Evaluation of a single injection PGF□2□a estrous synchronization system
by Charles Kent Higgins

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL SCIENCE
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Abstract:
Three years of breeding, calving, and production records were utilized to evaluate a single injection PGFgg system for estrous synchronization in range beef cattle. Reproductive performance, effects on calf weaning weight, effects of two years sequential treatment and costs for PGFgct-Synchronized (n=566) and non-synchronized control (n= 575) cattle were analyzed. Non-treated controls were artifically inseminated (Al) 8-12 hours after first observation of standing estrus. Treated cattle received PGF2α (25 mg free acid) either in the PM of day 4 (1975) or AM of day 5 (1976 and 1977) of breeding unless they had been observed in estrus prior to those times. Inseminations to detected estrus continued in treated cattle until 80 + 4 hr post-PGF2α when all remaining undetected animals were mass inseminated and recorded as nonestrus bred. Breeding seasons consisted of 25 days Al plus 20 days natural service (1975 and 1976) or 8.5 days Al plus 48.5 days natural service (1977). For two year treatment analysis cattle were allotted to one of four treatment sequences: (CC) nonsynchronized control for two consecutive years; (CT) control in year I and PGF2α system in year 2; (TC) PGF2α system in year I and control in year 2; (TT) PGF2α system for two consecutive years. Cost assumptions for a 10 day PGF2α and 21 day control system were $3.00/hr labor, $6.75/ unit semen cost, aid $4.50/25 mg PGF2α (treated cattle only) Results demonstrated that Al first service conception rates for control (62.9%) and treated (62.1%) cattle bred to an observed estrus were not significantly different, but were greater (P < .01) than that for treated cattle bred nonestrus (12.3%). Reduced conception rate in the non-estrus bred subgroup resulted in lower (P < .01) conception rates for all cattle in the PGF2α system (38.3%) vs controls (62.9%). Considerable proportions (14.0 to 35.7%) of nonestrus bred cattle were reinseminated three to four days after appointment breeding, indicating these cattle responed to PGF2α but were bred too early relative to ovulation. Pregnancy rate at day 10 (PR10) of breeding for treated cattle was greater (P < .01) than controls. Pregnancy rate at day 25 (PR25) and total pregnancy rate (TPR) were not different. Pregnancy rate at day 32 (PR32) was higher (P < .01) in treated cattle (65.9%) than in controls (56.7%) in the pooled analysis. A trend for an earlier (P < .05) average day of conception in treated cattle was observed. In the two year sequential treatment study, PR10 for CC, CT, TC, and TT were 21.1, 45.5, 24.8 and 43.7 percent, respectively, with CT and TT greater (P < ,01) than CC and TC. PR25, PR32 and total pregnancy rates were not significantly different. Comparison of a 10 day PGF2α and a 21 day control system demonstrated similar pregnancy rates but calves out of synchronized cows were older (P < .01) and heavier (P < .10) than those out of control dams. However, total breeding cost per treated cow ($12.48) was more than double the cost for controls ($6.12), despite reduced labor cost in the PGFgct system.

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EVALUATION OF A SINGLE INJECTION $PGF_{2\alpha}$ ESTROUS SYNCHRONIZATION SYSTEM

by

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A thesis submitted in partial fulfillment
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ABSTRACT

Three years of breeding, calving, and production records were utilized to evaluate a single injection $PGF_{2\alpha}$ system for estrous synchronization in range beef cattle. Reproductive performance, effects on calf weaning weight, effects of two years sequential treatment and costs for $PGF_{2\alpha}$-synchronized (n=566) and non-synchronized control (n=575) cattle were analyzed. Non-treated controls were artifically inseminated (AI) 8-12 hours after first observation of standing estrus. Treated cattle received $PGF_{2\alpha}$ (25 mg free acid) either in the PM of day 4 (1975) or AM of day 5 (1976 and 1977) of breeding unless they had been observed in estrus prior to those times. Inseminations to detected estrus continued in treated cattle until 80 $\pm$ 4 hr post-$PGF_{2\alpha}$ when all remaining undetected animals were mass inseminated and recorded as nonestrus bred. Breeding seasons consisted of 25 days AI plus 20 days natural service (1975 and 1976) or 8.5 days AI plus 48.5 days natural service (1977). For two year treatment analysis cattle were allotted to one of four treatment sequences: (CC) nonsynchronized control for two consecutive years; (CT) control in year 1 and $PGF_{2\alpha}$ system in year 2; (TC) $PGF_{2\alpha}$ system in year 1 and control in year 2; (TT) $PGF_{2\alpha}$ system for two consecutive years. Cost assumptions for a 10 day $PGF_{2\alpha}$ and 21 day control system were \$3.00/hr labor, \$6.75/unit semen cost, and \$4.50/25 mg $PGF_{2\alpha}$ (treated cattle only) Results demonstrated that AI first service conception rates for control (62.9%) and treated (62.1%) cattle bred to an observed estrus were not significantly different, but were greater ($P < .01$) than that for treated cattle bred nonestrus (12.3%). Reduced conception rate in the nonestrus bred subgroup resulted in lower ($P < .01$) conception rates for all cattle in the $PGF_{2\alpha}$ system (38.3%) vs controls (62.9%). Considerable proportions (14.0 to 35.7%) of nonestrus bred cattle were reinseminated three to four days after appointment breeding, indicating these cattle responed to $PGF_{2\alpha}$ but were bred too early relative to ovulation. Pregnancy rate at day 10 (PR10) of breeding for treated cattle was greater ($P < .01$) than controls. Pregnancy rate at day 25 (PR25) and total pregnancy rate (TPR) were not different. Pregnancy rate at day 32 (PR32) was higher ($P < .01$) in treated cattle (65.9%) than in controls (56.7%) in the pooled analysis. A trend for an earlier ($P < .05$) average day of conception in treated cattle was observed. In the two year sequential treatment study, PR10 for CC, CT, TC, and TT were 21.1, 45.5, 24.8 and 43.7 percent, respectively, with CT and TT greater ($P < .01$) than CC and TC. PR25, PR32 and total pregnancy rates were not significantly different. Comparison of a 10 day $PGF_{2\alpha}$ and a 21 day control system demonstrated similar pregnancy rates but calves out of synchronized cows were older ($P < .01$) and heavier ($P < .10$) than those out of control dams. However, total breeding cost per treated cow (\$12.48) was more than double the cost for controls (\$6.12), despite reduced labor cost in the $PGF_{2\alpha}$ system.

Introduction

Beef producers in the United States have been reluctant to accept artificial insemination (AI) mainly due to added labor requirements associated with estrus detection and required changes in overall management practices.

Techniques for estrous synchronization have been extensively studied during the last two decades in attempts to reduce or eliminate estrus detection while maintaining normal fertility. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been among the most successful agents for this purpose and is commercially available. However, data is scarce regarding carryover effect of successive years synchronization, effects on calf weaning weight, and economic comparisons between $PGF_{2\alpha}$ synchronization/AI and conventional AI breeding systems. The objectives of this study were to determine these relationships by comparing a single injection $PGF_{2\alpha}$/AI system with a conventional AI system.

Chapter 1

Artificial Insemination and Its Role in Herd Improvement

The advent of AI was one of the most important contemporary discoveries contributing to cattle improvement. The procedure has provided opportunity for progress by several means. Potential for genetic progress has been enhanced through the availability of proven AI sires. An inherent improvement in herd identification, record keeping, and other management practices has allowed producers to capitalize on that genetic potential.

Extensive evidence of increased use of AI and herd improvement in the dairy industry has been offered during the last three decades. From 1951 to 1959, production trends of a well managed dairy cow population in New York state showed a superiority of 28 and 48 percent in milk yield and butterfat production, respectively, for artificially sired cows over those naturally sired (Van Vleck and Henderson, 1963). Blom (1968) documented 35 and 46 percent increases in annual milk production for Red Danish and Danish Friesian breeds, respectively, during the period 1940-1965 in Denmark. Utilization of genetically superior sires through AI increased breeding values for Holstein cows in first lactation from an average of 6 kilograms (kg) per year for 1960-1965 to 28 kg per year for 1970-1975 (Powell et al., 1977). Additional evidence of improved production potential with AI was

offered by Britt (1979) who reported 277 kg higher breeding values for milk yield of sires produced by AI-sired Holstein cows over those produced by naturally sired dams in 1965. This advantage had increased to 527 kg by 1975. Use of proven AI sires coupled with improved nutrition and management practices has resulted in greater than 100 percent improvement in total milk production per dairy cow since 1945 in the United States (U.S. Dept. Ag., 1979). In 1945 less than one percent of lactating dairy cows in the U.S. were artificially bred as compared to an estimated 65 percent in 1980 (Freeman, 1980). Although not as well documented, beef producers have realized improved production through use of superior AI sires. In Montana, AI sired steer calves weighed 27 and 32 kg more at weaning than naturally sired calves in 1961 and 1962 (Mosher, 1964). In a Colorado beef herd, annual average weaning weights improved 61 kg (16 %) during a six year period following implementation of AI, with a 64 kg (24 %) increase in production per animal unit (a.u.)(Syntex, 1976). On several Washington ranches AI sired calves averaged 23 kg heavier than naturally sired calves (Rogers, 1973). In Wyoming, AI sired calves weighed 5.4 kg more at weaning then naturally sired calves (Stevens and Mohr, 1969).

Use of AI in beef cattle has been limited, as less than five percent of U.S. beef cows are artificially bred (Beverly, 1978). Evidence indicative of the lack of top sire use was offered by

Wallace (1980) who reported that offspring of the top 10 Angus and Hereford sires based on number of registered progeny represented only 3.6 and 2.7 percent of total registrations, respectively.

Estrus detection and associated problems have been repeatedly cited as the major deterrent to beef AI. Overemphasis of this problem has perhaps masked other reasons for restricted AI use. Producer resistance to necessary changes in overall management practices may be equally important (Tilton et al., 1973). Use of AI in the beef industry has been largely limited to enterprises characterized by small herd size. Range operations characterized by low cow densities per unit of pasture land have made little use of AI. With introduction of European breeds in the late sixties and early seventies, AI increased in popularity by providing a means of rapidly introducing seedstock whose offspring often commanded premium prices at that time (Black, 1972). Lack of widespread AI in range beef operations prompted research in estrous synchronization. An effective, economical means of controlling and grouping estrus and ovulation in range cows would likely make beef AI more acceptable (Britt, 1979).

Improved beef production resulting from AI has been partially attributable to factors other than superior genetics. Short breeding and calving periods (i.e. 45 to 60 days) have enhanced reproductive efficiency in beef herds (Roberts et al., 1970), provided proper management was maintained (Wiltbank, 1974). Improved management

necessary for quality AI has resulted in shorter breeding and calving seasons in beef herds. Between 1970 and 1975, AI management resulted in a 46 percent increase in proportion of yearling heifers bred during the first three weeks of the breeding season, subsequently resulting in 65 percent more heifers calving during the first 21 days of calving (Syntex, 1976). Pancake (1963) indicated that better management of the cow herd due to a progressive AI program resulted in 95 percent of 220 cows exhibiting estrus during a 25 day breeding period. Woodward (1963) reported 314 of 328 cows bred by AI in a 25 day breeding period.

First service pregnancy rates obtained in artificial breeding programs have varied with management and technician skill; estimates range from 25 to 95 percent, with averages of 60 to 70 percent (Gregory, 1966; American Breeders Service, 1974; Donaldson 1976; Donaldson, 1977a).

1.1 Cost/Return Comparisons

Change in income resulting from any operational transition in a given enterprise hinges on 1) additional costs and reduced returns or income-reducing factors and 2) additional returns and reduced costs or income increasing components (Herbst, 1976). Financial gain or loss encountered from implementation of an AI program is dependent on two broad factors; 1) change in breeding costs with AI versus natural ser-

vice and 2) change in value of AI calves. Highly accurate evaluation of cost-return differences would require use of individual sires both naturally and artificially within the same herd (Tilton et al., 1973).

Results from limited studies regarding economic comparisons of natural and artificial breeding vary widely. Reasons for this variation include ranch size and geographic location, differences in semen and labor charges, percentage calf crop, use of straightbreeding vs. crossbreeding systems, and year differences which include selling price variations (Herman, 1967; Stevens and Mohr, 1969; Learmonth, 1973; Rogers, 1973; Syntex, 1976, Tilton et al., 1973; and Sorensen, 1979).

Direct, additional cost factors encountered in AI over those of natural service include labor for estrus detection and insemination, semen, field tank rental and liquid nitrogen, breeding supplies, special facilities, and occasional expense for high quality feed during the breeding season (Stevens and Mohr, 1969 and Rogers, 1973). Stevens and Mohr (1969) reported 12 of 37 commercial cow calf ranches in Wyoming had lower breeding costs per calf weaned with AI versus natural service; herds with lower costs had higher detection and conception rates. Some sources indicate that breeding costs per AI calf are comparable with natural service, with semen and labor costs recognized as the two largest single items associated with AI

(Learmonth, 1973; Syntex, 1976, Tilton et al., 1973). Contrastingly, Sorensen (1979) reported less cost per cow bred AI compared to natural service when purchase costs of bulls used for natural breeding varied from 400-1000 dollars per head.

Increased value of AI calves primarily results from price premiums received and heavier weaning weights (Perry, 1968 and Stevens and Mohr, 1969). In the latter report with relatively low feeder calf prices existing (< 30 $/cwt.), purebred and commercial ranches average financial gain per AI calf weaned amounted to $30.02 and $3.31, respectively, over those breeding via natural service. Herman (1967) reported that 15 to 30 dollars per head increase in value of weaner calves could be obtained by AI use. Singleton and Petritz (1975) estimated net economic advantage per AI calf at $7.92.

Of significant importance, although difficult to accurately estimate, is increased value of replacement heifers due to superior genetic makeup. Wyoming producer estimates of added value obtained per AI replacement averaged $10.10 per head based on information from 37 commercial operations (Stevens and Mohr, 1969). These estimates were derived from market sales information and expressed producer willingness to pay extra for AI heifers.

Additional income may be derived from sale of calves and cull cows from increased cow numbers made possible by reduced bull numbers. Large ranches (> 750 a.u.) in Washington returned an additional 850

dollars from sale of additional steer and heifer calves and cull cows during years of depressed cattle prices (Rogers, 1973). Reduced bull maintenance expense was reported as an important contributing factor in net returns of ranches in the same study. Rogers (1973) stated that AI increased net ranch income by 3.2, 7.9, and 7.4 percent for small ($\leq$ 80 a.u.), medium ($\leq$ 356 a.u.) and large ranches ($\leq$ 754 a.u.), respectively.

The above evidence indicates that AI can increase profits over those from natural service in purebred and commercial beef operations. Good management and efficient, determined personnel are the keys to this potential. Reported causes of dollar losses in beef cattle AI programs involving over twelve thousand animals in order of importance were inadequate estrus detection, reduced inseminator efficiency, inadequate nutrition, and infectious reproductive diseases (Donaldson, 1977a). All of these factors can be controlled by management.

Chapter 2

Bovine Estrous Cycle

The bovine female reproductive system undergoes a rhythmical change referred to as the estrous cycle, which may be viewed as a series of events dependent on neuroendocrine pathways for successful completion and repeatability. Endogenous governing factors of reproduction include endocrine glands, reproductive hormones, target organs (i.e. ovary), and the nervous system.

Principal events of the cycle may be grossly divided into those associated with follicular growth and those with growth of the corpus luteum (CL). Well-known periods of the 20-21 day cycle occurring in a sequential manner are estrus (day 0-1), metestrus (2-4), diestrus (5-17), and proestrus (18-20).

2.1 Sequence of Events

The following review of cyclic sequences will consider post-ovulatory CL development as the initial event. Use of any initial step in description of sequential events of the cycle inevitably requires explanation of occurrences immediately preceding that step.

2.1.1. Post Ovulatory CL Development and the Luteal Phase

Days 2-4 (metestrus) following ovulation are characterized by conversion of granulosa and theca interna cells to progesterone-

secreting structures that eventually form functional CL (Manns and Hafs, 1976). LH is involved in this developmental period and evidence indicates that cyclic-Amp mediates LH action in inducing luteinization and progesterone synthesis (Espey, 1974). Following ovulation, progesterone levels rise gradually for two to three days coincident with CL development (Cupps, 1972), with some reports of slight but distinct depressed levels at day 4 of the cycle (Sprague et al., 1971 and Hansel and Echternkamp, 1972); CL begin functional progesterone secretion at approximately day 5. The principal function of the CL in nonpregnant cows is control of the length of diestrus (days 5-17) (Melampy and Anderson, 1968). Elevated progesterone during this period exerts negative feedback effects on the hypothalamo-hypophyseal system thereby inhibiting episodic gonadotropin release (Lamming, 1973). Short (1972) suggested that inhibition of gonadotropin release by progesterone might be mediated through suppression of the hypothalamo-hypophyseal system's ability to respond to estrogen of follicular origin during the luteal phase. Progesterone concentrations in peripheral plasma and luteal tissue generally parallel the growth curve of the CL throughout the luteal phase of the cycle, with peak levels (3.9-8.4 ng/ml) occurring at days 14-16 (Zolman et al., 1974; Glencross et al., 1973; Christensen et al., 1974; Chenault et al., 1975). Regulation of LH binding sites controls progesterone production by the CL and it is reported that LH may not autoregulate its own luteal

receptors (Spicer et al., 1980).

Waves of follicular growth occur during the luteal phase with reports of diphasic (Rajakoski, 1960) and/or triphasic (Bane and Rajakoski, 1961) growth periods throughout the cycle. It has been disclosed that often noted mid-cycle estrogen increases originate from an early wave (days 3-10) of increased follicular activity (Smith et al., 1975), although these mid-cycle follicles undergo atresia and are, therefore, not the ovulatory follicle for that particular cycle (Lamming, 1973). Dufour et al. (1972) reported that only after day 18 did the largest follicle ovulate in a trial involving observation of largest and second largest follicles throughout the cycle. Ireland et al. (1979) revealed extremely high concentrations (12-72 ng/ml) of estrogens in follicular fluid during days 5-10, suggesting that they may play integral physiological roles in mid-cycle follicular development.

The physiological significance of temporary mid-cycle estrogen peaks is uncertain. High progesterone levels during this period apparently do not prevent such a rise. Lamming (1973) conjectured that elevated estrogen during the luteal phase might be useful for uterine receptivity of the embryo or for transport of the fertilized ovum. Cupps (1972) recognized the large variations between cows and between cycles with respect to increased estrogen during diestrus and suggested a random pattern similar to that shown by FSH secretion.

Concannon (1972) postulated that this rise may initiate or precipitate normal luteolytic processes in the cow. Cowley et al. (1979) reported that midcycle follicles are important to initiation of luteolysis. It is suggested that estradiol levels during diestrus mediate tonic FSH secretion and subsequent follicular development and that this mechanism increases LH receptor sites within the ovary to facilitate luteotropic mechanisms and ovulation (Cumming, 1975). Mid-luteal estrogen peaks reportedly correspond to occurrence of "short cycles" and may reach sufficient levels for expression of behavioral estrus (Sorensen, 1979).

2.1.2. Luteolysis

The uterus plays a dominant role in regulation of CL lifespan and cyclic periodicity through local release of a luteolytic substance at days 17-19 in the non-pregnant, normally cycling cow. Hysterectomy and ovarian transplant studies with ewes indicate that uterine release of luteolysin is local. Prolongation of CL lifespan was observed when ewes and cows were hysterectomized (Wiltbank and Casida, 1956). Unilateral CL regression has been observed on the side of the conserved uterine horn in partially hysterectomized ewes (Inskeep and Butcher, 1966). Cumming (1975) provided the following evidence which indicates that uterus and ovary must be in close proximity for normal luteolysis in the ewe and cow: 1) uterine excision extends CL lifespan; 2) partial uterine removal extends CL lifespan in proportion to the amount

removed; this effect is confined to the CL adjacent to the portion of uterus removed; 3) transplantation of ovaries to other sites (i.e. neck) within the body lengthens the cycle; 4) uterine transplantation with ovary remaining *in situ* results in prolonged CL life; 5) transplantation of both ovary and uterus together as a single unit of tissue results in normal cyclical function and length; 6) infusion and cross-circulation experiments demonstrate luteolysin in utero-ovarian venous blood at the time of luteolysis.

Much evidence suggests that $PGF_{2\alpha}$ is "the" luteolysin in cows (as reviewed by Stabenfeldt *et al.*, 1978). Neurohormonal mechanisms initiating uterine release of $PGF_{2\alpha}$ at days 17-19 are not well understood, but estrogens appear to be involved (Inskeep, 1973). Goldberg and Ramwell (1975) and Warren *et al.* (1979) reported that estrogen appeared to stimulate uterine prostaglandin synthesis and postulated that rising estrogen levels from growing follicles in late luteal phase may initiate the luteolytic mechanism. McCracken *et al.* (1972) reported that prostaglandin secretion is under estrogen influence. Hansel and Echternkamp (1972) speculated that rises in plasma estrone observed prior to CL regression in cows might be involved in initiation of luteolysis, perhaps through its action on the endometrium. Furthermore, estrogens involvement in luteolytic mechanisms are supported by evidence that estradiol injections cause early regression of CL in heifers with intact uteri (Wiltbank *et al.*, 1961; Brunner *et*

al., 1969; Watson et al., 1980).

Mechanisms of $PGF_{2\alpha}$-induced luteolysis are not as yet unequivocably defined. Some plausible actions include a direct toxic effect on the CL (Henderson and McNatty, 1975), and reduction of ovarian blood flow through vasoconstrictive properties (Goldberg and Ramwell, 1975). More detailed discussion of $PGF_{2\alpha}$ and its effects is presented in Chapter 3.

$PGF_{2\alpha}$ from the uterus via counter-current mechanisms (McCracken et al., 1972), whereby $PGF_{2\alpha}$ diffuses directly from the uterine vein into the ovarian artery at days 17-19, cause CL regression and a concomitant rapid decline in systemic levels of progesterone (Robinson, 1977). Based on levels of plasma progesterone, no animal had lost luteal function by 5 days before estrus (Garverick et al., 1971); however, between day - 5 and - 4 a representative group of cows in this study exhibited more than a 50 percent decrease in plasma progesterone. Other documentation indicates that peak progesterone levels decline by at least 50 percent in 24-48 hours during luteolysis (Henricks et al., 1971; Wetteman et al., 1972; Robertson, 1972; Chenault et al., 1975). These reduced progesterone levels may directly stimulate gonadotropin release or remove a hypothalamo-hypophyseal block that permits release of gonadotropin during proestrus and estrus (Bearden and Fuquay, 1980).

2.1.3 Events Leading to Estrus and Ovulation

During proestrus (days 18-20), follicular growth is enhanced due to progesterone withdrawal following CL regression. Wise et al. (1980) reported that ovarian blood flow and progesterone changes were positively correlated, with lowest blood flow observed during proestrus. With CL demise and development of follicles, estrogen is produced in significant quantities (Wetteman et al., 1972), which is important in onset of sexual receptivity as well as initiation of events leading to preovulatory gonadotropin release (Manns and Hafs, 1976). Reciprocal changes in plasma levels of progesterone and estrogen determine the appearance of behavioral estrus and ovulation (Shemesh et al., 1972).

It is generally accepted that the pre-estrus estrogen peak triggers preovulatory surges of LH and FSH from the pituitary. Rising estrogen levels evoke positive feedback effects on the hypothalamo-pituitary axis stimulating GnRH release (Erb et al., 1971) which in turn cause LH and FSH release (Lamming et al., 1979 and Kesner and Convey, 1979). Pre-estrus estrogen peaks occur 12-24 hours prior to estrus onset (Christensen et al., 1974 and Smith et al., 1975) and precede pre-ovulatory LH and FSH release which coincide with estrus onset (Sprague et al.,1971 and Chenault et al., 1975). Preovulatory gonadotropin surges are usually of short duration, usually returning to basal levels within 18-24 hours (Geschwind, 1972).

Estrogen declines rapidly during estrus (Staigmiller et al., 1979), usually returning to basal levels during that period. Shemesh et al. (1972) reported that many cows permitted mating at a time when estrogen levels were at their lowest point during estrus.

Ovulation in the cow occurs approximately 10-12 hours following estrus completion (Thibault and Levasseur, 1974; Chenault et al., 1975). Espey (1974) described ovulation and follicular rupture. During ovulation, appropriate LH and FSH stimulation initiate a substantial increase in cyclic-Amp which result in significant elaboration of a zymogen enzyme (possibly collegenase). The connective tissue in the follicular wall is progressively degraded due to the action of this enzyne which results in gross reduction in tensile strength. The thin region at the apex of the follicle is most susceptible to distension under the stress of a small degree of intrafollicular pressure, with rupture imminent as follicle walls dissociate under this stress. Rondell (1970) reported that intrafollicular pressure is not involved in rupture of follicles.

If the preceding sequence of events fails to result in conception, they will be repeated in the next estrous cycle.

Chapter 3

Role of $PGF_{2\alpha}$ in Bovine Reproduction

Prostaglandins (PG's) are 20-carbon hydroxylated fatty acids containing cyclopentane rings at C-8 to C-12 (Hansel et al., 1976). Naturally occurring PG's are all derivatives of prostanoic acid, with six different series (A,B,C,D,E,F) exhibiting structural differences in the cyclopentane ring (Lehninger, 1977). Biosynthesis of PG's initiates with essential fatty acids, with $PGF_{2\alpha}$ originating from linoleic acid (Montgomery, 1977).

PG's were initally thought to originate in prostate glands, hence the term prostaglandin; Lauderdale (1974) reviewed evidence that the compounds occur in most mammalian tissues. The same author further indicated that bioactivity of PG's is primarily due to alteration of smooth muscle contractility and modulation of hormonal activity.

Babcock (1966) first suggested that PG might be the naturally occurring uterine luteolytic factor in bovine females. Since that speculation, evidence that maximal $PGF_{2\alpha}$ levels occur in endometrium, ovarian venous plasma, and uterine fluids at days 15 to 19 of bovine estrous cycles (Shemesh and Hansel, 1975 and Lamothe et al., 1977) coupled with documentation of exogenous $PGF_{2\alpha}$-induced luteolysis when administered during the luteal phase (days 5 to 17) of estrous cycles as previously discussed leaves little doubt of direct involvement of $PGF_{2\alpha}$ in normal CL regression in cycling bovine females.

3.1 Role of $PGF_{2\alpha}$ in Luteolysis

Precise mechanisms of $PGF_{2\alpha}$ involvement in events of CL regression have not been determined. Phariss et al. (1972) outlined five possible mechanisms by which $PGF_{2\alpha}$ mediates luteolysis.

The importance of hypothalamic-hypophyseal communication for control of ovarian activity and CL function led to the hypothesis that $PGF_{2\alpha}$ might initiate luteolysis by interfering/blocking normal luteotropic function at the hypothalamus and/or pituitary. Phariss et al. (1972) provided evidence that this is not the case in laboratory rodents, but little information was presented regarding bovines. Low dosages of $PGF_{2\alpha}$ when given systemically in the cow are ineffective in causing luteolysis but identical dosages administered in the ovarian artery cause CL demise (Lamond et al., 1973). Greene (1977) suggested that if $PGF_{2\alpha}$ mediates luteolysis through inhibition of hypothalamic/hypophyseal control mechanisms then the process (luteolysis) should occur throughout the estrous cycle. Although this theory is questionable, exogenous $PGF_{2\alpha}$ is known to cause regression of only functional CL (days 5 to 17). Previous discussion of local uterine control of CL lifespan in the cow along with proof of rapid metabolic clearance of $PGF_{2\alpha}$ (Karim, 1975) suggests that the compound does not direct its effects on the hypothalamo-pituitary axis in causing luteolysis. Evidence of normal LH release following $PGF_{2\alpha}$ (Chenault et al., 1975) further disproves this postulate.

Supposition that $PGF_{2\alpha}$ might cause uterine release of unidentified luteolysin/s (Phariss et al., 1972) was disproven upon demonstration that exogenous $PGF_{2\alpha}$ caused CL regression in hysterectomized cows (Lavoie et al., 1975 and Stellflug et al., 1975).

Induced biochemical alterations in bovine ovaries/CL simulating a "toxic" effect is a possible mechanism of $PGF_{2\alpha}$ involvement in luteolysis (Phariss et al. 1972). Henderson and McNatty (1975, 1977) reported inhibition of progesterone synthesis by $PGF_{2\alpha}$ in granulosa cells in vitro. In the earlier report, these authors hypothesized that $PGF_{2\alpha}$ interfered with gonadotropin stimulation of adenyl cyclase enzyme resulting in abolition of cyclic-Amp and, therefore, decreased progesterone synthesis. The more recent report suggested a "see-saw", antagonistic situation between ovarian receptors for LH and $PGF_{2\alpha}$. It was postulated that saturation of LH receptors by LH may prevent binding of $PGF_{2\alpha}$ to its respective sites and, conversely, occupation of $PGF_{2\alpha}$ sites inhibits interaction of LH with its receptors. Evidence of tight LH binding and a gradual dissociation (several days) was presented. It was proposed that following preovulatory LH surges, saturation of LH binding sites occurs, thereby masking $PGF_{2\alpha}$ receptors for a period of time. This represents plausible explanation as to why $PGF_{2\alpha}$ fails to cause luteal regression in bovines until approximately day 5 of the cycle. Gradual dissocation of LH from its receptors result in concomitant unmasking of $PGF_{2\alpha}$ receptors causing CL to

become increasingly susceptible to lytic action of the compound. These theories are supported by earlier reports that relative binding of $PGF_{2\alpha}$ to bovine CL and luteolysis were associated (Kimball and Lauderdale, 1975) and that $PGF_{2\alpha}$ may cause reduced gonadotropin binding capacity of CL (Hichens et al., 1974).

Evidence opposing LH saturation of luteal receptors in protecting CL from $PGF_{2\alpha}$ was provided by Gonzales-Mencio et al. (1977) who noted that LH infusions 4 hours prior to $PGF_{2\alpha}$ on days 10 to 12 in heifers failed to overcome lytic effects. Although it seems possible that by days 10 to 12 of the cycle the gradual dissociation mechanism of LH from its ovarian receptors as previously described might have progressed to conditions of irreversible $PGF_{2\alpha}$ binding, no reference was made in this regard. Additional opposition to an antiluteotropic action of $PGF_{2\alpha}$ (within ovary) was offered by Hansel et al. (1973) who reported luteotropic (increased progesterone synthesis) rather than luteolytic $PGF_{2\alpha}$ effects on bovine luteal tissue in vitro.

Another possible biochemical process of luteolysis induced by $PGF_{2\alpha}$ is lysosomal digestion of luteal cells. This has been demonstrated in ewes by McClellan et al. (1977), who reported structural degeneration of luteal cells due to increased activity of lysosomal enzymes during natural and exogenous $PGF_{2\alpha}$-induced luteolysis. These authors further indicated that activity of 3β-hydroxysteroid dehydrogenase, an enzyme required for progesterone synthesis, declined

rapidly following exogenous $PGF_{2\alpha}$.

Reduced ovarian blood supply due to vasoconstrictive effects has been postulated as another $PGF_{2\alpha}$ luteolytic mechanism (Phariss et al., 1972). Natural and exogenous $PGF_{2\alpha}$ reduces blood flow to CL-containing ovaries in ewes (Niswender et al., 1976). It was noted that CL receive a majority of blood entering entire ovaries and that during regression, along with reduced total blood volume, a shunting of blood within CL also restricts quantity available to luteal cells. Chamley and O'Shea (1976) supported vascular changes within the ovary as a component of $PGF_{2\alpha}$-induced luteolysis. Ford et al. (1977) reported that $PGF_{2\alpha}$ caused greater constriction of ipsilateral ovarian arteries than contralateral arteries and that $PGF_{2\alpha}$ potentiates vasoconstriction by facilitating release of norepinephrine from sympathetic nerve terminals rather than acting directly on smooth muscle cells.

Opposition to reduced blood flow as a direct component in luteolysis was presented by Fogwell et al. (1977) who showed total blockage of blood flow failed to result in luteal regression in ewes. Baird (as reviewed by Inskeep and Murdoch, 1980) concluded that reduction in blood flow may be a consequence of rather than a cause for CL demise.

Considering the presented evidence, it seems probable that $PGF_{2\alpha}$ plays a multi-faceted role in luteolysis. Most tenable of these elements include 1) anti-gonadotropism via receptor site competition

2) initiation of histochemical regression by lysosomes 3) reduced activity of enzymes involved in progesterone synthesis and 4) induced alterations in ovarian/CL vasculature.

Measurement of $PGF_{2\alpha}$ in blood is difficult because of rapid metabolic clearance and low concentrations; an alternative method has been to quantify the primary $PGF_{2\alpha}$ metabolite (15-keto-13, 14-dihydro-$PGF_{2\alpha}$), which has a longer half life than the parent compound (Stabenfeldt et al., 1978). Blood levels of this metabolite are elevated at the time of luteolysis (Peterson et al., 1974), and preliminary work indicates possible involvement in $PGF_{2\alpha}$-induced luteolysis (Milvae and Hansel, 1980).

An alternative hypothesis regarding CL regression is that endometrial tissue exerts its local luteolytic effects by providing the CL with one or more precursors (i.e. arachidonic acid) which are converted to $PGF_{2\alpha}$ by luteal tissue (Hansel et al., 1973). Hansel et al. (1976) provided evidence that the bovine ovary can convert arachidonic acid to $PGF_{2\alpha}$ and that sufficient levels are produced to elicit decreased progesterone and subsequent increased estrogen levels. However, complete CL regression did not occur in treated animals. Inskeep and Murdoch (1980) reviewed evidence that arachidonic acid (10 mg) injections administered into CL, follicles on ovaries with functional CL, or into lumens of ipsilateral uterine horns failed to cause luteal demise; the authors concluded that conversion of a uterine

compound to $PGF_{2\alpha}$ by CL subsequently resulting in luteolysis seemed unlikely.

3.2 Physiological Response to Exogenous $PGF_{2\alpha}$

$PGF_{2\alpha}$ has been administered via intrauterine, intravaginal, intraovarian, intramuscular, and subcutaneous routes.

3.2.1 Luteal Tissue Response

Hafs et al. (1974) found that 70 percent of original luteal tissue volume disappeared within 24 hours after 5 mg $PGF_{2\alpha}$ deposited in ipsilateral horns of Holstein cows on days 7, 11, and 15 of estrous cycles, with a majority of the remaining tissue lost during subsequent 24 hour periods. Luteal diameter (2.3 cm) in luteal phase heifers declined to 1.8, 1.2, and .6 cm at 1, 2, and 3 days following intramuscular injection of 30 mg $PGF_{2\alpha}$. Rate of luteolysis was retarded by one day and was more variable in heifers receiving 30 mg $PGF_{2\alpha}$, deposited intravaginally.

Subcutaneous administration of 30 mg $PGF_{2\alpha}$-Tham salt to cycling heifers between days 6 to 16 of estrous cycles caused CL regression within two to four days after injection (Lauderdale, 1972).

Louis et al. (1972) infused 5 mg $PGF_{2\alpha}$-Tham salt into contralateral uterine horns of cycling cows at day 11 of the cycle, resulting in decreased luteal diameter from 2.3 cm at treatment to 1.6 and .9 cm at 24 and 48 hours later, respectively. In a second trial, heifers

treated intravaginally with 30 mg $PGF_{2\alpha}$ on day 11 required 96 hours to exhibit comparable degrees of luteolysis.

Rowson et al. (1972) demonstrated that two 0.5 mg dosages $PGF_{2\alpha}$ 24 hours apart deposited in ipsilateral horns resulted in luteolysis; single dosages of 1 mg similarly infused were less effective.

Single intrauterine dosages of 2 mg $PGF_{2\alpha}$ caused complete luteolysis in the cow (Henricks et al., 1974; Fulka et al., 1975; Welch et al., 1975).

Stellflug et al. (1975) reported that single IM injections of 30 mg $PGF_{2\alpha}$-Tham salt or two 15 mg injections at six hour intervals were equally effective in causing luteolysis within similar time periods following treatment.

Incidence of luteolysis in diestrous lactating Holsteins administered 15, 25, or 35 mg IM was not different, but was greater than cows given 5 mg $PGF_{2\alpha}$ (Renegar et al., 1978).

3.2.2 Hormonal Response

Liehr et al. (1972) reported effects of $PGF_{2\alpha}$ on blood progesterone (P_4) levels in beef heifers. Small dosages (500 µg) of $PGF_{2\alpha}$ administered ipsilaterally on day 5 failed to markedly alter blood P_4 levels. Ipsilateral infusion of 6 mg $PGF_{2\alpha}$ on day 9 of estrous cycles reduced P_4 to nondetectable levels within two days post-treatment; P_4 levels in contralaterally treated heifers remained relatively high.

Renegar et al. (1978) found that P_4 levels declined to less than

1 ng/ml within 24 hours after luteolytic dosages of $PGF_{2\alpha}$ (15, 25, and 35 mg).

Thatcher and Chenault (1976) characterized plasma progestins, estradiol, and LH following either 30 mg intramuscular or 10 mg ipsilateral infusion of $PGF_{2\alpha}$ in cycling dairy animals. Progestins declined to basal levels associated with CL demise within 24 hours post-treatment. Plasma estradiol increased linearly by two-fold within 76 hours post-treatment, indicative of follicular growth and maturation following luteolysis and declining progestins. Transitory plasma LH oscillations were observed during the first two days following $PGF_{2\alpha}$, but normal preovulatory surges occurred between 72 and 96 hours following $PGF_{2\alpha}$. These authors concluded that $PGF_{2\alpha}$-treated animals demonstrated variablity in hormonal concentrations and precise timing of physiological events, but all responding animals exhibited similar sequences of hormonal events as animals undergoing natural luteal regression. Although variations in precise onset of response have been noted, other investigations substantiate normal hormonal patterns of progesterone, estradiol, and LH following luteolytic dosages of intrauterine and/or systemic $PGF_{2\alpha}$ (Nancarrow et al., 1974; Henricks et al., 1974; Chenault et al., 1976; Smith et al., 1979). Fogwell et al. (1978) reported that increases in both estradiol and LH are dependent on declining progesterone levels due to $PGF_{2\alpha}$-induced effects, suggesting an indirect effect of $PGF_{2\alpha}$ in eliciting these hormonal re-

sponses.

Intramuscular injections of 30 mg $PGF_{2\alpha}$ caused a 2.5-fold increase in blood prolactin within ten minutes of treatment, with elevated levels persisting for at least two hours (Hafs et al., 1974). Renegar et al. (1978) stated that prolactin increased from 38 ng/ml to peaks of 57, 84, and 95 ng/ml at 1.0 hour after 15, 25, or 35 mg intramuscular $PGF_{2\alpha}$, respectively; this pattern suggests a dose-response relationship of $PGF_{2\alpha}$ and prolactin. Smaller dosage (5 mg) failed to markedly alter prolactin levels. Louis et al. (1974) reported 5-fold increases in prolactin when heifers were given 15, 30, or 60 mg $PGF_{2\alpha}$.

Rapid increases (74 %) in somatotropin at 0.5 hours following 35 mg $PGF_{2\alpha}$ injections have been reported (Renegar et al., 1978); lower dosages (5, 15, or 25 mg) did not greatly modify growth hormone by 4 hours after treatment. Contrastingly, Hafs et al. (1974) reported 2.5, 7, and 26-fold increases in growth hormone within 30 minutes after 15, 30, and 60 mg $PGF_{2\alpha}$ injections, respectively, with levels remaining above basal levels for at least two hours.

Glucocorticoid levels increased linearly to peaks after one hour following 15, 25, and 35 mg $PGF_{2\alpha}$ (Renegar et al., 1978).

3.2.3 Other Physiological Effects

Thatcher and Chenault (1975) reported that 33.5 mg $PGF_{2\alpha}$ given intramuscularly caused no major alterations in blood pressure, heart rate, and uterine or aortic blood temperatures. However, identical

dosages administered by intravenous jugular infusion over a 2 minute period caused major changes in circulatory homeostasis and body temperature.

3.3 Early Stage CL of Pregnancy and $PGF_{2\alpha}$

Documentation regarding mechanisms of CL maintenance in early bovine pregnancy is scarce. In view of this, reference will be made to available knowledge of these aspects in ewes, though it is recognized that differences may exist between the two species.

It is known that in the ewe (Moor and Rowson, 1966) and cow (Betteridge et al., 1978) the conceptus must be in-utero by day 13 and 16 after mating, respectively, for CL maintenance and continued pregnancy. Presence of embryos at this time inhibits luteolysis in the ewe and cow (Hafez and Jainudeen, 1974), despite evidence that uterine venous $PGF_{2\alpha}$ levels are at least as high in early pregnant as in nonpregnant ewes (Inskeep and Murdoch, 1980). This would indicate that the conceptus produces or elicits action of some agent that overrides luteolytic effects of $PGF_{2\alpha}$.

Denamur (1974) demonstrated that luteal maintenance depends on luteotropic support of hypophyseal LH aside from antiluteolytic and/or luteotropic actions of the early conceptus in ewes.

Another prostaglandin, PGE_2, which elicits different effects than $PGF_{2\alpha}$, was studied as a possible antiluteolysin in ewes. Luteolysis has beeninhibited and/or postponed in nonpregnant ewes with PGE_2

(Henderson et al., 1977). Lewis et al. (1978) stated that overall mean concentrations of both PGE_2 and $PGF_{2\alpha}$ in plasma from utero-ovarian venous blood, endometrium, and ovarian arterial tissue were not significantly different between pregnant and nonpregnant ewes on day 14 post-estrus. However, blood concentrations of PGE_2 in ovarian arteries tended to be less in nonpregnant than in pregnant ewes ($P < .10$). The authors suggested that this may have been due to increased uptake and transport of PGE_2 to ovaries of pregnant ewes. It was further stressed that lack of significant differences in PGE_2 concentrations in other measured media between pregnant and nonpregnant ewes did not rule out that PGE_2 may be an antiluteolytic substance in ewes.

Godkin et al. (1978) provided evidence that ovine preimplantation embryos produce a luteotropic agent which contributes to the maintenance of early pregnancy in vitro. It was reported that the ovine embryos produced a substance which directly stimulated progesterone synthesis by both ovine and bovine CL, indicating that the unidentified material is not species specific.

Ford et al. (1979) suggested that the bovine conceptus may produce or stimulate synthesis of a factor which dilates ipsilateral utero-ovarian vasculature, thereby creating optimal conditions for pregnancy. Blood flow to gravid uterine horns of pregnant cows exhibited a transient, dramatic increase on days 14-18 after mating whereas blood flow to nongravid horns remained constant. Blood flow

in nonpregnant cows declined consistently within the same period. This suggests that preimplantation bovine embryos may directly or indirectly counteract vasoconstrictive effects of $PGF_{2\alpha}$ and thereby remove a plausible action of the compound in causing luteolysis as discussed earlier. It was not suggested that the unidentified substance thought to dilate utero-ovarian vasculature might be PGE_2.

Lewis et al. (1980) quantified PGE_2 and the primary $PGF_{2\alpha}$ metabolite (13, 14-dihydro-15- keto-PGF_2: PGFM) concentrations in blastocysts and endometrium of day 16 and 19 pregnant cows in vitro. Day 19 blastocysts produced more PGE_2 than either contralateral or ipsilateral endometrium on the same day ($P < .05$). Production of PGFM, on both days was less from blastocysts than endometrial tissue ($P < .05$). It was suggested that PG's are involved in blastocyst development and/or in maternal recognition of pregnancy.

Other attempts to identify antiluteolytic or luteotropic agents originating from the conceptus or from the uterus in response to a stimulus from the embryo are somewhat inconclusive (Chenault, 1979; Eley et al., 1979; Lewis et al., 1979; Godkin et al., 1980). Perhaps a search for known $PGF_{2\alpha}$ inhibitors such as indomethacin (Karim, 1975) would be of some value.

Chapter 4

Synchronization of Estrus and Ovulation

Knowledge of physiological mechanisms controlling events of the estrous cycle has led to means of artificial control and potential techniques for improvement of reproductive efficiency in commercial beef herds.

Natural and synthetic agents used in synchronization of estrus and ovulation procedures must meet several obvious criteria before they can be useful to beef producers. Foote (1978) reported that these compounds must: 1) effectively synchronize a large portion of estrous cycling females with no adverse effect on fertility; 2) comply with legislative restrictions governing their use in animals produced for human consumption; 3) be applicable with an acceptable amount of management effort; 4) provide for an effective cost/benefit ratio when used under varying production situations. The latter is perhaps the most important criteria to the majority of cattlemen but yet has received least attention.

The many advantages of estrous sychronization are well recognized, but perhaps the most important potential benefits for beef producers are facilitation of AI (Hansel, 1967), potentially shorter breeding and subsequent calving seasons (Lamming, 1973) and uniform calf crops (Foote, 1978). An important, inherent advantage is that management required for successful programs forces closer attention to cattle and

overall production techniques. All of these factors can contribute to more pounds of calf weaned (Wiltbank, 1970).

Two basic approaches have been employed for synchronizing estrus and ovulation in the bovine. The first involves administration of progesterone or synthetic progestagens which prevent estrus and ovulation until CL of treated animals regress, followed by withdrawal of treatment and subsequent estrus and ovulation in a large proportion of treated animals at approximately the same time. The second approach has relied on induction of luteolysis via use of estrogens, oxytocin, anti-LH preparations, uterine irritants and $PGF_{2\alpha}$. Efforts to enhance synchrony with both approaches have involved use of additional compounds such as estrogens and gonadotropins. Both in principle and practice, either basic method requires estrous cycling females in order to be effective.

4.1 Progesterone and Synthetic Progestagens

Progestins have been administered in feed, drinking water, intravaginal pessaries and coils, intramuscular or subcutaneous injections, and as subcutaneous implants. Review of various progestin compounds and routes of administration follows.

4.1.1 Progesterone Injections

Christian and Casida (1948) showed that progesterone (P_4) injections prolonged the diestrus phase of bovine cycles by preventing

estrus and ovulation. Heifers were injected daily (50 mg) over a 14 day period followed by cessation of suppressive treatment with a majority of animals exhibiting estrus within 5-6 days. Although reasonable synchrony was obtained, no fertility data were reported.

Willett (1950) injected 50-100 mg P_4 daily beginning on days 14-15 of the cycle and continued treatment for 13-17 days. Estrus occurred, on the average, 5 days after completion of the injection period. Eleven pregnancies resulted from twenty-two services at synchronized estrus.

Ulberg et al. (1951) conducted trials to determine effects of varying P_4 dosages administered at different times within the cycle and for varying durations on follicular development and onset of estrus. Cycling dairy heifers received 50, 25, 12.5, 6.25 or 3.125 mg P_4 in corn oil during injection intervals ranging from a single injection to 28 consecutive daily injections. The beginning of injection periods varied from day 15 of the cycle until the day of estrus. Results showed that time intervals between end of injections to estrus onset (3-7 days) decreased as dosage level decreased. Inhibition of follicular development was greatest in heifers receiving 50 mg P_4 on day 15 of the cycle (vs. days 17-19). Daily dosages of 25 or 12.5 mg usually prevented estrus and ovulation, but follicular development was not markedly affected. Dosages of less than 12.5 mg/day had little, if any, effect on reproductive parameters considered in this trial.

In a similar experiment Trimberger and Hansel (1955) reported that injected P_4 (50, 75, or 100 mg/day), administered predominantly on the 15th day and continuing for seven days, resulted in acceptable synchrony but 50 percent of the dairy cows treated exhibited abnormal ovarian conditions involving luteal development and extremely poor conception rates (12.5 %) as compared to controls.

In an effort to improve fertility and eliminate daily handling disadvantages required with a series of injections, Nellor and Cole (1956) administered single injections of P_4 (560 mg) to beef heifers followed 14-15 days later by single injections (750-2140 I.U.) of pregnant mare serum gonadotropin (PMSG). PMSG served to enhance follicular development following P_4 withdrawal. Estrus and ovulation were well synchronized as 90 percent showed estrus within 1-4 days after PMSG, but reduced conception (17%) resulted. In a similar trial, Ray et al. (1961) reported more variation (2-38 days) in time of estrus response following PMSG and poor fertility at synchronized breeding.

One hypothesis regarding reduced fertility at the synchronized estrus following P_4 injections was an altered hormone balance resulting from such treatment (Wiltbank et al., 1965). These authors suggested that introduction of estrogen concurrently with P_4 injections might cause less disruption and thus improve fertility. Hereford heifers received either 20 or 40 mg P_4 injections for 18-24 days alone or in