LUTEOLYSIS, GROWTH HORMONE, GLUCOCORTICOIDS, PROLACTIN AND MILK PRODUCTION IN LACTATING DAIRY COWS GIVEN PG $F_{2\alpha}$

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RANDALL HARRELL RENEGAR

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ABSTRACT

LUTEOLYSIS, GROWTH HORMONE, GLUCOCORTICOIDS, PROLACTIN AND MILK PRODUCTION IN LACTATING DAIRY COWS GIVEN $PGF_{2\alpha}$

By

Randall Harrell Renegar

The objectives of this thesis were to (1) determine the minimal dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which when given (im) to lactating dairy cows results in an incidence of luteolysis not significantly different from higher doses, (2) characterize changes in growth hormone, prolactin and glucocorticoids after $PGF_{2\alpha}$, and (3) determine changes in milk production after $PGF_{2\alpha}$.

Fifty-seven lactating (2-3 mo) Holstein cows were given (im) 25 mg $PGF_{2\alpha}$ 12 days before receiving at random a second dose of either 0 (saline), 5, 15, 25 or 35 mg $PGF_{2\alpha}$. Blood samples were collected via the coccygeal vein twice weekly from day -12 to day 0 (day of treatment injection), day 9 to 22 and days 24 and 26. Frequent blood samples were collected (jugular cannulae) at .5-hr intervals from -.5 to 2 hr then at 2-hr intervals for 16 hr, 4-hr intervals for 24 hr, 2-hr intervals for 54 hr and 12-hr intervals for 36 hours. Animals were observed for estrous behavior twice daily from day 2 to day 26, and ovaries were palpated twice

daily for 5 days after treatment and twice weekly thereafter to day 26. Milk samples were collected for protein and fat determinations and milk yield was recorded for each cow at AM and PM milkings 7 days before the first $PGF_{2\alpha}$ injection and for 7 days after treatment injection.

Luteolysis in response to $PGF_{2\alpha}$ (serum progesterone less than 1.0 ng/ml and no palpable corpus luteum within 132 hr) occurred in 0 of 11 cows given saline, 4 of 10 given 5 mg $PGF_{2\alpha}$, 9 of 11 given 15 mg, 8 of 10 given 25 mg and 10 of 10 given 35 mg. Incidence of luteolysis in cows given 15, 25 or 35 mg $PGF_{2\alpha}$ was significantly greater than that in cows given 0 or 5 mg. Incidence of luteolysis did not differ among animals given 15, 25 or 35 mg $PGF_{2\alpha}$ and averaged 88%. Progesterone declined to less than 1.0 ng/ml within 24 hr in animals with luteolysis after $PGF_{2\alpha}$. In animals given 5, 15 or 25 mg $PGF_{2\alpha}$ not having luteolysis, progesterone declined from 5.3 to 3.3 ng/ml within 12 hr, but returned toward pretreatment concentrations within 24 hours. Progesterone was unaltered in animals given saline. Following $PGF_{2\alpha}$-induced luteolysis, estrus began at 72 hr, peak of the LH surge occurred at 80 hr and ovulation occurred at 100 hours.

Growth hormone increased from 1.7 to 3.3 ng/ml within .5 hr after 35 mg $PGF_{2\alpha}$, but was unaltered in cows given 0, 5, 15 or 25 mg $PGF_{2\alpha}$. Glucocorticoid averaged 6.8 ng/ml prior to injection and was unaltered by saline or 5 mg $PGF_{2\alpha}$.

However, glucocorticoid increased to peaks of 22.3, 34.9 and 39.0 ng/ml at 1.0 hr following 15, 25 and 35 mg $PGF_{2\alpha}$, respectively. Prior to injection, prolactin averaged 37.8 ng/ml and was unaltered by saline or 5 mg $PGF_{2\alpha}$. Prolactin increased to peaks of 56.8, 84.5 and 95.6 ng/ml at 1.0 hr after 15, 25 and 35 mg $PGF_{2\alpha}$, respectively. The peak concentrations of glucocorticoid and prolactin were linearly related to the dose of $PGF_{2\alpha}$.

Average daily milk yield for the 7 days following $PGF_{2\alpha}$ was 22.4 kg and did not differ among treatments. Percent milk fat and percent total protein averaged 3.18 and 3.29, respectively, unaltered by dose of $PGF_{2\alpha}$.

These data indicate that 15 mg is the minimal intramuscular dose of $PGF_{2\alpha}$ for lactating dairy cows resulting in an incidence of luteolysis not significantly different from higher doses. $PGF_{2\alpha}$ also caused transient increases in serum growth hormone, glucocorticoid and prolactin, three hormones with important roles in galactopoesis and lactogenesis. However, milk yield, percent milk fat and percent total milk protein were unaltered following $PGF_{2\alpha}$.

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By

Randall Harrell Renegar

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TO MARILYN

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BIOGRAPHICAL SKETCH

Randall Harrell Renegar was born on April 12, 1952, in Winston-Salem, North Carolina. He attended primary schools in Charlotte, North Carolina, graduating from Independence High School in June, 1970. Pursuing his interest in biological science, he enrolled at North Carolina State University and completed requirements for Bachelor of Science degrees in Animal Science and Zoology in May, 1975. He accepted a research assistantship at Michigan State University studying under the directorship of Dr. Harold D. Hafs and completed the requirements for a Master of Science degree in August, 1977.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Artificial insemination of dairy cattle has made major contributions to improved milk production. However, some believe progress from artificial insemination (AI) has been retarded recently due to decreased estrus detection efficiency brought about by increased herd size. The need for accurate methods of estrus detection to enhance AI has prompted development of methods of ovulation control and estrus synchronization. Initial attempts to synchronize estrus involved delaying estrus with a 15- to 18-day progestogen treatment until the corpus luteum regressed. Subsequent removal of treatment resulted in reasonably synchronous occurrence of estrus; however, the fertility of an insemination following the synchronized estrus was low compared to controls. Recently, the use of progestogens for more limited periods (9-12 days) in conjunction with estrogens has been effective in synchronizing estrus with no apparent reduction in fertility.

Another method of estrus synchronization involves the use of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to promote regression of the corpus luteum. Injection, either intramuscularly (im) or subcutaneously (sc), or intrauterine (iu) infusion of $PGF_{2\alpha}$ results in demise of the corpus luteum. Normal

reproductive endocrine changes follow luteolysis, and estrus occurs in 2-6 days. Fertility of an insemination following estrus synchronization with $PGF_{2\alpha}$ is comparable to fertility following naturally occurring estrus, at least in heifers and beef cattle. However, $PGF_{2\alpha}$ has not been tested adequately in milking dairy cows because regulatory agencies required that milk be discarded for prolonged periods. Recent regulatory changes have made this work possible. Consequently, the research presented in this thesis was designed primarily to determine the minimal dose of $PGF_{2\alpha}$ which when given (im) to lactating dairy cows results in an incidence of luteolysis not significantly different from higher doses.

The second objective of this research was to characterise changes in blood concentrations of growth hormone, prolactin and glucocorticoids following $PGF_{2\alpha}$ treatment. Drastic shifts in secretion of these hormones could reflect unwanted physiological changes produced by exogenous $PGF_{2\alpha}$. Also, before it is approved for estrus synchronization in lactating dairy cows, we should know whether $PGF_{2\alpha}$ changes milk production and composition. Therefore, the third objective of this work was to determine milk yield, percent fat and percent protein in cows given $PGF_{2\alpha}$.

REVIEW OF LITERATURE

The Normal Bovine Estrous Cycle

The bovine estrous cycle is approximately 21 days although cycles with 19- to 22-day durations are considered normal (Chapman and Casida, 1937; Kazama and Hansel, 1970; Swanson et al., 1972). In this thesis, days of the estrous cycle will be numbered 0 to 21 with day 0 representing the day of estrus. Estrus averages 18 hr in duration ranging from 12-24 hr (Desjardins and Hafs, 1968). In sequence, the next three phases of the cycle are (1) Metestrus (day 1 to 4); (2) Diestrus (day 4 to 18); and (3) Proestrus (day 18 to 21). Slight variations may occur in the duration of the cycle phases among animals (Chapman and Casida, 1937). Here follows some of the major reproductive endocrine changes during the bovine estrous cycle.

Metestrus. Metestrus is characterized by ovulation and formation of the corpus luteum. The newly formed corpus luteum contains very little progesterone on day 2 (Hafs and Armstrong, 1968) but corpus luteum weight and function begin to increase by day 2-3 (Mares et al., 1962; Erb et al., 1971; Glencross et al., 1973; Hansel et al., 1973). With increased progesterone secretion in the developing corpus luteum, the peripheral blood concentrations of progesterone

begin to increase on day 3-5 and continue to increase reaching a peak on days 11-15 during diestrus (Stabenfeldt, 1968; Pope et al., 1969; Stabenfeldt et al., 1969; Hansel and Snook, 1970; Hansel et al., 1973). Plasma estrogen concentrations rise during metestrus from 2 pg/ml on day 1 to 6-7 pg/ml on day 4 or 5 (Wettemann et al., 1972; Glencross et al., 1973; Hansel et al., 1973). Hansel et al. (1973) reported that estrone makes up the major rise in estrogens during this period. Peripheral blood concentrations of luteinizing hormone are low during metestrus (Swanson et al., 1972; Hansel et al., 1973), ranging from 0.4 to 2.7 ng/ml (Schams et al., 1969; Wettemann et al., 1972). Pituitary concentrations of LH decrease from day 0 to day 2 (Hackett and Hafs, 1969) but no change in blood levels of LH occurs (Swanson et al., 1972). This would seem to indicate that pituitary synthesis of luteinizing hormone is depressed during this period. Pituitary FSH levels decline on day 4. If this reflects released FSH, it may stimulate the mid-cycle wave of follicle growth (Hackett and Hafs, 1969).

Diestrus. Corpus luteum growth and increased progesterone secretion characterize diestrus. Diestrus ends upon the initiation of luteal regression, which may occur anywhere from day 16 to 19 (Kazama and Hansel, 1970).

Corpus luteum growth continues until day 11-15 (Erb et al., 1971; Hansel et al., 1973; Hafs and Armstrong, 1968) of the cycle with no further changes in corpus luteum weight

until luteolysis. Erb et al. (1971) reported three periods of accelerated luteal growth which were associated with increased secretion of urinary estrogens. Corpus luteum concentration of progesterone increases significantly from day 4 to 7 (Hafs and Armstrong, 1968) with subsequent increases to day 17 (Mares et al., 1962). Plasma progesterone concentrations continue to increase during diestrus to a peak on day 11 to 13 of 5-9 ng/ml and remain high until luteolysis (Pope et al., 1969; Stabenfeldt et al., 1969; Wettemann et al., 1972; Hansel et al., 1973). Estradiol remains low during diestrus and averages about 3.6 pg/ml (Wettemann et al., 1972). Hansel et al. (1973) reported a rise in total estrogens on days 7 to 10 which may correspond with the mid-cycle wave of follicular development (Hackett and Hafs, 1969). The mid-cycle follicle growth is reflected by an increase in follicular wall weight during days 7 to 11 (Swanson et al., 1972). Plasma LH remains low during diestrus (Swanson et al., 1972; Hansel et al., 1973) while pituitary luteinizing hormone increases from day 2 to 20 (Hackett and Hafs, 1969). Pituitary follicle stimulating hormone concentrations decline to mid-cycle but are restored by days 10 to 13 with a subsequent decline prior to estrus (Desjardins and Hafs, 1968).

Proestrus. Regression of the corpus luteum characterizes proestrus, beginning between day 16 to 19 in

cows with 21-day cycles (Kazama and Hansel, 1970). Stabenfeldt et al. (1969) also reported a significant variation in day at which luteal regression occurs.

Progesterone concentrations fall precipitiously from peak values of 4-9 ng/ml about 3 days before estrus to less than 1.0 ng/ml 1-2 days before estrus (Pope et al., 1969; Stabenfeldt et al., 1969; Erb et al., 1971). Ayalon and Shemesh (1974) reported a proestrus peak of progesterone (2.3 ng/ml) at about 16 hr prior to the onset of estrus, but a subsequent study did not confirm this (Chenault et al., 1975). Pituitary FSH concentration decreases markedly from day 18-0 (Hackett and Hafs, 1969) coincided with a major increase in the number and size of follicles on the ovaries (Swanson et al., 1972).

Plasma estradiol concentrations begin to increase 3-4 days prior to estrus (Wettemann et al., 1972; Chenault et al., 1975). Chenault et al. (1975) reported slowly increasing estradiol concentrations (2.0 pg/ml to 4.0 pg/ml) from -12 to -4 hr with a more rapid increase up to a peak of 7.4 pg/ml at or about the time of the peak of the ovulatory surge of LH. Estradiol then decreases to baseline by 14 hr after the peak. Wettemann et al. (1972) and Glencross et al. (1973) reported similar estradiol changes. The ovulatory peak of LH occurs at or shortly before the beginning of estrus (Hansel and Snook, 1970; Swanson and Hafs, 1971) and remains above baseline for 6-10 hr (Schams, 1969; Swanson and Hafs, 1971). This peak is believed to be

stimulated by the increase in estradiol prior to the LH surge (Chenault et al., 1975). Swanson and Hafs (1971) reported maximum LH concentrations of 25.9 ng/ml. Snook et al. (1971) and Chenault et al. (1975) reported similar values. Chenault et al. (1975) reported a 22-hr interval from the peak of the ovulatory surge of LH to ovulation; however, Swanson and Hafs (1971) reported an interval of 32 hours. The variance in these reported intervals may reflect dissimilarity in the interval between consecutive palpations used for determining the time of ovulation.

Estrus. Estrus is the 12- to 24-hr period during which the cow is sexually receptive. Progesterone concentrations are less than 1 ng/ml during estrus, evidence of no functional corpus luteum (Stabenfeldt et al., 1969; Henricks et al., 1971; Glencross et al., 1973; Chenault et al., 1975). Estradiol concentrations decline from the peak at outset of estrus to baseline values well before the end of estrus (Chenault et al., 1975). Plasma LH also falls precipitously from the peak at the outset of estrus to basal values well before the end of estrus (Hansel et al., 1973; Chenault et al., 1975).

Luteolysis

Luteolysis is the process by which the corpus luteum becomes incapable of synthesis of progesterone and aids in returning the animal to sexual receptivity after a period of anestrum. Research principally on sheep indicated that

luteolysis is initiated by a blood born substance synthesized in the uterus (Caldwell et al., 1969; Goding et al., 1972). McCracken et al. (1972) autotransplanted ovaries to a location (neck) distant from the uterus of sheep, and found that the corpus luteum was retained for an extended period. Ovaries autotransplanted with accompanying uterine horn resulted in normal cyclic corpus luteum regression. When blood from the venous supply of a uterine horn in situ in one ewe was transfused to the arterial supply of an autotransplanted ovary in another ewe, luteolysis occurred in a normal cyclic pattern. Thus, the luteolytic influence of the uterus appears to act only when the uterus and ovary are located near each other. The uterus also is required for normal cyclic luteal regression in the cow (Wiltbank and Casida, 1956; Anderson et al., 1969). The local pathway for transport of the uterine luteolysin is venoarterial in nature (Ginther, 1976). Close apposition of the uterine vein and its adjacent ovarian artery in sheep (Ginther, 1974) and cattle (Ginther and Del Campo, 1974) allows diffusion of the luteolytic factor from the uterine drainage into arterial blood supplying the ovary (Mapletoft and Ginther, 1975; Mapletoft et al., 1976).

The mechanism of action of the uterine luteolytic agent is unknown. Pharriss (1970) purposed the hypothesis that the uterine luteolysin is acting to restrict blood flow to the ovary leading to the demise of the corpus luteum. Additional evidence indicates that luteolysis is brought

about by action of the luteolysin upon responsiveness of the corpus luteum to LH (Armstrong and Black, 1966; Lahav et al., 1976).

The identity of the substance responsible for luteolysis in cows is not known with certainty, although evidence strongly suggests that $PGF_{2\alpha}$ is the physiological luteolysin in sheep (Goding et al., 1972; McCracken et al., 1972). Recently, Scaramuzzi and Baird (1976) reported that sheep autoimmunized against $PGF_{2\alpha}$ fail to undergo a normal pattern of cyclic luteal regression.

Prostaglandins

Prior to reviewing the literature concerning $PGF_{2\alpha}$-induced luteolysis and estrus synchronization, I feel that a brief summary of work leading up to recognition of the potential of $PGF_{2\alpha}$ in reproductive physiology is needed.

The prostaglandins are a family of biologically active, unsaturated fatty acids widely distributed among animal tissues. Kurzrok and Lieb (1930) were the first to demonstrate the action of prostaglandins. They found that fresh human semen could cause either contraction or relaxation of uterine tissue in vitro. Goldblatt (1933) and von Euler (1935) demonstrated the ability of seminal fluid to cause smooth muscle contraction. Prostaglandin E_1 and prostaglandin F_2 were isolated from sheep vesicular gland in pure crystalline form by Bergstrom and Sjovall in 1957. Subsequently other prostaglandins were isolated and their

structures defined (Bergstrom et al., 1962a; Bergstrom et al., 1962b), thus stimulating increased effort in understanding the biological properties of the prostaglandins. It was not until Pharriss and Wyngarden (1969) demonstrated the luteolytic property of $PGF_{2\alpha}$ in pseudopregnant rats that its potential regarding application to controlled reproduction was recognized.

Luteolysis, Estrus and Fertility after $PGF_{2\alpha}$

A number of reviews of literature have recently appeared concerning the use of $PGF_{2\alpha}$ in estrus synchronization and controlled breeding (Inskeep, 1973; Arriola, 1975; Hafs et al., 1975a; Lamming et al., 1975; Manns and Hafs, 1976). For this reason, the following will be a brief summary of the literature including references to both reviews and original references.

Discovery of the luteolytic property of $PGF_{2\alpha}$ in rats (Pharriss and Wyngarden, 1969) prompted efforts to determine the effect of $PGF_{2\alpha}$ on luteal function in other species.

Subsequent work demonstrated the luteolytic capacity of $PGF_{2\alpha}$ in sheep (McCracken et al., 1972; Mellin and Busch, 1976), guinea pigs (Charchareon, 1974), horses (Hafs et al., 1974), beef cows (Hafs et al., 1975b) and dairy heifers (Louis et al., 1973; Louis et al., 1974; Elving et al., 1975; Stellflug et al., 1976). Louis et al. (1974) reported that changes in progesterone, estrogen and LH in heifers during and after $PGF_{2\alpha}$-induced luteolysis were not

significantly different from changes occurring after luteolysis in untreated heifers, and the interval from luteolysis to estrus or to the ovulatory surge of LH was not significantly different. Furthermore, the cycle following $PGF_{2\alpha}$ treatment did not differ endocrinologically from normal cycles in cows (Louis et al., 1974). This information indicates that $PGF_{2\alpha}$ has no residual effect on the endocrine events of the estrous cycle and probably modifies the cycle by effecting only on luteolytic mechanisms. Louis et al. (1973) gave $PGF_{2\alpha}$ to heifers during different phases of the estrous cycle and found that luteolysis occurred in heifers treated during diestrus but not metestrus or proestrus. A functional (progesterone secretion) corpus luteum (day 5 to 18 in the cow) is necessary for $PGF_{2\alpha}$ to initiate luteolysis. Hafs et al. (1975c) found a substantial degree of synchrony in interval to estrus following $PGF_{2\alpha}$-induced luteolysis in dairy heifers, with 88% of the animals in estrus 2-4 days after treatment. Lauderdale et al. (1974), Elving et al. (1975) and Kaneda et al. (1976) recorded similar results.

Lauderdale et al. (1974) treated beef cows and dairy heifers with $PGF_{2\alpha}$ to induce luteolysis and estrus with AI at either (1) 12 hr after observed estrus or (2) 72 and 90 hr after $PGF_{2\alpha}$ injection. Control cattle were inseminated 12 hr after observed estrus. Pregnancy at 35-60 days after AI did not differ among the three groups. Hafs et al. (1975c) also found no difference in fertility in animals inseminated at 72 and 88 hr when compared to controls.

Recent evidence reported by Stellflug et al. (1976) suggests that the minimum dose of $PGF_{2\alpha}$ to obtain maximum luteolytic response in dairy heifers is 15 mg when given (im) as Tromethamine salt.

In overview, reported evidence indicates that $PGF_{2\alpha}$ may be of great value for ovulation control to increase the use and efficiency of AI by eliminating the prerequisite of estrus detection. Aftificial insemination at a predetermined time should decrease the time necessary for estrus detection.

Anterior Pituitary and Adrenal Cortical Response to Exogenous $PGF_{2\alpha}$

Adrenal Corticotropin and Glucocorticoids. In 1969, de Wied et al. demonstrated the ability of prostaglandins of the E series but not F series to increase blood corticosterone in rats. Peng et al. (1970) found that the increase in adrenal corticoids associated with PGF_1 injection did not occur in hypophysectomized rats. In addition, PGE_1 injections given animals pretreated with morphine did not result in an adrenal cortical response (Peng et al., 1970). Stereotaxic injections of PGE_1 and $PGF_{2\alpha}$ into the median eminance area of the hypothalamus gave a far greater corticosterone response than injections into the anterior pituitary (Hedge and Hanson, 1972). In contrast, corticotropin releasing factor injections result in maximum response when given in the anterior pituitary. Louis et al. (1974) demonstrated that (im) injections or (iv) infusion of $PGF_{2\alpha}$

caused increased glucocorticoids in dairy heifers. This evidence indicates that prostaglandins of both the E and F series are capable of increasing adrenal corticoids via increased release of ACTH. The action of these prostaglandins appears to be on the hypothalamus, and not directly on the anterior pituitary (Hedge and Hanson, 1972).

Growth Hormone. Schofield (1970) incubated anterior pituitary slices with PGE_1 and reported increased growth hormone secretion. Cooper et al. (1972) reported that adenosine 3'5' monophosphate increased prior to increases in growth hormone in ox pituitaries incubated with PGE_2. Single injections of PGE_1 given to pentobarbitol treated rats increased blood growth hormone concentrations (Hertelendy et al., 1972). Hertelendy et al. (1972) also reported that single injections of PGE_1 increased growth hormone from 2 ng/ml to 14.4 ng/ml within 20 min in castrate male sheep. Louis et al. (1974) reported that 30 and 60 mg of $PGF_{2\alpha}$ given (im) to dairy heifers cause 7- and 26-fold increases in blood growth hormone levels, respectively. Tucker et al. (1975) observed similar increases in growth hormone with $PGF_{2\alpha}$ infusions into heifers at a rate of 30 mg/hour. In summary, prostaglandins E_1 and $F_{2\alpha}$ increase growth hormone secretion in vivo and in vitro. Evidence reported for PGE_1 suggests that prostaglandins exert their effect directly on the pituitary via cAMP. Prostaglandin $F_{2\alpha}$ is a potent inducer of growth hormone release in vivo in heifers.

Prolactin. Sato et al. (1974) first demonstrated the ability of prostaglandin E_1, E_2 and $F_{2\alpha}$ to increase serum prolactin in rats. Subsequently, Louis et al. (1974) showed that $PGF_{2\alpha}$ given (im) to heifers causes an increase in blood prolactin concentrations from 26 ng/ml prior to injection to 81 ng/ml by 10 min after injection. Tucker et al. (1975) demonstrated that infusions of $PGF_{2\alpha}$ dramatically increase blood serum prolactin in heifers.

In overview, the published evidence indicates that $PGF_{2\alpha}$ given in doses sufficient to cause luteolysis also elicits increased secretion of three hormones (glucocorticoid, growth hormone and prolactin) important in regulating intermediary metabolism (Hafs, 1975). Whether changes in these hormones induced by $PGF_{2\alpha}$ may affect milk production is unknown.

Milk Yield, Milk Fat and Milk Protein Response to GH, Prolactin and Glucocorticoids

Based upon the evidence outlined above, $PGF_{2\alpha}$ is an effective method of synchronizing estrus for controlled breeding in cattle. Before $PGF_{2\alpha}$ is widely used for controlled breeding in lactating cows, the possibility of undesirable side effects should be investigated. This is of special interest when one considers the increased release of growth hormone, prolactin and glucocorticoids following $PGF_{2\alpha}$ treatment to induce luteolysis (Hafs, 1975).

The following reviews relationships of growth hormone, prolactin and glucocorticoids with galactopoesis.

Growth Hormone. Asimov and Krouze (1937) first reported that anterior pituitary preparations relatively pure in growth hormone caused increased milk production in lactating cows. Chung et al. (1953) found that six daily injections of 100 mg of growth hormone caused a 50% increase in milk production (accompanied by substantial increases in percent milk fat) by the sixth injection in late lactating cows. In addition, milk production was maintained and percent milk fat increased in the face of a 40% reduction in TDN intake when accompanied by eight daily 100 mg injections of growth hormone. Wrenn and Sykes (1953) also reported increases in milk production after growth hormone treatment in heifers which had been hormonally induced into lactation. Bullis et al. (1965) compared a commercial preparation of growth hormone with a highly purified preparation and found increased milk production with both treatments although the response associated with the commercial product was significantly greater. All milk production increases were accompanied by increased percent fat and total solids. The reported work of Bullis et al. (1965) may indicate that the dramatic increases seen by Chung et al. (1953) and Wrenn and Sykes (1953) were partially due to contamination with other hormones in the preparations used, although it does not rule out the galactopoietic effect of growth hormone. Subsequent study with highly purified growth hormone indicated a significant galactopoietic effect (Machlin, 1973).

Machlin (1973) also reported that growth hormone induced increases in milk production without accompanying increases in feed consumption.

Glucocorticoids. In 1956 Shaw reported decreases in milk production and increases in blood glucose in cows during glucocorticoid treatment. Link et al. (1957) and Vigue (1958) reported similar results. Braun et al. (1970) administered (im) various types of synthetic glucocorticoids for 10 days to normal lactating Holstein cows. Milk production decreased over the 10-day period with the largest decrease occurring in the animals with the greatest increase in blood glucose. Bassett (1963) reported that cortisol fed to sheep caused increased blood glucose without increases in ketone bodies and plasma free fatty acids. This evidence may indicate that glucocorticoids decrease milk production by decreasing glucose utilization. In contrast, Swanson and Lind (1976) reported that prolonged glucocorticoid treatment (day 4 postpartum to end of lactation) resulted in increases in milk production when 5-10 μg was given orally each day. Higher doses of 20 μg and 50 μg caused no change and depression, respectively. In addition milk production was unchanged when 5 or 10 μg was given daily for 25 days. Head et al. (1976) reported no change in milk production but an increase in percent protein with 10 μg given orally from week 4 to week 44 of lactation.

Milk yield and composition responses to exogenous glucocorticoids appear to be related to synthetic preparation,

dosage, length of administration and possibly mode of administration. Although the results are not conclusive, evidence supports the view that large doses of glucocorticoids are detrimental to milk production while moderate levels of glucocorticoids are without effect or are slightly stimulatory when given chronically. Limited evidence suggests milk protein may be increased with moderate levels of glucocorticoid treatment.

Prolactin. Prolactin is galactopoietic in the rat (Johnson and Alfredson, 1958; Kumaresan et al., 1966), sheep (Cowie, 1969), rabbit (Cowie, 1969) and goat (Meites, 1961), but has minor galactopoietic activity in the cow (Karg and Schams, 1974). Experimentally induced reduction of prolactin secretion during lactation does not alter milk production significantly in cows (Karg and Schams, 1974; Smith et al., 1974). Similarly, Wrenn and Sykes (1953) found no change in milk yield in heifers given prolactin during hormonally induced lactation. Koprowski and Tucker (1973) found blood prolactin concentrations 2-4 hr before milking were not related ($r = -.03$) with milk yield. In contrast, Karg et al. (1972) reported depressed onset of lactation in cows given CB-154 (a prolactin release inhibitor) prior to lactation, indicating that prolactin is necessary for lactogenesis. Conclusive studies involving the galactopoietic effects of prolactin are not available in cows. However, the present evidence indicates that prolactin has no significant effect on milk production during established lactation in cows, but is required for initiation of lactation.

MATERIALS AND METHODS

This research was conducted primarily to determine the minimal intramuscular dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) required for luteolysis among lactating cows. Prolactin, growth hormone and glucocorticoid (indicator of adrenocorticotropin release) responses to $PGF_{2\alpha}$ were characterized for 4 hr after treatment. Additional information was obtained to determine acute (7 days following treatment) changes in milk production, percent milk fat and percent total protein following $PGF_{2\alpha}$.

Experimental Design

Fifty-seven lactating (2 to 3 mo) Holstein-Friesian cows were assigned at random to be given 0, 5, 15, 25 or 35 mg of $PGF_{2\alpha}$. All animals were given 25 mg $PGF_{2\alpha}$ 12 days prior to treatment injection to insure the existence of a functional corpus luteum at treatment. $PGF_{2\alpha}$ was diluted in 5 ml of .85% saline and given intramuscularly. Blood samples were collected from all animals via the tail vein twice weekly from day -12 to day 0 (day of treatment injection), day 9 to 22 and days 24 and 26. A limited number of animals (n = 30) were randomly selected to be bled at frequent intervals after treatment to determine anterior pituitary hormone and glucocorticoid changes after $PGF_{2\alpha}$

and to detect the ovulatory surge of LH. These blood samples were collected through indwelling jugular cannulae at .5-hr intervals from -.5 to 2 hr after treatment, then at 2-hr intervals for 16 hr, 4-hr intervals for 24 hr, 2-hr intervals for 54 hr and 12-hr intervals for 36 hr (last sample 132 hr after treatment). The remaining 27 animals were bled via tail vein at 0, 6, 12, 24, 48, 72, 96, and 132 hours. Blood samples were held at room temperature for 2-6 hr following collection and then held at 4 C for 48-72 hours. Following centrifugation at 3000 rpm for 30 min, serum was decanted and stored at -20 C until assayed. Animals were observed for estrous behavior twice daily beginning on day 2 and continuing to day 26. The ovaries and uterus were palpated twice weekly in each cow from day -12 to day 26 to detect changes in the size of the corpus luteum, ovulation and signs of estrus. Composite milk samples were collected for each cow at AM and PM milkings for 7 days prior to the first $PGF_{2\alpha}$ injection (pre-treatment samples) and 7 days after the treatment injection (post-treatment samples). Milk production was recorded for each milking.

Hormone Assays

Serum luteinizing hormone, prolactin and growth hormone were analyzed by double antibody radioimmunoassays (RIA) reported by Oxender *et al.* (1972), Tucker *et al.* (1971) and Purchas *et al.* (1970), respectively. The analysis of serum progesterone was by the single antibody radioimmunoassay

method described by Louis et al. (1973). Serum glucocorticoid concentrations were measured by protein-binding as reported by Smith et al. (1973).

Determination of Percent Milk Fat and Percent Total Protein

Milk samples were analyzed for percent milk fat using a Milko-Tester MK III by the method described by McGann et al. (1970). Total milk protein was measured using the dye-binding technique reported by Udy (1971).

Statistical Analysis

Treatment differences in the number of animals responding to $PGF_{2\alpha}$ (measured by luteolysis within 132 hr) were determined by chi-square statistics. Progesterone, prolactin, growth hormone and glucocorticoid data were analyzed by multivariate analysis of trends as described by Gill and Hafs (1971). Repeat-measure designs may result in heterogeneous variance of samples among periods within treatment group, and the statistical procedure used accounted for higher correlation between samples taken at close intervals than for samples taken more distant in time. Orthogonal contrasts were determined to locate significant differences among treatments. The difference between pre-treatment average and individual post-treatment data for milk yield, percent milk fat and total milk protein was analyzed by univariate split-plot. Interval to estrus was analyzed by one-way analysis of variance with orthogonal contrasts to test treatment differences.

RESULTS AND DISCUSSION

Reproductive Criteria

Five animals had serum progesterone less than 1.0 ng/ml and no palpable corpus luteum at the time treatment injections were given. Since they could not respond, they were eliminated from the analyses for luteolytic response, progesterone, and intervals to estrus, the ovulatory LH surge and ovulation.

Luteolysis. Luteolysis was determined by blood serum progesterone concentrations and ovarian palpation. I defined $PGF_{2\alpha}$-induced luteolysis as a decline in progesterone to less than 1.0 ng/ml and no palpable corpus luteum within 132 hr after treatment injection. No cows given saline had luteolysis within 132 hr following injection (Table 1). Luteolysis occurred in 4 of 10 cows given 5 mg $PGF_{2\alpha}$, 9 of 11 cows given 15 mg, 8 of 10 cows given 25 mg and 10 of 10 cows given 35 mg. The incidence of luteolysis in cows given 15, 25 or 35 mg $PGF_{2\alpha}$ was significantly greater ($P<.05$) than in cows given 0 or 5 mg $PGF_{2\alpha}$. No significant difference was detected among the cows given 15, 25 or 35 mg; however, there was some evidence ($P<.15$) that 35 mg $PGF_{2\alpha}$ was more effective in causing luteolysis. The average incidence of luteolysis for all animals given

Table 1. Luteolysis in lactating cows given (im) $PGF_{2\alpha}$.

$PGF_{2\alpha}$	No. cows responding / No. cows treated
(mg)	
0	$0/11^{a}$
5	$4/10^{b}$
15	$9/11^{c}$
25	$8/10^{c}$
35	$10/10^{c}$

^{a,b,c} Ratios with different superscripts differed significantly ($P<.025$)

15, 25 and 35 mg $PGF_{2\alpha}$ was 88%. Incidence of luteolysis in these lactating dairy cows is similar to reported incidences of 79 and 88% for suckled beef cows given (im) 30 or 60 mg $PGF_{2\alpha}$ and dairy heifers given (im) 20, 30 or 40 mg $PGF_{2\alpha}$, respectively (Hafs et al., 1975c). In contrast, Stellflug et al. (1976) found 100% incidence of luteolysis when six heifers were given (im) 15 mg $PGF_{2\alpha}$; however, the significance of this value is limited due to the number of animals used. Comparison of data from this thesis and previous work indicate that incidence of luteolysis for lactating dairy cows is similar to that reported for heifers and suckled beef cows, but conclusive comparisons must be done within one trial.

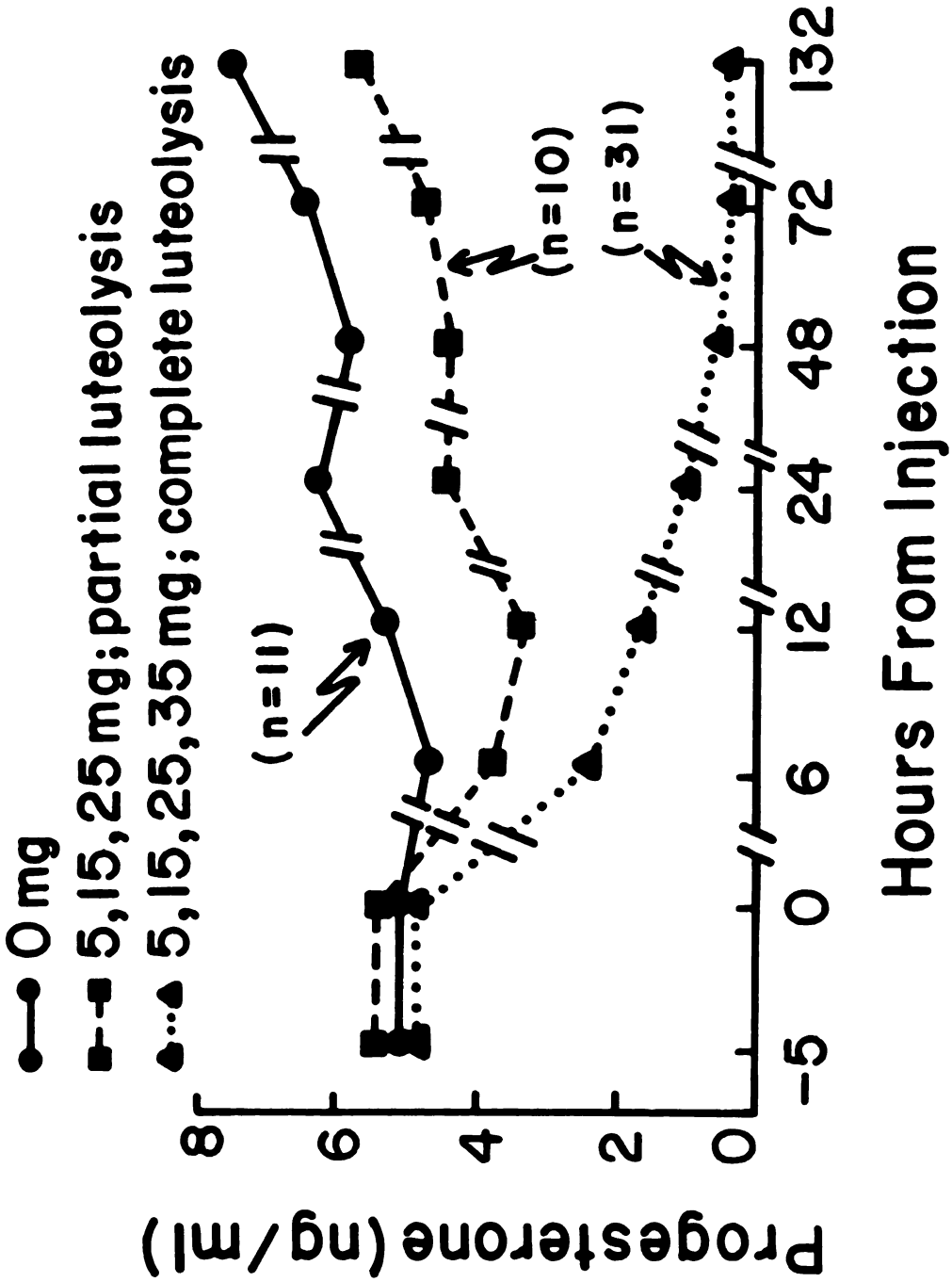
Progesterone. Average changes in serum progesterone among treatments were confounded with incidences of luteolytic response within teratments. Preliminary analysis indicated that serum progesterone changes for cows responding with luteolysis did not differ among treatments. Therefore, progesterone values for all cows (n = 31) responding with luteolysis were pooled. Progesterone data for the 10 cows without luteolysis following injection of 5, 15 or 25 mg $PGF_{2\alpha}$ likewise were pooled, as preliminary analysis indicated no difference in progesterone trend among treatments. Cows given saline (0 mg $PGF_{2\alpha}$) were designated as a control group (n = 11). Progesterone concentration did not differ among treatments prior to injections; it averaged 5.0 ± 0.3 ng/ml.

Serum progesterone was unaltered for 72 hr after saline injection, and it increased ($P<.05$) above pretreatment concentrations by 132 hr after treatment. Thus, the function of the corpus luteum was not reduced following saline injection.

Progesterone declined from 5.3 to 3.3 ng/ml ($P<.05$) within 12 hr after $PGF_{2\alpha}$ injection in cows without luteolysis, but returned toward pretreatment concentration by 24 hr following $PGF_{2\alpha}$ (Figure 1). Evidently, animals which did not have complete luteolysis following $PGF_{2\alpha}$ did have partial luteolysis. However, luteal function in these cows returned to near normal within 24 hours. Stellflug et al. (1976) reported similar changes in progesterone concentrations in heifers given doses of $PGF_{2\alpha}$ not sufficient for complete luteolysis. They found that progesterone concentrations declined by 12 hr following $PGF_{2\alpha}$ in heifers without complete luteolysis, but remained stable or increased during the remaining portion of a 108-hr sampling period.

Blood progesterone declined 50% ($P<.05$) from 4.9 to 2.4 ng/ml within 6 hr in cows with complete luteolysis after $PGF_{2\alpha}$. Then progesterone decreased ($P<.05$) to .8 ng/ml at 24 hr after injection and remained less than 1 ng/ml for the remainder of the 132-hr sampling period. Progesterone values for lactating cows with complete luteolysis in this thesis agree with previous work in heifers reported by Louis et al. (1973). They reported a 60% decrease in

Figure 1. Blood serum progesterone in lactating cows given (im) 0, 5, 15, 25 or 35 mg $PGF_{2\alpha}$. Standard errors ranged from 0.1 to 1.0 ng/ml and generally were proportional to the means.


0 mg
5,15,25 mg; partial luteolysis
5,15,25,35 mg; complete luteolysis
(n=11)
(n=10)
(n=31)
Progesterone (ng/ml)
8
6
4
2
0
-5
0
6
12
24
48
72
132
Hours From Injection

progesterone within 12 hr after $PGF_{2\alpha}$, and progesterone concentrations of less than 1 ng/ml within 24 hours. Louis et al. (1974) reported similar changes after $PGF_{2\alpha}$ treatment (im) in non-lactating dairy cows. Thus, the progesterone data for lactating cows in this thesis agree with previously reported data for non-lactating dairy cows and heifers.

Intervals to Estrus, the Ovulatory Surge of LH and Ovulation. All cows were observed twice daily for estrus behavior at approximately 10- to 14-hr intervals from day 1 to day 26 following injection. The beginning of estrus was assumed to have occurred at the time half way between the observation at which estrus was first detected and the preceding observation. In this experiment estrous behavior was considered a result of $PGF_{2\alpha}$-induced luteolysis only if it occurred within 6 days after treatment. One animal not observed in estrus (but with progesterone falling from 2.5 to .9 ng/ml within 12 hr) was eliminated from this analysis. No cows given saline were observed in estrus during the 6 days following injection. Estrus in response to $PGF_{2\alpha}$ was observed in 4 of 11 cows given 5 mg, 9 of 11 given 15 mg, 8 of 10 given 25 mg and 9 of 9 given 35 mg. Incidences of observed estrus were greater for cows given 15, 25 or 35 mg $PGF_{2\alpha}$. There were no significant differences in incidences of estrus among animals given 15, 25 or 35 mg $PGF_{2\alpha}$; however, there was some evidence ($P<.15$) that incidence of estrus was greater in cows given 35 mg $PGF_{2\alpha}$. The average interval to estrus was 72.8 ± 3.7 hr for all animals ($n = 30$) in estrus

as a result of $PGF_{2\alpha}$ injection. Animals (n = 11) not in estrus within 6 days of treatment (5, 15 or 25 mg $PGF_{2\alpha}$) averaged 272.3 ± 17.9 hr to the onset of estrus and this did not differ from the mean interval (280.9 ± 26.2) for saline treated controls (n = 10). The interval to estrus in these data is similar to that previously reported for heifers by Stellflug *et al.* (1975). They reported a 78-hr interval from $PGF_{2\alpha}$ injection to onset of estrus for heifers given 5, 10 or 15 mg (im) of $PGF_{2\alpha}$. Louis *et al.* (1973) reported a similar interval (74 hr) for heifers given 30 mg $PGF_{2\alpha}$. Following intrauterine infusion of $PGF_{2\alpha}$, Welch *et al.* (1975) reported that a majority of the suckled beef cows returned to estrus between 56 to 88 hr following treatment.

Interval from $PGF_{2\alpha}$ injection to peak of the ovulatory surge of luteinizing hormone (LH) was determined for cows with frequent blood sampling for 132 hr (n = 30). Only cows with luteolysis after $PGF_{2\alpha}$ had an LH surge within 132 hr (n = 18). Cows not responding to $PGF_{2\alpha}$ (n = 12) were designated as having an ovulatory surge greater than 132 hr after injection. The mean interval to the LH surge for cows with luteolysis after injection was 82.3 ± 6.1 hours. This value includes two cows which developed cystic follicles following injection. Excluding these two cows, the average interval was 80.3 ± 5.3 hours. The mean interval in this work is slightly longer than the interval (64 hr) previously reported for heifers given (im) 30 mg $PGF_{2\alpha}$ (Louis *et al.*, 1973), or the interval (67 hr) reported

for beef cows given (iu) 1 mg $PGF_{2\alpha}$ (Welch et al., 1975). Louis et al. (1974) reported an average interval to the LH surge of 71 hr in six non-lactating cows given (iu) 5 mg $PGF_{2\alpha}$, similar to the interval reported in this thesis. The mean interval to the LH surge reported in this work is not significantly greater than the interval from injection to the beginning of estrus (72.8 ± 4 hr) and agrees with previous reports that the ovulatory surge of LH occurs coincident with or shortly before the beginning of estrus (Hansel and Snook, 1970; Swanson and Hafs, 1971).

Ovulation was determined by palpation of the ovaries via the rectum. The time of ovulation was assumed to be midpoint between the last palpation without an ovulation and the first palpation indicating ovulation had occurred. The average interval from injection to ovulation for cows responding to $PGF_{2\alpha}$ injection was 100.1 ± 3.8 hr (n = 29). Two animals that developed persistent follicles following injection were not included in this calculation. Louis et al. (1973) reported an average interval to ovulation of 104 hr for non-lactating cows given (im) 30 mg injections of $PGF_{2\alpha}$. Welch et al. (1975) and Louis et al. (1974) reported similar intervals for lactating beef cows and dairy heifers given intrauterine $PGF_{2\alpha}$ infusion, respectively. Cows in the present study not responding with luteolysis following $PGF_{2\alpha}$ ovulated on the average 301.8 ± 13.7 hr after treatment (n = 21).

Ovulation in all cows occurred on the average 28.3 ± 3.7 hr after the onset of estrus (n = 48). Two cows with persistent follicles following luteolysis and one cow not detected in estrus were not included in the calculation. The interval from the beginning of estrus to ovulation reported in this work is similar to the interval of 32 hr reported by Swanson and Hafs (1971) for nontreated dairy heifers. Louis et al. (1973) and Elving et al. (1975) reported an average interval of 30.0 and 29.8 hr, respectively, for $PGF_{2\alpha}$-treated heifers.

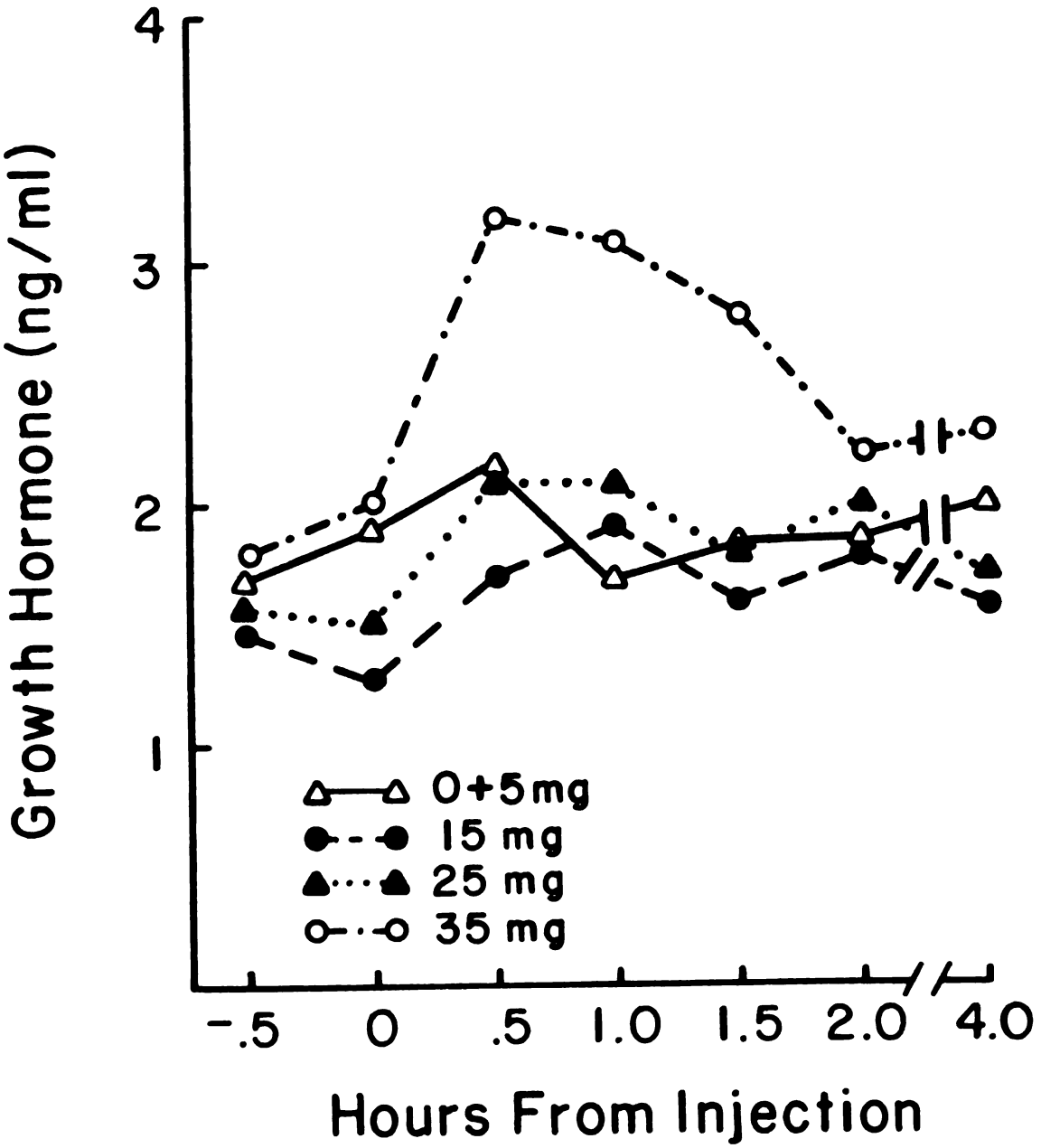
The data presented in this section indicate that $PGF_{2\alpha}$ is an effective method of synchronizing estrus and ovulation in lactating dairy cows. In addition, the intervals to estrus, the ovulatory surge of LH and ovulation resemble the intervals reported for treated dairy heifers, or from comparable values in untreated cattle.

Milk Production Criteria

The remaining portion of these results and discussion will be concerned with (1) the intermediary metabolic hormones; growth hormone, glucocorticoids and prolactin, following $PGF_{2\alpha}$, and (2) the effect of $PGF_{2\alpha}$ on milk yield and milk composition.

Growth Hormone. Blood serum growth hormone did not differ significantly among treatment groups prior to $PGF_{2\alpha}$ injection; it averaged 1.7 ng/ml (Figure 2). The average value was similar to baseline values previously reported for

Figure 2. Blood serum growth hormone in lactating cows given (im) $PGF_{2\alpha}$. Standard errors ranged from 0.2 to 1.9 ng/ml and generally were proportional to the means.


Growth Hormone (ng/ml)
4
3
2
1
0+5mg
15 mg
25 mg
35 mg
-.5
0
.5
1.0
1.5
2.0
4.0
Hours From Injection

lactating cows by Tucker et al. (1975). In contrast, Tucker et al. (1975) and Louis et al. (1973) reported baseline growth hormone concentrations in heifers of 5.7 and 6.7 ng/ml, respectively.

Growth hormone was unaltered for 4 hr after injection of saline or 5, 15 or 25 mg $PGF_{2\alpha}$. However, growth hormone increased ($P<.05$) 74% to a peak of 3.3 ng/ml at .5 hr in cows given 35 mg $PGF_{2\alpha}$. Growth hormone returned to near pretreatment concentration by 2 hours. The growth hormone increase in lactating cows after 35 mg $PGF_{2\alpha}$ reported in this study was much less than previously reported in heifers given similar doses of $PGF_{2\alpha}$. Louis et al. (1974) gave (im) 30 mg of $PGF_{2\alpha}$ to heifers and reported 7-fold increases in growth hormone within 30 minutes. In the same study, an intravenous injection of 5 mg increased growth hormone approximately 4-fold within 30 minutes.

Previous work by Hertelendy et al. (1972) indicates that prostaglandin-induced increases in growth hormone are mediated through the pituitary. They incubated ox pituitaries with PGE_1 and reported increased growth hormone synthesis. Thus, data from this thesis and earlier work suggest that changes accompanying lactation reduce $PGF_{2\alpha}$-induced growth hormone increases by decreasing pituitary response.

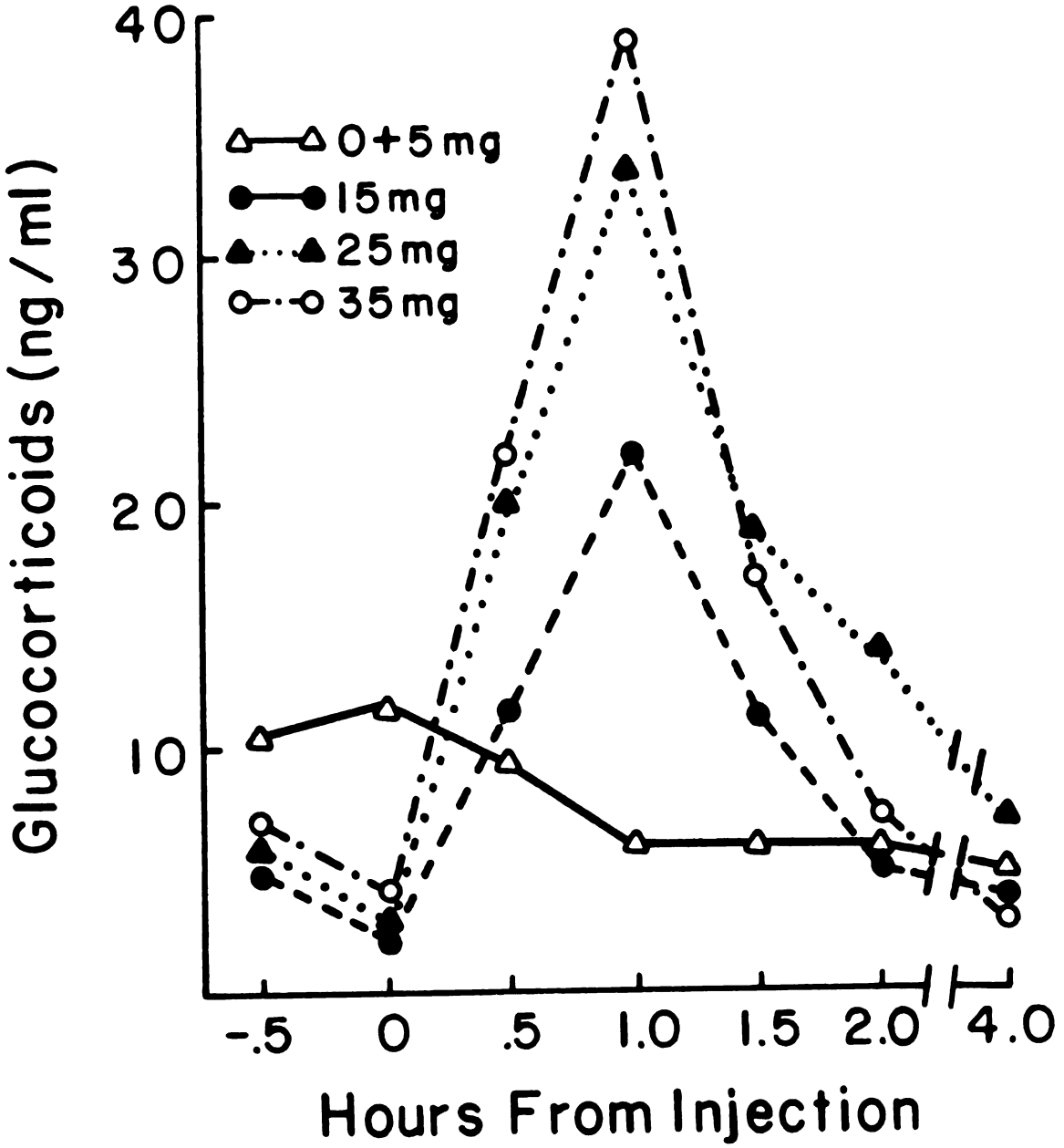
Glucocorticoids. Blood serum glucocorticoids averaged 6.8 ng/ml prior to injection. This agrees with earlier work by Koprowski and Tucker (1973), who measured glucocorticoid

concentrations in 26 cows at 2 to 4 hr prior to milking for an entire lactation. Glucocorticoids in samples taken from 8 to 20 weeks after calving (the approximate stage of lactation for cows sampled in this experiment) averaged 4.5 to 9.0 ng/ml.

In the current work, when cows were given saline or 5 mg $PGF_{2\alpha}$, glucocorticoid concentrations were not altered significantly (Figure 3). However, glucocorticoids increased rapidly to peaks of 22.3, 34.9 and 39.0 ng/ml at 1.0 hr following injection of 15, 25 and 35 mg $PGF_{2\alpha}$, respectively. Peak glucocorticoid concentrations were linearly related to dose of $PGF_{2\alpha}$ given. Glucocorticoids remained above pretreatment values until 4 hr after injection. In previous work, Louis et al. (1974) gave (im) 35 mg $PGF_{2\alpha}$ to heifers; glucocorticoids increased to 100 ng/ml at .5 hr and returned to pretreatment concentrations by 2.0 hr after injection. Hafs (1975) reported 2- and 4-fold increases of glucocorticoids (over basal concentrations of 3.0 ng/ml) in bulls given 20 and 40 mg of $PGF_{2\alpha}$, respectively. Peak concentrations were obtained at 1.0 hr after $PGF_{2\alpha}$ and glucocorticoids remained above pretreatment values for 4 hours.

The duration of this $PGF_{2\alpha}$-induced increase of blood serum glucocorticoids in lactating dairy cows is similar to that reported for bulls (Hafs, 1975), but is longer than values reported for heifers (Louis et al., 1974). Perhaps, glucocorticoids are cleared more readily in heifers than lactating cows, or the difference may be directly related

Figure 3. Blood serum glucocorticoids in lactating cows given (im) $PGF_{2\alpha}$. Standard errors ranged from 0.3 to 6.1 ng/ml and were generally proportional to the means.


40
30
20
10
Glucocorticoids (ng/ml)
0+5mg
15mg
25mg
35mg
-.5
0
.5
1.0
1.5
2.0
4.0
Hours From Injection

to ACTH secretion. In addition, the $PGF_{2\alpha}$-induced increase of glucocorticoids in lactating cows is intermediate in magnitude to reported increases for bulls and heifers after similar doses of $PGF_{2\alpha}$.

Work by Peng et al. (1970) indicates that $PGF_{2\alpha}$-induced increases in glucocorticoids are mediated by the hypothalamus in rats. Recent work in heifers (Stellflug et al., 1977) and bulls (Haynes et al., 1977) indicates that $PGF_{2\alpha}$ acts on the anterior pituitary or hypothalamus but not on the adrenal gland. Thus, the reduced glucocorticoid increase in lactating dairy cows observed in the present study as compared to that reported for heifers may be the result of a lactation-induced alteration of hypothalamic or anterior pituitary response to $PGF_{2\alpha}$. In bulls, steroid environment may have a role in modulating $PGF_{2\alpha}$-induced changes in blood glucocorticoids.

Prolactin. Prior to $PGF_{2\alpha}$ injection, blood serum prolactin averaged 37.8 ng/ml. In contrast, Tucker et al. (1975) reported pretreatment prolactin concentrations of 14.9 to 16.1 ng/ml in lactating cows 4 months post-partum. This dissimilarity is not surprising, since baseline prolactin concentrations are influenced by temperature (Wettemann and Tucker, 1974), photoperiod (Bourne and Tucker, 1975) and handling stress (Tucker, 1971); all factors which may have differed in these studies.

In this study, when cows were given saline or 5 mg $PGF_{2\alpha}$, prolactin concentrations were not altered within

4 hr (Figure 4). However, prolactin increased rapidly to peaks of 56.8, 84.5 and 95.6 ng/ml at 1.0 hr following injection of 15, 25 and 35 mg of $PGF_{2\alpha}$, respectively. As with glucocorticoids, peak prolactin concentrations were linearly related to the dose of $PGF_{2\alpha}$. Prolactin returned to pretreatment concentrations within 4 hr after injection. These changes in prolactin represent 1.5-, 2.0- and 2.5-fold increases over pretreatment values. Louis et al. (1974) reported 5-fold increases in prolactin when heifers were given (im) 15, 30 or 60 mg $PGF_{2\alpha}$; prolactin increased from 26 ng/ml to approximately 150 ng/ml .5 hr after $PGF_{2\alpha}$ injection. Hafs (1975) gave (im) 40 mg $PGF_{2\alpha}$ to bulls and reported a 2-fold increase in prolactin, from approximately 10 ng/ml to 20 ng/ml, within 1.0 hour. However, prolactin did not increase in bulls given 5 or 20 mg $PGF_{2\alpha}$.

As with glucocorticoids and growth hormone, differences in prolactin secretion among cows, heifers and bulls in response to $PGF_{2\alpha}$ may be the result of physiological changes accompanying lactation or differences in steroid environment. In addition, photoperiod and temperature effects on prolactin complicate comparisons among separate reports.

Milk Yield, Percent Milk Fat and Percent Total Milk Protein. The average post-treatment milk yield, adjusted by covariance for pretreatment milk yield, was 22.4 kg/day (Table 2). $PGF_{2\alpha}$ treatment did not significantly alter milk yield during the 7 days after injection. Adjusted post-treatment percent milk fat and percent total protein

Figure 4. Blood serum prolactin in lactating cows given (im) $PGF_{2\alpha}$. Standard errors ranged from 4.7 to 24.5 ng/ml and were generally proportional to the means.

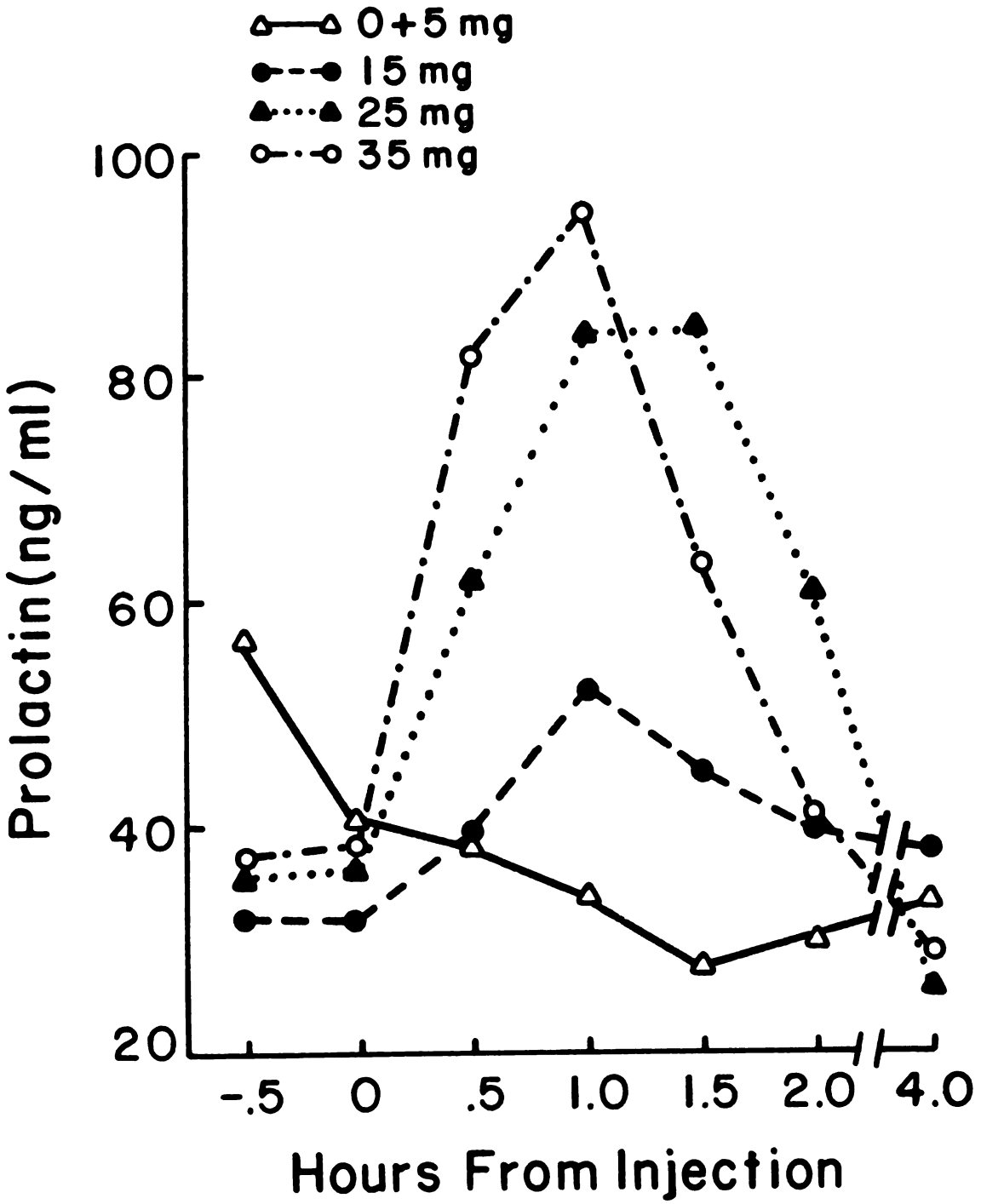

0+5 mg
15 mg
25 mg
35 mg
100
80
60
40
20
Prolactin(ng/ml)
-.5
0
.5
1.0
1.5
2.0
4.0
Hours From Injection

Table 2. Daily milk yield, milk fat and protein during 7 days after $PGF_{2\alpha}$.

$PGF_{2\alpha}$	Milk	Fat	Protein
(mg)	(kg)	(%)	(%)
0	22.2 ± .8[a]	3.09 ± .06	3.29 ± .03
5	22.7 ± .8	3.23 ± .06	3.25 ± .03
15	22.4 ± .5	3.18 ± .05	3.29 ± .02
25	22.7 ± .8	3.21 ± .06	3.32 ± .03
35	21.8 ± .8	3.17 ± .06	3.31 ± .03

[a] Entries are mean ± standard error for n = 11 to 13, adjusted for pretreatment values.

were 3.18 and 3.29, respectively. As with milk yield, $PGF_{2\alpha}$ did not significantly alter milk fat or total protein.

The data from this thesis do not provide significant evidence that $PGF_{2\alpha}$ has deleterious effects on milk yield, percent milk fat or percent total milk protein. Although serum concentration of glucocorticoids, growth hormone and prolactin were altered in lactating dairy cows given $PGF_{2\alpha}$, apparently these acute changes in hormone concentrations do not significantly alter milk production. Brush (1960) reported that glucocorticoids decreased milk production, but only when serum glucocorticoids reached very high concentrations (200 ng/ml). Likewise, Machlin (1973) reported increased milk production in lactating cows given growth hormone, only after relatively high doses (30 mg) administered for extended periods (10 days).

These milk production data also were analyzed to compare cows with luteolysis to cows with no luteolysis (Table 3). All cows with luteolysis exhibited estrous behavior, but no cows without luteolysis were observed in estrus during the 7-day milk sampling period. The data in Table 3 led to the conclusion that luteolysis and subsequent estrus did not significantly alter milk yield, percent milk fat or percent total milk protein.

McClandish (1926) reported a decrease in milk production on the day of estrus but a compensatory production 2 days prior to estrus. Similarly, Hooper and Brown (1919) reported decreased milk production on the day of estrus.

Table 3. Daily milk yield, milk fat and protein among cows with or without luteolysis in response to $PGF_{2\alpha}$.

Response	Milk	Fat	Protein
	(kg)	(%)	(%)
No Luteolysis (n=21)	22.7 ± .5[a]	3.06 ± .06	3.25 ± .03
Luteolysis (n=31)	22.4 ± .4	3.19 ± .05	3.20 ± .03

[a] Entries are mean ± standard error adjusted for pretreatment values.

However, in both studies some cows had no change in milk production on the day of estrus or had slight increases. Copeland (1929) found slight increases in milk production on the day of estrus. In addition, he reported an equally small decrease in butter fat. Thus, previously reported data indicate conflicting evidence of the effect of estrus on milk yield and composition. The data presented in this thesis suggest that any changes in milk yield and composition occurring at estrus are not sufficient to substantially alter average daily milk yield or milk composition.

GENERAL DISCUSSION

The results from this study indicate that 15 mg is the minimal dose of $PGF_{2\alpha}$ which when given to lactating dairy cows results in an incidence of luteolysis not significantly different from higher doses. Complete luteolysis occurred in 88% of the cows given 15, 25 or 35 mg $PGF_{2\alpha}$. This agrees with previously reported values for luteolytic response in suckled beef cows (Hafs et al., 1975b) and heifers (Louis et al., 1973). Analysis of the incidence of luteolysis for lactating dairy cows in the present study suggested ($P<.15$) that cows given 35 mg of $PGF_{2\alpha}$ had higher incidences of estrus. Additional work is necessary to determine the validity of this observation.

Blood serum progesterone in cows with complete luteolysis declined approximately 50% within 6 hr after injection. Following luteolysis in these cows (1) estrus began at 72.8 ± 3.7 hr, (2) the peak of an ovulatory surge of LH occurred at 80.3 ± 5.3 hr, and (3) ovulation occurred at 100.1 ± 3.8 hours. These results are similar to those previously reported for heifers by Louis et al. (1973) and Elving et al. (1975). Some cows given 5, 15 or 25 mg $PGF_{2\alpha}$ did not have complete luteolysis within 132 hours. Progesterone concentrations in these cows declined within

12 hr, but returned to normal values within 24 hr after $PGF_{2\alpha}$. Stellflug et al. (1976) reported similar progesterone changes when partially luteolytic doses of $PGF_{2\alpha}$ were given to heifers. Apparently, luteal biosynthesis of progesterone is reduced transiently following even non-luteolytic doses of $PGF_{2\alpha}$. In support of this observation, Lahav et al. (1976) reported that $PGF_{2\alpha}$ blocked LH stimulated cyclic AMP accumulation in isolated rat ovaries.

The results of this thesis indicate that $PGF_{2\alpha}$ is an effective method of estrus synchronization in lactating dairy cows. Hafs et al. (1975b) reported that fertility of heifers and suckled beef cows to inseminations at 70 and 88 hr after $PGF_{2\alpha}$ is comparable to fertility of animals inseminated at observed estrus. Since reproductive events following luteolysis (interval to estrus, LH surge and ovulation) in lactating dairy cows are similar to those reported for heifers and beef cows, breeding at a predetermined interval following $PGF_{2\alpha}$ is feasible for lactating dairy cows. Predetermined breeding would eliminate the need for estrous detection and encourage dairymen not using artificial insemination to do so.

$PGF_{2\alpha}$-induced increases of growth hormone, glucocorticoids and prolactin in lactating cows were less than increases reported for heifers (Louis et al., 1973), but greater than increases reported for bulls (Hafs, 1975) following similar doses of $PGF_{2\alpha}$. Differences in growth hormone, prolactin and glucocorticoid secretion among lactating cows, heifers and bulls following $PGF_{2\alpha}$ injection

may be due to altered hypothalamic or pituitary responses to $PGF_{2\alpha}$. Another possible explanation for the variance in reported $PGF_{2\alpha}$-induced secretion of these hormones is that weight differences among animals given similar doses of $PGF_{2\alpha}$ alter the concentration of $PGF_{2\alpha}$ that reaches the hypothalamus or pituitary. In addition, differences in reported peak concentrations of these hormones after $PGF_{2\alpha}$ treatment may be due to differences in clearance rate. Data from this thesis and previous work are not sufficient to determine factors responsible for these differences in $PGF_{2\alpha}$-induced changes of growth hormone, glucocorticoid and prolactin.

The results of this study indicated that milk yield, percent milk fat and percent total milk protein were unaltered following various doses of $PGF_{2\alpha}$. Apparently, the acute increases in growth hormone and glucocorticoids were insufficient to cause changes in milk production reported following large chronic doses of these hormones (Machlin, 1973; Braun, 1970).

$PGF_{2\alpha}$ given to lactating dairy cows is an effective method of synchronizing estrus to facilitate the use of artificial insemination. The acute changes in growth hormone, glucocorticoids and prolactin concentration following $PGF_{2\alpha}$ treatment fail to alter milk yield, percent milk fat or percent total milk protein.

SUMMARY AND CONCLUSIONS

Earlier reports indicate that $PGF_{2\alpha}$ is an effective luteolysin in dairy heifers and suckled beef cows. In addition, $PGF_{2\alpha}$ is an effective method of synchronizing estrus and ovulation for controlled breeding. $PGF_{2\alpha}$ given to heifers induces increases in blood serum growth hormone, glucocorticoids and prolactin; three hormones necessary for the initiation and maintenance of lactation. The purpose of this thesis was to (1) determine the minimal dose of $PGF_{2\alpha}$ which when given to lactating dairy cows results in an incidence of luteolysis not significantly different from higher doses, (2) characterize changes in blood serum growth hormone, glucocorticoids and prolactin after various doses of $PGF_{2\alpha}$ and (3) determine the acute response of milk yield, percent milk fat and percent total protein to various doses of $PGF_{2\alpha}$.

The results obtained in this thesis indicate that 15 mg is the minimal intramuscular dose of $PGF_{2\alpha}$ for lactating dairy cows resulting in an incidence of luteolysis not significantly different from higher doses. Following $PGF_{2\alpha}$-induced luteolysis the mean interval from injection to the beginning of estrus, peak of the ovulatory surge of LH

and ovulation were 72.8, 80.2 and 100.1 hr, respectively. The average interval from the beginning of estrus to ovulation was 28.3 hours.

Blood serum glucocorticoids and prolactin increased at 1.0 hr following injection of 15, 25 or 35 mg of $PGF_{2\alpha}$. Growth hormone increased slightly within .5 hr after injection of 35 mg $PGF_{2\alpha}$. All hormones returned to pre-treatment concentrations within 4 hr, so this effect of $PGF_{2\alpha}$ is transitory. Perhaps the short duration of the elevations in hormones explains why various doses of $PGF_{2\alpha}$ failed to significantly alter milk yield, percent milk fat or percent total milk protein in lactating dairy cows.

I conclude that $PGF_{2\alpha}$ is an effective method of estrous synchronization for lactating dairy cows, without deleterious effects on milk yield or gross milk composition.

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