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CONTROL OF THE PREOVULATORY LUTEINIZING HORMONE AND FOLLICLE STIMULATING

HORMONE SURGES IN CATTLE

presented by

JAMES STEPHEN KESNER

has been accepted towards fulfillment of the requirements for

PhD degree in Anymust Science

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CONTROL OF THE PREOVULATORY LUTEINIZING

HORMONE AND FOLLICLE STIMULATING

HORMONE SURGES IN CATTLE

Ву

James Stephen Kesner

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Animal Science

ABSTRACT

CONTROL OF THE PREOVULATORY LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE SURGES IN CATTLE

By

James Stephen Kesner

The objectives of the first series of studies were to determine: (1) the effect of estradiol on the ability of the bovine pituitary gland to release LH and FSH in response to LHRH, and (2) whether LHRH is required to trigger the preovulatory LH and FSH surges in cattle. Estradiol induces preovulatory-like LH and FSH surges in ovariectomized cows, but not steers. However, we found that preovulatory-like LH and FSH surges could be induced in steers if we supplied the missing component, i.e., an LHRH surge. Thus, 1 ug LHRH given every 20 min for 10 h beginning 12 h after injecting estradiol induced preovulatory-like LH and FSH surges. Furthermore, LH and FSH surges were induced in steers and ovariectomized cows by giving 1 ug LHRH every 20 min beginning 8, and in some cases 2 h after estradiol, i.e., 10 to 15 h before the endogenous surges would have occurred. Thus, estradiol maximally increased responsiveness of gonadotrophs to LHRH long before the time of the spontaneous gonadotropin surges. That a gonadotropin surge does not occur before

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James Stephen Kesner

12-20 h in estradiol-treated cows is evidence that LHRH is not being released in sufficient quantities to induce the surge until that critical period. In all cases, LH and FSH surges terminated despite continued injections of LHRH.

Decreasing the dose of LHRH injections from 1 ug/steer to \sim 0.3 ug/steer decreased the peak LH values 48 percent, but did not alter the FSH response. Furthermore, decreasing the frequency of LHRH injections from 25 to 50 min decreased LH release, but only in steers given estradiol. FSH release was not affected by decreasing the frequency of LHRH injections.

Despite giving estradiol and progesterone replacement at the time of ovariectomy, in amounts to mimic their concentrations during the luteal phase of the estrous cycle, basal LH and FSH concentrations increased in the serum 370 and 220 percent respectively, within 24 h after ovariectomy. When estradiol replacement was augmented to mimic the increase of blood estradiol concentrations that occurs at proestrus, preovulatory-like LH and FSH surges were induced. However, this was true only if progesterone replacement was stopped. Estradiol did not induce gonadotropin surges when estradiol replacement was maintained at low concentrations characteristic of the luteal phase, despite stopping progesterone replacement.

In conclusion, estradiol induces, whereas progesterone blocks the preovulatory LH and FSH surges in cows. Estradiol induces these surges by first increasing responsiveness of gonadotrophs to LHRH and then increasing the frequency and possibly the magnitude of pulsatile LHRH release. Despite the similarity between the control of LH and FSH surges, estradiol and LHRH induce greater changes in LH release, relative to the baseline, than in FSH. The gonadotropin surges terminate due to gonadotroph refractoriness to LHRH. Finally, estradiol and progesterone may act in conjunction with other ovarian factors to maintain low baselines of LH and FSH during the luteal phase of the estrous cycle.



ACKNOWLEDGMENTS

I wish to extend my sincere gratitude to my major professor, Dr. Edward M. Convey. Not only did Ed provide sound and expert guidance towards my scientific goals, but also demonstrated a personal concern and understanding that will be remembered always. I consider it a priviledge to have been able to work with him. I also wish to thank the members of my graduate committee, Dr. H. A. Tucker, Dr. R. S. Emery and Dr. F. Rottman, for their advice and cooperation throughout my Ph.D. program.

I wish to thank Dr. Vasantha Padmanabhan for all she taught me and for her friendship. I also extend a hearty thanks and acknowledgment to my fellow graduate students for their assistance in collecting and analyzing my data.

Finally, I wish to thank my parents for their love and support throughout the years.

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ABBREVIATIONS

BW	body weight
Ca ⁺⁺	calcium ion
CAMP	cyclic adenosine monophosphate
CGMP	cyclic guanosine monophosphate
E ₂	estradiol
FSH	follicle stimulating hormone
h	hour(s)
ID	inside diameter
im	intramuscular(-1y)
iv	intravenous(-ly)
к+	potassium ion
kg	kilogram(s)
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mΜ	millimolar
mm	millimeter(s)
ng	nanogram(s)
OD	outside diameter
P4	progesterone
þà	picogram(s)
RNA	ribonucleic acid
SC	subcutaneous(-ly)
SD	standard deviation
SE	standard error
ug	microgram(s)
vol	volume(s)
vs	versus

INTRODUCTION

The preovulatory surge release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), is responsible for triggering ovulation and contribute to subsequent ovarian follicular growth. It is generally believed that increasing amounts of estradiol, which are secreted by the preovulatory follicle(s), induce the gonadotropin surges. Yet it is not clear how estradiol accomplishes this task. In monkeys, estradiol acts primarily on the anterior pituitary gland to induce gonadotropin surges. But in rats estradiol acts on the hypothalamus as well as the anterior pituitary gland. Specifically, estradiol increases the ability of the anterior pituitary to release gonadotropins in response to luteinizing hormone releasing hormone (LHRH) stimulation from the hypothalamus. In addition, it increases the rate of LHRH secretion by the hypothalamus. At this time, little is known about how estradiol induces the preovulatory gonadotropin surge in cows.

Progesterone blocks the estradiol-induced gonadotropin surges in monkeys and rats. However, present evidence suggests that progesterone is not capable of exerting this action in cattle.



The objective of this dissertation is to determine how estradiol and progesterone control the preovulatory LH and FSH surges in cows. This includes determining the mechanism by which estradiol synergizes with LHRH to induce the preovulatory gonadotropin surge. The justification for investigating how the preovulatory gonadotropin surges are controlled is three-fold: (1) to obtain knowledge necessary for manipulating or predicting time of ovulation, (2) to obtain basic information on regulation of hypothalamo-hypophysial secretions, and (3) to satisfy my curiosity as to how estradiol can both stimulate and inhibit gonadotropin secretion.

REVIEW OF THE LITERATURE

Hormonal Profiles of the Bovine Estrous Cycle

A prerequisite to understanding how the secretion of gonadotropins is controlled, is to know how concentrations of gonadotropins and ovarian steriods change in peripheral blood throughout the estrous cycle. Given this knowledge, one may begin to manipulate hormone concentrations to study mechanisms by which they are controlled and to modify reproductive efficiency.

Estradiol

Estradiol in serum is lowest (2 to 8 pg/ml) during the luteal phase of the estrous cycle (Wettemann et al., 1972; Dobson & Dean, 1974; Kanchev et al., 1976). Synchronous with or shortly after luteal regression, estradiol increases to 10 to 20 pg/ml, reaching a peak shortly before, during or shortly after the preovulatory gonadotropin surges (Wettemann et al., 1972; Chenault et al., 1975; Dobson, 1978). Some investigators have reported that on day 4 to 7 of the estrous cycle (day 0 = estrus) estradiol concentrations increase to levels found at estrus (Glencross et al., 1973; Dobson & Dean, 1974). Superimposed upon this general pattern of concentrations of estradiol in serum are large

day-to-day fluctuations (Chenault et al., 1975; Kanchev et al., 1976).

Dobson and Dean (1974) reported that estrone averages about 5 pg/ml in serum with little variations throughout the estrous cycle. Estradiol-17a is present in serum in quantities 3 to 4 times greater than the 17B epimer, and increases slightly at estrus (Dobson & Dean, 1974).

Progesterone

Concentrations of progesterone in serum are 2 to 10 ng/ml during the luteal phase of the estrous cycle (Wettemann et al., 1972; Glencross et al., 1973; Kanchev et al., 1976). Progesterone concentrations decrease to undetectable levels 3 to 4 days before estrus as the corpus luteum regresses, and remain below 0.5 ng/ml until 3 to 4 days after estrus when a new corpus luteum becomes functional (Wettemann et al., 1972; Chenault et al., 1975; Kanchev et al., 1976; Convey et al., 1977). Progesterone is not secreted concurrent with the preovulatory gonadotropin surges in cows as it is in women (Abraham et al., 1972), monkeys (Knobil, 1974), dogs (Concannon et al., 1977), and rats (Butcher et al., 1974).

Luteinizing Hormone

Baseline concentrations of LH are low (0.5 to 1.0 ng/ml) throughout most of the estrous cycle. However, these low levels of LH are interrupted by a massive release of this hormone approximately 30 h before ovulation. This

preovulatory LH surge accompanies onset of behavioral estrus and causes ovulation to occur approximately 22 h later (Swanson & Hafs, 1971; Chenault et al., 1975). The duration of the LH surge is 6 to 10 h and its magnitude usually exceeds basal values by 50-fold or more. After the surge, LH concentrations become very low ($\sim l ng/ml$) and remain low throughout the luteal phase of the estrous cycle. As the corpus luteum regresses and progesterone levels decrease, LH concentrations increase slightly and remain elevated for 2 to 3 days until the preovulatory LH surge occurs (Chenault et al., 1975; Rahe et al., 1980; Roche & Ireland, 1981a).

LH is released from the anterior pituitary gland in pulses. The magnitude and frequency of these pulses vary throughout the estrous cycle (Rahe et al., 1980). On day 3 and 10 of the estrous cycle, LH pulses have magnitudes of \sim 1 and 4 ng/ml and occur at frequencies of \sim 1/h and 1/3h, respectively. The preovulatory LH surge is apparently the result of LH pulses that have increased in frequency (2 to 3/h) and magnitude (\sim 10 ng/ml) to the extent that LH concentrations in serum do not return to baseline between pulses.

Follicle Stimulating Hormone

FSH concentrations in bovine serum have been measured only sparingly. Consequently relatively little is known regarding details of its secretion pattern. Unlike LH, FSH concentrations do not increase 2 to 3 days prior to the

preovulatory gonadotropin surge (Roche & Ireland, 1981a). But a preovulatory FSH surge occurs synchronously with the LH surge (Dobson, 1978; Roche & Ireland, 1981a). These investigators also noted a secondary increase in FSH approximately 24 h later. However, this increase was smaller than the comparable secondary increase of FSH that occurs in ewes (Pant et al., 1977).

Autoregulation by LH and FSH

Because gonadotrophs become refractory to LHRH after the preovulatory gonadotropin surges, and because pulsatile secretion of gonadotropins may result from periods of gonadotroph refractoriness between bursts of gonadotropin release, some investigators have proposed that the gonadotropins regulate their own secretion. That is, following their release, gonadotropins may act either on the pituitary gland or the hypothalamus to induce a temporary refractory period with respect to further gonadotropin release.

This possibility has been studied by giving doses of gonadotropin that increase gonadotropin concentrations in peripheral blood approximately twofold. Using this approach, autoregulation of LH was not demonstrable in monkeys (Knobil, 1974) or sheep (Coppings & Malven, 1975). However, the increments in serum LH concentrations established by infusing LH were insignificant relative to the large concentrations of gonadotropins in the local vasculature of the pituitary gland or those reaching the hypothalamus via counter-current blood

flow through the hypophysial portal vasculature. Therefore, these results do not exclude the possibility that gonadotropin autoregulation occurs in these species.

On the other hand, similar studies showed that LH and FSH can autoregulate their own secretion in rabbits (Patritti-Laborde & Odell, 1978; Patritti-Laborde et al., 1981). These effects are dose-dependent and specific for each gonadotropin. This group (Patritti-Laborde et al., 1979) also showed that LH inhibits LH release by a direct action on the pituitary <u>in vitro</u>. However, this latter finding should be interpreted cautiously since the doses of LH given were insignificantly small relative to the large concentrations present in the local vasculature of the pituitary gland.

Effects of LHRH on LH and FSH Secretion

LHRH-Induced LH and FSH Release

In 1971-72, A. V. Schally and R. Guillemin and their coworkers (Matsui et al., 1971; Burgus et al., 1972) reported the structure of a decapeptide isolated from porcine and ovine hypothalami that caused release of LH and FSH in rats. This decapeptide, luteinizing hormone releasing hormone (LHRH), will elicit the release of LH and FSH in all mammalian species tested including domestic animals (Convey, 1973; Pelletier, 1976). Except for horses (Evans & Irvine, 1976), LHRH is normally a more potent secretagogue for LH than for FSH.

LHRH is necessary for the maintenance of basal secretion of LH and FSH. Thus, acute treatment of animals with antiserum to LHRH (McCormack et al., 1977; Lincoln & Fraser, 1979; Kawakami & Higuchi, 1979), anesthesia (Peet & Lincoln, 1977; Plant et al., 1978) or lesions of the medial basal hypothalamus (Bishop et al., 1972; Jackson et al., 1978; Plant et al., 1978) decrease LH and FSH concentrations in serum. The portion of the hypothalamus necessary for normal basal secretion of gonadotropins is the medial basal hypothalamus for monkeys (Plant et al., 1978), ewes (Jackson et al., 1978), and rats (Soper & Weick, 1980).

LHRH secretion is obligatory to induce the preovulatory surges of LH and FSH¹ in nonprimates. Evidence to support this view include the following. Concentrations of LHRH in hypophysial portal blood increase coincident with the preovulatory surges of LH and FSH in rats (Sarkar et al., 1976) and rabbits (Tsou et al., 1977). Gonadotropin surges can be blocked or delayed in rodents and ewes by giving antiserum to LHRH (Kawakami & Higuchi, 1979; Narayana & Dobson, 1979), anesthesia (Siegel et al., 1976; Dobson & Ward, 1977) or by severing rostral hypothalamic innervation to the medial basal hypothalamus (Halasz & Gorski, 1967; Jackson et al., 1978).

¹This primary FSH surge is to be distinguished from the secondary surge of FSH which occurs approximately 12 to 24 h after the primary FSH surge in rats and sheep.

The area of the brain which controls the preovulatory gonadotropin surges in rats probably lies within the medial preoptic area, suprachiasmatic nucleus, or anterior hypothalamic area (Hayashi et al., 1974; Clemens et al., 1976; Brown-Grant & Raisman, 1977; Gray et al., 1978). The corresponding site in sheep lies within the anterior hypothalamic area or perhaps in an extra-hypothalamic region which emits axons that pass through the anterior hypothalamic area (Domanski et al., 1980). Estes et al. (1977) established that biologically and immunologically active LHRH is localized primarily in two areas of the bovine hypothalamus. The major concentration of LHRH is in the pituitary stalk-median eminence area, with a small, but significant concentration of LHRH in the rostral hypothalamus at the level of the organum vasculosum of the stria terminalis.

LHRH concentrations in hypophysial portal blood increase concurrent with the LH and FSH surges in monkeys (Neill et al., 1977). However, it is not clear that LHRH is required for the gonadotropin surges in this species. For example, the gonadotropin surges are not blocked by cutting the pituitary stalk, which presumably removes endogenous LHRH secretion (Ferin et al., 1979). Furthermore, lesioning the medial basal hypothalamus, which removes the source of LHRH, does not block estradiol-induced LH and FSH surges (Wildt et al., 1980a). Thus, LHRH may not be essential for triggering the preovulatory LH and FSH surges in monkeys.

With regard to magnitude of LH release, LHRH is most efficacious just prior to and during preovulatory surges (Pickering & Fink, 1979b; Grimes et al., 1975; Hooley et al., 1974; Reeves et al., 1971). An increase in LHRH-induced FSH release, but not LH release, occurs 24 h subsequent to the preovulatory surges in hamsters (Shander & Goldman, 1978) coincident with the secondary FSH surge.

LH is secreted in a pulsatile manner (Dierschke et al., 1970; Yen et al., 1972; Foster et al., 1975b; Blake et al., 1980; Rahe et al., 1980), probably as a result of pulsatile LHRH release. Many studies have shown that this probably is true. Based on concentrations in hypophysial portal blood (Carmel et al., 1976) or hypothalamic interstitial fluid (Levine & Ramirez, 1980), LHRH appears to be released in pulses at a frequency consistent with pulses of LH secretion. LH pulses are extinguished by antiserum to LHRH (Snabes & Kelch, 1979; Lincoln & Fraser, 1979). Furthermore, pulsatile, but not continuous administration of LHRH reestablishes pulsatile LH release in vivo (Schuiling & Gnodde, 1976b) and in vitro (Osland et al., 1975). In fact, continuous infusion of a constant dose of LHRH to monkeys results in concentrations of LH and FSH in serum that are lower than are normally found throughout the estrous cycle (Belchetz et al., 1978). Some investigators have suggested that pulsatile gonadotrophin release may result from refractory periods of the gonadotrophs. But, this possibility seems unlikely in light of work by Malven (1975). He showed


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that the magnitude of LH release induced by electrically stimulating the hypothalamus was unrelated to the interval from the induced LH pulse and the previous, naturally occurring LH pulse. Collectively, these data are consistent with the hypothesis that LHRH secretion, and not the ability of the pituitary gland to respond to LHRH, is responsible for the pulsatile pattern of gonadotropin secretion.

At various times throughout the estrous cycle, patterns of FSH release diverge from that of LH. For this reason, investigators have searched for a hypothalamic factor distinct from LHRH that preferentially releases FSH. However, at present no such factor has been isolated. Alternatively, the divergent patterns of LH and FSH release may be caused by certain hormonal stimuli that alter the relative amounts of LH and FSH released by LHRH. For example, when rat pituitary cells are incubated with progesterone, LHRHinduced FSH release is augmented, whereas LHRH-induced LH release is not. Exposure of similar cultures to estradiol plus progesterone further augments LHRH-induced FSH release, but inhibits LHRH-induced LH release (Lagace et al., 1980; Drouin & Labrie, 1981). Furthermore, more FSH is released than LH when low doses of LHRH are infused into anesthetized proestrous rats (Wise et al., 1975).

LHRH Priming

LHRH is able to increase gonadotroph responsiveness to subsequent exposures of LHRH. This phenomenon, referred



to as LHRH priming, occurs in humans (Hoff et al., 1979), cattle (Foster, 1978; Padmanabhan et al., 1981), sheep (Crighton & Foster, 1977) and rats (Aiyer et al., 1974). The amount of LHRH needed to prime the pituitary may be less than that required to induce gonadotropin release. This observation is particularly important because it indicates that LHRH may be priming the gonadotrophs under conditions when LHRH is not stimulating gonadotropin release.

The efficacy of LHRH priming is a function of the interval between pulses of LHRH. The interval that yields maximum priming in species studies thus far is 1 to 2 h (cattle: Padmanabhan et al., 1981; Foster, 1978; sheep: Crighton & Foster, 1977; humans: Rommler, 1978; rat: Aiyer et al., 1974). The magnitude of LHRH priming, in terms of gonadotropin release, also depends on the stage of the menstrual or estrous cycle. Thus, priming is greatest just prior to and during the preovulatory surge in women (Hoff et al., 1977), ewes (Crighton & Foster, 1977) and rats (Aiyer et al., 1974).

The true reward of basic research is application of new-found knowledge. Investigators have begun to apply the basic knowledge of how small, repetitive doses of LHRH affects gonadotropin secretion to solve reproductive problems that result from hypothalamic deficiencies. Thus, ovulation can be induced in anestrous cows postpartum (Walters et al., 1980) and seasonally anestrous ewes (Domanski et al., 1977);



cyclicity can be established in monkeys that were prepubertal (Wildt et al., 1980b) or acyclic due to hypothalamic lesions (Knobil et al., 1980) or pituitary stalk section (Knobil, 1980); and hypogonadal girls and women can be treated so as to establish normal endocrine profiles (Valk et al., 1980; Marshall & Kelch, 1979). I believe these successful results reflect two general actions by LHRH. First, LHRH stimulates pulsatile gonadotropin release which in turn stimulates estradiol secretion. This process may require many days or weeks. Then in the presence of estradiol, LHRH primes the gonadotrophs. This process may occur over a period of hours or 1 to 2 days and climaxes with gonadotropin surges.

Mechanism of Action of LHRH

LHRH acts on the anterior pituitary gland to increase gonadotropin secretion (Pickering & Fink, 1979a); DeKoning et al., 1980; Labrie et al., 1979; Padmanabhan et al., 1978). The effects of LHRH includes priming of the gonadotrophs and synthesis and release of gonadotropins.

LHRH interacts with the gonadotrophs by binding to specific receptors located on the outer surface of the plasma membrane (Savoy-Moore et al., 1980; Clayton et al., 1980). Thus, the amount of LH released by LHRH is directly related to the amount of LHRH occupying binding sites (Naor et al., 1980a). Investigators have suggested that changes in responsiveness of the gonadotrophs to LHRH may be due to changes in the number of LHRH receptors. However, this proposal is



not yet supported by the literature. Thus, as gonadotroph responsiveness increases before or during the preovulatory gonadotropin surges, numbers of LHRH binding sites remain unchanged in cows (K. Leung, personal communication) and sheep (Wagner et al., 1979), or decrease in rats (Savoy-Moore et al., 1980; Clayton et al., 1980). However, these results may reflect the technical limitation of measuring LHRH binding sites in the total membrane fraction rather than only on the outer surface of the plasma membrane. Only a small percentage of all LHRH binding sites in gonadotrophs are located on the plasma membrane, and yet these probably represent the population of binding sites with which LHRH normally interacts to cause LH and FSH release.

High concentrations (40 to 59 mM) extracellular K⁺ mimic LHRH-induced LH and FSH release (Pickering & Fink, 1976; Carruthers et al., 1980; DeKoning et al., 1980), probably by depolarizing the plasma membrane of the gonadotrophs. Membrane depolarization permits extracellular Ca⁺⁺ to flow into the cell. This influx of Ca⁺⁺ is essential for LHRH- or K⁺-induced gonadotropin release (Naor et al., 1980b; Pickering & Fink, 1979a). Investigators have proposed that Ca⁺⁺ may act to facilitate movement of secretory vesicles along microfilaments or microtubules (Sherline et al., 1977) in a manner analagous to how Ca⁺⁺ facilitates myofilament movement. However, blocks of microfilament and microtubule function do not alter gonadotropin release (Pickering & Fink, 1979a).

Alternatively, Ca^{++} may participate in gonadotropin release by increasing cGMP production. Both, LHRH and Ca^{++} stimulate cGMP synthesis (Naor et al., 1980b; Snyder et al., 1980). Arguments that dissociation of cGMP or cAMP production from gonadotropin release disqualifies these nucleotides as effectors of gonadotropin release (Berault et al., 1980; Naor & Catt, 1980) are premature until similar studies are conducted on purified gonadotrophs. The reason for this view is that cyclic nucleotide production by gonadotrophs may increase coincident with LH release, but may be masked by basal production of cyclic nucleotides by other cell types in the pituitary that greatly outnumber the gonadotrophs.

 Ca^{++} may directly alter enzyme activity in the pituitary cells as it does in other systems (Fischer et al., 1971). Douglas (1974) proposed that the divalent positive charges of Ca^{++} may draw the secretory vesicles towards the plasma membrane to facilitate exocytosis. The secretory vesicles and plasma membranes both possess negative surface charges and therefore tend to repel each other in the absence of a mediator. Ca^{++} is not needed for binding of LHRH to its binding sites (Naor et al., 1980b).

LHRH priming may be mediated through specific LHRH receptors, though no evidence exists to support or refute this possibility. Many differences exist between the mechanisms governing the effects of LHRH on release and priming. For example, membrane depolarization per se will not cause LHRH priming. Thus, K⁺ induces gonadotropin release, but



does not prime the gonadotrophs to subsequent LHRH or K^+ (Pickering & Fink, 1976a). Furthermore, since K^+ -induced gonadotropin release leads to refractoriness of the gonadotrophs to LHRH or K^+ (DeKoning et al., 1980), it is reasonable to propose that refractoriness is not necessarily a consequence of priming.

LHRH priming also requires synthesis of protein(s), whereas immediate release of gonadotropins does not (DeKoning et al., 1980; Pickering & Fink, 1979a). These proteins are synthesized within 1 to 2 h after LHRH exposure. Newly synthesized LH and FSH may be the proteins required for priming since their rate of synthesis is also augmented 1 to 2 h after giving LHRH (Liu & Jackson, 1978; Khar & Jutisz, 1980). And while DeKoning et al. (1976b) noted no change in the rate of gonadotropin synthesis associated with LHRH priming, these findings should be verified by more sensitive methods, i.e., incorporation of radioactive precursors into gonadotropin.

Pituitary glands from chronically ovariectomized rats (Aiyer et al., 1976) or steers (Padmanabhan et al., 1981) cannot be primed by LHRH. Furthermore, the rate of LH release is similar for pituitary glands primed with LHRH and those from ovariectomized rats (DeKoning et al., 1980). For these reasons, it has been proposed that pituitary glands from ovariectomized rats are fully primed <u>in vivo</u>. This might be accomplished if secretion rates of LHRH increase after ovariectomy (Sarkar & Fink, 1980).



It also is not clear whether cyclic nucleotides are involved in LHRH priming. DeKoning and coworkers (1977, 1978) showed that cAMP mimicked LHRH priming, while Pickering and Fink (1979a) could not demonstrate such a relationship. Cyclic GMP production can be stimulated by LHRH, but requires extracellular Ca⁺⁺ (Naor et al., 1980b). Since extracellular Ca⁺⁺ is not required for priming (Pickering & Fink, 1979a; Bourne & Baldwin, 1980), it seems logical that LHRH-induced cGMP production does not initiate priming.

Finally, LHRH is capable of stimulating LH and FSH synthesis. This has been determined by measuring total hormone in an <u>in vitro</u> system (medium plus cells), (Apfelbaum & Taleisnik, 1976; Redding et al., 1972) and incorporation of radiolabeled amino acids and glucosamine (Azhar et al., 1978; Liu & Jackson, 1978; Liu & Jackson, 1979; Khar & Jutisz, 1980). However, disagreement exists as to whether LHRH-stimulated synthesis represents an increased rate of glycosylation (Azhar et al., 1978; Liu & Jackson, 1979) or protein synthesis (Khar & Jutisz, 1980). LHRH did not increase total LH (medium plus cells) in bovine pituitary cell cultures (Padmanabhan et al., 1978).

In summary, LHRH may affect gonadotrophs by binding to specific membrane associated receptors. From this association arises three actions. LHRH induces release of gonadotropins by depolarizing the plasma membrane, allowing extracellular Ca⁺⁺ to flow into the cell. The site of action for Ca⁺⁺ is unknown. Stimulation of gonadotropin release

for more than 2 to 4 h may eventuate in the plasma membrane, and thus the gonadotroph, becoming refractory to further stimulation. The second action of LHRH is to prime the gonadotrophs by stimulating synthesis of new protein, and possibly RNA. As a result gonadotrophs now are able to release more gonadotropin after subsequent LHRH stimulation. Gonadotrophs may be primed by LHRH in the absence of hormone release. This action apparently involves microfilaments and possibly microtubules. The intracellular messenger of this action if unknown, but appears not to be Ca⁺⁺. The third action of LHRH is to stimulate synthesis of gonadotropins. It is not clear whether this represents augmented protein synthesis, glycosylation or both. Nor is it known whether this action can occur independent of release or priming.

Negative Feedback of Estradiol and Progesterone on LH and FSH Secretion

Moore and Price (1932) were the first to note that ovarian factors exert negative feedback on gonadotropin release. The principle ovarian factors that exert negative feedback are estradiol and progesterone. These ovarian steriods maintain gonadotropin secretion at basal concentrations as a consequence of negative feedback.

Control of Basal Gonadotropin Secretion

Concentrations of LH and FSH increase in serum following ovariectomy of women (Yen & Tsai, 1971), monkeys (Karsch et al., 1973b), cows (Beck et al., 1976), ewes (Foster et al., 1975a) and rats (Brown-Grant, 1977). Replacement of estradiol alone reduces serum LH but not to precastration levels and progesterone has little or no effect. However, a combination of estradiol and progesterone synergizes to reduce serum LH to precastration concentrations (Wallach et al., 1970; Karsch et al., 1973b; Beck et al., 1976; Karsch et al., 1980; Goodman, 1978; Bieglmayer et al., 1980).

Inhibition of basal release of FSH by extradiol and progesterone has been studied only in primates and rodents. Concentrations of FSH in serum, like those of LH, increase after ovariectomy and are partially inhibited by extradiol, but reduced to precastration concentrations only by the combination of estradiol and progesterone in women (Wallach et al., 1970) and monkeys (Karsch et al., 1973). But this requirement for progesterone is inconsistent with the pattern of progesterone secretion one sees during the menstrual cycle. That is, concentrations of progesterone in serum are very low during the follicular phase of the menstrual cycle, yet gonadotropin levels are as low then as during the luteal phase. Certainly ovariectomy during either portion of the cycle leads to increased LH and FSH levels in blood (Yen & Tsai, 1971).

In ovariectomized rats, estradiol reduces FSH concentrations more than it reduces LH, though precastration levels of neither hormone are attained (Blake et al., 1972).



While progesterone does not alter serum LH concentrations in ovariectomized rats, FSH levels are reduced (Bieglmayer et al., 1980). Estradiol plus progesterone does not reduce serum concentrations of FSH more than estradiol alone (Blake et al., 1972). Judging from the literature, negative feedback of gonadotropin release by steroids occurs over a very narrow concentration range (Karsch et al., 1973; Goodman, 1978; Goodman et al., 1980).

Exogenous extradiol reduces the magnitude of the LH pulses in ovariectomized monkeys (Yamaji et al., 1972) and ewes (Goodman & Karsch, 1980), whereas progesterone decreases LH pulse frequency in ovariectomized ewes (Goodman & Karsch, 1980), but is without effect in ovariectomized monkeys (Yamaji et al., 1972).

Control of Preovulatory Gonadotropin Surges

Increased concentrations of estradiol in serum reduce the magnitude of the preovulatory LH surge in rats, in addition to inducing the surge. Thus, the magnitude of preovulatory LH surges are reduced if estradiol concentrations are maintained throughout the surge, rather than being allowed to decrease normally prior to the LH peak (Turgeon, 1979).

Progesterone blocks estradiol-induced surges of LH and FSH in monkeys (Dierschke et al., 1973) and LH surges in rats (Goodman, 1978). On the other hand, it is not clear whether progesterone blocks gonadotropin surges in cows and ewes. First, all pertinent studies of these species have been conducted on chronically ovariectomized animals. Whether these experimental models represent the mature, intact female with fidelity is dubious. Second, Rajamahendran et al. (1979) succeeded, while Short and coworkers (1973, 1979) and Hausler and Malven (1976) failed to block estradiol-induced LH surges with progesterone in ovariectomized cows. The variable(s) responsible for these divergent results is unknown. Finally, although estradiolinduced LH surges were blocked by giving progesterone to ovariectomized ewes (Scaramuzzi et al., 1971; Bolt et al., 1971; Howland et al., 1971), they were not blocked by endogenous progesterone in three of twelve diestrous ewes (Bolt et al., 1971; Howland et al., 1971).

In rats, a circadian rhythm endogenous to the hypothalamus signals gonadotropin release. This rhythm can be demonstrated by giving a single dose of estradiol to ovariectomized rats and observing the daily occurrence of preovulatory-like gonadotropin surges for the next three to five days. It is believed that the surge of progesterone that occurs simultaneously with the preovulatory gonadotropin surges is responsible for blocking the subsequent daily gonadotropin surges in cycling rats. This view is supported by the observation that progesterone given to estradiol-treated ovariectomized rats, concurrent with the gonadotropin surges on the first day, will block gonadotropin surges on subsequent days (Bank & Freeman, 1980).



Sites of Action

Exogenous estradiol causes a transient reduction of LH and FSH concentrations in serum and of LHRH-induced LH and FSH release in ovariectomized monkeys (Nakai et al., 1978) and rats (Debeljuk et al., 1977). Similar observations have been made for LH in ewes (Coppings & Malven, 1976) and cows (Beck & Convey, 1977). This transient period of inhibition of gonadotropin release is not normally seen before preovulatory surges, but this may be because gonadotropin levels are already low, i.e., not increased as in ovariectomized animals. This decreased ability of the pituitary to release LH has been interpreted as evidence for a direct action of estradiol on the pituitary gland. This interpretation is supported by two studies that demonstrate decreased LHRHinduced LH release from rat anterior pituitary tissue incubated in vitro with estradiol (Tang & Spies, 1975; DeKoning et al., 1976c).

Estradiol may also inhibit gonadotropin secretion via effects on the hypothalamus. This possibility is attractive in light of the paucity of evidence that estradiol acts directly at the pituitary to inhibit responsiveness of the gonadotrophs to LHRH. Estradiol may reduce responsiveness of the gonadotrophs to LHRH by altering LHRH secretion thereby reducing LHRH priming. In support of this view, Sarkar and Fink (1980) found that estradiol reduced the rate of LHRH secretion in rat hypophysial portal blood within 30 min. However, Carmel et al. (1976) were unable to detect a



change in the pulsatile LHRH secretion profile during the period when estradiol inhibits gonadotropin release in monkeys. Alternatively, estradiol may stimulate the secretion of a hypothalamic factor that inhibits gonadotropin secretion.

Progesterone apparently inhibits LHRH secretion. Thus, progesterone reduces the frequency of pulsatile LH release without altering LHRH-induced LH release in ovariectomized ewes (Goodman & Karsch, 1980). In addition, progesterone blocks daily gonadotropin surges in estradioltreated ovariectomized rats by blocking daily release of LHRH and not by inhibiting gonadotroph responsiveness (DePaolo & Barraclough, 1979).

Progesterone also can inhibit gonadotropin release via a direct effect on the pituitary gland. Thus, in rats (Lagace et al., 1980; Drouin & Labrie, 1981) and cows (Padmanabhan & Convey, 1980), progesterone blocks the stimulatory effect of estradiol on LHRH-induced LH release. In addition, progesterone inhibits the stimulatory effect of LHRH-induced LH release in ovariectomized rats in which the pituitary stalk is sectioned (Greeley et al., 1975). On the other hand, progesterone increases FSH release from rat anterior pituitary cells (Lagace et al., 1980; Drouin & Labrie, 1981), but had no effect on FSH release from bovine anterior pituitary cells (Padmanabhan & Convey, 1980). Finally, progesterone acts directly on the anterior pituitary gland to inhibit the stimulatory effect of estradiol on LHRH



priming with respect to LH secretion in cattle (Padmanabhan & Convey, 1981) and rats (Turgeon & Waring, 1981).

Control of the Secondary FSH Surge

In addition to the primary FSH surge which occurs coincident with the preovulatory LH surge, a secondary FSH surge occurs approximately 12 to 24 h later in ewes (Pant et al., 1977) and rats (Butcher et al., 1974). However, the stimulus that induces the secondary FSH surge is unknown.

A protein factor, inhibin, has been identified in follicular fluid that preferentially inhibits FSH release relative to LH release (DeJong & Sharpe, 1976). The loss of inhibin at or near ovulation may be responsible for the secondary FSH surge. In fact, inhibin activity in ovarian venous blood decreases on the evening of proestrus and early morning of estrus; the period that proceeds the initiation of the secondary FSH surge (DePaolo et al., 1979). Furthermore, ovarian follicular fluid will block the secondary FSH surge (Schwartz & Channing, 1977), LHRH-induced FSH release (Shander et al., 1980b) and FSH synthesis (Chowdhury et al., 1978) in rats. However, no one has determined whether the amount of inhibin present in the peripheral blood of rats is sufficient to inhibit FSH release.



Positive Feedback of Estradiol and Progesterone on LH and FSH Secretion

Control of Preovulatory Gonadotropin Surges

Considerable evidence indicates that increasing concentrations of estradiol are the primary stimuli responsible for inducing LH and FSH surges in spontaneously ovulating mammals. Thus, estradiol concentrations increase before gonadotropin surges in women (Yen et al., 1975), monkeys (Hotchkiss et al., 1971), cows (Chenault et al., 1975), ewes (Pant et al., 1977) and rats (Butcher et al., 1974). Neutralization of estradiol with antiserum blocks the LH surge and ovulation in rats (Ferin et al., 1969; Neill et al., 1971) and ovulation in ewes (Fairclough et al., 1976). Finally, replacement of estradiol reestablishes the LH and FSH surges in women (Monroe et al., 1972), monkeys (Helmond et al., 1980), cows (Beck & Convey, 1977; FSH not measured), ewes (Pant, 1973; Reeves et al., 1974) and rats (Brown-Grant, 1974; Goodman, 1978). Estradiol will not induce gonadotropin surges in steers (E. M. Convey, personal communication), wethers (Karsch & Foster, 1975) or orchidectomized rats (Neill, 1972), but will in orchidectomized men (Sterns et al., 1973) and monkeys (Steiner et al., 1976).

Concentrations of estradiol need not decrease to induce the gonadotropin surges in monkeys (Karsch et al., 1973a). This is also true for rats, though maintaining estradiol concentrations throughout the LH surge in this species reduces the magnitude of the surge (Turgeon, 1979). The relationship between the dose of estradiol and the magnitude of the LH surge is apparently not graded, but rather all-or-none for monkeys (Karsch et al., 1973a) and cows (Short et al., 1977a).

In species in which progesterone increases in serum concurrent with the preovulatory gonadotropin surges (primates, rats, dogs), progesterone synergizes with estradiol to augment the magnitude of the LH and FSH surges (Chang & Jaffe, 1978; Terasawa et al., 1980; Concannon et al., 1979; DePaolo & Barraclough, 1979). Indeed, preovulatory LH surges do not occur when ovarian and adrenal sources of progesterone are removed (Wilson et al., 1978). While progesterone has also been shown to induce (Mann & Barraclough, 1973) or hasten (Helmond et al., 1980; Concannon et al., 1979; Brown-Grant & Naftolin, 1972) onset of the gonadotropin surges under experimental conditions, these effects may have no physiological correlate since progesterone concentrations only begin to increase concurrent with or shortly after onset of the preovulatory LH and FSH surges. Thus, the proestrus increase in progesterone concentrations in serum of these species probably affects gonadotropin surges by augmenting their magnitude.

In rats, induction of the gonadotropin surges, whether by estradiol or progesterone, is tightly coupled to the light-dark cycle (Brown-Grant, 1974). This is not true for cows (Rzepkowski, 1981) or ewes (Jackson et al., 1975). However, Karsch (1978) reported that ovariectomized

ewes exhibited LH surges at the same time daily when given small, continuous quantities of estradiol. Whether the mechanism driving the latter observation exists in other domestic animals or primates, and whether it serves any physiological purpose is unknown.

Sites of Action

Estradiol increases the ability of LHRH to induce LH and FSH release in vivo in ovariectomized women (Lotz, 1975; Keye & Jaffe, 1975), monkeys (Nakai et al., 1978), cows (Beck & Convey, 1977), and rats (Libertun et al., 1974; Schuiling & Gnodde, 1976b). Estradiol may cause this increase by exerting a positive feedback effect directly upon the pituitary gland. Indeed, estradiol increases gonadotropin release in monkeys (Ferin et al., 1979) or LHRH-induced gonadotropin release in rats (Greeley et al., 1975; Fink & Henderson, 1977) when the hypothalamic influence on the pituitary gland is prevented by sectioning the hypophysial stalk. Furthermore, estradiol augments LHRH-induced LH and FSH release from pituitary cells in culture: cows (Padmanabhan et al., 1978; Padmanabhan & Convey, 1978), ewes (Moss & Nett, 1980; Huang & Miller, 1980). LHRH-induced gonadotropin release from rat pituitary cells in vitro is initially inhibited by estradiol (Tang & Spies, 1975; DeKoning et al., 1978), but then is increased beginning 5 to 10 h after adding estradiol (Drouin et al., 1976; Lagace et al., 1980; Drouin & Labrie, 1981). Miller and Wu (1981)

examined the effect of estradiol on basal FSH release <u>in</u> <u>vitro</u> across species. They discovered that the influence of estradiol on FSH release was inhibitory for pituitary glands from sheep, cattle, and pigs, stimulatory for rat pituitary glands and did not alter FSH release from rabbit pituitary glands.

Synthesis of LH or FSH increases when rat pituitary glands are incubated with estradiol for 2 to 6 h (Apfelbaum & Taleisnik, 1976; Liu & Jackson, 1977), but not for 48 h (Drouin & Labrie, 1981). Estradiol also increases LH synthesis by pituitary cells from cows (Padmanabhan et al., 1978) and ewes (Moss & Nett, 1980) by 28 and 18 h, respectively. On the other hand, when estradiol is given to ovariectomized cows, pituitary content of LH is not altered despite augmented LHRH-induced LH release <u>in vitro</u> and reduced concentrations of LH in serum (Convey et al., 1981). Thus, estradiol apparently stimulates gonadotroph responsiveness to LHRH in cows independent of increased synthesis of LH. Estradiol reduces synthesis of FSH by ovine pituitary cell cultures (Miller et al., 1977; Huang & Miller, 1980).

Estradiol augments LHRH priming (Lasley et al., 1975; DePaolo & Barraclough, 1979; Padmanabhan & Convey, 1981). The potential importance of estradiol-facilitated LHRH priming is exemplified by a report by Henderson et al. (1977). These investigators found that LHRH-induced LH release is increased in estradiol treated rats relative to those given oil. However, if endogenous LHRH secretion is

blocked by anesthesia 4 h prior to the exogenous LHRH challenge, then LHRH-induced LH release is not augmented by estradiol. It is not clear from this study whether estradiol augments LHRH priming by altering LHRH release or by enhancing the ability of gonadotrophs to respond to LHRH priming.

The effects of estradiol on the hypothalamus and central nervous system, as it pertains to preovulatory gonadotropin surges, have been studied primarily in monkeys, ewes, and rats. In rats, estradiol stimulates secretion of LHRH coincident with the gonadotropin surges (Sarkar et al., 1976; Sarkar & Fink, 1979). These gonadotropin surges are blocked or delayed by giving antiserum against LHRH (Kawakami & Higuchi, 1979). The medial preoptic area, suprachiasmatic nucleus, and anterior hypothalamic area are the principle hypothalamic sites of action for estradiol with regard to regulation of gonadotropin surges. This has been determined by implanting estradiol in (Goodman, 1978) or electrically stimulating (Sherwood et al., 1976) discrete areas of the hypothalamus. Furthermore, antiserum against estradiol prevents the increase in rate of protein synthesis in the preoptic area that normally occurs at the time of the gonadotropin surges (Ter Haar & MacKinnon, 1975).

Estradiol-induced LH and FSH surges in ewes are also dependent on LHRH secretion, though no evidence is available to indicate whether estradiol actually increases the rate of LHRH secretion. Thus, inhibition of LHRH secretion with

anesthesia (Radford & Wallace, 1974; Dobson & Ward, 1977) or neutralization of its biological activity with antiserum against LHRH (Narayana & Dobson, 1979) block or delay the estradiol-induced LH and FSH surges.

Judging from studies in which specific nerve tracts in the brain have been transected, neurons necessary for the expression of the estradiol-induced gonadotropin surges in ewes originate in the preoptic area, suprachiasmatic nucleus, or anterior hypothalamic area (Jackson et al., 1978; Radford, 1979; Domanski et al., 1980).

Whether estradiol acts on the hypothalamus to induce preovulatory gonadotropin surges in monkeys is controversial. Norman et al. (1976) reported that lesions in the anterior hypothalamus prevented normal cyclicity or estradiol-induced LH and FSH surges in Rhesus monkeys. Conceivably, these lesions prevented release of LHRH that normally follows estradiol treatment (Neill et al., 1977). But, when hypothalamic/pituitary communication was eliminated by sectioning the pituitary stalk (Ferin et al., 1979) or by lesioning the medial basal hypothalamus (Wildt et al., 1980a), estradiol still induced LH and FSH surges. We interpret these data as evidence that LHRH release is not needed to actually trigger the gonadotropin surges in monkeys. However, pulsatile LHRH is required up until a few hours before estradiol treatment (Wildt et al., 1980a). This trophic LHRH stimulation is required to maintain the secretory integrity of the gonadotrophs.



Progesterone acts directly on the rat pituitary gland to augment the stimulatory effect of estradiol on LHRHinduced LH and FSH release (Fink & Henderson, 1977; Lagace et al., 1980; Drouin & Labrie, 1981). When pituitary glands from proestrus rats were incubated with progesterone <u>in vitro</u>, LHRH-induced LH release was augmented (Turgeon & Waring, 1981). The augmentation of LH secretion by progesterone is short-lived and reverts to an inhibitory effect within 16 h.

Progesterone increases synthesis of FSH by rat pituitary cells in primary culture, and estradiol augments this action. Neither progesterone nor estradiol, alone or in combination, altered LH synthesis (Drouin & Labrie, 1981). FSH synthesis by ovine pituitary cultures is not altered by progesterone (Miller et al., 1977).

Progesterone apparently acts on the hypothalamus, at least in part, to increase gonadotropin release in rats. However, it is not clear how progesterone affects the hypothalamus. LHRH release is required for progesterone to induce gonadotropin surges in rats, since antiserum against LHRH blocks progesterone-induced gonadotropin surges (Lu & Yen, 1980; Kawakami & Higuchi, 1979). But while some investigators report that progesterone increases secretion of LHRH from the hypothalamus <u>in vivo</u> (Levine & Ramirez, 1980) and <u>in vitro</u> (Ramirez et al., 1980), others report that progesterone decreases the amount of LHRH release associated with gonadotropin surges (Sarkar & Fink, 1979; Sherwood et



al., 1976). Obviously, much more work is required to determine the effects of progesterone on LHRH secretion.

Progesterone and estradiol both act on the hypothalamus to augment gonadotropin surges in rodents. However, the mechanisms of action or potencies of the two steriods are different. In guinea pigs, anesthesia blocks gonadotropin surges induced by progesterone, but not by estradiol (Terasawa et al., 1979). In hamsters, anesthesia blocks spontaneous, preovulatory gonadotropin surges, but not surges induced by progesterone (Siegel et al., 1976). In rats, results obtained by deafferentating specific neural sites supports the hypothesis that neurons originating in or passing caudally through the organum vasculosum of the lamina terminals or preoptic area are necessary for expression of the stimulatory effect exerted by progesterone, whereas different neurons passing through a horizontal plane dorsal to the preoptic area permit expression of the estradiol-induced surges (Kawakami et al., 1978a,b; Samson & McCann, 1979).

Hormone Binding Sites and Control of LH and FSH Secretion

LHRH Binding Sites

The number of binding sites for LHRH in the rat anterior pituitary increases between diestrus and noon of proestrus (Savoy-Moore et al., 1980; Clayton et al., 1980). However, during the period prior to the gonadotropin surges when responsiveness of gonadotrophs is increasing rapidly,


the number of LHRH binding sites either decreases (rat: Savoy-Moore et al., 1980; Clayton et al., 1980) or does not change (cows: K. Leung, personal communications; ewe: Wagner et al., 1979). Thus, increased numbers of LHRH binding sites may not be the means by which estradiol increases gonadotroph sensitivity to LHRH. Alternatively, increased numbers of LHRH binding sites that are present before noon or proestrus may induce the increase in gonadotroph responsiveness that occurs after noon on proestrus. Clayton et al. (1980) showed that giving small doses of LHRH to rats increases the number of LHRH binding sites in the pituitary. Might this be a mechanism by which LHRH primes the gonadotroph?

Interpretation of these LHRH binding sites is difficult. One reason, is that the pituitary possesses two populations of binding sites (Clayton et al., 1979). One population has a higher affinity and is thought to represent the LHRH receptor. The second population of binding sites appears to be a proteolytic enzyme, perhaps an LHRHase. The presence of this proteolytic enzyme complicates quantification of LHRH receptors by degrading LHRH tracer as well as by binding to LHRH. This complication appears to have been solved by using an LHRH analogue that has a very low affinity for the proteolytic enzyme. Whether the proteolytic enzymes play a role in regulating the secretory status of gonadotrophs by degrading LHRH, and thus altering the effective concentration or binding half-life of LHRH relative to the gonadotrophs is unknown.

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Another reason why it is difficult to interpret the importance of number of LHRH binding site is that present methods quantify all LHRH binding sites in the cell. This is significant since only a very small percentage of all LHRH binding, as determined by immunohistochemistry, is located in the plasma membrane (Sternberger et al., 1978). Therefore, LHRH binding sites, as presently measured, may not reflect the population of binding sites that normally interacts with LHRH and transmits intracellular signals.

Estradiol Binding Sites

Estradiol translocates estradiol binding proteins from the cytoplasm to the nucleus where the hormone-binding site complex is believed to act. During the rat estrous cycle, number of estradiol binding sites in nuclei of anterior pituitary cells is greatest on the morning of proestrus and then decreases that afternoon. These changes in number of estradiol binding sites parallel changes of estradiol concentrations in serum (Sen & Menon, 1978). When exogenous estradiol is given to rats (Menon & Gunaga, 1976) or monkeys (Attardi et al., 1980), the number of estradiol binding sites in nuclei of the anterior pituitary cells increases during the period of low gonadotroph responsiveness to LHRH and remains high as gonadotroph responsiveness increases. The number of estradiol binding sites in nuclei of hypothalamic cells also increases (Menon & Gunaga, 1976).



The mechanism by which progesterone augments or blocks the estradiol-induced gonadotropin surges in rats is unknown. Some investigators have suggested that progesterone may alter the number of estradiol receptors in the pituitary gland or hypothalamus. However, Attardi (1981) showed that progesterone does not alter accumulation or retention of estradiol binding sites in nuclei of the pituitary or hypothalamic cells under conditions where progesterone either augments or blocks estradiol-induced gonadotropin surges.

Progesterone Binding Sites

Binding sites for progesterone are present in the anterior pituitary, hypothalamus, and preoptic area. Progesterone binding sites in all three of these areas increase after giving estradiol to rats (MacLusky & McEwen, 1980), and estrogen antagonists block this effect (Roy et al., 1979). In monkeys, estradiol increases the number of progesterone binding sites in the anterior pituitary and hypothalamus, but not in the preoptic area (MacLusky et al., 1980).

GENERAL MATERIALS AND METHODS

Three primary questions have been addressed in this dissertation. To answer the first question, three experiments were conducted and are presented consecutively under the heading of that question.

Dairy cows, heifers, and steers were the experimental animals in these studies. Blood was collected via jugular cannulae for determination of concentrations of LH (Convey et al., 1976) and FSH (Carruthers et al., 1980) in serum and concentrations of estradiol (Carruthers & Hafs, 1980) and progesterone (Louis et al., 1973) in plasma.

When LHRH was used, it was dissolved in saline and injected intravenously every 20, 25 or 50 min for 8 to 10 h as specified in each experiment. Saline, which was the vehicle for LHRH, was injected as a control. LHRH was generously supplied by Dr. R. Rippel (Abbott Labs, N. Chicago, Il).

Estradiol-17B (Sigma Chemical Co., St. Louis, Mo) was administered by one of two means. Estradiol was dissolved in safflower seed oil (Sigma Chemical Co., St. Louis, Mo) and given as a single injection (1 mg estradiol/3 ml oil) intramuscularly. To dissolve estradiol-17B in oil, it was dissolved in anhydrous ethyl ether (1 mg estradiol/ml ether;



Mallinckrodt, Inc., St. Louis, Mo). This solution was mixed with oil (1 mg estradiol/3 ml oil) and the ether evaporated. Safflower seed oil was injected into animals used as controls.

Alternatively, estradiol-17B was given via implants placed subcutaneously over the scapula. Implants consisted of polydimethylsiloxane (Silastic; Dow Corning) tubing (I.D., 3.35 mm; O.D., 4.65 mm; length, 55.0 mm) packed with crystalline estradiol-17B (Beck et al., 1976). Implants were placed in a "donor" steer for 24 h prior to use to allow the release rate of estradiol to approach a steady state. Empty implants were given to animals used as controls.

Progesterone (Sigma Chemical Co., St. Louis, Mo) was infused intravenously at a rate of 3 mg/9 ml/h. Progesterone was dissolved in ethanol:1.8% saline (1:1; vol:vol) to a concentration of 333 ug progesterone/ml diluent.

In some experiments, a model was required wherein endogenous LHRH release was inhibited or reduced below levels which cause LH and FSH release. We chose to use estradioltreated steers as our model. The rationale for this choice included the following considerations. Estradiol does not cause an LH surge in steers (E. M. Convey, unpublished observation). This contrasts with the ability of estradiol to induce gonadotropin surges after a 12 and 24 h latency period in ovariectomized cows (Short et al., 1979). Similar observations have been reported for orchidectomized rats (Neill, 1972) and wethers (Karsch & Foster, 1975). Instead, estradiol decreases serum LH concentrations, completely

eliminating the pulsatile LH release pattern which is characteristic of steers. We assume that this decrease in LH secretion results from decreased LHRH release and not a refractoriness of the pituitary to LHRH since exogenous LHRH will cause LH release in this model. In addition, we noted that pituitary glands of rats are not sexually differentiated (Harris & Jacobsohn, 1952). If we assume that sexual differentiation of the pituitary gland of cattle does not occur, then it seems reasonable to expect that effects of estradiol and LHRH on LH and FSH secretion that are mediated directly on pituitary gonadotrophs would be similar for steers and cows.

QUESTION 1

DOES ESTRADIOL-17B INDUCE THE PREOVULATORY LH AND FSH SURGES IN CATTLE BY INCREASING PITUITARY SENSITIVITY TO LHRH AND THEN INCREASING LHRH RELEASE?

Objectives

Experiments were designed to determine: (1) the ability of estradiol to alter the capacity of LHRH to induce LH and FSH release <u>in vivo</u> and (2) whether LHRH is required to cause the LH and FSH surge.

Experiment 1 Induced LH Surge in Steers

Objective

The objective was to determine whether multiple injections of LHRH beginning 12 h after estradiol induced a preovulatory-like LH surge in steers. I reasoned that if the LH surge, which occurs beginning 12 h after estradiol in ovariectomized cows, is due to LHRH released at this time, a preovulatory-like surge in steers should be induced by supplying the missing component, e.g., LHRH.

Materials and Methods

Sixteen Holstein steers, castrated at least five days earlier and weighing 320 to 400 kg, were assigned to one of four groups according to a 2 x 2 factorial arrangement of

treatments. Factors were oil or 1 mg estradiol in oil given at time zero and saline or 1 ug LHRH in saline given at 20 min intervals between 12 and 20 h after oil or estradiol.

Blood was sampled at 1 or 2 h intervals beginning before oil or estradiol and continuing until LHRH or saline injections began. Thereafter, blood was collected immediately before every other LHRH or saline injection, i.e., at 40 min intervals.

Data were transformed logarithmically to obtain homogenous variance and then analyzed by autoregressive split-plot analysis to remove autocorrelations of repeat measurements (Barr et al., 1979). This analysis does not require all correlations between times within a treatment to be equal, thus improving the accuracy of the error estimate. Specific comparisons were made using Bonferroni's t-test (Gill, 1978).

Results

Estradiol increased (P<0.005) concentrations of estradiol in serum from undetectable levels (<l pg/ml) to 166 pg/ml 2 h after injection. Concentrations were 144, 72, and 30 pg/ml at 4, 8, and 12 h, respectively, and were undetectable at 24 h.

Frequent injections of LHRH did not affect estradiol concentrations in the serum. Baseline concentrations of LH averaged 5 ng/ml and were not changed (P>0.05) in steers given oil and then frequent injections of saline (closed circles; Figure 1). LHRH injected at 20 min intervals for

8 h into steers given oil increased (P<0.0025) LH to 13 ng/ml at 40 min (first measurement; closed triangles), with no further increase thereafter. Estradiol reduced (P<0.0025) LH concentrations in serum by 2 h after it was injected (open circles and triangles) and this effect was still evident at 10 h. By 12 h after estradiol, LH concentrations had returned to values characteristic of untreated steers and were not affected (P>0.05) by injections of saline (open triangles). However, when LHRH was injected beginning 12 h after estradiol (open circles), LH increased (P<0.0025) to 23 ng/ml within 40 min and continued to increase linearly, reaching a peak of 49 ng/ml at 120 min after starting LHRH treatment. Thereafter, LH concentrations plummetted, despite continued LHRH injections. Thus, estradiol increased the capacity of these pituitary glands to release LH in response to LHRH between 12 to 18 h, i.e., the time when the estradiol-induced LH surges normally occur in ovariectomized cows.

Experiment 2 Change in LHRH-Induced LH and FSH Release With Time After Estradiol-17B in Steers

Objective

To determine changes in ability of the pituitary gland to release LH and FSH in response to LHRH with time after estradiol.

Figure 1.--LH concentrations in serum of steers given 3 ml oil or 1 mg estradiol in oil at time zero and then 5 ml saline or 1 ug LHRH in saline every 20 min for 12 to 20 h. Values represent the mean of four steers.



Materials and Methods

Twenty-four Holstein steers, castrated at least nine days earlier and weighting 190 to 300 kg, were assigned to one of six groups (n=4). Steers in four groups were given 1 ug LHRH at 20-min intervals for 10 h beginning either 2, 8, 12, or 20 h after 1 mg estradiol. Steers in two control groups were given either: (1) 1 ug LHRH at 20-min intervals for 10 h beginning 20 h after oil, or (2) saline at 20-min intervals for 10 h beginning 20 h after 1 mg estradiol.

Blood was collected and data were analyzed statistically as described in Experiment 1 (p. 40). However, FSH concentrations were determined on only every third sample (2-h intervals).

Results

LH release.--In steers treated with oil then LHRH (see inset), baseline LH averaged 4 ng/ml, increased (P<0.0025) to 19 ng/ml by 40 min (first measurement) after initiating LHRH injections and declined thereafter (Figure 2). In steers treated with estradiol followed by saline injections at 20 h (closed circles), LH concentrations were reduced (P<0.0025) beginning at 2 h and remained suppressed during frequent saline injections begun at 20 h. Repetitive LHRH injections begun at 8, 12, or 20 h after estradiol (open triangles, closed squares and open squares, respectively), caused more (P<0.025) LH to be released than in oil-treated controls. LH peaks averaged 30, 35, and

Figure 2.--LH concentrations in serum of steers given 3 ml oil (inset) or 1 mg estradiol in oil at time zero and then 5 ml saline or 1 ug LHRH in saline every 20 min beginning at 2, 8, 12 or 20 h and continuing for 10 h. Values represent the mean of four steers except those in the group receiving LHRH 8 h after estradiol where n = 3; see results.



38 ng/ml, respectively. LH concentrations in estradioltreated steers increased linearly during the first 120 min of LHRH treatment, then declined despite continued injections of LHRH. Peak LH concentration in steers given LHRH beginning 8, 12, and 20 h after estradiol, increased linearly (slope = .53 ng/h; P<0.001) with time after estradiol. In contrast, when LHRH injections were begun 2 h after estradiol (closed triangles), LHRH-induced LH release was less (P<0.025) than that of the oil-treated controls (Figure 2). For example, for oil treated steers, LH in the first and fourth serum samples collected after beginning LHRH injections averaged 14.4 and 13.2 ng/ml higher than baseline, while comparable values for steers given estradiol 2 h previously were only 1.0 and 8.4 ng/ml.

<u>FSH release</u>.--Baseline FSH in steers before estradiol averaged 219 ng/ml (open circles; Figure 3). When estradiol was given, FSH concentrations decreased linearly (P<0.05) at a rate of 9.8 \pm 2.3 ng/h for 12 h before stabilizing at approximately 100 ng/ml. LHRH given to steers every 20 min beginning 20 h after oil (open triangles) increased (P~0.07) FSH concentrations 40 ng/ml relative to the baseline which averaged 180 ng/ml. This LHRH-induced FSH increment was increased (P<0.05) to 77 ng/ml when steers were given estradiol 2 h prior to LHRH. This response was further increased (P<0.001) to 156, 153, and 145 ng/ml when estradiol was given 8, 12, or 20 h before LHRH. But note that the major effect of estradiol in modifying LHRH-induced FSH







release was to reduce the baseline from which the response began and had a slight effect on peak height (P<0.05).

Values for one steer that received LHRH 8 h after estradiol were deleted from analysis and Figures 2 and 3. The LH and FSH response of this steer was small (peak = 3.8 and 82 ng/ml, respectively) and found to be a statistical outlier (P<0.05).

Experiment 3 Change in LHRH-Induced LH and FSH Release <u>With Time After Estradiol-17B</u> in Ovariectomized Cows

Objective

This experiment was conducted to assess the underlying assumption that pituitaries of steers and cows respond to estradiol and LHRH in a qualitatively similar manner.

Materials and Methods

Ten ovariectomized Holstein heifers (BW = 325 to 410 kg) and ten ovariectomized cows (BW = 380 to 515 kg) were assigned to one of four treatment groups.¹ Ovariectomy was at least eighteen days before the start of this experiment. No differences in LH or FSH release were detected between heifers and cows. Hereafter, both groups will be collectively referred to as ovariectomized cows. Ovariectomized cows in three groups were given 1 mg of estradiol at time zero and

¹There were two cows and three heifers per treatment group, or vice versa. Of the ten ovariectomized cows, nine were Jerseys and one was Guernsey.



then a series of injections of: (1) LHRH beginning at 2 h; (2) LHRH beginning at 8 h; and (3) saline beginning at 12 h. The fourth group was given LHRH beginning at 2 h after oil. Saline or 1 ug LHRH were given every 20 min for 8 h.

Blood was collected and data were analyzed statistically as described in Experiment 1 (p. 40). However, FSH concentrations were determined on every second sample (80-min intervals).

Results

LH release.--In controls given estradiol then saline (closed circles), LH surges occurred with peak values at 17, 18, 22, 23, and 23 h after estradiol and peak heights which averaged 46 ng LH/ml (Figure 4). For comparison with LHRHinduced surges, estradiol-induced LH surges shown in Figure 4 have been centered about their peaks. When oil-treated ovariectomized cows were given LHRH (open triangles), LH increased (P<0.005) to 11 ng/ml at 40 min (first measurement), but did not increase further. Injections of LHRH beginning 8 h after estradiol (closed triangles) resulted in LH surges which resembled the estradiol-induced LH surges in terms of magnitude (P>0.10), duration, and shape. As was true for steers, LH release was decreased (P<0.005) between 2 and 4 h after estradiol (open circles) when compared with oil-treated controls. Thus, LH increased 6.8 and 6.6 ng/ml above baseline at 40 and 80 min after initiating LHRH in ovariectomized cows given oil, while the comparable values for cows given

Figure 4.--Serum LH concentrations in ovariectomized cows given LHRH beginning 2 h after oil (Δ), 2 h after estradiol (0), or 8 h after estradiol (Δ). Additional cows were given saline beginning 12 h after estradiol (0); the resulting LH surges have been centered about their peaks (right portion of figure). LHRH (1 ug) or saline was injected every 20 min for 8 h, while estradiol (1 mg) or oil was given at time zero. Values represent the mean of five animals except where indicated by bracketed numerals in the estradiol-saline group.



estradiol 2 h before starting LHRH were 0.9 and 1.9 ng/ml, respectively. However, the inhibition of LHRH-induced LH release in these steers was short-lived and followed within 3 h by LH surges (Figure 4). The magnitude of these latter surges were not different (P>0.10) from those induced by estradiol or by LHRH given 8 h after estradiol.

FSH release.--Baseline concentrations of FSH in ovariectomized cows before estradiol averaged 149 ng/ml (Figure 5; closed circles, open circles, open triangles). Estradiol decreased (P<0.01) FSH in serum of ovariectomized cows at a linear rate of 9.8 ± 1.6 ng/h for 6 h, after which FSH was maintained at ~80 ng/ml (Figure 5). FSH increased (P<0.025) 37 ng/ml above baseline upon giving LHRH to oiltreated controls. When LHRH injections began 2 h after estradiol (closed circles), FSH release was blocked (P<0.025) 80 min after beginning LHRH (first measurement). This inhibitory effect was transient, however, and FSH peaked 86 ng/ml above baseline 5 to 6 h after initiating LHRH. When LHRH was given beginning 8 h after estradiol (closed triangles), the LHRH-induced FSH increment (156 ng/ml) exceeded the comparable values for cows given oil and LHRH (P<0.001). The FSH release pattern of the latter treatment group resembled the preovulatory or estradiol-induced FSH surge in terms of magnitude (>180 ng/ml), duration (8-10 h), and general shape.



Figure 5.--F5H concentrations in serum of ovariectomized cows given 1 ug LHRH in saline every 20 min for 8 h beginning: 2 h after oil (Δ), 2 h after 1 mg estradiol in oil (0), or 8 h after 1 mg estradiol in oil (Δ). A fourth group of ovx cows was given saline every 20 min for 8 h beginning 12 h after 1 mg estradiol in oil (0). F5H surges in this latter group have been centered about their peaks (right portion of figure) and peaked 18, 20, 22, 22, and 22 h after estradiol in the individual cows. Values at each time represent the mean of determinations for five ovariectomized cows.

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Discussion

The present experiments illustrate the synergistic actions of LHRH and estradiol on the LH and FSH release. The effects of LHRH and estradiol on FSH release are similar in many ways to those on LH secretion, but less striking.

One objective of these studies was to determine the effect of estradiol on LHRH-induced LH and FSH release in cattle. To determine changes in pituitary responsiveness in vivo, it was necessary to minimize endogenous LHRH secretion, so as not to confound our test of LHRH-induced LH and FSH release. By giving estradiol to steers, we maintained serum LH and FSH concentrations below those characteristic of castrates for up to 30 h, despite the simultaneous increase in pituitary responsiveness to LHRH. These observations support our hypothesis that estradiol blocks LHRH secretion in steers. We recognize, however, that alternative explanations of these data are possible. For example, estradiol may change the pattern of LHRH secretion to one that releases LH and FSH less effectively, i.e., from a pulsatile mode to one in which secretion is continuous. Alternatively, LHRH secretion may not be inhibited, but reduced to levels below the threshold for LH and FSH release (Hoff et al., 1979).

These studies demonstrate that estradiol increases the capacity of the pituitary gland of steers and cows to release both LH and FSH in response to LHRH. Relative to this positive effect of estradiol, two points are noteworthy. First, it appears that this effect of estradiol is obligatory



to induction of the LH and FSH surge in cattle. For example, when LHRH was given to steers or ovariectomized cows treated with estradiol, a preovulatory-like LH and FSH surge was induced. But, when LHRH was given without estradiol, the amount of LH and FSH released was small, and in the case of LH, concentrations in the serum were maximal after the first LHRH injection. This is unlike the pattern of release which occurs during a preovulatory LH surge in which pulses of LH release build upon one another to create the surge. Second, the ability of pituitary glands to release LH and FSH after LHRH was nearly maximal 10 to 15 h prior to the time when estradiol usually induces LH and FSH surges in ovariectomized cows. Yet during this early phase of increase responsiveness to LHRH, serum LH and FSH concentrations, in animals not given LHRH, were equal to or less than control values. Based on these results, we suggest that during this period estradiol decreases LHRH secretion in ovariectomized cows as it does in steers. In ovariectomized cows, however, LHRH secretion apparently resumes approximately 12 h after estradiol and triggers an LH and FSH surge.

The mechanism by which estradiol increases the capacity of the pituitary gland to respond to LHRH is not understood. As responsiveness of the pituitary gland rapidly increases immediately prior to the gonadotropin surge, the number of LHRH receptors in the pituitary either decreases, as in rats (Savoy-Moore et al., 1980; Clayton et al., 1980) or does not change, as in ewes (Wagner et al., 1979) and cows


(K. Leung, personal communication). The affinity of LHRH for these receptors also did not change in any of these This would suggest that increased number of LHRH studies. receptors are not responsible for the rapid increase in gonadotroph responsiveness. Similarly, the increase in gonadotroph responsiveness to LHRH which occurs after estradiol in cows is not due to increased gonadotropin synthesis. Pituitary gland content and concentration of LH did not increase prior to the LH surge in estradiol-treated ovariectomized cows, even though the capacity of their pituitary glands to release LH in response to LHRH increased twofold (Convey et al., 1981). In fact, estradiol decreased FSH content of the pituitary glands in rats (Chappel et al., 1978) and decreased the rate of FSH synthesis in pituitary glands of anestrous sheep (Miller et al., 1977). Estradiol may increase pituitary sensitivity by acting within the gonadotroph to alter the afferent signal (amplification system) or efferent signal (secretory process) that governs gonadotropin release. This possibility appears to be the most likely mode of action, but represents a very general category and many biochemical and cytological processes.

Although there are many striking similarities between the control of FSH and LH secretion, at least one difference exists. The increment by which estradiol reduces basal FSH concentrations in castrated cattle ($\Delta = 40$ to 120 ng/ml) is nearly as great as the concentration increment of the ensuing FSH surge ($\Delta \approx 150$ ng/ml). In contrast, estradiol reduces



basal LH concentrations only 3 to 5 ng/ml, compared to an increment of 20 to 50 ng/ml for the ensuing LH surge. Since basal levels of FSH are reduced so dramatically by estradiol, retention of FSH in gonadotrophs, and its subsequent release by LHRH may account for the increase in magnitude of FSH release by LHRH following estradiol. FSH baselines also decrease prior to the preovulatory FSH surge in ewes, but to a lesser extent than seen herein (Pant et al., 1977). No decrease in FSH baseline was observed prior to the preovulatory FSH surge in cows (Roche & Ireland, 1981a).

Previous exposure of the pituitary gland to LHRH increases the quantity of LH or FSH released by a subsequent LHRH challenge. This phenomenon has been called "LHRH priming." Estradiol enhances this LHRH priming effect in rats (Aiyer et al., 1974; Lasley et al., 1975; Henderson et al., 1977) and cows (Padmanabhan & Convey, 1980). In the present experiment, estradiol may have increased pituitary gland sensitivity to LHRH by enhancing LHRH priming. Consider LH release induced by LHRH after oil or 8 h or more after beginning LHRH injections were similar in animals given oil or estradiol. Thus, at 40 min there was no evidence of an estradiol-induced increase in pituitary sensitivity to But, in response to continued LHRH injections, quan-LHRH. tity of LH released continued to increase only in estradioltreated animals.

Present results suggest that the LH and FSH surge terminates because the pituitary becomes refractory to LHRH.



This phenomenon also occurs in women (Jewelewicz et al., 1974) and anestrous ewes (Chakraborty et al., 1974). In contrast, FSH release in rats did not decline after 2 h of continuous LHRH infusion (Blake & Garner, 1980). Refractoriness to LHRH is not due to depletion of LH content in the pituitary gland since LH stores are only reduced 30 to 50 percent following the estradiol-induced LH surge in heifers (Convey et al., 1981). Refractoriness may, however, be due to depletion of a releasable pool of LH and FSH. The number of pituitary LHRH receptors in rats are reduced on the day of estrus (Savoy-Moore et al., 1980; Clayton et al., 1980) suggesting that reduction in receptor availability may be responsible for this refractory period.

In addition to estradiol acting on the pituitary gland to increase LHRH-induced LH and FSH release, it also exerted a potent, albeit transient negative effect on gonadotroph responsiveness to LHRH. Relative to oil-treated controls, responsiveness of pituitary gland to LHRH was low 2 h after estradiol, yet was high beginning 5 to 8 h after estradiol. The reason the transitory inhibition of LHRHinduced FSH release was not observed in estradiol-treated steers may have been because we first measured FSH 2 h after beginning LHRH. The inhibitory action of estradiol may be exerted directly on the pituitary as appears to be the case in monkeys (Nakai et al., 1978) and rats (Schuiling & Gnodde, 1977; Apfelbaum & Taleisnik, 1976). However, Padmanabhan and Convey (1978) were unable to inhibit LHRH-induced



gonadotropin release with estradiol <u>in vitro</u>. This observation is consistent with the idea that estradiol may inhibit LH and FSH release indirectly by causing release of an inhibitory agent. Estradiol will also inhibit LHRH-induced gonadotropin release in ewes and humans, but a negative effect of estradiol <u>in vitro</u> has been shown only with rat pituitary glands (DeKoning et al., 1976c).

Relatively little is known about LHRH secretion around the time of the preovulatory gonadotropin surge. The present studies emphasize the necessity of LHRH for triggering and maintaining the LH and FSH surge in cattle. Estradiol increased pituitary gland responsiveness to LHRH, but no gonadotropin surge occurred in steers unless exogenous LHRH was provided. In ovariectomized cows, a preovulatory-like LH and FSH surge was induced by giving exogenous LHRH beginning at 2 or 8 h after estradiol. Presumably the LH and FSH surge that occurs beginning 12 to 24 h after estradiol in cows results from endogenous LHRH release. Thus, cows are similar to ewes (Dobson & Ward, 1977; Narayana & Dobson, 1979), and rats (Sarkar & Fink, 1979; Kerdelhue et al., 1976), which also require LHRH to elicit the preovulatory LH and FSH surge. Estradiol will induce LH and FSH surges in monkeys with transected pituitary stalks (Ferin et al., 1979) suggesting that LHRH is not needed to induce the LH and FSH surge in this species. However, in that study it was not clear how much of the LHRH-rich infundibulum was left attached to the pituitary gland raising the possibility



that LHRH was still available. LHRH secretion increases in monkeys coincident with the preovulatory gonadotropin surge (Neill et al., 1977).

The present studies suggest that both LHRH secretion and increased pituitary gland responsiveness to LHRH are necessary for the estradiol-induced preovulatory LH and FSH surge in cows. Figure 6 depicts the dynamic effects of estradiol on these two factors relative to the changes in LH release. In ovariectomized cows, LH and FSH release are inhibited by estradiol for at least 12 h. The first phase of this inhibitory period (2 to 4 h after estradiol) appears to be due, at least in part, to relatively low gonadotroph responsiveness to LHRH. LHRH secretion may also be blocked at this time, but we have no evidence to support this view. The second phase of low gonadotropin release (4 to 12 h after estradiol) occurs at a time when the pituitary gland is more responsive to LHRH than pituitary glands of control animals. Thus, we deduce that LHRH release during this phase must be below the threshold that will cause detectable gonadotropin release. Apparently, LHRH secretion resumes to trigger and maintain the LH and FSH surge, although from these studies, we are unable to compare the rate of LHRH secretion during the gonadotropin surge with that occurring prior to estradiol treatment. Nevertheless, it is clear that increased LHRH secretion, not pituitary gland responsiveness, determines the timing of the preovulatory LH and FSH surge. Furthermore, in



Figure 6.--Diagram depicts estradiol-induced changes in serum LH concentrations, LHRH release, and responsiveness of the anterior pituitary to LHRH. Note that estradiol initially inhibits LH secretion by reducing pituitary responsiveness, then maintains LH at low concentrations by inhibiting LHRH secretion. Pituitary responsiveness increases several hours before the LH surge commences. Resumption of the LHRH secretion triggers and maintains the LH surge, yet the surge terminates despite continued LHRH stimulation.



HOURS AFTER ESTRADIOL



these studies LH and FSH surges terminated due to refractoriness of the pituitary gland to LHRH.



QUESTION 2

HOW DOES DOSE AND FREQUENCY OF INJECTION OF LHRH AFFECT RELEASE OF LH AND FSH IN CATTLE TREATED WITH ESTRADIOL-17B

Objective

The objective of this study was to determine how changing dose and frequency of LHRH injections, in the presence or absence of estradiol, affect LH and FSH in cattle.

Materials and Methods

Thirty-two Holstein steers were assigned to groups arranged as a 2 x 2 x 2 factorial experiment. Groups were balanced for BW, which averaged 297 kg (range = 230 to 355 kg). Main effects included: estradiol (0 vs 1 mg); dose of LHRH (1000 ng/steer vs 1 ng/kg BW);¹ and frequency of LHRH injections (25 vs 50 min). In previous experiments we determined that: (1) 1000 ng LHRH/animal given every 25 min causes preovulatory-like LH and FSH surges in estradiol-treated steers and cows (Question 1); and (2) 1 ng LHRH/kg BW given every 50 min reestablishes and maintains normal pulsatile LH secretion in steers in which endogonous LHRH release has been blocked via anesthesia. A description of the titration of the latter dose is presented in an Appendix to this dissertation.

¹Note that 1 ng LHRH/kg BW equates to 230 to 355 ng LHRH/steer.



At time zero, steers were given 1 mg estradiol or oil vehicle alone via im injection. LHRH was injected via jugular cannulae in 2.3 to 5.0 ml saline every 25 or 50 min from 12 to 19.5 h after giving estradiol. This interval corresponds to the time of the LH surge in estradiol-treated ovariectomized cows (Beck & Convey, 1977). Blood was sampled via jugular cannulae every 25 min from 11 h 35 min to 19 h 55 min relative to time of injection of estradiol. Blood was collected immediately before the next scheduled LHRH injection.

Data were transformed (logarithm of LH and square root of FSH) to adjust for heterogenous variance and then analyzed by autoregressive split-plot analysis to remove autocorrelations of repeat measurement (Barr et al., 1979). The significance of main factors and their interactions were determined by orthogonal contrasts (Gill, 1978).

Results

Estradiol increased (P<0.001) LHRH-induced LH release relative to that of oil-treated steers, irrespective of dose or injection frequency of LHRH (Figure 7). After the first injection of LHRH basal LH (3.3 ng/ml) increased to 7.4 and 8.3 ng/ml serum in steers given oil and estradiol, respectively. Thus, amount of LH release by the first injection of LHRH was not affected (P>0.10) by estradiol. However, subsequent LHRH injections increased LH release in steers given estradiol such that LH averaged 14.4 ng/ml 3 h after



Figure 7.--Effect of presence of estradiol as a main factor on concentrations of LH in serum of Holstein steers. Thus, each response curve represents the pooled means of four treatment groups (n = 4 steers per treatment) which received LHRH at different doses (1 ng/Kg BW vs 1000 ng/steer) and frequencies (25 vs 50 min). Estradiol (1 mg) or oil vehicle was injected im at time zero and LHRH given repetitively iv from 12 to 19.5 h.



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beginning LHRH injections. In contrast, in steers injected with oil, LH concentrations decreased immediately despite continued LHRH injections.

Increasing the dose of LHRH from 1 ng/kg BW (\sim 300 ng/steer) to 1000 ng/steer increased (P<0.001) the overall peak LH value from 7.5 to 14.4 ng/ml (Figure 8).

The two-way interaction between frequency of LHRH injection and presence of estradiol was significant (P<0.001; Figure 9). Thus, increasing the frequency of LHRH injections from 50 to 25 min intervals increased (P<0.01) LH release, but only in steers given estradiol. When LHRH was given at 25 and 50 min intervals, peak LH values averaged 7.9 and 7.4 ng/ml for steers given oil and 16.1 and 12.8 ng/ml for those given estradiol, respectively. Finally, only the combination of 1000 ng LHRH/steer given every 25 min to steers pretreated with estradiol induced LH release in a pattern that was similar to the preovulatory LH surge in terms of magnitude (21.4 ng/ml), duration (>8 h), and general profile (Figure 10).

Estradiol affected (P \simeq 0.08) FSH release in two ways (Figure 11). First, estradiol reduced FSH baselines to 119 ng/ml compared to 184 ng/ml for steers injected with oil. Second, the increase in FSH release with time after beginning LHRH treatment was greater in steers given estradiol (Δ = 53 ng/ml) than in those given oil (Δ = 17 ng/ml). Dose or frequency of LHRH injections did not significantly (P>0.10) affect FSH release. Only in steers given estradiol followed





Figure 8.--Effect of LHRH dose as a main factor on concentration of LH in serum of Holstein steers (230 to 355 kg BW). Thus, each response curve represents the pooled means of four treatment groups (n = 4 steers per treatment) which received LHRH at different frequencies (25 vs 50 min) and estradiol (0 vs 1 mg). Estradiol (1 mg) or oil vehicle were injected im at time zero and LHRH given repetitively v from 12 to 19.5 h.





Figure 9.--Effect of presence of estradiol and frequency of LHRH injections on concentrations of LH in serum of Holstein steers. Thus, each response curve represents the pooled means of two treatment groups (n = 4 steers per treatment) which received LHRH at different dose (1 ng/Kg BW vs 1000 ng/steer). Estradiol (▲) or oil vehicle (□) were injected im at time zero and LHRH given iv every 25 (---) or 50 (---) min from 12 to 19.5 h.





Figure 10.--Concentrations of LH (•---•) and FSH (0----0) in serum of steers 1 mg estradiol followed 12 h later by 1000 ng LHRH every 25 min for 7.5 h (n = 4).





Figure 11.--Effect of presence of estradiol as a main factor on concentrations of FSH in serum of Holstein steers. Thus, each response curve represents the pooled means of four treatment groups (n = 4 steers per treatment) which received LHRH at different doses (1 ng/Kg BW vs 1000 ng/steer) and frequencies (25 vs 50 min). Estradiol or oil vehicle was injected im at time zero and LHRH given repetitively iv from 12 to 19.5 h.




by 1000 ng LHRH steer every 25 min did FSH release resemble the normal preovulatory FSH surge in terms of magnitude (199 ng/ml), duration (>8 h), and general profile (Figure 10).

Discussion

Evidence is accumulating to suggest that LHRH is released in a pulsatile manner (Carmel et al., 1976; Kao et al., 1977; Belchetz et al., 1978; Blake et al., 1980; Levine & Ramirez, 1980). Furthermore, indirect evidence supports the idea that the frequency and/or amplitude of these LHRH pulses vary throughout the bovine estrous cycle (Rahe et al., 1980). Thus, we studied the efficacy of various doses and frequencies of LHRH injections with regard to their ability to release LH and FSH. These factors were examined in steers with and without estradiol pretreatment.

We have shown that estradiol increases the amount of LH and FSH released by LHRH in cattle (Question 1). That finding was confirmed in this study despite changing the dose and injection frequency of LHRH. However, the amount of LH and FSH released by the first injection of LHRH was not affected by estradiol, whereas release in response to subsequent injections were augmented (Figure 7). This observation is consistent with the hypothesis that estradiol augments responsiveness of gonadotrophs to LHRH, at least in part, by increasing the ability of LHRH to prime the pituitary to subsequent LHRH exposure (Fink & Pickering, 1980). LHRH priming has been demonstrated in cattle (Foster, 1978)



as well as women (Hoff et al., 1979), sheep (Crighton & Foster, 1977), and rats (Aiyer et al., 1974). Furthermore, LHRH priming is demonstrable <u>in vitro</u> using cells from pituitary glands of diestrous cows (Padmanabhan et al., 1981) and LHRH-priming of these cells is augmented by estradiol (Padmanabhan & Convey, 1981).

Doubling the frequency of LHRH injections increased LH secretion in steers given estradiol, but not in those given oil. It is not clear how increasing the frequency of LHRH injections augmented LH release. One possibility is that the shorter intervals between LHRH stimuli increases the priming ability of LHRH (Padmanabhan et al., 1981; Foster, 1978). Alternatively, the increased release of LH may not have been due to increased injection frequency per se, but rather of doubling the amount of LHRH administered, i.e., giving a constant LHRH dose twice as often. Our study was not designed to distinguish between these possibilities.

Changing the dose and frequency of LHRH injections did not significantly alter FSH release. In fact, LHRH increased FSH release only slightly and primarily in steers pretreated with estradiol. This relatively low efficacy of LHRH to increase serum concentrations of FSH releative to LH may reflect a difference in the ability of the gonadotrophs to release FSH and LH in response to LHRH. On the other hand, LHRH may stimulate release of similar quantities of FSH and LH, and we are just unable to detect these increments of FSH. This situation could arise if basal concentrations of

FSH in serum were high relative to the increment of FSH released by LHRH. This, in turn, could result from a high basal release rate of FSH or a relatively long half-life of FSH in blood (Akbar et al., 1974). This explanation is supported by our observation, and those of others (Ajika et al., 1972; Borrel et al., 1978), that anesthesia only slightly reduces concentrations of FSH in serum (see Appendix). If LHRH-induced FSH release is responsible for only a small percentage of total FSH in blood, then blocking LHRH release with anesthesia should have only a slight effect on FSH concentrations in serum.

Increasing dose and frequency of LHRH injections augments the LHRH-induced increment of LH, independent of changes in FSH release. Thus, the pattern and magnitude of LHRH release may be an important determinant of the ratio of concentrations of LH to FSH in serum. Frequency of LHRH pulses has been shown to affect the ratio of LH to FSH in serum of monkeys (Knobil, 1980).

One objective of the present study was to examine whether secretion rates of LHRH that mimicked those found in steers would be sufficient to trigger preovulatory-like LH and FSH surges in steers pretreated with estradiol. Our results suggest they are not. Only after increasing the dose of LHRH 3- to 4-fold and the frequency of LHRH injections from every 50 min to every 25 min was the LH and FSH release pattern in estradiol-treated steers similar to preovulatory LH and FSH surges in terms of magnitude, duration, and



general shape. If one may extrapolate these results obtained from steers and apply them to cows, the implication is that the magnitude and frequency of LHRH release must increase during proestrus-estrus in order for estradiol to induce preovulatory gonadotropin surges in cows. We recognize that an extrapolation of this extent is risky. However, the observation by Rahe et al. (1980) that frequency of LH pulses increases from once every 3 to 4 h to every 20 to 40 min during the LH surge supports our contention that LHRH is secreted more frequently than every 50 min during the preovulatory surges. Our proposal that the magnitude of LHRH secretion also increases during the preovulatory gonadotropin surges can only be tested by direct measurement of LHRH release.

QUESTION 3

DOES ESTRADIOL-17B INDUCE AND PROGESTERONE INHIBIT THE PREOVULATORY LH AND FSH SURGES IN HEIFERS?

Objectives

The objectives of this study were to determine: (1) whether estradiol, given via implants in amounts to stimulate a proestrus increase, induces preovulatory-like LH and FSH surges; and (2) whether progesterone, given via infusion in amounts to simulate concentrations found in blood during the luteal phase, inhibits gonadotropin surges.

Materials and Methods

Fourteen Holstein heifers were used. These heifers weighed 345 to 445 kg and had displayed at least two estrous cycles of normal length (18-23 days). On day 11 to 15 of an estrous cycle (estrus = day 0) heifers were ovariectomized via vagina and estradiol and progesterone replacement was begun simultaneously (less than 1 min delay). All heifers were initially given one estradiol implant and intravenous infusion of progesterone. This replacement regimen was designed to maintain concentrations of estradiol and progesterone in blood at levels existing before ovariectomy.

Twenty-four h after ovariectomy, steroid replacement was adjusted to conform to one of three treatment regimens:



(1) progesterone infusion was terminated and two additional estradiol implants were given every 12 h for 36 h (n = 5); (2) progesterone infusion was maintained and two additional estradiol implants were given every 12 h for 36 h (n = 3); and (3) progesterone infusion was terminated and two empty implants were given every 12 h for 36 h (n = 6). Steroid replacement was maintained through the sixth day postovariectomy. When progesterone infusion was terminated, infusion of the vehicle was continued.

Blood was collected via cannulae from a jugular vein contralateral to that used for infusion of progesterone. Concentrations of LH and FSH were determined in serum collected every 2 h beginning the day before and continuing six days after ovariectomy. In addition, LH was measured in samples collected every 15 min for 6 h on the day before and on each of the four days after ovariectomy to monitor changes in the pulsatile secretory pattern of LH. Additional blood was collected every 4 h (first 4 days) or 12 h (last 3 days) to determine concentrations of estradiol and progesterone.

For statistical analyses, hormone data were subdivided into periods within the experiment. Three periods were defined for the estradiol and progesterone data, relative to ovariectomy: (1) the day before ovariectomy; (2) the day after ovariectomy when heifers were infused with progesterone and had one estradiol implant; and (3) days 2 through 6 when steroid replacement differed among treatment groups.



Four periods were defined for the LH and FSH data:¹ (1) the period of low, stable gonadotropin baselines before and immediately after ovariectomy; (2) the period of rapidly increasing gonadotropin baselines after ovariectomy; (3) the period of elevated gonadotropin baselines just prior to the gonatropin surges; and (4) the period after the gonadotropin surges. These periods were analyzed by linear regression within treatments (Gill, 1978). Specific comparisons of y-intercepts and slopes the results obtained from regression were conducted using Bonferroni's t-test (Gill, 1978).

In addition, changes in the frequency and magnitude of pulsatile LH release were examined. A pulse release of LH was defined as an increase in LH concentration that exceeded the variation within an LH assay by three standard deviations (3 x SD = 0.39 ng) and that peaked within 30 min of the previous nadir. Differences in the frequency of LH pulses were determined by analysis of variance, while differences in the magnitude of LH pulses were analyzed by linear regression using a generalized linear model (Alvey et al., 1977).

Results

Progesterone concentrations in plasma averaged 7.2 ng/ml during diestrus on the day before ovariectomy, and

¹Relative to ovariectomy, the four periods were defined for LH as: (1) -24 to 10 h, (2) 12 to 16 h, (3) 18 to 78 h, and (4) 94 to 144 h and for FSH as: (1) -12 to 8 h, (2) 10 to 24 h, (3) 26 to 78 h, and (4) 94 to 144 h, respectively.



progesterone replacement maintained this mean concentration (5.9 ng/ml; P>0.10) on the day after ovariectomy (Figure 12). Progesterone concentrations decreased during the six days when progesterone was infused (slope = $-0.9 \pm .7$ ng/day; P<0.005), but did not fall below 1 ng/ml, as occurred when infusion of progesterone was stopped.

Estradiol concentrations in plasma averaged 2.3 pg/ml on the day before ovariectomy, and one estradiol implant maintained this mean concentration (2.5 pg/ml; P>0.10) during the six day experimental period after ovariectomy (Figure 12, bottom panel). In contrast, estradiol concentrations in plasma increased at a rate of 1.0 ± .2 pg/day when heifers were given two additional estradiol implants every 12 h for 36 h. These additional implants established levels of 5 to 6 pg estradiol/ ml plasma 70 to 90 h after ovariectomy when preovulatory-like LH and FSH surges were induced (Figure 12, top and middle panel). The profile of estradiol in plasma established by these supplemental estradiol implants is similar to that of nonsynchronized, cycling heifers in terms of rate of increase, variability and absolute values (Figure 13).

LH (Figure 14) and FSH (Figure 15) in serum of heifers averaged 0.6 and 43 ng/ml, respectively, on the day before ovariectomy during the luteal phase of the estrous cycle. Ten to 12 h after ovariectomy was performed and steroid replacements were begun, LH and FSH concentrations in serum increased linearly (P<0.005) at rates of 0.4 \pm .1 and 2.6 \pm .7 ng/h, respectively. Frequency and magnitude of



Figure 12.--Concentrations of estradiol $(\bullet - \bullet)$ and progesterone (O--O) in plasma of heifers relative to ovariectomy and replacement of estradiol and progesterone. Simultaneously with ovariectomy, all heifers received one estradiol implant sc (E2) and progesterone (P4) infusion iv (horizontal bar). Twenty-four h later, treatments were altered: (Top) progesterone infusion was stopped and two additional estradiol implants were given every 12 h for 36 h, n = 5; (Middle) progesterone infusion was continued and two additional estradiol implants were given every 12 h for 36 h, n = 3; and (Bottom) progesterone infusion was stopped and two empty implants were given every 12 h for 36 h, n = 6.





Figure 13.--Concentrations of estradiol in plasma of heifers
 before the onset of the LH and FSH surges.
 Heifers were either untreated and cycling natu rally (0----0, n = 3) or ovariectomized and given
 nine estradiol implants sc as described in figure
 l2. (o----0, n = 8, groups 1 and 2 in figure 12.)





Figure 14.--Concentrations of LH in plasma of heifers relative to ovariectomy and replacement of estradiol and progesterone. Simultaneously with ovariectomy, all heifers received one estradiol implant sc (E2) and progesterone (P4) infusion iv (horizontal bar). Twenty-four h later, treatments were altered: (1) progesterone infusion was stopped and two additional estradiol implants were given every 12 h for 36 h (•_____•, n = 5); (2) progesterone infusion was continued and two additional estradiol implants were given every 12 h for 36 h (x····x, n = 3); and (3) progesterone infusion was stopped and two empty implants were given every 12 h for 36 h (o----o, n = 6).





Figure 15.--Concentrations of FSH in plasma of heifers relative to ovariectomy and replacement of estradiol and progesterone. Simultaneously with ovariectomy, all heifers received one estradiol implant sc (E2) and progesterone (P4) infusion iv (horizontal bar). Twenty-four h later, treatments were altered: (1) progesterone infusion was stopped and two additional estradiol implants were given every 12 h for 36 h (----, n = 5); (2) progesterone infusion was continued and two additional estradiol implants were given every 12 h for 36 h (x...x, n = 3); and (3) progesterone infusion was stopped and two empty implants were given every 12 h for 36 h (o----o, n = 6).

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pulsatile LH release averaged 0.9 pulses/6 h and 1.7 ng/ml, respectively, on the day before ovariectomy, and 4.5 pulses/6 h and 2.7 ng/ml on the day after ovariectomy. Thus, the increase in LH concentrations was due primarily to an increase (P<0.005) in the frequency of pulsatile LH release, and to a lesser extent to an increase (P<0.05) in the magnitude of the LH pulses (Table 1).

Gonadotropin concentrations had plateaued by 24 h after ovariectomy at which time LH averaged 2.6 ng/ml and FSH averaged 96 ng/ml (Figures 14 & 15). After estradiol and progesterone replacements were adjusted 24 h after ovariectomy, no consistent differences were discernable for LH or FSH concentrations or pulsatile LH release among treatment groups.

LH and FSH surges were induced in all heifers in the treatment group in which progesterone infusion was stopped and additional estradiol implants were given. These surges resemble preovulatory surges in terms of magnitude (>20 ng LH/ml; 200 ng FSH/ml), duration (10 to 12 h), and general shape, peaking 61.8 ± 4 h (mean ± SE) after progesterone infusion was terminated. No gonadotropin surges occurred in heifers in the other two treatment groups.

After the preovulatory-like gonadotropin surges, serum LH and FSH concentrations (ng/ml) were significantly lower (LH = 0.9; FSH = 31; P<0.005) than comparable values for heifers from the other treatment groups (LH = 1.7; FSH = 120). The lower LH concentrations were a result of



















Treatment ^b	Days After Ovariectomy								
	0	1	2	3	4				
	Frequency ^d								
1	0.6	5.0	5.6	6.0	1.0**				
2	1.3	4.0	2.3	5.0	3.3				
3	0.7	4.5	5.5	5.0	5.2				
Combined ^C	0.9	4.5*							
	Magnitude ^e								
1	1.3	3.4	2.0	1.8	0.9**				
2	1.8	2.7	2.4	1.3	1.3				
3	2.0	2.0	1.8	1.4	1.8				
Combined ^C	1.7	2.7*							

Table	1Frequency	and	Magnitude	of	\mathbf{LH}	Pulses	s in	Heife	ers
	Before and	l Aft	cer Ovaried	ctom	iy a	and Rej	place	ement	of
	Estradiol	and	Progester	one.	ā				

^aSee <u>Materials and Methods</u> for criteria for LH pulses.

^bAll heifers received one estradiol implant sc and progesterone infusion iv at the time of ovariectomy. Twentyfour h later, treatments were altered: (1) progesterone infusion was stopped and two additional estradiol implants were given every 12 h for 36 h, n = 5; (2) progesterone infusion was continued and two additional estradiol implants were given every 12 h for 36 h, n = 3; and (3) progesterone infusion was stopped and two empty implants were given every 12 h for 36 h, n = 6.

^CValues were averaged across treatment groups within days when heifers were still treated similarly.

^dNumber of LH pulses during a 6 h period.

eMagnitude (ng/ml) is the mean of LH pulses during a
6 h period.

*Greater than the comparable value for day 0 (P<0.01).

**Values differ from those of other treatment groups on day 4 (P<0.01).



reduced (P<0.005) frequency (1.0 pulses/6 h) and magnitude (0.9 ng/ml) of pulsatile LH release (Table 1).

Discussion

Results of the present study demonstrate that increasing concentrations of estradiol in serum, similar to that which occurs during proestrus, will induce preovulatorylike surges of LH and FSH in heifers. Lower concentrations of estradiol, similar to those found during the luteal phase of the estrous cycle, were not sufficient to induce gonadotropin surges, even when progesterone concentrations were decreased to concentrations observed during proestrus.

These results from heifers concur with similar studies of monkeys (Karsch et al., 1973a) and rats (Goodman, 1978). In those studies, gonadotropin surges were also induced by giving estradiol at doses that reestablished concentrations of estradiol in serum similar to those seen immediately before the onset of the preovulatory gonadotropin surges. In addition, monkeys and rats were studied during the follicular phase of the menstrual cycle or immediately after ovariectomy, respectively, to avoid changes in the responsiveness of the hypothalamo-hypophysial axis to estradiol that may result from chronic ovariectomy. Goodman and coworkers (1981) gave estradiol and progesterone replacement to ewes immediately after ovariectomy and mimicked the concentrations of these steroids found at proestrus. While this replacement regimen induced FSH and LH surges, the



magnitude of the LH surges was substantially less than that of the preovulatory LH surge. Thus, induction of the LH surge in ewes may require more than decreasing progesterone and increasing estradiol concentrations in blood.

In the present study, we have demonstrated that progesterone is an effective blocker of LH and FSH surges in heifers. In fact, even when progesterone concentrations were as low as 1 to 2 ng/ml serum, gonadotropin surges were blocked. The mechanism by which progesterone blocks the gonadotropin surges is unknown. However, Padmanabhan and Convey have shown, using bovine anterior pituitary cell cultures, that progesterone blocks the stimulatory effects of estradiol on LHRH-induced LH release (1980) and LHRH priming (1981).

Others (Short et al., 1973; Hausler & Malven, 1976; Short et al., 1979) have reported that progesterone will not block estradiol-induced gonadotropin surges in chronically ovariectomized cows. One reason for this discrepancy may be that the hypothalamus and/or pituitary of chronically ovariectomized heifers have become refractory to progesterone. Alternatively, progesterone plus estradiol may need to be present simultaneously for more than a few hours to establish negative feedback sufficient to block the gonadotropin surges.

Despite replacing estradiol and progesterone in amounts to achieve concentrations similar to those found during the luteal phase of the estrous cycle, LH and FSH concentrations in serum increased within 10 to 12 h after



ovariectomy. This may have occurred because replacement of estradiol or progesterone did not perfectly mimic normal secretion of these steroids by the ovaries. Thus, even though steroid replacement maintained concentrations of estradiol and progesterone in plasma within the normal range for the luteal phase of the cycle, decreasing concentrations of estradiol and progesterone or differences in the minuteto-minute concentration profiles may have been responsible for the increased baselines of LH and FSH in serum. In the present study, estradiol and progesterone concentrations were more variable after steroid replacement than before. Indeed, the gonadal steroids inhibit LH release more efficaciously when steroids are delivered continuusly as opposed to intermittently (McCarthy & Swanson, 1976; Haynes et al., 1977; Desjardins, 1981). Beck and coworkers (1976), using concentrations of estradiol and progesterone greater than those of the present study, were also unsuccessful at maintaining low concentrations of LH in serum of ovariectomized heifers. Alternatively, LH and FSH baselines may have increased in the presence of estradiol and progesterone replacement because of the absence of other inhibitory factors from the ovary. This possibility has also been proposed for the control of basal secretion of FSH in sheep (Goodman et al., 1981). Estradiol and progesterone can totally account for negative feedback on gonadotropin release in monkeys (Karsch et al., 1973b) and rats (Goodman, 1978).


In the present experiments, concentrations of estradiol and progesterone fluctuated widely in plasma during the luteal and proestrous phases of the estrous cycle as well as after ovariectomy when estradiol and progesterone were administered. In preliminary investigations, replacement of estradiol and progesterone by either intravenous infusion or subcutaneous implantation resulted in fluctuating concentrations in the peripheral plasma (J. S. Kesner and E. M. Convey, unpublished observation). We were unable to explain these fluctuations in steroid concentrations on the basis of assay variation, fluctuations of infusion rate or handling of blood samples. Therefore, one must consider the possibility that these acute changes in steroid concentrations may result from variability in their rate of clearance from plasma.

Possibly one of the most important observations of the present study, in terms of practical application, was the high degree of synchrony between gonadotropin surges among heifers. LH and FSH surges peaked between 60 and 63 h after progesterone withdrawal (n = 5 heifers). In comparison, LH surges peaked 44 to 72 h after removing progesterone pessaries from heifers in which the corpora lutea had previously regressed (n = 10 heifers), (Roche & Ireland, 1981b). Thus, comparing these two experimental approaches, the intervals from progesterone disappearance to gonadotropin surges were similar, but the synchrony of the gonadotropin surges was much better when concentration of estradiol was controlled



by replacement therapy. This comparison leads us to suggest that asynchrony of gonadotropin surges, relative to spontaneous regression of the corpus luteum, is due primarily to variation in the rate of estradiol secretion rather than variations in the responsiveness of the hypothalamohypophysial axis.



GENERAL DISCUSSION

The overall objective of this dissertation was to determine how the preovulatory gonadotropin surges are controlled in cows. The work described herein contributes significantly towards attaining this goal. Based on our results, we conclude that physiological concentrations of estradiol, progesterone, and LHRH play principle roles in controlling the preovulatory LH and FSH surges. Thus, estradiol induces LH and FSH surges. Furthermore, LHRH must be present in order for estradiol to exert this action. Progesterone alone, or in synergy with estradiol, blocks estradiol-induced gonadotropin surges.

Based on results of our studies, we suggest that estradiol induces the LH and FSH surges by similar mechanisms. This is not surprising, however, since the two gonadotropin surges are synchronized temporarily and similar in their profiles. Based on our results, we suggest that estradiol induces the gonadotropin surges by acting on both the anterior pituitary and also the hypothalamus. At the anterior pituitary, estradiol increases the ability of the gonadotrophs to respond to LHRH. The mechanism by which estradiol increases gonadotroph responsiveness to LHRH may consist of one or more of the following: (1) a direct action on the



pituitary gland to increase responsiveness to LHRH stimuli; (2) a direct action on the pituitary gland to increase the ability of LHRH to prime gonadotrophs to subsequent LHRH stimuli; or (3) alteration of hypothalamic secretions to indirectly increase gonadotroph responsiveness. This third mechanism may increase gonadotroph responsiveness by either one or both of the first two mechanisms.

In vitro studies conducted in our laboratory (Padmanabhan et al., 1978, 1981) demonstrate that estradiol is capable of acting on bovine pituitary cells to increase responsiveness to LHRH as well as to increase the ability of LHRH to prime the gonadotrophs (mechanisms 1 & 2). The studies described herein were not designed to differentiate between these mechanisms. However, our observation that pituitary responsiveness in estradiol-treated cattle did not excede that of oil-treated controls until after the initial LHRH stimulus was given is consistent with the possibility that estradiol acts by increasing the ability of LHRH to prime the gonadotrophs. Alternatively, estradiol may not increase gonadotroph responsiveness to LHRH per se, but rather prevent the decay of responsiveness that occurs after LHRH stimulation in the absence of estradiol. Thus, the rate at which concentrations of gonadotropins increase in serum is maintained for 3 to 4 h in estradiol-treated cattle, but abruptly decreases in the absence of estradiol.

Estradiol must also increase LHRH release to induce gonadotropin surges. Thus, when estradiol is given to



steers, gonadotropin surges do not occur probably because LHRH release is not augmented. However, gonadotropin surges are induced in estradiol-treated cows by giving LHRH before the time of the expected gonadotropin surges. These results lead us to propose that estradiol initially reduces LHRH release, then restores or augments LHRH release to trigger the LH and FSH surges.

Little is known about the mechanism by which estradiol augments LHRH release. Since the magnitude of the estradiol-induced gonadotropin surges is apparently all-ornone, the magnitude of the proposed LHRH surge, required to trigger the gonadotropin surges, may also be released in an all-or-none fashion is as follows. Presumably a finite population of neurons is responsible for secreting LHRH that, in turn, triggers the gonadotropin surges. Perhaps the release of LHRH by these neurons is synchronized by the spread of action potentials from one neuron to another via collateral axons. Thus, of all neurons stimulated by estradiol, the first to exceed the threshold of excitation would experience an action potential as well as trigger action potentials in other neurons. Thus, the first neuron to undergo an action potential determines the timing of a burst of LHRH release. After a period of refractoriness, the neurons would fire again and release another pulse of LHRH, and so forth. This mechanism of coordinated neural excitation and LHRH release is analogous to the one governing excitation and contractility of myocardial cells.

It is not clear what cellular processes occur during the latent period between estradiol injection and onset of the gonadotropin surges. This latent period functions as a neural clock and probably represents a functional latency during which requisite products are produced, i.e., synthesis of new RNA or transport of protein the length of an axon.

We propose that estradiol induces preovulatory gonadotropin surges by first increasing gonadotroph responsiveness to LHRH then by increasing the frequency and possibly magnitude of pulsatile LHRH release. The gonadotropin surges terminate due to refractoriness of the gonadotrophs to LHRH, and not because LHRH secretion terminates or because the pituitary becomes depleted of gonadotropin (Convey et al., 1981). Unfortunately, the bases for this mechanism were results of experiments using supraphysiological doses of estradiol. This mechanism should be reexamined under conditions similar to those used in our final experiment in which chronic ovariectomy was avoided and doses of estradiol were given to reestablish estradiol concentrations in blood similar to those at proestrus.

The hypothesis that we have put forward for action of estradiol in cows is similar to one proposed for rats (Turgeon, 1980). In rats, estradiol increases responsiveness of gonadotrophs to LHRH as well as release of this decapeptide. On the other hand, the mechanism by which estradiol induces the gonadotropin surges in monkeys, and likely women, is apparently quite different from those



proposed for cows and rats. Even though LHRH release may increase coincident with the gonadotropin surges (Neill et al., 1977), estradiol appears to be capable at eliciting LH and FSH surges independent of hypothalamic involvement (Ferin et al., 1979; Wildt et al., 1980). Little is known about how estradiol exerts this action on the monkey pituitary.

The site at which progesterone blocks preovulatory gonadotropin surges is unknown. Padmanabhan and Convey have shown that progesterone blocks the actions of estradiol to increase: (1) LHRH-induced LH release (1980) and (2) the ability of LHRH to prime gonadotrophs with respect to LH release (1981). Whether progesterone also blocks these effects of estradiol on FSH secretion in vitro remains to be determined. Progesterone may inhibit estradiol-induced gonadotropin surges in monkeys by altering a hypothalamic secretion (E. Knobil, personal communication). Goodman and Karsch (1980) provided indirect evidence that progesterone may reduce the frequency of LHRH secretory pulses. This latter effect would not only reduce the amount of LHRH affecting the gonadotrophs, but may also reduce LHRH priming. I am unaware of evidence that progesterone blocks gonadotropin surges by reducing LHRH release. Taken together, these observations on progesterone mechanism of action suggest that progesterone may act through multiple modes to block preovulatory gonadotropin surges.

All research should have a purpose. There were three reasons why I chose to study the control of the



preovulatory gonadotropin surges in cattle. The first was to generate basic information on neuroendocrine regulation. It is difficult to predict how our information may be useful in this respect, but some possibilities follow. Information regarding gonadotropin control in cattle could be extrapolated for future studies with humans. Our experimental models, i.e., giving castrate males replacement LHRH in pulses or replacing steroids immediately after ovariectomy to mimic their physiological concentrations may be adapted by other researchers. Our results may be a useful example of hormonal control principles for researchers studying other hormonal axes.

The second justification for this dissertation was to use the information regarding control of preovulatory gonadotropin surges in ovulation and apply this information so as to manipulate the hormonal secretions and regulate fertility in cattle and other domestic animals. Examples of fertility management include hastening the first preovulatory gonadotropin surges in pubertal heifers and postpartum cows and synchronizing or predicting the time of ovulation.

The final reason for studying control of gonadotropin surges, and likely the least consequential to mankind, was my intrigue with the concept that estradiol can exert both negative and positive feedback on gonadotropin secretion. How does estradiol do this? The work presented



herein contributes substantially towards answering that question. Yet my fascination with this question has not been quenched. Instead, I am more intrigued than ever by the new questions arising from our work and my readings. For instance, what are the intracellular processes involved in gonadotropin secretion; or LHRH secretion? And what are the mechanisms by which hormones alter these secretory processes? What are the physiological ramifications of changing the pattern of pulsatile secretion of hormones?



SUMMARY AND CONCLUSIONS

The studies presented in this dissertation examine the control of the preovulatory LH and FSH surges in cattle by LHRH, estradiol, and progesterone. Based on these studies, I conclude that increasing concentrations of estradiol in serum induce the preovulatory gonadotropin surges in cows by acting on the anterior pituitary gland and hypothalamus. Estradiol affects the pituitary by increasing the ability of gonadotrophs to release LH and FSH in response to LHRH. This action occurs long before the onset of the gonadotropin surges.

The ability of estradiol to augment LHRH-induced LH release is increased when the dose and/or the frequency of LHRH injections are increased. Furthermore, increasing the frequency of LHRH injections augments LH release only if estradiol is present. FSH release was not significantly affected by changing the dose or frequency of LHRH injections.

Maintaining plasma estradiol and progesterone at concentrations characteristic of the luteal phase of the estrous cycle does not prevent gonadotropin concentrations from increasing in serum after ovariectomy. LH and FSH surges are induced when concentrations of progesterone are



reduced and concentrations of estradiol are increased in a manner to simulate proestrus. Gonadotropin surges are blocked when progesterone concentrations are maintained at concentrations characteristic of the luteal phase of the estrous cycle.

In conclusion, estradiol induces and progesterone blocks the preovulatory LH and FSH surges in cattle. Our studies indicate that estradiol induces the preovulatory gonadotropin surges by first increasing gonadotroph responsiveness to LHRH, and then increasing the frequency and possibly magnitude of pulsatile LHRH release.



APPENDIX

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APPENDIX

TITRATION OF LHRH

Three experiments were conducted to determine the dose of LHRH required to reestablish and maintain normal pulsatile LH release in steers in which endogenous LHRH release had been blocked. Different steers (weighing 175 to 239 kg) were used for each experiment. Steers were anesthetized with sodium pentobarbital (W. A. Butler Co., Brighton, MI) for approximately 5 h. Pentobarbital inhibits LHRH secretion without altering the ability of the pituitary gland to release gonadotropin after LHRH (Blake & Sawyer, 1972; Radford & Wallace, 1974). Beginning 15 to 30 min after onset of anesthesia, injections of LHRH were given iv at 50-min intervals. Fifty min is the mean interval between pulses of LH release in steers (Kesner et al., 1981).

First we gave three doses of LHRH (0.3, 0.8, and 2.4 ng/kg BW). Each dose was given twice in succession, with the sequence for the doses for each steer arranged as for a Latin square. In a second and third study LHRH was given as a series of six injections at concentrations of 1.3 and 1.0 ng/kg BW, respectively. In all three studies, blood was collected every 10 min via jugular cannulae. When blood

sampling and LHRH injecting were scheduled simultaneously, blood was collected immediately prior to LHRH injection.

LH and FSH profiles in serum are shown for one representative steer from each study (Figure 16). LH was immediately released after most LHRH stimuli, whereas distinct pulses of FSH release were often not discernable after LHRH. Additionally, anesthesia quickly reduced serum concentrations of LH, but not FSH. For these reasons, LH provided a more sensitive index for titrating LHRH.

In the first study, average height of the LH pulses before anesthesia was 3.2 ng/ml above baseline. After anesthesia, LH pulse height averaged 0.2, 1.4, and 5.8 ng/ml above baseline after giving 0.3, 0.8, and 2.3 ng LHRH/kg BW, respectively. The magnitude of LH pulses before anesthesia in the second and third studies averaged 1.9 and 1.3 ng/ml. LH pulses induced by injecting 1.3 or 1.0 ng LHRH/kg BW averaged 4.9 and 1.2 ng/ml, respectively. Based on these studies, 1.0 ng LHRH/kg BW given every 50 min reestablished normal pulsatile LH secretion in steers, i.e., the magnitude of LH pulses after this dose of LHRH most closely mimicked (P<0.05) preanesthesia pulses of all doses tested. Thus, this dose of LHRH, when given every 50 min, mimics endogenous release of LHRH in steers.

Figure 16.--LH and FSH concentrations in serum of Holstein steers (175 to 239 kg BW) anesthetized with sodium pentobarbital (black bar) and then given various doses of LHRH (arrows) at 50-min intervals. Figure depicts one of the three animals examined in each of the studies. Studies differ in the LHRH dose given. In the first study (top), three doses of LHRH were given to each steer; each dose given twice in succession. These three paired injections were given in sequences following a Latin square design.





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