EFFICACY OF SYNTHETIC GONADOTROPIN RELEASING HORMONE ANALOGS FOR CONTROL OF OVULATION DURING ESTRUS SYNCHRONIZATION PROTOCOLS

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ABSTRACT

Two experiments were conducted to determine efficacy of GnRH analogs, Cystorelin (CYS, gonadorelin diacetate tytrahydrate) and Factrel (FAC, gonadorelin hydrochloride), for use in beef timed AI synchronization. In Experiment one 342 beef cows from 7 herds were assigned CYS or FAC treatment as part of the Ovsynch protocol (GnRH d 0 and 9, Lutalyse d 7). Cattle treated with FAC had greater tendency (P=.09) to be pregnant at d 45. One individual herd demonstrated FAC-treated cows had more pregnancies at day 45. In Experiment two, 18 beef cows received either CYS or FAC as part of the Ovsynch protocol, intensive blood samples, from time –30 to 525 min post GnRH, were collected at each GnRH injection. Ultrasounds were conducted daily over the course of the protocol. A treatment by phase interaction (P=.03) was found for the time to maximum LH concentration, where CYS-treated follicular cows had a shorter interval than did FAC treated follicular or luteal cows. The duration of detectable LH response showed a treatment by phase interaction (P = .02) where follicular and luteal CYS-treated cows had shorter interval than follicular or luteal FAC-treated cows. The variables maximum LH concentration, and area under LH curve did not differ. Cows treated with CYS had more (P=.02) non-dominant follicles. In Experiment three, 16 ewes randomly received either CYS, FAT or Fertagyl (FER; gonadorelin diacetaate tytrahydrate), and FAT's induced LH maximum concentration occurred sooner (P=.02) than CYS. We conclude that either product may be used in beef cows without compromising fertility.

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Chapter I
INTRODUCTION

The evolution of the beef cattle industry has been dynamic ever since man first sought the cow for a constant supply of milk, meat, clothing, and power. Since that time, the cooperation of both farmers and scientists have yielded continuously increasing agriculture production efficiency. In the past, revolutionary strategies to maximize agricultural yields and profitability were implemented which allowed fewer farmers to meet the food demands of the human population. Thereafter, many people turned to occupations other than agriculture which gave rise to the Industrial Revolution (Taylor, 1984). Thus, directly and indirectly through advances in agriculture, an increase in the standard of living for society was possible. The objective of this research was to increase the production efficiency of beef cattle through advancing reproductive strategies.

The United States Beef Cattle Industry

In the recent past, the efficiency of beef production has increased (Koch and Algeo, 1993), and there is no obvious reason why the trend will slow. Fifty years ago one farmer in the United States was able to provide enough food to meet the demands of eleven consumers; this number has risen in excess of eighty today. Unfortunately, this tendency is not global. Farmers in the former Soviet Union during the mid 1980's only had the ability to feed four consumers each (Taylor, 1984). The major difference between these two countries is the utilization of technologies. The United States is among the leading nations that are implementing novel strategies to increase agriculture efficiency.

Beef cattle production is a major industry in the United States, and has a dramatic impact on the agriculture economy. In 1980, there were 111,242,000 cattle in the United States, 21,469,000,000 pounds of beef were produced, and production per cow was 449 pounds (USDA, 2001). Today the number of cattle has fallen by 13% to 97,309,000 head, however beef production has increased by over 17% to 26,000,000,000 pounds, and production per cow has increased 26% to 610 pounds per cow (see Figure 1-1, USDA, 2001).

This dramatic increase in beef production using fewer cattle is due to advances in management strategies, including artificial insemination (AI).

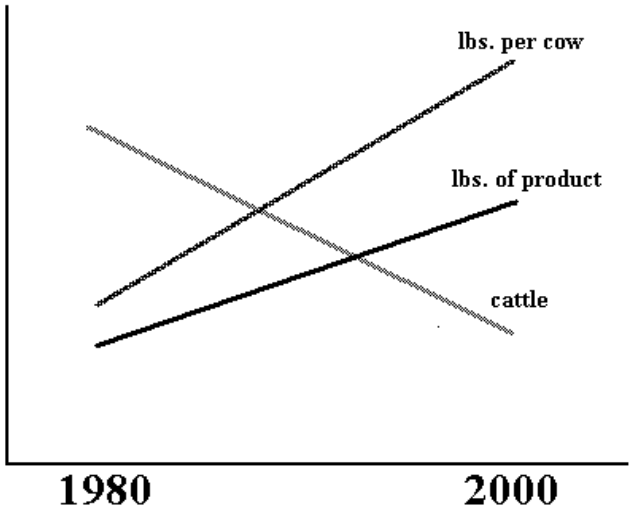

Figure 1-1. United State Beef Cattle Trends. From 1980 to 2000 the number of beef cattle and total pounds of beef produced in the United States dropped. However, during the same interval quantity of beef produced per cow increased greatly.

Value of Artificial and Timed Insemination to the Industry

In no other species of farm animal is reproductive efficiency more important than in the cow. Gestation length is relatively long in the cow (283 d) compared to the sow (114 d), doe (150 d) and ewe (148 d), and so any loss of production time is amplified many times over. At best, a cow can produce only one calf per year. Thus, the rate of cattle genetic progress is likely to be relatively slow compared to other domestic farm species (Peters and Ball, 1995). The use of AI in combination with effective synchronization protocols can reduce the number of d open and accelerate genetic progress.

Artificial insemination allows use of superior genetics from one individual to be utilized abroad. Farmers, who in the past only had access to a limited gene pool, now can chose from a plethora of genes which have been carefully screened for many qualities. The potential of AI has been fully realized within the last 40 years by the dairy industry. Dairy

farmers that use frozen/thawed semen from proven bulls have caused a rapid and substantial increase in milk yields over time (Roy and Greenwald, 1991). The beef industry has been slower to adopt AI strategies due to labor and time constraints. New methods of synchronization that eliminate estrus detection (Pursley et al., 1995) are attracting more beef producers each year.

Aitchison (1982) provides a fundamental equation for animal breeding that summarizes the four basic components that influence genetic progress over time (see below equation). The use of AI in conjunction with an effective estrus synchronization program can directly effect each of the four variables.

$$\text{Genetic Progress Per Year} = \frac{\text{Selection Accuracy} \times \text{Genetic Variation} \times \text{Selection Intensity}}{\text{Generation Interval}}$$

Artificial insemination has several advantages as well as disadvantages. The advantages of AI have been summarized by Noakes (1997):

- Superior sires can be used extensively
- Long term storage of semen
- Usage of semen after bulls have been progeny tested
- Better control of venereal diseases
- Hazard of handling potentially dangerous bulls is eliminated, and
- Less cost devoted to feeding and maintaining a bull

Noakes (1997) also recognized some disadvantages:

- Estrus detection necessary in some protocols
- Importance of correctly timed insemination
- Increased potential of inbreeding after extensive use of a limited number of sires
- Potential of extensive transfer of undesirable genetic traits, and
- Potential to spread infectious diseases

For most producers, the advantages far outweigh the disadvantages. Recent synchronization protocols have eliminated the need for estrus detection, and animals can be

mass inseminated (Pursely et al, 1995). Today all reputable bull studs screen their semen for infectious disease and abnormalities. The potential to introduce disease into a herd through AI is very low. In the recent past less than 5% of beef cattle in the United States are artificially inseminated (Odde, 1989). Artificial insemination after the use of an estrus synchronization treatment is one of the reproductive management tools that will aid in advancing animal agriculture into the new century.

Research Objectives

During interactions with producers and veterinarians, we have discovered some favor one GnRH analog over another based on previous experiences. We conducted a thorough review of current literature to determine if any research had been conducted in this area. We found only one abstract (Bentley et al., 1998) in which GnRH analogs had been compared within the same study. Since there is a lack of research in this area, three experiments were conducted to determine if differences exist between two popular GnRH analogs, Cystorelin (gonadorelin diacetaate tytrahydrate, Merial Limited, Iselin, NJ) and Factrel (gonadorelin hydrochloride, Fort Dodge Animal Health, Fort Dodge, IA).

Experiment one consists of nine replicates in a large field trial (n = 496) comparing Cystorelin and Factrel. In experiment two and three we investigated the bio-efficacy of the two GnRH analogs by comparing their resulting LH profiles in vivo using cattle (n = 19) and ewes (n = 16), respectively. We rationalize that the most effective treatment is the one with highest pregnancy rates, and greatest LH response. Our aim was to give producers the tools required to make informed management decisions based on scientific evidence rather than popular opinion.

Chapter II
REVIEW OF LITERATURE

A complete and scientifically detailed understanding of any physiological process is vital for its successful manipulation. The basic premise of any estrus synchronization protocol pivots around the predictable control of events associated with the animal's reproductive physiology. Control of reproductive events can be achieved through pharmacological administration of biologically active agents. Usually the agents used in synchronization protocols are either based on or are the hormones that occur in the female at various times during her cycle. Therefore, it is paramount to review basic endocrinology and physiology of the female for a full appreciation of the mechanisms involved during a synchronization treatment.

The Bovine Estrous Cycle

In the beef cow, estrus has a duration of 6 to 24 h (mean 15 h) and is designated as d 0, or the start of the cycle. During the estrus phase of the cycle the predominant reproductive hormone is estrogen (E, Hansel and Echternkamp, 1972). The cow's estrous cycle reoccurs every 21 (range 17-24) d, and no period of seasonal anestrus is observed as is the case in sheep. The cycle fluctuates between periods of E to progesterone (P_4) dominance. Progesterone predominates during the luteal phase and contributes to preparation of the uterus for a potential conceptus. Progesterone also prevents a return to estrus prior to and after maternal recognition of pregnancy has occurred.

Proestrus, the follicular phase

The growing follicles on the ovary produce E in proportion to their size (Falck, 1959). The increased E concentration causes a positive feedback on the hypothalamus that results in increased gonadotropin releasing hormone (GnRH) release (Reeves et al., 1971; Kesner et al., 1981). Gonadotropin releasing hormone also can self-prime the anterior pituitary causing additional gonadotropin release (Crighton and Foster, 1977). Throughout the estrous cycle, cohorts of follicles grow, regress, and are replaced continuously (Smeaton and Robertson,

1971; Matton et al., 1981). For an unknown reason, one follicle will start to dominate E production, will experience accelerated growth, and produce inhibin (Hansel and Convey, 1983). During this time the dominant follicle has switched from follicle stimulating hormone (FSH)- to luteinizing hormone (LH)-dependence for sustained survival (Hansel and Convey, 1983). Inhibin serves as an inhibitor of FSH release from the anterior pituitary thus retards the growth of FSH-dependant non-dominate follicles (Hansel and Convey, 1983), and soon these follicles will undergo atresia (Rajakoski, 1960; Ireland and Roche, 1983). At a threshold concentration of E, with low levels of P_4, GnRH sequentially increases the magnitude of LH pulse secretion, and eventually causes a preovulatory LH surge (Hansel and Convey, 1983). Behavioral estrus is observed during this time due to the effects of E.

Metestrus, the early luteal phase

Following the LH surge, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), E, and P_4 are released from the ovary and aid in ovulation. Afterwards, luteinization occurs due to the action of LH. The newly formed CL produces oxytocin, relaxin, and most importantly, P_4. The increased P_4 once again reestablishes the negative feedback on GnRH secretion and prevents a premature return to estrus.

Diestrus, the mid-luteal phase

During the mid cycle (see Figure 2-2) is when P_4 is the predominate hormone governing the female reproductive tract (Hansel and Echternkamp, 1972). Progesterone and 20-β-hydroxyprogesterone concentrations associated with the corpus luteum (CL, Hafs and Armstrong, 1968) and in peripheral blood (Hansel et al., 1973) increase during the luteal phase and peak near d 10. Also during the luteal phase, LH is secreted in a pulsatile manner characterized by Rahe et al. (1980). Progesterone serves as a negative feedback retarding cyclic GnRH release from the lateral portions of the external layer of the median eminence adjacent to the pituitary stalk, preventing a return to estrus. Neurons that secrete GnRH reside in the preoptic area of the anterior hypothalamus with terminals at the median eminence.

The late luteal phase

Around d 17, if a conceptus is not present, the non pregnant bovine uterus produces $PGF_{2\alpha}$ which causes luteolysis. Progesterone concentration decreases and GnRH secretion increases once the negative feedback influence has been removed. Gonadotropin releasing hormone stimulates the release of FSH and LH from the anterior pituitary. Follicular growth and maturity occur when FSH binds to its specific receptors on the growing follicle. Luteinizing hormone induces final follicle maturation and initiates the ovulation process.

Luteinizing hormone pulse amplitude and frequency are influenced by steroid hormone concentrations (Rahe et al., 1980). During periods of high E concentration a high frequency, low amplitude LH pulse occurs. In contrast a low frequency, high amplitude LH pulse occurs under P_4 dominance (Rahe et al., 1980)

It should be noted that the cow's estrous cycle progresses under a complex series of hormonal interactions, all of which may not be realized and have not been discussed here. For the reason of simplicity, only the major hormones have been described. For an in-depth review of the estrous cycle see Hansel and Convey (1983). With this overview in mind specific mechanisms in the estrous cycle pertaining to our research will be reviewed in greater detail.

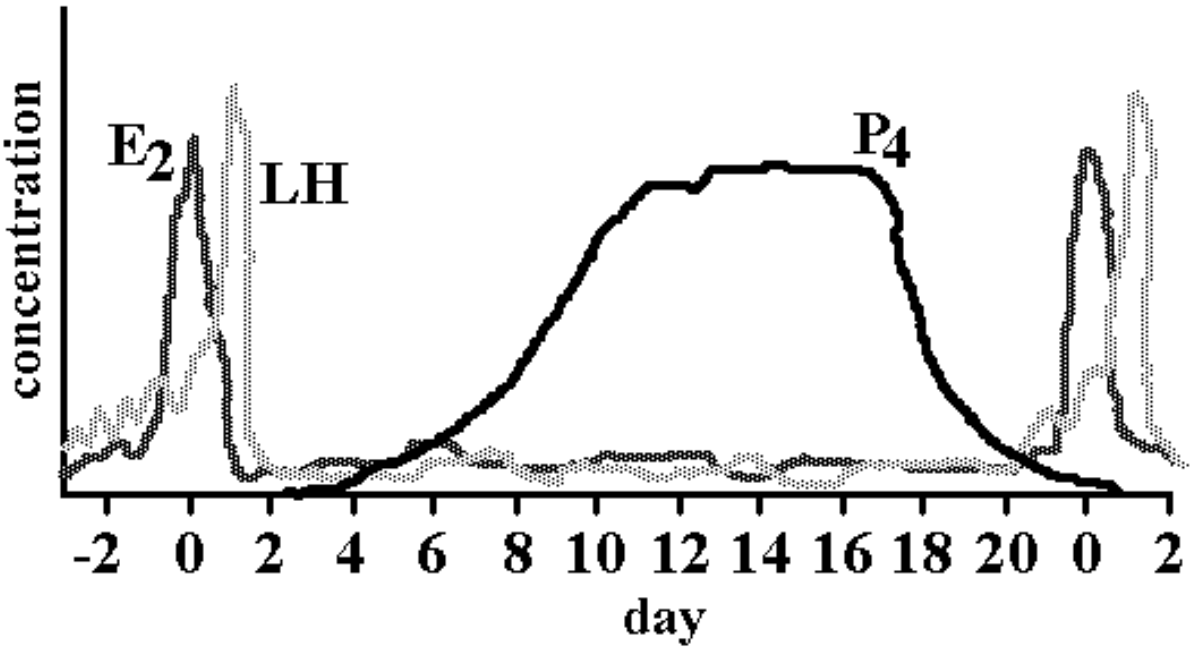

Figure 2-1. Major Hormones of the estrous cycle. Notice that a rise in E concentrations occur just prior to the LH surge. After the LH surge and ovulation, the dominant follicle has been removed, and so E concentrations are reduced. Progesterone then dominates until around d 18 (cow) when luteolysis occurs. Adapted from Hansel and Convey (1983).

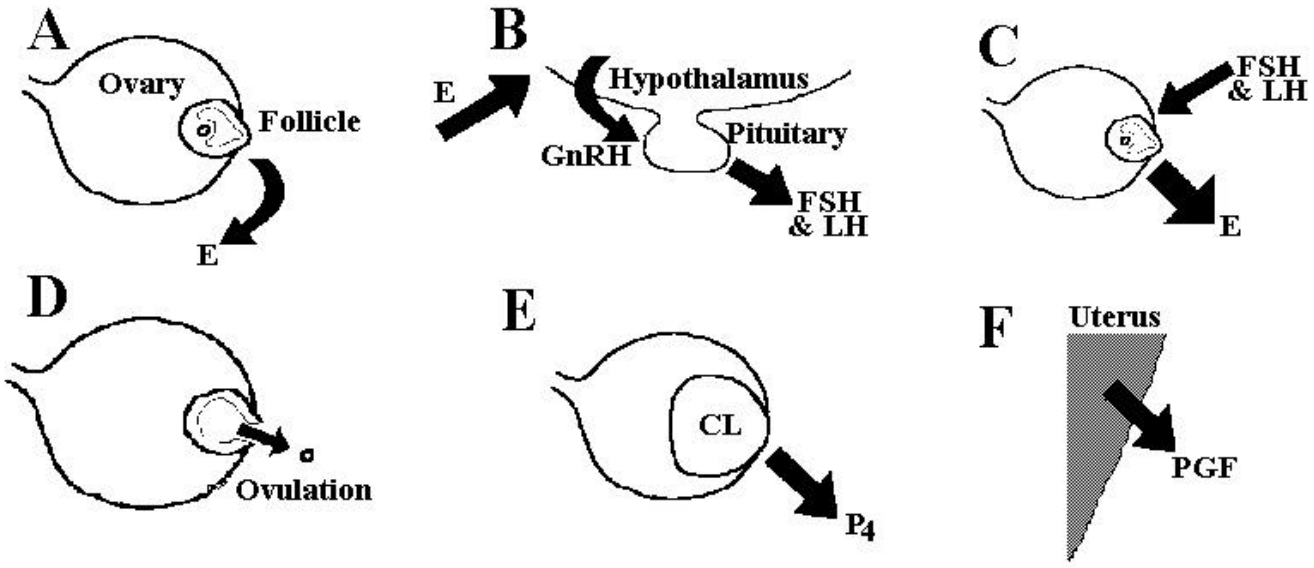

Figure 2-2. Basic Bovine Estrous Cycle. A dominant follicle on the ovary secretes E in proportion to its size (d 0, A). This increased concentration of E speeds firing of the GnRH pulse generator which induces release of FSH and LH from the pituitary (d 0, B). Low E concentrations retard the frequency of the GnRH pulse generator. Gonadotropins are responsible for follicular growth and maturation, and cause further release of E, creating a positive feed back (d 0, C). At a threshold level of E secretion the LH surge occurs and causes ovulation (d 1, D). After ovulation a CL forms in the void where the follicle once existed. The CL produces P_4 which hinders E production (d 4-17, E). When E levels are low and P_4 levels are high, a negative feedback is placed upon the preovulatory center which prevents high amplitude pulses of GnRH. If no conceptus is present the uterus produces $PGF_{2\alpha}$ which destroys the functional CL, and removes P_4 negative influence on GnRH secretion (d 18, F).

Release of LH from the Anterior Pituitary

The release of LH from the anterior pituitary is under the control of complex interactions, all of which are not fully understood. External and internal cues, as well as both negative and positive feedback mechanisms govern LH release. External cues from the environment exert their effects by means of the central nervous system. Such factors include but are not limited to photoperiod, availability of food, temperature, and sexual receptivity of the opposite sex (Fink, 1988). The body's internal environment also manipulates LH release. Warren (1983) reported metabolism, bodyweight and body fat percentage, and several nutrition associated diseases all exert feedback loops within the body that modulate gonadotropin release. Diseases which result in malnutrition can restrict LH secretion. The precise mechanisms of the internal environment and disease modulation of LH are not known; however, it appears that the central gonadotropin regulatory mechanism is affected which leads to altered pulse frequency (Warren, 1983).

Classically, LH is thought to be synthesized and released from the anterior pituitary under control of its secreteoguge GnRH (Schally et al., 1973). In the past, there was speculation that LH and FSH secretion were directed by two independent secretoguges. However, the work of White (1970) disproved this notion and concluded both were under the control of a common secretoguge termed GnRH. Gonadotropin releasing hormone is synthesized by specific neurons in the hypothalamus (Silverman, 1987), and is released into the hypophysial portal blood system (see Figure 2-3). Synthesis and release of GnRH is controlled by neurons in the fore-, mid-, and hind-brain and regulated by steroid hormones. In the ovariectomized animal, release of LH is not continuos, but rather pulsatile with a frequency of 1 h in cows (Forrest et al., 1980; Rache et al., 1978) and sheep (Butler et al., 1972).

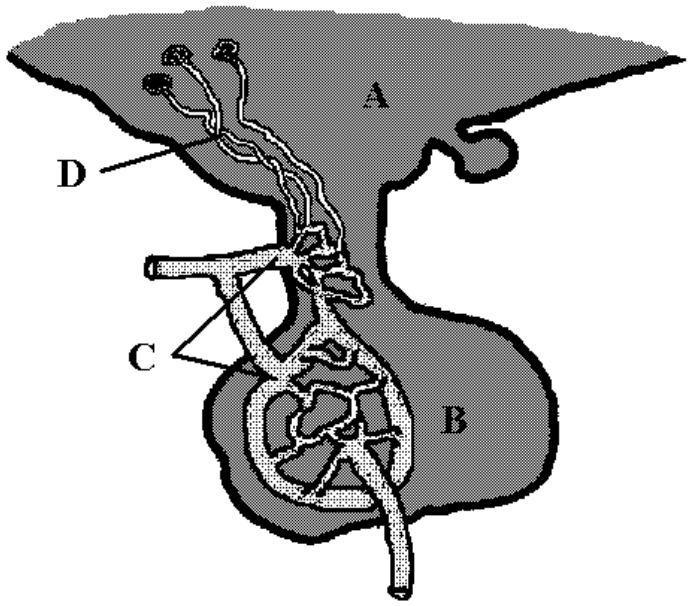

Figure 2-3. Anatomy of lower hypothalamus and pituitary pertaining to gonadotropin release. A, hypothalamus; B, pituitary; C, hypophyseal portal blood system; D, GnRH secreting neurons. Gonadotropin releasing hormone is synthesized in the hypothalamus and transported down its respective neurons via neurophysin. The neurons synapse with the blood vessels of the hyposeal portal blood system, and release GnRH into this specialized capillary bed. Blood laced with GnRH then circulates directly to and throughout the anterior pituitary and causes release of gonadotropins. Adapted from Greenspan and Strewler (1997).

Estrogens and P_4 can exhibit either a stimulatory or inhibitory effect on LH release; this relationship is thought of as the classical control mechanism. Luteinizing hormone response is largely dependent upon the immediate steroid surroundings. Increased production of E from the follicles, especially the dominant follicle, is responsible for initiating the cascade of events leading to the LH surge (Knobil, 1974; Legan et al., 1975; Fink, 1979a). Release of GnRH occurs daily throughout the cycle (Legan et al, 1975; Henderson et al, 1977a; MacKinnon et al., 1978;), but only at sub-threshold levels of what is required to induce the LH surge. It has been demonstrated that high circulating concentrations of E is required for occurrence of the natural LH surge and subsequent ovulation (Sarkar et al., 1976; Sherwood et al., 1980; and Ching, 1982). Sarkar and Fink (1979a) found ovariectomized rats did not experience a release of GnRH at the pituitary stalk. However, when estradiol

benzoate was administered, the GnRH release occurred as normal. Ovariectomized cattle treated with E implants have higher blood mean LH concentrations than non-treated cows (Crister et al., 1983; Day et al., 1986; Stumpf et al., 1988b; Kinder et al., 1991). Kinder et al. (1991) reported these elevated LH levels were a result of increased LH pulse amplitude. Estrogen levels are critical for LH pulse activity. During periods when E is low, LH pulse frequency is low, when E is high, frequency of LH pulses increases. Thus, background E enhances the pituitary's responsiveness to GnRH. Several laboratories have demonstrated that at first E exerts a negative influence on GnRH secretion, but after 8 to 12 h the effect is positive (Vilchez-Martinez et al., 1974; Cooper et al., 1974; Henderson et al., 1977b). Vilchez-Martinez et al. (1974) demonstrated the biphasic response in rats. Two to 9 h after pretreatment with 20 μg estradiol benzoate, administration of GnRH did not increase serum LH concentration. After 14 h, however, administration of estradiol benzoate caused serum LH concentration to be elevated. Cooper et al. (1974) reported rats that where treated for 3 h with E demonstrated an inhibitory effect on LH secretion. However, after 9 h continuos E administration the pituitary responsiveness was enhanced (Cooper et al., 1974). When E concentration was elevated above physiological levels, a biphasic effect was also observed in cows (Kesner et al., 1981; Butler et al., 1983). Luteinizing hormone pulse frequency was enhanced by increasing serum E concentration which caused the LH surge prior to ovulation (Stumpf et al., 1989, 1991; Cupp et al., 1995). Normal physiological levels of E during the follicular phase modulated LH secretion through increasing LH pulse amplitude (Day et al., 1986; Stumpf et al., 1988a, 1989; Kinder et al., 1991). Wolfe et al. (1992) showed that when E was administered at levels similar to that which is found during late gestation frequency and amplitude of LH release was retarded.

Scaramuzzi et al. (1971) determined that high levels of circulating P_4 hindered the positive feedback effects of E in ovariectomized ewes. In the early luteal phase P_4 concentration was low and LH pulse frequency was elevated over mid-luteal levels (Rahe et al., 1980; Peters et al., 1994). Research from several laboratories have established that when natural P_4 levels are high, such as during the luteal phase, E can not induce an LH surge in the ewe (Bolt et al., 1971; Symons et al., 1973). Short et al. (1979) demonstrated that a LH surge similar to the preovulatory surge is not possible during the luteal phase due to elevated P_4

levels. Roberson et al. (1989) demonstrated that cows treated with P_4 at levels found during the mid-luteal phase showed a larger interval between LH pulses (i.e. reduced frequency). When P_4 levels are reduced, such as during the follicular phase (Bolt et al., 1971) or anestrus (Symons et al., 1973), E effectively elicits an LH surge. Yuthasastrakosol et al. (1974) and Howland et al. (1978) showed that P_4 prevents positive feedback on GnRH centers even when E is administered in supra-pharmacological doses (4 mg estradiol benzoate) to ewes. Scaramuzzi et al. (1971) and Jackson et al. (1975) injected E into ovariectomized ewes that had and had not been treated with P_4 prior to injection and found the magnitudes of LH surges were similar. Research from several independent laboratories have that shown that P_4 blocks the E induced LH surge in cattle (Bolt et al., 1971; Short et al., 1973; and Kesner et al., 1981, 1982). Bergfeld et al. (1995) administered large and small doses of P_4 to cattle and observed that when the treatments were exchanged, the frequency of LH release was dramatically affected during the first 6 h after treatment.

Karsch and Foster (1975) and Paint (1977) also found that repeated injections of E over the course of several d had the ability to elicit LH surges upon each injection, thus P_4 has no role in preventing refractoriness of the LH surge (Martin, 1984). Martin et al. (1984) found small doses of E and P_4 have little effect on frequency of LH pulses in ovariectomized ewes during the normal breeding season, however, when administered in combination an inhibitory effect on GnRH release became apparent (see Figure 2-4).

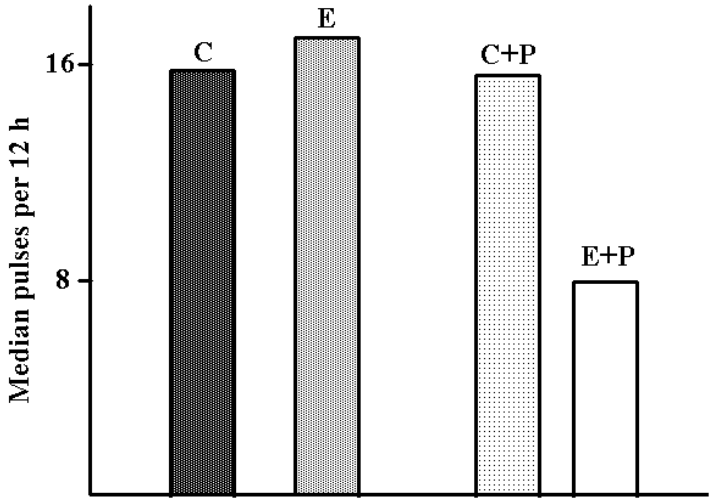

Figure 2-4. Median frequency of LH pulses in ovariectomized ewes during the breeding season as modulated by E and P_4. Ewes received subcutaneous implants designed to release either 3 to 4 μg E daily or 1.5 ng P_4 per d or E + P_4 combination. (C, non treated ewes, Martin et al., 1984)

Schams et al. (1977) showed mean concentrations of LH were constant the first week post-ovulation. However, after 11-13 d post-ovulation, concentrations are reduced (see Table 2-1, Cupp et al., 1995). Cupp et al. (1995) also reported that during the early luteal phase when P_4 concentration is reduced, LH pulse frequency is greater, and E concentrations are elevated as compared to the mid-luteal phase. The precise relationship between changing pattern of LH and pattern of follicular development is not understood (Rathbone et al., 2001). Cupp et al. (1995) supplied data on LH, FSH, P_4 and E_2 concentrations during the luteal phase of cows (see Table 2-1). During the process of luteolysis, and soon after, there was a greater serum LH concentration and increased LH pulsatile frequency resulting from reduced P_4 concentration (Imakawa et al., 1986; Cupp et al., 1995).

Table 2-1. Mean concentration of LH, FSH, E, and P_4 during the luteal phase of the bovine estrous cycle. Values with different superscripts within a column differ (P<0.05) Adapted from Cupp et al. (1995).

Day of cycle	LH amplitude ng/ml	LH frequency pulses/12hr	LH mean conc. ng/ml	FSH ng/ml	P_4 ng/ml	E pg/ml
4	.50^{b}	4.48^{b}	.70^{b}	1.56^{b}	3.38^{b}	7.97^{b}
5	.68^{bc}	4.88^{b}	.81^{bd}	1.86^{cd}	5.94^{c}	6.36^{bc}
6	.80^{bc}	4.11^{b}	.64^{bc}	1.53^{b}	7.49^{cd}	5.74^{c}
7	.65^{b}	2.68^{c}	.69^{b}	1.97^{c}	9.90^{c}	4.36^{cd}
8	1.28^{d}	4.00^{b}	.72^{b}	1.67^{ce}	8.82^{de}	4.79^{cd}
9	1.31^{d}	2.78^{c}	.79^{bd}	2.01^{d}	10.86^{e}	4.44^{cd}
10	1.04^{cd}	3.44^{bc}	.64^{bc}	1.66^{ce}	6.61^{e}	4.08^{cd}
11	1.09^{cd}	3.11^{bc}	.74^{bd}	1.72^{c}	12.21^{fg}	3.67^{de}
12	.66^{b}	2.89^{c}	.55^{c}	1.52^{b}	11.18^{eg}	3.72^{de}
13	.80^{bc}	2.78^{c}	.64^{bc}	1.56^{b}	13.08^{f}	2.73^{e}
14	.82^{bc}	3.89^{bc}	.60^{bc}	1.50^{b}	11.63^{fg}	3.88^{de}
15	.97^{bc}	4.56^{b}	.86de	1.63^{be}	12.15^{fg}	3.52^{de}
16	1.24^{d}	6.90^{d}	.99^{ef}	1.45^{b}	8.45d^{e}	5.30^{c}
17	1.03^{cd}	4.19^{b}	.94^{e}	1.69^{ce}	10.43^{e}	5.20^{c}
18	.71^{b}	4.47^{b}	.70^{b}	1.32^{b}	7.18^{c}	5.11^{c}
19	1.10^{cd}	7.57^{d}	1.29^{f}	1.62^{be}	3.16^{b}	10.89^{f}
P.S.E.	.20	.99	.09	.09	1.23	.80

Beck et al. (1976) and Stumpf et al. (1993) found P_4 given to ovariectomized cows caused reduced release of LH (see Table 2-2).

Table 2-2. Pulse frequency of LH in ovariectomized cows treated with E and P_4. Values with different superscripts differ (P > 0.05). Adapted from Stumpf et al. (1993).

Treatment	LH pulse frequency (pulses/h)
E	0.97 ± 0.07^{a}
P_4	0.52 ± 0.08^{b}
P_4 + E	1.40 ± 0.07^{c}

Sarkar and Fink (1979a) found that administration of P_4 12.5 h before the expected time of proestrus induced lower concentrations of LH in mice that had been pretreated with estradiol benzoate, and that the response was dose dependent. Progesterone may stimulate LH release but only when the pituitary has been exposed to E for several h (E priming; Fink, 1988). In the rat, P_4 switched from an inhibitory to a stimulatory role for LH secretion at the initiation of proestrus (Aiyer and Fink, 1974; Nishizuka et al., 1984). Rats injected with 2.5 mg P_4 at 10 h of metestrus then followed the next d with two injections of GnRH spaced 1 h apart showed increased LH release (Aiyer and Fink, 1974). The second GnRH injection caused LH release that was significantly greater than the first. This effect is likely due to GnRH receptor self up-regulation.

The responsiveness of the pituitary to GnRH increases dramatically just prior to and during the natural LH surge (Eskay et al., 1977), and this effect has been demonstrated in several species including rat, hamster, sheep, and humans (Fink, 1979a). Without this 20 to 50 fold up regulation in responsiveness, the full LH surge necessary for ovulation would not be obtainable (Fink et al., 1982). The increased responsiveness of the pituitary to GnRH may be due to GnRH self-priming or to the stage of the estrous cycle, likely correlated with estradiol 17-β concentrations (Aiyer et al., 1974).

The termination of the LH surge is due primarily to a decline in portal plasma GnRH concentration (Sarkar et al., 1976 Sherwood et al., 1980). Sarkar et al. (1976) demonstrated that the LH surge occurs shortly after the GnRH surge, and afterward plasma LH concentrations fall in direct correlation with GnRH. Blake (1976) suggests GnRH may stimulate a decline in pituitary responsiveness.

Several man-made agents are known to block the LH surge by hindering GnRH release. Sherwoood et al. (1980) found the naturally occurring LH surge was blocked in rats that were under the influence of the anesthetics alpha-chloralose, ketamine hydrochloride, and urethane. Several other anesthetics including alphaxalone and alphadolone acetate have been shown not to affect the LH surge in rats (Sherwood et al., 1980). There are several other known regulators of LH release other than the classical mechanisms of GnRH, P_4, and E and shall be discussed here briefly.

Catecholamines

Continuous infusion of 0.3 or 1.8 μg of norepinephrine into the jugular vein of rats decreased the firing rate of the LH pulse generator, and reduced LH secretion while not affecting pulse amplitude (Gallo, 1984). Desensitization to the negative effects of norepinephrine had not occurred after 20 continuous h of administration. Leipheimer et al. (1985) also found a decrease in pulse frequency of LH in ovariectomized rats after norepinephrine administration, in addition to have demonstrated by push pull perfusion the effect is mediated partly at the medial preoptic area. Norepinephrine is released from the medial preoptic nucleus in a pulsatile manner, but there is no known correlation between LH and norepinephrine pulsatility (Jarry et al., 1986). When norepinephrine access to the hypothalamus was blocked in the monkey, pulsatile LH release was not altered (Krey et al., 1975). However, Kordon et al. (1972) has reviewed several studies that demonstrate catecholamines containing neurons, including norepinephrine and epinephrine, are involved in the propagation of the ovulatory LH surge. Hardin and Randel (1983) showed that the magnitude of LH release was reduced by epinephrine injection, and area under the LH curve was reduced by norepinephrine in prepuberal beef heifers. Molnar and Barraclough (1993) demonstrated norepinephrine amplified LH release in the rat.

Li (1989) incubated isolated swine pituitary cells with epinephrine, norepinephrine and L-isoproterenol (a nonselective beta-agonist) and found that GnRH stimulated LH secretion was reduced after 30 min. Propranolol (beta-antagonist) reversed the inhibitory effect of epinephrine (Li, 1989). Meyer and Goodman (1986) concluded that steroid dependent and independent actions of the ovine anestrus period are mediated through catecholaminergic and serotonergic neurons. They found dopaminergic (pimozide and fluphenazine) and alpha-andernergic (phenoxybenzamine and clonidine) antagonists increased circulating LH levels in anestrus ewes, while agonist for dopaminergic (apomorphine) and alpha-adrenergic (clonidine) receptors retarded LH secretion (Meyer and Goodman, 1986). Pimozide and phenoxybenzamine (catecholamines) did not increase LH pulse frequency in these ovariectomized ewes during the anestrus period (Meyer and Goodman, 1986). Shahab et al. (1993) demonstrated that when N-methyl-D-aspartate (NMDA, and excitatory amino acids) are injected into Holstein bull calves an acute release of LH followed. Estienne et al.

(1989) demonstrated that NMDA reduced LH concentrations in wethers 2 d post injection. Popwell et al. (1996) reported that excitatory amino acids inhibit and stimulate LH secretion in swine. Estienne et al. (1998) reported NMDA decreased LH pulse frequency in ovariectomized guilts, however, no effect was detected in intact guilts (Estienne et al., 1995).

Serotonin

Spontaneous ovulation in rats was suppressed when hypothalamic levels of serotonin were elevated (Labhsetwar, 1972). Ovulation, the LH surge, and estrus behavior were suppressed when serotonin was infused into the medial basal hypothalamus in sheep (Domanski et al., 1975). Przekop et al. (1975) infused serotonin into the third cerebral ventricle of 24 rabbits of which 15 failed to ovulate and form a CL. Hery et al. (1976) discovered that serotonin producing neurons in the suprachiasmatic nucleus play an important role in the control of cyclic LH secretion. When serotonin synthesis was blocked in rats, the normal rise in LH during proestrus did not occur (Hery et al., 1976), and ovulation failed (Labhsetwar 1972).

Estrogen can modulate the effects of serotonin. Becu de Villalobos et al. (1984) treated female E primed rats with 12.5 μg estradiol benzonate twice a wk for 2 wk. Afterward the rats responded with LH release in a dose dependent manner after administration of 2.5 or 5 mg/kg of serotonin creatinin sulfate. Female rats, which were not E primed, did not respond. Thus, administration of synthetic serotonin induced LH release under the proper steroid influence. These findings are supported by evidence that hypothalamic levels of serotonin peak near the time of the natural LH surge (Hery et al., 1982). Leonardelli et al. (1974) concluded after research with guinea pigs that the reduction in LH concentration induced by serotonin is a not a result of synthesis, but rather decreased release.

g-aminobutyric acid (GABA)

γ-aminobutyric acid (GABA) or its receptor agonist have been found to inhibit LH pulsatile release in the rat (Fuchs et al., 1984; Lambert et al., 1984). Release of GABA has been demonstrated to be inversely correlated with circulating LH levels (Demling et al., 1985; Jarry et al., 1988). Some studies indicated GABA neurons may direct E negative feedback

influence on LH release. Jarry (1986) found that elevated levels of E influenced GABA pulsatility. Shivers et al. (1983) reported that GnRH neurons were not direct targets for E action. Thus, there was an unknown component which mediated the actions of E. Flugge et al. (1986) demonstrated that E-receptive neurons of GABAergic nature existed in the medial preoptic/anterior hypothalamic area. Demling and colleagues (1985) further concluded these neurons might interact with terminals of the catecholaminergic systems and cause the release of catecholamines, which may affect activity of the GnRH neuron network. Studies have also demonstrated that GABA producing neurons may also directly influence GnRH release. Leranth et al. (1985) showed GABAergic neurons synapse directly on GnRH neurons, and may modulate activity of GnRH producing cells.

Histamine

Luteinizing hormone concentrations are elevated after histamine is injected into the cerebral ventricles of ovariectomized E primed rats (Libertun and McCann, 1976). Donoso (1978) administered 5 μg histamine in the third ventricle of the brain of female rats. An increased concentration of LH in proestrus rats, but not at any other stage of the estrous cycle (Donoso, 1978). However, i.v. administration of histamine had no effect (Libertun and McCann, 1976). Knigge et al. (1984) administered 50 μg histamine systematically to 10 men. There was an augmentation of the GnRH response, however, LH nor FSH secretion were altered. It was unclear how histamine mediates its effects on GnRH and LH release.

Follicular Dynamics, Waves and Dominant Follicles

Ginther et al. (1989a, b, 2001) described follicular waves in detail and several other reviews are available (Roche et al., 1991; Driancourt et al., 1991; Lucy et al., 1992; Fortune et al., 1994; Campbell et al., 1995). The initiation of the first follicular wave of the cycle is distinguishable when a cohort of 4 mm follicles have emerged and continue growth (recruitment). A few d after recruitment, one of the follicles achieves dominance while the remainders become subordinate (selection). The dominant follicle may progress to the ovulatory stage, or may experience atresia depending on the immediate steroidal environment. The second wave emerges around 10 d post-estrus, and if a cow experiences three waves, the

final emergence will occur around d 16. The mechanism by which one follicle achieves dominance over its subordinates is poorly understood (Ginther et al., 1996).

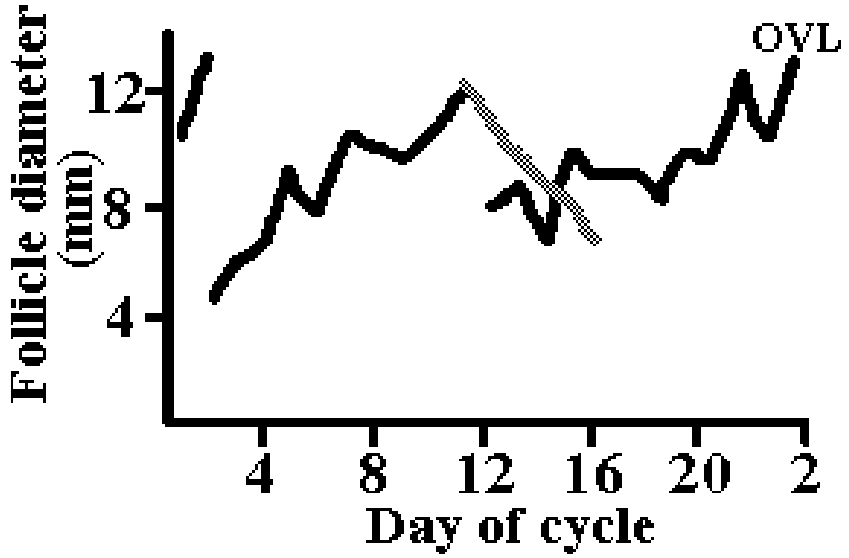

Figure 2-5. Follicular Dynamics. The growth, atresia and ovulation of the dominant follicle during the estrous cycle. Ovulation occurs 2 d post-estrus. The gray line depicts a follicle that underwent atresia. (OVL = ovulation, Rajakoski, 1960)

Follicular wave phenomenon was demonstrated over forty years ago, using Swedish Red and White breeds of cattle (Rajakoski, 1960). Researchers concluded that the cow experienced two such follicular waves over the course of the estrous cycle by examining ovaries postmortem (see Figure 2-4, Rajakoski, 1960). Histological evidence later revealed that three rather than two waves occurred during the cycle, and that each of these waves produced one dominant follicle (Ireland and Roche, 1983). When ultrasound technology became available, many laboratories utilized this technique to resolve the questions of how many follicular waves occur. Pierson and Ginther (1984) judged ultrasound an effective tool

for monitoring and evaluating ovarian follicles and CL in normal and superovulated heifers. Pierson and Ginther (1984) reported that locating the reproductive structures was not difficult after several practice sessions, however, a clear image was not always obtainable. Cattle were evaluated trans-rectally and for best results all fecal material needed to be removed from the rectum to allow complete contact between the transducer and rectal wall. The instrument was deemed practical after ultrasound data was consistent with reports generated by marking structures with India ink and examination postmortem. Utilizing ultrasound technology, Ginther et al. (1989b) found 81% of cattle scanned exhibited two follicular waves per cycle, the remainder experienced three waves. Other investigators found a predominance of cattle with three follicular waves. Savio et al. (1988) found 81% of their herd had three dominant follicles per cycle, 15% had two, and the remaining 4% had one. When all data were combined, it was concluded that over 95% of cows experience either 2 or 3 follicular waves.

Some cattle of *Bos indicus* origin may have a total of four waves per cycle. Rhodes et al. (1995) studied 17 Brahman heifers using ultrasound. The dominant follicle and CL of these heifers was smaller than *Bos taurus* breeds, but the overall pattern of development was similar. Also, one heifer on this study experienced four follicular waves per cycle (see Figure 2-6). Zeitoun et al. (1996) reported that cattle of *Bos indicus* origin that experienced four waves per cycle also experienced a longer estrous cycle. However, most of these four wave cycles were the result of extended interovulatory intervals resulting from delayed luteolysis or ovulation failure (Ko et al., 1991; Adams et al., 1992a). Ko et al. (1991) induced a four wave cycle in a heifer by performing a cautery on the dominant follicle on d 3 (See Figure 2-7).

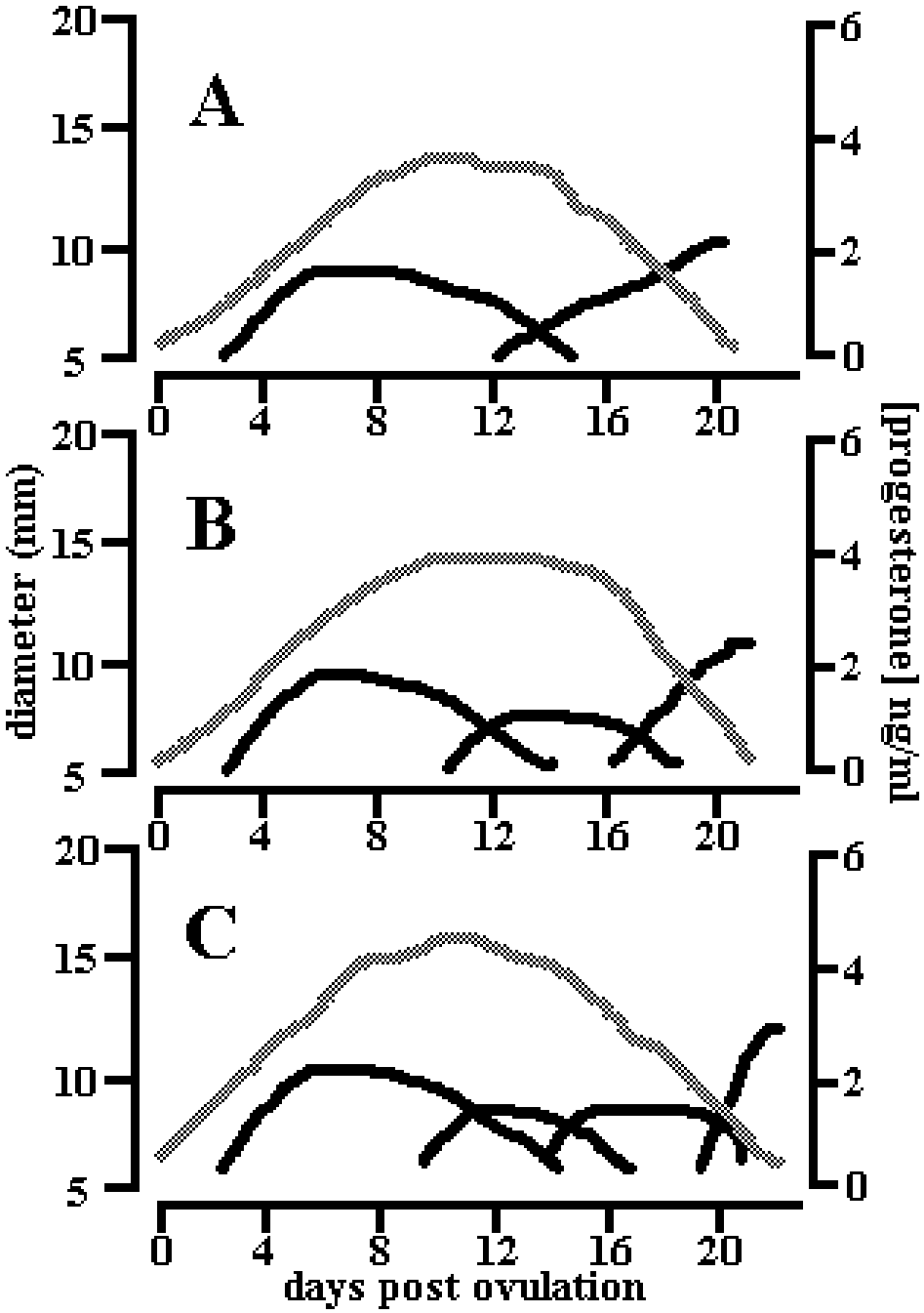

Figure 2-6. Follicular Dynamics. Depicted is data from a two wave; A, three wave; B, and four wave; C cows. The solid line represents the dominant follicle diameter, the gray line designates plasma P_4 concentrations (Rhodes et al., 1995).

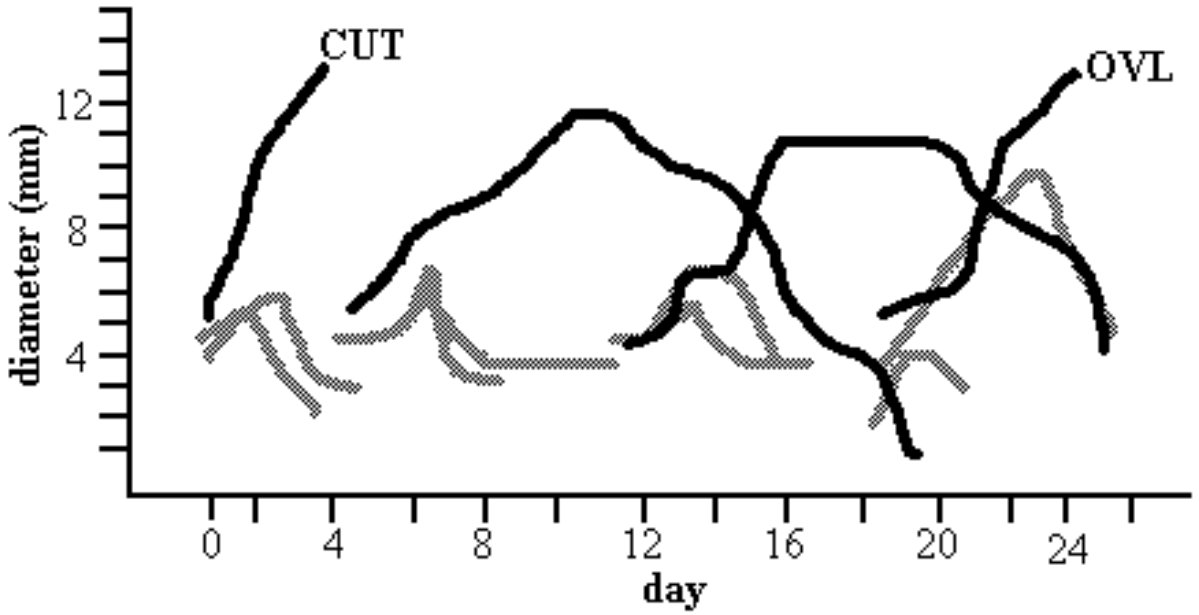

Figure 2-7. Induced four follicular wave cow. Depicted are diameters of largest follicles from pretreatment ovulation to post treatment ovulation. A cautery was performed on d 4 (CUT), the next ovulation occurred on d 24 (OVL, Ko et al., 1991).

Adams et al. (1992b) found that heifers treated with P_4 on d 0-5 had smaller dominant follicles (12.7 ± 0.9 vs. 15.3 ± 0.7 mm) than non-treated heifers. They found that P_4 treatment did not suppress circulating FSH levels, but the second FSH surge occurred earlier. Adams et al. (1992b) also found heifers supplemented with P_4 (30 mg/d) had continued growth and maintenance of the dominant follicle during d 6 through 20 of the cycle.

From these experiments it can be concluded a two-wave cycle cow experienced a shorter estrous cycle duration (20 d) than did a three wave cow (23 d). The 21 d cycle existed only as an average (Adams and Mapletoft, 1998).

Murphy et al. (1991) found heifers (Fresian x Herford) fed low levels of dry matter had similar diameters of dominant follicles (11.8 ± 0.1 mm) than did cows fed intermediate (13.7 ± 0.2 mm) or adequate (13.2 ± 0.3 mm) levels of nutrition. Growth rate was not affected, but persistence of the dominant follicle was shorter in low (9.8 ± 0.2 d) compared to intermediate (11.9 ± 0.3 d) or adequate (12.7 ± 0.4 d) dry matter intake (Murphy et al., 1991). The mechanism for this action is not well understood. Lucy et al. (1992) fed lactating dairy

cows supplemental energy and found cows had fewer small follicles (3 to 5 mm) and more large follicles (6 to 9 or >15 mm). Thus, supplementing energy needs and lactational status may play a role to aid in follicular growth.

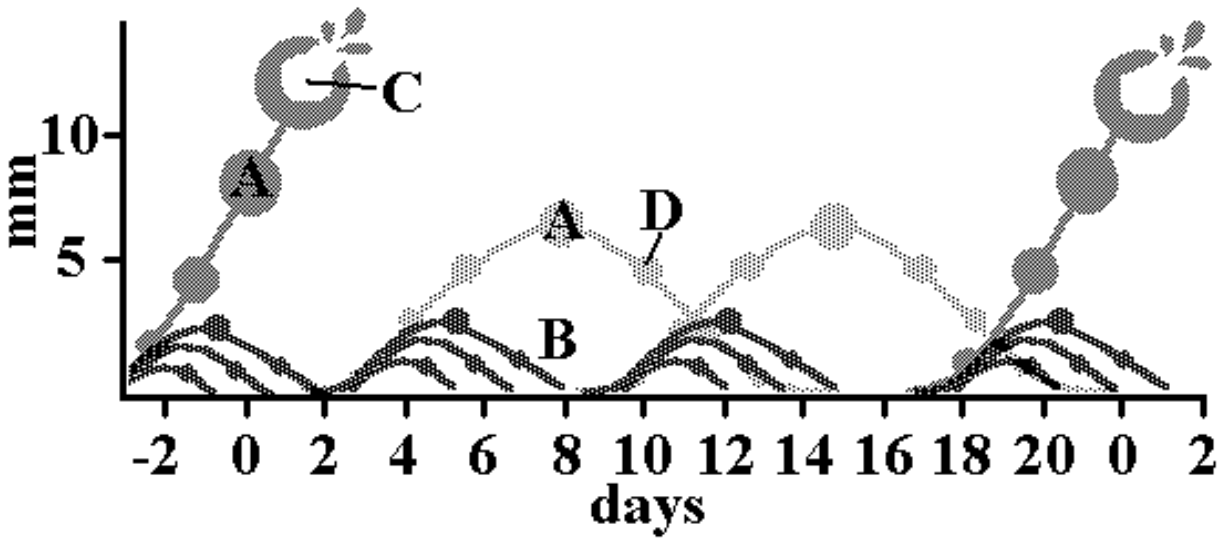

Figure 2-8. Follicular wave. The follicular wave depicted represents a three wave cow.

Wave Stimulation

Several independent laboratories have established that in the perpubertal animal follicles grow and regress comparable to mature animals (Roche et al., 1991; Evans and Rawlings, 1993; Hopper et al., 1993; Evans et al 1994; Melvin et al., 1999). Evans and Rawlings (1993) demonstrated that 2 wk old heifers experienced follicular waves. Adams et al. (1992a) showed a relationship between FSH surges and follicular wave emergence. Two and 3 wave heifers had respectively 2 and 3 apparent FSH surges during the inter-ovulatory interval. These surges occurred 2 to 4 d before detectable emergence of a follicular wave and decreased near the time when the follicles began to diverge into dominate and subordinates (Adams et al., 1992a). This relationship has been confirmed by several other independent laboratories (Sunderland et al., 1994; Gong et al., 1995; Bodensteiner et al., 1996).

The factors which precisely govern regulation of circulating FSH concentrations during a FSH surge are not understood (Ginther et al., 1996). Inhibin may modulate FSH activity during the FSH surge (Kastelic et al., 1990b; Turzillo et al., 1993; Kaneko et al.,

1995). Kaneko et al. (1995) injected inhibin antiserum into cows and found increased plasma concentrations of FSH. Inhibin antiserum also induced the growth of several small (≥ 4 < 7 mm in diameter), medium (≥ 7 < 10 mm), and large (≥ 10 mm) follicles in cows (Kaneko et al., 1995). Martin et al. (1991) found that intercellular concentrations of inhibin in cows decreased during the growth phase of dominant follicles, but increased during the same phase in non-ovulatory follicles. Therefore, inhibin may play a role in the growth and atresia of dominant follicles throughout the estrous cycle (Martin et al., 1991; Guilbault et al., 1993; Ireland et al., 1994). However, the timing of inhibin release from dominant follicles is not precisely understood (Ginther et al., 1996).

Steroids may be involved in inhibin release since low levels of E are known to be present in small follicles (5 mm, Echternkamp et al., 1994), and pharmacological doses of E caused a suppression in FSH secretion (Bolt et al., 1990; Bo et al., 1993). Bo et al. (1993) showed that the mean d of maximum FSH concentration occurred earlier in heifers treated with 5 mg estradiol valerate on the d after ovulation rather than 3 or 6 d later. Alternately, Ginther et al. (1996) reported that FSH surges might occur with an inherent rhythm, and occur with frequency of every 5.5 d.

Melvin et al. (1999) showed that the size of the dominant follicle increased as onset of puberty approached. The period of maximal follicular size increase occurred 30 d pre-puberty, when LH pulses increased in frequency, (Melvin et al., 1999). According to McDougall et al. (1995), the size of the dominant follicle increased as the end of anestrus postpartum period approached. Rathbone et al. (2001) proposed increased LH pulse frequency was responsible for large dominant follicle development.

Follicular dominance

Current theory on follicular dominance states that every follicle has the potential to achieve dominance and to suppress the development of subordinate follicles. Adams et al. (1993a) supplemented cattle with FSH injections, and found all follicles in the cohort present at injection were able to achieve dominant follicle diameter. Exogenous FSH is used to induce superovulation to provide a large number of embryos for use in embryo transfer procedures. Gibbons and coworkers (1996) used ultrasound-guided ablation to pre-select a

dominant follicle at random by removing all other members of its cohort. Research from this laboratory demonstrated that any follicle has the potential, and can be forced to become the dominant follicle. Ko et al. (1991) and Adams et al. (1993b) destroyed the dominant follicle of a cohort and showed that a subordinate follicle may inherit dominance if deviation had occurred within two d. Adams et al. (1992a) cauterized the dominant follicle three d before expected ovulation and found that the largest subordinate follicle reached a larger diameter than controls (11.7 vs. 8.0 mm), and reached maximum diameter later (9.2 vs. 3.1 d). The emergence of the secondary wave is also accelerated (6.4 vs. 9.3 d, Adams et al., 1992a).

Role of FSH and LH in production of dominant follicles

The suppression of FSH allows for the deviation and emergence of a single dominant follicle in cattle. During the deviation process, the dominant follicle switches from FSH- to LH-dependency for continued growth (Hansel and Convey, 1983). There is an abundance of evidence that the dominant follicle acts to suppress its subordinates. The dominant follicle may secrete factors that directly inhibit subordinate follicles since follicular fluid obtained from dominant follicles administered systematically slowed follicular growth (Ginther et al., 1996). Law et al. (1992) injected Herford x Friesian heifers with bovine follicular fluid, that was devoid of inhibin, which failed to suppress peripheral FSH concentrations and follicular development. Wood et al. (1993) reported similar results using similar techniques and concluded that non-steroid factors in bovine follicular fluid were responsible for delayed ovulation. When the dominant follicle was removed 3 d after ovulation an immediate surge in FSH was observed (Adams et al., 1992a). Adams et al. (1992a) injected FSH for 2 d after the dominant follicle reached 6 mm in diameter and deviation was postponed 2 d.

After achieving dominance, the follicle is no longer dependent on FSH for growth, but rather switches to LH dependence. Several studies point to the importance of both LH and P_4 for continued dominant follicle maturation (Sirois and Fortune, 1990; Fortune et al., 1991; Adams et al., 1992b; Smith and Stevenson, 1995). Sirois and Fortune (1990) used vaginal P_4 releasing devices, designed to deliver 0.9 to 2.1 ng/d, and found prolonged growth and a 1.4 fold greater size of the ovulatory follicle. Gong et al. (1995) injected 5 μg of buserelin (a GnRH analog) to heifers twice a d for 3 wk. After ovulation the dominant follicle of the new

follicular wave did not obtain a diameter greater than 7 to 9 mm. The authors concluded LH pulsatile secretion was blocked and thus LH must be necessary for post-deviation development. In agreement, Fortune et al. (1991) showed life span of the dominant follicle could be extended by artificially increasing LH pulse frequency.

Role of estrogen in production of dominant follicles

Guilbault et al. (1993) demonstrated that dominant follicles experiencing the growing phase of development were E active. However, during the regressing phase dominant follicles were histologically atretic and E inactive. Ireland et al. (1984) showed the utero-ovarian vein in cows with the greatest E content during the LH surge corresponded to the ovary that produced maximal P_4 concentrations after the LH surge. Ireland et al. (1984) also demonstrated the utero-ovarian vein carrying the greatest concentrations of E 1 to 24 h after $PGF_{2\alpha}$ injection corresponded to the ovary with maximal E production during estrus.

Persistent follicles

Several independent laboratories (Roberson et al., 1989; Sirois and Fortune, 1990; Kojima et al., 1992, 1995; Sanchez et al., 1993, 1995; Wehrman et al., 1993) have reported cattle that were treated with progestins for estrus synchronization had elevated E blood levels greater than what is normally found during the luteal phase. Rathbone et al. (2001) speculated that increased E levels may be due to a persistent follicle. Elevated E levels during this period are likely due to increased frequency of LH pulses (Roberson et al., 1989; Kojima et al., 1992, 1995; Savio et al., 1993a), and are likely similar to the follicular phase (Rathbone et al., 2001).

Persistent follicles reduce fertility

When typically used commercial doses of synthetic progestins, specifically, melengestrol acetate (MGA) or norgestomet, were used for estrus synchronization, pregnancy rates were reduced compared to administration of levels two to three times greater (Savio et

al., 1993b; Wehrman et al., 1993). Mihm et al. (1994), reported that as the time a persistent follicle existed increased (over 4 d), pregnancy rate declined. Wehrman et al. (1996) reported oocytes exposed to increased amounts of E for longer periods times than normal, and exposure of the uterus, may be the mechanism of reduced fertility.

Another theory regarding reduced fertility associated with persistent follicles involves LH. The first meiotic division of an oocyte is induced by the LH surge during estrus (Rathbone et al., 2001). Revah and Butler (1996) proposed that the oocyte in persistent follicles may be exposed to an increased frequency of LH pulses which induces start of the first meiotic division prematurely. Wehrman et al. (1996) reported that conception rates are similar for normal and persistent follicles, however, in the latter early embryonic death occurred at a greater frequency.

Manipulation of Reproductive Events

In the recent past, there has been an abundance of research conducted regarding control of reproductive events in cattle. Several review articles are available on the subject (Odde, 1990; Larson and Ball, 1992; Seguin, 1997; Wiltbank, 1997; Roche et al., 1997). The number of reviews reflects the level of interest for the subject. Recent research advances, primarily the use of ultrasound (Pierson and Ginther, 1984), have brought new insight for the mechanisms involved in normal ovarian function. These advances allowed for novel ideas concerning reproductive function to be implemented. With this new information, researchers have devised revolutionary ways of approaching the task of estrus synchronization.

Production Estrus Synchronization Protocols

Modern estrus synchronization protocols involve either lengthening or shortening the animal's estrous cycle to achieve synchrony. A variety of techniques are available for producers to utilize, and all are based on several strategies of hormonal supplementation including progestins or P_4, $PGF_{2\alpha}$, gonadotropins, and E, as well as follicle ablation. Several reviews are available for dairy and beef cattle synchronization options (Odde and Holland, 1994; Ryan et al., 1995; Kinder et al., 1996). Early methods of estrus synchronization have been reviewed by Hansel and Beal (1979).

Progesterone

Melengestrol acetate (6alpha-methyl-6dehydro-16methylene-17alpha-acetoxy-preng-4,6-diene-3,20-dione; Pharmacia Upjohn, Kalamazoo, MI) is one of the most commonly used synthetic progestin sources for estrus synchronization (Odde, 1990). It was first commercially available to improve rate of gain in feedlot heifers (Zimbelman and Smith, 1966a, b; Bloss et al, 1966; Newland and Henderson, 1966; Zimbelman, 1966; O'Brein et al., 1968; Young et al., 1969; Purchas et al., 1971). Several laboratories confirmed that MGA suppressed estrus when orally administered (Zimbelman and Smith, 1966; Roussel and Beatty, 1969; DeBois and Bierschwal, 1970; Randel et al., 1972). It has been known for several years that the level of MGA supplemented is related to the time of observed estrus upon withdraw (Zimbelman and Smith, 1966a). Cattle which received lower levels of MGA expressed estrus activity sooner upon withdraw (Zimbelman and Smith, 1966b; Hill et al., 1971; Randel et al., 1972).

Reports indicate that fertility rates are decreased after long term exposure to progestogens (Wiltbank et al., 1967). Guthrie et al. (1970) and Lamond et al. (1971) demonstrated follicular growth and an increase in atretic follicles after MGA treatment. Hawk (1971) showed sperm transport was altered in the ewe after artificial insemination and P_4 treatment. Rates of cleavage are also reduced when embryos are exposed to pharmacological doses of P_4 (Wishart and Young, 1974).

Christian and Casida (1948) injected 25 and 50 mg of P_4 daily to yearling heifers starting on d 14 of their estrous cycle. After 14 d treatment the higher level of P_4 suppressed estrus and prevented ovulation in these heifers (Christian and Casida, 1948). Heifers treated with 50 mg P_4 experienced estrus 5 to 6 d after treatment had ceased, and all subsequent estrous cycles were of normal duration (Christian and Casida, 1948). However, 25 mg P_4 did not suppress estrus in all heifers (2/4), and the researchers concluded this dose appeared to be close to the threshold of concentration needed to prevent ovulation in yearling heifers. Savio et al. (1993b) treated non-lactating Holstein cows with 6 mg norgestomet implants (synthetic progestin) d 8 through 23 of the estrous cycle, and found high concentrations of P_4 retarded LH pulse frequency which gives rise to turnover of the dominant follicle. Adams et al.

(1992b) also concluded that P_4 inhibited the dominant follicle in a dose dependant manner but did not affect FSH secretion. Thus, P_4 prevents ovulation, but does not have an affect on the emergence of follicular waves. The recommended dose of P_4 used in synchronization protocols is less than normal physiological levels during the luteal phase. Lower than physiological levels of P_4 resulted in development of oversized follicles of which some persisted and contributed to development of follicular cysts (Odd, 1990). Due to these potential problems few regimens are currently used which rely solely on P_4.

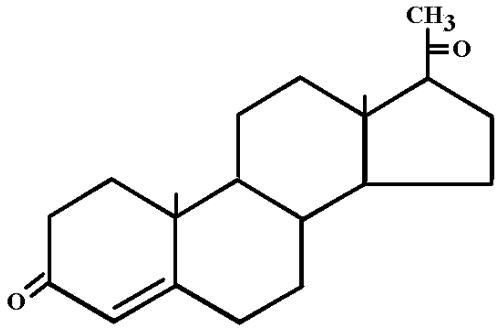

Figure 2-9. Structure of progesterone. Progesterone is a cholesterol derivative. Cholesterol is reduced to pregenolone. Progesterone is formed via the delta-4 pathway from pregenolone.

Progesterone Regimens

Cattle were efficiently synchronized by feeding MGA for 14-18 d at 0.5-1mg per cow with expected estrus 3 to 7 d after discontinuing treatment (Zimbelman and Smith 1966a; Roussel et al., 1969; De Bois and Bierschwal, 1970; Zimbelman et al., 1970; Wettemann et al., 1971). Wiltbank and Kasson (1968) and Roche (1974b, 1976) found that cattle treated with progestogens for less than 14 d did not have reduced conception rates. Many protocols involving MGA and additional pharmacological agents including estradiol (Smith and Zimbelman, 1968b), estradiol cypionate (Smith and Zimbelman, 1968a, b), gonadotropins (Smith and Zimbelman, 1968b, c), human chorionic gonadotropin (Smith and Zimbelman,

1968b, c; Roche and Crowley, 1973), and pregnant mare serum gonadotropin (Smith and Zimbelman, 1968b) were affective for estrus synchronization. Roche (1976) created an estrus synchronization protocol utilizing P_4 releasing intrauterine devices (PRID). The PRIDs were inserted for 12 d with injections of 5 mg estradiol benzonate and 50 mg P_4 at time of insertion. Fertility using this protocol were similar to untreated cows (Roche, 1976; Hansel and Beal 1979; and Roche et al., 1981). Another device, controlled intervaginal drug releasing devices (CIDRs) which are similar to PRIDs, are commercially available and are used mainly for dairy herd synchronization (Rathbone et al., 2001).

Progesterone implants can be used as a delivery vector. A useful protocol is depicted in Figure 2-10.

Figure 2-10. A progesterone Synchronization Protocol. All cows receive P_4 containing implants on d 0. Seven d later the implants are removed, and cows are inseminated at estrus detection. The majority of cows that respond to this treatment exhibited estrus on d 11 (62%, Macmillan and Peterson, 1993).

Prostaglandin

McCracken (1972) demonstrated that $PGF_{2\alpha}$ is the natural luteolysin in sheep. Several other laboratories have demonstrated $PGF_{2\alpha}$ is the natural luteolysin in cattle (Rowson et al., 1972; Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972) and since that time it has become the most commonly used pharmacological agent for estrus synchronization in cattle (Inskeep, 1973; Odd, 1990; Larson and Ball, 1992). Prostaglandin $F_{2\alpha}$ causes luteolysis and in doing so removes the negative feedback influence of P_4 on GnRH secretion. Induced

luteolysis is the oldest means of effectively manipulating the estrous cycle (Teige and Jakobsen, 1956). Inskeep (1973) reported that $PGF_{2\alpha}$ administered in the early stages (d 5 and 6) of the estrous cycle in cattle was not as effective in inducing luteolysis as later administration. It was speculated that early administration was not affective because $PGF_{2\alpha}$ receptors were not yet present on the CL. This theory was discredited by Wiltbank et al. (1995). Wiltbank et al. (1995) removed ovaries from heifers on d 2, 4, 6, and 10 of the estrous cycle and found high-affinity $PGF_{2\alpha}$ receptors present at all d sampled. Wiltbank (1997) later speculated the 4 to 6 d CL established a positive feedback loop for intraluteal P_4 production after exogenous $PGF_{2\alpha}$ treatment.

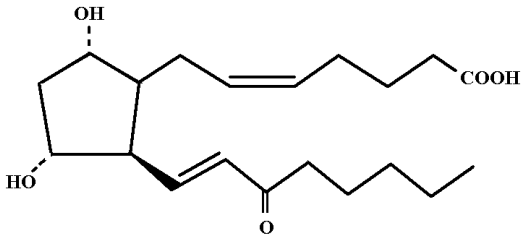

Figure 2-11. Structure of prostaglandin F_{2a}.

Nearly 43% of treated cattle respond to treatment with $PGF_{2\alpha}$ (Burfenings et al., 1978). Of cattle that respond, the resulting estrus was scattered over a 6 d period for the entire herd (Seguin, 1987). Tanabe and Hann (1984) treated dairy heifers on d 7, 11, and 15 of their cycle with 25 mg dinoprost and found estrus occurred with frequency 86.0%, 90.0%, and 98.0% respectively. However, estrus occurred within a 48 h interval for heifers treated on d 7, but required 72 h for d 11 and 15 (Tanabe and Hann, 1984). Much of the variability associated with $PGF_{2\alpha}$ is due to follicular status and stage of estrous cycle at treatment (Kastelic and Ginther, 1990a, 1991; Savio et al., 1990).

Prostaglandin Regimens

Many protocols involving exogenous administration of $PGF_{2\alpha}$ have been developed (Cooper, 1974; Roche, 1974a). The normal treatment regimen for $PGF_{2\alpha}$ consists of two injections spaced 10 to 14 d apart (see Figures 2-12 and 2-13). The theory behind this treatment is at least one injection will be administered during the middle stage of the estrous cycle, and in theory, all cattle should be responsive to $PGF_{2\alpha}$ at this time. When cattle were injected with $PGF_{2\alpha}$ during d 5-16 of the estrous cycle a return to estrus was observed within 2 to 4 d (Rowson et al., 1972; Liehr et al., 1972; Lauderdale, 1972). Several factors may influence the return to estrus interval include age, breed, and physiological factors (Moore, 1975; Britt, 1979; Burfening et al., 1987). The main factors effecting synchrony when using $PGF_{2\alpha}$ treatment was the stage of the estrous cycle (Dobson et al., 1975; Jackson et al., 1979; Refsal and Seguin, 1980; King et al., 1982; Stevenson et al., 1984a; Tanabe and Hann et al., 1984). The sensitivity of the CL to $PGF_{2\alpha}$ administration was greatest on d 10 (King et al., 1982; Tanabe and Hann, 1984). A common method to synchronize estrus using $PGF_{2\alpha}$ is two injections spaced 10 to 12 d apart (Lauderdale, 1973; Lauderdale, 1975; Cooper and Rowson, 1975; Britt et al., 1978; Hansel and Beal, 1979; Britt, 1979). This protocol resulted in acceptable conception in the mid 1970's (35%) with fixed time AI at 80 h post second injection (Cooper and Rowson, 1975). However, different strategies are now available that result in higher conception rates (Pursley et al., 1995).

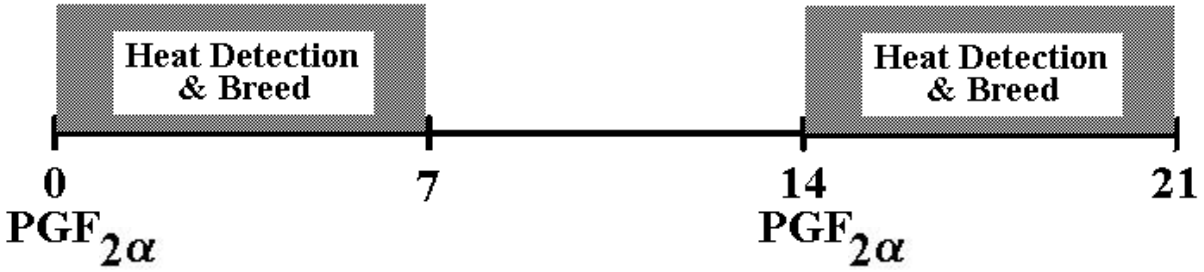

Figure 2-12. Modified two injection prostaglandin treatment. On d 0 all cows receive $PGF_{2\alpha}$ and estrus detection is necessary for 7 d. Animals found in estrus are bred. Cows that are not observed in estrus after the first $PGF_{2\alpha}$ injection receive a second injection 14 d later. After injection, estrus detection is necessary for 7 d and animals are bred at estrus detection.

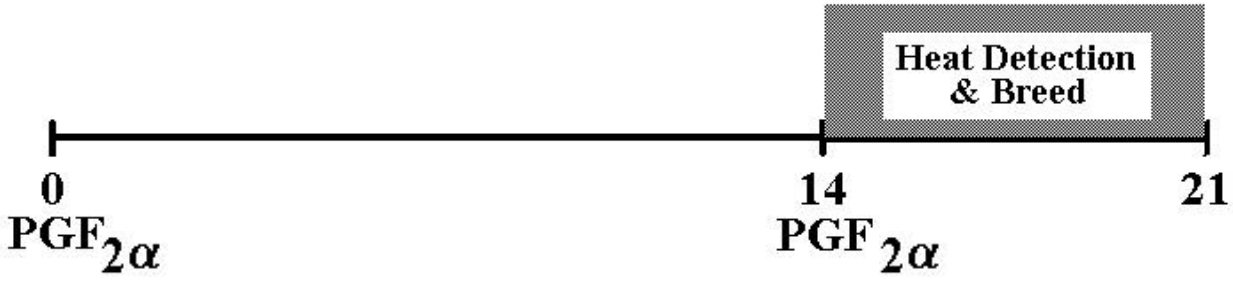

Figure 2-13. Two injection prostaglandin treatment. If labor availability is a constraint, all cows can be bred after second $PGF_{2\alpha}$ injection. On d 0 all cows receive $PGF_{2\alpha}$. Fourteen d later all cows receive another $PGF_{2\alpha}$ injection. After injection estrus detection is necessary for 7 d and animals are bred at estrus detection.

Several alternatives to the previously described approches exist. If labor is not a limiting factor, hormone cost can be reduced by breeding at natural estrus for six d. Any cattle that have not been bred after six d should receive $PGF_{2\alpha}$ injection, followed by estrus detection and insemination at estrus (see Figure 2-14).

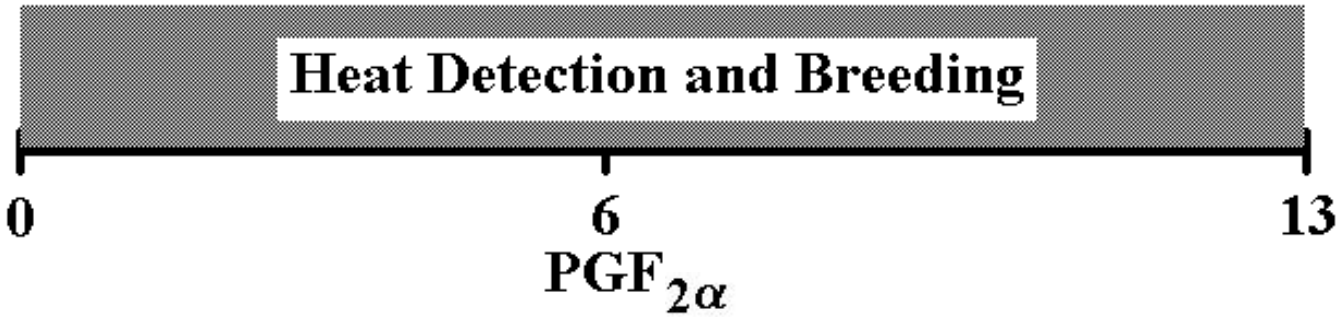

Figure 2-14. Modified one injection PGF_{2a} treatment. In order to reduce hormone costs cattle can be inseminated on natural estrus for 6 d. After 6 d any cattle that have not been bred should receive a $PGF_{2\alpha}$ injection, followed by insemination at estrus detection.

Gonadotropin Releasing Hormone

Gonadotropin releasing hormone is a decapeptide which has the same structure in several species including sheep, pigs, baboons, dogs, and man (Ory, 1983). The amino acid sequence is depicted in Figure 2-15. Histidine and tryptophan, the second and third amino acids are vital for activation of the adenyl cyclase system and for gonadotropin release (Coy and Schally, 1978; Stewart, 1981). The decapeptide is inactivated when cleavage occurs between positions 6 and 7 by proteolytic brain enzymes (Stewart, 1981). Substitutions at the C-terminus and position six increase resistance to enzymatic degradation, and may prolong half-life (Fujino et al., 1972; Monaha et al., 1973; Ory, 1983;).

Gonadotropin Releasing Hormone is synthesized in the cytoplasm of the dienchphalon and packaged for transport in the Golgi apparatus (Hurne and Lambalk, 2001). Gonadotropin

releasing hormone RNA has been found in the pituitary, placenta, ovary, myometrium, endometrium, and prostate (Chegini et al., 1996; Krsmanovic et al., 2000; Kang et al., 2000). Stojikovic et al. (1994) revealed a seven transmembrane G-protein coupled receptor for GnRH. Unlike most G-protein receptors, the GnRH receptor lacks a common carboxyl-terminal cytoplasmic domain and has a short intercellular third loop (Reinhart et al., 1992). When GnRH binds to specific receptors on gonadotropes coupling occurs to Gp_s11 proteins which activates the second messengers diacylglycerol, which then activates protein kinase C, and inositol-4,5-triphosphate leading to the production of cyclic AMP and release of calcium ions (Stojilkovicc et al., 1994; Kaiser et al., 1997; Huirne and Lambalk, 2001). Naor et al. (1998) also demonstrated GnRH receptor stimulation activates phospholipase A_2 and D.

In order to be effective, the estrus synchronization treatments discussed thus far require a substantial amount of time and labor, especially for estrus detection. These programs also fall short of producer's expectations in regard to the precision of estrus synchronization and conception rates. The cattle industry demanded a method of synchronization that reduced labor and time costs while not sacrificing fertility. Fogwell and associates (1986) reported in order to provide for precise estrus synchrony manipulation of both the CL and follicular waves were necessary. In addition, all animals should either have at least one, or no E active follicles to achieve homogeneity. Thatcher et al. (1989) demonstrated injection of 10 µg Buserelin, a potent GnRH agonist, modulates ovarian follicular waves and CL function in cattle. Macmillan et al. (1985a) reported similar results using 5µg buserelin. Thus, the effectiveness of GnRH analogs have been demonstrated for manipulation of reproductive events.

Figure 2-15. Amino acid sequence naturally occurring of GnRH.

Gonadotropin releasing hormone was first synthesized in the 1970's (Matsuo et al. 1971a). It first made its appearance on the agricultural commercial market as a treatment for cattle follicular cysts. Kittok et al. (1973) reported that repeated or prolonged exposure to GnRH may be required to mimic the preovulatory LH surge in cattle, and repeated doses caused a resumption of normal estrous cycles when administered to cattle with ovarian follicular cysts. Brown and Reeves (1983) recovered follicular, luteal, and pituitary tissue from cows, ewes, sows, and rats and preformed analysis to detect the presence of GnRH receptors on each. They found high affinity GnRH receptors in the pituitaries of all species studied and on the ovary of the rat. No GnRH receptors were found on the ovary tissues from any of the domestic farm animal studied. Thus, the actions of GnRH at the ovary must be brought about due to induced release of FSH and LH from the anterior pituitary.

It has been demonstrated that the actions of both FSH and LH are initiated by different but specific receptors on follicular and luteal cells (Twagiramungu et al., 1995a). Chenault et al. (1990) treated Holstein heifers with various doses (10 to 500μg) of fertirelin acetate, buserelin, and gonadorelin and found levels of FSH and LH to be elevated up to 2 to 5 h after administration. Stevenson et al. (1993) found similar results when cattle were injected with 8 μg receptal and elevated levels of LH were maintained 1 to 5 h later. Rettmer et al. (1992) also found that 200 μg fertirelin acetate elevated LH levels 1 to 4 h after administration.

Natural Mechanism of E & GnRH

Sirois and Fortune (1988) demonstrated the occurrence of estrus is associated with the presence of a large dominant follicle. Richards (1980) reported that E is responsible for promoting folliculogenesis during the estrus phase, and for initiating hormonal cascades necessary for normal reproduction including further gonadotropin release. Gonadotropins enhanced steroidogenic enzyme activity in granulosa and theca cells through cAMP dependant processes (Ireland, 1987). The dominant follicle produced 17β-estradiol in proportion to its size. The 17β-estradiol fed back on the hypothalamus and induced estrus activity through synthesis of androgens by facilitating the delta-5 pathway in addition to inhibiting P_4 production (Fortune et al., 1988). Estrogen, through its positive feedback on the

hypothalamus, caused further release of GnRH which acted on the gonadotrophs and induced pulsatile LH release and finally the LH surge leading to ovulation of the dominant follicle (Clarke, 1987; Nett, 1987).

GnRH Agonist Actions

Twagiramungu et al. (1992c) treated beef cattle with 8 µg buserelin. Six d later any cattle that had not exhibited spontaneous estrus were given 500 µg cloprostenol ($PGF_{2\alpha}$ analog). The occurrence of estrus in cows treated with buserelin was reduced up to 6 d after treatment. Also, percentages of estrus occurring between d 6 and 10 after buserelin were greater, and conception rate and interval from $PGF_{2\alpha}$ injection to estrus did not differ. The authors concluded that GnRH treatment followed 6 d later with $PGF_{2\alpha}$ allowed the elimination of estrus detection from d 0 to 6, without sacrificing pregnancy or conception rate. Similar findings have been reported using Cystorelin (Twagiramungu 1995a). Twagiramungu et al. (1994a, b) utilized ultrasonography and histological techniques to investigate the reason for delays in estrus. Experiments showed the dominant follicle either ovulated or underwent atresia. Also, the number of class two (6 to 9 mm) follicles experiencing atresia increased. Behavioral estrus did not occur since the dominant follicle was not allowed to reach its maximal diameter prior to ovulation. Inducing ovulation prevented E concentrations from reaching levels to produce estrus. Treatment with GnRH analogs induced ovulation and luteinization in luteal beef cows, and cyclic beef cows that did not have a functional CL at treatment (Twagiramungu et al., 1994a, b). Similar results were obtained using buserelin in non-lactating Holstein cows (Schmitt et al., 1994). Several authors have reported the disappearance of the largest follicle after treatment with a GnRH analog (Thatcher et al., 1989; Guilbault et al., 1990; Macmillan and Thatcher, 1991).

Silcox et al. (1993) conducted a study to determine if the ability of the dominant follicle to ovulate is dependent upon its developmental stage at the time of GnRH analog treatment. Ovulation occurred in all Holstein cows in which a dominant follicle was in the growth phase at the time of treatment. Thirty-three percent of cows in the static phase ovulated, and no follicles were ovulated which were in the regression phase of development.

Silcox et al. (1993) also concluded estrous cycle length (19.9 ± 3 d) was not affected by treatment.

Rollosson et al. (1994) speculated GnRH receptor concentrations decrease during the static and regression phase of follicle development. Concentration of hCG receptors was greater on follicles experiencing the growth phase than static and regression phases. Dominant follicles had greater hCG binding than did subordinate follicles. Also, there were no differences in hCG binding to the CL during any phases. At the onset of the atresia process, the number of gonadotropin receptors on the follicle diminished (Guilbault et al., 1993), which may trigger the onset of this process. Efficacy of ovulation by GnRH analogs may be controlled by receptor numbers and affinity (Macmillan and Thather, 1991). Follicle diameter and duration of growth stage follicles existence was decreased in cows administered a GnRH analog, however, this was not the case during the static or regression phases (Prescott et al., 1992).

Previous exposure of the dominant follicle to high P_4 concentrations may cause atresia and ovulation failure (Twagiramungu et al., 1994a). Stock and Fortune (1993) and Ginther et al. (1989a, b) extended the estrous cycle by administering intravaginal P_4-releasing devices that maintained sub-luteal levels of P_4. They concluded that atresia of large follicles occurred through feedback effects of elevated luteal P_4, and that prolonged follicular growth was associated with reduced fertility. Thus, low P_4 concentrations prevent atresia and induce persistent follicles. The actions of P_4 may be due to decreased LH pulse frequency. In contrast, Roberson et al. (1989) administered subphysiological levels of P_4 to cows and found that the mean LH concentration was increased and pulse frequency was reduced. Ireland and Roche (1983) found specific binding of hCG to E active or inactive follicles experiencing atresia after the natural LH surge. Prescott et al. (1992) showed that once a dominant follicle enters into atresia it can not be induced to ovulated by GnRH analog treatment.

A new cohort of follicles emerged within 2 d after GnRH analog treatment regardless of ovarian structures at the time of treatment (Twagiramungu et al., 1995). Macmillan and Thatcher (1991) monitored 5 cow's ovaries with ultrasound for 5 d after treatment with buserelin. Results from the study are summarized in Table 2-3.

Follicular stimulation after GnRH is most likely due to the release of FSH that occurs shortly after treatment. Rettmer et al. (1992) measured concentrations of FSH after fertirelin acetate in dairy heifers and found FSH was increased within 15 min of administration and remained elevated for 300 min (see Figure 2-16). Follicle stimulating hormone levels were elevated after disappearance of the dominant follicle (Ko et al., 1991; and Adams et al., 1992a). Adams et al. (1992a) found that FSH levels were elevated 2 to 4 d prior to emergence of a follicular wave, the period after dominant follicle regression or ovulation (see Figure 2-17).

A complete proposed GnRH receptor agonist model is summarized in figure 2-16.

Table 2-3. Follicles present after buserelin treatment. Average number of class 1, 2 and 3 follicles per cow after treatment with buserelin (10μg, Macmillan and Thatcher 1991).

Days post Treatment	Class 1 (3-5 mm)	Class 2 (6-9 mm)	Class 3 (> 9 mm)	Total (> 3 mm)
0	4.6	1.8	1.0	7.4
1	5.4	2.2	0.4	8.0
2	3.4	1.2	0.2	4.8
3	3.6	.06	0.4	4.6
4	3.4	1.4	0.4	5.2
5	2.8	0.4	1.0	4.2
Total	3.9	1.3	0.6	5.8

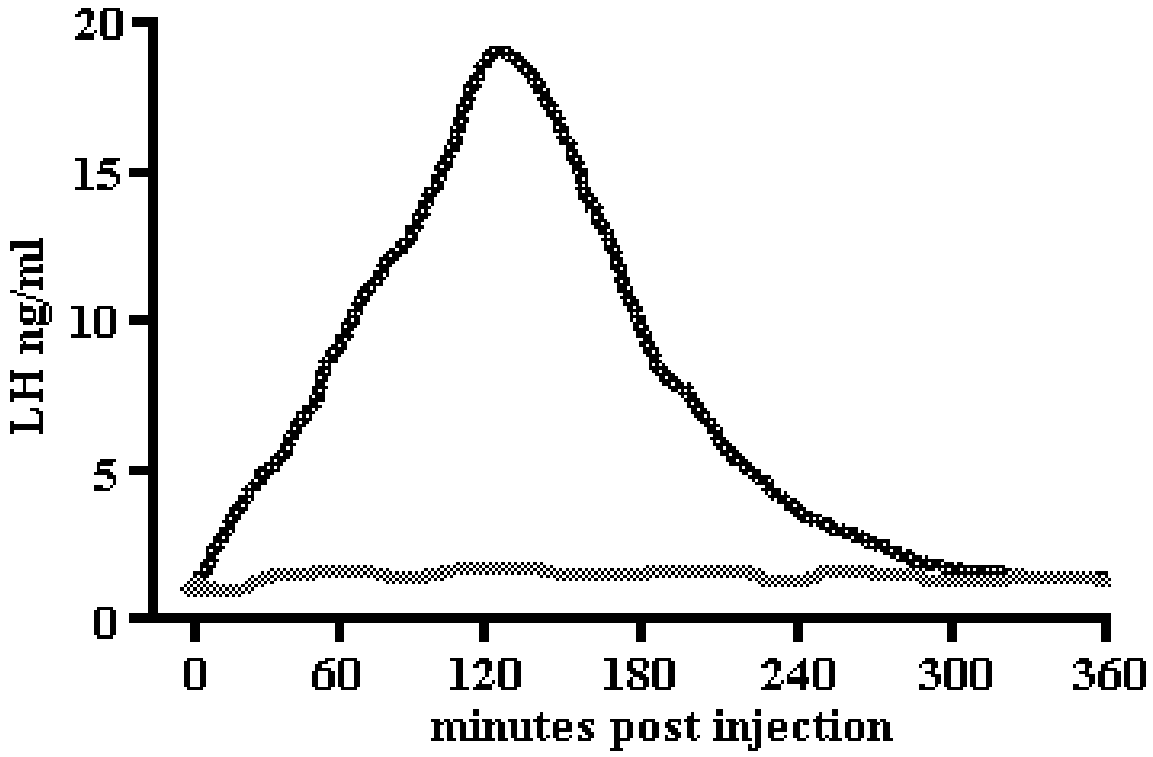

Figure 2-16. Luteinizing Hormone Concentration. Serum LH concentration from dairy heifers after 200 μg fertirelin acetate (solid black line) or saline (gray line, Rettmer et al., 1992).

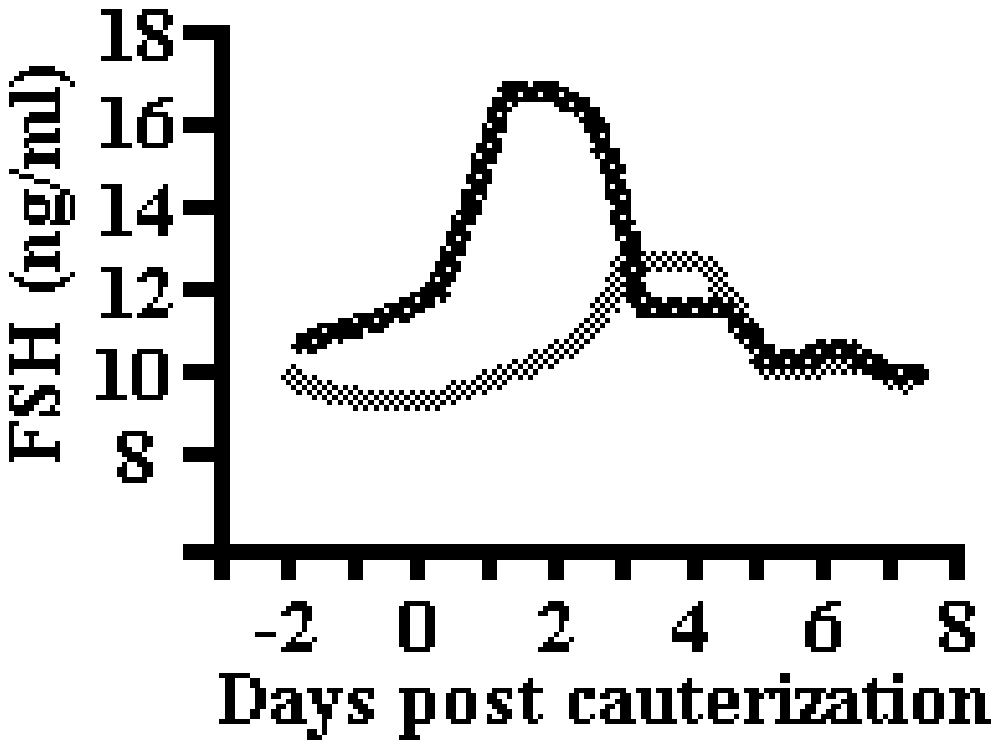

Figure 2-17. Follicle Stimulating Hormone Concentration. Follicle stimulating hormone concentrations after cauterization on d 0. Treatment group depicted in black, controls in gray (Adams et al., 1992a).

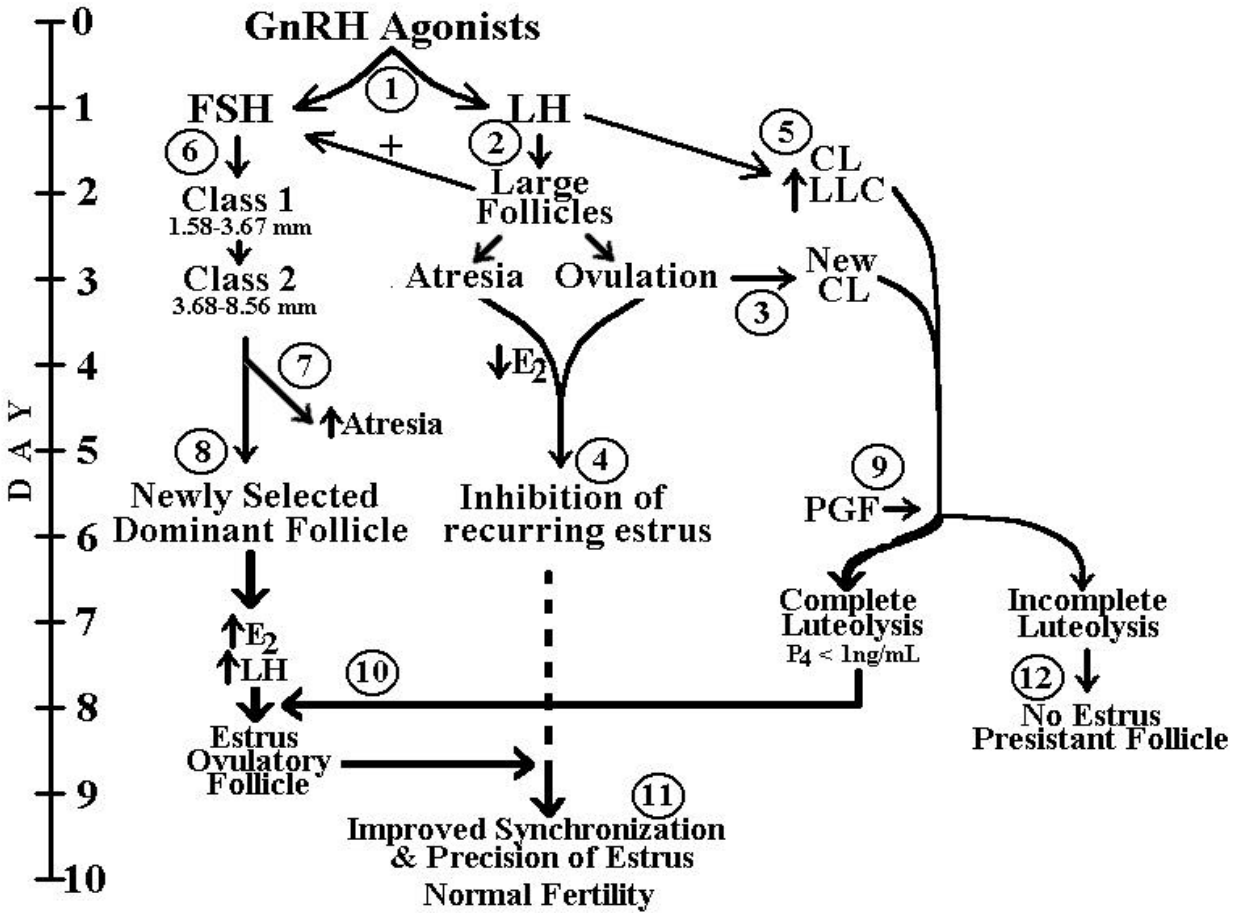

Figure 2-18. Proposed GnRH receptor agonist model. The model is based on a 10 d program for cattle. Treatment with a GnRH agonist on d 0 causes release of gonadotropins [1]. Large follicles ovulate due to the effects of LH [2] and a new CL is formed [3] or the follicles experience atresia. In either case, increasing E levels and recurring estrus are inhibited between d 0 and 6 [4]. Large luteal cells (LLC) increase in number on the CL present at treatment [5]. FSH stimulates turnover of follicles from class 1 to 2 [6]; however, increased atresia in class 2 [7] retards additional growth. A new dominant follicle is selected [8] from the synchronized wave 3 to 4 d after treatment. Complete luteolysis occurs after injection of prostaglandin $F_{2\alpha}$ on d 6 [9]. Estrogen levels and LH pulse frequency increase, the LH surge occurs after estrus and the selected dominant follicles ovulates [10]. Between d 7 and 10 synchronization rate and precision of estrus are improved and results in normal fertility [11]. In a small minority of cows, estrus is blocked due to incomplete luteolysis, and the selected dominant follicle becomes persistent [12]. (Twagiramungu et al., 1995a)

Chemistry of GnRH analogs

After the sequence of naturally occurring GnRH determined (Matsuo et al., 1971b; Amoss et al., 1971) thousands of analogs both agonist and antagonist have been produced for pharmacological manipulation of reproductive events (Hahn et al., 1985). Reviews of GnRH localization, metabolism and enzyme inactivation are available (Griffiths, 1967; Marks, 1977). Manipulation of two residues of GnRH increased its releasing activity up to 50X (Coy et al., 1985). Fujino et al. (1972) replaced glycine with an ethylamide group at the C-terminus. Monahan et al. (1973) replaced glycine at position 6 with alkyl D-amino acids. Coy et al. (1976) and Konig et al. (1975) found D-amino acids with bulky side chains, D-Phe and D-Trp, and D-Ser(tBu) caused even greater biological activity. Coy et al. (1974) and Fujino et al. (1974) discovered a C-terminal ethylamide group in combination with D-Ala or D-Leu at position 6 also increased biological activity.

GnRH Synchronization Regimens

In mid-luteal phase cows, GnRH analogs caused disruption of normal follicular dynamics by decreasing the number of large follicles through luteinization and or atresia (McNatty et al., 1981; Thatcher et al., 1989; Guilbault et al., 1990). When follicular fluid was injected into cows, a delay in estrus activity was observed (Quirk and Fortune, 1986). Analogs of GnRH extended CL duration and protected against spontaneous luteolysis (Henderson and McNatty, 1975; Macmaillan et al., 1985b). Gonadotropin releasing hormone agonist treatment did not inhibit estrus behavior when estrus was imminent at administration time (Peters and Ball, 1995). Thatcher et al. (1989) reported GnRH analogs used prior to $PGF_{2\alpha}$ treatment increased synchrony of estrus response. Twagiramuneu et al. (1991) reported pretreatment with burserlin 6 d prior to $PGF_{2\alpha}$ treatment (see Figure 2-17) eliminated the need for estrus detection without surrendering pregnancy nor conception rates. Chenault et al. (1990) and Guilbault et al. (1990) reported after burserlin administration large follicles were replaced by 4 to 6 mm and 7 to 9 mm follicles. Twagiramungu et al. (1992c) demonstrated GnRH agonist in combination with $PGF_{2\alpha}$ treatment did not decrease conception and fertility rates. Pursley et al. (1995) demonstrated an additional injection of GnRH analog 2 d post $PGF_{2\alpha}$ injection eliminates the need for estrus detection.

There are several synchronization protocols in existence utilizing GnRH analogs (Twagiramungu et al., 1992c; Wolfenson et al., 1994; Pursley et al, 1995). For simplicity only the major treatments will be discussed here.

Select Synch

Select-synch (see Figure 2-19) was the first protocol established utilizing GnRH analogs. On d 0 all cows receive an injection of a GnRH analog. Starting 6 d after injection, and for 6 successive d afterwards the herd is monitored for estrus activity, and bred 8 to 12 h after estrus detection. On d 7 cows that have not been detected in estrus are given an injection of $PGF_{2\alpha}$ to induce luteolysis. This is an effective protocol, but the need for estrus detection is still present (Twagiramungu et al., 1992b), and pregnancy rates can range (20.8%, Lemaster et al., 2001; 53%, Stevenson et al., 2000; 40%, Kojima et al., 2000).

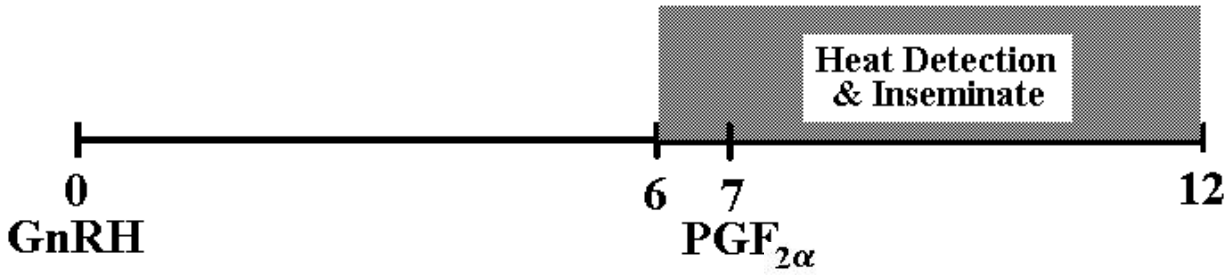

Figure 2-19. Select Synch Protocol. An injection of GnRH followed 6 d later with $PGF_{2\alpha}$ provided an effective means of synchronizing estrus (Twagiramungu et al., 1991).

Cosynch

Cosynch (see Figure 2-20) was modeled after Select Synch, however, the need for

estrus detection was eliminated with a second GnRH injection. On d 0 all cows receive a GnRH analog. Seven d later all cows receive $PGF_{2\alpha}$ to induce luteolysis. Two d after $PGF_{2\alpha}$ injection, all cows receive a second GnRH injection followed by immediate insemination. Pregnancy rates after Cosynch were 40 to 54% (Lamb et al., 2001; Geary et al., 2001a, b). This system effectively reduces labor costs, however, higher levels of fertility are achievable. The second GnRH injection at breeding may cause premature ovulation and reduce pregnancy rates. Recent reports indicate insemination at GnRH injection may not be ideal. Dalton et al. (2000) reported AI of superovualted cattle 24 h after estrus increased fertilization rates compared with insemination at 0 or 12 h after estrus. Dalton et al. (2001) demonstrated AI 12 h post estrus optimized fertility of dairy cattle.

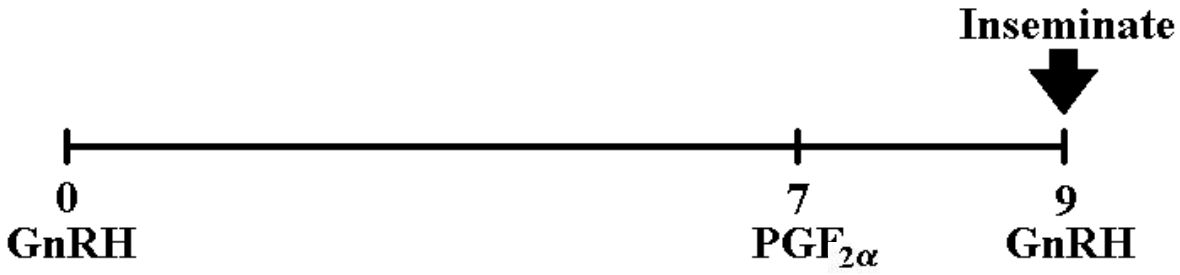

Figure 2-20. Cosynch Protocol. An injection of GnRH followed 7 d later with $PGF_{2\alpha}$ and insemination at an additional GnRH analog injection on d 9 resulted in 40 to 50% conception rates (Lamb et al., 2001).

Ovsynch

The Ovsynch protocol (see Figure 2-21) is a very popular synchronization treatment for beef and dairy cattle utilizing GnRH analogs, in combination with $PGF_{2\alpha}$, since the need for estrus detection is eliminated. Conception rates are comparable to untreated cattle after this protocol. Pregnancy rates after timed AI with this protocol were similar for beef

(Twagiramungal et al. 1992a,b, 1995) and dairy cattle (Pursley et al., 1995; Schmitt et al., 1996; and Wiltbank et al., 1996) to unsynchronized estrus.

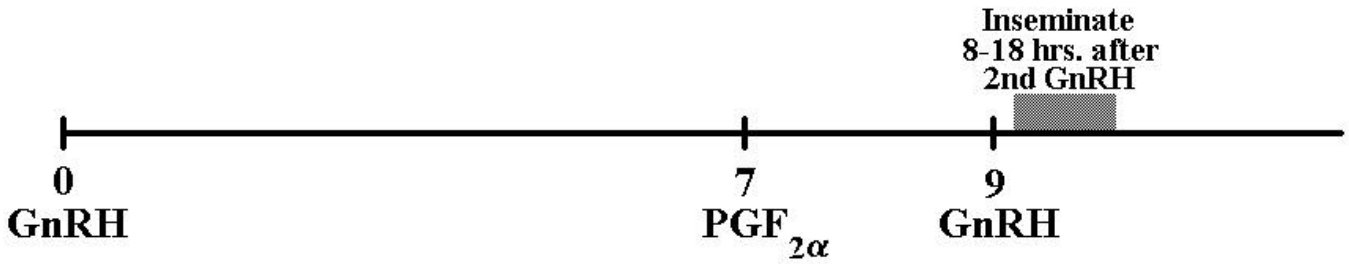

Figure 2-21. Ovsynch Protocol. The Ovsynch protocol as described by Pursley et al. (1995) demonstrated an additional injection of a GnRH analog 2 d post $PGF_{2\alpha}$ injection eliminated the need for estrus detection. Cattle are injected with a GnRH analog followed 76 d later with $PGF_{2\alpha}$ treatment. Two d post $PGF_{2\alpha}$ treatment cattle receive a secondary GnRH analog injection followed by insemination 8 to 18 h later without estrus detection.

Estrogens

Wiltbank et al. (1961) demonstrated 5mg E induced luteal regression in cows. Other researchers discovered the duration of a P_4 treatment for estrus synchronization can be shortened with an injection of E at initiation of treatment (Odde, 1990; Wiltbank et al., 1997). Bo et al. (1995b) showed E in conjunction with P_4 treatment suppressed the growth of dominant follicles. Bo et al. (1995a) demonstrated when estradiol-17β was administered to P_4 implanted cows on d 3, 6, or 9 a new follicular wave emerged 4 d later. Barros et al. (2000) and Fernandes et al. (2001) have shown the second GnRH injection in the Ovsynch protocol may be replaced with E without affecting synchrony. It has been shown that estradiol benzoate administered during periods of relatively low P_4 induces an LH surge approximately 16 to 24 h after administration (Bo et al., 1994; Hanion et al., 1996; Lammoglia et al., 1998). Currently, GnRH and E combination synchronization protocols are

not widely used in production agriculture in the United States since only estradiol cypionate is approved for use in cattle.

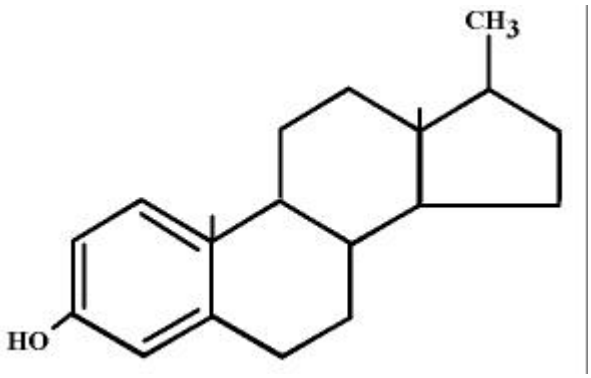

Figure 2-22. Structure of estradiol 17-b

Chapter III
QUESTIONS AND OBJECTIVES

The purpose of this research is to establish which GnRH analog, Cystorelin or Factrel, is most effective for use as part of an estrus synchronization treatment for use with timed AI. Both products are GnRH analogs that differ slightly in their chemical organization. These two products represent the major two chemical arrangements utilized today in animal agriculture. We have several objectives to accomplish in order to reach our purpose:

- To determine which analog will result in the greatest number of pregnancies which are maintained to at least 45 d.

- To determine which, if any, factors might play a role in the efficacy of the agents under standard production (field) conditions.

- To analyze the LH profile induced by the two analogs.

- To determine the temporal profile of the emergence, propagation, and establishment of a dominant follicle and regression of ovarian follicular waves under the influence of the two analogs.

- To compare endocrine and ovarian responses to each of these analogs.

Chapter IV
EFFICACY OF CYSTORELIN VERSES FACTREL: FIELD TRIALS

Introduction

When utilizing $PGF_{2\alpha}$ for synchronization protocols, estrus detection is necessary for acceptable conception rates (Archibald et al., 1992; Lucy et al., 1986). Synchronization of estrus occurs over a 5 d period after $PGF_{2\alpha}$ treatment (Lauderdale et al., 1974; Hafs and Manns, 1975; Seguin et al., 1985). Thatcher et al. (1989) and Twagiramungu et al. (1992c) decreased the expected estrus interval after $PGF_{2\alpha}$ treatment by utilizing a GnRH analog 6 d which resulted in higher conceptions rate. Pursley et al. (1995), Burke et al. (1996) and Twagiramungu et al. (1995) enhanced the precision of estrus by treating with GnRH analogs 2 d post estrus by synchronizing ovulation. Pregnancy rates after timed AI using this protocol were similar for beef (Twagiramungal et al. 1992a,b, 1995) and dairy cattle (Pursley et al., 1995; Schmitt et al., 1996; and Wiltbank et al., 1996) to unsynchronized estrus.

After a extensive review of current literature we are unable to discover any field trials comparing GnRH analogs. Several scientists have conducted trials utilizing different GnRH preparations, however, not within the same experiment. Field experiments were conducted to determine if any treatment differences exist for Cystorelin or Factrel.

Materials and Methods

Since any estrus synchronization experiment requires a large number of animals for to detect statistically differences, we utilized cattle from several operations and institutions within the state of Virginia. Overall, data were collected on 496 cows that were treated with three different synchronization protocols using 100μg doses of Cystorelin (gonadorelin diacetate tetahydrate, Merial Limited, Iselin, NJ) or Factrel (gonadorelin hydrocholoride, Fort Dodge Animal Health, Ford Dodge, IA). The protocol utilized was the decision of the respective herdsman. The majority of the data was gathered using the Ovsynch protocol.

We cooperated with five different organizations for this experiment. The locations, treatments, and number of animals are listed below in Table 4-1.

Table 4-1. Location and number of cattle for field trial experiments.

Herd	Location	Treatment	n
1	Pulaski, Virginia	Ovsynch	125
2	Newport, Virginia	Ovsynch	61
3	Bland, Virginia	Ovsynch	30
4	Bland, Virginia	Ovsynch	21
5	Bland, Virginia	Ovsynch	26
6	Bland,Virginia	Ovsynch	29
7	Buckingham, Virginia	Ovsynch	32
8	Raphine, Virginia	Cosynch	120
9	Buchingham, Virginia	Select-Synch	44
TOTAL			488

In exchange for use of the cattle, each organization received all drugs, insemination labor, and pregnancy checking procedures at no cost. Semen was selected and supplied by the herdsman.

The majority of animals on this experiment received the Ovsynch protocol. The present research is part of an ongoing larger study in which all three of the major protocols are being examined. Presently, sufficient animal numbers are not available for the Cosynch and Select-synch protocols, but are included here as an indication of what the larger experiment with more animal numbers may demonstrate.

Synchronization Protocols Utilized

The three major synchronization protocols in production use today were utilized for the completion of this research, Ovsynch, Cosynch, and Select-Synch. Estimated cost of the synchronization protocols are as follows on a per cow basis; Ovsynch, $12.00; Co-synch, $10.00; and Select-synch, $7.00.

Ovsynch protocol

Cattle in herd 1 through 6 received the Ovsynch protocol (total n = 324). Cows were randomly assigned to receive either Cystorelin or Factrel. On d 0, all cows received an i.m. injection of 100 μg of their pre-assigned GnRH analog. Seven d following GnRH analog injection all cows received of 25 mg Lutalyse® (Pharmacia and Upjohn, Kalamazoo, MI) to induce luteolysis. On d 9, all cows received an additional 100 μg of their assigned GnRH product i.m. Fourteen to 18 h following the second GnRH injection, all cows were mass inseminated by a skilled AI technician. Cattle were mixed with proven bulls 14 d after insemination for clean-up purposes.

Co-synch protocol

Cattle at indicator 5 received the Cosynch protocol (total n = 120). Cows were randomly assigned to receive either Cystorelin or Factrel. On d 0, all cows received an i.m. injection containing 100 μg of their pre-assigned GnRH analog. Seven d following GnRH analog injection all cows received 25 mg of Lutalyse to induce luteolysis. On d 9 all cows received an additional 100 μg of their assigned GnRH product intramuscularly and were inseminated by a skilled AI technician. Any cattle which exhibited estrus early were inseminated 12 h after observed estrus. Cattle were mixed with proven bulls 14 d after insemination for clean-up purposes.

Select-synch protocol

Cattle at herd 5 received the Select-synch protocol (total n = 44). Cows were randomly assigned to receive either Cystorelin or Factrel. On d 0, all cows received an i.m. injection containing 100 μg of their pre-assigned GnRH analog. Seven d following GnRH

analog injection all cows received of 25 μg of Lutalyse to induce luteolysis. On d 9 all cows received an additional 100 mg of their assigned GnRH product i.m. Cattle were checked for estrus and inseminated by a skilled AI technician 8-12 h after observed estrus. Cattle were mixed with proven bulls 14 d after insemination for clean-up purposes.

Ultrasonography

Forty-five d post-insemination trans-rectal ultrasonogrophy was performed by a veterinarian to determine pregnancy status. Animals with a 45-d fetus were considered to have conceived in response to AI. Any cows with a fetus less than 40 d were considered not to have conceived to the synchronization treatment.

Statistical Analysis

Data were analyzed using the GLM procedures of SAS (SAS, 1999). The full model included age (AGE), body condition score (BCS), d post partum (DPP), location (LOC), treatment (TRT) and the interactions TRTxAGE, TRTxDPP, TRTxBCS, and TRTxLOC. Backwards elimination was used to reduce the model until only significant interactions were included. The final reduced model included LOC, TRT, and TRTxLOC. After recognizing significant factors in the model Chi-squared data analysis was performed using the FREQ procedures of SAS. Data were analyzed grouped by treatment, and location within treatment for locations where Ovsynch was utilized.

Results

This study demonstrated a tendency ($P = .09$) for more cows treated with Factrel to conceive following AI using the Ovsynch protocol (see Table 4-2). There were no significant ($P < .1$) treatment effects in 6 of the 7 herds from which data was collected (see Table 4-2), except for herd 4 ($P = .03$).

Table 4-2. Summary of Ovsynch results. More cows in herd 4 which were treated with Factrel had pregnancies at d 45 as compared to Cystorelin treated cows (P=. 03)*. Cows at the remaining locations did not have treatment differences. When all herds were combined there was a tendency for more cows treated with Factrel to be pregnant at d 45 as compared to Cystorelin treated cows (P = .09)$^{+}$.

Herd 1	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.9 ± .1	35.48 (22/62)	64.52 (40/62)
Factrel	5.9 ± .1	33.33 (21/63)	66.67 (42/63)
Total	5.9 ± .1	34.40 (43/125)	65.60 (82/125)
Herd 2	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.6 ± .1	50.00 (14/28)	50.00 (14/28)
Factrel	5.5 ± .1	30.30 (10/33)	69.70 (23/33)
Total	5.6 ± .1	39.34 (24/61)	60.66 (37/61)
Herd 3	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.1 ± .1	73.33 (11/15)	26.67 (4/15)
Factrel	5.1 ± .1	53.33 (8/15)	46.67 (7/15)
Total	5.1 ± .1	63.33 (19/30)	36.67 (11/30)
Herd 4*	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.7 ± .3	70.00 (7/10)	30.00 (3/10)
Factrel	5.5 ± .2	18.18 (2/11)	81.82 (9/11)
Total	5.6 ± .2	42.89 (9/21)	57.14 (12/21)
Herd 5	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.2 ± .2	20.00 (2/10)	80.00 (8/10)
Factrel	5.0 ± .2	37.50 (6/16)	62.5 (10/16)
Total	5.1 ± .2	30.77 (8/26)	69.23 (18/26)
Herd 6	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.6 ± .2	50.00 (8/16)	50.00 (8/16)
Factrel	5.6 ± .3	61.54 (8/13)	38.46 (5/13)
Total	5.6 ± .2	55.17 (16/29)	44.83 (13/29)
Herd 7	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.4 ± .1	43.75 (7/16)	56.25 (9/16)
Factrel	5.3 ± .1	31.25 (5/16)	68.75 (11/16)
Total	5.4 ± .1	37.50 (12/32)	62.50 (20/32)
All Herds Combined^{+}	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.6 ± .1	45.22 (71/157)	54.75 (86/157)
Factrel	5.6 ± .1	35.93 (60/167)	64.07 (107/167)
Total	5.6 ± .1	40.43 (131/324)	59.57 (193/324)

A tendency (P = .07) was found for more cows treated with Cystorelin to maintain pregnancy at least to d 45, rather than Factrel, when using the Cosynch protocol (see Table 4-3). However, variation within this analysis is high, and more animal numbers are necessary for significance.

Table 4-3. Summary of Cosynch results. Cows that were treated with Cystorelin, as part of the Cosynch protocol, tended to have more pregnancies at d 45 compared to Factrel treated cows (P = .07).

	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.4 ± .1	45.90 (28/61)	54.10 (33/61)
Factrel	5.4 ± .2	62.71 (37/59)	37.29 (22/59)
Total	5.4 ± .1	54.17 (65/120)	45.83 (55/120)

Another tendency (P = .06) was found for more cows treated with Factrel to conceive to AI using the Select-Synch protocol (see Figure 4-2). However, variation within this analysis is high and more animal numbers are necessary for increased significance.

Table 4-4. Summary of Select-synch results. Cows that were treated with Factrel, as part of the Select-synch protocal, tended to have more pregnancies at d 45 compared to Cystorelin treated cows (P = .06).

	BCS	Open (%, n/n)	Pregnant (%, n/n)
Cystorelin	5.3 ± .1	76.19 (16/21)	13.81 (3/21)
Factrel	5.3 ± .2	45.45 (10/22)	54.55 (12/22)
Total	5.3 ± .1	60.47 (26/43)	34.88 (15/43)

Chapter V

EFFICACY OF CYSTORELIN VERSES FACTREL: BOVINE LUTEINIZING HORMONE BLOOD PROFILE

Introduction

In the previous field experiments, a tendency was found for Factrel to be more effective when used in the Ovsynch protocal for maintaining pregnancy to at least d 45 after insemination. To better understand this response, LH release and follicular dynamics were measured after administration of the two analogs. Bentley et al. (1998) conducted an experiment that compared several characteristics of Cystorelin-, Factrel- and Fertagyl-induced LH release in diary cows. Bentley et al. (1998) concluded Cystorelin induced the highest LH release and ovulation of the dominant follicle after administration. The work of Bentely et al. (1998) is the only investigation to our knowledge comparing GnRH analogs for use in AI protocols within the same experiment. Several other studies have been conducted on the efficacy of GnRH analogs (Matsuo et al. 1971a, b; Mori et al., 1974, 1979; Coleman et al., 1988), however, none make comparisons within the same experiment. Thus, this investigation was conducted to gather more information in this area.

Materials and Methods

Animal Maintenance and Preparation

For this experiment, 19 healthy crossbred cows of predominately Angus breeding from 2 to 5 years of age were utilized. Animals were maintained in two paddocks with concrete surfaces covered with sawdust. Cattle were fed quality hay daily with ad libitum access to water. Body condition scores for these animals ranged from 5 to 7. Prior to experimentation cows were fitted with Kamar patches (Kamar, Inc, Steamboat Springs, CO) to aid in twice daily visual estrus detection. Animals that exhibited estrus were assigned to a treatment.

In preparation for the experiment, all cows that had previously exhibited estrus received of 25 mg of Lutalyse® (Pharmacia and Upjohn, Kalamazoo, MI) i.m. to synchronize

the animals' estrous cycles. After injection, cattle were monitored twice daily (08:00 and 18:00) for estrus. Seven of 10 cows exhibited preparatory estrus between 2.5 and 3 d post Lutalyse injection, and were assigned to a receive 100 μg Cystorelin (n = 3) or 100 μg Factrel (n = 4) injection as part of the Ovsynch protocol. In replicate two, 12 of 15 responded between 2.5 and 3.5 d post Lutalyse injection, and were randomly assigned to receive 100 μg Cystorelin (n = 7) or 100 μg Factrel (n = 5) injection as part of the Ovsynch protocol. The cows that failed to respond, were not detected, or responded at a time other than the mean window, and were not used for research purposes.

Placement of Catheters

Twelve d after Lutalyse injection, cattle were fitted with either 14 gauge x 127.0 or 76.2 mm jugular catheters (Abbacath, Town USA) with dead volumes of less than .5 mL. Prior to catheter placement, each cow was haltered, restrained, and the neck was clipped and sterilized with iodine. Catheters were held in position by suturing directly to the animal's neck. To facilitate blood collection, a 610 mm extension set with a dead volume of 2.5 mL was fitted to the catheter. To protect the extension set and catheter, a pouch (100 x 100 mm) was affixed to the animal. Once the catheter and extension set were placed in the pouch, the entire assembly was secured to the animal's neck with adhesive paste and several wraps of adhesive tape. Cattle were allowed at least 30 min of rest time after catheter placement before the start of blood collection. Cattle were tied via their halters in a chute or individual stalls for the duration of the experiment dependant on resource availability.

Blood Collection, and Storage

Blood samples were collected every 15 min from -30 to 525 min post GnRH analog injection. Prior to each blood sample collection at least 4 mL of fluid was collected from the catheter plus extension and discarded. The samples (4 to 8 mL) were collected and placed in 16 x 100 mL glass tubes containing no additive or anticolaguant resting in an ice bath. After each collection, the catheter and extension were flushed with at least 3 mL heparanized saline (15 units per mL) to prevent clotting. The samples were allowed to clot for at least 1 h prior to being refrigerated at 4° C overnight. The following d, the samples were centrifuged at

3,000 x g for 20 min. The serum portion was collected in 12 x 75 mm polypropylene tubes, capped and stored at -20° C until assays were conducted. An ultra-low freezer (-120° C) became available during the first collection of replicate two, and samples were stored within. However, the freezer failed and samples were found in a cold (~5-15° C), yet liquid state, and were immediately transferred to a different freezer (-20° C).

Ultrasonography

Ovarian dynamics were monitored as described by Pierson et al. (1988) by ultrasound evaluation using an Aloka 210 ultrasound machine (Corometrics Medical Systems, Inc., Wallingford, CT.) with a 7.5 MHz linear array transducer. Ultrasound evaluations were performed 1 d prior to first GnRH analog injection and until after disappearance of the dominant follicle after second GnRH analog injection. Each ultrasographic evaluation was recorded in total on VHS tape and was later analyzed. All follicles in excess of 3 mm in antral diameter were measured and mapped individually for each cow. Presence of a CL was also noted and mapped for each cow. The measurement and time of disappearance of the ovulatory follicle and emergence of new follicular waves were recorded.

Assays

Progesterone

Progesterone concentrations were quantified by solid-phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA) to determine progesterone levels in samples –30 and –15 for each animal. Sensitivity of the assay was .02 ng/mL. Intra-assay coefficient of variation was 3.42%.

Luteinizing Hormone

A double antibody radioimmunoassay was validated in order to determine LH concentration in blood serum samples. Specific rabbit anti-bovine LH antiserum was provided by USDA (USDA-309-684P) and served as the first antibody. Second antibody consisted of sheep anti-rabbit gammagloublin and used at a dilution of 1:10 in 0.5 M PBS-EDTA (pH=7.0). Pure bovine LH (National Hormone and Pituitary Program - [AFP-8614B])

was dissolved in assay buffer to prepare a standard curve. The curve consisted of seven points counted in triplicate. Lyophilized primary antiserum (USDA-309-684P) was initially reconstituted in 0.05 M PBS-EDTA at a dilution of 1:800 and was further diluted with PBS-EDTA containing NRS at 1:200 dilution. Primary antiserum was used at a final dilution of 1:230,000 and bound 35-45% of I^{125}-LH. Secondary antiserum was prepared by diluting with sheep anti-rabbit gammagloublin with 1:200 NRS to reach a final 1:10 dilution. Tracer was composed of pure bovine LH iodinated with I^{125} (half life = 60 d) which resulted in activity of 30,000 CPM/100μL. Tracer remained stable for a maximum of 35 d before results were compromised. Inter- and intra-assay coefficients of variation were 16.97 and 7.32 % respectively.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS, 1999). Significant differences were considered to have a P value less than 0.05. When significant differences were detected, Tukey's procedure was used to determine differences among treatment least squared means. Duration of LH response was defined as the interval in which LH levels were in excess of .0309 ng/mL, the lowest detectable level of the assay. Any value less than the lower limit of the assay was assigned the value .0309 ng/mL. Total area under the LH curve was calculated by multiplying all detectable LH concentrations by 15 and summing, since blood samples were collected with frequency of 15 min. Progesterone concentrations (P_4[]) and P_4[] x TRT interaction were analyzed as a covariant for each response. Backward elimination was utilized to eliminate variables which did not significantly contribute to the model. In all cases P_4[] and P_4[] x TRT interaction were not significant contributors to the model and were removed from the analysis.

Ultrasound variables analyzed were quantity of non-ovulatory follicles (QNOV), total follicles (TOTFOL), follicle size at first injection (FSZ), d of wave emergence after first GnRH analog injection (EMP), growth rate of ovulatory follicle after wave emergence (GWR), peak size of ovulatory follicle (PSZ), and size of dominant follicle at second GnRH analog injection (SSZ).

Results

For the variable maximum concentration (MAXCON), no treatment (TRT) differences were detected (P = .62, Figure 5-1). However, differences were detected between phases (PHS, P < .0001, Figure 5-2). During the follicular phase, maximum concentration of LH was greater than during mid-luteal (14.33 ± 1.1 vs. 3.73 ± 1.1 ng/mL respectively). The PHS x TRT interaction for MAXCON was not significant (P = .24). There was a significant (P = .003) TRT x PHS interaction for MAXCONTIM (Figure 5-3). The MAXCONTIM for Cystorelin (114.0 ± 10.1 min) did not differ (P = .89) from Factrel (125.0 ± 10.6 min) during the luteal phase. However, Cystorelin's (73.1 ± 11.3 min) MAXCONTIM was shorter (P = .0037) than Factrel (133.1 ± 11.3) during the follicular phase. A TRT x PHS interaction was found for DDR (P = .05, Figure 5-4). The DDR for Cystorelin (286.5 ± 24.1 min) differed (P = .02) from Factrel (393.3 ± 25.4 min) during the luteal phase, and Cystorelin (191.3 ± 27.0 min) differed (P < .0001) from Factrel (408.8 ± 27.0 min) during the follicular phase.

When area under the LH curve (AUC, see Figure 5-5) was calculated, no differences (P = .55) were detected between Cystorelin (318.7 ± 49.6 ([ng/mL]/min)*15) and Factrel (263.3 ± 53.9 ([ng/mL]/min)*15). Differences (P < .0001) were detected between the luteal (100.1 ± 51.2 [ng/mL]/min) and follicular (481.9 ± 52.4 [ng/mL]/min) phases after GnRH analog administration for AUC (see Figure 5-6). The TRT x PHS interaction for AUC was not significant (P = .49)

Treatment differences (P = .02) between Cystorelin (3.21 ± .24) and Factrel (2.43 ± .22) were found for total number of non-ovulatory follicles (all ultrasound data presented in Table 5-1). Cows treated with Cystorelin (3.79 ± .26) had more (P = .03) follicles per day during ultrasound examinations than did Factrel treated cows (3.01 ± .23). The size of the pre-ovulatory follicle did not differ (P = .39) among Cystorelin (11.6 ± .90 mm) nor Factrel (10.5 ± .82) treatment. The d of follicular wave emergence did not differ (P = .30) between Cystorelin (2.0 ± .4 d) and Factrel (2.6 ± .4 d) treatments. The rate of pre-ovulatory follicle growth between Cystorelin (1.2 ± .5 mm/d) and Factrel (.7 ± .4 mm/d) also did not differ (P = .47). No differences between peak size of ovulatory follicles after Cystorelin (13.3 ± 1.2 mm) or Factrel (12.7 ± 1.11 mm) were detected.

Pre-ovulatory follicle size did not differ (P = .59) between Cystorelin (13.2 ± 1.3 mm) or Factrel (12.3 ± 1.1 mm) treated cows. The d the ovulatory follicle disappeared after Cystorelin (2.20 ± .29 d) and Factrel (2.29 ± .25 d) treatment were similar (P = .83). Only one cow, which was treated with Cystorelin, failed to ovulate after the first injection. All cattle responded to the first injection by initiation of a new follicular wave. The d of new wave emergence ranged from 1 to 4 d post GnRH analog injection. All cattle responded to second GnRH analog treatment by ovulation within 4 d after treatment.

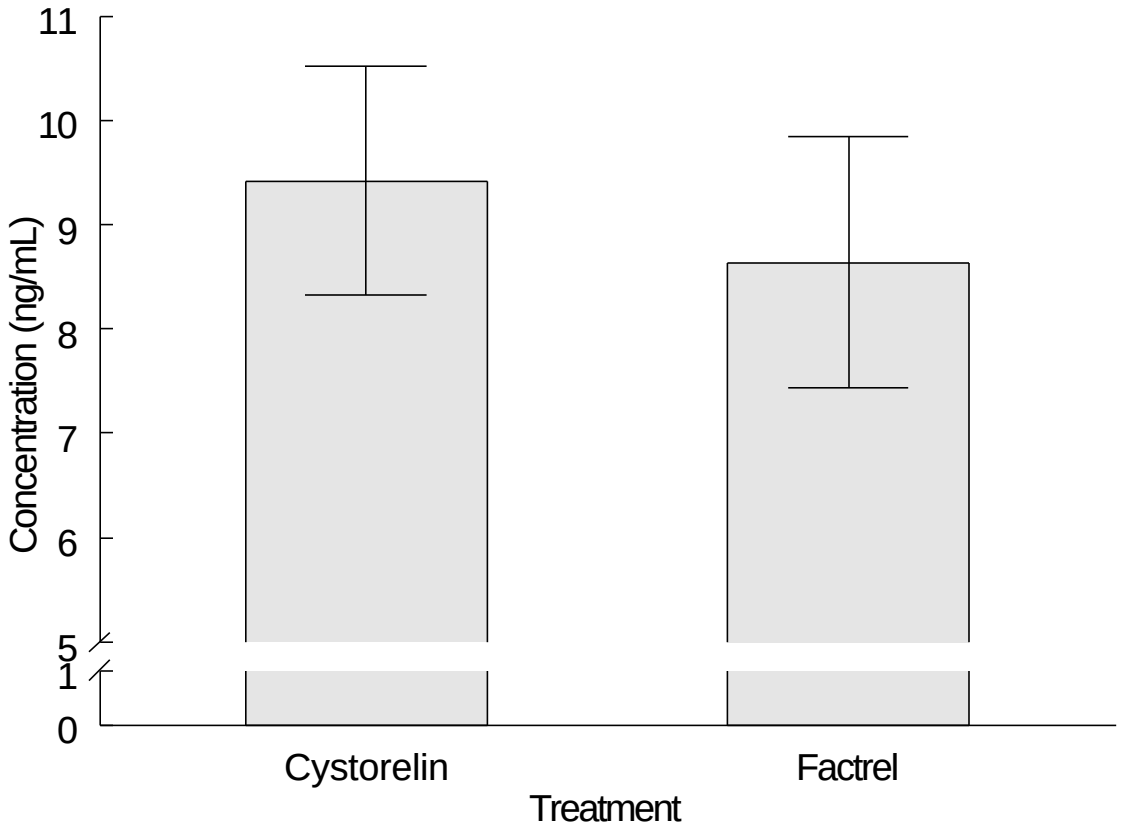

Figure 5-1. Maximum LH concentration after administration of Cystorelin and Factrel in beef cows. Maximum concentration of LH after GnRH analog administration did not differ among treatments (P = .62). Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA.

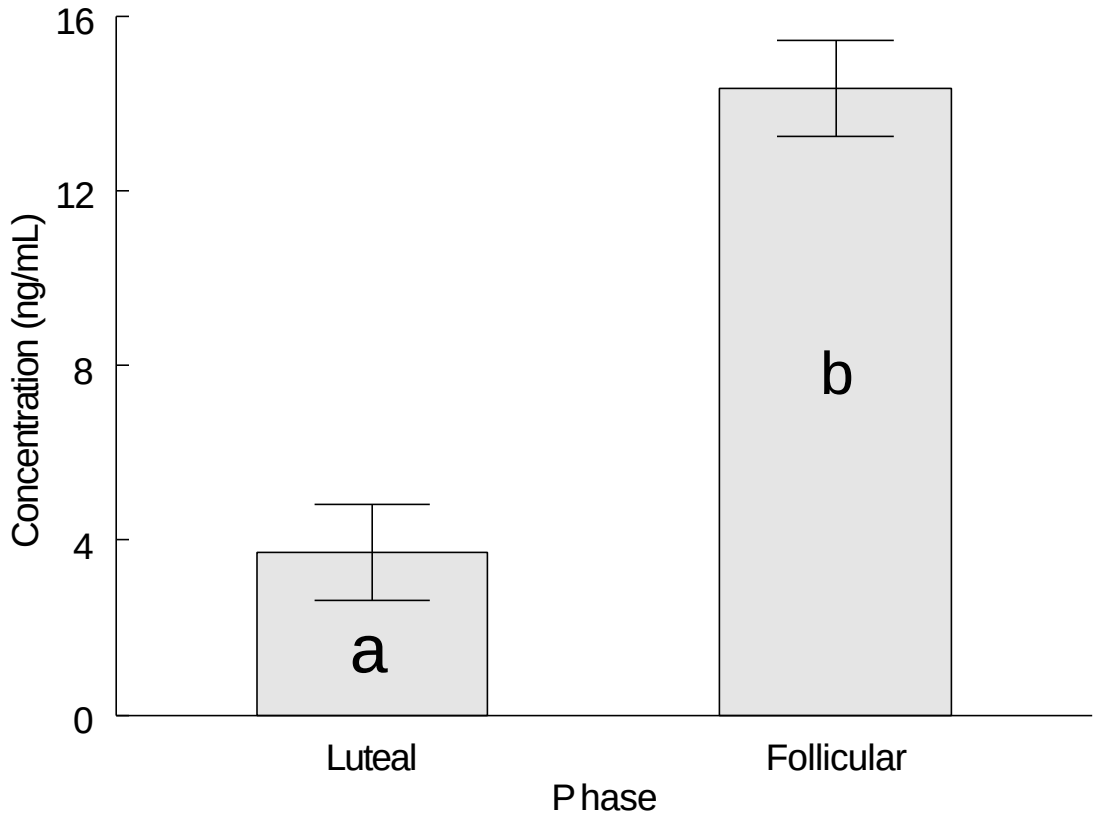

Figure 5-2. Maximum LH concentration after GnRH analog administration during luteal and follicular phases in beef cows. The maximum concentration of LH (LS means ± SEM) after GnRH analog administration was effected ($P < .0001$) by stage of estrus cycle. Means represented by bars with different letters (a,b) differ.

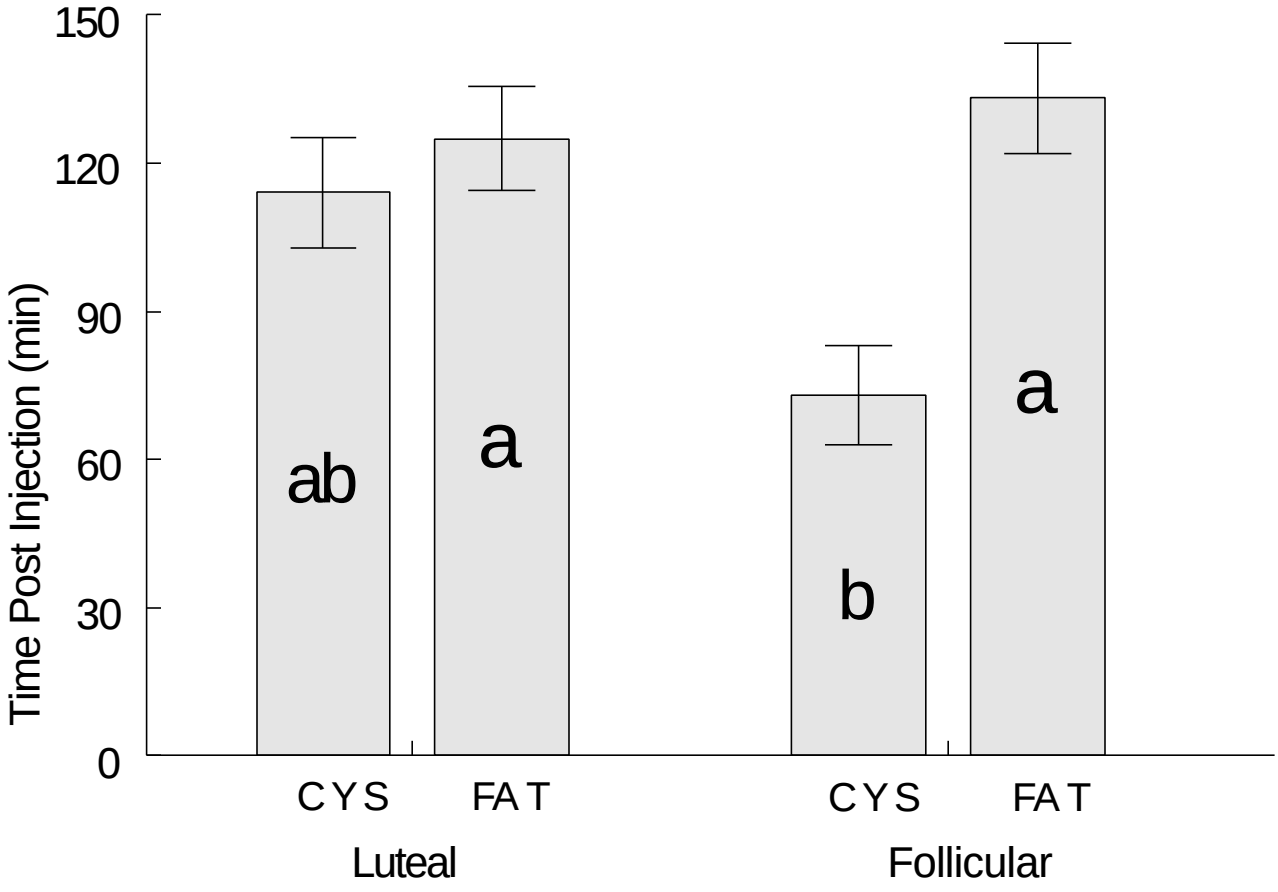

Figure 5-3. Treatment by phase interaction for time to maximal LH concentration after GnRH analog administration in beef cows. A TRT x PHS interaction was noted for time to maximum concentration of LH (LS means ± SEM) after GnRH analog administration (P = .03). The time of the LH peak in response to Cystorelin and Factrel was similar in the luteal phase but differed in the follicular phase during which time the response to Cystorelin was shorter. Means represented by bars with different letters (a,b) differ.

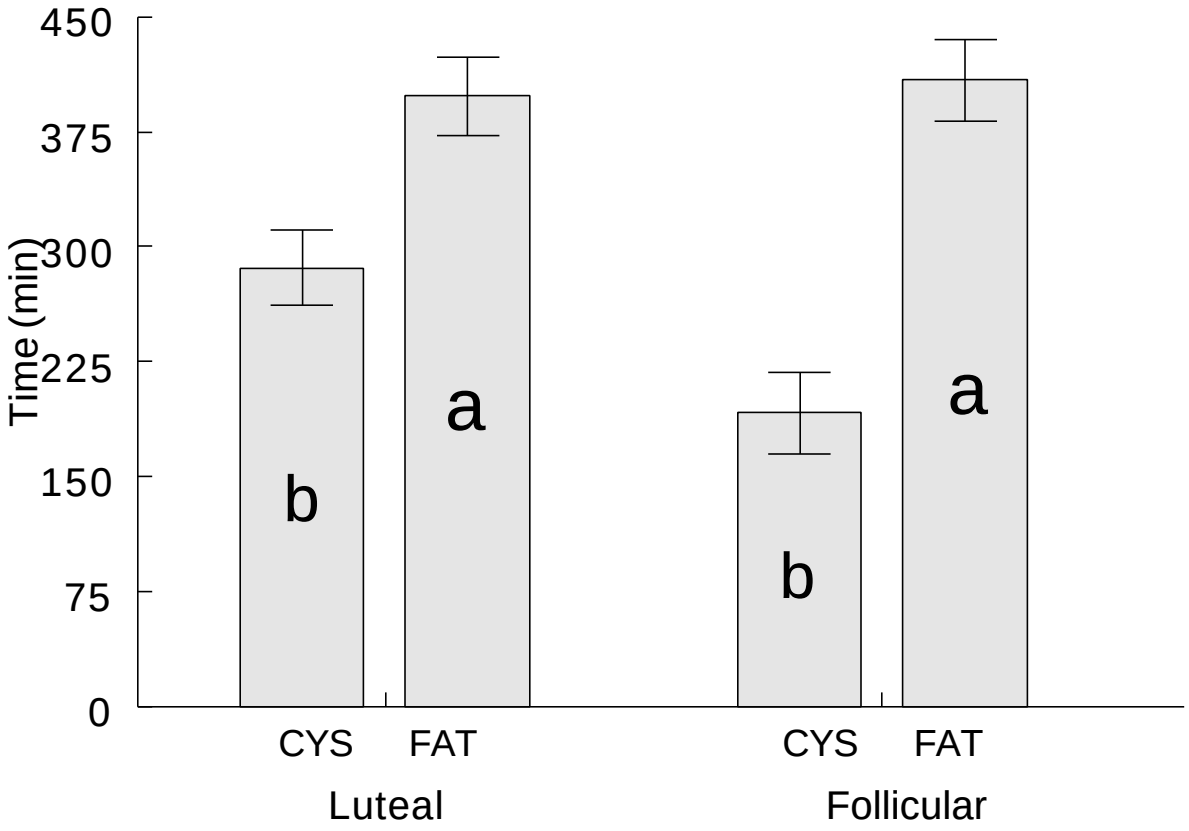

Figure 5-4. Treatment by phase interaction for duration of detectable LH response after GnRH analog administration in beef cows. A TRT x PHS interaction was noted for duration of detectable LH response (LS means ± SEM) after GnRH analog administration (P = .05). Cystorelin treated cows differed (P < .02) than Factrel treated during the luteal phase. Means represented by bars with different letters (a,b) differ.

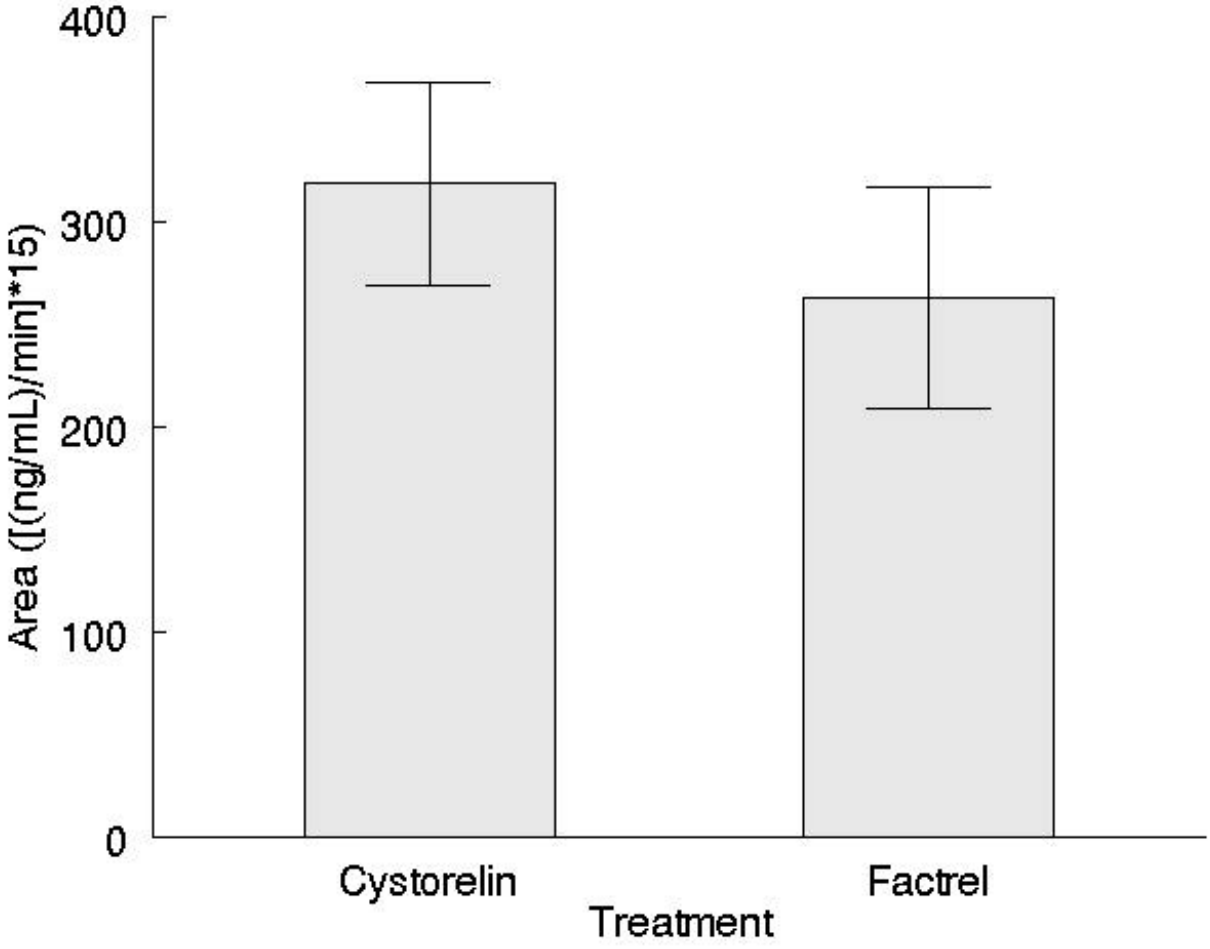

Figure 5-5. Calculated area beneath the LH curve during detectable response after Cystorelin and Factrel administration in beef cows. No differences (P = .55) were detected between Cystorelin and Factrel treated cows for area beneath the LH curve. Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA.

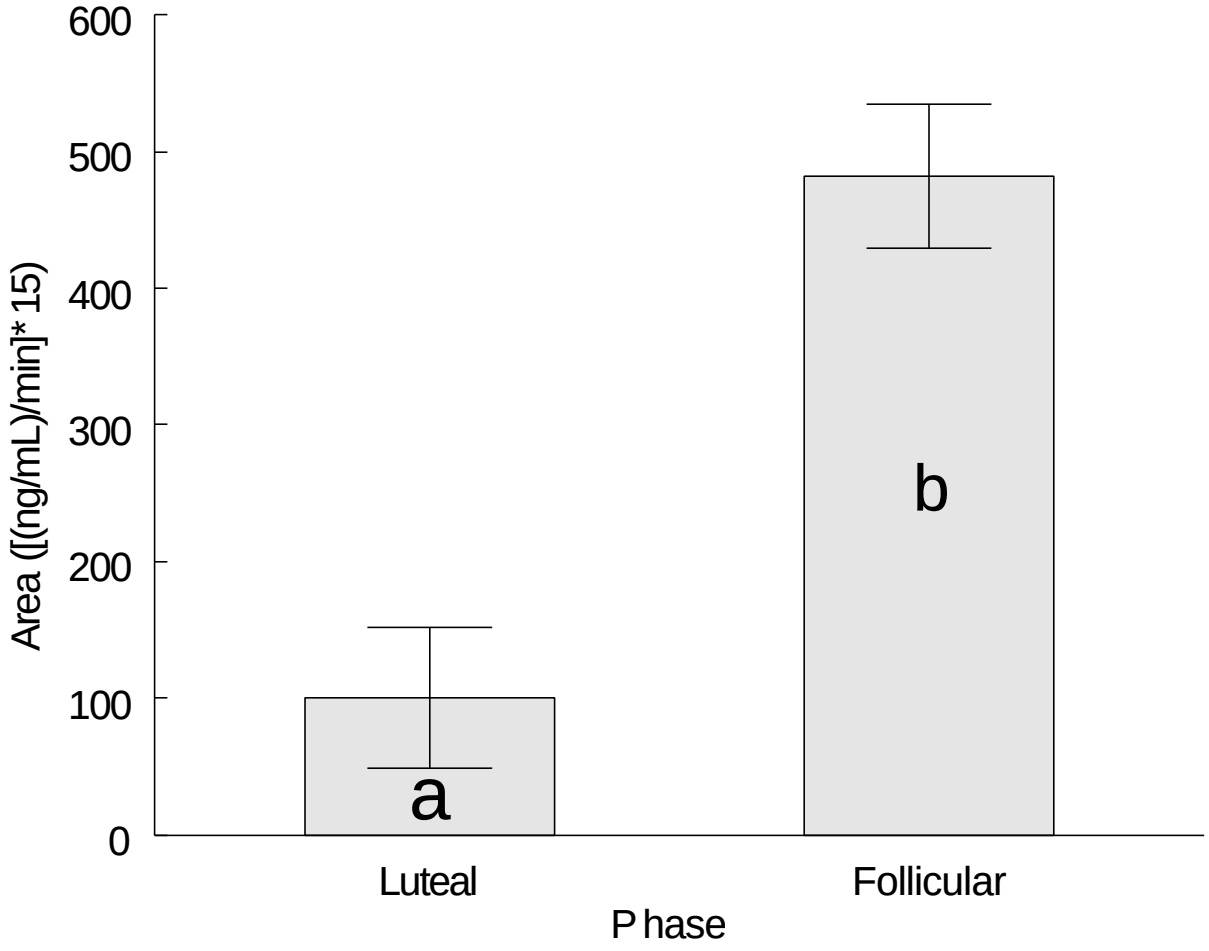

Figure 5-6. Calculated area beneath the LH curve during detectable response after GnRH analog administration in beef cows. Differences ($P < .0001$) were detected between the luteal and follicular phases after GnRH analog administration. Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA. Means represented by bars with different letters (a,b) differ.

Table 5-1. Summary of ultrasound results. Differences were detected for variables quantity of non ovulatory follicles and total follicles in excess of 3 mm per d. In both cases Cystorelin treated cows had greater numbers than Factrel treated cows. The remainder of variables analyzed did not differ.

Summary of Ultrasound Data

Response Variable	Cystorelin	Factrel	P value
Quantity of non-ovulatory follicles	3.21 ± .24	2.43 ± .22	**.02**
Total follicles in excess of 3 mm present per day	3.79 ± .26	3.01 ± .23	**.03**
Ovulatory follicle size at first GnRH analog injection	11.6 ± .9 mm	10.5 ± .82 mm	.39
Day of new follicular wave emergence post first GnRH analog injection	2.0 ± .4 d	2.6 ± .4 d	.30
Rate of ovulatory follicle growth	1.19 ± .46 mm/d	.711 ± .43 mm/d	.47
Ovulatory follicle peak size	13.3 ± 1.2 mm	12.7 ± 1.1 mm	.71
Ovulatory follicle size at second GnRH analog injection	13.2 ± 1.3 mm	12.3 ± 1.1 mm	.59
Day ovulation had occurred post second GnRH analog injection	2.2 ± .3 d	2.3 ± .3 d	.83

Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA.

Chapter VI

EFFICACY OF CYSTORELIN VERSES FACTREL VERSES FERTYGAL: OVINE LUTENIZING HORMONE BLOOD PROFILE

Introduction

An additional experiment utilizing 16 crossbred ewes of various ages and all in good body condition was conducted to determine if the LH releasing response between Cystorelin, Factrel, and Fertagyl differed. While Fertagyl and Cystorelin are chemically the same compound, they are supplied by different manufactures. The manufacture of Cystorelin utilized sodium phosphate for pH adjustment, the Factrel preparations has potassium phosphate added for the same purpose.

Materials and Methods

Animal Maintenance and Preparation

Seven d prior to blood sample collection, 16 ewes were placed at random into one of three treatment groups (Cystorelin, n = 5; Factrel, n = 5; Fertagyl, n = 6), and were transported from the Virginia Tech Sheep Center to the Animal Science building to allow acclimation to the environment. Ewes were maintained in crates .75 by 2 m long. Water was available ad libitum prior to and during the collection process and feed was available once per d in a quantity according to the Stockman's Handbook.

All ewes exhibited at least two distinct periods of estrus as checked by vasectomized rams. On d 0, all ewes received a 3 mg norgestomet implant (i.e., one half of a 6-mg Syncro-Mate-B implant; Rhone Merieux, Athens, GA) in the dorsum of the ear. Six d following implantation all ewes were injected with $PGF_{2\alpha}$ (5mg + 5mg 4h later; Lutalyse, Pharmacia and Upjohn, Kalamazoo, MI.) to induce luteolysis. Implants were removed on d 9 and ewes were observed for estrus as checked by vasectomized rams. Ewes were fitted with jugular catheters d –1 to GnRH analog injection. Ewes were injected with either 100 µg Cystorelin,

Factrel or Fertagyl 11 d following synchronized estrus. Blood samples were collected at –30, -15, 0 and at 15 min increments for a total of 585 min. After collection, blood samples were centrifuged at 2600 x g for 30 min at 4°C. The serum portion was collected and stored at –150° C and –20° C until assays were conducted.

Blood Collection, Storage, and Assays

Blood collection, storage, and assays producers were conducted in the same manner as described in Chapter V.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS, 1999). Significant differences were considered to have a P value less than 0.05. When significant differences were detected, Tukey's procedure was utilized to determine differences among treatment least squared means. Duration of LH response was deemed the interval in which LH levels were in excess of .0309 ng/mL, the lowest detectable levels of the assay. Any value less than the lower limit of the assay was assigned the value .0309 ng/mL. Total area under the LH curve was calculated by multiplying all detectable LH concentrations by 15 and summing.

Results

Maximum concentration of LH after GnRH analog administration was different among treatments. Maximum concentrations for Cystorelin, Factrel, and Fertagyl were 32.55 ± 5.55, 17.01 ± 5.55, and 37.53 ± 5.07, respectively. Factrel and Fertagyl differed (see Figure 6-1). Time to maximum concentration post GnRH analog administration for Cystorelin, Factrel and Fertagyl were 102.0 ± 11.9, 108.0 ± 11.9, and 115.0 ±10.9 min respectively (see Figure 6-2). No effects were observed for duration of detectable response (concentrations greater than .0309 ng/mL, the lowest limit of the assay). Durations were 330.0 ± 30.4, 255.0 ± 30.4, and 327.5 ± 27.7 min for Cystorelin, Factrel, and Fertagyl, respectively (Figure 6-3). A tendency (P = .08) for treatment differences was observed for total area under the resulting

LH curve. Areas were 1117 ± 213, 617 ± 213, and 1309 ± 194 [ng/mL]/min, for Cystorelin, Factrel and Fertagyl, respectively. Factrel and Fertagyl differed (see Figure 6-4).

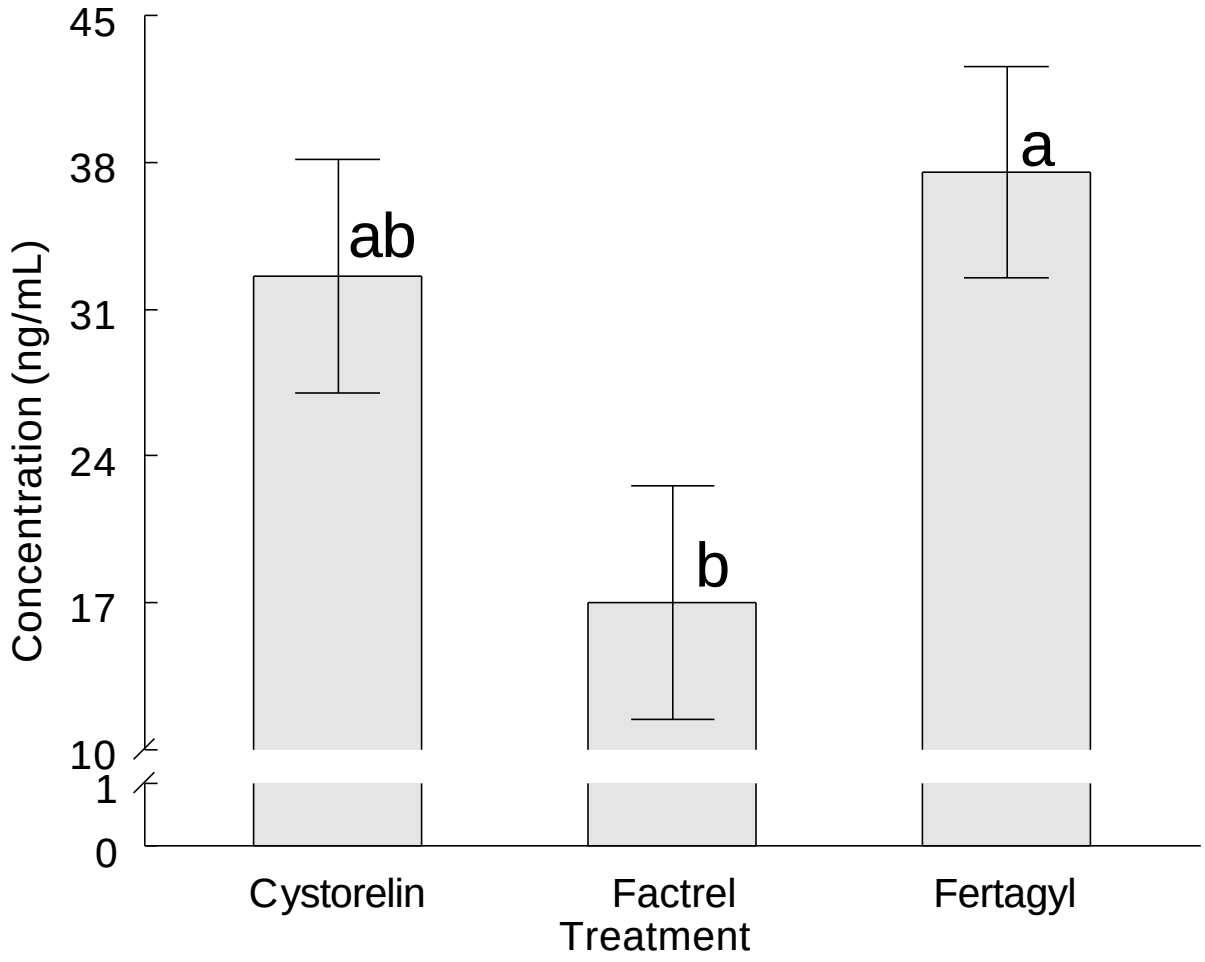

Figure 6-1. Maximum concentration of LH after GnRH analog administration in sheep. Maximum concentration of LH (LS means ± SEM) after GnRH analog administration differed among treatments (P = .05)[a,b]. Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA. Means represented by bars with different letters (a,b) differ.

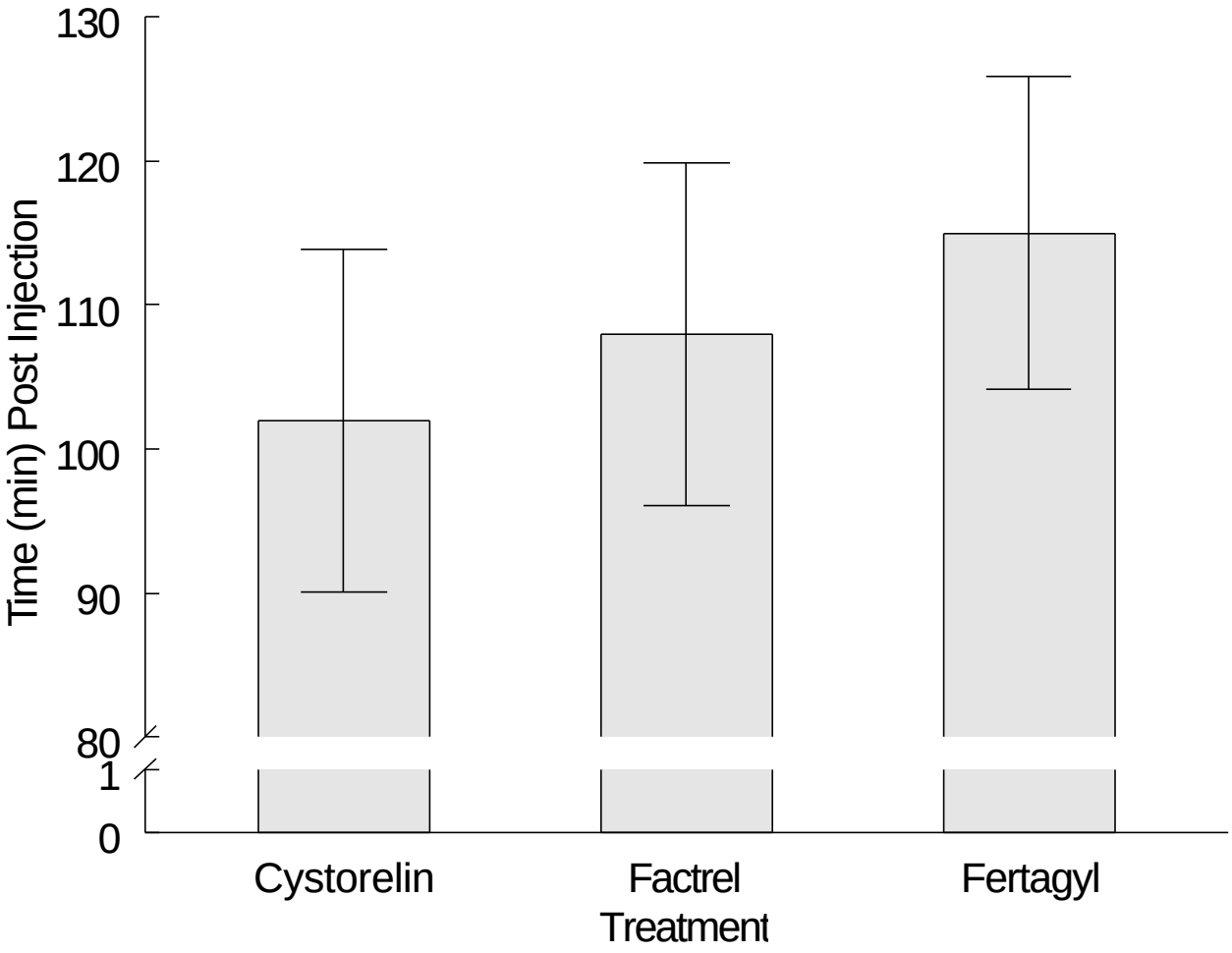

Figure 6-2. Time to maximum concentration post GnRH analog administration in sheep. Time to maximum concentration (LS means ± SEM) post GnRH analog administration did not differ among treatments (P = .73). Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA. Means represented by bars with different letters (a,b) differ.

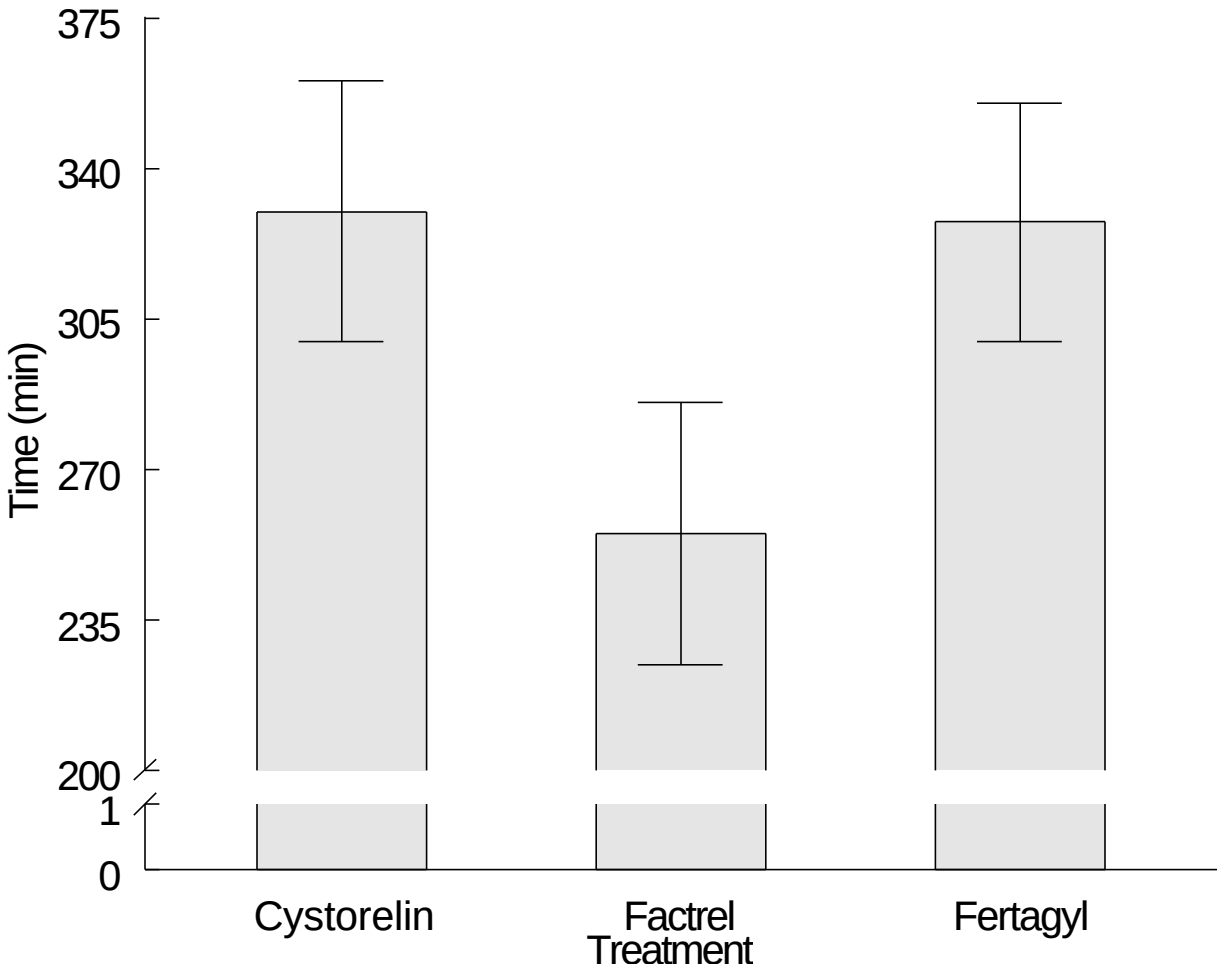

Figure 6-3. Duration of detectable LH response after GnRH analog administration in sheep. No treatment effects (P = .1722) were observed for duration of detectable (concentrations > .0309 ng/mL) response. Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA. Means represented by bars with different letters (a,b) differ.

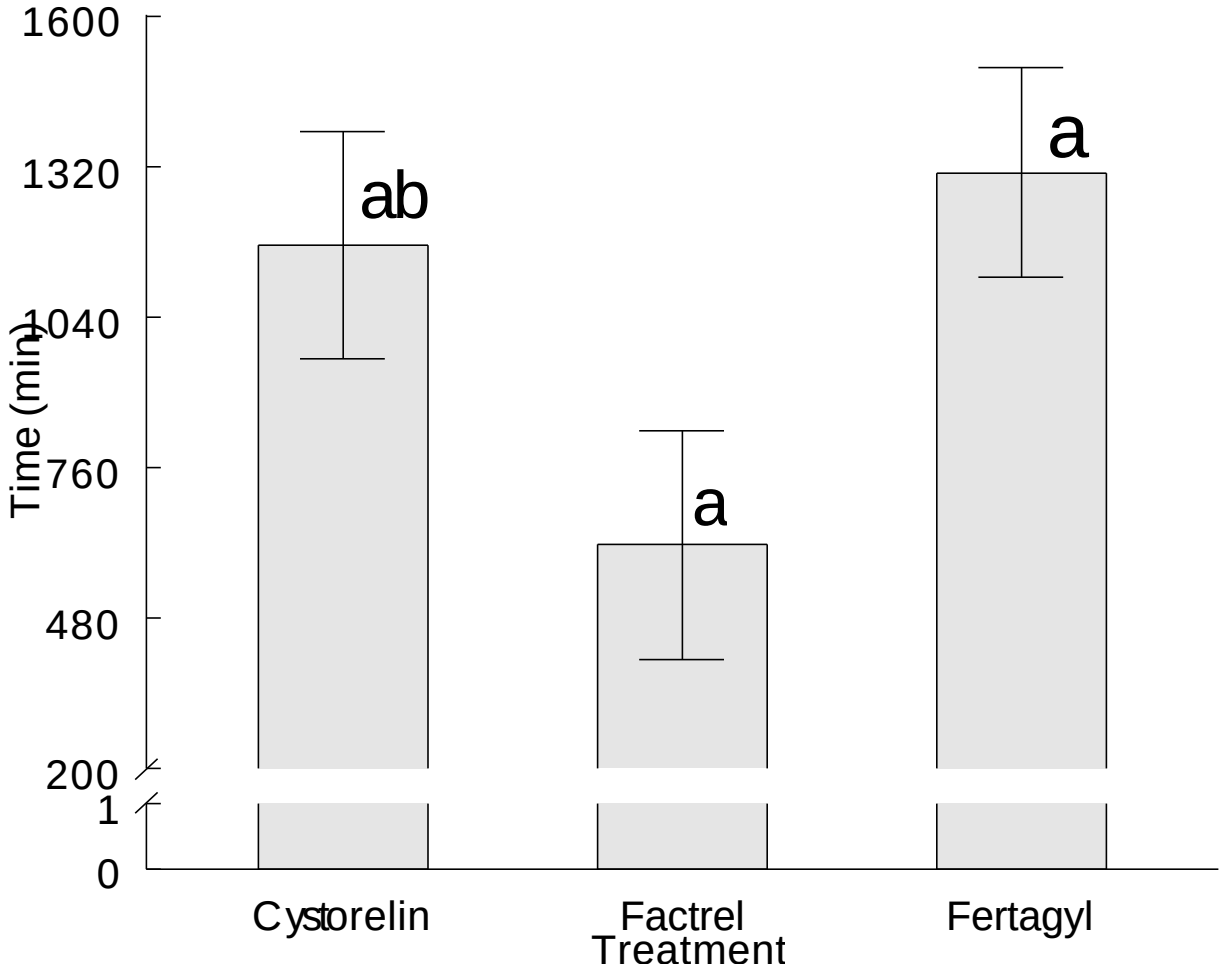

Figure 6-4. Area under LH curve after GnRH analog administration in sheep. A tendency (P = .08) for treatment differences was observed for total area (LS means ± SEM) under the resulting LH curve. Factrel and Fertagyl differed (P = .03). Values (LS means ± SEM) are represented and were analyzed with two-way ANOVA. Means represented by bars with different letters (a,b) differ.

Chapter VII
DISCUSSION AND IMPLICATIONS

Several conclusions can be drawn from these experiments. First, cattle treated with Factrel, as part of the Ovsynch protocol, had a greater tendency (P = .09) to establish and maintain pregnancy to at least d 45. Treatment by phase interactions were found for the time to maximum LH concentration, and for the duration of detectable response. From the ultrasound data, it can be concluded that cows treated with Cystorelin had a greater quantity of non-ovulatory follicles and more total follicles than Factrel treated cows. Data collected from the sheep study complimented the cow data in all responses except that LH maximum concentration occurred earlier for ewes treated with Factrel. Based upon these data, it is concluded that either GnRH analog, Cystorelin or Factrel, may be utilized for synchronization of ovulation without compromising fertility.

Prior to the advent of synchronization protocols utilizing GnRH analogs and $PGF_{2\alpha}$, conception rates after timed breeding were inconsistent. Timed breeding after $PGF_{2\alpha}$ treatment apparently had variable results due to fluctuations in ovulation time (Kaim et al., 1990; Risco et al., 1998) or even ovulation failure (Stevenson et al., 1987; Tenhagen et al., 2000). Our results demonstrate use of the Ovsynch protocol provides an acceptable ovulation interval for timed AI. Chenault et al. (1990) demonstrated an injection of a GnRH analog induced a LH and FSH surge. The first commercial GnRH analogs were utilized as a treatment for follicular cysts and to improve early-postpartum reproductive efficiency (Britt et al., 1977; Nash et al., 1980; Kesler and Garverick, 1982; Benmrad and Stevenson, 1986). Our results do not dispute the finding of Macmillan and Thatcher (1991) who reported ovulation or luteinization occurs to large follicles present at the time of GnRH analog administration.

Several early reports indicated GnRH administration at first breeding does not improve conception rates (Gunzler, et al., 1974; Kazmer et al., 1981; Echternkamp and Maurer., 1983; Lee et al., 1983, 1995; Stevenson et al, 1984b; and Graves et al., 1985). Others report the contrary for repeat breeder cows (Gunzler et al., 1974; Schels and Mostafawi, 1978; Lee et al., 1983; Lucy and Stevenson, 1986; and Stevenson et al., 1984b). Coleman et al. (1988) demonstrated GnRH had no effect on conception rate (76.7%), with

administration at AI. Mori and Takahashi (1978) found GnRH analog administration at time of insemination increased conception rate (75.0%) over controls (61.3%), which is similar to our results.

More recent reports indicate synchronization protocols that utilize GnRH, followed 7 d later with $PGF_{2\alpha}$, improve estrus detection rates and provide tighter synchrony of estrus (Thatcher et al., 1993; Wolfenson et al., 1994; and Twagiramungu et al., 1995). Vasconcelos et al. (1999) found 87% of cows ovulated after the second GnRH injection, and 64% after the first GnRH injection. In our experiment only 1 out of 20 cows failed to ovulate after the first GnRH analog injection, and all ovulated after the second GnRH analog injection. This may be due to the presynchornization protocol we implemented. Thompson et al. (1999) and Stevenson et al. (2000) demonstrated that GnRH protocols result in fertile estrus in cycles, in estrual well as anestrual cows. The establishment of the Ovsynch protocol (Pursley et al., 1995) improved conception rates after timed AI by synchronizing ovulation (Burke et al., 1996; and Pursley et all, 1997a,b). Risco et al. (1998) demonstrated that Ovsynch increased net revenue per dairy cow, hence one reason we chose to use this protocol. Conception rates after timed AI can be improved by breeding at the time of observed estrus (Stevenson et al., 1996, 1999; and Pursley et al., 1997b). After treatment, the peak estrus response occurs around 60 h post $PGF_{2\alpha}$ injection (Geary et al., 2000; Stevenson et al., 2000; and DeJarnett et al., 2001). Thus, drug costs and pregnancy rates may be improved by inseminating cows at observed estrus 3 d after $PGF_{2\alpha}$ injection followed by insemination of all untreated cows in addition to a GnRH injection (Stevenson et al., 2000; Lemaster et al., 2001).

Results from the field studies indicate a tendency for Factrel to be more effective than Cystorelin in establishing pregnancy to at least d 45 when utilizing the Ovsynch protocol. The pregnancy rates at d 45 overall for Cystorelin (54.75%), and Factrel (64.07%), are similar to Pursley et al's. (1995) results when using Cystorelin and obtaining 50% pregnancy rates, and Twagiramungu et al. (1992c) using buserelin with 87.0% pregnancy rates. Geary et al. (1998) found 54% percent of random cows conceived while 59% of cyclic cows became pregnant. In our experiment, to account for these effects, cows were grouped by body condition score and days post partum. Lemaster et al. (2001) found conception rates after

Select-synch were 50.5% and pregnancy rates were 20.8%. Thus, our results are typical and did not compromise our ability to detect differences.

Our data show an effect of location by treatment for cattle undergoing the Ovsynch protocol when Cystorelin and Factrel are utilized as GnRH analogs. At only one location, out of seven, was GnRH analog treatment significant. Due to the low number of cows at each location, we conclude that treatment can not account for all of the differences. Days postpartum and body condition score were analyzed as covariates and found to be insignificant. Greater effects such as days postpartum, body condition score, plane of nutrition, or age may account for the differences detected. Our results demonstrate with low animal numbers, such as is common with most beef herds within Virginia, that inaccurate conclusions can bias results. Perceived differences may be due to simple random chance.

Such variation in results may be attributed to variation in follicular status at time of treatment. Analogs of GnRH have been administered at random stages of the estrous cycle and it was found that the stage of follicular development affects synchrony of a new wave (Prescott et al., 1992; Silcox et al., 1993; Pursley et al., 1995; and Twagiramungu et al., 1995). Martinez et al. (1999) found ovulation occurred in 89%, 56%, and 22% of cows treated with GnRH analogs on d 3, 6, and 9 of the estrous cycle.

De Jarnette et al., (2001) reports 8 to 10% of treated beef cows exhibit premature estrus behavior due to GnRH's inability to turnover older dominant follicles. In the present experiment, any cows which were observed in estrus were bred 12 h later. Augmentation of protocols utilizing GnRH and prostaglandin $F_{2\alpha}$ with progestin treatment can prevent premature estrus (Thompson et al., 1999; Stevenson et al., 2000). A limitation of this approach is that during times of treatment with progestins development of persistent follicles can occur (Patterson et al, 1989; Kinder et al., 1996). Despite this, several laboratories report success using GnRH, $PGF_{2\alpha}$ and progestin combinations (Ryan et al., 1995; and Stevenson et al., 2000; DeJarnette et al., 2001).

Some producers may be discouraged from using GnRH- $PGF_{2\alpha}$ synchronization due to costs. Publications from Britt and Gaska (1998) and Nebel and Jobst (1998) indicate most of the hormone cost associated with the Ovsynch protocol is associated with GnRH analogs.

Fricke et al. (1998) and Whittier and Hall (unpublished data) demonstrated the dosage of GnRH may be reduced by half without compromising the efficacy of the treatment.

The LH profile experiments demonstrated similarities and differences from a similar experiment reported by Bently et al. (1998). Bentley et al. (1998) found peak LH release during the luteal phase occurred at either 1 or 2 h post GnRH analog injection. Cystorelin's peak at 6.6 +/- 4.3 ng/mL at 2 h Fertagyl and Factrel were 4.7 +/- 2.6 ng/mL at 1 h and 3.8 +/- 1.7 ng/mL at 2 h. Coleman et al. (1988) found heifers treated with GnRH analog at 6 rather than 12 h post estrus exhibited the greatest LH response, and also found administration did not have an effect on subsequent P_4 production. Mori et al. (1974) found peak LH response to Des-Gly-$NH^{10}{}_2$, Pro-ethylamide^9-LH-RH occurred 20-30 min after administration and remained elevated for 3 h. Mori et al. (1979) found peak level of LH after Des-Gly-$NH^{10}{}_2$, Pro-ethylamide^9-LH-RH administration occurred between 90 and 105 min. Mori et al. (1981) found when GnRH analogs were injected into male calves, LH concentrations peaked at 120 min post injection. Williams et al. (1982) and Chenault et al. (1990) demonstrated administration of a GnRH analog resulted in a LH surge within 2 to 3 h after administration in cattle. Our results did not demonstrate differences among time of maximum concentration.

Bentley found ovulation occurred more frequently after Cystorelin treatment (90%) than in Fertagyl (50%) or Factrel (40%). No difference in ovulation rate was found since all cows ovulated post treatment.

The peak size of ovulatory follicles are similar between Bently et al. (Cystorelin 13.9 +/- 2.5 mm, Fertagyl 10.3 +/- 4.8 mm, Factrel 11.7 +/- 4.6 mm, 1998) and our data. Pursley et al. (1995) found peak size after GnRH treatment was 15.7 +/- 0.48 mm in cows. Pursley et al. (1995) found growth rate from emergence until secondary GnRH injection in Ovsynch was 1.34 +/- 0.09 mm/d, similar to our results. Our results also correspond to Pursley et al's. (1995) data for size of ovulatory follicle on d of GnRH analog injection (13.9 +/- 0.51). Day of wave emergence did not differ from our study and Pursley et al. (2.1 +/- 0.31 d; 1995). The d of new wave emergence was later in the present study than Bentley et al. (Cystorelin 1.1 +/- 1.4, Fertagyl 1.4 +/- 2.0, Factrel 1.4 +/- 1.2 d, 1998). Ginther et al. (1989a) reported that dominant follicles grow linearly for 6 d, maintain a constant diameter for 6 d, then regress.

In summary, the following differences among treatments (excluding phase differences) were found. 1.) A tendency for Factrel treatment to result in more pregnancies maintained to at least d 45 than Cystorelin treatment when used in the Ovsynch protocol. 2.) A treatment by phase interaction was found for the time to maximum LH concentration. Cystorelin administered during the follicular phase resulted in a shorter time to maximal concentration than did any other treatment during any phase. 3.) The duration of detectable response was greater after Cystorelin treatment. 4.) A treatment by phase interaction was observed for the duration of a detectable response. Cystorelin-induced LH release during the luteal phase was detectable for a longer time than Factrel. However, during the follicular phase Factrel-induced LH response was detectable for a longer duration. 5.) Area under the LH curve was greater for Cystorelin, however not statistically different. 6.) Treatment with Cystorelin resulted in greater quantity of non-ovulatory follicles. 7.) Total follicles observed after Cystorelin treatment was greater. 8.) The sheep results complimented the cow results for maximum concentration and duration of detectable response. However, Factrel treated ewes' maximum concentration occurred later than Cystorelin treated ewes. Other variables measured for these studies did not differ among treatments.

If producers are unsatisfied with pregnancy rates after GnRH-$PGF_{2\alpha}$ induced synchrony and timed AI, several management factors can be implemented to increase effectiveness. Fertility may be improved after synchronization of ovulation protocols with 48 h calf removal d 7 to 9 of treatment (Geary et al., 2001). Conception rates after Ovsynch in dairy cows can be affected by month of insemination, d of milk production, heat stress, and body condition score (Burke et al., 1996; De la Sorta et al., 1998; and Moreia et al., 2000). Klindworth et al. (2001) demonstrated body condition score had an effect on first service conception rate in North German dairy cows. Moreira reported dairy cows in low body condition had reduced pregnancy rates to the Ovsynch protocol. Vasconcelos et al. (1999) demonstrated cows undergoing Ovsynch treatment, which was initiated during mid, cycle had smaller ovulatory follicles and greater pregnancy rates.

Based on these observations, more in-depth research is necessary to understand the rational for observed differences, and more animal units are necessary to determine if

treatment differences exist between Cystorelin and Factrel. Using these observations, producers can base GnRH analog choice upon personal preference without affecting fertility.

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APPENDICES

Appendix A
Luteinizing Hormone Radioimmunoassay Reagents

Phosphate Buffered Saline (PBS) Stock Solution (0.14 M NaCl, 0.01 M PO_4, pH 7.0)

Place 143 g NaCl in 1000 mL beaker

Add 8.2806 gm NaH_2 PO_4 H_2O (monobasic sodium phosphate)

Add 17.0352 gm Na_2HPO_4 (dibasic sodium phosphate, anhydrous)

Add 1.75 gm sodium ethylmercurithiosalicylate (Thimersol)

Dissolve in approximately 800 mL H_2O

Dilute to 1000 mL in volumetric flask

Transfer to storage container and store at 4^o C

Phosphate Buffered Saline Working Solution

Dilute 400 mL PBS stock to 7 L H_2O

Adjust pH to 7.0

Store at 4^o C

Assay Buffer (0.1 % PBS Gel)

Place 1.0 g gelatin in 1000 mL beaker

Add 10 mL 1:100 sodium ethylmercurithiosalicylate

Add approximately 800 mL PBS

Stir on hot plate/stir until dissolved

Add PBS to bring volume to 1000 mL

Stir

Freeze in 100 mL aliquots in plastic containers

PBS-EDTA (0.05 M EDTA-PBS pH 7.0)

Place 18.6125 g ethylene dinitrilotetraacetic acid, disodium salt (disodium EDTA) in 1000 mL beaker

Add 10 mL 1:100 merthiolate

Add approximately 800 mL PBS

Stir and heat until dissolved

Adjust pH to 7.0

Transfer to volumetric flask and bring to 1000 mL with PBS

Store at 4^{o} C

Normal Rabbit Serum (NRS)

Dilute NRS to 1:200 in PBS-EDTA

Store at 4^{o} C

Luteinizing Hormone for Calibration Curve

Purified lyophilized LH is reconstituted with assay buffer for final concentration of 5.0 ng/100 µL

Store at –20 ^{o} C

Appendix B
Luteinizing Hormone Radioimmunoassay Validation Procedure

0:00 hours

1. Dilute 100 mL/mL in H_20 oLH to 50 ng/mL with assay buffer
2. Make the following standard dilutions from 5.00 ng/100 µL standard:

Standard	Formula
2.50 ng/100 µL	1600 µL 5.00 ng/100 µL combined with 1600 µL assay buffer
1.25 ng/100 µL	1600 µL 2.50 ng/100 µL combined with 1600 µL assay buffer
0.625 ng/100 µL	1600 µL 1.25 ng/100 µL combined with 1600 µL assay buffer
0.313 ng/100 µL	1600 µL 0.625 ng/100 µL combined with 1600 µL assay buffer
0.156 ng/100 µL	1600 µL 0.313 ng/100 µL combined with 1600 µL assay buffer
0.078 ng/100 µL	1600 µL 0.156 ng/100 µL combined with 1600 µL assay buffer
0.039 ng/100 µL	1600 µL 0.078 ng/100 µL combined with 1600 µL assay buffer

Spiked Standard	Formula
2.50 ng/100 µL	800 µL 5.00 ng/100 µL standard and 800 µL low pool LH
1.25 ng/100 µL	800 µL 2.50 ng/100 µL standard and 800 µL low pool LH
0.625 ng/100 µL	800 µL 1.25 ng/100 µL standard and 800 µL low pool LH
0.313 ng/100 µL	800 µL 0.625 ng/100 µL standard and 800 µL low pool LH
0.156 ng/100 µL	800 µL 0.313 ng/100 µL standard and 800 µL low pool LH
0.078 ng/100 µL	800 µL 0.156 ng/100 µL standard and 800 µL low pool LH
0.039 ng/100 µL	800 µL 0.078 ng/100 µL standard and 800 µL low pool LH

Volumes	Formula
25 µL	25 µL high pool LH combined with 75 µL assay buffer
50 µL	50 µL high pool LH combined with 50 µL assay buffer
75 µL	75 µL high pool LH combined with 25 µL assay buffer
100 µL	100 µL high pool LH

3. Pipette assay buffer, standards, quality control samples, and volume samples according to the below chart. All are run in triplicate.

Tube #	ID	Standard	Spiked	Volumes	Buffer
1-3	TC	-	-	-	-
4-6	NSB	-	-	-	500 µL
7-9	Bo	-	-	-	500 µL
10-12	0.039 ng	100 µL	-	-	400 µL
13-15	0.078 ng	"	-	-	"
16-18	0.156 ng	"	-	-	"
19-21	0.313 ng	"	-	-	"
22-24	0.625 ng	"	-	-	"
25-27	1.25 ng	"	-	-	"
28-30	2.50 ng	"	-	-	"
31-33	0.019 ng	-	100 µL	-	"
34-36	0.039 ng	-	"	-	"
37-39	0.078 ng	-	"	-	"
40-42	.156 ng	-	"	-	"
43-45	.313 ng	-	"	-	"
46-48	.625 ng	-	"	-	"
49-51	1.25 ng	-	"	-	"
52-54	50 ng	-	"	-	"
55-57	25 µL	-	-	100 µL	"
58-60	50 µL	-	-	"	"
61-63	75 µL	-	-	"	"
64-66	100µL	-	-	"	"

4. Add 200 µL 1:200 NRS to tubes 4-6
5. Add 200 µL of 1:115,000 first antibody to all tubes except 1-6

6. Vortex lightly
7. Incubate at room temperature

06:00 hours

1. Add 100 µL 125I-LH tracer to all tubes
2. Vortex lightly
3. Incubate at room temperature

72:00 hours

1. Add 200 µL 1:100 second antibody to all tubes except 1-3
2. Vortex lightly
3. Incubate at room temperature

96:00 hours

1. Add 2 mL PBS at 4o C to all tubes except 1-3
2. Immediately centrifuge for 30 minutes, 4^{o} C, 2600 x g, no break
3. Decant liquid, invert only once
4. Count

Appendix C
Luteinizing Hormone Radioimmunoassay Validation Volumes

Tube #	ID	Standard	Spiked Volumes		Buffer	1st Ab	NRS	Tracer	2nd Ab
1-3	TC	-	-	-	-	-	-	100 µL	-
4-6	NSB	-	-	-	500 µL	-	200 µL	"	200 µL
7-9	Bo	-	-	-	500 µL	200 µL	-	"	"
10-12	0.039 ng	100 µL	-	-	400 µL	"	-	"	"
13-15	0.078 ng	"	-	-	"	"	-	"	"
16-18	0.156 ng	"	-	-	"	"	-	"	"
19-21	0.313 ng	"	-	-	"	"	-	"	"
22-24	0.625 ng	"	-	-	"	"	-	"	"
25-27	1.25 ng	"	-	-	"	"	-	"	"
28-30	2.50 ng	"	-	-	"	"	-	"	"
31-33	0.019 ng	-	100 µL	-	"	"	-	"	"
34-36	0.039 ng	-	"	-	"	"	-	"	"
37-39	0.078 ng	-	"	-	"	"	-	"	"
40-42	0.156 ng	-	"	-	"	"	-	"	"
43-45	0.313 ng	-	"	-	"	"	-	"	"
46-48	0.625 ng	-	"	-	"	"	-	"	"
49-51	1.25 ng	-	"	-	"	"	-	"	"
52-54	2.50 ng	-	"	-	"	"	-	"	"
55-57	25µL	-	-	100 µL	"	"	-	"	"
58-60	50µL	-	-	"	"	"	-	"	"
61-63	75µL	-	-	"	"	"	-	"	"
64-66	100µL	-	-	"	"	"	-	"	"

Appendix D
Luteinizing Hormone Radioimmunoassay Procedure

Tubes

1-30 Standard curve in triplicate

31-34 Quality control samples

35- Samples in duplicate

0:00 hours

1. Dilute 100 mL/mL in H_20 oLH to 50 ng/mL with assay buffer
2. Make the following standard dilutions from 5.00 ng/100 µL standard:

Standard	Formula
2.50 ng/100 µL	1600 µL 5.00 ng/100 µL combined with 1600 µL assay buffer
1.25 ng/100 µL	1600 µL 2.50 ng/100 µL combined with 1600 µL assay buffer
0.625 ng/100 µL	1600 µL 1.25 ng/100 µL combined with 1600 µL assay buffer
0.313 ng/100 µL	1600 µL 0.625 ng/100 µL combined with 1600 µL assay buffer
0.156 ng/100 µL	1600 µL 0.313 ng/100 µL combined with 1600 µL assay buffer
0.078 ng/100 µL	1600 µL 0.156 ng/100 µL combined with 1600 µL assay buffer
0.039 ng/100 µL	1600 µL 0.078 ng/100 µL combined with 1600 µL assay buffer

3. Pipette assay buffer, standards, quality control samples, and volume samples according to the following chart:

Tube #	ID	Standard	Pool	Buffer	Sample
1-3	TC	-	-	-	-
4-6	NSB	-	-	500 µL	-
7-9	Bo	-	-	“	-
10-12	0.039 ng	100 µL	-	400 µL	-
13-15	0.078 ng	“	-	“	-
16-18	0.156 ng	“	-	“	-
19-21	0.313 ng	“	-	“	-
22-24	0.625 ng	“	-	“	-
25-27	1.25 ng	“	-	“	-
28-30	2.50 ng	“	-	“	-
31-32	High pool	-	100 µL	“	-
33-34	Low pool	-	“	“	100 µL
35-	Samples	-	-	“	“

4. Add 200 µL 1:200 NRS to tubes 4-6
5. Add 200 µL of 1:115,000 first antibody to all tubes except 1-6
6. Vortex lightly
7. Incubate at room temperature

06:00 hours

1. Add 100 µL ^{125}I-LH tracer to all tubes
2. Vortex lightly
3. Incubate at room temperature

72:00 hours

1. Add 200 µL 1:100 second antibody to all tubes except 1-3
2. Vortex lightly
3. Incubate at room temp

96:00 hours

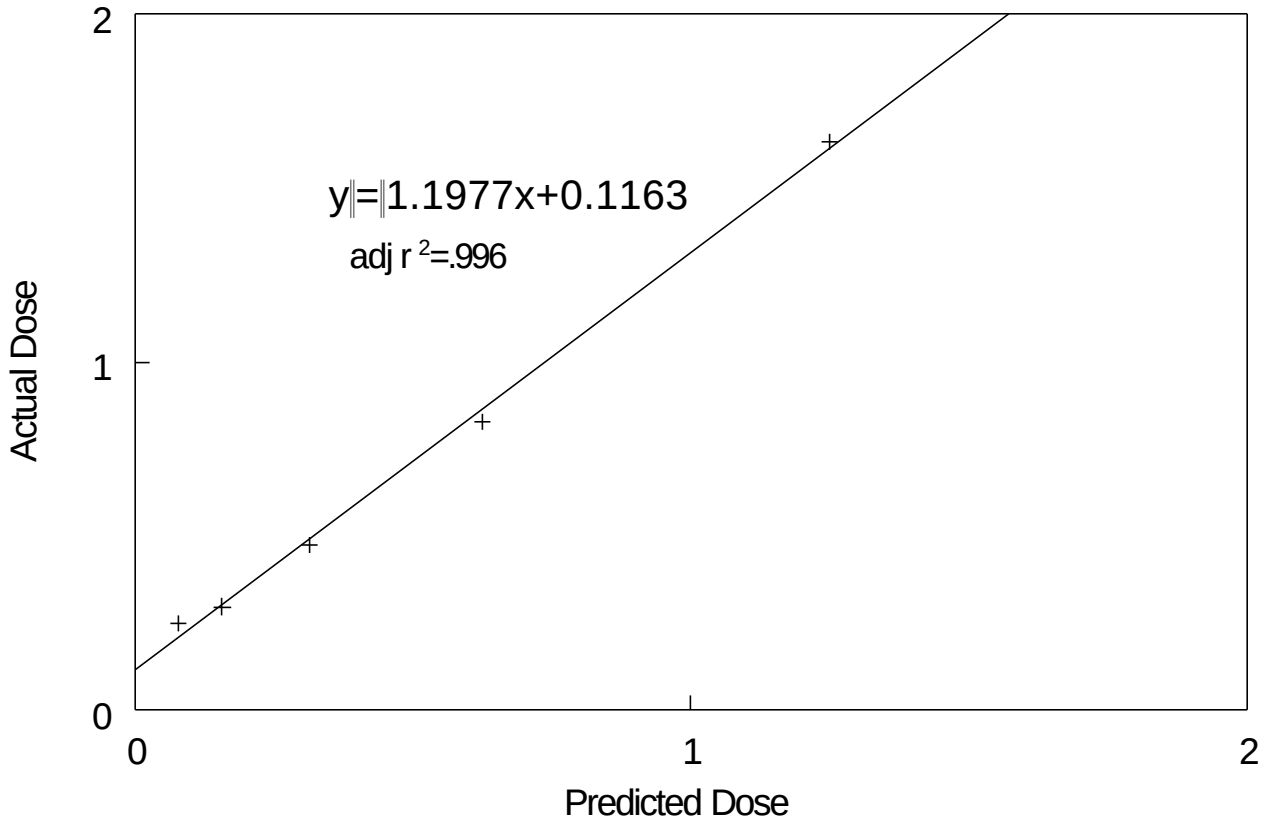
1. Add 2 mL PBS at 4o C to all tubes except 1-3
2. Immediately centrifuge for 30 minutes, 4^{o} C, 2600 x g, no break
3. Decant liquid, invert only once, pellet is very fragile
4. Count

Appendix E
Luteinizing Hormone Radioimmunoassay Validation Volumes

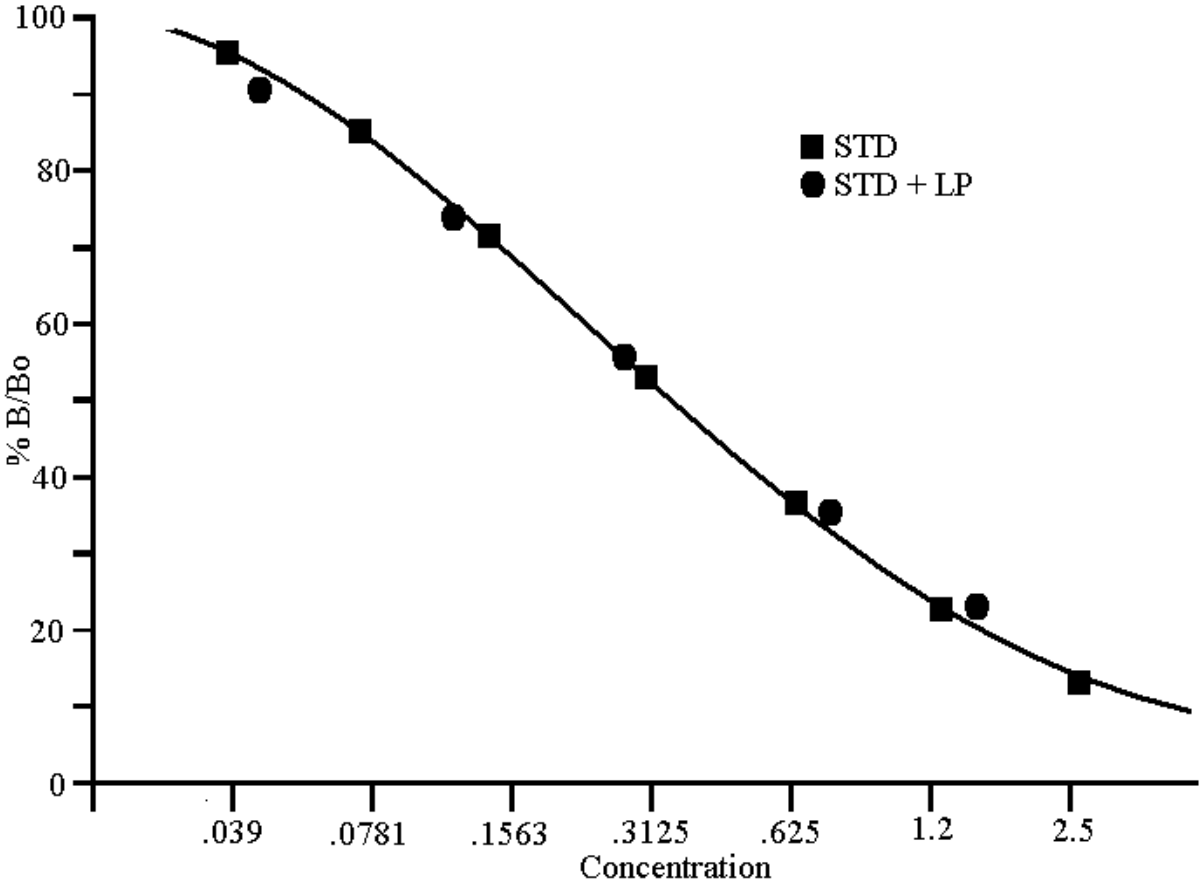
Tube #	ID	Standard	Pool	Sample	Buffer	1st Ab	NRS	Tracer	2nd Ab
1-3	TC	-	-	-	-	-	-	100 µL	-
4-6	NSB	-	-	-	500 µL	-	200 µL	"	200 µL
7-9	Bo	-	-	-	500 µL	200 µL	-	"	"
10-12	0.039 ng	100 µL	-	-	400 µL	"	-	"	"
13-15	0.078 ng	"	-	-	"	"	-	"	"
16-18	0.156 ng	"	-	-	"	"	-	"	"
19-21	0.313 ng	"	-	-	"	"	-	"	"
22-24	0.625 ng	"	-	-	"	"	-	"	"
25-27	1.25 ng	"	-	-	"	"	-	"	"
28-30	2.50 ng	"	-	-	"	"	-	"	"
31-32	High pool	-	100 µL	-	"	"	-	"	"
33-34	Low pool	-	"	100 µL	"	"	-	"	"
35-	Samples	-	-	"	"	"	-	"	"

Appendix F
Luteinizing Hormone Radioimmunoassay Validation Data

Appendix F. Figure 1. Luteinizing hormone radioimmunoassay validation - actual dose vs. predicted dose.

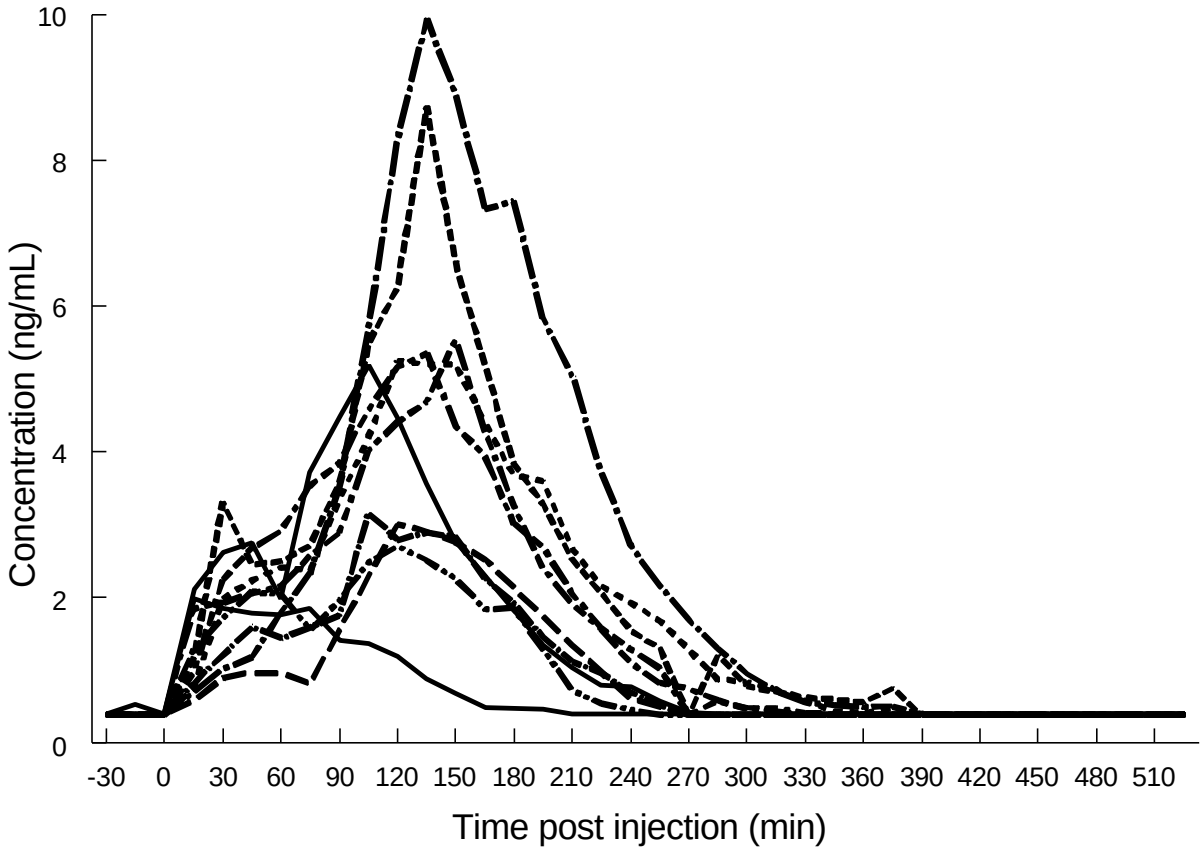


Appendix F. Figure 2. Luteinizing hormone radioimmunoassay - validation %B/Bo vs. concentration. STD, standard; STD+LP, standard + blood serum from mid luteal dairy cow

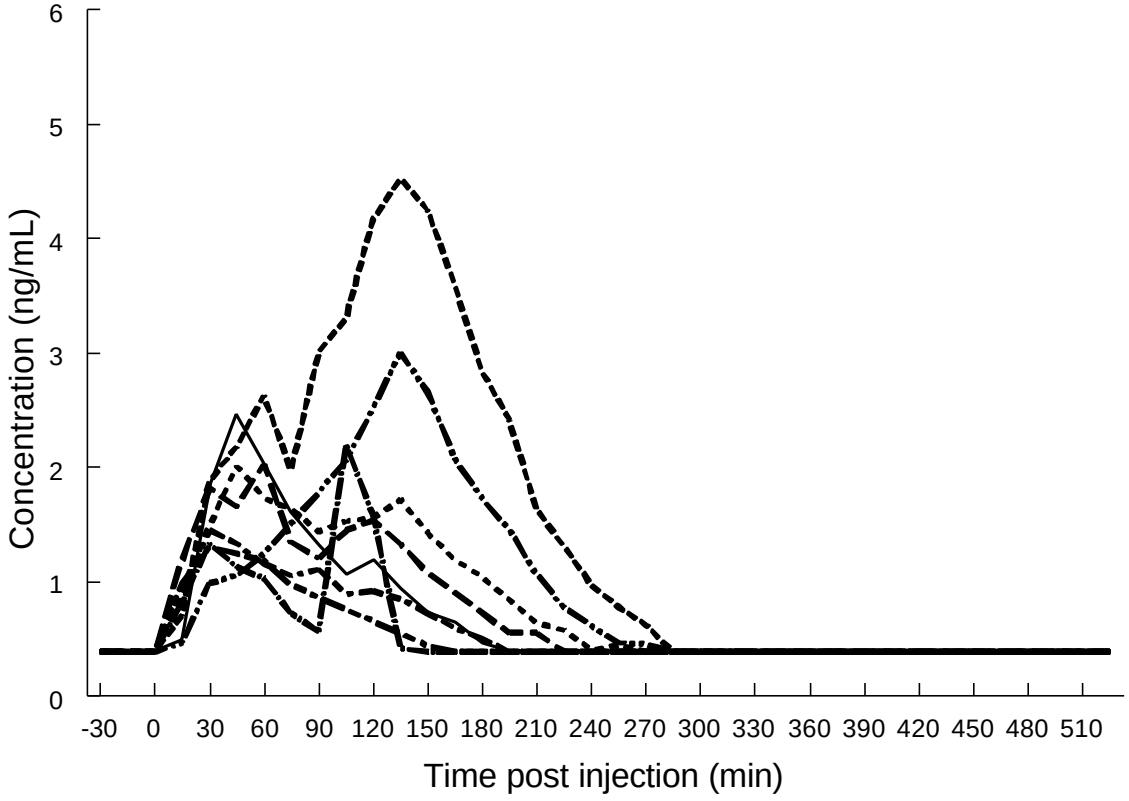


Appendix G
Luteinizing Hormone Profile Data

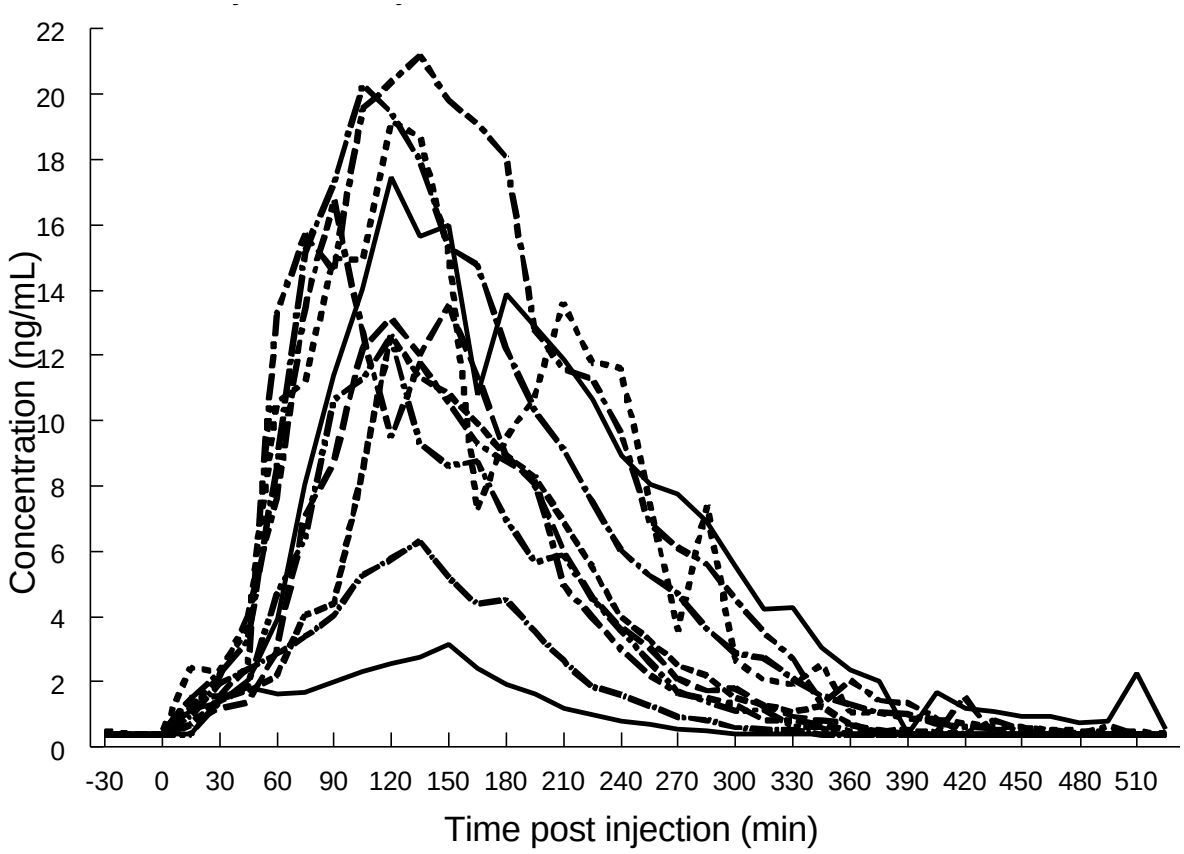
Appendix G. Figure 1. Serum luteinizing hormone concentration after Cystorelin injection in luteal phase beef cows (n = 10).



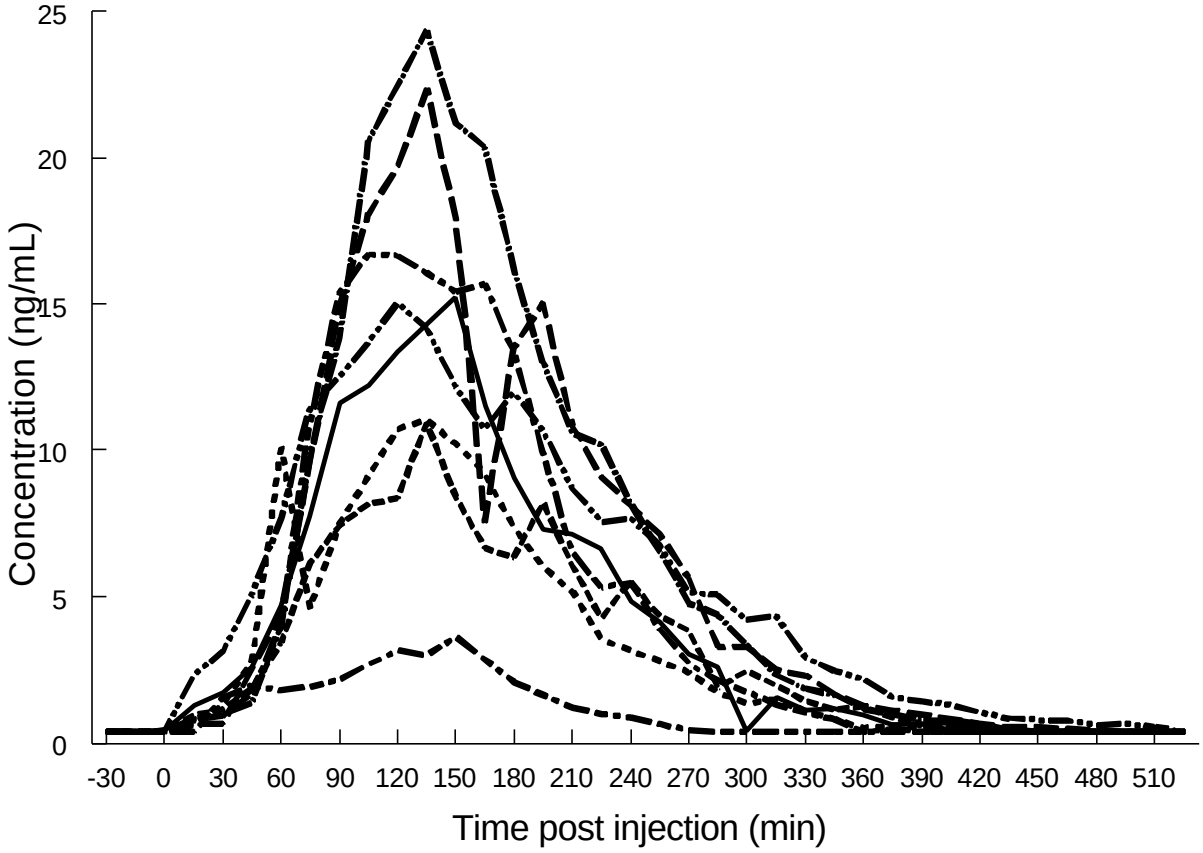
Appendix G. Figure 2. Serum luteinizing hormone concentration after Factrel injection in luteal phase beef cows (n = 8).



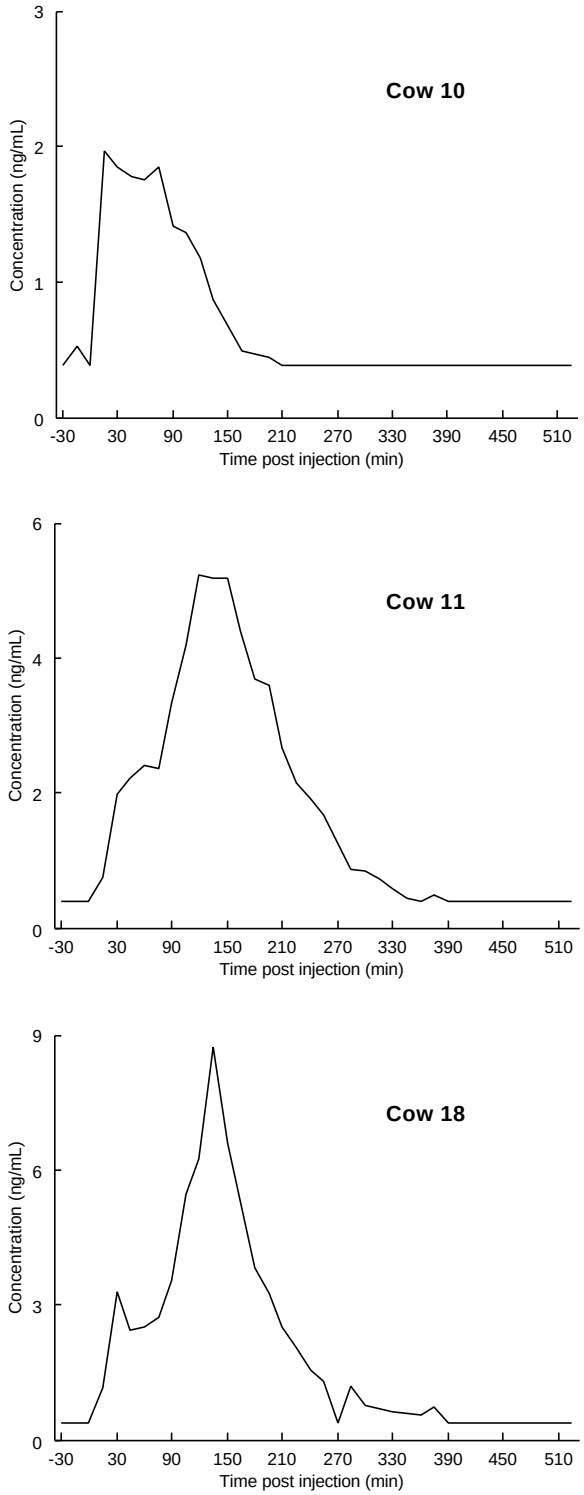
Appendix G. Figure 3. Serum luteinizing hormone concentration after Cystorelin injection in follicular phase beef cows (n = 10).



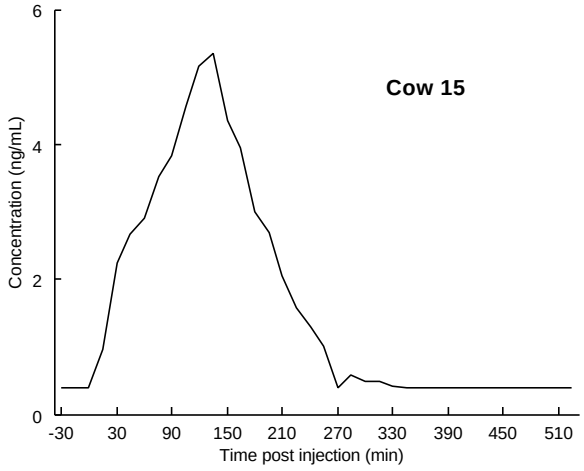
Appendix G. Figure 4. Serum luteinizing hormone concentration after Factrel injection in follicular phase beef cows (n = 8).

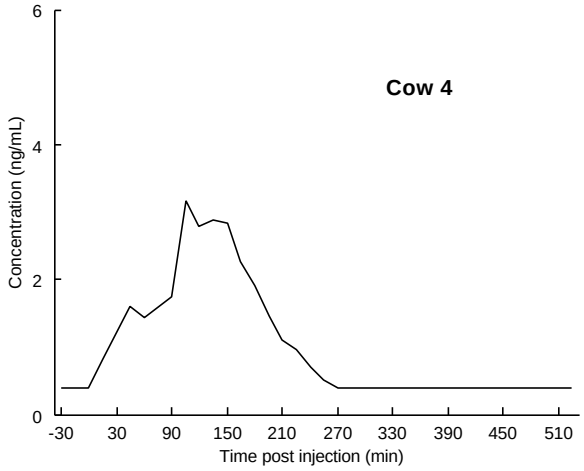


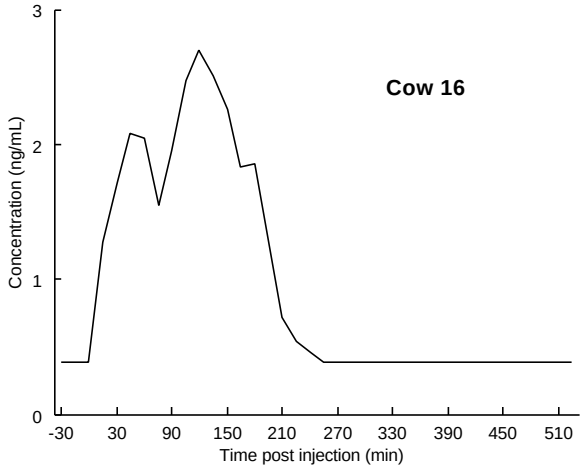
Appendix G. Figure 5. Serum luteinizing hormone concentration profiles of individual cows after Cystorelin injection during the luteal phase (n = 10).



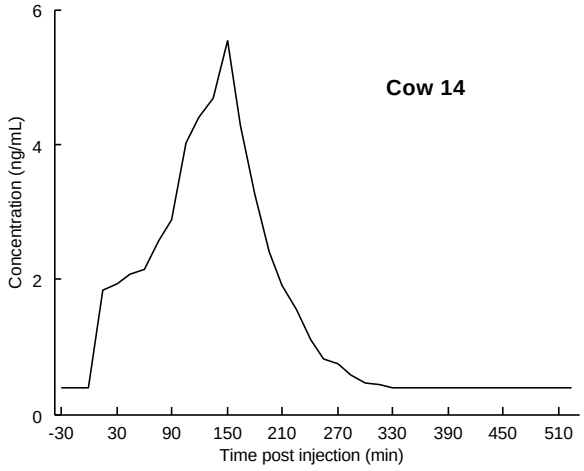
Appendix G. Figure 5. Continued.

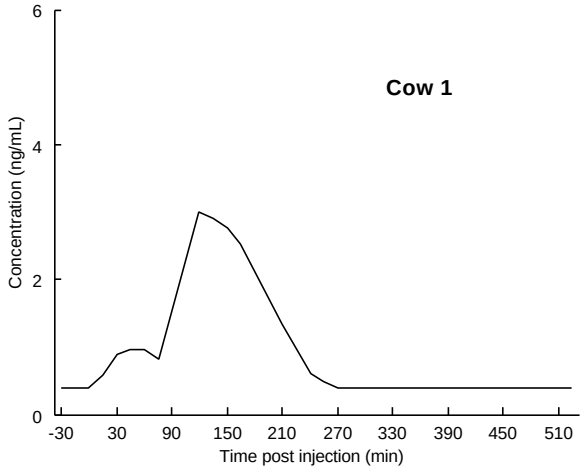


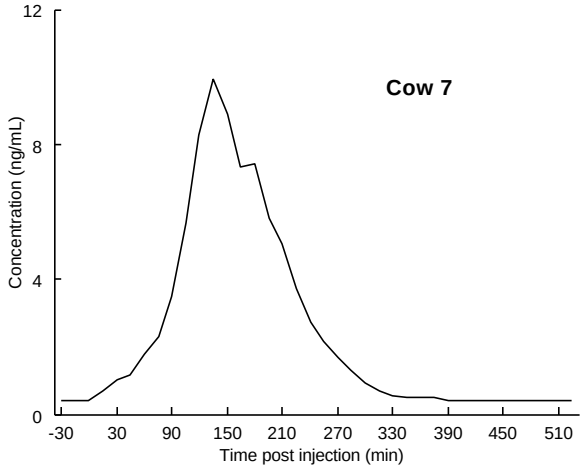




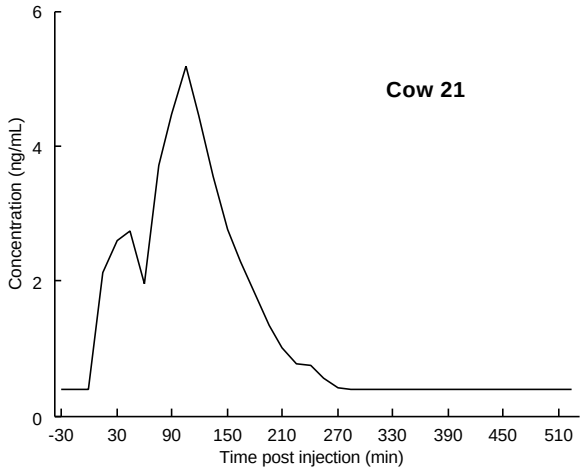
Appendix G. Figure 5. Continued.



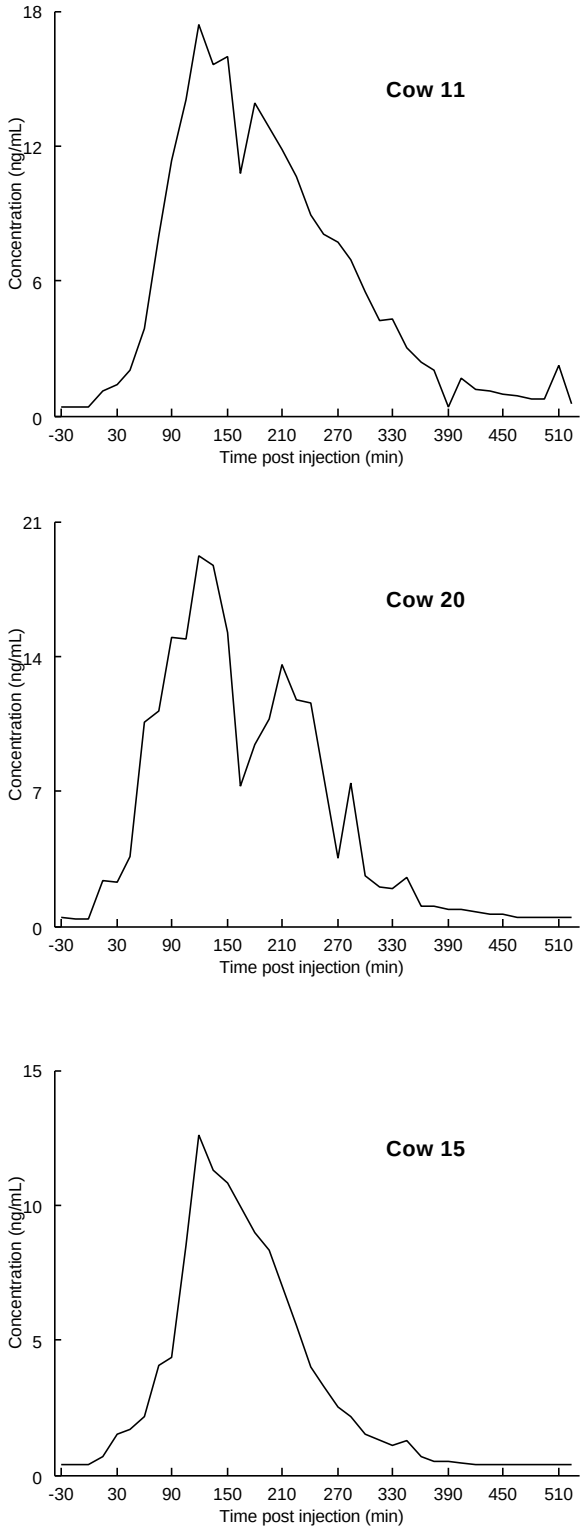




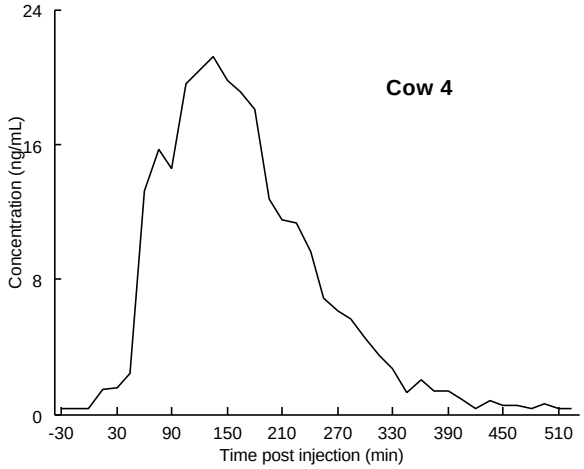
Appendix G. Figure 5. Continued.

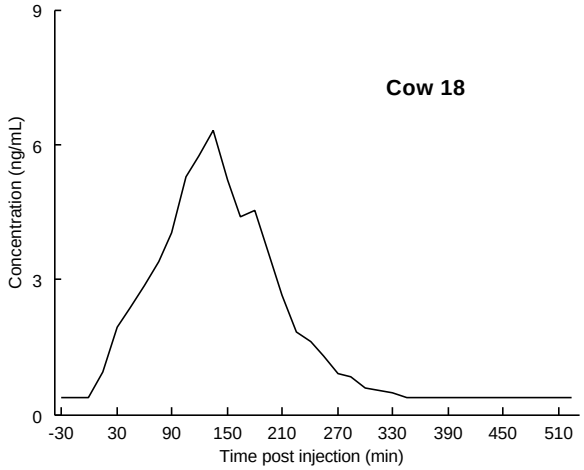


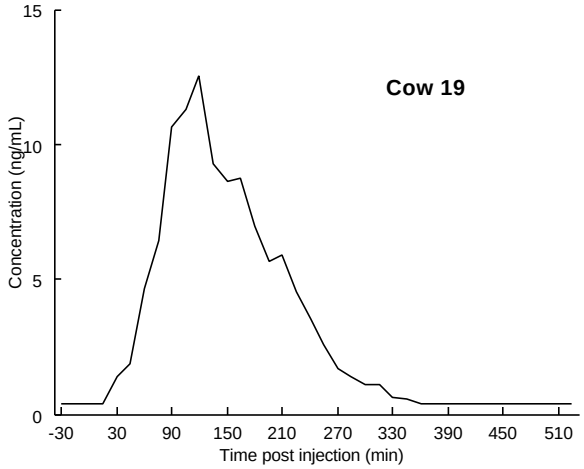
Appendix G. Figure 6. Serum luteinizing hormone concentration profiles of individual cows after Cystorelin injection during the follicular phase (n = 10).



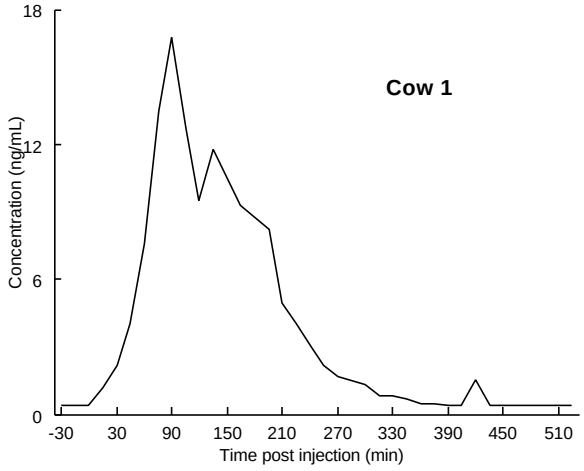
Appendix G. Figure 6. Continued.

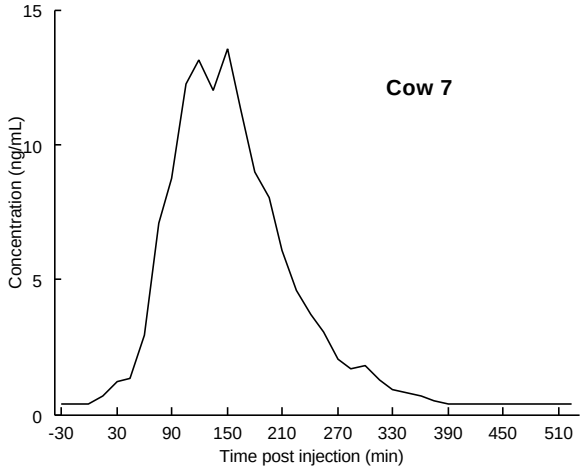


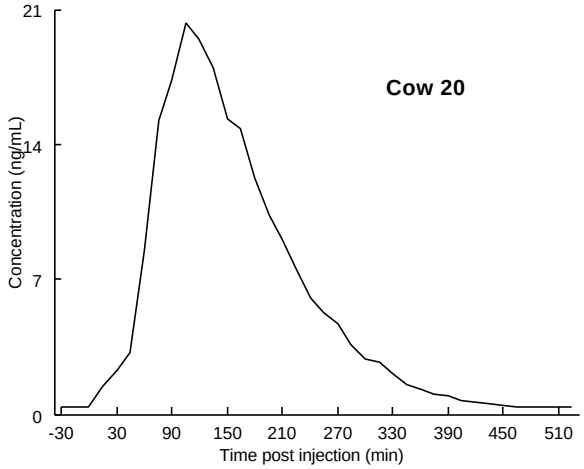




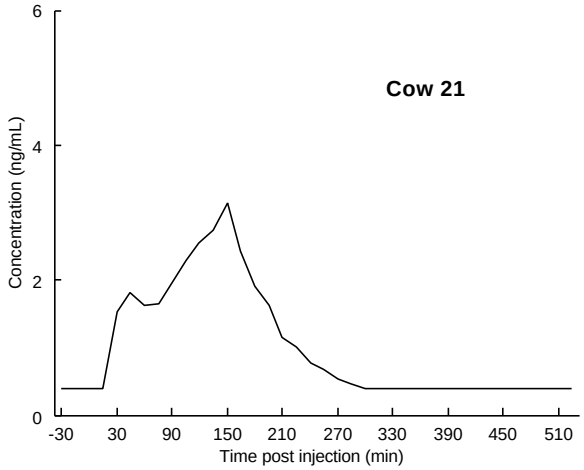
Appendix G. Figure 6. Continued.



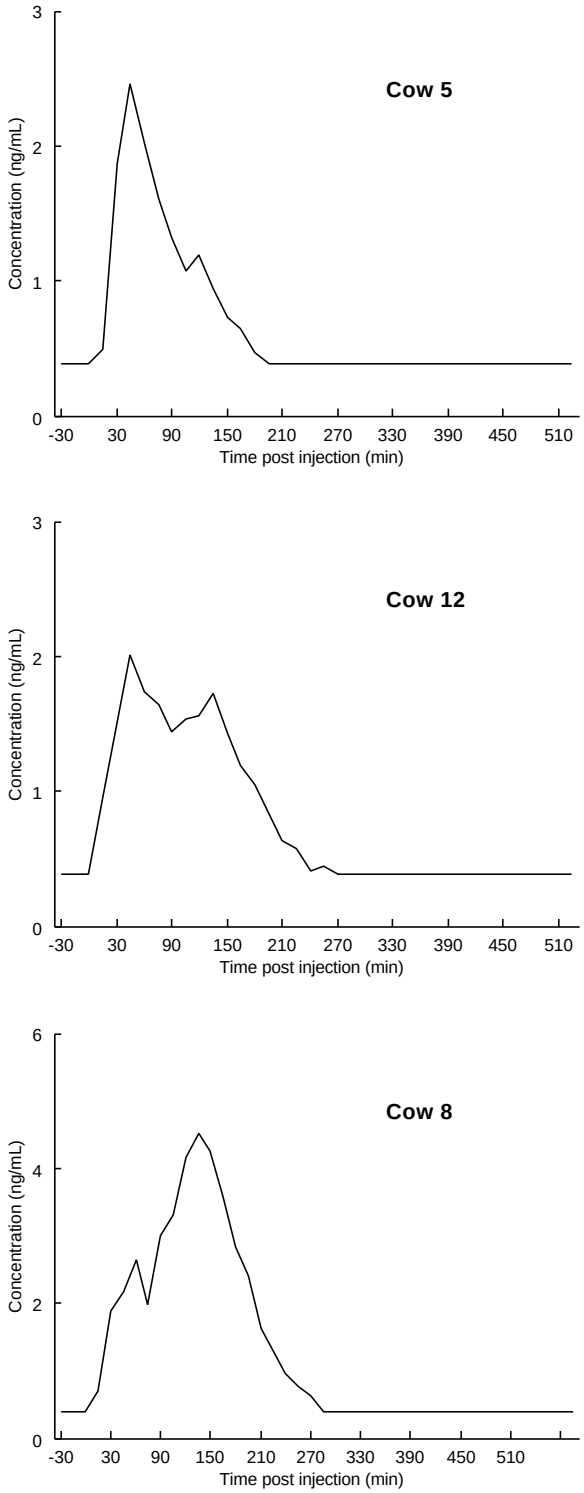




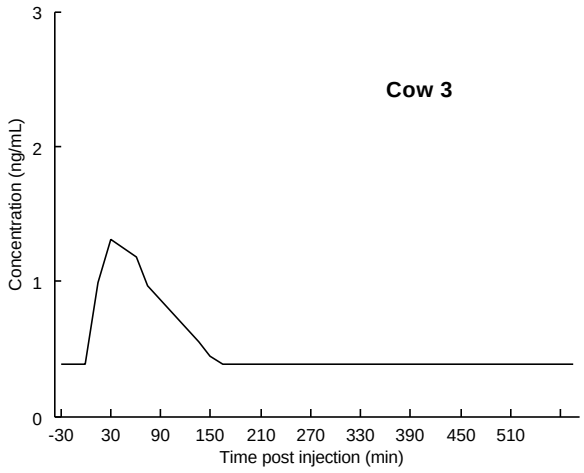
Appendix G. Figure 6. Continued.

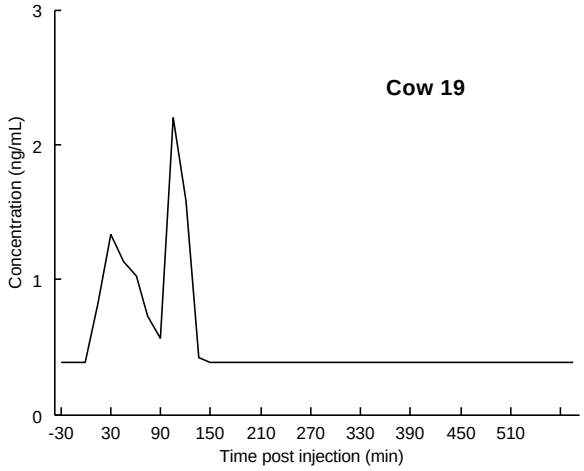


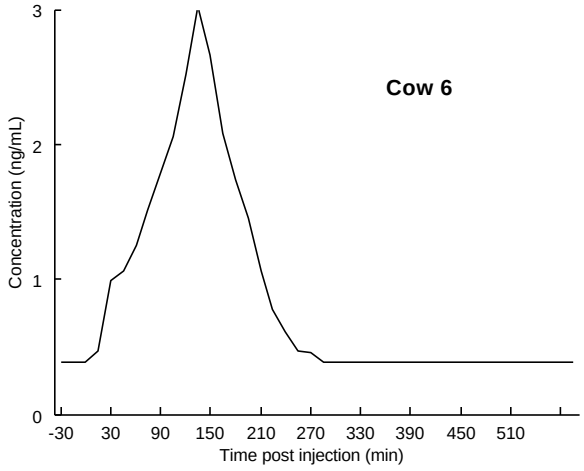
Appendix G. Figure 7. Serum luteinizing hormone concentration profiles of individual cows after Factrel injection during the luteal phase (n = 8).



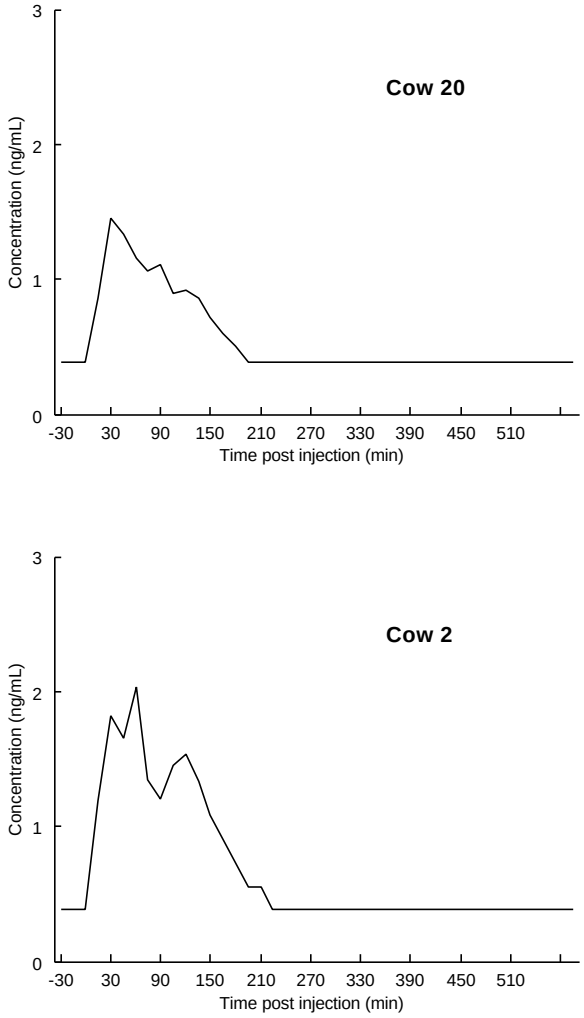
Appendix G. Figure 7. Continued.



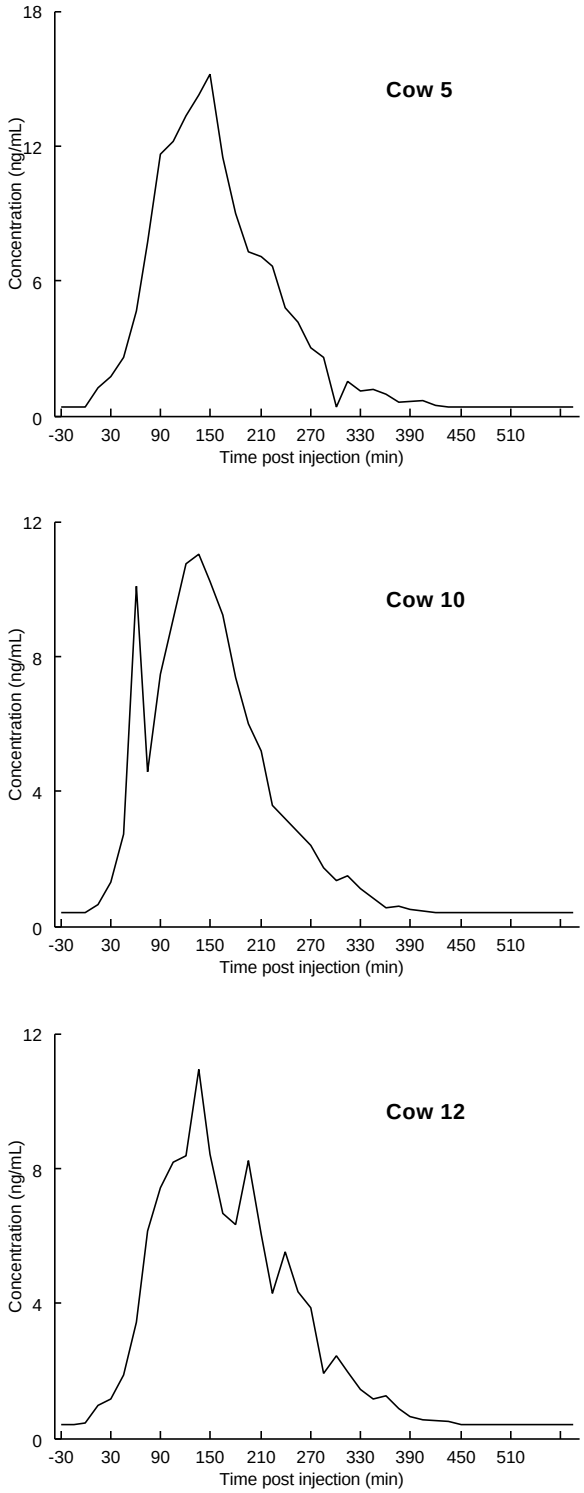




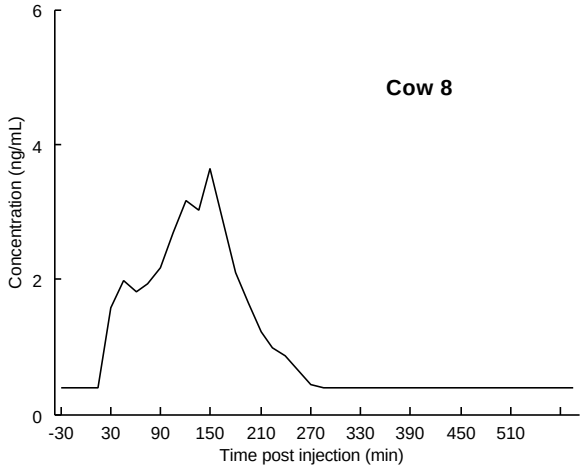
Appendix G. Figure 7. Continued.

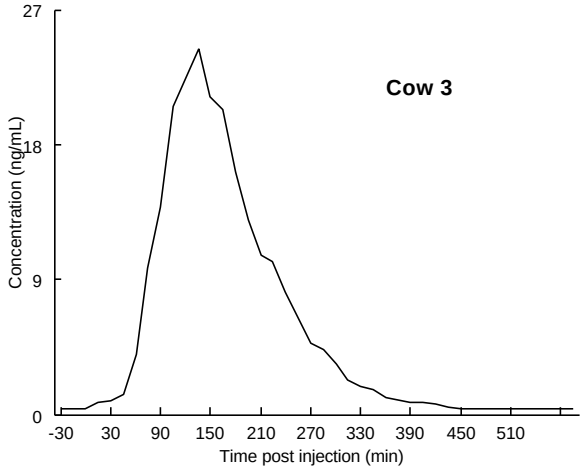


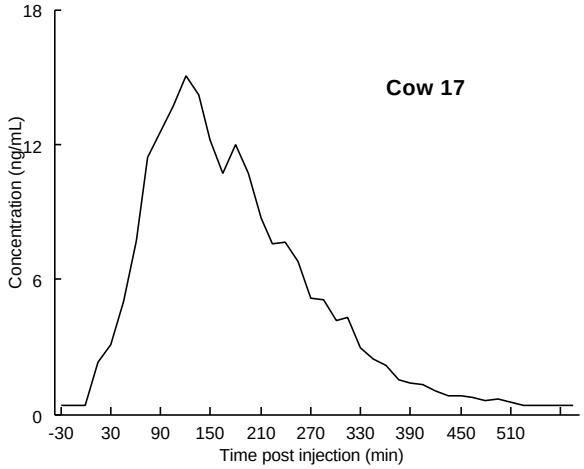
Appendix G. Figure 8. Serum luteinizing hormone concentration profiles of individual cows after Factrel injection during the follicular phase (n = 8).



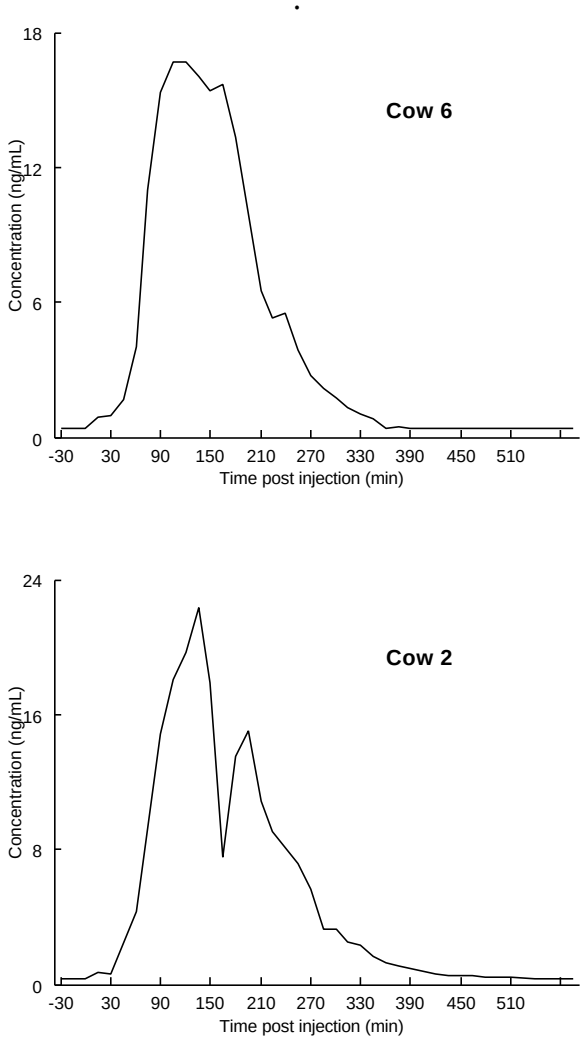
Appendix G. Figure 8. Continued.







Appendix G. Figure 8. Continued.



Appendix H
Ultrasound Data

Appendix H. Table 1. Summary of ultrasound data collected from day –1 until ovulation had occurred during the Ovsynch protocol. Trans-rectal ultrasounds using an Aloka 210 ultrasound machine were conducted daily.

COW	TRT[a]	OV1[b]	SZ1[c]	NWE[d]	NWD[e]	SX2[f]	ROG[g]	PKS[h]	OV2[i]	DOV[j]
1	1	1	15	1	1	17	0.93	17	1	2
2	2	1	10	1	1	13	0.31	13	1	2
3	2	1	-	1	3	13	0.59	14	1	2
4	1	0	12	1	3	15	0.41	18	1	2
5	2	1	11	1	3	13	0.78	13	1	2
6	2	1	10	1	4	12	1.70	12	1	2
7	1	1	-	1	1	11	1.50	12	1	2
8	2	1	10	1	3	8	-0.20	10	1	4
12	2	1	10	1	2	15	2.50	15	1	2
18	1	1	12	1	3	-	0.06	10	1	-
15	1	1	7	1	2	8	0.81	8	1	2
17	2	1	12	1	2	12	-0.70	12	1	2

[a] TRT, Treatment. 1 = Cystorelin, 2 = Factrel

[b] OV1, Occurrence or regression or ovulation after first GnRH analog injection; 0 = no change, 1 = regression or ovulation

[c] SZ1, Size of dominant follicle at time of first GnRH analog injection (expressed in mm)

[d] NWE, New wave emergence after the fist GnRH analog injection 1 = a new follicular wave emerged

[e] NWD, Day of new follicular wave emergence post first GnRH injection

[f] SX2, Size of prevoulatory follicle at second GnRH analog injection (expressed in mm)

[g] ROG, Rate of prevoulatory follicle growth from emergence to second GnRH analog injection (expressed in mm)

[h] PKS, Peak size of prevoulatory follicle after second GnRH analog injection (expressed in mm)

[i] OV2, Occurrence of ovulation after second GnRH injection, 1 = ovulated

[j] DOV, Day by which ovulation had occurred post second GnRH analog injection (expressed in days)

Appendix I
Statistical Languages

Appendix Ia. Field Trial Data Language:

```
data OVSYNCH;
  input IDN $ AGE BCS DPP LOC TRT PGC CLV;
  datalines;
run;
data OVSKLOC; set OVSYNCH;
If LOC=5 then LOC=4;
If LOC=6 then LOC=4;
If LOC=7 then LOC=4;
proc glm data=OVSYNCH;
  class AGE BCS LOC TRT;
  model PGC=AGE BCS DPP LOC TRT TRT*AGE TRT*DPP TRT*BCS TRT*LOC
/solution;
  lsmeans AGE TRT/STDERR PDIFF;
  lsmeans LOC/STDERR PDIFF;
  run;
proc glm data=OVSYNCH;
  class AGE BCS LOC TRT PGC;
  model PGC=AGE BCS LOC TRT TRT*AGE TRT*BCS TRT*LOC;
  lsmeans AGE TRT/STDERR PDIFF;
  lsmeans LOC/STDERR PDIFF;
  run;
proc glm data=OVSYNCH;
  class BCS LOC TRT PGC;
  model PGC=BCS LOC TRT TRT*BCS TRT*LOC;
  lsmeans TRT/STDERR PDIFF;
  lsmeans LOC/STDERR PDIFF;
  run;
 proc glm data=OVSYNCH;
  class LOC TRT PGC;
  model PGC=LOC TRT TRT*LOC;
  lsmeans TRT/STDERR PDIFF;
  lsmeans LOC/STDERR PDIFF;
  run;
 proc glm data=OVSKLOC;
  class LOC TRT PGC;
  model PGC=LOC TRT TRT*LOC;
  lsmeans TRT/STDERR PDIFF;
  lsmeans LOC/STDERR PDIFF;
  run;
```

```
proc catmod data=OVSYNCH;
  response 1 0;
  model PGC=LOC TRT TRT*LOC / freq prob nodesign;
  run;
proc freq data=OVSYNCH;
  tables LOC*TRT*PGC/CHISQ;
  run;
proc freq data=OVSYNCH;
  tables TRT*PGC/CHISQ;
  run;
proc freq data=OVSKLOC;
  tables LOC*TRT*PGC/CHISQ;
  run;
proc freq data=OVSKLOC;
  tables TRT*PGC/CHISQ;
  run;
quit;
```

Appendix Ib. Bovine LH Assay Data Language:

```
data LHPROF;
  input IDN UNT TRT PHS TIM CON ARE;
        datalines;
data LHARE;
  input UNT TRT PHS ARE;
        datalines;
data PROGEST;
  input UNT    PHS    TIM    PRG;
        datalines;
proc sort data=progest; by unt phs;
proc means noprint; by unt phs;
        var prg;
        output out=progout mean=mprg;
proc sort data=lhprof;
        by unt phs; run;
proc sort data=lhprof;
        by unt phs con;
data lhmax; set lhprof; by unt phs con;
        if last.phs;
        maxcon=con-.3906;
        maxcontim=tim;
proc sort data=lhmax; by unt phs;
proc sort data=progout; by unt phs;
data lhmax1; merge lhmax progout; by unt phs;
proc glm data=lhmax1;
        class trt phs;
        model maxcon maxcontim=trt|phs;
        lsmeans trt|phs /stderr;
        lsmeans trt/pdiff adjust=tukey;
        lsmeans phs/pdiff adjust=tukey;
        lsmeans trt*phs/pdiff adjust=tukey;
        MANOVA / printe;
data lh2; set lhprof;
        if con=.3906 then con=.;
        if con=. then delete;
proc sort data=lh2; by unt phs tim;
data lhminout lhmaxout; set lh2; by unt phs tim;
        if first.phs then do;
                starttim=tim;
                startcon=con;
                output lhminout; end;
        else if last.phs then do;
                endtim=tim;
                endcon=con;
```

```
                output lhmaxout; end;
data lhmin2; set lhminout;
        drop endtim endcon;
data lhmax2; set lhmaxout;
        drop starttim startcon;
data lhminmax; merge lhmin2 lhmax2;
        length=endtim-starttim;
        keep endtim starttim endcon startcon unt idn trt phs length;
proc sort data=lhminmax; by unt phs;
data lhminmax1; merge lhminmax progout; by unt phs;
proc glm data=lhminmax1;
        class trt phs;
        model length=trt | phs;
        lsmeans trt phs trt*phs /stderr;
        lsmeans trt/pdiff adjust=tukey;
        lsmeans phs/pdiff adjust=tukey;
        lsmeans trt*phs/pdiff adjust=tukey;
proc sort data=lhare; by unt phs;
data lhare1; merge lhare progout; by unt phs;
proc means data=lh2; by unt phs;
        var are trt;
        output out=lhmnout mean(trt)=trt sum(are)=totarea;
proc glm data=lhmnout;
        class trt phs;
        model totarea=trt | phs;
        lsmeans trt phs trt*phs /stderr;
        lsmeans trt/pdiff adjust=tukey;
        lsmeans phs/pdiff adjust=tukey;
        lsmeans trt*phs/pdiff adjust=tukey;
proc glm data=lhare1;
        class trt phs;
        model are=trt | phs;
        lsmeans trt | phs /stderr;
        lsmeans trt/pdiff adjust=tukey;
        lsmeans phs/pdiff adjust=tukey;
        lsmeans trt*phs/pdiff adjust=tukey;
run;
quit;
```

Appendix Ic. Ultrasound Data Language:

```
data ULTRA;
        input IND UNT TRT DAY JDTE LQNOV LASNO LSSF LOVPR LOVSZ LCL
        RQNOV RASNO RSSF ROVPR ROVSZ RCL TCH;
        if lovpr=1 and day>4 then ovsz=lovsz;
        else if rovpr=1 and day>4 then ovsz=rovsz;
        qnov=lqnov+rqnov;
        totfol=lqnov+rqnov+lovpr+rovpr;
        datalines;
data ULTRA2;
        input IDN UNT TRT FOV FSZ NWW EMD SSZ GWR PSZ SOV OVD;
        datalines;
proc sort data=ultra; by trt day;
proc means data=ultra n mean std min max stderr; by TRT DAY;
        var ovsz qnov totfol; run;
proc glm data=ULTRA;
        class trt day;
        model qnov totfol=trt | day /solution;
        lsmeans trt | day /pdiff adjust=tukey stderr;
proc glm data=ULTRA;
        class trt day tch;
        model ovsz=trt day /solution;
        lsmeans trt day / pdiff adjust=tukey stderr;
proc glm data=ultra2;
        class trt;
        model fsz emd ssz gwr psz ovd = trt;
        lsmeans trt / pdiff adjust=tukey stderr; run;
quit;
```

Appendix Id. Ovine LH Assay Data Language:

```
data LHPROF;
  input IDN UNT TRT TIM CON ARE;
        datalines;
data LHARE;
  input UNT TRT ARE;
        datalines;
data PROGEST;
  input UNT TIM       PRG;
        datalines;
proc sort data=progest; by unt;
proc means noprint; by unt;
        var prg;
        output out=progout mean=mprg;
proc sort data=lhprof;
        by unt con;
data lhmax; set lhprof; by unt con;
        if last.unt;
        maxcon=con-.3906;
        maxcontim=tim;
proc sort data=lhmax; by unt;
proc sort data=progout; by unt;
data lhmax1; merge lhmax progout; by unt;
proc glm data=lhmax1;
        class trt;
        model maxcon maxcontim=trt;
        lsmeans trt /stderr pdiff;
        MANOVA / printe;
data lh2; set lhprof;
        if con=.3906 then con=.;
        if con=. then delete;
proc sort data=lh2; by unt tim;
data lhminout lhmaxout; set lh2; by unt tim;
        if first.unt then do;
        starttim=tim;
        startcon=con;
        output lhminout; end;
        else if last.unt then do;
        endtim=tim;
        endcon=con;
        output lhmaxout; end;
data lhmin2; set lhminout;
        drop endtim endcon;
data lhmax2; set lhmaxout;
        drop starttim startcon;
```

```
data lhminmax; merge lhmin2 lhmax2;
        length=endtim-starttim;
        keep endtim starttim endcon startcon unt idn trt length;
proc sort data=lhminmax; by unt;
data lhminmax1; merge lhminmax progout; by unt;
proc glm data=lhminmax1;
        class trt;
        model length=trt;
        lsmeans trt/stderr pdiff;
proc sort data=lh2; by unt;
proc means data=lh2; by unt;
        var are trt;
        output out=lhmnout mean(trt)=trt sum(are)=totarea;

        data lhmnout1; merge lhmnout progout; by unt;
proc glm data=lhmnout1;
        class trt;
        model totarea=trt;
        lsmeans trt/stderr pdiff; run;
quit;
```

Appendix Ie. Linear Regression Language:

```
data REG;
  input DAY SIZ;
        datalines;
proc plot data=REG;
plot SIZ*DAY; run;
proc glm data=REG;
model SIZ=DAY/p clm;
output out=new p=yhat; run;
quit;
```

Appendix J
ANOVA Tables

Appendix J. Table 1. Full model analysis of variance for the effects of age (AGE), body condition score (BCS), days post partum (DPP), location (LOC), and treatment (TRT) with Cystorelin or Factrel on pregnancies maintained to at least d 45 in beef cows after AI using the Ovsynch protocol.

Source	df	Mean Square	F Value	Pr > F
AGE	12	0.145	0.61	0.8341
BCS	9	0.324	1.36	0.2090
DPP	1	0.165	0.69	0.4061
LOC	5	0.330	1.39	0.2315
TRT	1	0.366	1.54	0.2168
AGE*TRT	9	0.215	0.90	0.5233
DPP*TRT	1	0.020	0.08	0.7716
BCS*TRT	6	0.258	1.08	0.3744
LOC*TRT	5	0.150	0.63	0.6770

Using type III means squared

Appendix J. Table 2. Reduced model analysis of variance for the effects of location (LOC), and treatment (TRT) with Cystorelin or Factrel on pregnancies maintained to at least d 45 in beef cows after AI using the Ovsynch protocol.

Source	df	Mean Square	F Value	Pr > F
LOC	3	0.436	1.82	0.1437
TRT	1	0.798	3.33	0.0690
LOC*TRT	3	0.113	0.48	0.6995
Error	316	0.240		

Using type III means squared

Appendix J. Table 3. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 1 (n = 125).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	22
Left-sided Pr <=F	0.6704
Right-sided Pr >= F	0.4741
Table Probability (P)	0.1446
Two-sided Pr <= P	0.8519

Appendix J. Table 4. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 2 (n = 61).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	14
Left-sided Pr <= F	0.9668
Right-sided Pr >= F	0.0957
Table Probability (P)	0.0625
Two-sided Pr <= P	0.1881

Appendix J. Table 5. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 3 (n = 30).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	11
Left-sided Pr <= F	0.9359
Right-sided Pr >= F	0.2249
Table Probability (P)	0.1608
Two-sided Pr <= P	0.4497

Appendix J. Table 6. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 4 (n = 21).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	7
Left-sided Pr <= F	0.9983
Right-sided Pr >= F	0.0242
Table Probability (P)	0.0225
Two-sided Pr <= P	0.0300

Appendix J. Table 7. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocal for herd 5 (n = 26).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	2
Left-sided Pr <= F	0.3121
Right-sided Pr >= F	0.9185
Table Probability (P)	0.2307
Two-sided Pr <= P	0.4198

Appendix J. Table 8. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 6 (n = 29).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	8
Left-sided Pr <= F	0.4037
Right-sided Pr >= F	0.8403
Table Probability (P)	0.2441
Two-sided Pr <= P	0.7107

Appendix J. Table 9. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for herd 7 (n = 32).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	7
Left-sided Pr <= F	0.8633
Right-sided Pr >= F	0.3580
Table Probability (P)	0.2213
Two-sided Pr <= P	0.7160

Appendix J. Table 10. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Ovsynch protocol for all herds combined (n = 324).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	71
Left-sided Pr <= F	0.9654
Right-sided Pr >= F	0.0558
Table Probability (P)	0.0213
Two-sided Pr <= P	0.0911

Appendix J. Table 11. Reduced model analysis of variance for the effects treatment (TRT) with Cystorelin or Factrel on pregnancies maintained to at least d 45 in beef cows after AI using the Co-synch protocol.

Source	df	Mean Square	F Value	Pr > F
TRT	1	0.84751551	3.46	0.0655
Error	118	0.24528942		

Using type III means squared

Appendix J. Table 12. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Co-synch (n = 120).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	28
Left-sided Pr <= F	0.0478
Right-sided Pr >= F	0.9791
Table Probability (P)	0.0269
Two-sided Pr <= P	0.0703

Appendix J. Table 13. Reduced model analysis of variance for the effects of location (LOC), and treatment (TRT) with Cystorelin or Factrel on pregnancies maintained to at least d 45 in beef cows after AI using the Select-synch protocol.

Source	df	Mean Square	F Value	Pr > F
LOC	2	0.042	0.19	0.8299
TRT	1	1.046	4.67	0.0373
LOC*TRT	2	0.444	1.98	0.1526
Error	37	0.224		

Using type III means squared

Appendix J. Table 14. Chi squared analysis for the effect of Cystorelin or Factrel treatment on pregnancies maintained to d 45 in beef cows after AI using the Select-synch protocol (n = 43).

Fisher's Exact Test	
Cell (1,1) Frequency (F)	16
Left-sided Pr <= F	0.9918
Right-sided Pr >= F	0.0394
Table Probability (P)	0.0312
Two-sided Pr <= P	0.0618

Appendix J. Table 15. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on the maximum serum luteinizing hormone concentration in beef cows on d 9 and 0 of the estrous cycle (n = 19).

Source	df	Mean Square	F Value	Pr > F
TRT	1	5.274	0.25	0.6234
PHS	1	975.939	45.52	<.0001
TRT*PHS	1	32.180	1.50	0.2298
Error	31	21.441		

Using type III means squared

Appendix J. Table 16. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on time to reach maximum serum luteinizing hormone concentration in beef cows on d 9 and 0 of the estrous cycle (n = 19).

Source	df	Mean Square	F Value	Pr > F
TRT	1	2326.039	2.29	0.1406
PHS	1	10932.289	10.75	0.0026
TRT*PHS	1	5206.988	5.12	0.0308
Error	31	1017.218		

Using type III means squared

Appendix J. Table 17. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on duration of serum luteinizing hormone response in beef cows on d 9 and 0 of the estrous cycle (n = 19).

Source	df	Mean Square	F Value	Pr > F
TRT	1	15607.289	2.68	0.1117
PHS	1	235215.421	40.40	<.0001
TRT*PHS	1	24214.217	4.16	0.0500
Error	31	5821.855		

Appendix J. Table 18. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on total calculated area beneath the resulting lutenizing hormone profile for beef cows on d 9 and 0 of the estrous cycle (n = 19).

Source	df	Mean Square	F Value	Pr > F
TRT	1	8912.487	0.37	0.5488
PHS	1	1153149.995	47.51	<.0001
TRT*PHS	1	11534.838	0.48	0.4956
Error	32	4271.926		

Appendix J. Table 19. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on quantity of non-ovulatory follicles (n = 15).

Source	DF	Mean Square	F Value	Pr > F
TRT	1	21.519	5.75	0.0179
DAY	13	4.991	1.33	0.2019
TRT*DAY	13	4.297	1.15	0.3256
Error	128	3.743		

Using type III means squared

Appendix J. Table 20. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on total follicle quantity (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	21.285	5.15	0.0250
DAY	13	7.645	1.85	0.0423
TRT*DAY	13	4.349	1.05	0.4073
Error	128	4.136		

Using type III means squared

Appendix J. Table 21. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on ovulatory follicle peak size (mm, n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	35.862	4.17	0.0449
DAY	9	15.443	1.79	0.0842
Error	72	8.610		

Using type III means squared

Appendix J. Table 22. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on size of pre-ovulatory follicle at time of first GnRH analog injection (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	3.300	0.81	0.3918
Error	9	4.078		

Using type III means squared

Appendix J. Table 23. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on day of emergence of a new follicular wave after first GnRH analog injection (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	1.055	1.19	0.2978
Error	11	0.883		

Using type III means squared

Appendix J. Table 24. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on growth rate of follicle until time of second GnRH analog injection (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	0.725	0.56	0.4703
Error	11	1.296		

Using type III means squared

Appendix J. Table 25. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on peak size of ovulatory follicle after second GnRH analog injection (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	1.238	0.14	0.7118
Error	11	8.615		

Using type III means squared

Appendix J. Table 26. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on size of ovulatory follicle at time of second GnRH analog (n = 15).

Source	df	Mean Square	F Value	r > F
TRT	1	2.438	0.30	0.5936
Error	10	8.023		

Using type III means squared

Appendix J. Table 27. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and day (DAY) on day that ovulation had occurred by following secondary GnRH analog injection (n = 15).

Source	df	Mean Square	F Value	Pr > F
TRT	1	0.0214	0.05	0.8264
Error	10	0.423		

Using type III means squared

Appendix J. Table 28. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel on the maximum serum luteinizing hormone concentration in ewes treated during the mid luteal phase (n = 16).

Source	df	Mean Square	F Value	Pr > F
TRT	2	606.701	3.93	0.0461
Error	13	154.229		

Using type III means squared

Appendix J. Table 29. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on time to reach maximum serum luteinizing hormone concentration in ewes treated during the mid luteal phase (n = 16).

Source	df	Mean Square	F Value	Pr > F
TRT	2	232.500	0.33	0.7260
Error	13	708.461		

Using type III means squared

Appendix J. Table 30. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on duration of serum luteinizing hormone response in ewes treated during the mid luteal phase (n = 16).

Source	df	Mean Square	F Value	Pr > F
TRT	2	9328.125	2.02	0.1722
Error	13	4618.269		

Using type III means squared

Appendix J. Table 31. Analysis of variance for the effects of treatment (TRT) with Cystorelin or Factrel and phase (PHS) on total calculated area beneath the resulting lutenizing hormone profile for beef cows on d 9 and 0 of the estrous cycle (n = 19).

Source	df	Mean Square	F Value	Pr > F
TRT	2	706918.758	3.12	0.0783
Error	13	226741.510		

Using type III means squared

VITA

Mark A. Cline

Education

2002 Accepted as a Ph.D. candidate. Virginia Polytechnic Institute and State University, Blacksburg. Preceptor - Dr. Michael Denbow, Ph.D. Area of specialization - neurophysiology.

2002 MS, Reproductive Physiology - Virginia Polytechnic Institute and State University, Blacksburg. Areas of specialization - Estrous synchronization via GnRH in the bovine and ovine, and ovine embryo transfer; Preceptors - Dr. John B. Hall, Ph.D. and Dr. Greg S. Lewis, Ph.D.

1999 BS, Animal Science, minor Biology - Virginia Polytechnic Institute and State University, Blacksburg.

1996 Associate in Arts and Sciences - Blue Ridge Community College, Weyers Cave, Virginia. Cum Laude.

1994 Advanced Studies - Fort Defiance High School, Fort Defiance, Virginia.

Teaching Experiences

Teaching Assistant: Principles of Biology (BIOL 1106, Virginia Tech), 3 credits, Spring 2002

This course deals with the study of animal and plant anatomy and physiology, ecology, and animal behavior. Students enrolled in this course are from various majors working towards a four year degree.

Instructor: Animal Agriculture (AT 0164, Virginia Tech), 4 credits, Fall 2001

This course deals with the study of animal products, production methods and management systems for beef, sheep, horses, dairy, swine, goats, and poultry. Classroom instruction, demonstrations and hands-on experience with university livestock and poultry. Students enrolled in this course are working towards an Associate degree in Agriculture Technology.

Laboratory Instructor: Animal Anatomy and Physiology laboratories (ALS 2304, Virginia Tech), Fall 1999-2001

Topics covered in this course include: anatomy and physiology of domestic animals including cell, neural, skeletal, muscular, respiratory, cardiovascular, urinary, and

endocrine systems. Students enrolled in this course are from various majors (Animal Science, Dairy Science, Human Nutrition, Education, Biology, …) and are working towards a Bachelors of Science degree in a life sciences field.

Undergraduate Teaching Assistant: Animal Anatomy and Physiology (ASL 2304, Virginia Tech), Fall 1997-1998

Responsibilities included assisting the graduate TA with teaching laboratories, administering quizzes and lab practicals, and developing visual teaching aids. Topics covered in this course include: anatomy and physiology of domestic animals including cell, neural, skeletal, muscular, respiratory, cardiovascular, urinary, and endocrine systems.

Guest Lecturer: Agriculture Technology courses. (1999-2000)

Many guest demonstrations and lectures for two-year Agriculture Technology students. Topics include reproductive technologies, animal handling, animal health, and reproductive cycles and management.

Graduate Mentor - Undergraduate Research Projects:

1) Bovine reproductive exams as an indicator of reproductive health. (2001)

2) Validation of luteinizing hormone assay. (2001)

3) Efficacy of three synthetic GnRH products via their induced luteinizing hormone profile in the ovine. (2000-2001)

4) Estrous synchronization in ewes via prostaglandin F2? and GnRH. (2000)

5) Relationship between body condition score and superovulation in the ewe. (1999)

6) Ultrasonography after superovulatory treatment in the ewe. (1999)

Research Experience

Lead Investigator:

1) Field trials: Comparison of ovulation synchronization protocols utilizing synthetic GnRH products in the bovine. (2000-2001)

2) Efficacy of two synthetic GnRH products via induced luteinizing hormone profiles in the bovine. (2001)

3) Site of embryo deposition in the ovine for embryo transfer. (1999-2000)

4) Determination of ovulation time in sheep after injection of PMSG or P.G. 600®. (1997)

Research Assistant:

Assisted several other graduate students with their thesis projects. (1996-2000)

Experiments:

- Oxytocin induced cervical dilation for ovine artificial insemination.
- Rate of oxytocin clearance in the ewe.
- Modulation of the uterine immune system by prostaglandins.
- Induced cervical dilation in sheep: evaluation of the effects on fertilization rates and embryonic development.
- Uterine response to multiple exposures of Escherichia coli and Arcanobacterum pyogenes in nulliparous ewes.

Professional Publications and Presentations

Cline M. A., J. B. Hall, and W. D. Whittier. 2001. Efficacy of commercial GnRH preparations for use in the Ovsynch synchronization protocol: beef cattle field trials. NCR-87 2001 Annual Report.

Cline M. A., J. N. Ralston, R. C. Seals and G. S. Lewis. 2001. Intervals from norgestomet withdraw and injection of equine chorionic gonadotropin or P.G. 600® to estrus and ovulation in ewes. Journal of Animal Science. 79:589-594.

Cline M. A., J. N. Ralston, R. C. Seals and G. S. Lewis. 1997. Determination of ovulation time in sheep after injection of PMSG or P.G. 600®. Journal of Animal Science. 75(Supplement 1):25.

Cline M. A., J. N. Ralston, R. C. Seals and G. S. Lewis. Determination of Ovulation Time in Sheep After Injection of PMSG or P.G. 600®. Paper presented at the Southern Section of the American Society of Animal Science Annual Meetings, Feb. 1998.

Laboratory and Reproductive Techniques

Artificial insemination
Blood collection and processing
Catheterization: Jugular and Saphenous vein
Embryo transfer: non-invasive and surgical
Enzyme Immunoassay
Laparoscopy
Mid-ventral laparotomy
Radioactive material handling
Radioimmunoassay
Radioimmunoassay validation
Rectal palpation of reproductive tract
Statistical analysis of data
Ultrasonography in cattle and sheep

Awards

Animal and Poultry Sciences Graduate Student Scholarship. Excellence in teaching, research, academics and service to agriculture. (2001)

Graduate Teaching Assistantship. Department of Animal and Poultry Sciences. (2001)

Southern Section of the American Society of Animal Scientists. Undergraduate research presentation - 3rd place. Cline M. A., J. N. Ralston, R. C. Seals and G. S. Lewis. Determination of Ovulation Time in Sheep After Injection of P.G. 600®. (1997)

Virginia Tech 13th Annual Research Symposium undergraduate category - 2nd place. Cline M. A., J. N. Ralston, R. C. Seals and G. S. Lewis. Determination of Ovulation Time in Sheep After Injection of P.G. 600®. (1997)

Virginia Farm Bureau Outstanding Young Agriculturist Award. (1994)

National FFA Degree. (1994)

Star Agri-businessman, Fort Defiance High School. (1994)

Professional Affiliations, Organizations and Societies

American Association for Higher Education
American Dairy Science Association
American Society of Animal Scientists
Association of College and University Biology Educators
Society for Amateur Scientists
Phi Theta Kappa
Virginia Cattleman's Association

Production Agriculture Experiences

Family Farm Experiences: Our family farm operation consists of 150 dairy and 150 breeding beef cows, located in the Shenandoah Valley of Virginia. Crops enterprise includes: corn, sorghum, soybeans, hay, alfalfa, barley, rye, wheat, and oats. Personal interest and experience with sheep and horses.

Large Animal Veterinary Experiences: 1995-1997. Employed as a large animal veterinary assistant. Veterinary animal experiences include: beef and dairy cattle, horses, mules, sheep, goats, and companion animals. Gained knowledge of various veterinary techniques.

Relevant Computer Skills

Proficient on PCs (Windows 3.1, 95, 98, 2000, NT and ME interfaces). Experience with Microsoft Office Suite, Quarto Pro, Netscape Navigator, Internet Explorer, newsreaders, FTP, HTML programming, JAVA scripts, animated image creation, image manipulation, Adobe Assistant, SAS, and remedying basic computer problems. Easily learn new applications and technologies with minimal effort. Experienced web master for several web pages.