OVULATION, FERTILITY AND ENDOCRINE RESPONSE AFTER PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
THOMAS MICHAEL LOUIS
1975

This is to certify that the

thesis entitled

Ovulation, Fertility and Endocrine Response after Prostaglandin $F_{2\alpha}$ in Cattle

presented by

Thomas Michael Louis

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Dairy Science

Harold D. Hafs
Major professor

Date May 1, 1975

O-7639

ABSTRACT

OVULATION, FERTILITY AND ENDOCRINE RESPONSE AFTER PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

By

Thomas Michael Louis

Even with the substantial increase in genetic progress from artificial insemination (AI) of cattle, a relatively small percentage of dairy heifers and beef cattle are artificially inseminated because estrous detection is required. Proper estrous detection requires a substantial investment of labor. Effective estrous synchronization would obviate the need for estrous detection, and increase the utilization of artificial insemination because of higher fertility.

The major objective of this dissertation research was to determine whether $PGF_{2\alpha}$ was luteolytic in cattle and whether it was effective for synchronization of ovulation with a view to insemination at a predetermined time without estrous detection. A secondary objective was to compare the same reproductive endocrine events during normal luteolysis with those after treatment with $PGF_{2\alpha}$. Thus, the endocrine, behavioral, and ovarian changes induced by

$PGF_{2\alpha}$ as well as fertility of cattle after $PGF_{2\alpha}$ treatment were studied in four experiments.

To assess luteotropic activity after intrauterine $PGF_{2\alpha}$ in cows, each of six Holstein cows was given 5 mg $PGF_{2\alpha}$ (Tham salt) in the uterine born ipsilateral to the corpus luteum on day 11 of the estrous cycle. With an intervening control estrous cycle between each $PGF_{2\alpha}$ treatment, the same cows were given 5 mg $PGF_{2\alpha}$ in the ipsilateral horn on day 15 and on day 7 of the estrous cycle, and five of the six were given 5 mg $PGF_{2\alpha}$ into the contralateral uterine horn on day 11. The response of each measured endocrine or reproductive criterion was similar regardless of day of the cycle $PGF_{2\alpha}$ was administered or whether $PGF_{2\alpha}$ was deposited into the ipsilateral or contralateral uterine horn; (1) luteal diameter decreased ($P<.01$) from 2.5 to 1.6 cm within 24 hours, (2) serum progesterone fell ($P<.01$) from 3.6 ng/ml to 1.7, 1.2 and 1.0 ng/ml at 12, 24 and 48 hours, respectively, (3) serum estradiol increased ($P<.01$) from 5.0 pg/ml to 6.1, 11.3, 12.7 pg/ml at 12, 24 and 48 hours, respectively, (4) a surge of LH occurred ($P<.01$) with a peak at 71 hours, (5) estrus began at 72 hours, and (6) ovulation occurred at 95 hours. Based upon changes in blood serum LH, progesterone and estradiol and upon interestrual intervals, the estrous cycle which followed $PGF_{2\alpha}$ treatment did not differ significantly from control cycles in the same cows.

To determine if $PGF_{2\alpha}$ would have a luteolytic effect if given systemically, 30 mg $PGF_{2\alpha}$ was injected either im into five heifers during diestrus (days 9 to 13 of the estrous cycle), im into six heifers during metestrus (day 3) or intravaginally into six heifers during diestrus. After im $PGF_{2\alpha}$ during diestrus; (1) luteal diameter decreased ($P<.05$) 2.3 cm to 1.8 cm at 24 hours and to 0.6 cm at 72 hours, (2) blood serum progesterone fell ($P<.01$) from 4.0 ng/ml to 1.5 ng/ml at 12 hours and to 1.0 ng/ml at 72 hours, and (3) estrus began at 74 hours and ovulation at 104 hours. These responses resembled those after intrauterine $PGF_{2\alpha}$ administration. After intravaginal $PGF_{2\alpha}$ during diestrus, one heifer failed to respond; the other five responded similarly to those given im $PGF_{2\alpha}$ during diestrus except that luteolysis was more variable and delayed 1 to 2 days compared to that after im $PGF_{2\alpha}$. $PGF_{2\alpha}$ im given during metestrus was neither luteolytic nor luteostatic.

To assess acute changes in progesterone and estradiol and to determine if luteolysis proceeded more rapidly after two 15 mg injections at 6 hour intervals or after one injection of 60 mg than after a 30 mg injection, blood samples were obtained at 10 minute intervals for the first hour and then at 1.5, 2, 4, 6, 12, and 18 hours. On the average, blood progesterone declined ($P<.01$) continuously from 3.9 ng/ml before $PGF_{2\alpha}$ 2.4, 2.1, and 1.7 ng/ml at 1, 6, and 12 hours respectively. The second 15 mg

injection at 6 hours after initial injection did not alter the rate of decline in progesterone. Estradiol averaged 1.4 pg/ml at the time of $PGF_{2\alpha}$ injection in the 16 heifers, then increased ($P<.05$) to 2.6 pg/ml at 1 hour and continuously to an average of 6.2 pg/ml at 12 hours after $PGF_{2\alpha}$. Evidently, blood plasma progesterone began to fall within 10 minutes and continuously after $PGF_{2\alpha}$, and estradiol increased continuously possibly beginning within 1 hour. Relative to 30 mg $PGF_{2\alpha}$ im, two injections of 15 mg at 6 hour intervals or 60 mg $PGF_{2\alpha}$ did not hasten luteolysis or onset of estrus. Thus a single 30 mg im injection of $PGF_{2\alpha}$ is ample to cause luteolysis in heifers.

To deternine if $PGF_{2\alpha}$ could be used to synchronize estrus to allow artificial insemination at a predetermined time, which would result in high fertility, two trials were initiated to determine if cattle with palable corpora lutea given $PGF_{2\alpha}$ and inseminated either after showing signs of estrus or at predetermined intervals (72 and 90 hours) would have ferility equivalent to control cows which had not been treated with $PGF_{2\alpha}$.

In the first trial, seven of 13 controls and 6 of 12 $PGF_{2\alpha}$-treated heifers were diagnosed pregnant at 40 days from the first insemination. In the second trial, percent pregnancy diagnosed by palpation at 35 to 60 days after insemination, among controls was 62%/25 no greater than that for cattle inseminated during estrus after $PGF_{2\alpha}$

(90%/21) or that for those inseminated 72 and 90 hours after $PGF_{2\alpha}$ (79%/19).

In overview, exogenous $PGF_{2\alpha}$ given during diestrus caused dramatic luteolysis beginning possibly within 10 minutes in cows and heifers, accompanied by increased estrogen secretion, an ovulatory surge of LH coincidental with onset of estrus, and ovulation in synchrony. But $PGF_{2\alpha}$ was neither luteolytic nor luteostatic in metestrus. Since fertility of cows inseminated by appointment at 72 and 90 hours after $PGF_{2\alpha}$ was not reduced compared to that of controls, one may speculate that $PGF_{2\alpha}$ may be used in the future to allow breeding with normal fertility at predetermined times, independent of estrous detection.

OVULATION, FERTILITY AND ENDOCRINE RESPONSE AFTER PROSTAGLANDIN $F_{2\alpha}$ IN CATTLE

By

Thomas Michael Louis

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Departments of Dairy Science and Physiology

1975

TO MY WIFE

BIOGRAPHICAL SKETCH

I was born on December 27, 1944, in Pensacola, Florida. I attended primary schools in Port au Prince, Haiti and Blacksburg, Virginia, graduating in June, 1963. Pursuing an interest in biological sciences I enrolled in Virginia Polytechnic Institute and State University and graduated with a B.S. in biological sciences in June, 1968. After graduation from college I worked as a management trainee for Allstate Insurance Company until I returned to graduate school in the fall of 1969. At that time, I accepted a graduate teaching assistantship in the department of zoological sciences at Virginia Polytechnic and Institute State University. In June, 1971, I received the M.S. degree. My theses was entitled "The effect of DOM on the compound eye of Manduca sexta." Pursuing an interest in reproductive endocrinology, I enrolled at Michigan State University in the fall of 1971 studying under the directorship of Dr. Harold D. Hafs. I completed the requirements for the Ph.D. in May, 1975. I will join Drs. John Challis and Geoffrey Thorburn as a Lalor Fellow in June at the Nuffield department of Obstetrics and Gynecology,

University of Oxford, Oxford, England for postdoctoral studies on the role of prostaglandins in parturition.

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Harold Hafs, for thoughtful leadership and financial support, which has been accompanied by his enthusiastic assistance with with even the most menial of task. The facilities and assistance provided by Dr. C. A. Lassiter and Department of Dairy Science are greatly appreciated.

I am greatful to the advice and consent given by Dr. S. A. Aust, Dr. J. L. Gill, Dr. J. Meites, and Dr. H. A. Tucker.

I wish to thank my colleagues, J. N. Stellflug, Dr. B. E. Seguin, D. Vines, Dr. K. Mongyunpunya, and R. Kittok for their assistance at the barn and with laboratory task. I would like to offer special thanks to Dr. D. A. Morrow, and Dr. W. D. Oxender for assistance in performing rectal palpations. The able laboratory assistance of P. Kaneshiro, J. Mapes, and C. Rankin is greatly appreciated.

A very special note of gratitude to Dr. R. G. Saacke, whose interest and enthusiasm for reproductive biology, kindled my interest in this fascinating field. His example and encouragement played a fundamental role

in leading to my decision to pursue a carrier in reproductive endocrinology.

And finally, a word of graditude for my wife, Mary, to whom this dissertation is dedicated, for her encouragement, understanding, and financial assistants.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Artificial insemination has dramatically improved milk production of dairy cattle, because of greater use of genetically superior bulls. However, infertility has limited genetic progress. United States dairymen cull over 850,000 cows annually because of infertility, resulting in an estimated loss of 600 million dollars annually.

There are many causes of infertility, but ineffective estrous detection is probably the most important in herds utilizing artificial insemination. But proper estrous detection requires a substantial investment of labor. Effective estrous synchronization would obviate the need for estrous detection, and probably greatly increase the utilization of artificial insemination.

For almost two decades, it has been possible to synchronize the estrous cycle in cattle with progestagens. Estrus and ovulation are delayed for as long as progesterone is given, usually 14 to 18 days until the corpora lutea of all animals have regressed. Unfortunately, daily progestagen administration for 14 to 18 days poses some management problems. For instance, the fertility of cattle

inseminated at the first estrus 3 to 7 days following progestagens withdrawal is reduced from that of untreated controls, and estrous synchronization is not sufficiently precise to allow inseminations at a predetermined time without regard to estrus.

In 1969 and 1970, several researchers demonstrated that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induced luteolysis in several laboratory species. This observation suggested a new approach to synchronization of the estrous cycle. Since there were no reports of $PGF_{2\alpha}$'s luteolytic effect in cattle, the present studies were undertaken to determine whether $PGF_{2\alpha}$ was luteolytic in cattle and whether it was effective for synchronization of ovulation with a view to inseminations at a predetermined time without estrous detection. To accomplish these objectives I determined the intervals from $PGF_{2\alpha}$ to estrus and ovulation, and the fertility of cows after $PGF_{2\alpha}$ treatment.

REVIEW OF LITERATURE

Approaches to Ovulation Control

Over the past two decades, researchers have tried two different approaches to estrous synchronization in cattle, but none have gained widespread practical application. The first delays estrus and ovulation with gonadotropin inhibitors, such as progesterone or progestagens, until the corpora lutea of all treated animals have regressed. After withdrawal of the inhibitor, the cattle are artificially inseminated at the next estrus. The second approach involves induction of luteolysis with treatments such as corpus luteum enucleation or intrauterine infusion of iodine solution, followed by insemination at the subsequent estrus. Unfortunately, it is now generally accepted that neither of these approaches produce estrous synchronization which is sufficiently precise to allow insemination at a predetermined time without regard to estrus.

Progress in estrous synchronization research accelerated when recent advances in methodology for measuring reproductive hormones allowed accurate determination of blood and tissue hormones related to the

endocrine events of the estrous cycle. This knowledge provided a better understanding of the consequences of these various estrous synchronizing treatments.

The Bovine Estrous Cycle

Before entering into the body of this review, I think it wise to present initially a brief overview of the normal events in the bovine estrous cycle to provide a basis for understanding subsequent discussion of estrous synchronization.

Behavior

Most mature dairy cattle have a 21-day estrous cycle. According to Trimberger (1948), heifers have slightly shorter estrous cycles than cows; the mature cow remains in estrus for a longer period (18 hours) than heifers (16 hours), and cows and heifers which began estrus in the afternoon remained in estrus longer than those which began estrus in the morning. But neither cows nor heifers exhibited seasonal changes in estrus duration or inter-estrual intervals. Nalbandov (1964) reported that older cows exhibit more intensive estrus activity than heifers. Morrow (1969) confirmed this trend towards more intense libido in older cows, and also observed that the incidence of standing heat increased from the first to the third post-pubertal estrous cycle.

Anatomical Changes in the Ovary

Rajakoski (1960) studied the distribution of sizes of follicles throughout the estrous cycle. He found that more small (<5 mm) follicles were present during winter and spring, and concluded that two waves of follicular growth occur during the estrous cycle. Cole et al. (1969) confirmed these observations and reported that the first wave of follicular growth begins about 3 to 4 days after estrus, but these follicles persist no longer than until day 11 when they atrophy. Then a second wave begins during mid-cycle, resulting in a large Graafian follicle which ovulates about 12 to 18 hours after estrus. In agreement with these observations, Hackett and Hafs (1969) found largest follicular wall weight at the outset of estrus, and another peak during mid-cycle (day 7 to 11). Rajakoski (1960) found twice as many large follicles (greater than 5 mm) on the right ovary. In regard to time of ovulation relative to estrus, Trimberger (1948) reported that ovulation occurred on the average 10.2 hours after the end of estrus. Swanson et al. (1972) confirmed this observation when they reported ovulation at 29 hours after the onset of estrus.

Corpus luteum weight and volume increase rapidly between day 3 and 12 of the estrous cycle in the cow and then remain relatively constant until day 16 to 18 when corpus luteum regression begins (Erb et al., 1971).

Endocrinology

The ovulatory surge of LH peaks near the onset of estrus and persists about 6 to 8 hours (Swanson and Hafs, 1971). Hansel and Snook (1970) and Schams and Karg (1969) also reported peak LH at or shortly before the beginning of estrus and both of these laboratories reported smaller peaks in serum LH around day 8 to 10 of the cycle. Both the proestrous and the mid-cycle peaks in serum LH correspond with the decreases previously observed in pituitary LH (Hackett and Hafs, 1969 and Rakha and Robertson, 1965).

Rakha and Robertson (1965) reported a decrease in pituitary FSH between onset and end of estrus. Subsequently, Desjardins and Hafs (1968) reported that pituitary FSH was depressed from day 6 to 9 after estrus and during the week before estrus. However, Hackett and Hafs (1969) found the most pronounced decrease in pituitary FSH commenced on day 18 and the decline continued until the day of estrus. If low pituitary FSH concentration reflects release of FSH, then one would expect high blood FSH during periods of low pituitary FSH. This concept was not entirely substantiated in the only paper I have found on variations in blood FSH during the estrous cycle in cows by Akbar et al. (1974). For instance, these authors reported no significant changes in FSH during the cycle, although there were tendencies for elevated FSH from days 6 to 10 after estrus and especially on the day of estrus.

Pituitary prolactin decreased significantly around estrus in heifers (Sinha and Tucker, 1969), and Swanson and Hafs (1971) reported a corresponding increase in blood serum prolactin during estrus in cows. On the other hand, Schams and Karg (1970) found no relationship between blood prolactin concentrations and stage of the estrous cycle. The functional relationship of prolactin to estrus, ovulation and luteal growth in the cow is unclear; the estrual rise in prolactin (Swanson and Hafs, 1971) may merely reflect increased activity of the animals at this time.

Until about 5 years ago, studies of progesterone changes during the bovine cycle were limited to corpora lutea concentration because the spectrophotometric assay was too insensitive to detect blood progesterone. For instance, there is a significant increase in progesterone and 20 β-hydroxy-pregn-4-en-3one (20 B-ol) concentration in bovine CL (beginning as early as day 4 after estrus) then remaining high until day 15 after estrus, followed by a decrease beginning at day 17 (Mares et al., 1962).

Recently, radioimmunoassays (RIA) have allowed measurement of peripheral blood progesterone. For example, serum progesterone averages less than 0.5 ng/ml during estrus and increases to 5 to 7 ng/ml from day 15 to 18 (Stabenfeldt et al., 1969, and Swanson et al., 1972). Erb et al. (1971) found a highly significant correlation between venous progesterone and luteal progesterone. They

suggested that ovarian venous and peripheral plasma progesterone provide accurate estimates of luteal function since they are highly correlated with luteal weight, morphology and progesterone content.

Estradiol begins to increase in peripheral serum as early as 2 days before estrus to a peak of about 10 pg/ml about 12 hours before the onset of estrus; then estradiol falls to 3-4 pg/ml by 2 days after estrus and remains near these low values throughout the luteal phase of the estrous cycle (Wetteman et al., 1973). In addition to the peak before estrus reported by Wetteman et al. (1973), Hansel and Echternkamp (1972) also reported increased estradiol during metestrus and during diestrus with a third peak of estradiol just before complete luteal regression. But Wetteman et al. (1973) and Henricks et al. (1971) did not find estrogen peaks during diestrus in cattle. It is generally believed that during proestrus the rapidly growing Graafian follicle stimulated by the gonadotropins (FSH and LH) secreted high levels of estrogen. The LH surge which occurs around the onset of estrus may be estrogen induced since serum estradiol reaches highest concentrations around the time of LH peak (Henricks et al., 1971).

In summary, with the possible exception of small spikes of estradiol during metestrus and diestrus, blood estrogen remains low during the luteal phase of the cycle but increases markedly beginning 3 days before estrus and

decreases rapidly beginning at the onset of estrus. Blood progesterone is low at estrus; increases starting about the second day after estrus in parallel with the development of the corpus luteum and decreases at the end of the luteal phase during proestrus (Henricks et al., 1971; Wettemann et al., 1972; Swanson et al., 1972; Henricks et al., 1970). Under the stimulation of rapidly rising estrogen, LH is released for 6-8 hours with a peak near the beginning of estrus. This ovulatory surge of LH precedes ovulation by 29-31 hours (Henricks et al., 1970; Swanson et al., 1971). Changes in blood FSH concentrations are less well-defined but appear to follow the changes in LH around estrus.

Estrous Synchronization With Progestagens

The objective of estrus synchronization is to manipulate the reproductive cycle so that the cow may be bred during a predetermined interval with normal fertility. Most estrous synchronization research in the cow has been done with progestagens.

Makespeace et al. (1937) was the first to demonstrate that injections of progesterone inhibited ovulation after mating in rabbits. Applying this knowledge, Willett (1950) and Ulberg and Casida (1951) used progesterone to inhibit estrus and ovulation until the corpora lutea of all animals had regressed and thus synchronized estrus and ovulation in cattle. Since progesterone is not orally active in ruminants, orally active analogues were

administered in feed, water, or pessaries, as intramuscular or subcutaneous injections or as implants (reviewed by Hansel, 1967).

When progestogens were administered for 12 to 14 days and then withdrawn, estrus began 3 to 7 days later. Unfortunately, the interval from progestogen withdrawal to onset of estrus usually was too variable to allow timed inseminations without regard to estrus detection. But the major practical restriction to progestagens has been the reduced conception rate from an insemination at the synchronized estrus (Hansel, 1967). This reduced fertility has been associated with low fertilization rate, low embryonic survival, reduced sperm transport, abnormal cervical mucus patterns, altered patterns of uterine contractions and abnormally high estradiol (Lauderdale, 1973; Wettemann et al., 1973).

Many researchers attempted to improve both the fertility and the degree of synchronization attained following progestagen treatment, with little success (Hansel, 1967). Thus, some researchers began to investigate alternative methods for estrous synchronization of cows, mostly focusing on the normal uterine control of luteolysis. By controlling the functional life of the animal's corpus luteum, one might theoretically expect to attain a more nearly normal conception rate, since one would be changing only endogenous hormone concentrations during luteal regression.

Utero-Ovarian Research and Control of Luteolysis

Loeb (1923) was the first to show that the uterus controlled the corpus luteum; he reported that hysterectomy in guinea pigs greatly prolonged the life span of the corpus luteum. Subsequent studies have extended these findings to include the rabbit, rat, hamster, cattle, sheep, swine, and horses (Anderson et al., 1969; Dobrowolski and Hafez, 1971; and Ginther and First, 1971).

Further evidence of uterine control of luteal function was shown by Ginther (1966). After he insterted a plastic coil into the uterine lumen ipsilateral to the ovary with a functional corpus luteum on day 3 of the estrous cycle, cows returned to estrus within 14 days, but when the plastic coil was placed into the contralateral uterine lumen, cows had a normal (21 day) interestrual interval. Evidently, a foreign body placed into the uterus adjacent to the ovary with the corpus luteum caused premature luteal regression, but the foreign body was not luteolytic when placed in the uterine horn opposite the ovary with the corpus luteum. Similar luteolytic effects of other foreign substances have also been reported in cattle. For instance, distention of the bovine uterus during the first 8 days of the estrous cycle (Hansel and Wagner, 1960) also inhibited corpus luteum development and resulted in abbreviated cycle. When Yamauchi et al. (1967) injected a viscous gel-like substance through the

cervix into the uterus of cows at various stages of the estrous cycle, the duration of the cycle was reduced when the gel was injected into the uterus during the early luteal phase, but treatment in the late luteal phase increased the interestrual interval 24 to 28 days.

Administration of uterine irritants such as Lugols solution (dilute iodine) given between days 4 to 10 also inhibit corpus luteum development and result in an abbreviated estrous cycle. For instance, Seguin et al. (1973) reduced the interestrual interval to 11 days by irritation of the uterus with Lugols solution on day 4 of the cycle, but increased the interval to 25 days with a similar treatment on day 15. Results of this study indicated that intrauterine infusion of Lugols may have destroyed the source of the luteolytic factor and that synthesis of luteolysin probably resumed during the 10-day period of endometrial repair, since synthesis of the endometrium and concomitant synthesis of the luteolysin was followed by normal luteolysis and estrus. These authors also demonstrated that altered duration of the estrous cycle was associated with altered blood progesterone and corpora luteal maintenance in each of these treatments. In further support for an endometrial synthesis of the luteolysin, pyometra and endometitis also modify the life-span of the corpus luteum (Kendrick and McEntee, 1965).

Early luteal regression also occurs in cows given large (100 IU) doses of oxytocin daily on days 2 to 6 of

the estrous cycle (Woody and Ginther, 1968). But this effect of oxytocin does not occur in hysterectomized cows (Armstrong and Hansel, 1959). Wiltbank (1966) established that exogenous estradiol causes regression of the corpus luteum in both intact and hysterectomized cows. But Brunner et al. (1969) reported that 5 mg of estradiol 17 β/day administered daily from day 6 to 15 caused luteal regression in intact heifers and produced only a nonsignificant decline in luteal progesterone of hysterectomized cows. These results suggest that like the comparable action of oxytocin, the luteolytic effect of estrogen is mediated through the uterus.

Following the lead of Loeb (1923), Wiltbank and Casida (1956) reported that hysterectomy prevented corpus luteum regression, for as long as 154 days in cows. Similarly, Anderson et al. (1962) reported that hysterectomy in diestrous heifers prevented corpus luteum regression for at least 270 days. But unilateral removal of the uterine horn adjacent to the ovary with the corpus luteum caused maintenance of a corpus luteum located in the adjacent ovary, while a corpus luteum located on the contralateral ovary regressed normally (Ginther, 1966). Similar results have been reported for sheep (McCracken and Caldwell, 1969) and guinea pigs (Bland, 1970).

Further evidences for this local communication between uterine horn and adjacent ovary follows: (1) McCracken and Baird (1969) demonstrated that selective

ligation of vascular connections between uterus and ovary prevented normal luteal regression in sheep. (2) McCracken et al. (1971) reported that when the ovary was removed from the local influence of the uterus by transplantation to the neck, the corpus luteum did not regress and the progesterone content of the ovarian effluent did not change over a prolonged (30-36 day) period. But when they transplated both ovary and uterus to the neck, normal estrous cycles were maintained. Hence, the ovary and uterus must be in close proximity for normal luteolysis. Snook and Hansel (1970) carried out similar ovarian transplant experiments in cows and found virtually identical results to those reported by McCracken et al. (1971) in sheep. (3) When uterine blood was cross-cannulated from the uterine vein of an intact proestrus ewe to the ovarian supply of another ewe which had its ovary transplanted to the neck, both animals underwent a normal luteolysis together and began estrus concurrently (McCracken et al., 1971). (4) In cows, Ginther and Delcampo (1974) reported significantly more prominent utero-ovarian vein and ovarian arterial anastomosis on the side without a corpus luteum. Lending further support to the hypothesis that the local pathway for uterine induced luteolysis is a venoarterial pathway.

This evidence indicates that the uterus normally produces substances which cause corpus luteum regression in ewes and cows. But the nature of the uterine factor(s) and

the route by which it is transported from the uterus to the adjacent ovary remain unclear.

While the exact nature of the uterine luteolysin is unclear, one possible candidate has been suggested and seriously studied. This research began when Babcock (1966) suggested that the prostaglandins might be uterine luteolysin since they are found in abundance in uterine endometrium and they have venoconstrictor properties. This suggestion and subsequent research led to the conclusion by Goding et al. (1972) that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the uterine luteolysin in sheep. Evidence for and against this conclusion is discussed below.

Prostaglandins

Before discussing the possible role of prostaglandin $F_{2\alpha}$ in control of the bovine corpus luteum, I would like to give a very brief historical overview and explain the chemical nomenclature of the prostaglandins.

Historical Overview

Prostaglandin is the generic name of a family of biologically active lipids. The first indication of their existence came in 1930 when two New York gynecologists (Kurzrok and Lieb, 1930) published that human seminal fluid produced contraction and relaxation when applied to isolated strips of human uterus. A few years later Goldblatt (1933) and Von Euler (1935) independently described the muscle contractile activity of human seminal

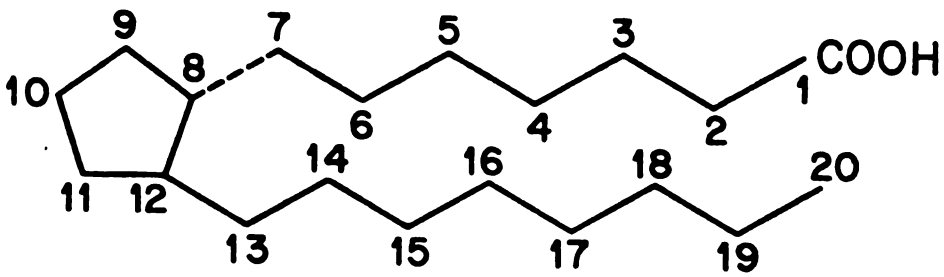
plasma, and in a subsequent report Von Euler (1935) associated this activity with an acidic lipid which he named prostaglandin. This was a misnomer since the prostate gland contains little prostaglandin; the highest concentration is found in the seminal vesicle.

Further research was delayed by the lack of sufficiently sophisticated scientific methods and instrumentation to proceed with the isolation and characterization of these acidic lipids. In the late 1950s after mass spectrophotometric and chromatographic techniques came available, Bergstrom and coworkers collected sheep vesicular glands for extraction and identification of prostaglandins. This culminated with Bergstrom et al. (1962) illucidating the chemical structures of three naturally occurring prostaglandins, and generated new interest concerning the physiological and pharmacological properties of these fatty acid derivaties.

Prostaglandin Nomenclature

The term prostaglandin is as general as steroid. Like the steroids, all prostaglandins share a basic structure. Usually they are abbreviated PG, with the chemical names of the various PGs derived from a hypothetical molecule, prostanoic acid. All natural PG possess the same basic 20-carbon structure with a cyclopentane ring, the carbon atoms numbered as in Figure 1, with substituents above the plane of the ring designated as alpha and those

Figure 1. Structure of prostanoic acid.


9
7
5
3
COOH
10
8
1
6
4
2
14
16
18
20
11
12
13
15
17
19

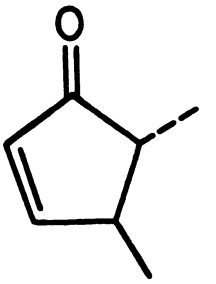
below as beta. There are 4 major groups of prostaglandins identified by the letters E, F, A, and B. Each letter corresponds to a particular ring structure (Figure 2). The numerical subscripts, which follow the letter, indicate the degree of unsaturation in the alkyl and carboxylic side chains. All naturally occurring prostaglandins contain an alpha hydroxyl group at C-15 and a trans double bond between C-13 and C-14.

Prostaglandins and Luteolysis

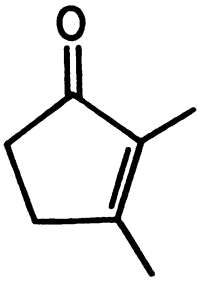
As previously mentioned, Babcock suggested as early as 1966 that prostaglandin might be the uterine luteolysin. However, interest in $PGF_{2\alpha}$ as a luteolysin began in earnest with a report on the hypertensive effect of $PGF_{2\alpha}$ (Ducharme et al., 1968) which indicated that $PGF_{2\alpha}$ constricts primarily veins with little effect on arteries. This effect fitted an hypothesis which suggested that local uterine control of the corpus luteum involved changes in blood flow through the utero-ovarian vein and ovarian artery (between a uterine horn and its adjacent ovary), and that secretion of a venoconstrictor substance from the uterus could restrict blood flow in the utero-ovarian vein and thereby reduce ovarian perfusion and thus induce luteolysis (Pharriss and Wyngarden, 1969, and Pharriss, 1970).

Pharriss chose $PGF_{2\alpha}$ to test this hypothesis since it has potent venoconstrictor properties (Ducharme et al.,

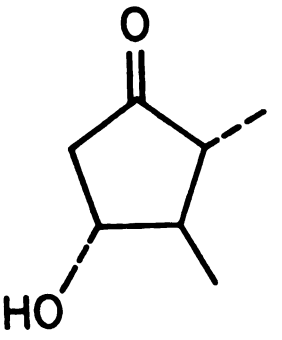
Figure 2. Cyclopentane Rings of Prostaglandins.



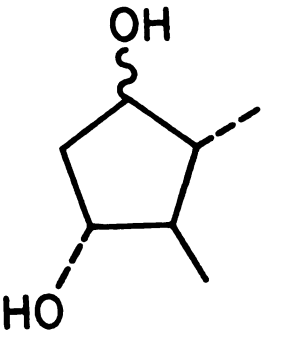
"A"



"B"



"E"



"F"

1968) and it was present in the uterine endometrium (Pickles, 1967). In these preliminary experiments, Pharriss and Wyngarden (1969) infused $PGF_{2\alpha}$ into pseudo-pregnant rats for 48 hours and $PGF_{2\alpha}$ induced luteolysis effectively. Similar effects were observed in the hysterectomized hamster (Hansel, 1969) in the hysterectomized guinea pig (Blatchley and Donovan, 1969) and in sheep ovaries autotransplanted to the vessels of the neck (McCracken et al., 1970).

$PGF_{2\alpha}$ caused luteal regression *in vivo* in every species of mammal tested, except humans, ferrets and pigs (reviewed by Poyser, 1973). In contrast, studies undertaken to describe the mechanism of luteolysis, $PGF_{2\alpha}$ did not inhibit progesterone synthesis *in vitro* and is in keeping with a possible vascular effect (Speroff and Ramwell, 1970). However, there is some evidence which indicates that *in vivo* and *in vitro* effects are not disparate. For instance, when McCracken et al. (1970) infused $PGF_{2\alpha}$ directly into the sheep ovaries, ovarian venous progesterone initially increased and then fell rapidly. Moreover, Demers et al. (1972) reported that $PGF_{2\alpha}$ did inhibit progesterone production when they incubated corpora lutea in vitro for 6 hours, 4 hours longer than used in previous studies wherein $PGF_{2\alpha}$ was not luteolytic, but it should be pointed out that Demers et al. (1972) unfortunately did not include a 2-hour control so this effect could be due to other factors besides the time of incubation.

As one explanation for the mechanism of luteolytic action, Behrman et al. (1971) proposed the $PGF_{2\alpha}$ may exert its luteolytic activity by inhibiting the enzyme cholesterol ester synthetase in rats, thus restricting the amount of sterol available for progesterone synthesis. On the other hand, Novy (1972) provided evidence using radioactive microspheres that prostaglandins may cause a regional redistribution of blood flow through the rabbit ovary without causing consistent changes in total ovarian blood flow. Using similar techniques, Thorburn and Hales (1972) demonstrated a selective reduction in blood flow through the sheep corpus luteum after giving $PGF_{2\alpha}$. In overview, there is evidence for both a biochemically mediated change in progesterone synthesis during luteolysis and evidence for redistribution of blood flow in the ovary; which results in shunting blood away from the corpus luteum. Possibly both take place during luteolysis.

Prostaglandin $F_{2\alpha}$--the Uterine Luteolytic Hormone?

In the two species most studied so far (sheep and guinea pig), the uterine venous blood contained higher concentrations of PGF during luteal regressions 2 or 3 days before estrus than at other periods during the cycle (Bland et al., 1971; McCracken et al., 1971; Blatchley et al., 1972). Sheep and guinea pig uteri also contain higher concentrations of $PGF_{2\alpha}$ and obtained highest $PGF_{2\alpha}$ synthetic activity during proestrus (Wilson et al., 1972

and Poyser, 1972). Recently, Poyser (1973) found that when indomethacin, a blocker of Prostaglandin synthesis, was placed in the guinea pig uterus in a slow release medium, it prevented luteal regression; progesterone remained elevated and the duration of the interestrual interval was greatly prolonged.

Estrogen may stimulate the release of uterine $PGF_{2\alpha}$. For instance, single injections of estradiol-17β late in the guinea pig cycle stimulated uterine $PGF_{2\alpha}$ (Blatchley et al., 1971), and estrogen secretion increases at the time of luteolytic release of uterine $PGF_{2\alpha}$ in sheep (Smeaton and Robertson, 1971). X-irradiation (Karsch et al., 1970) or mechanical destruction (Ginther et al., 1970) of follicles (and thus concomitant estrogen secretion) retards luteolysis in cows and ewes. This provides further support for the obligatory role of estrogen in normal luteal regression. Caldwell et al. (1972) found that peripheral PGF did not increase in response to an estrogen injection in ovariectomized ewes unless they were pretreated with progesterone. Infusion of physiological amounts of estradiol-17β (1 ng/min) in the uterine artery of ewes in diestrus resulted in at least a ten-fold increase of PGF in uterine vein blood, beginning 1-2 hours after the start of the infusion (McCracken et al., 1973).

Pharriss's venoconstrictor theory, which states that PGs may possibly be the uterine luteolysin which passes through the utero-ovarian vein into the adjacent

ovarian artery is attractive since it might explain how $PGF_{2\alpha}$ released from the uterus arrives in the ovary without passing through the systemic circulation. This local transfer is essential since PGs are rapidly metabolzied; 90% being removed in one pass through the lung (Piper and Vane, 1969). In support of this hypothesis, McCracken et al. (1971) infused $^{3}H\text{-}PGF_{2\alpha}$ into the uterus; radioactivity in the ovarian artery was 30 times that in the internal iliac artery. To explain these data, they proposed a "countercurrent mechanism" by which $PGF_{2\alpha}$ is actively transferred from the utero-ovarian vein into the ovarian artery. Thus, $PGF_{2\alpha}$ released from the uterus could arrive in the ovary in high concentration and cause luteolysis. In further support of this hypothesis, Barrett et al. (1971) found that surgical separation of the utero-ovarian vein and ovarian artery in sheep prevented lutel regression. However, the notion that uterine luteolysin is transferred directly from utero-ovarian vein to the ovarian artery (Pharriss, 1970; McCracken, 1971) has come under criticism. For instance, Coudert et al. (1974) showed that neither a single injection nor infusion of $^{3}H\text{-}PGF_{2\alpha}$ led to appreciable transfer of radioactivity into the ovarian artery. These carefully controlled experiments on a relatively large number of sheep showed conclusively that diffusion, filtration, counter-current exchanges and active transport do not occur between the anterior uterine vein or the utero-ovarian vein and the ovarian artery.

One possible explanation of the difference between results reported by McCracken et al. (1971) and Coudert et al. (1974) is that the Coudert technique prevents recirculation of radioisotope by draining the utero-ovarian venous blood out of the abdomen. In this way, any appearance of radioactivity in the ovarian artery cannot be accounted for by contamination due to recirculation but must be due to some local transfer mechanism.

I conclude that a complete explanation for the luteolytic action of $PGF_{2\alpha}$ and its possible role as the uterine luteolysin must await further studies. While the above mentioned studies have not delineated prostaglandins physiological role as the uterine luteolysin, there was ample evidence at the time I began this dissertation that $PGF_{2\alpha}$ was luteolytic in a number of different species (reviewed by McCracken et al., 1973). However, I found no reports of luteolysis after $PGF_{2\alpha}$ treatment in cattle. In view of the potential significance of effective ovulation control in cattle artificial insemination, I undertook this dissertation research.

MATERIALS AND METHODS

Experimental Objectives

This dissertation was designed to study whether intrauterine or systemic administration of $PGF_{2\alpha}$ would cause luteolysis in cows during various phases of the estrous cycle. It was my intent to determine the temporal relationships between changes in hormone secretion (progesterone, estradiol and luteinizing hormone), estrous behavior and ovulation after $PGF_{2\alpha}$, and to compare these with the comparable events during normal luteolysis. As a practical adjunct to the reproductive and endocrine studies, I also compared fertility of cattle inseminated during the estrus induced with prostaglandin $F_{2\alpha}$ with that of control cattle.

Four experiments were conducted.

Experiment 1

Luteolysis after intrauterine administration of prostaglandin $F_{2\alpha}$

At the outset, I was unaware of any report that $PGF_{2\alpha}$ was luteolytic if given systemtically. In view of the possible local transfer of luteolysin from uterine vein to ovarian artery and given the limited supply of

$PGF_{2\alpha}$, I administered it into the uterus to maximize chances of detecting an effect in the first experiment.

The first experiment was designed to determine whether $PGF_{2\alpha}$ caused luteolysis in cows during different phases of diestrus. The sequence of changes in blood serum progesterone, estradiol, and LH, and estrous behavior and ovulation after $PGF_{2\alpha}$was compared with comparable changes during control estrous cycles of the same cows.

Six primiparous nonlactating Holstein cows were housed in stanchions and observed for signs of estrus twice daily in a lot with other cows. After it was established that each cow had normal estrous cycles, on day 11 after estrus, 5 mg $PGF_{2\alpha}$ (Tham salt[1] dissolved in 0.5 ml saline) was deposited through an 18 inch blunt 17 ga needle into the lumen of the uterine horn ipsilateral to the corpus luteum. Blood was withdrawn from a jugular cannula at 6-hour intervals until a cow showed signs of proestrus, then at 2-hour intervals until estrus, at 6-hour intervals thereafter until ovulation, and then on days 2, 4, 7, 11, and 15 and twice daily from day 18 through a control estrus. Corpus luteum diameter and ovulation were monitored by palpation of the ovaries twice daily from $PGF_{2\alpha}$ treatment until ovulation and at weekly intervals thereafter. With intervening control estrous cycles between each $PGF_{2\alpha}$ treatment, the same cows were given

---

[1]Supplied by Dr. J. W. Lauderdale, The Upjohn Company, Kalamzoo, Michigan.

5 mg $PGF_{2\alpha}$ into the ipsilateral uterine horn on day 15 and on day 7 of the estrous cycle, and five of the six cows were given 5 mg $PGF_{2\alpha}$ into the contralateral uterine horn on day 11. Since the four treatments were applied sequentially, treatment effects were confounded with time. This confounding should not have affected the major conclusions, because luteal function in cows does not differ greatly among the fall and winter months (Trimberger, 1948) when this research was conducted.

Sera were assayed for LH, estradiol and progesterone using radioimmunoassay procedures which are outlined in the steroid and protein hormone assay procedures below.

Since repeated measurements were made within animals, a split plot statistical analysis was used (Gill and Hafs, 1971). Analysis of variance of each criterion of response in this experimental also included predetermined orthogonal contrasts within 3 degrees of freedom for the four $PGF_{2\alpha}$ treatments as follows: (1) treatment in ipsilateral uterine horn vs. contralateral horn (1 degree of freedom) and (2) among the three ipsilateral horn treatments (2 degrees of freedom). To test for carryover of the $PGF_{2\alpha}$ effect on subsequent control estrous cycles, the cycle initiated by each treatment with $PGF_{2\alpha}$ was compared with the following control cycle and the three control cycles were compared with each other by analysis of variance for each criterion.

Experiment 2

Progesterone, LH, estrus and ovulation after intramuscular prostaglandin $F_{2\alpha}$ in heifers

From a practical point of view, intrauterine administration of $PGF_{2\alpha}$ may be difficult or ill advised under many farm conditions. Therefore, the principal objective of the second experiment was to determine if $PGF_{2\alpha}$ could be given systemically in large doses (30 mg Tham salt) either im or intravaginally to heifers during diestrus. Also, for comparison, $PGF_{2\alpha}$ was injected into heifers on day 3 of the cycle before the corpus luteum is fully formed and functional.

Thirty mg prostaglandin $F_{2\alpha}$ Tham salt ($PGF_{2\alpha}$) in 1.5 ml saline was injected im into each of five heifers during diestrus (8-14 days after estrus) and into six heifers during metestrus (3 days after estrus). In another six heifers during diestrus (10-14 days after estrus), 30 mg $PGF_{2\alpha}$ in 1.5 ml saline was deposited in the vagina adjacent to the cervix. The heifers were observed twice daily for signs of behavioral estrus, and corpus luteum diameter and ovulation were monitored by daily palpation. Heifers were bled by jugular puncture at 12-hour intervals until onset of estrus and on days 2, 4, 7, and 11 after the estrus induced by $PGF_{2\alpha}$, or at 12-hour intervals for 5 days if estrus was not induced by $PGF_{2\alpha}$. Sera were assayed for LH, and progesterone using

radioimmunoassay procedures which are outlined in the steroid and protein hormone procedure section below.

As in experiment 1, a split plot repeat measurement statistical analysis was used (Gill and Hafs, 1971). Analysis of variance of each criterion of response in this experiment also included predetermined contrast with 1 degree of freedom for the three $PGF_{2\alpha}$ treatment as follows: (1) treatment on day 3 im vs day 11 im (1 degree of freedom) and (2) treatment on day 11 im vs. vaginal on day 11 (1 degree of freedom).

Experiment 3

Acute changes in progesterone and estradiol after $PGF_{2\alpha}$

The first two experiments were designed principally to describe the overall pattern of luteolysis, and determine the intervals to estrus and ovulation after $PGF_{2\alpha}$. They were not intended to look at the possible acute changes in gonadal hormones after $PGF_{2\alpha}$. Since during the course of the first two experiments I became aware of a report of increased progesterone synthesis when bovine corpora lutea were incubated with $PGF_{2\alpha}$ *in vitro* (Sellner and Wickersham, 1970), I speculated that a part of the luteolytic action of $PGF_{2\alpha}$ *in vivo* may involve a brief surge in progesterone possibly exhausting the corpora luteas supply of progesterone precursor before the rapid fall observed in the two previous experiments. Another possibility was that injected $PGF_{2\alpha}$ may effect luteolysis

indirectly through a follicular action resulting in a brief surge in blood estrogen. A secondary objective of this experiment was to determine if a larger dose (60 mg) or repeated injections of a smaller dose (15 mg) of $PGF_{2\alpha}$ Tham salt at 6-hour intervals would have different effect than 30 mg im on the rate of decline of progesterone or rate of increases in estradiol. Consequently, acute changes in blood progesterone and estradiol were determined from samples obtained in the following experimental regime.

Holstein heifers in diestrus were given (a) an im injection of 30 mg $PGF_{2\alpha}$ dissolved in 2 ml sterile saline (six heifers); (b) an im injection of 15 mg $PGF_{2\alpha}$ in 2 ml saline followed 6 hours later by a second im injection of 15 mg (four heifers); or (c) an im injection of 60 mg $PGF_{2\alpha}$ in 2 ml saline (six heifers). Blood was collected from jugular cannulae immediately before $PGF_{2\alpha}$ injections. After $PGF_{2\alpha}$ injections blood was collected at 10-min. intervals for 1 hour, and then at 1.5, 2, 4, 6, 12, and 18 hr. and assayed for determination of the acute changes in progesterone and estradiol during $PGF_{2\alpha}$-induced luteolysis. Blood was assayed for estradiol and progesterone using radioimmunoassay procedures which are outlined in steroid and protein assay hormone procedures below.

Again as in experiments 1 and 2 a split plot repeat measurement statistical analysis was used. It should be pointed out that since the statistical analysis in experiments 1, 2, and 3 involved repeatment measurement the

probability of type 1 error may be artificially low. This over estimation of statistical significance is caused by the high correlation of errors associated with heterogeneity of the pattern of variance and covariance of samples taken at several sampling points on the same subject. In other words, the actual type I error rate may exceed the nominal type I error if measurements on the same animal adjacent in time are more highly correlated than those taken at a point more distant in time.

Experiment 4

Fertility of cattle following $PGF_{2\alpha}$ injection

Since the major potential practical application of $PGF_{2\alpha}$ was synchrony of ovulation to improve efficiency of artificial insemination, I conducted two trials to determine fertility of cattle inseminated at the estrus which followed $PGF_{2\alpha}$ treatment.

In the first trial with 25 Holstein heifers, 13 controls were inseminated at 12 hours after onset of estrus and 12 were inseminated without regard to onset of estrus at 72 and 90 hours after im injection of 30 mg $PGF_{2\alpha}$. The heifers were palpated for pregnancy at 40 days after insemination.

In the second trial, 66 crossbred beef cattle with palpable corpora lutea were assigned at random to be inseminated (1) without treatment at 12 hours after onset of control estrus during an 18 to 21 day period, (2) at

12 hours after onset of estrus detected during a 7-day period following injection of 30 mg $PGF_{2\alpha}$, or (3) twice without regard to estrus at 72 and 90 hours after injection of 30 mg $PGF_{2\alpha}$. Pregnancy was determined by palpation at 35 to 60 days after insemination. The Chi-square contingency table for difference between means (Li, 1964).

Blood Collection and Hormone Assay Procedures

The procedural details for collecting and processing blood and for radioimmunoassay for progesterone, estradiol and LH are described in this section.

Blood Collection and Handling

In the 1st and 3rd experiments, blood was obtained through jugular cannula. In prepartion for cannulation, needles and cannulae were soaked in 70% ethanol for 6 hours before use. The cannulation region on the neck was scrubbed and wetted with tincture of iodine. Twenty-four hours before $PGF_{2\alpha}$ treatment, the jugular vein was punctured with a 2 inch 13 ga thin wall stainless steel needle. Then a 24-inch (Bolab V10, 1.57 x 2.08 MM) length of poly vinyl chloride medical grade cannula (Bolab, New Hampshire) was introduced through the needle and passed down the jugular vein until 6 inches remained exposed. The needle was then removed and a 1-inch piece of Elastoplast (Duke Lab, Hartford, Conn.) tape was applied around the catheter with the adhesive side applied to the neck

to keep the cannula from moving. The exposed part of the catheter was then passed through a 1 cm^2 hole in the middle of a piece (3 x 5 inches) of Elastoplast tape. Tag Cement (Allweather Cement Co., Lincoln, Neb.), applied to the neck around the puncture and to the back of the tape was used to fix this piece of tape on the neck. A second piece of Elastoplast (3 x 5 inches) was then applied adhesive side down to hold the exposed cannula in place. Then the cannula was flushed with 3.5% sodium citrate and plugged with a blunted 16 ga needle with a cannula adapter. To sample blood, one could remove the plug and discard the first 5 ml of Na citrate and blood, collect a sample and then flush the cannula with 3.5% sodium citrate and replace the plug.

The blood was transferred to polypropylene centrifuge tubes, kept at room temperature for 2 to 4 hours and transferred to 5°C for 1 to 2 days to allow clotting. Within 2 days, the blood was centrifuged at 6500 x g for 15 minutes and the serum transferred to 7-dram plastic vials and stored at -20°C until assay for hormones.

Radioimmunoassay Procedures

Radioimmunoassay (RIA) of Luteinizing Hormone (LH).

--The bovine LH assay has been described elsewhere (Oxender, 1972a; Oxender et al., 1972b). The details of the procedure, including some minor changes, are as follows:

LH antiserum was developed by repeated injections of NIH-LH-B5 into guinea pigs (Appendix I.C.1). Purified bovine LH (LER-1072-2) used for iodination was supplied by Dr. L. E. Reichert, Jr. (Emory University, Atlanta, Georgia). This preparation had an LH potency of 1.66 NIH-LH-Si units/mg and showed no FSH activity when tested at 3600 ug in the Steelman-Pohley assay. It had a thyroid stimulating hormone (TSH) contamination estimated at 0.021 USP units/mg.

Radioiodination--Purified bovine LH (LER-1072-2) had been previously dispensed into 1 ml vials (2.5 ul of a 1 ug/ul solution in glass distilled water) and stored at -20°C. These vials were thawed immediately before iodination and iodination was performed at room temperature. Twenty-five ul of 0.5 M sodium phosphate buffer at pH 7.5 (Appendix I.A.1) was added to the hormone and mixed. One mCi of ^{125}I (80-140 mCi/ml, Amersham/Searle Corporation, Arlington Heights, Illinois) was added, and the contents were mixed gently.

Forty ug chloramine-T (Appendix I.A.3, Eastman Organic Chemicals, Rochester, New York) was added to the vial, the vial was stoppered, and the contents were gently mixed by finger tapping. The reaction was stopped at exactly 2 minutes by adding 125 ug sodium metabisulfite (Appendix I.A.4). After thorough mixing, 25 ul of 2.5% bovine serum albumin (BSA, Nutritional Biochemicals, Inc., Cleveland, Ohio) in 0.01 M phosphate buffered saline (PBS)

pH 7.0 (PBS-2.5% BSA) was added to minimize the loss of hormone adhering to the glass vial.

A 1x12 cm glass column packed with Bio Gel P-60 (Bio Rad Labs., Richmond, California) was equilibrated previously by flushing with 0.05 M sodium phosphate buffer pH 7.5 (Appendix I.A.2), and then 2 ml PBS-2.5% BSA was added and eluted with buffer to reduce non-specific binding of the protein hormone to glass. One hundred ul of transfer solution (Appendix I.A.5) was added to the vial with iodinated LH and the contents of the vial were layered beneath the buffer on the surface of the column. Seventy ul of rinse solution (Appendix I.A.6) was added to the hormone vial, recovered, and also layered beneath the buffer on the column. The iodinated LH was eluted from the column under gravity with 0.05 M sodium phosphate buffer and 15 ml were collected in 1 ml aliquots from the column in 12x75 mm disposable glass tubes containing 1 ml of 2% Knox gelatin in PBS (Appendix I.B.4). The elution profile was determined by quantifying the radioactivity of 10 ul from each of the 15 tubes in an automatic gamma counter (Nuclear Chicago Corp., Des Plaines, Illinois). The first column peak of radioactivity represented iodinated LH and the second peak represented free ^{125}I. The peak ^{125}I-LH tube was used in the LH RIA. The iodinated LH was quite stable; stored at 4°C, it could be used up to 2 weeks.

Radioimmunoassay--Each unknown was assayed in dilution duplicate (50, 100 or 200 ul). Two selected

dilutions (50, 100 or 200 ul) made in PBS-.1% Knox gelatin (Appendix I.B.4) of each unknown were added to disposable glass culture tubes (12x75 mm) with a Hamilton microliter syringe (Hamilton Co., Whittier, California). PBS with 0.1% Knox gelatin was then added to give a total of 500 ul. As discussed by Hunter (1967), use of two dilutions provided evidence of the specificity of the assay which is not provided with duplicate determinations of the same dilution. Each lot of 100 tubes included 10 tubes containing 0, 0.8, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, and 20.48 ng of standard LH (NIH-LH-B8, National Institutes of Health, Endocrinology Study Section, Bethesda, Maryland; Appendix I.B.5).

Two hundred ul of LH antibody (Appendix I.B.7), hereafter referred to as first antibody, was added at a dilution of 1:200,000 to each of the assay tubes and the tubes were incubated at 4°C for 24 hours. The stock ^{125}I-LH was diluted with PBS containing 0.1% Knox gelatin so that 100 ul contained about 200,000 CPM. One hundred ul of ^{125}I-LH solution was then added to each tube. Incubation was continued at 4°C for 24 hours.

Sheep anti-guinea pig gamma globulin (SAGPGG, Appendix I.C.2), later referred to as the second antibody, was diluted to a titer which would optimally precipitate the guinea pig gamma globulin. The second antibody formed an antigen-antibody-antibody complex large enough to be precipitated by centrifugation. Two hundred ul of SAGPGG

was added to each tube and incubation continued for 72 hours. After each addition, the tubes were vortexed gently and covered during the incubation to retard evaporation.

Following the final incubation, 3 ml of cold PBS (Appendix I.B.1) was added to each tube to dilute the unbound ^{125}I-LH. The antibody bound ^{125}I-LH was precipitated by centrifugation at 2500 g for 30 minutes in a refrigerated centrifuge with a swinging bucket rotor (Sorvall Model RC-3, Ivan Sorvall, Inc., Norwalk, Connecticut). The supernatant fluid was decanted and the tubes allowed to drain for 30 minutes and remaining fluid adherent to the neck and lip of the tube was removed with absorbent tissue. The bound ^{125}I-LH of the precipitate was then quantified in an automatic gamma counter. Samples usually were counted for 10 minutes or for a total of 4000 or 10,000 counts, whichever accumulated first. This information was punched automatically on paper tape by a Teletypewriter (Teletype Corp., Skokie, Illinois). The standard curve was calculated by multiple regression analysis on a CDC 3600 computer. The values for standard LH assay fit linear, quadratic and cubic components of the regression equation. These regression coefficients were entered manually into an Olivetti computer (Programma 101, Olivetti Underwood, New York, New York). The counting time for each unknown was entered into the computer from the punched tape through a Punched Tape Editor (Beckman Model 6912 Tape Editor,

Beckman Instruments, Inc., Fullerton, California), and LH concentrations in the unknown were computed automatically.

Control tubes were included in each assay to determine background radioactivity (tube containing 1:400 control guinea pig serum in place of the first antibody), total counts added (tube containing only ^{125}I-LH) and counts in the precipitate (tube containing no unknown or standard). Values for the duplicate standards were averaged and plotted as the percent of ^{125}I-LH precipitated at each dose of LH standard.

Oxender et al. (1972b) reported that the assay detected as little as 0.1 ng LH (NIH-LH-B5). Mean recoveries of 0.1, 0.5, 2.0 and 8.2 ng of bovine LH added to 100 ul of serum were 0.1, 0.4, 1.9 and 8.4 ng, although precision was reduced at the high level.

Radioimmunoassay (RIA) of Progesterone.--At the outset of the first experiment, progesterone was quantified by the radioimmunoassay described by Kittok et al. (1973), but this progesterone assay described by Kittok was laborious; only 30-40 unknowns could be completed daily. A more efficient procedure was desirable because of the large numbers of samples in this research. Thus, the following procedure for RIA of progesterone (Louis et al., 1973) was developed whereby more than 150 unknowns could be assayed within 1 day.

Aliquants (100 ul) of each unknown were placed in two disposable extraction tubes (15x85 mm). To account for procedural losses, 3,000 dpm ^{3}H-progesterone (80-100 Ci/mmole) was added to a third aliquant from a representative number (10 to 20 within each assay) of unknowns. Each tube was vortexed with 2 ml benzene-hexane (1:2) for 30 seconds, then stored at -20°C for at least 1 hour to freeze the aqueous phase. The organic solvent from the tube with ^{3}H-progesterone was decanted into 10x75 mm disposable culture tubes for radioimmunoassay. The solvent was evaporated and 200 ul antibody[2] (diluted 1:4500 in 1:400 normal rabbit serum in 0.1 M phosphate-buffered saline, pH 7.1) (Appendix I) was added to each tube. Two sets of standard tubes containing 0, 25, 50, 100, 200, 500, 1000, 1500, 2000 pg progesterone was included in each assay and treated similarly to unknowns. After addition of antibody, each tube was vortexed 10 sec and incubated 30 minutes at room temperature. Then about 24,000 cpm ^{3}H-progesterone (1, 2, 6, 7, ^{3}H-progesterone, 80-100 Ci/mmole diluted in 200 ul in 0.1% Knox gelatin in 0.1 M phosphate-buffered saline, pH 7.1) (Appendix I.4.B) was added to each tube, and the tubes were vortexed 10 seconds and incubated at

---

[2]The rabbit antiprogesterone prepared against 6--succinylprogesterone conjugated to bovine serum albumen was supplied by G. D. Niswender, Dept. of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado.

4°C for 4-20 hours. To separate free from antibody-bound progesterone, 0.5 ml of dextran-coated charcoal (0.5 g neutral Norit and 1 g Dextran T-70 in 100 ml distilled water) was added at 4°C, and each tube was vortexed for 10 seconds, equilibrated at 4°C for 10 minutes and centrifuged (2,500 g) for 10 minutes at 5°C. Antibody bound ^{3}H-progesterone in 0.5 ml of the supernatant fluid was measured in a liquid scintillation spectrometer (Nuclear-Chicago Mark II). Preliminary experiments revealed little variance in procedural losses (80 $\pm$ 1% extraction efficiency, n=20). Hence the mass of progesterone determined in each unknown was corrected for the average loss of tracer. The sensitivity of this assay was less than 25 pg progesterone; this amount displaced 22 $\pm$ 6% of the ^{3}H-progesterone bound to antibody.

I determined progesterone in aliquants of 12 cow sera by my modified RIA and by the radioimmunoassay described by Kittok et al. (1973); results from the two methods were highly correlated (r=0.94), and the means did not differe significantly (2.13 vs. 2.17 ng/ml). However, the within-sample coefficient of variation for the 12 progesterone determinations by our method was 8%, less than the 36% for the other assay. Among various steroids tested, Niswender et al. (1973) reported that this antiserum cross reacted significantly only with 5-β pregnan-3,20-dione to the extent of 30%.

Radioimmunoassay (RIA) of Estradiol.--Serum estradiol initially was quantified by radioimmunoassay as described by Wetteman et al. (1972). Later during the first experiment, I modified the estradiol assay along the same lines as the progesterone assay to increase the number of unknowns from 30-40 per week to more than 150 a week. Thus, this assay (Hafs et al., 1973) was modified as follows.

Serum duplicates (0.5 ml) were placed in 15x125 mm glass extraction tubes (which had been washed twice with distilled methanol) and vortexed for 1 minute with 3 ml benzene. The solvent fraction was transferred by disposable pipette to 12x85 mm glass culture tubes and the solvent evaporated under nitrogen. Estradiol standards (Sigma Chemical) were dissolved in redistilled absolute ethanol and three sets of 10 standard tubes (0.0, 0.5, 1, 2, 4, 7, 10, 20, 50, and 100 pg estradiol) were incorporated into each assay. Two hundred ul of anti-estradiol[3] (1/4,000) in 0.1% Knox gelatin in 0.01 M phosphate buffered saline (Appendix I.4.B) was added to each tube containing standards and unknowns, and the contents were vortexed for 10 minutes and the tubes incubated at room temperature for 30 minutes. Then 200 ul PBS phosphate (0.01 M) buffered (pH 7.1) saline

---

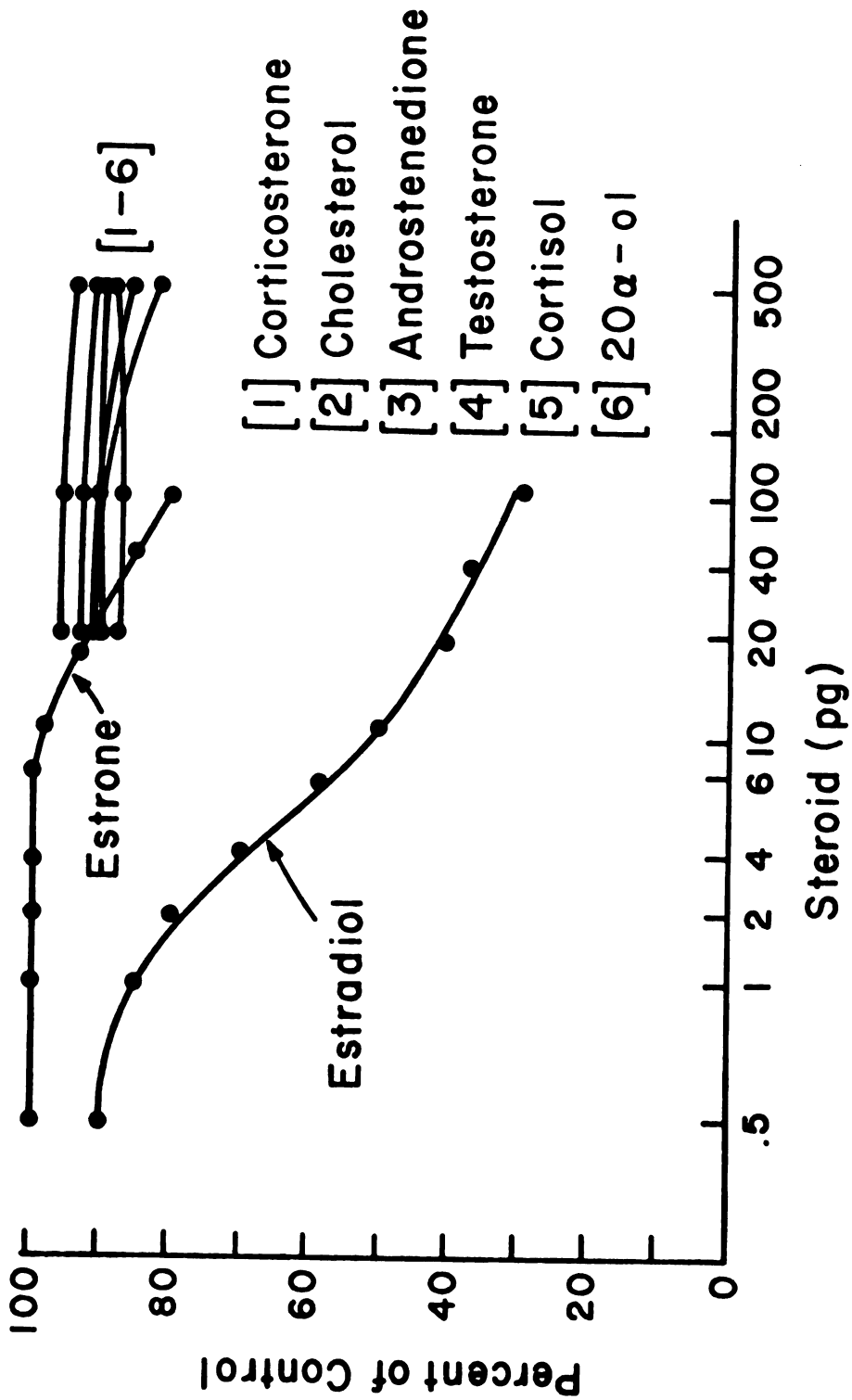
[3]Antiestradiol prepared against 6-oxime estradiol conjugated to bovine serum albumin (rabbit antiserum #825) was generously supplied by Dr. G. D. Niswender, Dept. of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado.

(0.9%) with 0.1% Knox gelatin containing 5000 cpm ^{3}H 1,2,4,6,7,-estradiol was added to each tube, the contents were mixed by vortexing for 10 seconds and the tubes were incubated at 5°C for 3 hours. Five hundred ul of 1% Dextran T-70 and 0.5% neutral Norit in distilled water (Pharmacia, Uppsula, Sweden) was added to each tube and the contents mixed for 30 seconds. Then 0.5 ml of the aqueous phase was transferred to scintillation vials and diluated with liquid scintillation fluid (PCS Solubilizer, Amersham Searle Co.) for quantification of radioactivity in a liquid scintillation spectrometer. Recovery of estradiol was determined by adding to 10 ul (800 cpm) ^{3}H-1,2,4,6,7-estradiol to a third aliquant of a representative number (10 to 20 within each assay) of serum samples, and the ^{3}H-estradiol was allowed to equilibrate with the serum before extraction with benzene. The extracts from these tubes were decanted directly into liquid scintillation vials and radioactivity quantified to determine average procedural losses for that assay was obtained. This extraction procedure recovered approximately 92% $\pm$ 1 of the added estradiol.

To validate this modified estradiol radioimmunoassay, I determined the recovery of estradiol added to steer sera. When 5, 10, and 20 pg of 17-estradiol was added to 1 ml serum samples, 111% $\pm$ 8 of added estradiol was recovered. The sensitivity of the assay was less than 1 pg estradiol which displaced 10 $\pm$ 1% of the ^{3}H-estradiol

bound to the antibody. A standard curve for this assay and the crossreaction (about 2%) with estrone is illustrated (Figure 3). The other steroids tested failed to cross-react detectably.

Figure 3. Standard curve and crossreactions of the radioimmunoassay for estradiol.


Percent of Control
100
80
60
40
20
0
.5
1
2
4
6
10
20
40
100
200
500
Steroid (pg)
Estrone
Estradiol
[1-6]
[1] Corticosterone
[2] Cholesterol
[3] Androstenedione
[4] Testosterone
[5] Cortisol
[6] 20α-ol

RESULTS AND DISCUSSION

Experiment 1

Luteolysis after intrauterine administration of prostaglandin $F_{2\alpha}$

The first experiment was designed to determine whether administration of $PGF_{2\alpha}$ caused luteolysis in cows during different phases of diestrus. The sequence of changes in blood serum progesterone, estradiol, and LH, and estrous behavior and ovulation after $PGF_{2\alpha}$ was compared with comparable changes during control estrous cycles of the same cows.

On the basis of corpus luteum diameter, the rates of luteal involution after the four $PGF_{2\alpha}$ treatments did not differ significantly (Table 1). On the average, more than 70% (Figure 4) of the original luteal volume was lost within 24 hours after administration of $PGF_{2\alpha}$, and a similar portion of the remainder disappeared during the second 24-hour period. The decline in blood progesterone during the first 24 hours was more rapid ($P<.02$) after $PGF_{2\alpha}$ on day 11 than on days 7 and 15 (Table 2), and consequently the interaction between $PGF_{2\alpha}$ treatments and interval after treatment was significant. Apparently the

Table 1.--Corpus luteum diameter after intrauterine $PGF_{2\alpha}$ (5 mg) in cows.

$PGF_{2\alpha}$ treatment		Hours after $PGF_{2\alpha}$			
Uterine horn	Day of cycle	0	24	48	72
		(cm)			
Ipsilateral[a]	7	2.4±2[c]	1.5±.2	0.9±.3	UP[d]
	11	2.7±1	1.7±.1	0.9±.1	UP
	15	2.8±1	1.7±.2	0.9±.1	UP
Contralateral[b]	11	2.3±2	1.6±.2	0.9±.2	UP
Avg.		2.5±1	1.6±.1	0.9±.2	UP

[a]Six cows.

[b]Five cows.

[c]Mean ± SE.

[d]Unpalpable.

Figure 4. Corpus luteum diameter in cows after intrauterine $PGF_{2\alpha}$ (5 mg).

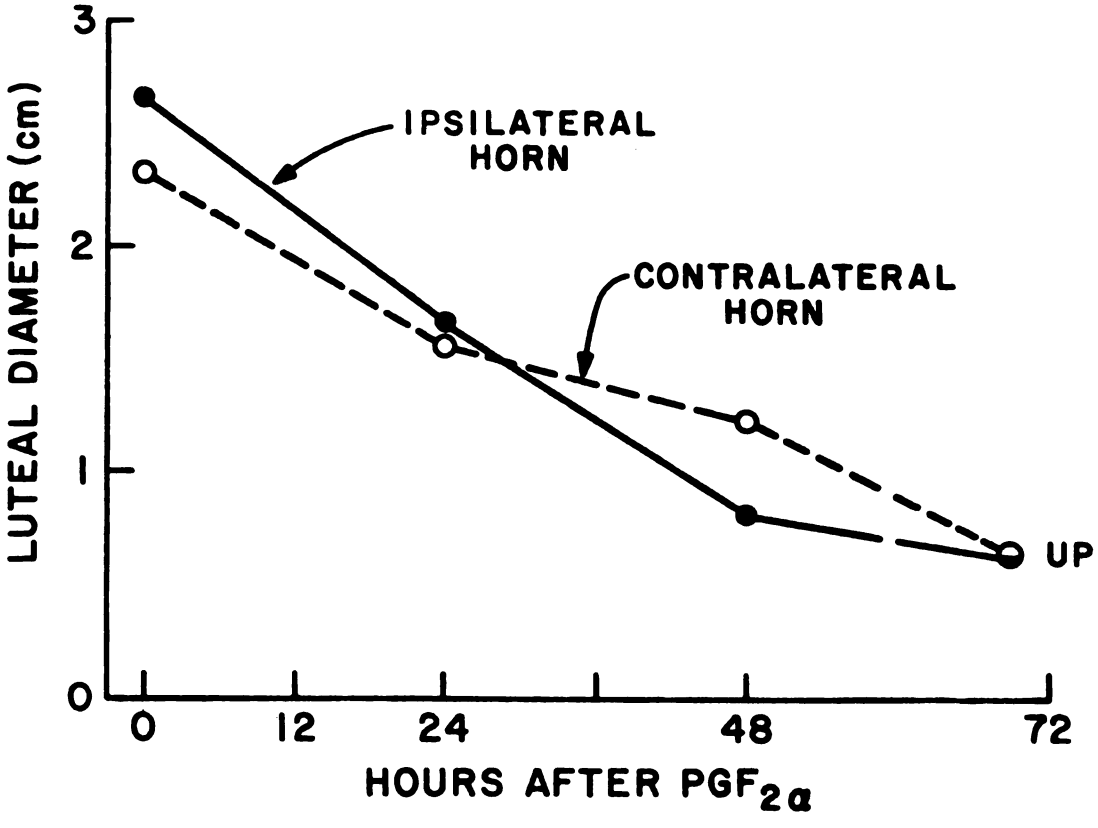

3
2
1
0
LUTEAL DIAMETER (cm)
IPSILATERAL HORN
CONTRALATERAL HORN
UP
0
12
24
48
72
HOURS AFTER PGF2α

Table 2.--Blood serum progesterone after intrauterine $PGF_{2\alpha}$ (5 mg) in cows.

$PGF_{2\alpha}$ treatment		Hours after $PGF_{2\alpha}$				
Uterine horn	Day of cycle	0	12	24	48	72
		(ng/ml)				
Ipsilateral[a]	7	2.6±.6[c]	1.3±.2	1.0±.1	1.2±.2	0.9±.2
	11	4.0±.6	1.5±.2	0.9±.1	0.8±.2	0.8±.1
	15	3.4±.4	2.1±.4	1.9±.5	1.0±.2	1.2±.4
Contralateral[b]	11	4.2±.3	2.0±.3	0.7±.2	0.7±.2	0.7±.1
Avg.		3.6±.3	1.7±.2	1.2±.2	1.0±.1	0.8±.1

[a]Six cows.
[b]Five cows.
[c]Mean ± SE.

corpus luteum of the mid-diestrus (day 11) cow is more sensitive to the luteolytic effects of $PGF_{2\alpha}$ than the corpora lutea of either the early diestrus (day 7) or the late diestrus (day 15) cows. Administration of $PGF_{2\alpha}$ into the contralateral uterine horn on day 11 resulted in a decline in progesterone similar to that after $PGF_{2\alpha}$ in the ipsilateral uterine horn on day 11 (Figure 5). Averaged across the four $PGF_{2\alpha}$ treatments, blood progesterone (Table 2) decreased ($P<.01$) to less than 50% of the original value within 12 hours. Furthermore, the patterns of changes in blood progesterone during the cycles which immediately followed treatment with $PGF_{2\alpha}$ did not differ significantly from those in the subsequent control estrous cycles (Figure 6). Both of the patterns of rising progesterone during metestrus and diestrus resembled data from untreated heifers (Swanson et al., 1972) and cows (Henricks et al., 1972, and Wetteman et al., 1972).

The rapid decline in progesterone after intrauterine $PGF_{2\alpha}$ was similar to that reported by Chamley et al. (1972) in sheep with ovarian transplants that were infused with $PGF_{2\alpha}$. They reported that intraarterial infusion of $PGF_{2\alpha}$ caused a dramatic fall in progesterone secretion to 50% of the control values within 3 hours after beginning infusion of $PGF_{2\alpha}$. In cows, Nancarrow et al. (1974) reported progesterone fell to 50% of initial values within 6-7 hours after the first intrauterine administration of 0.5 mg $PGF_{2\alpha}$. My findings agree with these reports that

Figure 5. Serum progesterone in cows after intrauterine $PGF_{2\alpha}$ (5 mg).

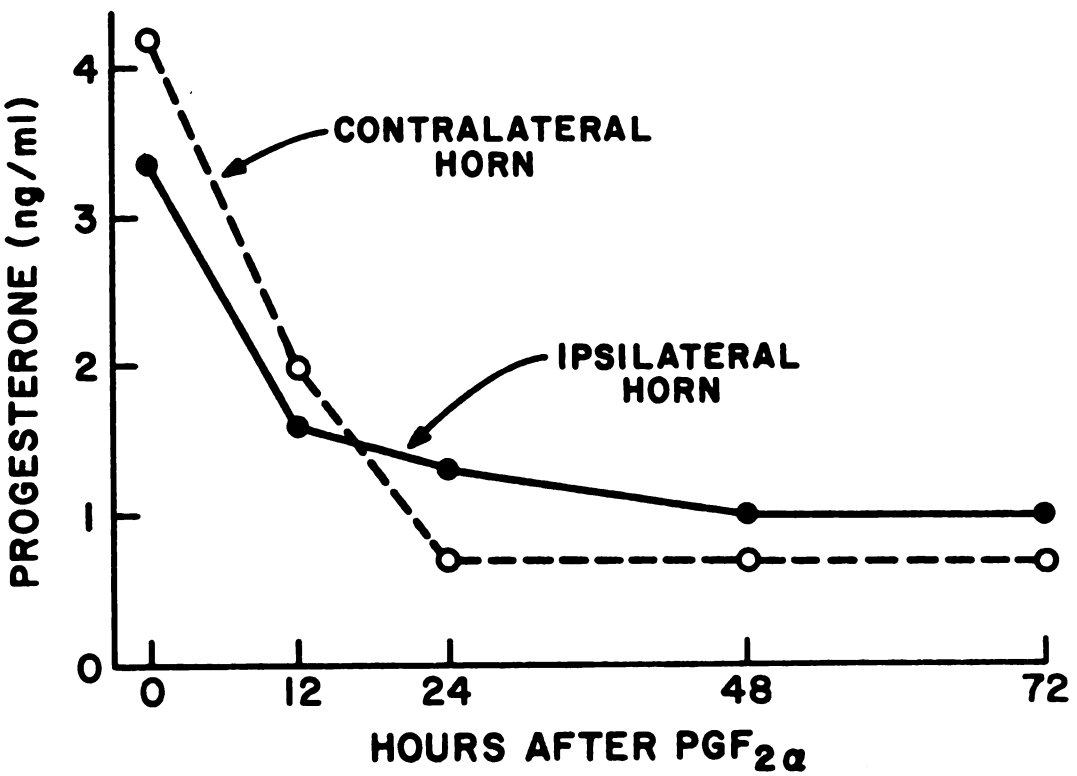
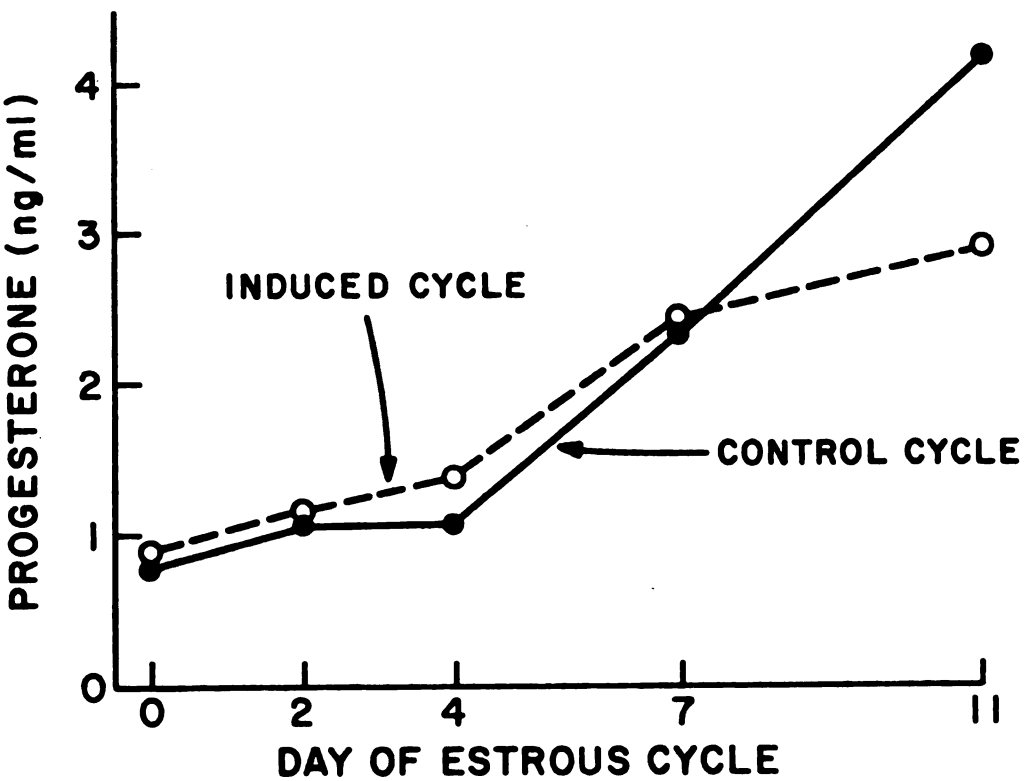

PROGESTERONE (ng/ml)
4
3
2
1
0
CONTRALATERAL HORN
IPSILATERAL HORN
0
12
24
48
72
HOURS AFTER PGF2α

Figure 6. Serum progesterone in cows after $PGF_{2\alpha}$ 5mg, im on day 15 and in control cycles.


PROGESTERONE (ng/ml)
0
1
2
3
4
INDUCED CYCLE
CONTROL CYCLE
0
2
4
7
11
DAY OF ESTROUS CYCLE

progesterone declines most rapidly during the first 12 hours after treatment with $PGF_{2\alpha}$. But, since I sampled blood only every 12 hours, I had no way of determining when the largest decline occurred from the results of this experiment. So future experiments might be indicated to more precisely determine changes in progesterone after $PGF_{2\alpha}$.

Whether $PGF_{2\alpha}$ was deposited in the ipsilateral uterine horn on day 7, 11, or 15, or into the contralateral horn on day 11 of the estrus cycle, blood estradiol doubled ($P<.01$) within 24 hours after $PGF_{2\alpha}$ administration and then increased ($P<.01$) to 15.5 pg/ml of 72 hours, near the onset of estrus (Figure 7). The precipitous fall in blood progresterone after $PGF_{2\alpha}$ administration preceded by about 12 hours the increase in estradiol, similar to comparable data reported by Barrett et al. (1971) in sheep. Moreover, the concentrations of blood estradiol during the estrous cycle which immediately followed treatment with $PGF_{2\alpha}$ did not differ significantly from the subsequent control cycle (Figure 8), whether $PGF_{2\alpha}$ was given on day 7, 11, or 15. On the average, estradiol fell from a peak 19.3 pg/ml 12 hours after the onset of estrus following $PGF_{2\alpha}$ to 14.8 and 6.7 pg/ml at day 2 and 4 of the control cycle, respectively. These values were similar to those reported by Wetteman et al. (1972) in untreated heifers.

Regarding the equivalent luteolysis of $PGF_{2\alpha}$ in ipsilateral or contralateral uterine horns, the 5 mg $PGF_{2\alpha}$

Figure 7. Serum estradiol in cows after intrauterine $PGF_{2\alpha}$ (5 mg).

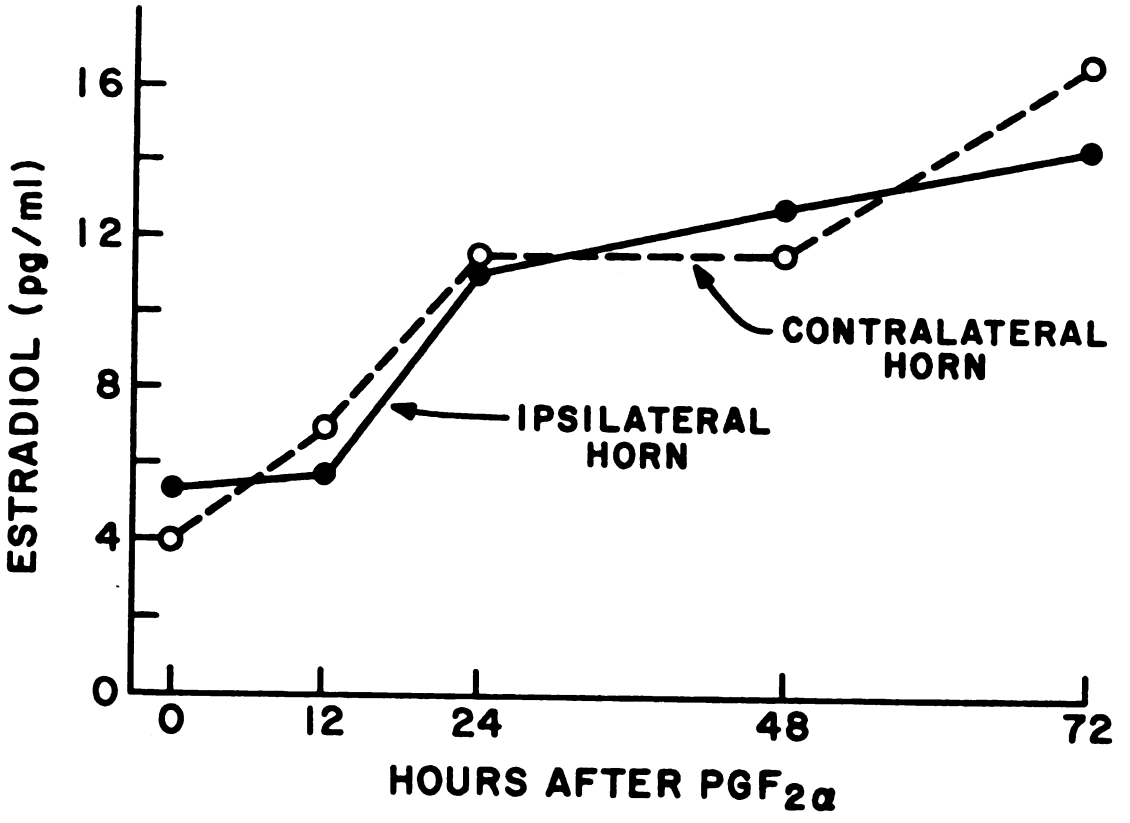
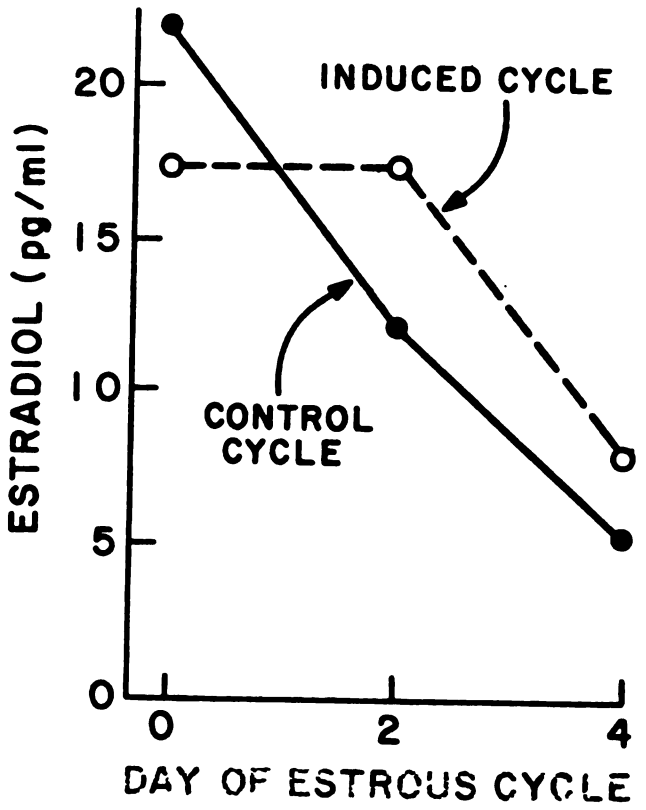

ESTRADIOL (pg/ml)
16
12
8
4
0
0
12
24
48
72
HOURS AFTER PGF2α
CONTRALATERAL HORN
IPSILATERAL HORN

Figure 8. Serum estradiol in cows after $PGF_{2\alpha}$ (5 mg i.u.) and in control cycles.


INDUCED CYCLE
CONTROL CYCLE
ESTRADIOL (pg/ml)
20
15
10
5
0
0
2
4
DAY OF ESTROUS CYCLE

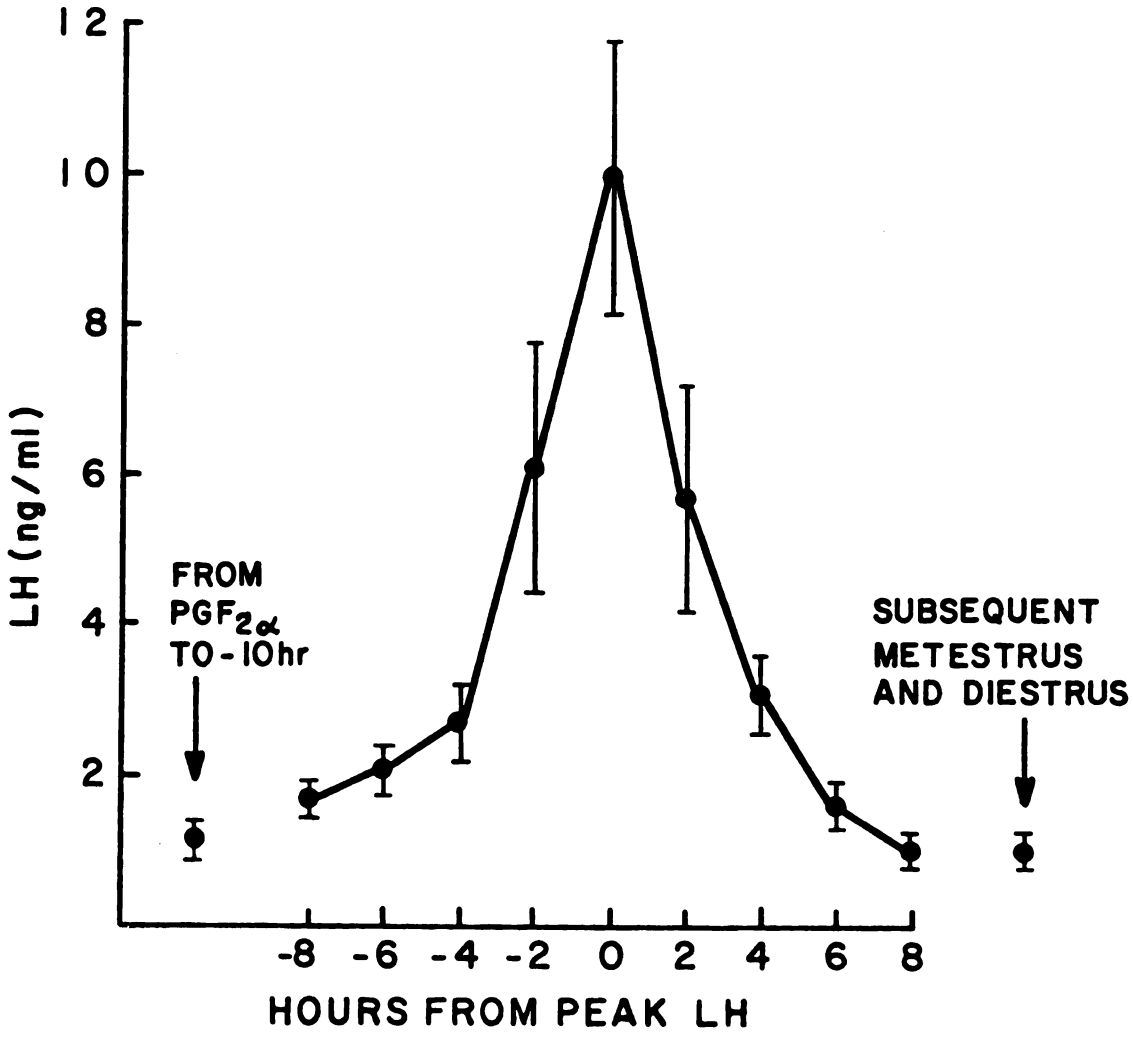
deposited into the contralateral uterine horn probably acted locally on the corpus luteum of the contralateral ovary, because large doses which have been tested subsequent to this experiment, are required for effective luteolysis when $PGF_{2\alpha}$ is administered systemically (Stellflug et al., 1974). Possibly the $PGF_{2\alpha}$ deposited in the contralateral horn was transferred from the contralateral utero-ovarian vein to the ipsilateral utero-ovarian vein through the venous shunts found in the area of the uterine horns (Ginther and Delcampo, 1974). Alternatively, regardless of site of deposition, the 0.5 ml saline used as a carrier for the $PGF_{2\alpha}$ may have been distributed rapidly carrying the $PGF_{2\alpha}$ throughout both uterine horns as a consequence of normal uterine contractions or result of the smooth muscle contractile properties of $PGF_{2\alpha}$ (reviewed by Bergstrom et al., 1968). Recently, Hixon and Hansel (1974) confirmed these results. They found equivalent concentrations of exogenous PGF in the ipsilateral and contralateral utero-ovarian vein after intrauterine administration of 6 mg $PGF_{2\alpha}$.

Based upon the results of this experiment, the fall in blood progesterone after $PGF_{2\alpha}$ appeared to precede the increase in estradiol by about 12 hours. But as previously mentioned, I cannot be sure of the exact time of this increase in this experiment since blood progesterone and estradiol were measured only at 12 hour intervals. Progesterone had decreased to 1 ng/ml or below by 72 hours;

estrogen doubled within 24 hours and continued to rise toward a peak 12 hours after the onset of estrus. I found a similar temporal relationship between progesterone, estradiol and time to estrus in these same cows during the control cycles. Furthermore, this temporal relationship of estradiol to estrus resembles that reported by Henricks et al. (1971) in untreated cows. It differs slightly from previous reports from our laboratory which indicated that the increase in estradiol normally may accompany, not follow the decline in progesterone (Wetteman et al., 1972) in untreated cows. Future experiments are required to define precisely these temporal changes in progesterone and estradiol within the first 18-24 hours after $PGF_{2\alpha}$. But overall, the pattern of increasing estradiol and decreasing progesterone during the 3 days after $PGF_{2\alpha}$ administration resembled that during the 3 days before estrus in untreated cows.

Blood serum LH averaged 1.3 ng/ml from $PGF_{2\alpha}$ administration until approximately 14 hours before the peak of the ovulatory surge of LH. During this monitoring period, no samples of serum contained LH greater than 4 ng/ml. LH concentrations rose slowly from 12 to 14 hours before LH peak (Figure 9) then rapidly increased ($P<.01$) to a peak of 10.4 ng/ml around the time of onset of estrus. On the average, the surge of LH persisted for at least 8 hours, similar to that in untreated cows (Schams and Karg, 1969; Henricks et al., 1970; Swanson and Hafs, 1971); then

Figure 9. Blood serum LH after ipsilateral uterine $PGF_{2\alpha}$ (5 mg) on day 7, 11, and 15.


12
10
8
6
4
2
LH (ng/ml)
FROM
PGF2α
TO-10hr
SUBSEQUENT
METESTRUS
AND DIESTRUS
-8 -6 -4 -2 0 2 4 6 8
HOURS FROM PEAK LH

LH returned to basal values and averaged 1.1 ng/ml during the subsequent diestrus. At the peak of the surge of LH following $PGF_{2\alpha}$, blood serum estradiol averaged 18.8 pg/ml and progesterone averaged 0.8 ng/ml. None of these LH criteria was influenced significantly by day (7, 11, or 15) of administration of $PGF_{2\alpha}$ in the ipsilateral or contralateral uterine horn. The LH peak at the estrus following $PGF_{2\alpha}$ was greater ($P<.05$) than that at the subsequent control estrus (8.3 ng/ml). However, I probably underestimated the LH peak in the control estrus because blood was sampled only twice daily during the control estrus while blood was sampled at 2 hour intervals following $PGF_{2\alpha}$. In other words, the peak of the ovulatory surge of LH persists for no more than 2 hours, and I probably missed it during the control estrus in these cows.

The interval from $PGF_{2\alpha}$ treatment to peak LH averaged 71 hours (Table 3), and was not influenced significantly by day or uterine site of $PGF_{2\alpha}$ treatment. The onset of estrus (Table 3) was estimated to have occurred 6 hours before the first observation of standing estrus by checking for signs of estrus twice daily (morning and evening) and then estimating that estrus had begun 6 hours after the detection 12 hours previously which did not indicate estrus and thus 6 hours before the detection which indicated standing estrus. Estrus began within 24-138 hours after $PGF_{2\alpha}$ without exception in these cows. Of the 23 intervals from $PGF_{2\alpha}$ to onset of estrus, 19

Table 3.--Intervals from intrauterine $PGF_{2\alpha}$ (5 mg) to peak LH, onset of estrus and ovulation in cows.

$PGF_{2\alpha}$ treatment		Interval from $PGF_{2\alpha}$ to			Duration of cycle after $PGF_{2\alpha}$
Uterine horn	Day of cycle	Peak LH	Onset estrus	Ovulation	
		----------(hr)---------			(days)
Ipsilateral[a]	7	69±7[c]	71±7	86±11	21.5±.4
	11	68±15	68±15	94±13	20.8±.3
	15	72±6	70±7	97±5	20.6±.7
Contralateral[b]	11	71±7	76±10	100±13	---
Avg.		71±4	72±5	95±5	21.0±.3

[a]Six cows.

[b]Five cows.

[c]Mean ± SE.

occurred within 42-96 hours (Table 3). Ovulation, estimated at 6 hours before the palpation which revealed a collapsed follicle, occurred an average of 95 hours after $PGF_{2\alpha}$ (Table 3). Thus, the intervals from $PGF_{2\alpha}$ to onset of estrus and to ovulation were not influenced by day or uterine site of $PGF_{2\alpha}$ treatment.

On the average, the interval from $PGF_{2\alpha}$ to onset of estrus was 72 hours with an interval from LH peak to ovulation after $PGF_{2\alpha}$ of 24 hours. This interval was similar to 29 hours reported by Swanson and Hafs (1971) in untreated heifers and indicated the interval from onset of estrus to ovulation in $PGF_{2\alpha}$ cows closely coincided with interval from onset of estrus to ovulation in normal cycling animals. This information accompanied by the high degree of synchrony of estrus and ovulation (Table 3) indicated that $PGF_{2\alpha}$ could possibly be used to synchronize cattle for artificial insemination without regard to estrus detection. The duration of the estrous cycle following treatment with $PGF_{2\alpha}$ averaged 21 days (Table 3), and was not influenced by the day of the previous cycle when $PGF_{2\alpha}$ was administered. In other words, there was no evidence for a carry-over effect of $PGF_{2\alpha}$ on the subsequent control cycle.

In overview, after a single intrauterine injection of 5 mg $PGF_{2\alpha}$ into cows on day 7, 11, or 15 of the estrous cycle, (1) blood progesterone fell 50% within 12 hours, (2) estradiol more than doubled within 24 hours, (3) the

peak of an ovulatory surge of LH occurred at 71 hours, (4) estrus began at 72 hours, and (5) ovulation occurred at 95 hours. Moreover, the reproductive and endocrine criteria measured after $PGF_{2\alpha}$ were not influenced by day of treatment of $PGF_{2\alpha}$. Nor did the site of injection, (in the ipsilateral or contralateral uterine horn) have an effect on any of the measured criteria. The high degree of synchrony of ovulation after treatment of diestrus cattle with $PGF_{2\alpha}$ suggested that estrus detection prior to artificial insemination may be unnecessary after $PGF_{2\alpha}$ treatment. Consequently, cattle with palpable corpora lutea could be injected and inseminated either after they show signs of estrus or at a predetermined interval (between 72-90 hours after $PGF_{2\alpha}$).

This experiment left the following questions unanswered. (1) Must the $PGF_{2\alpha}$ be administered locally via the uterus or can it be administered systemically? (2) Will the animal without a mature, functional corpus luteum (day 3 of estrous cycle) respond to a luteolytic dose of $PGF_{2\alpha}$? (3) What are the actue (within 18 hours after $PGF_{2\alpha}$) changes in gonadal hormones (progesterone and estradiol)? (4) Does the good synchrony of estrus and ovulation illustrated in this experiment (Table 3) indicate the possibility of good fertility when cows are inseminated after synchronization with $PGF_{2\alpha}$ and can this synchrony be obtained when $PGF_{2\alpha}$ is given systemically? I have attempted to answer these questions in the following three experiments.

Experiment 2

Progesterone, LH, estrus and ovulation after intramuscular prostaglandin $F_{2\alpha}$ in heifers

Since intrauterine administration of $PGF_{2\alpha}$ may be difficult or ill advised under many farm conditions I performed the second experiment to determine if $PGF_{2\alpha}$ in large doses (30 mg Tham salt) could be administered systemically either im or intravaginally to heifers during diestrus. I also wished to determine if an animal with a non-functional corpus luteum (day 3 of the estrous cycle) would respond to a luteolytic dose of $PGF_{2\alpha}$, so I injected 30 mg $PGF_{2\alpha}$ into heifers on day 3 of the cycle before the corpus luteum was fully formed and functional.

After im injection of 30 mg $PGF_{2\alpha}$ (Tham salt) during diestrus, blood serum progesterone fell about 60% within 12 hours and to basal values (1.0-1.5 pg/ml) at 24 hours and remained at these low concentrations for 72 hours (Table 4). In contrast, im $PGF_{2\alpha}$ on day 3 of the cycle had no apparent effect on corpus luteum function because progesterone increased continuously during the next 5 days (Table 4) as expected of untreated cattle at this stage of the cycle (Swanson et al., 1972). The duration of the cycle during which $PGF_{2\alpha}$ was given on day 3 was 19 days, near that expected in untreated heifers. This observation is in agreement with the report (Rowson et al., 1972) that $PGF_{2\alpha}$ was not luteolytic before day 5 of the estrous cycle in cattle.

Table 4.--Blood serum progesterone after intravaginal or intramuscular $PGF_{2\alpha}$ (30 mg Tham salt) in heifers.

Hr. after $PGF_{2\alpha}$	Site of $PGF_{2\alpha}$		
	Intramuscular		
	Diestrus[a]	Metestrus[b]	Vaginal diestrus[a,c]
	(ng/ml)		
0	4.0±0.4	0.6±0.1	4.6±0.4
12	1.5±0.2	0.6±0.1	2.4±0.3
24	0.8±0.1	0.9±0.3	1.5±0.4
48	1.0±0.2	1.1±0.3	1.1±0.4
72	1.0±0.2	1.3±0.3	0.6±0.1
120	0.5±0.5	2.1±0.2	0.6±0.1
Day 11 of next cycle	5.0±0.5	3.7±0.6	3.9±0.6

[a]Five heifers.

[b]Six heifers.

[c]A sixth heifer did not respond.

Among the six heifers given $PGF_{2\alpha}$ intravaginally, one apparently failed to respond, her blood progesterone averaged 3 ng/ml during the entire 19 day monitoring period. The duration of the estrous cycle during which $PGF_{2\alpha}$ was given was 19 days for this heifer, apparently unaltered by $PGF_{2\alpha}$. Hence, she is omitted from further discussion. In the remaining five heifers given $PGF_{2\alpha}$ intravaginally, progesterone dropped 47% (2.2 ng/ml within 12 hours and continuously for 72 hours (Table 4). The decline in blood progesterone after intravaginal $PGF_{2\alpha}$ appeared parallel to but delayed relative to that after im $PGF_{2\alpha}$ during diestrus, but the difference was not significant. Corpus luteum diameter declined continuously during the 72 hours after both im and intravaginal $PGF_{2\alpha}$ was given during diestrus (Table 5), in parallel with changes in blood progesterone. The decrease was slightly but not significantly faster after im $PGF_{2\alpha}$ than after intravaginal $PGF_{2\alpha}$. Luteal diameter was too small for accurate assessment when $PGF_{2\alpha}$ was given on day 3 of the cycle, however it increased to 1.4 and 1.8 cm at 2 to 5 days later, and to 2.4 cm on day 11 of that estrous cycle (Table 5) parallel with the increasing progesterone in the same animals.

All five heifers given $PGF_{2\alpha}$ im on day 11 of the estrous cycle exhibited estrus beginning about 3 days later (Table 6). All five ovulated about 1 day after onset of estrus. After intravaginal $PGF_{2\alpha}$ on day 11, the onset of estrus was retarded (p .08) and more variable relative to

Table 5.--Corpus luteum diameter after intravaginal or intramuscular $PGF_{2\alpha}$ (30 mg Tham salt) during diestrus or metestrus in heifers.

Days after $PGF_{2\alpha}$	Site of $PGF_{2\alpha}$		
	Intramuscular		
	Diestrus[a]	Metestrus[b]	Vaginal diestrus[a,c]
	(cm)		
0	2.3±0.1	. .	2.5±0.1
1	1.8±0.1	. .	2.2±0.1
2	1.2±0.1	. .	1.5±0.2
3	0.6±0.3	. .	0.6±0.4
5	UP[d]	. .	UP[d]
8	UP[d]	. .	UP[d]

[a]Five heifers.

[b]Six heifers.

[c]A sixth heifer did not respond.

[d]Too small for precise stimation.

Table 6.--Intervals to onset of estrus, peak LH and ovulation after intravaginal or intramuscular $PGF_{2\alpha}$ (30 mg Tham salt) during diestrus in heifers.

Interval from $PGF_{2\alpha}$ to	Site of $PGF_{2\alpha}$	
	Intramuscular[a]	Vaginal[a,b]
	(hours)	
Onset of estrus	74±3	117±18
Peak LH	64±4[c]	128±19
Ovulation	104±6	138±20

[a]Five heifers.

[b]A sixth heifer did not respond.

[c]Four heifers.

that after im $PGF_{2\alpha}$. Similarly, the interval from $PGF_{2\alpha}$ treatment to LH peak was longer ($P<.01$) after intravaginal than im $PGF_{2\alpha}$, but the comparable differences in intervals from administration of $PGF_{2\alpha}$ to ovulation were more variable and did not differ significantly. Blood LH remained near values typical of diestrus (1-2 ng/ml) until 10-12 hours before estrus in heifers given $PGF_{2\alpha}$ on day 11 (Table 7). Then an LH surge occurred for about 6-8 hours near the onset of estrus as normally occurs in untreated cows (Henricks et al., 1970, Swanson and Hafs, 1971) and similar to the LH surge in $PGF_{2\alpha}$ treated cows (Experiment 1). I found no LH surge in one heifer given

Table 7.--Blood serum LH after intravaginal or intramuscular $PGF_{2\alpha}$ (30 mg Tham salt) during diestrus in heifers.

Time after $PGF_{2\alpha}$	Site of $PGF_{2\alpha}$	
	Intramuscular[a]	Vaginal[a,b]
	(ng/ml)	
From $PGF_{2\alpha}$ to LH surge	0.6±0.1	0.7±0.1
At LH peak	4.1±1.2[c]	5.8±2.0
Subsequent diestrus	0.5±0.1	0.7±0.1

[a]Five heifers.

[b]A sixth heifer did not respond.

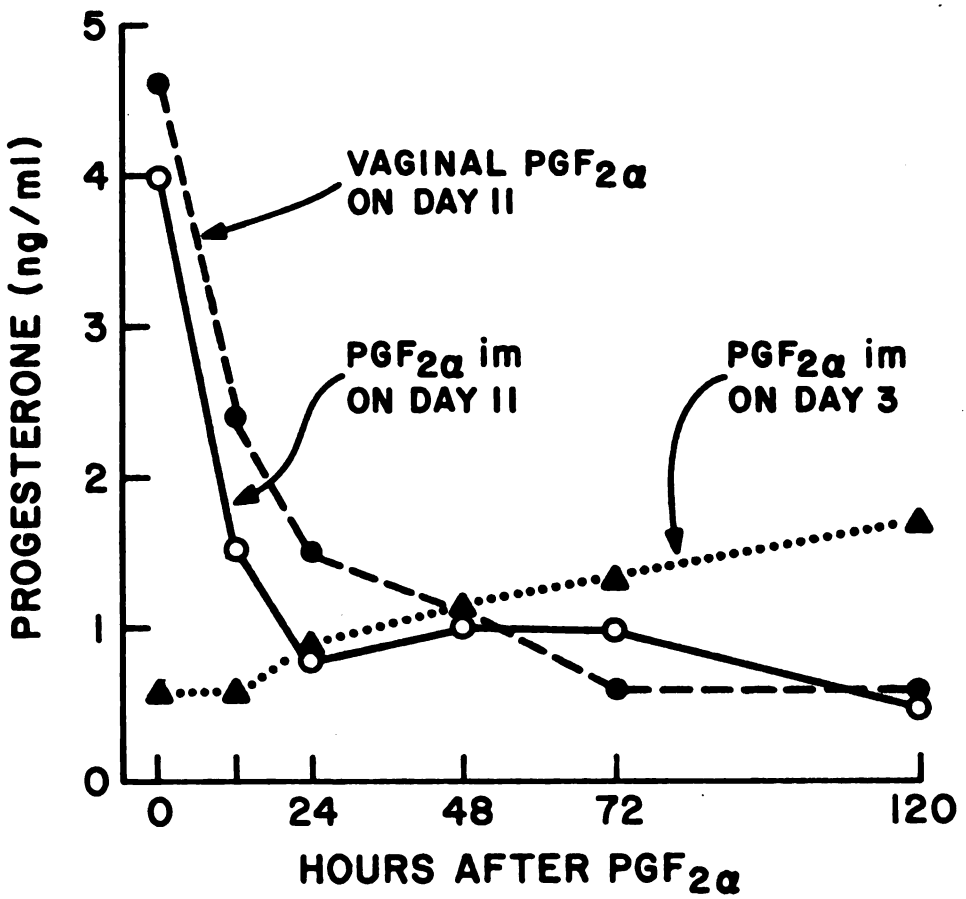
[c]Four heifers.

$PGF_{2\alpha}$ im on day 11 while I cannot be sure the LH surge possibly occurred in between the 12 hour bleedings because this heifer began estrus at 74 hours after $PGF_{2\alpha}$.

The rapid decline in progesterone after 30 mg $PGF_{2\alpha}$ given systemically has recently been confirmed by Nancarrow et al. (1974). In these experiments, progesterone fell from 5.1 ng/ml to 2.7 ng/ml within 9 hours and to basal values by 24 hours. Both my original study and the report by Nancarrow et al. (1974) show that $PGF_{2\alpha}$, 30 mg administered systemically caused a rapid and synchronous decline in progesterone indicative of rapid and synchronous luteolysis.

In summary, after im $PGF_{2\alpha}$ during diestrus, (1) luteal diameter decreased from 2.3 cm to 1.8 cm at 24 hours and to 0.6 cm at 72 hours, (2) blood serum progesterone fell from 4.0 ng/ml at 12 hours to 1.0 ng/ml at 72 hours (Figure 10, and (3) estrus began at 74 hours and ovulation occurred at 104 hours. After intravaginal $PGF_{2\alpha}$ during diestrus, one heifer failed to respond, and the other five responded similarly to those given im $PGF_{2\alpha}$ during diestrus except that luteolysis was more variable and delayed by 1 day compared to that after im $PGF_{2\alpha}$ (Figure 10). $PGF_{2\alpha}$ was neither luteolytic nor luteostatic (Figure 10) in heifers during metestrus (day 0-5 of the estrous cycle). I conclude that 30 mg $PGF_{2\alpha}$ is luteolytic during diestrus in heifers, with synchrony of estrus and ovulation comparable to that after intrauterine administration in cows

Figure 10. Serum progesterone in heifers after systemic $PGF_{2\alpha}$ (30 mg).


PROGESTERONE (ng/ml)
5
4
3
2
1
0
VAGINAL PGF2α
ON DAY 11
PGF2α im
ON DAY 11
PGF2α im
ON DAY 3
0
24
48
72
120
HOURS AFTER PGF2α

(Experiment 1). The results of this experiment with $PGF_{2\alpha}$ im on day 3 further substantiates Rowson et al.'s (1972) report that $PGF_{2\alpha}$ is not luteolytic when given before day 5 and indicates the inadvisability of using $PGF_{2\alpha}$ to synchronize cattle that do not have a fully formed corpus luteum. Intravaginal administration was not as successful as im $PGF_{2\alpha}$ because luteolysis was delayed 1 day and the other indices measured (LH, progesterone, interval to estrus and interval to LH peak) were considerably more variable than after im $PGF_{2\alpha}$ during diestrus, and intravaginal $PGF_{2\alpha}$ apparently was not luteolytic in one heifer.

Thus, two of the questions (1) must the $PGF_{2\alpha}$ be administered locally via the uterus or can it be administered systemically and (2) will the animal without mature, functional corpus luteum (day 3 of the estrous cycle) respond to a luteolytic dose of $PGF_{2\alpha}$ posed after experiment 1 were answered by experiment 2. Because the results of this experiment indicated that $PGF_{2\alpha}$ im was neither luteolytic nor luteostatic in metestrus and that 30 mg im $PGF_{2\alpha}$ was luteolytic during diestrus but im administration was more effective than intravaginal administration.

Experiment 3

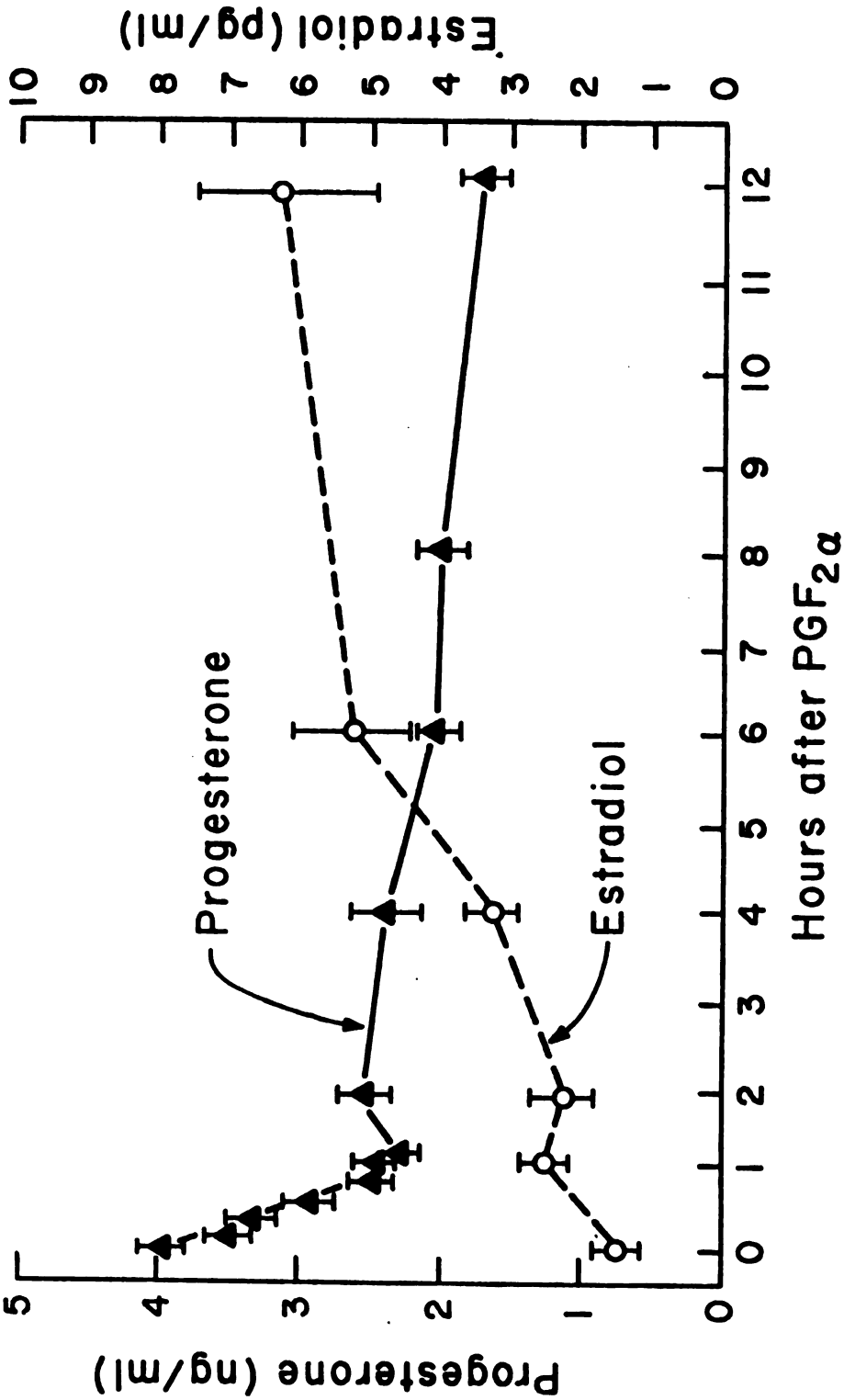
Acute changes in progesterone and estradiol after $PGF_{2\alpha}$

Since I had measured progesterone and estradiol only at 12-hour intervals in experiment 1, and recent reports indicated increased progesterone synthesis when

bovine corpora lutea were incubated with $PGF_{2\alpha}$ *in vitro* (Sellner and Wickersham, 1970), I speculated that measurement of acute changes in progesterone and estradiol may contribute to our understanding of changes in gonadal hormones after $PGF_{2\alpha}$. I speculated that the mechanism of luteolytic action *in vivo* may involve a brief surge in progesterone secretion (missed during 12 hour sampling in experiment 1 and 2) before the fall. Thus, the principal objective of this experiment was to measure acute changes (within 18 hours of $PGF_{2\alpha}$) in blood progesterone and estradiol. A second objective was to determine whether luteolysis, as measured by the progesterone decline, proceeded more rapidly after two 15 mg injections at 6 hour intervals or after an injection of 60 mg than after an injection of 30 mg $PGF_{2\alpha}$ im in heifers.

In experiment 3, neither the declines in blood progesterone nor the increases in estradiol differed after 15, 30 or 60 mg im of $PGF_{2\alpha}$. On the average, blood progesterone declined continuously from 3.9 ng/ml before $PGF_{2\alpha}$ to 2.1 and 1.7 ng/ml at 6 and 12 hours (Figure 11). The second 15 mg injection at 6 hours after the initial injection did not alter the rate of decline in progesterone. The results in Figure 7 indicate blood progesterone may begin to fall within 10 minutes after im injection of $PGF_{2\alpha}$, and there was no evidence in any of these 16 heifers for increased progesterone synthesis before the fall. The rapid fall in progesterone to 50% of the initial values by

Figure 11. Average blood plasma progesterone and estradiol after 15 mg (2 X), 30 mg, or 60 mg (im) $PGF_{2\alpha}$ in heifers.


Progesterone (ng/ml)
Estradiol (pg/ml)
Hours after PGF2α
Progesterone
Estradiol
0
1
2
3
4
5
6
7
8
9
10
11
12

12 hours agreed with the progesterone declines after $PGF_{2\alpha}$ in experiments 1 and 2. But compared to the results in experiment 1 estradiol, increased much more rapidly than expected. It averaged 1.4 pg/ml at the time of $PGF_{2\alpha}$ injection in these 16 heifers, then increased ($P<.05$) to 2.6 pg/ml at 1 hour and continuously to an average of 6.2 pg/ml at 12 hours after $PGF_{2\alpha}$ (Figure 11).

The rapid progesterone decline observed is in close agreement with a report by Baird et al. (1975) in sheep; they also reported a precipituous fall within 1 hour of the $PGF_{2\alpha}$ injection. But my data does not agree with reported values for protesterone measured in uterine venous serum in cows. For example, Hixon and Hansel (1974) reported that the initial response to $PGF_{2\alpha}$ was increased progesterone in uterine-venous blood in every animal studied, lasting no longer than 30 minutes. But since these studies involve samples taken from the utero-ovarian vein and not the jugular vein, possibly there is a dilution of the progesterone increase that they measured in the uterine vein blood by the time it reaches the jugular vein.

There appeared to be at least two phases to the progesterone decline. After 15, 30 and 60 mg $PGF_{2\alpha}$, jugular progesterone fell rapidly ($P<.05$) from the pre-treatment concentrations by 30 minutes. Thereafter, the decline in progesterone was slower over the next 48 hours. The rapid first 30 minute decline closely reflects the half-life of progesterone reported by Sandberg and

Slaunwhite (1958). One possible explanation for these results is that there is a rapid initial shut down of systemic progesterone concentration possibly attributable to changes in blood flow through the ovary which by-passes the corpus luteum (Thorburn and Hales, 1972) followed by a slower decline which may be due to demunition of the venoconstrictive action combined with a second luteolytic action directly on the corpus luteum or alternatively another luteolytic factor induced by $PGF_{2\alpha}$ to cause a decline in the synthesizing ability of the moribund corpus luteum.

The continuous rise in jugular blood estradiol concentration, which may begin as early as 1 hour after $PGF_{2\alpha}$ is in contrast to uterine vein concentrations found by Hixon and Hansel (1974). They reported a rapid rise in estradiol concentrations to a peak by 9 hours followed by a decline and then a slow rise to a peak around estrus. In our studies as well as Hansel and Hixon's study, however, estrogen began to increase slightly before progesterone had declined to less than 1 ng/ml but high proestrus concentrations of estrogen were not observed in either study until after progesterone had reached basal concentrations (1 ng/ml). Possibly estrogen may play a role in the second phase of $PGF_{2\alpha}$ mediated luteolysis which follows the rapid initial shut down of systemic progesterone concentration, possibly by directly initiating synthetic changes in the follicle, or alternatively

inducing aramotizing enzymes (which induce estrogen synthesis) in the moribund corpus luteum. Yet another possibility is that $PGF_{2\alpha}$ may have directly modified release of pituitary hormones which affected follicular steroidogenesis.

Overall, the interval to onset of estrus for the 16 heifers averaged 68.9 hours and resembled the interval reported after injection of 5 mg $PGF_{2\alpha}$ into the uterus of cows in experiment 1. Changes in blood progesterone indicated that there was no luteolytic advantage to repeated 15 mg injection at 6 hour intervals or to the larger 60 mg injection. I had anticipated that the second injection of 15 mg $PGF_{2\alpha}$ (6 hours after the first) might increase the rate of luteolysis, but it did not modify the decline of progesterone which was initiated by the first injection. Evidently, a second injection of $PGF_{2\alpha}$ is required only when the first is too small to cause complete luteolysis (Rowson et al., 1972).

In conclusion, in answering to the question as to what acute changes in blood progesterone and estradiol occurred after $PGF_{2\alpha}$. Blood plasma progesterone may begin to fall within 10 minutes after im injection of $PGF_{2\alpha}$ and there was no evidence for increased progesterone synthesis before the fall. Blood estradiol increased continuously beginning as early as 1 hour after $PGF_{2\alpha}$. Relative to 30 mg $PGF_{2\alpha}$ im, two injections of 15 mg at 6 hour intervals

or 60 mg $PGF_{2\alpha}$ did not hasten luteolysis. Thus, a single 30 mg im injection of $PGF_{2\alpha}$ is ample to cause luteolysis.

Experiment 4

Fertility of cattle following $PGF_{2\alpha}$ injection

In view of the synchrony of ovulation after treatment of diestrus cattle with $PGF_{2\alpha}$ and since the major practical goal of this dissertation research was the use of $PGF_{2\alpha}$ to synchronize estrus to allow artificial insemination at a predetermined time, two trials were initiated.

In the first trial, 7 of 13 controls and 6 of 12 $PGF_{2\alpha}$-treated heifers were diagnosed pregnant at 40 days from the first insemination. One of the six Holstein heifers pregnant from inseminations after $PGF_{2\alpha}$ aborted on day 68, apparently due to an outbreak of Infectious Bovine Rhinotracheitis (IBR). The others averaged 283 days gestation; the five calves (four males and one female) resulting from these inseminations averaged 43 kg at birth and veterinary examinations of the calves revealed no physical abnormalities. Hence a second larger trial was undertaken with 66 cross-bred beef cattle randomly assigned to one of three treatments. (1) controls (i.e., without treatment) inseminated at 12 hours after onset of control estrus during and 18 to 21 day period (N=25), (2) $PGF_{2\alpha}$ A.I. inseminated at 12 hours after onset of estrus detected during a 7 day period following injection of

30 mg $PGF_{2\alpha}$ (N=25), or (3) $PGF_{2\alpha}$ inseminated twice without regard to estrus at 72 and 90 hours after $PGF_{2\alpha}$ (N=19).

Most cows began estrus on days 3 and 4 after $PGF_{2\alpha}$ injection. Four of the 25 cows which were to be inseminated at estrus detected during the first 7 days after $PGF_{2\alpha}$ were not observed in estrus during the seven day period and are thus excluded from the data in Table 8. Of the 21 cows that were observed and inseminated after $PGF_{2\alpha}$, 14 exhibited estrus within 3.5 days following $PGF_{2\alpha}$ and the other 7 averaged longer intervals (5.2 days) to estrus. Pregnancy, diagnosed by palpation at 35 to 60 days after insemination (Table 8), among controls was 62%, no greater than that for the cattle inseminated during estrus after $PGF_{2\alpha}$ (90%) or that for those inseminated 72 and 90 hours after $PGF_{2\alpha}$ (79%). Thus, since fertility of cows inseminated by appointment at 72 and 90 hours after $PGF_{2\alpha}$ was not reduced compared to that of controls $PGF_{2\alpha}$ may be used in the future to allow breeding with normal fertility at predetermined times, independent of estrous detection.

Thus, experiment 4 answered the most important question posed by this dissertation research (Does the good synchrony of estrus and ovulation illustrated in experiments 1 and 2 indicate the possibility of good fertility when cows are inseminated after synchronization with $PGF_{2\alpha}$ and can this synchrony be obtained when given systemically?). Since results of this experiment indicate good fertility in both trials with insemination twice at

Table 8.--Fertility[a] of cows inseminated after $PGF_{2\alpha}$.

Treatment	(% pregnant/number cows)
Controls, AI 12 hr. after onset of estrus	62/25
$PGF_{2\alpha}$, AI 12 hr. after onset of estrus	90/21
$PGF_{2\alpha}$, AI at 72 and at 90 hr.	79/19

[a]Fertility based upon uterine palpations at 35 to 60 days after inseminations.

72 and 90 hours after $PGF_{2\alpha}$ without regard to estrus, I conclude that $PGF_{2\alpha}$ can be used to synchronize estrous for time insemination. And further one can expect fertility after double insemination not to be reduced from that of controls.

GENERAL DISCUSSION

While this research was in progress, Rowson et al. (1972) reported that intrauterine administration of 0.5 mg $PGF_{2\alpha}$ on each of 2 consecutive days (at 24-hour intervals) caused return to estrus 3-4 days later in cows treated between day 5 to 16 of the estrous cycle. After a single injection of 1 mg (free acid) into the uterine horn ipsilateral to the corpus luteum, estrus occurred at 60-80 hours in 17 of 28 cows (Inskeep, 1973). When 1.5 or 2.0 mg $PGF_{2\alpha}$ was administered in a subsequent experiment, estrus occurred 60-72 hours later in 16 of 19 cows. Lier, Marion, and Olson (1972) observed estrus in five heifers at an average of 2-4 days after depositing 6 mg $PGF_{2\alpha}$ into the uterine horn ipsilateral to the corpus luteum, but six consecutive hourly intra-uterine injections of 0.5 mg $PGF_{2\alpha}$ did not alter the estrous cycle.

The results on the luteolytic efficacy of $PGF_{2\alpha}$ obtained during this dissertation research compare favorably with the above studies. For instance, after a single intrauterine injection of 5 mg $PGF_{2\alpha}$ Tham salt (equivalent to 3.5 mg mg free acid) into cows on days 7, 11, or 15 of the estrous cycle (1) blood progesterone fell 50% within

12 hours, (2) estradiol more than doubled within 24 hours, (3) the peaks of an ovulatory surge of LH occurred at 71 hours, (4) estrus began at 72 hours, and (5) ovulation occurred at 95 hours. These changes did not differ significantly from the comparable changes during control cycles in the same cows. An im injection of 30 mg $PGF_{2\alpha}$ Tham salt (21 mg free acid) during diestrus caused similar results.

The rapid synchronous decline in luteal diameter and blood progesterone as well as the synchronous interval to estrus observed in my studies and confirmed in the literature cited above, indicate that $PGF_{2\alpha}$ can be used to synchronize cattle for artificial insemination under farm conditions. But since $PGF_{2\alpha}$ will cause abortion in cattle (Lauderdale, 1972) its widespread indiscriminant use would be contraindicated in cattle that may be pregnant. This might be a problem in large herds of beef cattle that have been only recently separated from bull and thus would have a mixture of pregnant and non-pregnant animals. To avoid prostaglandin induced abortions, prostaglandin should only be used in herds that have not been with bulls or that have been pregnancy tested. It also should not be used to synchronize estrus for repeated breedings.

Several recent reports indicate some promising results from modifications of previously tested models for estrous synchronization with progesterone. For instance, Roche (1974) gave heifers a subcutaneous progesterone

implant and an im injection of 5 mg estradiol benzoate plus 50 mg progesterone at the outset, and implants were removed 9 to 12 days later; the majority of the heifers began estrus on the second day after implant removal, but only 41% of 51 heifers conceived from a single insemination at 48 hours. The success of this synchronization regimen (as judged by the acceptable fertility) apparently is attributable largely to the short duration of progesterone or progestogen treatment. Estrous synchronization after short-term progestogen treatment appears to be promising and should be tested more extensively. But while the fertility trials look promising the overall verdict will be out until much larger trials have been performed to test the commercial efficacy of short term progesterone synchronization.

The same can be said for the fertility trials reported in this dissertation. But in the case of prostaglandin, these larger trials have been completed. For instance, Hafs et al. (1975a, b) injected prostaglandin twice 12 days apart into heifers and suckled cows. At the first injection, 65% of cattle at random stages of the cycle would be espected to have functional corpora lutea. They reasoned that these animals should respond to prostaglandin and begin estrus about 3 days later. The other 35% which did not respond would be in diestrus 12 days later. Thus, virtually all cows would be in diestrus when the second injection was given. In these

trials, cows or heifers were inseminated either twice at 70 and 88 hours after prostaglandin (Hafs et al., 1975a), and once at 80 hours for comparisons with twice at 70 and 88 hours (Hafs et al., 1975b). With over 900 cows and heifers in 36 commercial herds involved in these trials, the authors concluded that insemination without regard to behavioral signs of estrus at 80 hours after the second injection of prostaglandin resulted in normal fertility in heifers and cows. Thus, the fertility resulting from A.I. at predetermined intervals after prostaglandin treatment without regard to onset of estrus appears to warrant application under commercial conditions. Ovulation synchronization with $PGF_{2\alpha}$ could lead to greatly expanded inplementation of artificial insemination, especially among range heifer and cows and dairy heifers where estrous detection has been a problem in the past.

With regard to other effects of $PGF_{2\alpha}$ on cattle, Dr. Wayne Oxender and Dr. Brad Sequin recorded rectal temperature, heart rate, breathing rate and uterine contractions and found no change after $PGF_{2\alpha}$ administration. However we (Louis et al., 1974) found that blood prolactin GH and glucocorticoids in heifers increased several fold within 5-15 minutes after im injections of 15, 30, or 60 mg $PGF_{2\alpha}$ or after a single 5 mg iv injection of $PGF_{2\alpha}$. Blood LH increased at least twofold over basal estimates but not until 1.5 to 6 hours after im $PGF_{2\alpha}$. Constant iv infusion of $PGF_{2\alpha}$ at the rate of 0.5 mg/min for 30 minutes

produced greater concentrations of prolactin, GH glucocorticoids and LH than those found after im injection. Blood glucose increased 59-67% above pretreatment values between 30 and 60 minutes after iv administration of $PGF_{2\alpha}$. Plasma insulin increased more than twofold over basal estimated at 45 minutes after $PGF_{2\alpha}$ administration, but these increases only approached significant ($P \sim 0.10$). And free fatty acids did not change significantly ($P<0.05$) after $PGF_{2\alpha}$ administration. Thus, exogenous $PGF_{2\alpha}$ administration causes marked increase in several plasma hormones and glucose. The mechanism of action is unclear and requires future research.

While the practical potential of prostaglandin for estrous synchronization has been established in this dissertation and the subsequent studies cited above, the physiological role or action has not been established. The previously well accepted hypothesis that $PGF_{2\alpha}$ reaches the ovary without entering the systemic circulation by passing locally from the utero-ovarian vein to the closely associated ovarian artery is highly controversial, since several investigators have provided strong evidence disputing this hypothesis. For instance, Coudert et al. (1973) showed conclusively that diffusion, filtration, counter current exchanges and active transport do not occur between the anterior uterine vein, the utero-ovarian vein and the ovarian artery in sheep. Shemesh and Hansel (1975) reported that PGF concentrations in the ovarian

artery were not significantly higher than those of the peripheral blood, even when very high concentrations of PGF were found in the uterine vein, nor did PGF concentration in the ovarian artery rise significantly at any time during the cycle. Thus, both these reports provide evidence that challenges the hypothesis that uterine $PGF_{2\alpha}$ is transferred from uterus to ovary via a local veno-arterial system. These data and other evidence in the literature review section of this dissertation (Prostaglandin, the uterine Luteolytic Hormone? p. 23 case doubt on the physiological role of $PGF_{2\alpha}$ as "the uterine luteolysin."

Further research is needed in this area and should include rapid (possibly 1 minute) sampling and measurement of possible precursors to $PGF_{2\alpha}$ in the ovarian arterial blood of normal animals before the onset of luteal regression to characterize the normal rapid changes in ovarian arterial concentrations of $PGF_{2\alpha}$ and its precursors. Blood should also be monitored from more than one of the many branches of the ovarian artery which leads to the corpus luteum to assure that $PGF_{2\alpha}$ is not being selectively transferred through a branch which might possibly be missed by single blood sampling. It is possible that $PGF_{2\alpha}$ is transferred by an alternate pathway such as the lymphatics, or a transperitoneal approach.

While the role of $PGF_{2\alpha}$ in normal luteolysis is unclear, it seems reasonable to suggest that luteolysis

after $PGF_{2\alpha}$ administration is instigated by a decline in blood flow to the corpus (Novy, 1973, Thorburn and Hales, 1972). This change in blood supply may deny the corpus luteum the cholesterol and other steroid precursors needed for progesterone synthesis, thus causing an immediate shut down of progesterone synthesis. This may explain the rapid 30-minute decline in progesterone observed in experiment 3, followed by the slower decline which may be due to demunition of the venoconstrictive action combined with a second luteolytic action induced by $PGF_{2\alpha}$ to cause a decline in synthesizing ability of the moribund corpus luteum (Behrman, 1971).

In conclusion, although we do not know the normal physiological role of prostaglandin $F_{2\alpha}$ in luteolysis, the evidence presented and reviewed (Hafs et al., 1975a, b) in this dissertation allows one to envision that in the future prostaglandin will be injected twice (10 to 12 days apart), and then inseminated at 80 hours without regard to behavioral signs of estrus with resulting normal fertility.

SUMMARY AND CONCLUSIONS

Since earlier reports indicated that prostaglandin $F_{2\alpha}$ was luteolytic in several laboratory animals, the purpose of this dissertation was to determine: (1) if $PGF_{2\alpha}$ was luteolytic in cows and heifers and (2) whether fertility was normal after synchronization with prostaglandins.

In the first experiment, after 5 mg of prostaglandin $F_{2\alpha}$ (Tham salt) was deposited in the uterus of cows during diestrus, progesterone decreased 50% within 12 hours, estradiol more than doubled within 24 hours, luteinizing hormone peaked at 71 hours, estrus began at 72 hours and ovulation occurred at 95 hours. These changes did not differ significantly from the comparable changes during control cycles in the same cows.

In another experiment, after intramuscular (im) injection of 30 mg $PGF_{2\alpha}$ (Tham salt) in heifers during diestrus, the heifers responded similarly to those given intrauterine $PGF_{2\alpha}$ in the first experiment. But im $PGF_{2\alpha}$ on day 3 of the estrous cycle was not luteolytic, and intravaginal with 30 mg $PGF_{2\alpha}$ resulted in luteolysis which

was more variable and retarded by approximately 1 day compared with that after im $PGF_{2\alpha}$.

In a third experiment, after 30 or 60 mg im injection of $PGF_{2\alpha}$ (Tham salt) blood estradiol increased continuously possibly beginning with 1 hour to a peak just before estrus. Neither 15 mg given twice, 30 mg or 60 mg im injection changed the rate of decline of progesterone or the rate of increase in estradiol so there would be no advantage to larger doses or split doses of im $PGF_{2\alpha}$. Blood progesterone began to fall within 10 minutes after $PGF_{2\alpha}$ with no evidence for increased progesterone synthesis before the fall.

Since the synchronization of estrus observed during the first 3 trials looked good, I decided to test the fertility of cows inseminated during estrus after $PGF_{2\alpha}$ was given to test whether the resulting fertility was equivalent to untreated controls. Two trials were conducted. In the first trial, seven of 13 controls and six of 12 $PGF_{2\alpha}$ treated heifers were diagnosed pregnant from the first insemination. In the second, the average percent pregnant for 40 cattle inseminated after $PGF_{2\alpha}$ (85%) was not reduced compared to the percent pregnant for 24 controls (62%). Since the fertility of the animals inseminated at 72 and 90 hours after $PGF_{2\alpha}$ was not reduced after $PGF_{2\alpha}$, possibly ovulation control with $PGF_{2\alpha}$ may allow commercial artificial insemination in some herds where detection of estrus is difficult or impossible.

APPENDICES

APPENDICES

I. Composition of reagents used in radioimmunoassay

A. Reagents for radioiodination

1. 0.5 M sodium phosphate buffer, pH 7.5

Monobasic (0.5 M)

Add 69.005 g $NaH_2PO_4 \cdot H_2O$ to distilled water. Dissolve, dilute to 1 liter.

Dibasic (0.5 M)

Add 70.98 G Na_2HPO_4 to distilled water. Heat to dissolve, then dilute to 1 liter.

Mix monobasic and dibasic to give pH 7.5.
Dispense in 1 ml portions, store at -20°C.
Store the monobasic and dibasic buffers at 4°C.

2. 0.05 M sodium phosphate buffer, pH 7.5

Solution A

$NaH_2PO_4 \cdot H_2O$---------- 2.78 g
Merthiolate---------- 0.01 g
Dilute to 100 ml with distilled water.

Solution B

$NaHPO_4/7H_2O$----------26.825 g
Merthiolate---------- 0.05 g
Dilute to 500 ml with distilled water.

Use 16 ml Solution A, 84 ml Solution B, dilute to 400 ml with distilled water.
Adjust pH to 7.5 with NaOH, if necessary.
Store at 4°C.

3. Chloramine-T

Upon receiving chloramine-T, dispense into small, tightly sealed vials, cover with foil, and store at -20°C.
Dilute 10 mg* chloramine-T to 10 ml with 0.05 M $NaPO_4$, pH 7.5 buffer. Use within 30 minutes of preparation. Discard chloramine-T remaining in vial.
*30 mg for GH

4. Sodium metabisulfite, 2.5 ug/ul
   Dilute 25 mg $Na_2S_2O_5$ to 10 ml with 0.05 M $NaPO_4$, pH 7.5 buffer. Use within 30 minutes of preparation.

5. Transfer solution
   Sucrose---------- 1.6 g
   KI---------- 0.1 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at -20°C.

6. Rinse solution
   Sucrose---------- 0.8 g
   KI---------- 0.1 g
   Bromphenol blue---------- 0.001 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at 20°C.

B. Reagents for radioimmunoassay

1. 0.01 M phosphate buffered saline, pH 7.0 (PBS)
   NaCl---------- 143 g
   Monobasic phosphate---------- 100 ml
   (See Appendix A.1)
   Dibasic phosphate---------- 260 ml
   (See Appendix A.1)
   Merthiolate---------- 1.75 g
   Dissolve in distilled water and transfer to a large container.
   Dilute to 17.5 liters with distilled water.
   Adjust pH to 7.0, if necessary, store at 4°C.

2. 0.05 M EDTA - PBS, pH 7.0
   Disodium EDTA---------- 18.612 g
   Add approximately 950 ml PBS.
   Adjust pH to 7.0 with 5 NaOH while stirring.
   Dilute to 1 liter, store at 4°C.

3. PBS - 1% egg white albumin (PBS - 1% EWA)
   or PBS - 1% bovine
   Serum albumin (PBS - 1% BSA).
   Add 990 ml PBS to beaker.
   Add 10 g EWA (Sigma Chemical Corp.) or 10 g BSA.
   Mix over magnetic mixer.
   Filter through Whatman No. 1 filter paper.
   Store at 4°C.

4. PBS - 0.1% Knox gelatin (PBS - 0.1% Knox)
   Weigh about 1 gm of Knox gelatin.
   Using graduate cylinder, add appropriate volume of PBS to make 0.1%
   Mix over magnetic mixer.
   Store at 4°C.

5. LH standard
   Weigh 5-10 ug of NIH-LH B8 on Cahn Electro-balance.
   Using 10 ml pipette, add PBS - 0.1% Knox at appropriate volume to make a dilution of 1 ug/ml
   Then, with volumetric flask, further dilute to 40 ng/ml
   Store at -20°C in small semen vial, 4 ml each.

6. 1:400 normal guinea pig serum (NGPS).
   Obtain blood from guinea pig that has not been used to develop antibodies.
   Allow blood to clot, recover serum and store the serum in convenient quantities at -20°C.
   Add 2.5 ml of appropriate serum to a 1 liter volumetric flask, dilute to 1 liter with 0.05 M PBS-EDTA, pH 7.0
   Divide into 100 mg portions and store at -20°C.

7. Guinea pig anti-bovine LH (GPABLH, identified in our laboratory as antibody I).
   Dilute the antisera to 1:400 with 0.05 M PBS-EDTA, pH 7.0.
   Dispense in small quantities, store at -20°C.
   On day of use, dilute the 1:400 antisera to the required concentration using 1:400 NGPS as diluent.

8. Anti-gamma globulin
   Use sheep angi-guinea pig gamma globulin (SAGPGG) in LH assay.
   Dilute antisera to required concentration with 0.05 M PBS-EDTA, pH 7.0.
   Store at 4°C or at -20°C.

C. Antibody and anti-gamma globulin production

1. Guinea pig anti-LH
   - 0.5 or 1.0 mg NIH-LH-B5 was dissolved in water and Freund's complete adjuvant added (1:1 ratio).
   - 1.1 or 0.6 ml of the emulsion per guinea pig was injected subcutaneously in 4 scapular region sites.
   - The above procedure was repeated 15 and 30 days later substituting Freund's incomplete adjuvant for adjuvant.
   - Antisera was collected by cardiac puncture 46 and 78 days after the initial injection.

2. Sheep anti-guinea pig gamma globulin
   - Guinea pig gamma globulin (Fraction 11, Pentex, Inc., Kankakee, Illinois) (40 mg), steptomycin (100 mg) and penicillin (1000 I.U.) was emulsified in 5 ml of water plus 5 ml Freund's complete adjuvant.
   - 10 ml was subcutaneously injected in 8 scapular sites of a 75 kg goat.
   - The above procedure repeated 15 days later substituting Freund's incomplete adjuvant for adjuvant.
   - Antisera was collected 30 days after the second antigen injection by jugular vein puncture.

II. Preparation of liquid scintillation fluids

A. Steroid scintillation fluid

Naphthalene	480 g
PPO	30 g
POPOP	0.3 g
Xylene	2000 ml
p-dioxzne	2000 ml

Mix until dissolved.

B. Bray's solution

Naphthalene	240 g
PPO	16 g
Dimethyl POPOP	0.8 g
Ethylene Glycol	80 ml
Methanol	400 ml
p-dioxane	3264 ml

Mix until dissolved.

BIBLIOGRAPHY

BIBLIOGRAPHY

Akbar, A. M., L. E. Reichert, Jr., T. G. Dunn, C. C. Kaltenback, and G. D. Niswender. 1974. Serum levels of follicle-stimulating hormone during the bovine estrous cycle. J. Anim. Sci. 39:360.

Anderson, L. L., F. C. Neal, and R. M. Melampy. 1962. Oxytocin on ovarian function in cycling and hysterectomized heifers. A. Anim. Sci. 24:964.

Anderson, L. L., K. P. Bland, and R. M. Melampy. 1963. Comparative aspects of uterine luteal relationships. Recent Prog. Hormone Research 25:57.

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533.

Babcock, J. C. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. In: "Ovarian Regulatory Mechanisms." J. Reprod. Fertil. Suppl. 1, p. 47.

Baird, D. T. and R. J. Scaramuzzi. 1975. The role of prostaglandins in luteal regression. Ann. Biol. Biochem. and Biophys. 15:2.

Barrett, M. A., de B. Blockey, J. M. Brown, I. A. Cumming, J. R. Goding, B. J. Mole, and J. M. Obst. 1971. Initiation of the oestrous cycle in the ewe by infusion of $PGF_{2\alpha}$ to the autotransplanted ovary. J. Reprod. Fertil. 25:136.

Behrman, H. R., G. J. McDonald, and R. O. Greep. 1971. Luteinizing hormone stimulation of ovarian prostaglandin biosynthesis. Lipids 6:791.

Bergstrom, S., R. Ryhage, B. Samuelsson, and J. Stovall. 1962. The structure of prostaglandin E, F_1 and F_2. Acta Chem. Scand. 16:501.

Bergstrom, S., L. A. Carlson, and J. R. Weeks. 1968. The prostaglandins: A family of biologically active lipids. Pharm. Rev. 20:1.

Bland, K. P. 1970. Congenital abnormalities of the uterus and their bearing on ovarian function. Vet Rec. 86:44.

Bland, K. P., E. W. Horton, and N. L. Poyser. 1971. Levels of prostaglandin F_2 in the uterine venous blood of sheep during the oestrous cycle. Life Sciences 10:509.

Blatchley, F. R. and B. T. Donovan. 1969. Luteolytic effect of prostaglandin in the guinea pig. Nature 221:1065.

Blatchley, F. R., B. T. Donovan, N. L. Poyser, E. W. Horton, C. J. Thompson, and M. Los. 1971. Identification of prostaglandin $F_{2\alpha}$ in the utero-ovarian blood of guinea pigs following oestrogen treatment. Nature 230:243.

Blatchley, F. R., B. T. Donovan, E. W. Horton, and N. L. Poyser. 1972. The release of prostaglandins and progesterone into the utero-ovarian venous blood of guinea pigs during the oestrous cycle and following oestrogen treatment. Journal of Physiology 228:69.

Brunner, M. A., L. E. Donaldson, and W. Hansel. 1969. Exogenous hormones and luteal function in hysterectomized and intact heifers. J. Dairy Sci. 52:1849.

Caldwell, B. V., S. A. Tellson, W. A. Brock, and L. Speroff. 1972. The effects of exogenous progesterone and estradiol on prostaglandin $F_{2\alpha}$ levels in ovariectomized sheep. Prosta. 1:217.

Chamley, W. A., J. M. Buckmater, M. D. Cain, J. Cerini, M. E. Cerini, I. A. Cumming, and J. R. Goding. 1972. The effect of prostaglandin $F_{2\alpha}$ on progesterone, oestradiol and luteinizing hormone secretion in sheep with ovarian transplants. J. Endo. 55:253.

Cole, H. H. and R. B. Snook. The estrous cycle. Proc. 5th Intern. Congr. Animal Reproduction, Trento II, 143 (1964).

Coudert, S. P., G. D. Phillips, C. Faiman, C. Chernecki, and M. Palmer. 1974. Infusion of tritiated prostaglandin $F_{2\alpha}$ into the uterine vein of the ewe: Absence of local venous-arterial transfer. J. Reprod. Fertil. 36:333.

Demers, L. M., H. R. Behrman, and R. O. Greep. 1972. Effects of prostaglandins and gonadotrophin on luteal prostaglandin and steroid biosynthesis. Adv. Biosci. (Suppl.) 9:114.

Desjardins, C. and H. D. Hafs. 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Animal Sci. 27:472.

Dobrowoloski, W. and E. S. E. Hafez. 1971. The uterus and control of ovarian function. Acta Obstet. Gynec. Scand., Suppl. 12:1.

Ducharme, D. W., J. R. Weeks, and R. G. Montgomery. 1968. Studies on the mechanism of hyptertensive effect of prostaglandin $F_{2\alpha}$. J. Pharmacol. Exp. Therap. 160:1.

Erb, R. E., R. D. Randel, and C. J. Callahan. 1971. Female sex steroid changes during the reproductive cycle. J. Anim. Sci., Suppl. 1, 32:80.

Gill, J. L. and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Animal Sci. 33:331.

Ginther, O. J. 1966. The influence of the uterus on the lifespan of the corpus luteum. Vet. Med. Small Anim. Clinic 32:1199.

Ginther, O. J. 1970. Effect of destruction of follicles on corpus luteum. J. Anim. Sci. 31:233.

Ginther, O. J. and N. L. First. 1971. Maintenance of the corpus luteum in hysterectomized mares. Am. J. Vet. Res. 32:1687.

Ginther, O. J. and C. H. Delcampo. 1974. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus in cattle. Amer. J. Vet. Res. 35:193.

Goding, J. R., M. D. Cain., J. Cerini, M. Cerini, W. A. Chamley, and I. A. Cumming. 1972. Prostaglandin $F_{2\alpha}$ "the" luteolytic hormone in the ewe. J. Reprod. Fertil. 28:146.

Goldblatt, M. W. 1933. A depressor substance in seminal fluid. J. Soc. Chem. Ind., Lond. 52:1056.

Hackett, A. J. and H. F. Hafs. 1969. Pituitary and hypothalamic endocrine changes during the bovine estrous cycle. J. Anim. Sci. 28:531.

Hafs, H. D. and D. T. Armstrong. 1968. Corpus luteum growth and progesterone synthesis during the bovine estrous cycle. J. Anim. Sci. 27:134.

Hafs, H. D., T. M. Louis, P. A. Noden, and W. D. Oxender. 1973. Control of estrous cycle with $PGF_{2\alpha}$ in cattle and horses. J. Anim. Sci. Suppl:10.

Hafs, H. D., J. G. Manns, and B. Drew. 1975a. Onset of oestrus and fertility of dairy heifers and suckled beef cows treated with prostaglandin $F_{2\alpha}$. Animal Prod. In Press.

Hafs, H. D., J. G. Manns, and G. E. Lamming. 1975b. Synchronization of oestrus and ovulation in cattle. Animal Prod. In Press.

Hansel, W. and W. C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilatation and intrauterine infusions of seminal and preputial fluids. J. Dairy Sci. 43:796.

Hansel, W. 1967. Control of the ovarian cycle in cattle: A review. Aust. Vet. Journal 43:441.

Hansel, W. 1969. Personal communication.

Hansel, W. and R. B. Snook. 1970. Pituitary ovarian relationships in cows. J. Dairy Sci. 50:1948.

Hansel, W. and S. E. Ecternkamp. 1972. Control of ovarian function in domestic animals. Amer. Zool. 12:225.

Henricks, D. M., J. F. Dickey, and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346.

Henricks, D. M., J. F. Dickey, and J. R. Hill. 1971a. Plasma estrogen and progesterone levels in cows prior to and during estrus. Endocrinol. 89:1350.

Henricks, D. M., D. R. Lamond, J. R. Hill, and J. F. Dickey. 1971b. Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. J. Anim. Sci. 33:450.

Hixon, J. E. and W. Hansel. 1974. Evidence for preferential transfer of prostaglandin $F_{2\alpha}$ to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11:543.

Hunter, W. M. 1967. The preparation of radioiodinated proteins of high activity, their reaction with antibody in vitro: The radioimmunoassay. In: D. M. Wier (ed.), Handbook of Experimental Immunology, p. 605-654, F. A. Davis Co., Philadelphia, Pa.

Inskeep, E. K. 1973. Potential uses of prostaglandins in control of reproductive cycles of domestic animals. J. Anim. Sci. 36:1149.

Karsch, F. J., J. W. Noveroske, J. F. Roche, H. W. Norton, and A. V. Nalbandov. 1970. Maintenance of ovine corpora lutea in the absence of ovarian follicles. Endocrin. 87:1228.

Kendrick, J. W. and K. McEntee. 1967. The effect of artificial insemination with serum contaminated with IBR-IPV virus. Cornell Vet. 57:3.

Kittok, R. J., J. H. Britt, and E. M. Convey. 1973. Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cyst. J. Anim. Sci. 36:985.

Kurzrok, R. and C. C. Lieb. 1930. Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med. 26:268.

Lauderdale, J. W. 1972. Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246.

Lauderdale, J. W. 1973. Fertility and sperm transport following prostaglandin or progestogen treatment of cattle. Inserm. 28:499.

Li, J. C. R. 1964. Statistical Inference I, p. 467-469. Edwards Brothers, Inc., Ann Arbor, Mich.

Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effect of prostaglandin on cattle estrus cycles. J. Anim. Sci. 35:247.

Loeb, L. 1923. The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. Proc. Soc. Exp. Biol. and Med. 20:441.

Louis, T. M., H. D. Hafs, and B. E. Seguin. 1973. Progesterone, LH, estrus and ovulation after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 143:152.

Louis, T. M., J. N. Stellflug, H. A. Tucker, and H. D. Hafs. 1974. Plasma prolactin, growth hormone, luteinizing hormone and glucocorticoids after prostaglandinfed in heifers. Proc. Soc. Exp. Biol. Med. 147:128.

Makepeace, A. W., G. L. Weinstein, and M. H. Friedman. 1937. The effect of progestin and progesterone on ovulation in the rabbit. Amer. J. Physiol. 119:512.

Mares, S. E., R. G. Zimbelman, and L. E. Casida. 1962. Variation in progesterone content of the bovine corpus luteum of the estrual cycle. J. Anim. Sci. 21:226.

McCracken, J. A. and D. T. Baird. 1969. The study of ovarian function by means of transplantation of the ovary in the ewe. The Gonads, K. Mckerns (ed.), Applteon-Century-Crofts, New York, Chap. 7, p. 175.

McCracken, J. A. and B. V. Caldwell. 1969. Corpus luteum maintenance in a ewe with one congenitally absent uterine horn. J. Reprod. Fertil. 20:139.

McCracken, J. A., M. E. Glen, and R. J. Scaramuzzi. 1970. Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. J. Clin. Endocrinol. Metab. 30:544.

McCracken, J. A., D. T. Baird, and J. R. Goding. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent Progr. Horm. Res. 27:537.

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

McCracken, J. A., B. Barcikowski, J. C. Carlson, K. Green, and B. Samuelsson. 1973. The physiological role of prostaglandin $F_{2\alpha}$ in corpus luteum regression. Adv. Bio. Sci. 9:599.

Morrow, D. A. 1969. Estrous behavior and ovarian activity in prepubertal and post-pubertal dairy heifers. J. Dairy Sci. 52:224.

Nalbandov, A. V. 1964. Reproductive physiology. 2nd ed., W. H. Freeman, San Francisco, p. 316.

Nancarrow, C. D., H. Hearnshaw, P. E. Mattner, P. J. Connell, and B. J. Restal. 1974. Hormonal changes occurring in cattle following the administration of prostaglandin $F_{2\alpha}$. J. Reprod. Fertil. 36:484.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr., and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinol. 84:1166.

Niswender, G. D. 1973. Influence of site of conjugation on the specificity of antibodies to progesterone. Steroids 20:1041.

Novy, M. J. 1972. Distribution of ovarian blood flow in rabbits as measured by radioactive microspheres. Biol. Reprod. 7:105.

Oxender, W. D., H. D. Hafs, and L. A. Edgerton. 1972a. Serum growth hormone, LH and prolactin in the pregnant cow. J. Anim. Sci. 35:51.

Oxender, W. D., H. D. Hafs, and W. G. Ingalls. 1972b. Serum growth hormone, LH and prolactin in the bovine fetus and neonate. J. Anim. Sci. 35:56.

Pharriss, B. B. and L. J. Wyngarden. 1969. The effect of $PGF_{2\alpha}$ on the progesterone content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92.

Pharriss, B. B. 1970. The possible vascular regulation of luteal function. Perspect. Biol. Med. 13:434.

Pickles, V. R. 1967. Prostaglandins in the human endometrium. Int. J. Fert. 12:335.

Piper, P. J. and J. R. Vane. 1969. Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature 223:29.

Poyser, N. L. 1972. Production in prostaglandins by the guinea pig uterus. J. Endocrin. 54:147.

Poyser, N. L. 1973. The formation of prostaglandins by the guinea pig uterus and the effect of indomethacin. Adv. Biosci. 9:631.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variation. Acta Endocrinol. 34, Su-pl. 52:1.

Rakha, A. M. and H. A. Robertson. 1965. Changes in levels of follicle stimulating hormone and luteinizing hormone in the bovine pituitary gland at ovulation. J. Endocrinol. 31:245.

Roche, J. F. 1974a. Effect of short-term progesterone treatment on oestrous response and fertility in heifers. J. Reprod. Fertil. 40:433.

Roche, J. F. 1974b. Synchronization of oestrus in heifers with implants of progesterone. J. Reprod. Fertil. 41:337.

Rowson, L. E. A., R. Tervit, and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J. Reprod. Fertil. 29:145.

Sandberg, A. A. and W. R. Slaunwhite, Jr. 1958. Disappearance of radioactive progesterone in plasma. Endocrinol. Metabolism 18:253.

Schams, D. and H. Karg. 1969. Radioimmuno-logische LH--gestimmung im blatserum von rind unter besonderer berucks ichtigung des brunstzyklus. Acta Endocrinol. 61:96.

Schams, D. and H. Karg. 1970. Untersuchungen uber prolactin im rinderblut mit einer radioimmunologischen bestimmungsmethode. 2 bl. Vet. Med. 17:193.

Seguin, B. E., D. A. Morrow, and T. M. Louis. 1974. Luteolysis, luteostatis, and the effect of prostaglandin $F_{2\alpha}$ in cows after endometrial irritation. Am. J. Vet. Res. 35:57.

Sellner, R. G. and E. W. Wickersham. 1970. The effects of prostaglandins on steroidogenesis. J. Anim. Sci. 31:230.

Shemesh, M. and W. Hansel. 1975. Levels of prostaglandin F (PGF) in lovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. Proc. Soc. Exp. Biol. Med. 148:123.

Sinha, Y. N. and H. A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507.

Smeaton, T. C. and H. A. Robertson. 1971. Studies on growth and atresia of graafian follicles in the ovary of the sheep. J. Reprod. Fertil. 25:243.

Snook, R. M. and W. Hansel. 1970. Personal communication.

Speroff, L. and P. W. Ramwell. 1970. Prostaglandin stimulation of in vitro progesterone synthesis. J. Clin. Endocrinol. Metab. 30:345.

Stabenfeldt, G. H., L. L. Ewing, and L. E. McDonald. 1969. Peripheral plasma progesterone levels during the bovine oestrous cycle. J. Reprod. Fert. 19:433.

Stellflug, J. N., T. M. Louis, H. D. Hafs, and B. E. Seguin. 1975. Luteolysis, estrus and ovulation and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglandin $F_{2\alpha}$. Prostaglandins. In Press.

Swanson, L. V. and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038.

Swanson, L. V., H. D. Hafs, and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone, and glucocorticoids from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284.

Thorburn, G. D. and J. R. S. Hales. 1972. Selective reduction in blood flow to the ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into a uterine vine. International Union of Physiological Science Meeting, August, 1972.

Thornburn, G. D. and D. H. Nicol. 1971. Regression of the ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into the ovarian artery and uterine vein. J. Endocrinol. 51:785.

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Univ. of Nebraska Agr. Exp. Sta. Research Bull. 153.

Ulberg, L. C. and L. E. Casida. 1951. Ovarian response in heifers to progesterone injections. J. Anim. Sci. 10:752.

Von Euler, V. S. 1935. A depressor substance in the vesicular gland. J. Physiol., Lond. 84:21.

Wetteman, R. P., H. D. Hafs, L. A. Edgerton, and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020.

Wetteman, R. P. and H. D. Hafs. 1973. Pituitary and gonadal hormones associated with fertile and non-fertile insemination at synchronized and control estrus. J. Anim. Sci. 36:716.

Willett, E. L. 1950. The fertility of heifers following administration of progesterone to alter the estrual cycle. J. Dairy Sci. 33:381.

Wilson, L., Jr., R. J. Cenella, R. L. Butcher, and E. K. Inskeep. 1972. Levels of prostaglandins in the uterine endometrium during the ovine oestrous cycle. J. Anim. Sci. 34:93.

Wiltbank, J. N. and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134.

Wiltbank, J. N., J. E. Ingalls, and W. W. Rowden. 1961. Effects of various forms and levels of estrogens alone or in combinations with gonadotrophins on the estrous cycle of beef heifers. J. Anim. Sci. 20:341.

Wiltbank, J. N. 1966. Modification of ovarian activity in the bovine following injection of oestrogen and gonadotropin. In: "Ovarian Regulatory Mechanims." J. Reprod. Fertil. Suppl. 1, pp. 1-8.

Wishart, D. F. and I. M. Young. 1974. Artificial insemination of progestin (SQ1009)-treated cattle at predetermined times. Vet. Rec. 95:503.

Woody, C. O. and O. J. Ginther. 1968. Effect of exogenous progesterone on corpora lutea in unilaterally hysterectomized heifers. J. Anim. Sci. 27:1387.

Yamaudi, M., T. Nakahara, Y. Kaneda, and S. Inui. 1967. Effect of uterine distention on the oestrus cycle of the cow. J. Reprod. Fertil. 13:379.