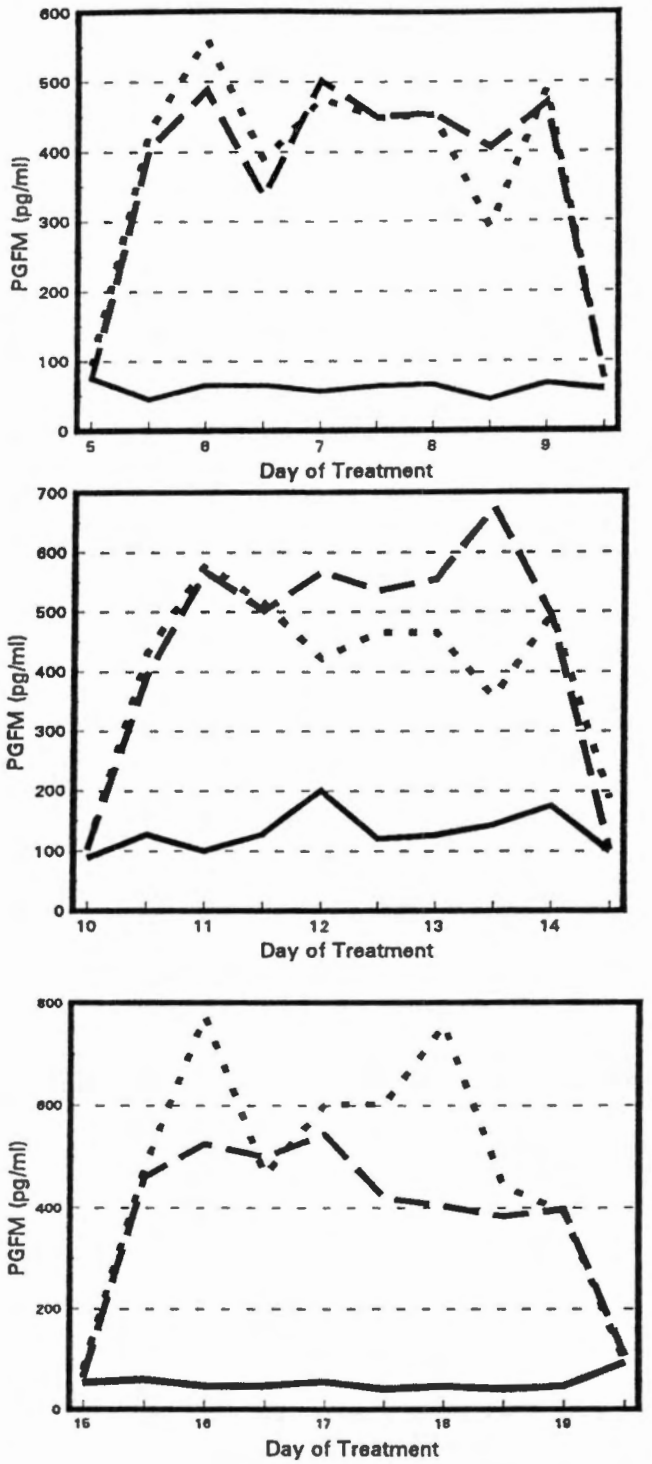

Figure 5: Mean concentrations of PGFM for Days 5-8 (Panel A), 10-13 (Panel B), and 15-18 (Panel C) for Experiment 1. Pooled SEM = 80 pg/ml. CON (———), PGF (— — —), and P+L (• • • • • •).

Oxytocin secretion thirty minutes after initiation of treatment was increased in intact cows (PGF vs. CON; $P < .07$ for Days 5-8; $P = .0001$ for Days 10-13 and 15-18). Furthermore, this increase was prevented by lutectomy (P+L vs. PGF; $P < .05$ for Days 5-8; $P = .0001$ for Days 10-13 and 15-18; Figure 6).

Secondary Experiment

Concentrations of P_4 and PGFM

Concentrations of P_4 were significantly reduced in animals administered PGF and P+L by 2200 on the first day of treatment when compared to the first sample collected on Day 5 (1.9 ± .2 vs .5 ± .2 and 2.1 ± .3 vs .3 ± .3 ng/ml for PGF and P+L, respectively; $P < .01$).

Mean concentrations of PGFM were significantly higher in cows administered PGF and P+L treatments (1368 ± 219 and 1443 ± 233 pg/ml, respectively) when compared to CON cows 46±248 pg/ml; $P < .01$). Furthermore, mean concentrations of PGFM immediately prior to flushing was significantly higher in cow receiving PGF and P+L treatments (2836 ± 287 and 1673 ± 335 pg/ml, respectively) when compared to CON cows (21 ± 385 pg/ml; $P < .001$). Also, mean concentrations of PGFM immediately prior to flushing were significantly

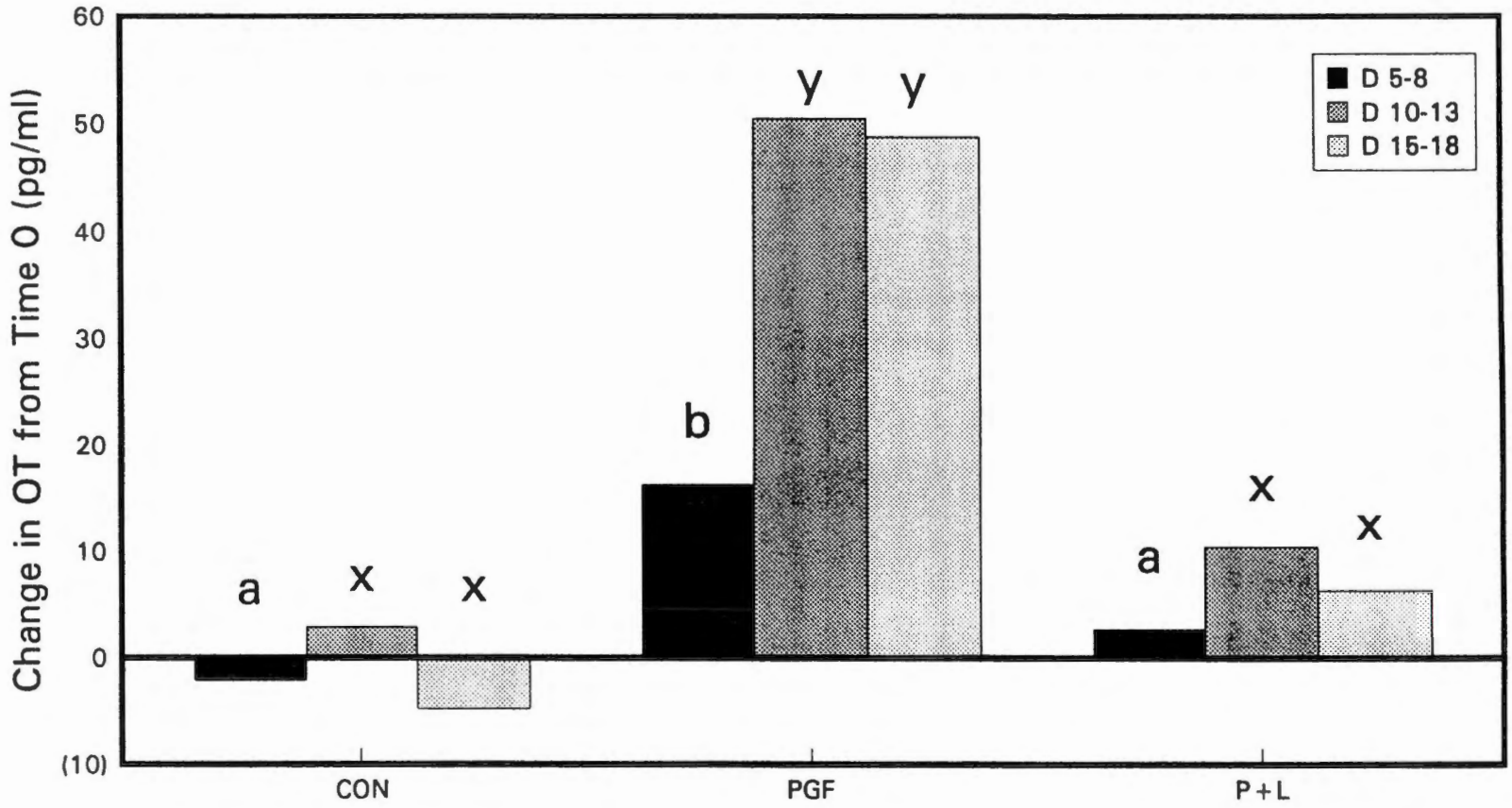

Figure 6: Mean change in oxytocin concentrations in Experiment 1. [a,b]Treatments tend to differ (P < .07) when letters above the bars are different. [x,y]Treatments differ (P = .0001) when letters above the bars are different. Pooled SEM = 5.7 pg/ml.

higher in the PGF group when compared to the P+L group (P = .001). Furthermore, mean concentrations of $PGF_{2\alpha}$ in uterine flush samples were 172 ± 62, 201 ± 41, and 207 ± 53 pg/ml for CON, PGF and P+L, respectively (P > .6).

DISCUSSION

The objective of this study was to determine if embryo survival in the presence of luteolytic concentrations of $PGF_{2\alpha}$ differed with developmental age of the embryo and if the presence of the CL was required to manifest the effects of $PGF_{2\alpha}$ on lowered embryonic survival. The results from this study indicate the embryo developing from a morula to a expanded blastocyst (Days 5-8) is the most susceptible to the negative effects of $PGF_{2\alpha}$. Furthermore, removal of luteal oxytocin tends to increase pregnancy rates within this daygroup. Moreover, the embryo becomes less susceptible to the effects of $PGF_{2\alpha}$ as the embryo ages. This finding was expected since uterine concentrations of $PGF_{2\alpha}$ are known to be elevated from Days 16 and 19 of pregnancy (Bartol et al., 1981) which suggests as the embryo gets older it becomes more resistent to the detrimental effects of $PGF_{2\alpha}$. The results from this experiment as well as data from Buford et al. (1996) strongly suggest the regressing CL is required to manifest the negative effects of elevated concentrations of $PGF_{2\alpha}$ on lowered embryonic survival in cattle from Days 5-8 of pregnancy.

Several explanations are plausible to explain why

bovine embryos become resistent to $PGF_{2\alpha}$ as they age. Keefer and Rice (1992) reported that co-culture of 1 cell bovine embryos with 4-8 cell bovine embryos improved the blastocyst formation in the cell embryos. Thus, these "helper" or older embryos may produce a factor that is beneficial to younger developing embryos. Furthermore, Hernandez-Hedezma et al. (1992) reported that bovine embryos begin producing IFN-τ or bTP-1 as early as the expanded to hatched blastocyst stage of development. IFN-τ is the anti-luteolytic protein produced by the trophoblast to prevent pulsatile release of $PGF_{2\alpha}$.

Several mechanisms could be involved in the reduction of embryonic survival in the presence of elevated concentrations of $PGF_{2\alpha}$. First, the CL may be releasing a embryotoxic factor during luteolysis. Several products are released from the regressing CL including one (Smith et al., 1993, 1994) or two (Juengel et al., 1994) tissue inhibitors of metalloproteinases-1 and -2, tumor necrosis factor-α (TNFα; McIntush et al., 1996), interleukins , interferon-γ (as reviewed by Pate, 1995) and oxytocin (Flint et al., 1990).

At the initiation of luteolysis, several immune cells

including macrophage and neutrophils invade the CL and release several products. These products include TNF-α and several interleukins, which stimulate $PGF_{2\alpha}$ production (Pate, 1995) by *in vitro* cultured bovine luteal cells (Bagavandoss et al., 1988, 1990), but did not affect steroidogenesis (Nothnick and Pate, 1990). TNF-α is at its highest concentrations at luteolysis (McIntush et al., 1996; Bagavandoss et al., 1990) and caused fetal death in pregnant mice (Silver et al., 1994). Therefore, several mechanisms may be involved during the regression of the CL and possibly lowering embryonic survival in cattle.

Second, the embryotoxic effect may be due to elevated concentrations of $PGF_{2\alpha}$. Embryonic development was retarded in rat embryos cultured with .1-10 ng/ml $PGF_{2\alpha}$ (Breuel et al., 1993a). Furthermore, rabbit embryos cultured with $PGF_{2\alpha}$ prevented development to the hatched blastocyst stage (Maurer and Beier, 1976). However, if only elevated concentrations of $PGF_{2\alpha}$ were required to lower embryonic survival, pregnancy rates should not have increased in the P+L groups. Although, more frequent blood sampling may have indicated a more sustained release of $PGF_{2\alpha}$ in PGF cows as seen in the secondary experiment. Even though uterine

concentrations of $PGF_{2\alpha}$ did not differ, PGFM levels collected immediately prior to flushing the uterus indicated that $PGF_{2\alpha}$ was higher in PGF treated cows compared to P+L (2836 ± 287 and 1673 ± 335 pg/ml, respectively; $P < .001$). Furthermore, the nondifferences in uterine $PGF_{2\alpha}$ concentrations may be due to the release of $PGF_{2\alpha}$ during the flushing procedure as well as the low numbers of cows per treatment group. Thus, the regressing CL is required to manifest the effects of elevated concentrations of $PGF_{2\alpha}$ on lowering embryonic survival in cattle.

Thirdly, prostaglandins may cause lowered embryonic survival indirectly through the actions of E_2 (Thatcher et al., 1989). Ayalon (1973) reported that in cows with degenerated embryos, plasma concentrations of E_2 were higher on the day of estrus and Days 3 and 4 after insemination in comparison to cows with normal embryos. Continued infusion or twice daily injections of $PGF_{2\alpha}$ increased size and number of large follicles in postpartum cows and ewes (Villeneuve et al., 1988, 1989). Villeneuve (1990) observed increased concentrations of E_2 following $PGF_{2\alpha}$ treatment of early postpartum cows. Administration of gonadotropin releasing hormone agonists 11 to 13 days postestrus, which results in

follicular atresia, increased pregnancy rates of cows inseminated at the previous estrus (MacMillan et al., 1986), probably through a reduction in secretion of E_2 when follicles underwent atresia (Thatcher et al., 1989). Furthermore, cows with higher concentrations of E_2 during Days 14-17 had lower pregnancy rates than cows with lowered concentrations of E_2 (Pritchard et al., 1993).

Exogenous E_2 will increase uterine concentrations of OT receptors (Hixon and Flint, 1987) and result in luteal regression (Stormshak et al., 1969). Administration of E_2 will not induce complete luteal regression in hysterectomized cows (Kaltenbach et al., 1964) indicating that the involvement of E_2 in luteal regression is via the uterus. However, elevated concentrations of E_2 were not observed during Days 5-8 in the present study which suggests that the reduction in embryonic survival is due to the effects of $PGF_{2\alpha}$.

Lastly, the embryotoxic effect may be due to the release of oxytocin from the regressing CL and thus, stimulating further release of $PGF_{2\alpha}$. Oxytocin administration is known to increase concentrations of $PGF_{2\alpha}$ and shorten estrous cycle length in cattle (Armstrong and

Hansel, 1959). The most convincing evidence that OT may be acting as an embryotoxin was obtained by Lemaster et al. (1996). Briefly, cattle supplemented with exogenous progestogen were administered either saline (CON), 100 IU OT + 1 g flunixin meglumine (OT+FM; a prostaglandin inhibitor), 100 IU OT (OT) or 100 IU OT + lutectomy (OT+LUT) on Days 5-8 of pregnancy. PGFM was significantly elevated in animals receiving OT+LUT when compared to CON and OT+FM 30 minutes after the initial injection. Furthermore, 50% of OT animals had luteal phases of less than 14 d. Pregnancy rates at 30 days were 80%, 80%, 33% and 30% for CON, OT+FM, OT and OT+LUT animals, respectively. Thus, the additional secretion of $PGF_{2\alpha}$ due to release of OT from the CL may be having an effect on embryonic survival in cattle.

In summary, a model for studying embryonic survival in cycling cattle was established with multiple injections of $PGF_{2\alpha}$ at three different periods of embryonic development. The results from this study as well as from Buford et al. (1996) indicate that the regressing CL is required to manifest the effects of $PGF_{2\alpha}$ in lowering embryonic survival in cattle supplemented with exogenous progestogen. Removal of the regressing CL (i. e., removal of luteal OT) may

either interfere with the positive feedback loop of luteal OT and uterine $PGF_{2\alpha}$ and prevent even higher concentrations of uterine luminal concentrations of $PGF_{2\alpha}$. Moreover, the embryo becomes resistant to the effects of $PGF_{2\alpha}$ as the embryo ages.

LITERATURE CITED

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. Journal of Dairy Science 42:553-542.

Ayalon, N. 1978. A review of embryonic mortality in cattle. Journal of Reproduction and Fertility 54:483-493.

Ayalon, N. 1973. Annual report of research No. 2. Comparative studies of repeat breeders and normal cow. Kimron Veterinary Institute, Beit Dagan (Israel), pp. 1-51.

Bagavandoss, P., S. L. Kunkel, R. C. Wiggins and R. L. Keyes. 1988. Tumor necrosis factor-α (TNF-α) production and localization of macrophages and T lymphocytes in the rabbit corpus luteum. Endocrinology 122:1185-1187.

Bagavandoss, P., R. C. Wiggins, S. L. Kunkel, D. G. Remick and R. L. Keyes. 1990. Tumor necrosis factor production and accumulation of inflammatory cells in the rabbit corpus luteum of pseudopregnancy and pregnancy in rabbits. Biology of Reproduction 42:367-376.

Balapure, A. K., I. C. Caicedo, K. Kawanda, D. S. Watt, C. E. Rexroad and T.A. Fitz. 1989. Multiple classes of $PGF_{2\alpha}$ binding sites in subpopulations of ovine luteal cells. Biology of Reproduction 41:385-392.

Bartol, F. F., W. W. Thatcher, F. W. Bazer, F. A. Kimball, J. R. Chenault, C. J. Wilcox and R. M. Roberts. 1981. Effects of the estrous cycle and early pregnancy on bovine uterine, luteal, and follicular responses. Biology of Reproduction 25:759-776.

Bazer, F. W., J. L. Vallet, R. M. Roberts, D. C. Sharp and W. W. Thatcher. 1986. Role of conceptus secretory products in establishment of pregnancy. Journal of Reproduction and Fertility 76:841-850.

Bazer, F. W., R. D. Geisert and M. T. Zavy. Fertilizaion, cleavage and implantation. In: E.S.E. Hafez (Ed.), Reproduction in Farm Animals Sixth Edition, Philadelphia,: Lea and Febiger; 1993: 188-212.

Bazer, F. W., T. L. Ott and T. E. Spencer. 1994. Pregnancy recognition in ruminants, pigs, and horses: signals from the trophoblast. Theriogenology 41:79-94.

Bellows, R. A., R. E. Short and R. B. Staigmiller. Research areas in beef cattle reproduction. In: H. W. Hawk, A. Osum (Ed.), Animal Reproduction: BARC Symposium 3. Montclair; 1979: 3-18.

Biggers, J. D., B. V. Leonov, J. F. Baskar and J. Fried. 1978. Inhibition of hatching of mouse blastocysts in vitro by prostaglandin antagonists. Biology of Reproduction 19:519-533.

Bjurulf, E. and G. Selstam. 1996. Rat luteinizing hormone receptor messenger ribonucleic acid expression and luteolysis: Inhibition by prostaglandin $F_{2\alpha}$. Biology of Reproduction 54:1350-1355.

Booth, W. D., R. Newcomb, H. Strange and L. E. A. Rowson. 1975. Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. Veterinary Record 97:366-369.

Boyd, J., P. Bacsigh, A. Young and J.A. McCracken. 1969. Fertilization and embryonic losses in dairy cattle. British Veterinary Journal 125:87-97.

Breuel, K. F., A. Fukuda and F. N. Schrick. 1993a. Effect of prostaglandin $F_{2\alpha}$ on development of 8-cell rat embryos in vitro. Biology of Reproduction 48 (Supplement 1):173.

Breuel, K. F., P. E. Lewis, F. N. Schrick, A. W. Lishman, E. K. Inskeep and R. L. Butcher. 1993b. Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception rate. Biology of Reproduction 48:655-661.

Buford, W. I., F. N. Schrick, E. K. Inskeep. 1996. Embryotoxicity of a regressing corpus luteum in beef cows supplemented with progestogen. Biology of Reproduction 54:531-537.

Butcher, R. L. 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy in rats. Endocrinology 101:830-840.

Butcher, R. L., J. E. Reber, A. W. Liskman, K. F. Breuel, F. N. Schrick, J. C. Spitzer and E. K. Inskeep. 1992. Maintainance of pregnancy in postpartum beef cows having short-lived corpora lutea. Journal of Animal Science 70:3831-3837.

Carver, D. A. 1989. Ovarian regulation of uterine secretion of $PGF_{2\alpha}$ in nonpregnant cows. Masters thesis, West Virginia University.

Chang, M. C. 1952. Development of bovine blastocyst with a note on implantation. Anatomy Record 113:143-161.

Chang, M. C. 1966. Transport of eggs from the fallopian tube to the uterus as a function of oestrogen. Nature 212:1048-1049.

Committee on Reproductive Nomenclature. 1972. Recommendations for standardizing bovine repoductive terms. Cornell Vererinarian 62:216-237.

Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia and E. K. Inskeep. 1991. Effects of progestogen treatment on concentrations of prostaglandins and oxytocin in plasma from the posterior vena cava of postpartum beef cows. Journal of Reproduction and Fertility 91:411-421.

Cullor, J. S. 1990. Mastitis and its influence upon reproductive performance in dairy cattle. International symposium on bovine mastitis; Indianapolis, Indiana. pp. 176-180.

Diskin, M. G. and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. Journal of Reproduction and Fertility 59:463-468.

Dunlap, S. C. and C. K. Vincent. 1971. Influence of postbreeding thermal stress on conception rat in beef cattle. Journal of Animal Science 32:1216-1218.

El-Banna, A. A., B. Sacher and E. Schilling. 1976. Effect of indomethacin on egg transport and pregnancy in the rabbit. Journal of Reproduction and Fertility 46:375-378.

Ellington, J. E. 1991. The bovine oviduct and its role in reproduction: A review of the literature. Cornell Veterinarian 81:313-328.

Elsden, R. P. and G. E. Siedel. 1982. Procedures for recovery, bisection,freeaing and transfer of bovine embryos. Bulletin 2, Animal Reproduction Laboratory, Colorado State University, Fort Collins.

Evans, C. A. and T. G. Kennedy. 1978. The importance of prostaglandin synthesis for the initiation of blastocyst implantation in the hamster. Journal of Reproduction and Fertility 54:255-261.

Farin, P. W. and C. T. Estill. 1993. Infertility due to abnormalities of the ovaries in cattle. Veterinary Clinics of North America Food Animal Practice 9:291-308.

Flint, A. P. F. and E. L. Sheldrick. 1982. Ovarian secretion of oxytocin as stimulated by prostaglandin. Nature 297:587-588.

Flint, A. P. F., E. L. Sheldrick, T. J. McCann and D. S. C. Jones. 1990. Luteal oxytocin: characteristics and control of synchronous episodes of oxytocin and $PGF_{2\alpha}$ secretion at luteolysis in ruminants. Domestic Animal Endocrinology 7:111-124.

Fuchs, A. 1987. Prostaglandin $F_{2\alpha}$ and oxytocin interactions in ovarian and uterine function. Journal of Steriod Biochemistry 27:1073-1080.

Fuchs, A. 1988. Oxytocin and ovarian function. Journal of Reproduction and Fertility Supplement 36:39-47.

Geisert, R. D., J. W. Brookman, R. M. Roberts and F. W. Bazer. 1982. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biology of Reproduction 27:941-955.

Gerritts, R. J., T. H. Blosser, H. G. Purchase, C. E. Terrill and E. J. Warwick. 1979. Economics of improving reproductive efficiency in farm animals. In: H. W. Hawk and A. Osum (Ed.), Animal Reproduction: BARC Symposium 3. Montclair,: 1979: 413-422.

Goding, J. R. 1974. The demonstration that $PGF_{2\alpha}$ is the uterine luteolysin in the ewe. Journal of Reproduction and Fertility 38:161-271.

Godkin, J. D., F. W. Bazer, F. W. Moffatt, J. Sessions, and R. M. Roberts. 1982. Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts on Days 13-21. Journal of Reproduction and Fertility 65:141-150.

Godkin, J. D., F. W. Bazer, and R. M. Roberts. 1984. Ovine trophoblast protein-1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein sysnthesis. Endocrinology 114:120-130.

Gorewit, R.C. 1979. Methods for determining oxytocin concentrations in unextracted sera: characterization in lactating cattle. Proceedings for the Society for Experimental Biology and Medicine 160:80-87.

Graves, P.E., K.L. Pierce, T.J. Bailey, B.R. Rueda, D.W. Gill, D.F. Woodward, A.J. Yool, P.B. Hoyer and J.W. Regan. 1995. Cloning of a receptor for prostaglandin $F_{2\alpha}$ from the ovine corpus luteum. Endocrinology 136:3430-3436.

Gross, T. S., W. W. Thatcher, P. J. Hansen, J. W. Johnson and S. D. Helmer. 1988. Presence of an intracellular endometrial inhibitor of prostaglandin synthesis during early pregnancy in the cow. Prostaglandins 35:359-378.

Hamilton, W. J. And J. A. Laing. 1946. Development of the egg of the cow up to the stage of blastocyst development. Journal of Anatomy 80:194-204.

Hansel, W. and E. M. Convey. 1983. Physiology of the estrous cycle. Journal of Animal Science 57(Supplement 2):404-424.

Hansel, W., P. W. Concannon and J. H. Lukaszewska. 1973. Corpora lutea of large domestic animals. Biology of Reproduction 8:222-245.

Harper, M. J. K. And R. C. Skarnes. 1972. Inhibition of abortion and fetal death produced by endotoxin or prostaglandin $F_{2\alpha}$. Prostaglandins 2:295-309.

Henderson, K. M. And K. P. McNatty. 1977. A possible relationship between corticotropin stimulation and prostaglandin $F_{2\alpha}$ inhibition of steroidogenesis by granulosa cells in vitro. Journal of Endocrinology 73:71-78.

Henricks, D. M., J. F. Dickey, J. R. Hill and W. E. Johnston. 1972. Plasma estrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. Endocrinology 90:1336-1342.

Hernandez-Ledezma, J. J., J. D. Skies, C. N. Murphy, A. J. Watson, G. A. Schultz and R. M. Roberts. 1992. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. Biology of Reproduction 47:374-380.

Hixon, J. E. and A. P. F. Flint. 1987. Effects of a luteolystic dose of oestradiol on uterine oxytocin receptor concentrations , phosphoinositide turnover and prostaglandin $F_{2\alpha}$ secretion in sheep. Journal of Reproduction and Fertility 79:457-467.

Hoffman, L. H. 1978. Antifertility effects of indomethacin during early pregnancy in the rabbit. Biology of Reproduction 18:148-153.

Homanics, G. E. And W. J. Silvia. 1988. Effects of progesterone and estradiol-17β on uterine secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin in ovariectomized ewes. Biology of Reproduction 38:804-811.

Howard, H. J., R. G. Scott and J. H. Britt. 1990. Associations among progesterone, estradiol-17β, oxytocin and prostglandin in cattle treated with hCG during diestrus to extend corpus luteum function. Prostaglandins 40:51-70.

Inskeep, E. K. and J. B. Peters. Economic benefits of reproductive management, synchronization of estrus and artificial insemination in beef cattle and sheep. In: B. G. Brackett, G. E. Seidel and S. M. Seidel (Ed.), New Technologies in Animal Breeding. New York: Academic Press; 1982: 243-254.

Ireland, J. J. and J. F. Roche. 1983. Development of nonovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 112:150-156.

Jacobs, A. L., G. E. Homanics and W. J. Silvia. 1991. Activity of phospholipase C in ovine luteal tissue in resronse to $PGF_{2\alpha}$, PGE and luteinizing hormone. Prostaglandins 41:495-500.

Jenner, L. J., T. J. Parkinson, and G. E. Lamming. 1991. Uterine oxytocin receptors in cyclic and pregnant cows. Journal of Reproduction and Fertility 91:49-58.

Juengel, J. L., G. W. Smith, M. F. Smith, R. S. Youngquist and H. A. Gaverisck. 1994. Patern of protein production by bovine corpora lutea during luteolysis and characterization of expression of two major secretory products of regressing corpora lutea. Journal of Reproduction and Fertility 100:515-520.

Kaltenbach, C. C., B. Cook, G. D. Niswender and A. V. Nalbandov. 1967. Effect of pituitary hormones on progesterone synthesis by ovine luteal cells in vitro. Endocrinology 81:1407-1409.

Keefer, C. L. and S. Stice. 1992. In vitro culture of bovine nucleus transfer embryos. Biology of Repoduction 46(Supplement 1):461.

Kindahl, H., L. E. Edquist, A. Bane and E. Granstrom. 1976. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal oestrous cycle and early pregnancy in heifers. Acta Endocrinologica 82:134-149.

Kotwica, J. and D. Skarzynski. 1993. Influence of oxytocin removal from the corpus luteum on secretory function and duration of the oestrous cycle in cattle. Journal of Reproduction and Fertility 97:411-417.

Lafrance, M. and A. K. Goff. 1990. Control of uterine prostaglandin $F_{2\alpha}$ release in vitro. Biology of Reproduction 42:288-293.

Lahav, M., A. Freud and H. R. Lindner. 1976. Abrogation by prostaglandin $F_{2\alpha}$ of LH stimulated cyclic AMP accumulation in isolated rat corpora lutea of pregnancy. Biochemical and Biophysical Research Communications 68:1294-1300.

Lauderdale, J. W. 1986. A review of patterns of change in luteal function. Journal of Animal Science 62 (Supplement 2):79-91.

Lee, J. S. and W. J. Silvia. 1994. Cellular mechanisms mediating the stimulation of ovine endometrial secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin: role of phospholipase A_2. Journal of Endocrinology 141:491-496.

Lemaster, J. W., F. M. Hopkins and F. N. Schrick. 1996. Effect of oxytocin on embryonic survival in cattle. Biology of Reproduction 54 (Supplement 1):103.

Lewis, G. S. 1989. Prostaglandin secretion by the blastocyst. Journal of Reproduction and Fertility Supplement 37:261-267.

Macmillan, K. L., V. K. Taufa and A. M. Day. 1986. Effects of an agonist of gonadotrophin releasing hormone (Beserelin) in cattle. III: pregnancy rates after a post-insemination injection during metoestrus or dioestrus. Animal Reproduction Science 11:1-10.

Malayer, J. R., P. J. Hansen, T. S. Gross and W. W. Thatcher. 1990. Regulation of heat shock-induced alteratioons in the release of prostaglandins by the uterine endometrium of cows. Theriogenology 34:219-230.

Maurer, R. R. and H. M. Beier. 1976. Urterine proteins and development in vitro of rabbit preimplantation embryos. Journal of Reproduction and Fertility 48:33-41.

Maurer, R. R. and J. R. Chenault. 1976. Fertilization failure and embryonic mortality in parous and nonparous beef cattle. Journal of Animal Science 56:1186-1189.

McCracken, J. A., D. T. Baird and R. J. Scaramuzzi. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent Progress in Hormone Research 27:537-582.

McCracken, J. A., J. C. Carlsson, M. E. Glen, J.R. Goding, D. T. Baird, and B. Samuelsson. 1972. Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in the sheep. Nature 238:129-134.

McCracken, J. A., W. Schramm and W. C. Okulicz. Hormone receptor control of pulsitile secretion of $PGF_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. In: L. E. Edquist and H. Kindahl (Ed.), Prostaglandins in Animal Reproduction II. Amsterdam,: Elsevier; 1984: 31-56.

McIntush, E. W., J. D. Pletz, G. W. Smith, D. K. Long, H. R. Sawyer and M. F. Smith. 1996. Immunolocalization of tissue inhibitor of metalloprosteinases-1 within ovine periovulatory follicular and luteal tissues. Biology of Reproduction 54:871-878.

Milvae, R. A. 1986. Role of luteal prostaglandins in the control of bovine corpus luteum functions. Journal of Animal Science 62 (Supplement 2):7-15.

Moore, L. G., V. J. Choy, R. L. Elliot, and W. B. Watkins. 1986. Evidence for the pulsitile release of $PGF_{2\alpha}$ inducing release of ovarian oxytocin during luteolysis in the ewe. Journal of Reproduction and Fertility 76:159-166.

Moore, P. K. Biosynthesis and catabolism of prostaglandins, thromboxanes and leukotrienes. In: Prostanoids: pharmacological, physiological and clinical relevance. Cambridge,: Cambridge University Press; 1985: 1-40..

Muldoon, T. G. 1980. Regulation of steroid hormone receptor activity. Endocrine Reviews 1:339-364.

Newcomb, R. and L. E. A. Rowson. 1975. Conception rate after uterine transfer of cow eggs, in relation to synchronization of oestrus and age of eggs. Journal of Reproduction and Fertility 43:539-541.

Nishizuka, Y. 1984. The role of protein kniase C in cell surface signal transduction and tumor promotion. Nature 308:693-698.

Niswender, G. D. and T. M. Nett. The corpus luteum and its control. In: E. Knobil and J. D. Neill (Ed.), The Physiology of Reproduction. New York,: Raven Press, Ltd.; 1988: 489-525.

Niswender, G. D., D. E. Suter and H. R. Sawyer. 1981. Factors regulating receptors for LH on ovine luteal cells. Journal of Reproduction and Fertility Supplement 30:183-189.

Nothnick, W. B. and J. L. Pate. 1990. Interleukin-1β is a potent stimulator of prostaglandin synthesis in bovine luteal cells. Biology of Reproduction 43:898-903.

Oettel, M., M. Koch, A. Kurischko and K. Schubert. 1979. A direct evidence for the involvement of prostaglandin $F_{2\alpha}$ in the first step of estrone-induced blastocyst implantation in the spayed rat. Steroids 33:1-8.

Ostrowski, J., M. A. Kjelsberg, M. G. Maron and R. J. Lefkowitz. 1992. Mutagenesis of the β_2-adrenergic receptor: how structure elucidates function. Annual Reviews of Pharmacology and Toxicology 32:167-183.

Pate, J. L. 1995. Involvement of immune cells in regulation of ovarian function. Journal of Reproduction and Fertility Supplement 49:365-377.

Plata, N. I., J. C. Spitzer, C. E. Thompson, D. M. Henricks, M. P. Reid and T. J. Newby. 1990. Synchronization of estrus after treatment with luprositol in beef cows and in beef and dairy heifers. Theriogenology 33:943-952.

Pratt, B. P., J. G. Berardinelli, L. P. Stevens and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. I: Comparison of gonadotropin and effects of progesterone and estrogen. Journal of Animal Science 54:822-829.

Pritchard, J. Y. and E. K. Inskeep. 1993. Relationship of conception rate to perpheral concentrations of progesterone and estradiol. Bilogy of Reproduction 48(Supplement 1):88.

Ramirez-Godinez, J. A., G. H. Kiracofe, D. L. Carnahan, M. F. Spire, K. B. Beeman, J. S. Stevenson and R. R. Schalles. 1982a. Evidence for ovulation and fertilization in beef cows with short estrous cycles. Theriogenology 17:409-414.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles and R. J. Kittok. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 15:613-623.

Rao, C. V., V. L. Estergreen, F. R. Carman, Jr. And G. E. Moss. 1979. Receptors for gonadotropin and prostaglandin $F_{2\alpha}$ in bovine corpora lutea of early, mid and late luteal phase. Acta Endocrinologica 91:529-537.

Rasmussen, H. And P. Q. Barrett. 1984. Calcium messenger system: an integrated view. Physiology Reviews 64:938-984.

Raw, R. E., T. E. Curry and W. J. Silvia. 1988. Effects of progesterone and estradiol on the concentrations and activity of cyclooxygenase in the ovine uterus. Biology of Reproduction 38:(Supplement 1):167.

Rawson, L. E. A., R. Trevit and A. Brand. 1972. The use of prostaglandins for synchronization of estrus in cattle. Journal of Reproduction and Fertility 29:145 (abstract).

Roberts, J. S., B. Barcikowski, L. Wilson, R. C. Skarnes and J. A. MacCracken. 1975. Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. Journal of Steroid Biochemistry 6:1091-1097.

Roberts, R. M., J. C. Cross and D. W. Leaman. 1992. Interferons as hormones of pregnancy. Endocrine Reviews 13:432-452.

Roche, J. F., M. P. Boland and T. A. McGeady. 1981. Reproductive wastage following artificial insemination of heifers. Veterinary Record 109:401-403.

SAS. 1989. The MIXED Procedure: User's Guide Statistics. Statistical Analysis Sytems Institute, Cary, NC

Schallenberger, E., D. Schamms and H. H. D. Meyer. 1989. Sequences of pituitary, ovarian and uterine hormone secretion during the first 5 weeks of pregnancy in dairy cattle. Journal of Reproduction and Fertility Supplement 37: 277-286.

Schramm, W., L. Bovaird, M. E. Glew, G. Schramm and J.A. McCracken. 1983. Corpus luteum regression induced by ultra-low pulses of prostaglandin $F_{2\alpha}$. Prostaglandins 26:347-364.

Schrick, F. N., E. K. Inskeep and R. L. Butcher. 1993. Survival of embryos from early postpartum beef cows in normally cycling recipients. Biology of Reproduction 49:617-621.

Seguin, B. E., D. A. Morrow and T. M. Louis. 1974. Luteolysis, luteostasis, and the effect of prostaglandin $F_{2\alpha}$ in cows after endometrial irritation. American Journal of Veterinary Research 35:57-61.

Silver, R. M., W. S. Lohner, R. A. Daynes, M. D. Mitchell and D. W. Branch. 1994. Lipopolysaccharide-induced fetal death: the role of tumor necrosis factor alpha. Biology of Reproduction 50:1108-1112.

Silvia, W. J., G. S. Lewis, J. A. McCracken, W. W. Thatcher and L. Wilson, Jr. 1991. Hormonal regulation of uterine secetion of prostaglandin $F_{2\alpha}$ during luteolysis in ruminants. Biology of Reproduction 45:655-663.

Silvia, W. J., R. E. Raw, D. S. Trammell and S. H. Hayes. 1991. Changes in oxytocin-induced activity of phospholipase C in caruncular endometrium during the estous cycle and early pregnancy in ewes. Journal of Animal Science 69 (Supplement 1):411.

Silvia, W. J. and R. E. Raw. 1993. Activity of phospholipase C and release of prostaglandin $F_{2\alpha}$ by endometrial tissue from ewes during the estrous cycle and early pregnancy. Journal of Reproduction and Fertility 97:529-537.

Silvia, W. J. and M. L. Taylor. 1988. Relationship between secretion of prostaglandin $F_{2\alpha}$ induced by oxytocin and endogenous concnetrations of estradiol and progesteron at three different stages of the bovine estrous cycle. Journal of Animal Science 67:2347-2353.

Silvia, W. J., J. S. Lee, D. S. Trammell, S. H. Hayes, L. L. Lowberger and J. A. Brosckman. 1994. Cellular mechanism mediating the stimulation of ovine endometrial secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin: role of phospholipase C and diacylglycerol. Journal of Endocrinology 141:481-490.

Silvia, W. J., J. S. Lee, D. S. Trammell, S. H. Hayes, L. L. Lowberger and J. A. Brockman. 1994. Cellular mechanisms mediating stimulation of ovine endometrial secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin: role of phospholipase C and diacylglycerol. Journal of Endocrinology 141:481-490.

Smith, G. W., T. L. Goetz, R. V. Anthony and M. F. Smith. 1994. Molecular cloning of an ovine ovarian tissus inhibitor of metallaproteinases: Ontogeny of messenger ribonucleic acid expression and in situ localization within preovulatory follicles and luteal tissue. Endocrinology 134:344-352.

Smith, G. W., R. M. Moor and M. F. Smith. 1993. Identification of a 30,000 M_r polypeptide secreted by cultured ovine granulosa cells and luteal tissue as a tissue inhibitor inhibitor of metalloproteinases. Biology of Reproduction 48:125-132.

Spitzer, J. C. 1986. Influence of nutrition on reproduction in beef cattle. In D. A. Morrow (Ed.) Current Therapy in Theriogenology (2nd Ed.) W.B. Saunders Co. pp. 320-341. Philadelphia, PA.

Sreenen, J. M. and M. G. Diskin. 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Veterinary Record 112:517-521.

Stormshak, F. and L. E. Casida. 1965. Effect of LH and ovarian hormones on corpora lutea of pseudopregnant and pregnant rabbits. Endocrinology 77:337-342.

Stormshak, F., H. E. Kelly and H. W. Hawk. 1969. Suppression of ovine luteal function by 17β-estradiol. Journal of Animal Science 29:476-478.

Thatcher, W. W., F. W. Bazer, D. C. Sharp and R. M. Roberts. 1986. Inter-relationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pigs and horses. Journal of Animal Science 62 (Supplement 2):25-46.

Thatcher, W. W., K. L. MacMillan, P. J. Hansen and M. Drost. 1989. Concnepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 31:149-164.

Thatcher, W. W., M. D. Meyer and G. Danet-Desnoyers. 1995. Maternal recognition of pregnancy. Journal of Reproduction and Fertility Supplement 49:15-28.

Villeneuve, P., J. J. Dufour and L. A. Guilbault. 1988. Influence of infusion of prostaglandin $F_{2\alpha}$ and weaning on surface and histologic populations of ovarian follicles in early postpartum beef cows. Journal of Animal Science 66:3174-3184.

Villeneuve, P, R. A. Dailey, E. K. Inskeep, L. A. Guilbault and J. J. Dufour. 1989. Effect of multiple injections of $PGF_{2\alpha}$ on follicular development in early postpartum ewes. Journal of Animal Science 67 (Supplement 1):406.

Wegner, J. A., R. Martinez-Zaguilan, M. E. Wise R. J. Gilles and P. B. Hoyer. 1990. $PGF_{2\alpha}$-induced calcium transient in ovine luteal cells. I. Alterations in cytosolic free calcium levels and calcium influx. Endocrinology. 127:3029-3037.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. Journal of Animal Science 34:1020-1024.

Wiltbank, M. C., M. G. Diskin, J. A. Flores and G. D. Niswender. 1990. Regulation of the corpus luteum by protein kinase C II: Inhibition of lipoprotein-stimulated steroidogenesis by prostaglandin $F_{2\alpha}$. Biology of Reproduction 42:239-245.

Wiltbank, M. C., T. F. Shiao, D. R. Bergfelt and O. J. Ginther. 1995. Prostaglandin $F_{2\alpha}$ receptors in the early corpus luteum. Biology of Reproduction 52:74-78.

APPENDIX

Feed used in Experiments 1 and 2

Ingredients

Processed grain by-products, plant protein products, grain products, forage products, cane molasses, calcium carbonate, salt, lignin sulfonate, potassium sulfate, magnesium sulfate, zinc, oxide, manganese oxide, ferrous sulfate, magnesium oxide, copper sulfate, cobalt carbonate, ethylenediamine dihydroiodide, calcium iodate, sodium selenite, vitamin A acetate, vitamin D-3, vitamin E supplement and mineral oil.

Guaranteed Analysis

Crude protein (min.)	25.00%
Crude fat (min.)	2.50%
Crude fiber (max.)	9.75%
Calcium (min.)	1.25%
Phosphorus (min.)	0.55%
Salt (min.)	1.30%
Potassium (min.)	1.00%
Vitamin A (min.)	5,000 IU/lb
Vitamin D (min.)	1,500 IU/lb

Active Ingredient

Melengesterol Acetate	2,000 mg/ton

Manufactured By
Tennessee Farmers Cooperative
200 Waldron Road
Lavergne, TN 37086

Vita

Richard Seals was born on July 28, 1972 in Morristown, Tennessee. He attended Hamblen County public schools in Morristown, where he graduated from Morristown-Hamblen High School East in June of 1990. Richard attended Walters State Community College in the summer of 1990 and then transferred to the University of Tennessee, Knoxville in the Fall of 1992 majoring in Animal Science. In the Spring of 1994, Richard graduated from the University of Tennessee with a B.S. degree and entered graduate school within the same department in the Fall of 1994 under the direction of Dr. F. Neal Schrick. In the Summer of 1996, he graduated with a M.S. degree in animal science with a concentration in reproductive physiology. Richard accepted a Ph. D. position at Virginia Tech under the direction of Dr. Greg Lewis to begin in the Fall of 1996.