ABSTRACT

OVARIAN STEROID CONTROL OF SERUM LUTEINIZING HORMONE CONCENTRATIONS IN HOLSTEIN HEIFERS

By

Thomas Wesley Beck

The objectives of these experiments were to characterize serum luteinizing hormone (LH) concentrations in ovariectomized Holstein heifers and to determine how serum LH concentrations are modified by ovarian steroids (estradiol-17β and progesterone) and gonadotropin releasing hormone (GnRH).

Holstein heifers were ovariectomized 60 h after treatment with: 1) no steroid (n=3), 2) four estradiol-17β implants (n=4), 3) a progesterone pessary (n=4), or 4) four estradiol implants plus a progesterone pessary (n=4). Serum steroid concentrations achieved after administration of these devices were within physiological ranges for heifers during the estrous cycle (estradiol, 15 to 30 pg/ml; progesterone, 1.1 to 4.2 ng/ml).

Serum LH concentrations increased after ovariectomy in heifers given no exogenous steroids. Estradiol or progesterone each depressed this post-castration increase in serum LH, but only the combination of estradiol and progesterone maintained serum LH at concentrations characteristic of intact heifers during diestrus (<2 ng/ml).

In a second experiment, serum LH concentrations were monitored at 10-min intervals for 4 h in 12 chronically (1.5 months) ovariectomized heifers. Frequent sampling revealed an oscillating pattern of LH secretion in which serum LH concentrations continuously increased and decreased by at least .5 ng/ml. The frequency of these oscillations in serum LH ranged from 25 to 100 min between heifers, but occurred at constant intervals within each heifer.

In a third experiment, yearling Holstein heifers ovariectomized 1.5 months were given either no treatment (n=5) or four estradiol implants (n=5). Serum LH concentrations did not change in non-treated heifers. Serum LH concentrations were decreased between 2 and 6 h after estradiol treatment, then increased between 14 and 22 h post-treatment (peak at 18.5 h).

In a fourth experiment, 10 Holstein heifers, ovariectomized six months previously, were given 40 µg of GnRH followed by four estradiol implants 2 days later. The heifers were then randomly assigned to one of two groups to be given 40 µg of GnRH at 6 or 12 h after estradiol. Serum LH concentrations increased 40 to 90 ng/ml after each GnRH injection, but the GnRH induced LH release was greater after estradiol than before. Differences in LH responses were attributed to an increased duration of GnRH induced increase in serum LH concentrations after estradiol rather than an increase in the maximum concentration of serum LH. Post-estradiol LH responses to GnRH were adjusted for each heifer's pre-estradiol LH response to GnRH. The LH response to GnRH at 12 h after estradiol was greater than at 6 h after estradiol. In addition, in heifers

given GnRH 6 h after estradiol, serum LH concentrations increased to 20 ng/ml between 17 and 22 h after estradiol while heifers given GnRH 12 h after estradiol had unchanged serum LH concentrations between 17 and 60 h after estradiol.

In a fifth experiment, 12 heifers were aborted by cesarian section and ovariectomized at 120 days of pregnancy. Jugular blood was collected at 10-min intervals for 70 min at .25 days before and .25, .5, 1, 2, 4, and 8 days after ovariectomy. Serum LH concentrations fluctuated greater than .5 ng/ml as early as .5 days after ovariectomy and regular fluctuations appeared to be established at 8 days after ovariectomy.

In a sixth experiment, 12 heifers ovariectomized 4.5 months previously were given four estradiol implants. Four of these heifers were given progesterone pessaries for 12 h preceding estradiol treatment, four heifers were given progesterone pessaries at 12 h before until 48 h after estradiol treatment, and four heifers were given progesterone pessaries from 6 to 36 h after estradiol treatment. Serum LH concentrations were not different among the three groups of heifers for the first 20 h of the experiment (8 h after estradiol). Serum LH concentrations increased at 14 h after estradiol treatment in heifers which had progesterone pessaries removed prior to estradiol treatment. Serum LH concentrations continued to increase in these heifers until 20 h after estradiol. This is the time when serum LH concentrations increase after estradiol treatment alone. Serum LH concentrations did not increase in the second and third groups of heifers over this time period (8 to 36 h after estradiol). When progesterone was removed from

the second and third groups of heifers at 36 h after estradiol treatment, serum LH concentrations increased but stayed within the range of those for ovariectomized heifers.

Data from these experiments indicated that basal serum LH concentrations increase in ovariectomized heifers as a function of pulsatile increases in LH release which begin as early as 12 h after ovariectomy and become more frequent and regular with passage of time. It was also shown that estradiol and progesterone each have suppressing effects on basal serum LH concentrations, but only the combination of estradiol and progesterone at physiological concentrations, maintained serum LH concentrations similar to those in intact diestrous heifers.

Estradiol treatment initially decreases elevated basal LH concentrations in ovariectomized heifers, but serum LH subsequently increases to concentrations 10 times that of ovariectomized heifers. Additionally, the serum LH response to GnRH increases with time after estradiol exposure, but no single injection of GnRH produced a release of LH with a duration equal to that of the estradiol induced LH release. Therefore, estradiol probably has a negative effect on basal LH secretion while stimulating increased pituitary responsiveness to GnRH and a sustained release of LH-releasing hormone.

Progesterone administration simultaneous with or 6 h after estradiol blocked the estradiol-induced LH release in ovariectomized heifers. Therefore, progesterone does not need to be present at the time of estradiol exposure to block the estradiol-induced LH release. This would indicate that progesterone is not merely a competitive

inhibitor of estradiol but specifically interferes with the processes set in motion by estradiol to block the release of LH in heifers.

Gonadotropin releasing hormone administered 12 h after estradiol treatment also prevents the estradiol-induced LH release in heifers. The mechanism by which GnRH blocks the estradiol-induced LH release is equivocal. Possibly, GnRH acts to decrease releasable pituitary LH stores and/or pituitary LH-releasing hormone receptors.

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By

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TO CAROL

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

FSH	follicle stimulating hormone
g	gram
GnRH	gonadotropin releasing hormone
h	hours
kg	kilogram
LH	luteinizing hormone
LH-RH	luteinizing hormone releasing hormone
min	minute
ml	milliliter
ng	nanogram
pg	picogram
µg	microgram

INTRODUCTION

Many advances in reproductive physiology have been made in the past two decades, particularly with regard to our understanding of neuroendocrine control mechanisms. The role of the hypothalamus as the controlling influence on the pituitary was first proposed in the 1950's. The purification of hypothalamic extracts containing releasing factors in the mid 1960's was followed closely by methods for measuring minute quantities of pituitary hormones in the peripheral plasma (radioimmunoassays). With these tools researchers have constructed working models for changes which occur physiologically. Briefly, the hypothalamus collects endogenous and exteroceptive stimuli, integrates these stimuli, and relays messages to the pituitary which release specific hormones to effect a physiological response at the target organs. With regard to reproduction, the serum concentration of ovarian hormones are a part of the stimuli received and processed by the hypothalamus in control of gonadotropin secretion. Recent evidence has indicated that estrogens have both stimulatory and inhibitory effects on LH secretion. Estrogens are also reported to have a direct effect on pituitary LH release.

Experiments described here were designed to evaluate the role of ovarian steroid hormones in control of LH secretions in Holstein heifers.

REVIEW OF LITERATURE

The Estrous Cycle

Endocrine changes that occur during the estrous cycle of mammals have been described in several excellent reviews, to which the reader is referred (Hansel and Snook, 1970; Geschwind, 1972; Brennan and West, 1975). Endocrine changes specific to the bovine estrous cycle are briefly described below.

An estrous cycle in heifers is approximately 21 days in length, and the day of estrus (standing heat) is designated as day zero. Progesterone is present in sera of heifers at 2 to 4 ng/ml during the majority of the luteal phase (days 4 to 18) of an estrous cycle (Wettemann et al., 1972). Blood progesterone begins to increase coincident with functional organization of the corpus luteum, from .9 ng/ml on day 4 to a broad plateau (4.6 ng/ml) which persists at least from day 7 after an estrus until approximately 3 days before the next estrus (Wettemann et al., 1972). Ovarian follicles also increase in size during the luteal phase of a cycle. Serum estrogen concentrations increase on day 9 of a bovine estrous cycle as a consequence of this follicular growth (Hackett and Hafs, 1969). However, these midcycle follicles normally regress without ovulating. Therefore, in heifers, both estrogens and progesterone are present during the luteal phase of an estrous cycle.

Pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) content increase gradually during the luteal phase of an estrous cycle (Hackett and Hafs, 1969), while serum LH and FSH concentrations remain low (<1 ng/ml) and relatively stable (Akbar et al., 1974b).

Beginning two or three days before estrus, several important changes take place in rapid succession. First, the corpus luteum regresses and serum progesterone concentrations decrease, while follicle size and serum estradiol concentrations increase (Hansel et al., 1973; Hackett and Hafs, 1969). Hackett and Hafs (1969) reported that pituitary FSH content starts to decrease between days 18 and 20 of an estrous cycle, whereas a decrease in pituitary LH content occurs after day 20. In the sera, however, LH and FSH increase simultaneously around the onset of estrous behavior (Akbar et al., 1974b). Elevated serum estrogen concentrations one or two days before estrus eventually cause the ovulatory discharge of LH (Hobson and Hansel, 1972a,b). Rupture of the Graafian follicle then follows the LH "surge" by approximately 24 h.

Background Research

The study of ovarian hormones and their effects on reproduction have ranged from observations on estrous symptoms to monitoring neurochemical changes in the brain after treatment with ovarian steroids. In the following discussion the author will attempt to review the development of knowledge concerning hypothalamo-pituitary-ovarian interactions. Particular emphasis will be given to effects of ovarian steroids on: 1) estrus behavior, 2) indirect measures of

hormone dynamics, i.e., pituitary LH content and ovulation, and 3) serum gonadotropin concentrations.

Behavior

Involvement of ovarian secretions in sexual behavior can be demonstrated simply by removal of the ovaries and noting cessation of estrous behavior (Hafez, 1968). Progesterone and estrogens have been shown to be important components of ovarian control of estrous behavior. For example, the estrogen antagonist MER-25 (Shirley et al., 1968) and estrogen antibodies (Ferin et al., 1969) block estrous behavior in rats if given on the second day of diestrus. In addition, estradiol can induce estrous behavior in ovariectomized cows (Asdell et al., 1945); however, large doses of estradiol are required (600 rat units/day for 3 days).

Progesterone can either enhance or block estrous behavior depending upon the time of administration. Pretreatment of ovariectomized ewes (Robinson et al., 1956) or cows (Melampy et al., 1957) with progesterone decreased the dose of estradiol required to induce behavioral estrus and increased the intensity of estrous symptoms. In contrast, progesterone given simultaneously with estradiol blocked all manifestations of behavioral estrus in ovariectomized heifers (Foote and Waite, 1965).

Pituitary Luteinizing Hormone Content and Ovulation

In 1958, Parlow developed a specific and sensitive bioassay for quantifying LH. This refined technique made possible investigations

of the effects of gonadal steroids on pituitary LH content and concentration.

Estrogens have been shown to decrease pituitary LH concentration. For example, estradiol (2 mg) given to ewes on day 5 of an estrous cycle caused pituitary LH content to be reduced relative to controls, 48 h after treatment (Piper and Foote, 1968). Decreased pituitary LH content that occurs after estradiol in ewes probably reflects LH release since ovulation followed estradiol injections (Howland et al., 1968). Subsequently, Labstewar (1970) demonstrated a causal relationship between increasing serum estradiol, LH release and ovulation. That is, anti-estrogen given to rats two days prior to estrus inhibited ovulation, while exogenous LH, ovine hypothalamic extract, or mating overcame the effect of anti-estrogen. Estradiol implanted in the basal tuberal region of the rat hypothalamus decreased pituitary LH concentration (Kanematsu and Sawyer, 1964). These data indicate that estradiol may decrease pituitary LH via an effect on the hypothalamus.

The role of progesterone in control of LH release appears to be confined to an inhibitory influence on estradiol induced LH release. Thus, Labhsetwar et al. (1964) demonstrated that progesterone (400 mg) given to ovariectomized heifers did not decrease pituitary LH and FSH content. Exogenous progesterone blocks estradiol-induced ovulation in ewes (Piper and Foote, 1968). This suggests that progesterone blocks LH release after estradiol.

From results of experiments reviewed above, a general concept of how ovarian steroids interact to control LH secretion and estrous behavior can be proposed. Estradiol causes a decrease in pituitary

LH concentration, ovulation and estrous behavior. Progesterone has no effect on pituitary LH concentration or behavior in the absence of estradiol but can inhibit estradiol-induced estrous behavior and ovulation when administered with estradiol. Conversely, progesterone augments estradiol-induced behavioral estrus if given before estradiol. In the context of the normal bovine estrous cycle, it is likely that the decrease in progesterone that occurs prior to estrus permits rising serum estradiol concentrations to induce the preovulatory surge of LH release.

Serum Gonadotropin Concentrations

Development of radioimmunoassays capable of quantitating LH in serum of sheep (Geschwind et al., 1968; Niswender et al., 1968; Wheatly and Radford, 1969) and cattle (Niswender et al., 1969) made possible studies concerning control of LH release in these species.

Serum concentrations of LH and FSH in heifers are low (<1 ng/ml) and stable during the first 20 days of an estrous cycle (Akbar et al., 1974b). Removal of ovarian hormone feedback by ovariectomy causes serum LH concentrations to increase in cattle (Hobson and Hansel, 1972a,b; Short et al., 1973). Similar increases in serum LH concentration have been reported after ovariectomy of rats (Samli and Geschwind, 1967), ewes (Butler et al., 1971), and monkeys (Dierschke et al., 1970).

Radford et al. (1969) suggested that ewes required serum progesterone concentrations to be increased and then decreased sharply before estradiol could stimulate the preovulatory increase in serum LH concentrations. However, estradiol-17β given to anestrous ewes

in the absence of prior progesterone caused marked increases in serum LH concentrations (Goding et al., 1969). The magnitude of increase in serum LH concentrations observed by the latter authors was similar to the spontaneous LH "surge" observed 4 to 16 h after the onset of estrus in cycling ewes. Subsequently, Scaramuzzi et al. (1971) described changes in serum LH concentrations which occurred after treatment of ewes with estradiol benzoate and progesterone. In ewes pretreated with progesterone and subsequently given estradiol benzoate, serum LH concentrations were decreased 0 through 12 h post-injection to values characteristic of intact ewes. However, at 12 to 24 h after estradiol, serum LH concentrations increased in a surge of comparable magnitude and duration as the preovulatory LH surge of this species. However, if progesterone was administered with estradiol benzoate, serum LH concentrations decreased to values characteristic of intact ewes but did not increase 12 to 24 h later. Similarly, Hobson and Hansel (1972a,b) and Short et al. (1973) demonstrated that estradiol causes an initial decrease in serum LH concentrations, then a marked increase in serum LH concentrations in ovariectomized heifers. Hobson and Hansel (1972a) were able to block the estradiol-induced increase in serum LH concentrations in ovariectomized heifers with progesterone, but Short et al. (1973) did not.

Control of Baseline Luteinizing Hormone Concentrations

If the influence of ovarian hormones is eliminated by ovariectomy, serum LH increases within 30 h in heifers (Hobsen and Hansel, 1972b), but which ovarian factor(s) maintain serum LH at low

concentrations, which characterize the major portion of an estrous cycle, are unknown. Because estrogens and progesterone are the major hormones secreted by the ovary, research designed to determine factors controlling basal LH release have focused primarily on effects of these hormones.

Effect of Estrogens on Baseline Luteinizing Hormone Concentrations

Serum estrogen concentrations are low (less than 10 pg/ml) over the majority of a heifer's estrous cycle, increasing only at mid-cycle and again before the preovulatory surge of LH (Hansel et al., 1973). Estradiol decreases serum LH concentrations 6 to 9 h after injection in chronically ovariectomized heifers but does not prevent an increase in serum LH when given immediately after ovariectomy (Hobsen and Hansel, 1972a,b). Thus, with increasing time after ovariectomy, heifers may have a decreased threshold for estrogen negative feedback on LH secretion.

In ovariectomized rats, low doses (.1 µg/100 g body weight) of estradiol-17β increased pituitary LH concentration without affecting serum LH concentrations. Higher doses of estradiol (.5 and 14.6 µg/100 g body weight) caused increased pituitary LH concentration but decreased serum LH concentrations (Barraclough and Haller, 1970; Blake et al., 1972). These authors concluded that the threshold for estrogen stimulation of LH synthesis is lower than the threshold for estrogen inhibition of LH release.

Rhythmic discharges of LH which are characteristic of LH secretion in ovariectomized monkeys are blocked within minutes after a single intravenous injection of estradiol-17β or initiation of

estradiol infusions (Yamaji et al., 1972). Sensitivity to negative feedback effects of estradiol on LH secretion in monkeys may also change with time after ovariectomy. Thus, Karsh et al. (1973c) observed that estradiol implants which maintained constant physiological concentrations of estradiol-17β (50 to 80 pg/ml) were ineffective in preventing the post-ovariectomy increase in serum LH and FSH when implants were inserted at ovariectomy. However, similar implants given to monkeys ovariectomized for 14 months caused serum LH to decrease to concentrations characteristic of intact female monkeys (Karsch et al., 1973c).

In the prepubertal pig, estradiol has been reported to be inhibitory to pituitary LH release. Estradiol-17β implants, in place for three days, decreased basal LH secretion in intact prepubertal pigs (Foxcraft et al., 1975; Pomerantz et al., 1975). Additionally, basal LH secretion was decreased immediately after (1 to 12 h) 5 μg intravenous (iv) injection of estradiol-17β.

In spayed ewes, Cumming et al. (1971) found no combination of estradiol-17β, estrone and progesterone which reduced serum LH concentrations to values comparable to those of intact ewes (<2 ng/ml). Brown et al. (1972), however, demonstrated that infusion of estradiol-17β (.1 μg/h) decreased serum LH concentrations in castrate ewes if the infusions were maintained for long periods (21 to 27 days). In contrast, Diekman and Malven (1973) reported that estradiol decreased serum LH concentrations in ovariectomized ewes to values comparable with those of intact ewes within only 4 days. In addition, Diekman and Malven (1973) reported that estradiol immediately eliminated the pulsatile LH releases characteristic of the castrate ewe.

As evidenced by the previous reports, it is hard to distinguish between procedural and species differences in the effects of estradiol on basal LH secretion. However, several effects of estradiol are consistent across species. In ovariectomized rats, monkeys, ewes, and heifers, estradiol can decrease serum LH concentrations. However, in heifers and monkeys, estradiol was not effective in decreasing serum LH concentrations immediately after ovariectomy. In ovariectomized monkeys and ewes and prepubertal pigs, estradiol blocked the pulsatile releases of LH shortly after administration.

Effect of Progesterone on Baseline Serum Luteinizing Hormone Concentrations

During the major portion of the bovine estrous cycle, progesterone is the predominant ovarian hormone in peripheral plasma. However, in ovariectomized monkeys, progesterone has no effect on LH secretion unless given in doses which result in blood concentrations 100 times greater than those observed during the luteal phase of a menstrual cycle (Yamaji et al., 1972). Although ovariectomized monkeys do not show changes in serum LH concentrations when given progesterone, progesterone can synergize with estradiol to suppress LH secretion. For example, estradiol plus progesterone, at doses which result in serum concentrations comparable to those normally occurring during the menstrual cycle, decrease serum LH concentration to values comparable to those of intact female monkeys (Karsch et al., 1973c).

In ovariectomized rats, progesterone augments both the inhibitory effects of estradiol on serum LH concentrations and the stimulatory effects of estradiol on pituitary LH concentrations

but has no effect when given alone (Blake et al., 1972). In contrast, LH release by luteinizing hormone releasing hormone (LH-RH) in ovariectomized rats is increased after an injection of estrogen plus progesterone relative to untreated controls (Samli and Geschwind, 1967). These results suggest that progesterone synergizes with estrogen to enhance LH-RH release in rats.

In ewes, progesterone alone does not affect serum LH concentrations but, in combination with estradiol, decreases mean serum LH concentrations and inhibits rhythmic LH release (Diekman and Malven, 1973). On the basis of these reports, it would appear that progesterone alone does not decrease basal serum LH concentrations in ovariectomized animals but can synergize with estrogen to suppress LH secretion.

Control of the Preovulatory Surge of Luteinizing Hormone

Recently, Chanault et al. (1975) described changes in serum LH, progestin and estradiol concentrations which occur just before ovulation in cattle. The timing and sequence of these endocrine events are helpful in evaluating experimentally induced LH releases. Briefly, progestins were 5.7 ng/ml at 6 days before estrus and decreased to .07 ng/ml coincident with the preovulatory LH surge. Conversely, estradiol increased from 2 pg/ml 4 days before estrus to 7.4 pg/ml at the peak of the LH surge and was decreased to 3.7 pg/ml at 5 h thereafter. Luteinizing hormone concentrations increased continually for 8 h, then decreased to baseline within 2.5 hours.

Effects of Estrogens on Luteinizing Hormone Release

In ovariectomized heifers, several estrogens produce similar effects on serum LH concentrations. Estradiol benzoate (800 μg), estradiol-17α, estradiol-17β, estradiol (2 or 4 mg) or diethylstilbestrol (800 μg) produced an initial decrease in serum LH concentration followed by an LH "surge" which reached a peak at 20 h post-injection (Hobson and Hansel, 1972a). Estrone (4 mg) produced an LH peak without initially suppressing baseline LH (Hobson and Hansel, 1972a). Estradiol benzoate given to heifers 0 and 12 h after corpus luteum removal caused an LH peak approximately 40 h post-surgery (Hobsen and Hansel, 1972b). However, if estradiol was not given to heifers after corpus luteum removal, LH peaks occurred 90 h post-surgery. Estradiol benzoate given to intact (day 3 menstrual cycle) or ovariectomized Rhesus monkeys produced an LH surge only if plasma estradiol was maintained at concentrations greater than 135 pg/ml for more than 12 h (Yamaji et al., 1971).

Karsch et al. (1973b) demonstrated that the ability of estradiol to cause an LH surge was dependent upon duration of estradiol exposure and concentration of serum estradiol and these factors were inversely related. For example, serum estradiol exposure had to exceed 100 to 200 pg/ml for 42 h, 200 to 400 pg/ml for 36 h, or 1200 to 2000 pg/ml for 24 h in order to elicit LH surges, while serum estradiol at less than 100 pg/ml for up to 120 h did not elicit an LH surge.

Weick et al. (1972) induced an LH surge with estradiol benzoate on day 2 of a menstrual cycle in monkeys, while a second injection

of estradiol benzoate induced another LH surge if given 4 or 8 but not 2 days after the first injection. In contrast, a second surge of LH release could be induced by estradiol in anestrous ewes only 24 h after the first estradiol-induced LH release (Symons et al., 1973). In the latter experiment, however, the LH peaks were reduced from 139 to 26 ng/ml after the second estradiol injection, suggesting that the refractory period may be a function of releasable LH stores.

The time of the estrous cycle at which estradiol is administered will also affect the serum hormone response. A single injection of estradiol-17β (1 mg or 100 μg) on day 3 of an estrous cycle caused an LH surge 20 h later in ewes (Bolt et al., 1971; Pant, 1973). However, serum FSH concentration did not increase as they do normally on the day of estrus (Pant, 1973). Symons et al. (1973) reported that estradiol-17β (50 μg) on day 1 or 6 of an estrous cycle did not cause an LH surge in ewes. However, the same dose of estradiol given to anestrous ewes caused an LH surge. Follicle stimulating hormone was also released by estradiol in anestrous ewes (Reeves et al., 1974). In addition, FSH release occurred coincident with the LH surge (Jonas et al., 1973; Beck and Reeves, 1973).

The method of estradiol administration can also modify its effect on LH secretion. Chronic estradiol implantation in ovariectomized monkeys decreased plasma LH concentrations, but increasing estradiol concentration via a single injection of estradiol caused a discharge of LH similar to the spontaneous, preovulatory LH surge (Karsch et al., 1973a).

Effects of Progesterone on Luteinizing Hormone Release

The preovulatory LH surge, which occurs normally in monkeys, can be blocked by progesterone injected during the late follicular phase of a menstrual cycle (Spies and Niswender, 1972). Progesterone will also block estradiol-induced LH and FSH release in monkeys if given with or 12 h after estradiol benzoate (Dierschke et al., 1973).

In ewes, progesterone also blocks estradiol-induced LH release. For example, if serum progesterone concentrations are high (day 10), estradiol-17β (1 mg) does not cause an LH surge (Bolt et al., 1971). Additionally, progesterone injected with estradiol blocked the estradiol-induced LH surge in ovariectomized ewes (Scaramuzzi et al., 1971).

In ovariectomized heifers, progesterone (100 or 1000 mg) caused slight decreases in basal serum LH concentration and no LH surge was detected (Hobsen and Hansel, 1972a). Progesterone given with estradiol benzoate or estrone prevented estradiol-induced LH peaks and basal serum LH concentrations were decreased. In contrast, Short et al. (1973) reported that progesterone did not prevent the estradiol-induced LH release in ovariectomized heifers.

Luteinizing Hormone-Releasing Hormone Induced Gonadotropin Release

McCann (1962) first demonstrated that acid extracts of the stalk median eminence region of the hypothalamus caused LH release in immature rats. Subsequently, others have confirmed this observation and demonstrated LH releasing activity in crude and highly purified hypothalamic extracts in rats (Ramirez and McCann, 1963a,b;

Chowers and McCann, 1965; Piacsek and Meites, 1966; Arimura et al., 1972), sheep (Domanski and Kochman, 1968; Reeves et al., 1970a,b, 1971a,b), and cattle (Zolman et al., 1973). Purified LH-RH isolated from porcine hypothalami was characterized as a decapeptide (pyro Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) by Matsuo et al. (1971a). This decapeptide was then synthesized by Matsuo et al. (1971b) and found to be comparable to the natural porcine LH-RH in its ability to release LH in the rat (Arimura et al., 1972). Synthetic LH-RH has been shown to release LH in ewes, cows and gilts (Reeves et al., 1973a), bitches (Nett et al., 1975), mares (Downey et al., 1974) and hens (Reeves et al., 1973b) and both LH and FSH in the rat (Schally et al., 1971), sheep (Reeves et al., 1972b) and heifers (Kaltenbach et al., 1974).

Effects of Estrogens on Luteinizing Hormone-Releasing Hormone Induced Gonadotropin Release

Reeves et al. (1971a) injected ewes with a standard dose (3 µg) of purified porcine LH-RH at several stages of an estrous cycle and found the LH response (peak minus baseline) was greater prior to ovulation than at other stages of the cycle. Thomas et al. (1973) found similar results in women, as did Cooper et al. (1974a) and Zeballos and McCann (1975) in rats. Zolman et al. (1974) did not find an effect of stage of the estrous cycle in heifers but did report a significant correlation between serum estradiol concentrations at the time of LH-RH injection and magnitude of the LH increase.

Arimura and Schally (1971) found that estradiol benzoate pretreatment of intact diestrous rats greatly increased the LH response to purified porcine LH-RH. In ovariectomized rats (Cooper et al.,

1974b), estradiol-17β had a biphasic effect on pituitary responsiveness to synthetic LH-RH. That is, magnitude of LH release by LH-RH was decreased at 3 h, unchanged at 6 h, and increased at 9 h after estrogen relative to pretreatment responses.

Legan and Karsch (1975) questioned the view that estrogens induce an increased responsiveness of the pituitary to LH-RH and suggested that it is some other ovarian hormone. This theory is based on failure of estradiol to fully restore the "proestrus-like" LH response to exogenous LH-RH in ovariectomized rats. Moreover, in prepubertal gilts, estradiol had a depressing rather than stimulatory effect on pituitary responsiveness to LH-RH (Foxcraft et al., 1975).

Most investigators agree that exogenous estrogen can cause LH release in several species. However, the mechanism by which estrogens exert this effect is incompletely understood. Evidence supports the view that estrogens either directly or indirectly induce changes in the pituitary which enhance responsiveness of the pituitary to LH-RH. For example, the LH response to exogenous LH-RH in ewes was augmented by treatment with estradiol benzoate 24 h before LH-RH administration (Reeves et al., 1971b). In addition, Hobson and Hansel (1974) reported that estradiol-17β did not change hypothalamic LH-RH concentration but decreased pituitary LH concentration in ovariectomized heifers. This would indicate that estradiol-induced LH release is due to a change at the pituitary rather than released LH-RH from the hypothalamus. They also found increased LH release from pituitaries exposed to hypothalamic extracts *in vitro* when pituitary donors had been given estradiol-17β 18 h prior to slaughter.

In contrast, estradiol-17β added directly to incubation media did not affect magnitude of LH release by hypothalamic extracts. These results indicate that the effect of estradiol on increasing magnitude of LH release by LH-RH is not directly on the pituitary. However, estradiol may not have been present for a sufficient period of time to cause required changes in the pituitary, or pituitary cells may not be able to respond to the estradiol stimulus *in vitro*.

Estrogens may also cause a surge release of LH-RH which causes LH and FSH to be released prior to ovulation. For example, both estradiol and LH-RH cause LH and FSH release and therefore it is probable that LH-RH is the mediator of the estradiol-induced release of these gonadotropins in ewes (Jonas et al., 1973).

Effects of Progesterone on the Luteinizing Hormone-Releasing Hormone Induced Gonadotropin Release

Effects of progesterone on LH release are poorly understood. Libertun et al. (1974) reported that pituitary responsiveness to LH-RH was unchanged 3 days after progesterone (25 mg) treatment in ovariectomized rats. However, progesterone (1 mg/rat) injected at noon of diestrus-3 into rats with 5-day estrous cycles increased pituitary responsiveness to LH-RH, while progesterone (1 mg/100 g) given at noon of diestrus-2 to rats having 4-day estrous cycles decreased the LH-RH induced LH release on the following day (Martin et al., 1974). Debeljuk et al. (1972) reported that 5 mg progesterone given on the morning of estrus in rats blocked LH release induced by 0.05 but not 1.0 μg LH-RH. On the other hand, Cumming et al. (1972) reported that infusions of progesterone (500 μg/h) for 24 h did not decrease LH-RH induced LH release in anestrous ewes. In

rats and in intact anestrous ewes, a combination of progesterone and estradiol clearly decreased the magnitude of LH release by LH-RH (Debeljuk et al., 1972). Based on current literature, the effects of progesterone in modifying LH-RH induced LH release are not clear. The effect of this hormone on LH-RH induced gonadotropin release apparently depends upon dose and stage of the reproductive cycle in which it is given.

Effects of Ovarian Steroids on the Luteinizing Hormone-Releasing Hormone Induced Luteinizing Hormone Release from Pituitaries *in vitro*

Several investigators have evaluated the effect of a number of steroids on LH-RH induced LH release *in vitro* to determine whether these steroids exert their action via a direct effect on the anterior pituitary. Schally et al. (1973) reported no effect of .036 to .08 g/ml estradiol-17β or 1.1 to 3.3 μg/ml progesterone on LH-RH induced LH release by rat pituitaries *in vitro*. However, ethinyl-estradiol (.00016 to .5 μg/ml), 5α-dihydroxyprogesterone (1.1 to 10 μg/ml), 17α-hydroxyprogesterone (1.1 to 10 μg/ml) and higher doses of estradiol-17β (.5 to 25 μg/ml) with progesterone (10 μg/ml) inhibited the LH response to LH-RH more effectively than any of these steroids alone. Tang and Spies (1975) found a significant inhibition of the LH-RH induced secretion of LH when 2.7 ng/ml of estradiol-17β was added to rat pituitary cell cultures. In this system, progesterone (3.14 ng/ml) had no effect and 20α-hydroxypregn-4-ene-3-one augmented the LH-RH induced secretion of LH.

MATERIALS AND METHODS

General Methods

Animals

Holstein heifers of unknown pedigree were purchased for these experiments, and all were exhibiting regular estrous cycles at the time they were used.

To facilitate collection of blood samples, cannula were placed in a jugular vein of conscious heifers using the following procedure. A jugular vein was punctured with a 12-gauge needle and 15 to 20 cm of intravenous vinyl tubing[1] was threaded through the needle into the vein. Thereafter, the needle was removed and the exposed cannula end fitted with a 16-gauge tubing adaptor. Each cannula was filled with a solution of 3.5% sodium citrate and the cannulae sealed by fitting the adaptor with a stopper. The cannula was passed through a reclosable pouch made from a 7.6 x 12.7 cm adhesive tape which was affixed to the neck with Allweather tag cement.[2] The cannula was coiled and placed within the pouch for storage between sampling periods. Blood samples were collected as follows: 1) a

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[1] BB317 stock number V10, Bolab, Inc., 6 Tinkham Ave., Derry, NH 03038.

[2] C2283N-FO-617, Nasco, 901 Jonesvill Ave., Fort Atkinson, WI 53538.

mixture of sodium citrate and blood was withdrawn and discarded; 2) the blood sample (10 ml) was collected; and 3) the cannula was refilled with sterile 3.5% sodium citrate.

Administration of Steroids

Pessaries used in these experiments consisted of a flat (.02 cm) rectangular piece of spring steel (25.0 x 3.6 cm) which was twisted into a spiral and coated with polydimethylsiloxane containing 10% progesterone (Figure 1). These spirals were compressed, inserted into the vagina, and released. The pessaries were held in the vagina by the pressure of the spring against the vaginal wall. A 30-cm string, attached to one end of the spiral, extended through the vaginal opening when the pessary was in place. This string provided a means to quickly check that the pessaries remained in place and also provided a convenient method for pessary removal. The surface area of the pessary in direct contact with the vaginal wall was 85.8 square centimeters.

Implants consisted of polydimethylsiloxane (Silastic)[3] tubing (I.D., 3.35 mm; O.D. 4.65 mm; length, 55 mm) packed with crystalline estradiol-17β (Figure 2). Each end was sealed with Silastic Medical Adhesive Type A.[3] Briefly, the method of placing these estradiol implants was as follows. A small incision in the skin was made at the tip of the ear and two subcutaneous channels were created by blunt dissection with a metal probe (diameter, 5 mm). An implant was placed in an implant cannula (I.D., 4.7 mm; Figure 3) and the

[3]Dow Corning, USA.

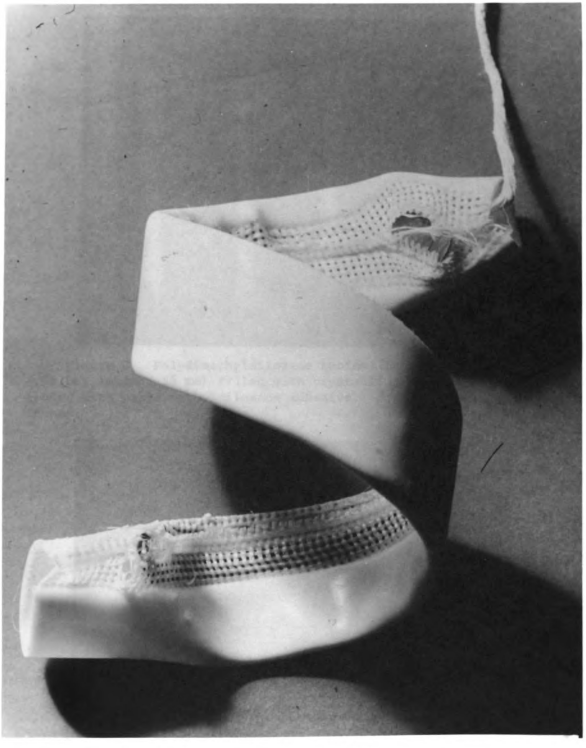

Figure 1. Pessary. Polydimethylsiloxane sheath containing 10% progesterone, 25.0 x 3.6 cm.

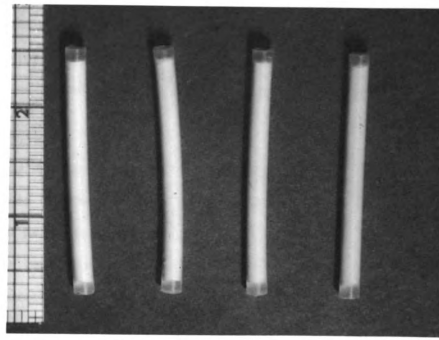

Figure 2. Polydimethylsiloxane tubing (I.D., 3.35 mm; O.D., 4.65 mm; length, 55 mm) filled with crystalline estradiol-17β and sealed with polydimethylsiloxane adhesive. Scale at left is in inches.

Figure 3. Implant cannula and trocar. Scale at top is in inches.

cannula was inserted into one subcutaneous channel. As the cannula was withdrawn, the implant was pushed from the cannula and into the channel with a trocar (Figure 3). Using this technique, two implants were placed in each ear.

Hormone Assay

Serum samples were assayed by specific radioimmunoassays for luteinizing hormone (Oxender et al., 1972), estradiol (Hafs et al., 1974) and progesterone (Louis et al., 1973) as previously reported from this laboratory. Briefly, these assays are based on the ability of the hormone being assayed to displace a radioactively labeled form of the same hormone from an antibody. An important property of the antibody is that it recognizes and binds in significant quantities only the hormone being assayed. Hormone concentration is determined by comparing antibody bound radioactivity in tubes containing unknown amounts of hormones with those containing known amounts of a standard. The standards for the assays reported here were NIH-LH-B5 (National Institutes of Health), estradiol-17β (Sigma Chemical Company) and progesterone (Sigma Chemical Company).

Gonadotropin Releasing Hormone

Gonadotropin-releasing hormone (GnRH) used in these experiments is the brand name of a synthetic decapeptide produced by Abbott Laboratories. The chemical structure of this decapeptide is identical to that of porcine luteinizing hormone-releasing hormone (LH-RH) as determined by Matsuo et al. (1971a). Abbott GnRH is equivalent in potency to the natural purified porcine LH-RH. The GnRH

supplied by Drs. W. F. White and R. Rippel of Abbott Laboratories was 98-100% pure decapeptide deacetate tetrahydrate.

Specific Objectives and Experimental Procedures

Experiment I: Serum Luteinizing Hormone Concentrations in Heifers Given Steroid Implants and Subsequently Ovariectomized

Objectives. The objectives of this experiment were to: 1) determine if constant serum concentrations of estradiol, progesterone or estradiol and progesterone could inhibit the post-castration rise in serum LH concentrations in heifers and 2) assess the practicality of using estradiol implants and progesterone pessaries to effect constant physiological serum concentrations of estradiol and progesterone in heifers.

Design. Fifteen heifers averaged 308 kg body weight when assigned to one of four treatment groups. All heifers had shown at least two consecutive estrous cycles of normal duration at the time of steroid treatment; however, treatments were given without regard to stage of the estrous cycle. No steroid treatment was given to three heifers. Each of four heifers was given a progesterone pessary, four were given four estradiol implants, and four were given progesterone pessaries plus four estradiol implants. All heifers were ovariectomized at 60 h after beginning steroid treatment. Ovariectomy was performed through a high lumbar incision in the paralumbar fossa on standing animals. Procaine was used to effect a local anesthetic block for the incision.

Blood was collected from each heifer by jugular puncture immediately prior to the time steroid treatments were administered. Sixty hours later (just prior to ovariectomy), each heifer was cannulated and a second blood sample taken. Subsequent blood samples were taken via cannula at 2-h intervals until 48 h after ovariectomy and then twice daily for four days. The implants and/or pessaries were removed and again blood was collected at 2-h intervals for 48 h and then daily for four days.

Serum samples were assayed for LH, and selected samples for estradiol and progesterone.

Analysis. Serum hormone data were statistically analyzed as a 2 x 2 factorial, split-plot, repeat-measure design with unequal replication. All data were tested for heterogeneous variance using Hartly's F max test of variances (Sokal and Rohlf, 1969). If the variance was heterogeneous, a conservative F value with 1 degree of freedom for the numerator was used as the test statistic. Hartly's F max test also was used to test treatment effects on variation among serum LH concentrations during time periods defined in the results.

Experiment II: Serum Luteinizing Hormone Concentrations in Ovariectomized Heifers

Objectives. The objective of this experiment was to establish the post-castration pattern of LH secretion in Holstein heifers.

Design. One and one-half months after ovariectomy, ten heifers were cannulated and jugular blood was collected at 10-min intervals

for 4 h (1300 to 1700 h). Serum was assayed for LH concentration.

Analysis. Serum LH concentrations were plotted against time for individual heifers. The resulting graphs were visually assessed for changes in serum LH concentrations of greater than .5 ng/ml. The time interval for serum LH concentrations to decrease from a maximum to a minimum and increase to a maximum again was also recorded.

Experiment III: Estradiol Induced Changes in Serum Luteinizing Hormone Concentrations

Objectives. The objectives of this experiment were to quantify the changes in serum LH concentrations in Holstein heifers after treatment with estradiol implants and to define the time course of these changes.

Design. Ten heifers were ovariectomized for 1.5 months when cannulated and randomly assigned to one of two groups. Each heifer in one group was given four estradiol implants while heifers in the other group were untreated. Jugular blood was sampled 30 and 5 min prior to implantation of estradiol, then at 2-h intervals for 36 h after estradiol. To monitor basal LH fluctuations, blood was collected at 10-min intervals for 1 h beginning 2 and 5 h after treatment. Additionally, blood samples were collected at 30-min intervals from 16 to 24 h after implantation of estradiol.

Analysis. These data were analyzed statistically as a split plot, completely randomized design with repeat measure.

Experiment IV: Estradiol and Gonadotropin Releasing Hormone Induced Luteinizing Hormone Release

Objectives. The objectives of this experiment were: 1) to determine the effect of 40 mg of GnRH on serum LH concentrations in ovariectomized heifers; 2) to determine if estradiol affects the pituitary LH response to 40 μg of GnRH in heifers; and 3) to determine if serum LH response to 40 μg of GnRH changes with time after estrogen exposure.

Design. Ten heifers, ovariectomized for seven months at the time this experiment was conducted, were fitted with jugular cannulae and randomly assigned to one of two treatment groups. Each heifer was given an intramuscular (im) injection of GnRH (40 μg) followed two days later by four estradiol implants. A second GnRH injection (40 μg) was given to one group of heifers at 6 h after estradiol and to the remaining heifers at 12 h after estradiol. Blood was collected at 10-min intervals for 1 h prior to implanting estradiol and at 2-h intervals for 60 h after estradiol. In addition, blood was taken at 10-min intervals from 1 h before until 2 h after GnRH, then at 30-min intervals for 2 h, then at hourly intervals for two additional hours. Thus, each LH concentration determined for serum collected after the first GnRH injection could be compared with the respective value obtained after the second GnRH injection during estradiol treatment.

Analysis. Statistical analysis revealed that magnitude of LH release by GnRH given prior to estradiol appeared greater in heifers scheduled to receive a second injection of GnRH at 12 h

after estradiol than in heifers to be given GnRH at 6 h after estradiol. Therefore, change in serum LH concentrations resulting from GnRH given after estradiol was adjusted on the basis of pre-treatment response. Analysis of variance was then performed on the differences. Additional non-orthogonal contrasts were performed after inspection of the data.

Experiment V: Serum Luteinizing Hormone Concentration after Abortion and Ovariectomy in Heifers

Objectives. The objectives of this experiment were to monitor serum LH concentrations before and after ovariectomy and describe the pattern of LH secretion responsible for the post-castration rise in basal serum LH concentrations in heifers.

Design. The 12 heifers used in this experiment were purchased for an experiment not reported herein. That experiment involved fetal cannulation and abortion by cesarean section at 120 days of pregnancy. Following abortion each dam was ovariectomized.

Eight blood samples were taken at 10-min intervals before surgery began and at .25, .5, 1, 2, 4, and 8 days after ovariectomy. Resulting serum samples were assayed for LH concentration.

Analysis. The data for individual heifers were plotted against time. These graphs were assessed for fluctuations in serum LH concentrations by inspection.

Experiment VI: Progesterone Effect on the Estradiol Induced Changes in Serum Luteinizing Hormone Concentrations

Objectives. The objectives of this experiment were to determine the effects of progesterone exposure before, during, or after estradiol on the estradiol-induced changes in serum LH concentrations.

Design. Twelve heifers, ovariectomized for four months, were cannulated and assigned to one of three treatment groups. Each of four heifers was given a progesterone pessary for 12 h, then four estradiol implants for 60 h. Four heifers were each given a progesterone pessary fof 48 h and four estradiol implants were given when these pessaries had been in place for 12 h. Finally, four heifers were given progesterone by pessary beginning 6 h after the start of estradiol treatment and pessaries were removed 36 h after the estradiol was implanted. All estradiol implants were in place for 60 h. Five blood samples, taken at 15-min intervals, were collected before and after initiation of estradiol treatment. Thereafter, blood was collected at 2-h intervals for 60 h. All serum samples were assayed for LH with selected samples assayed for estradiol and progesterone.

Analysis. Serum hormone concentrations were analyzed as a split plot repeat measure design by analysis of variance. Significant treatment effects were further analyzed by orthogonal contrasts.

RESULTS AND DISCUSSION

Experiment I: Serum Luteinizing Hormone Concentrations with Steroid Implants and Subsequent Ovariectomy

Serum LH concentrations increase after ovariectomy in monkeys (Dierschke et al., 1970), rats (Blake and Sawyer, 1972), ewes (Roche et al., 1970) and heifers (Hobson and Hansel, 1972b; Short et al., 1973), presumably because negative feedback by ovarian steroids is eliminated. Exogenous estrogens suppress serum LH concentrations in ovariectomized monkeys (Yamaji et al., 1972) and ewes (Diekman and Malven, 1973). However, estradiol benzoate did not prevent serum LH concentrations from increasing after ovariectomy in cattle (Hobson and Hansel, 1972). Thus, ovarian steroid involvement in negative feedback control of basal LH secretion in cattle has not been determined. The objective of this experiment was to determine whether estradiol, progesterone or a combination of these steroids when given at physiological concentrations could prevent the increase in serum LH after ovariectomy.

Serum Steroids

Average estradiol and progesterone concentrations in serum collected just prior to steroid treatment were not different among treatment groups (Figures 4 and 5). Average serum estradiol 60 h after administration of steroid implants, but before ovariectomy,

Figure 4. Serum estradiol concentrations of Holstein heifers treated with no steroid (controls), four estradiol-17β implants (E_2), a progesterone pessary (P), or both four estradiol-17β implants and a progesterone pessary (E_2+P). All steroids were administered immediately after the blood sample taken on day 0 relative to steroid treatment. All heifers were ovariectomized immediately after the blood sample taken on day 0 relative to ovariectomy. All steroid implants and/or pessaries were removed 8.5 days after they were administered.

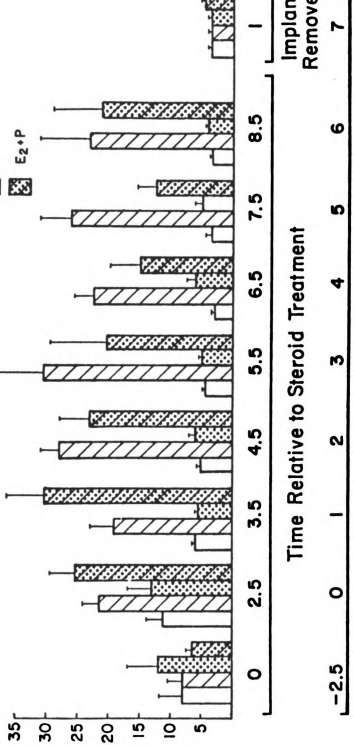
CONTROL
E2
P
E2+P
SERUM ESTRADIOL (pg/ml)
35
30
25
20
15
10
5
0
2.5
3.5
4.5
5.5
6.5
7.5
8.5
1
Implant Removed
Time Relative to Steroid Treatment
-2.5
0
1
2
3
4
5
6
7
Time Relative to Ovariectomy (Days)

Figure 4

Figure 5. Serum progesterone concentrations in Holstein heifers. Treatment was the same for Figure 1.

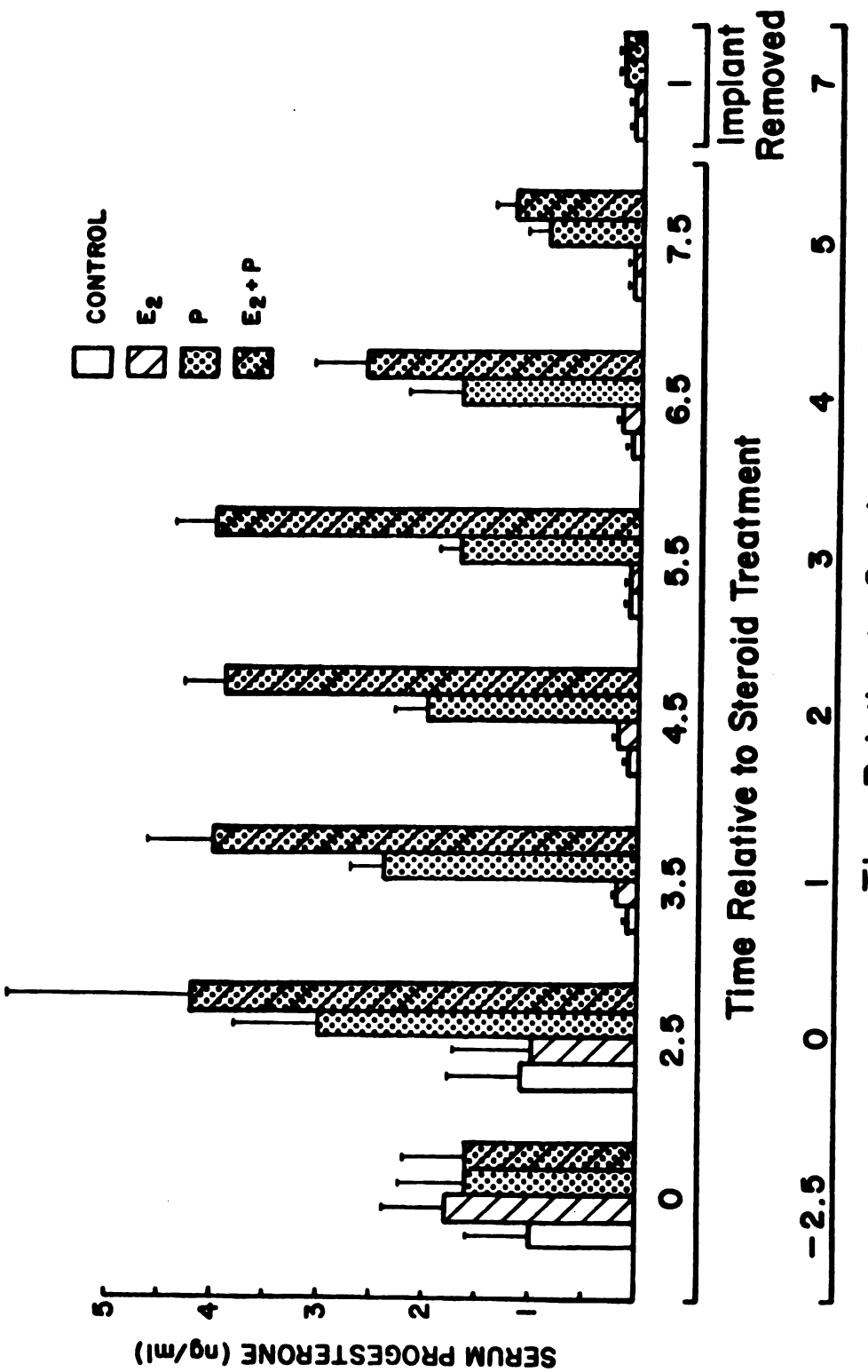
SERUM PROGESTERONE (ng/ml)
5
4
3
2
1
CONTROL
E_2
P
E_2+P
0
2.5
3.5
4.5
5.5
6.5
7.5
Implant Removed
Time Relative to Steroid Treatment
−2.5
0
1
2
3
4
5
7
Time Relative to Ovariectomy (Days)

Figure 5

averaged 24 ± 2 pg/ml in those heifers bearing four estradiol implants. This estradiol concentration was approximately three times greater ($P<.01$) than before estradiol treatment and two times greater ($P<.01$) than the comparable average (12 ± 2 pg/ml) for heifers without estradiol implants. The serum estradiol concentration in heifers given four estradiol implants was approximately twice the 9.7 pg/ml previously reported for proestrous heifers (Wettemann et al., 1972). Ovariectomy reduced ($P<.01$) serum estradiol concentrations to 6.0 ± .3 pg/ml at 24 h post-surgery in heifers not given exogenous estradiol. But, in heifers bearing implants, serum estradiol was unchanged (25 ± 4 pg/ml) at 24 h after ovariectomy relative to pre-surgery concentrations. This suggests that exogenous estradiol given for 60 h may suppress endogenous ovarian secretion of estradiol. At 24 h after removal of the estradiol implants, average serum estradiol concentration was reduced to 4.0 ± .3 pg/ml, which was not different ($P>.05$) from that of heifers which had not received estradiol implants (Figure 4).

Average progesterone concentration in serum collected at 60 h after insertion of pessaries, but before ovariectomy, was increased ($P<0.05$) relative to the comparable average for heifers not receiving progesterone (3.6 vs 1.0 ng/ml; Figure 5). By 24 h after ovariectomy, serum progesterone of heifers not given pessaries was reduced to less than 0.2 ng/ml. In contrast, in heifers given pessaries there was no significant decrease in serum progesterone concentrations. Average serum progesterone remained at approximately 3.0 ng/ml for the 6 days following ovariectomy. This suggests that exposure to exogenous progesterone for 60 h may suppress the

endogenous ovarian secretion of progesterone. Average serum progesterone (2.9 ng/ml) in heifers bearing pessaries is comparable to mean progesterone concentrations previously reported from this laboratory (2.6 ng/ml) for heifers in the progestational phase (day 7) of an estrous cycle (Wettemann et al., 1972). Removal of the pessaries 6 days after ovariectomy resulted in a marked decrease ($P<0.01$) in serum progesterone from 2.5 ng/ml just before removal to less than 0.2 ng/ml 24 h after pessary removal.

Analysis of variance of serum progesterone revealed a significant estradiol by progesterone interaction ($P<0.05$; Figure 5); average serum progesterone in heifers treated with estradiol plus progesterone was greater ($P<0.05$) on days 1, 2 and 3 after ovariectomy than that of heifers receiving progesterone alone. Maintenance of higher serum progesterone concentrations in heifers given estradiol relative to that of heifers given progesterone alone may have been due to estrogen-induced vaginal hyperemia. Alternatively, higher serum estradiol concentration in heifers bearing estradiol implants may have decreased the metabolic clearance rate of progesterone. Additionally, serum progesterone decreased ($P<0.05$) with time so that by 6 days after insertion of pessaries (day 4 post-ovariectomy) progesterone concentrations were comparable to pretreatment values but were still greater ($P<0.05$) than comparable values for heifers not given progesterone.

Serum Luteinizing Hormone

During 48 h following ovariectomy serum LH concentration gradually increased ($P<.01$) in all heifers except those receiving

estradiol plus progesterone (Figure 6). Analysis of variance of serum LH in samples collected at 12-h intervals through 6 days post-ovariectomy (Figure 7) revealed that average serum LH concentrations of heifers treated with estradiol (3.1 $\pm$ 0.3) or progesterone (3.4 $\pm$ 0.3) were less than comparable values for untreated control heifers (4.4 $\pm$ 0.5). Average serum LH concentrations of heifers given estradiol plus progesterone (1.5 $\pm$ 0.1) were lower than the averages of each of the other groups. Serum LH concentrations increased ($P<.05$) after removal of progesterone pessaries and/or estradiol implants in the three groups of heifers given these steroid treatments (Figure 8). Thus, 4 days after steroid withdrawal the serum LH concentrations were not different ($P>.05$) among the four groups of heifers.

During 0.5 to 3 days following ovariectomy average variations (S^2) in serum LH for heifers treated with estradiol plus progesterone was 0.2, which was less ($P<0.05$) than comparable values for control heifers (12.4), or heifers treated with estradiol (8.4) or progesterone (5.8) alone (Table 1). This trend persisted during days 4 to 6 after ovariectomy, after which the depot steroids were removed. Variation in serum LH concentrations after implant removal increased ($P<.05$) with time in heifers which had received steroid treatment such that differences in variance between groups for the period 4 to 6 days post-implant removal (Table 1) were not significant ($P>.05$). Since estradiol or progesterone alone maintained serum LH at concentrations below that of untreated control heifers, it is possible that either steroid at higher concentrations might reduce serum LH in ovariectomized heifers to concentrations which

Figure 6. Serum luteinizing hormone concentrations of heifers receiving depot steroids and subsequently ovariectomized. Two hour samples for 48 h.

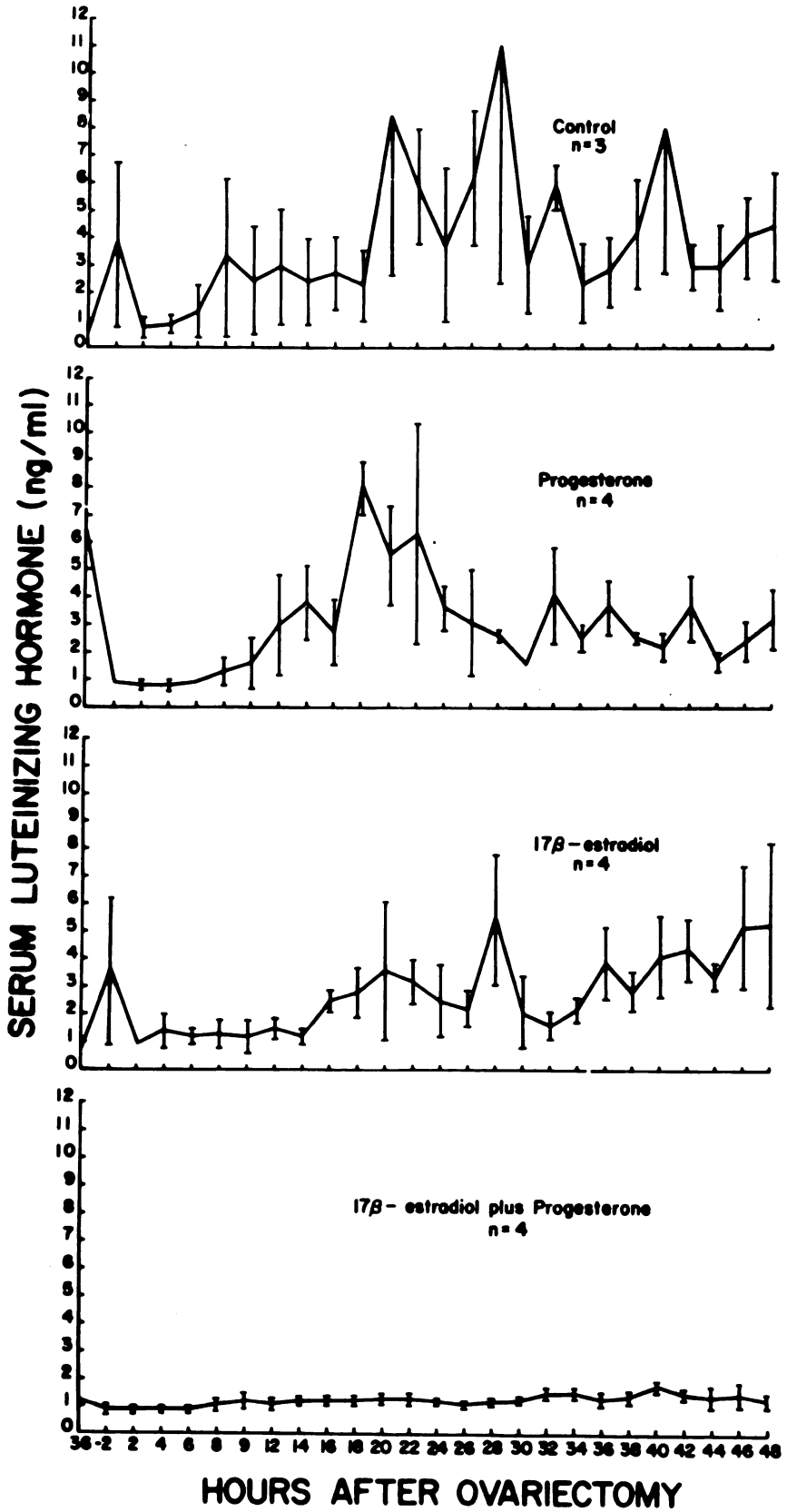
Control
n=3
Progesterone
n=4
17β-estradiol
n=4
17β- estradiol plus Progesterone
n=4
SERUM LUTEINIZING HORMONE (ng/ml)
HOURS AFTER OVARIECTOMY
36 -2 2 4 6 8 9 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48

Figure 6

Figure 7. Serum luteinizing hormone concentrations of heifers receiving depot steroids and subsequently ovariectomized. Twelve hour samples for 6 days.

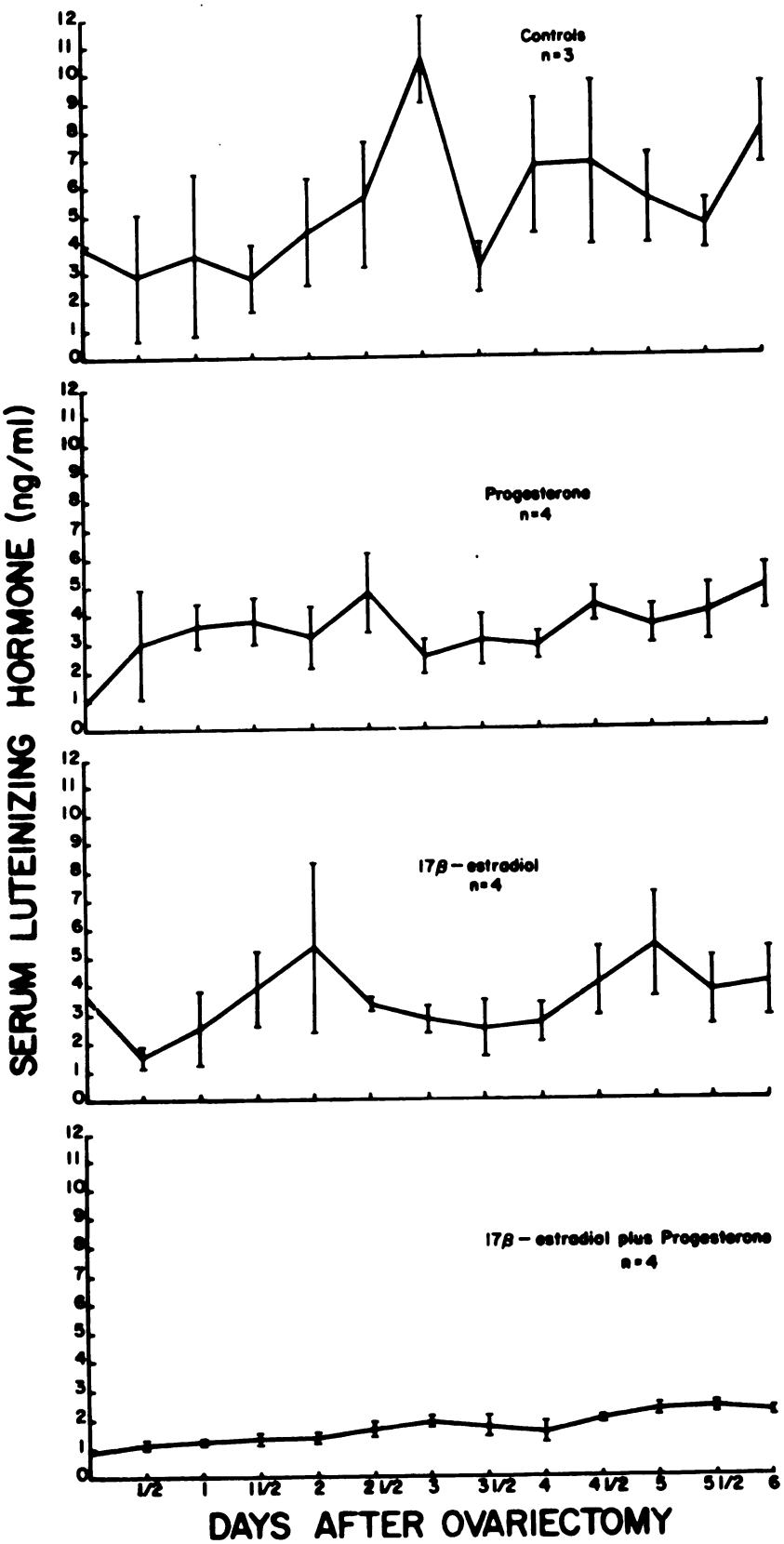
Controls
n=3
Progesterone
n=4
17β – estradiol
n=4
17β – estradiol plus Progesterone
n=4
SERUM LUTEINIZING HORMONE (ng/ml)
DAYS AFTER OVARIECTOMY
1/2
1
1 1/2
2
2 1/2
3
3 1/2
4
4 1/2
5
5 1/2
6

Figure 7

Figure 8. Serum luteinizing hormone concentrations after removal of depot steroids.

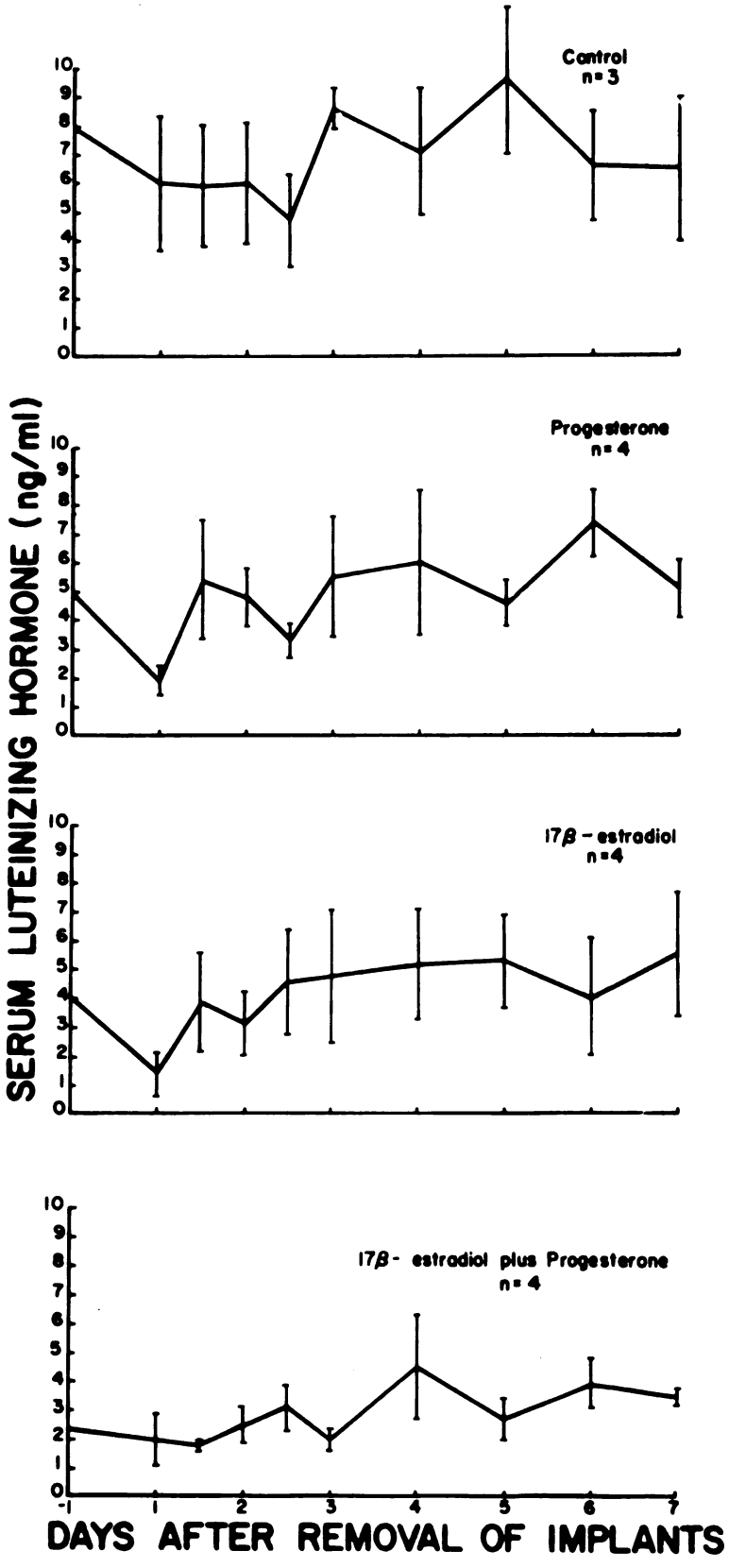
Control
n=3
Progesterone
n=4
17β - estradiol
n=4
17β - estradiol plus Progesterone
n=4
SERUM LUTEINIZING HORMONE (ng/ml)
DAYS AFTER REMOVAL OF IMPLANTS

Figure 8

Table 1. Between animal variances [$(ng/ml)^2$] of mean serum LH concentrations averaged over days

Time	Control	Estradiol-17β	Progesterone	Estradiol-17β plus progesterone
Post-ovariectomy				
.5-3 days	12.4[a]	8.4[a]	5.8[a]	0.2[b]
4-6 days	10.1[a]	6.0[a]	2.5[b]	0.3[c]
Post-implant removal[d]				
.5-3 days	3.9[a]	9.2[a]	6.8[a]	1.0[b]
4-6 days	14.0[a]	11.3[a]	11.0[a]	6.8[a]

[a,b,c]Variances within one time block (row) which have different superscripts resulted in a significant F max ratio at the 5% level.

[d]Implants removed 6 days after ovariectomy.

approximate those found in intact heifers. However, within the limits of serum estradiol and progesterone concentrations approximating those normally found during the bovine estrous cycle, only the combination of estradiol plus progesterone was effective in maintaining serum LH concentrations and variance at precastration levels. These data support results obtained with monkeys (Karsch et al., 1973c). The latter authors observed that serum estradiol at concentrations equal to precastration values did not prevent a post-castration increase in serum LH concentrations unless progesterone was also given. Others (Scaramuzzi et al., 1971; Diekman and Malven, 1973) have reported estradiol alone reduced serum LH in

castrate ewes to concentrations characteristic of intact diestrous ewes. However, serum estradiol was not quantified in these studies and the possibility that serum concentrations of estradiol exceeded those normally found during the reproductive cycle of the ewe makes it difficult to ascribe physiological significance to those data.

Progesterone alone does not consistently lower serum LH concentrations in ovariectomized ewes (Scaramuzzi et al., 1971; Diekman and Malven, 1973), rats (Debeljuk et al., 1972) or monkeys (Karsch et al., 1973c). In fact, progesterone was effective in reducing LH concentrations in ovariectomized monkeys only when given in "massive doses" (Yamaji et al., 1972). However, increased serum progesterone concentrations in heifers given progesterone plus estradiol relative to those given progesterone alone raises the possibility that the greater efficacy of the combined steroid treatment in preventing the post-castration increase in serum LH concentration and variance may be attributable to a higher serum progesterone concentration alone. If this had been the case, one would expect serum LH concentrations and variance to be similar between heifers given estradiol and progesterone and those given progesterone alone on days 4 to 6 post-ovariectomy, since serum progesterone concentrations were not statistically different between these two groups of heifers during that period. Therefore, these data strongly suggest that negative feedback of ovarian steroids on LH release in heifers is a synergistic effect of progesterone and estradiol.

Experiment II: Serum Luteinizing Hormone Concentrations in Ovariectomized Heifers

Butler et al. (1971) reported transient surges in LH concentrations in serum of ewes ovariectomized 3 weeks. These surges occurred at approximately 1-h intervals. Reeves et al. (1972a) described a cyclic pattern in serum LH secretion which averaged 52 minutes in ewes ovariectomized 50 days. Diekman and Malven (1973) reported "rhythmical fluctuations" in serum LH concentrations as early as 20 h after ovariectomy with a mean cycle interval of 75 minutes. Similarly, in castrate male and female monkeys plasma LH concentrations fluctuated with a mean period of 75 minutes (Dierschke et al., 1970). Although serum LH concentrations have been reported to increase after ovariectomy in heifers (Hobson and Hansel, 1972a,b; Short et al., 1973), cyclic rhythms in LH secretion have not been reported for this species. This experiment was designed to investigate serum LH concentrations in heifers 1.5 months after ovariectomy.

Individual serum LH concentrations for 10 ovariectomized heifers are shown in Figure 9. Each heifer exhibited continuously oscillating serum LH concentrations over the 4-h sampling period. Duration of these oscillations varied greatly between heifers but were constant within an individual heifer (Table 2). The average duration of these oscillations was 46 $\pm$ 2.5 minutes.

In an effort to explain fluctuations in serum LH after castration, Knobil (1974) proposed that castration removed negative feedback control of LH secretion, leaving only "short loop" feedback to control LH release. Under these circumstances, serum LH concentrations resulting from each pulsatile discharge of LH would inhibit

Figure 9. Serum luteinizing hormone concentrations in 10 heifers ovariectomized 1.5 months. Ten minute samples taken for 4 h.

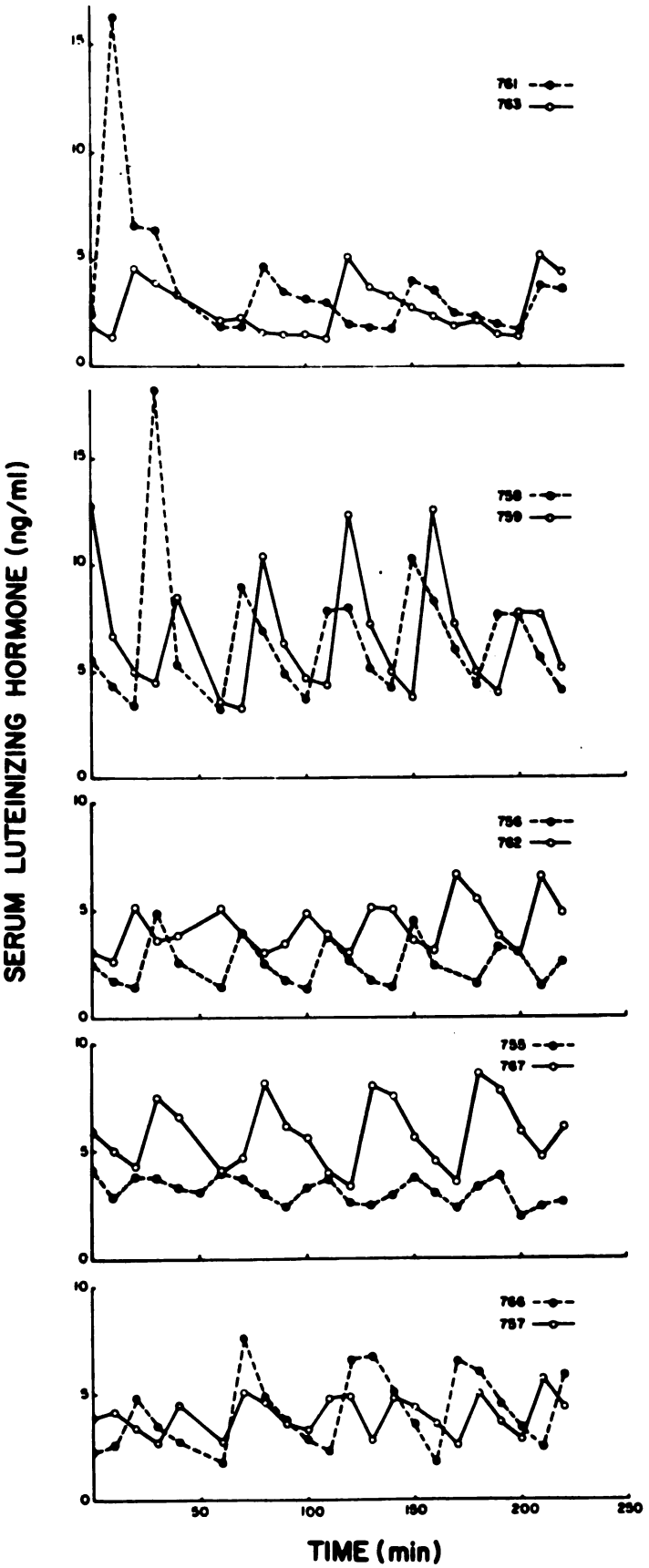
SERUM LUTEINIZING HORMONE (ng/ml)
TIME (min)
761
763
756
759
756
762
755
767
766
757
0
5
10
15
50
100
150
200
250

Figure 9

Table 2. Interpeak intervals[a] of serum LH oscillations

Heifer number	Interval				
	1	2	3	4	5
761	70	70	60	--	--
763	100	90	--	--	--
758	40	45	35	45	--
759	40	40	40	45	--
756	40	40	40	35	--
762	40	40	35	35	40
755	35	50	40	40	--
767	50	50	50	--	--
766	50	55	50	--	--
757	30	45	25	40	30

[a]Interval duration was measured in minutes.

further LH release until plasma LH declined to concentrations below threshold, at which time LH would be released again. This on-off mechanism would cause serum LH concentrations to oscillate throughout a sampling period. As a test of this hypothesis, Knobil (1974) infused human chorionic gonadotropin, ovine LH or human LH into ovariectomized monkeys. Oscillations of serum LH concentrations continued in the face of elevated serum concentrations of these hormones. However, since nonsimian LH was utilized in this study, the possibility exists that exogenous rhesus LH could inhibit endogenous LH secretion in ovariectomized monkeys. Coppings and Malven (1975) infused ovine LH into castrate ewes and reported that the oscillations of serum LH concentrations continued in the face of serum LH concentrations averaging 123 ng/ml. Therefore, it would appear that oscillations in serum LH concentrations of castrates are the result of an endogenous rhythm of the pituitary or hypothalamus.

Experiment III: Estradiol-Induced Changes in Serum Luteinizing Hormone Concentrations

Estradiol elicits a surge of LH similar to the preovulatory LH surge in anestrous ewes (Goding et al., 1969). Estradiol injections have been shown to cause an initial decrease in serum LH concentrations and a subsequent surge of LH in ovariectomized ewes (Scaramuzzi et al., 1971), monkeys (Yamaji et al., 1971), and heifers (Hobson and Hansel, 1972a). Karsch et al. (1973b) reported that estradiol implants could induce an LH release in ovariectomized monkeys. The present experiment was designed to monitor serum estradiol and LH

concentrations in ovariectomized heifers after administration of four estradiol implants.

Serum Estradiol

Four estradiol implants were administered to ovariectomized heifers, and serum estradiol and LH concentrations were quantified. In heifers given no steroid treatment, serum estradiol ranged from 5 to 10 pg/ml throughout the 36-h sampling period (Figure 10). However, in heifers given estradiol implants, serum estradiol ranged from 5 to 10 pg/ml in pretreatment samples, increased to 160 pg/ml at 30 min after estradiol administration, then decreased to 70 and 27 pg/ml at 3 and 36 h, respectively.

Serum Luteinizing Hormone

Serum LH fluctuated between 2 and 10 ng/ml during the 36-h sampling period in control heifers (Figure 11). However, in estradiol-treated heifers, average serum LH concentration was decreased ($P<0.01$) between 2 and 6 h after estradiol treatment but then increased ($P<0.01$) to a peak of 57 ng/ml at 18.5 hours. Average duration of this increase in serum LH was 8 hours.

Estradiol implants provide a constant source of estrogen over a long period of time (Karsch et al., 1973a,b,c). However, there is an initial period after insertion when serum estradiol increases rapidly (30 min) to extremely high concentrations (160 pg/ml) before decreasing to the steady state concentration (27 pg/ml). Karsch et al. (1973a) soaked estradiol implants in buffer prior to insertion to remove estradiol buildup on the surface of the implant. Pre-soaked implants did not cause a sharp spike in serum estradiol

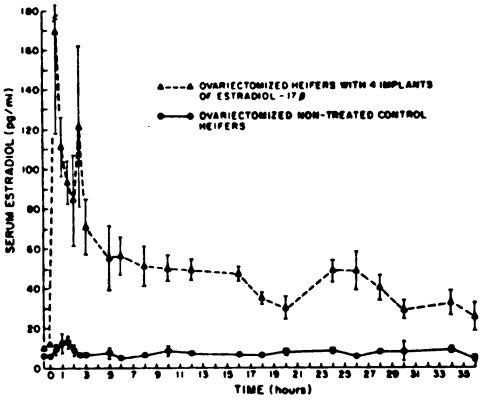

Figure 10. Serum estradiol concentrations in ovariectomized heifers given 4 estradiol implants or no treatment. n = 5 for each group.

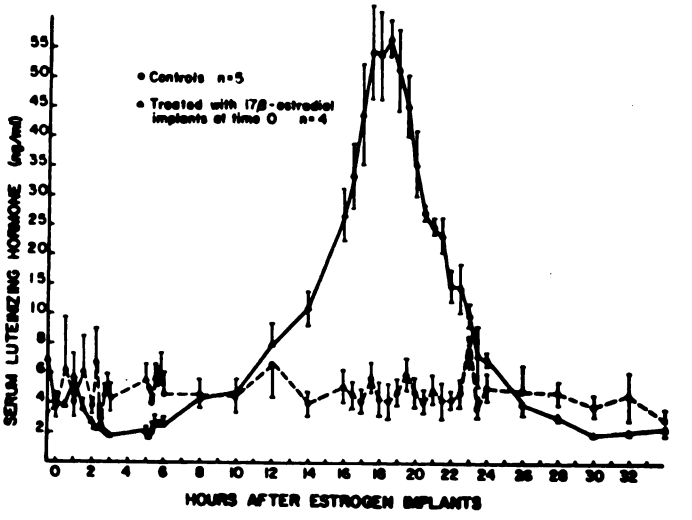

Figure 11. Serum luteinizing hormone concentrations in ovariectomized heifers given 4 estradiol implants or no treatment.

concentrations after insertion but rather a gradual increase to steady state concentration. These authors further reported that the transient increase in serum estradiol concentrations which occurred with unsoaked implants was required to cause a surge release of LH in the monkeys. Since the positive effects of estradiol on serum LH concentrations were a desired effect in these studies, non-soaked implants were used in subsequent experiments.

Constant elevated concentration of serum estradiol did not maintain serum LH concentrations at precastration concentrations in the first experiment reported herein. However, in heifers given estradiol in the present experiment, serum LH concentrations were decreased. The difference in these LH responses may be due to the abrupt increase in serum estradiol concentrations in the present experiment or the higher initial estradiol concentrations achieved. Alternatively, a change in pituitary sensitivity to estrogen may have occurred with increasing time after castration. In support of this view, Karsch et al. (1973c) observed that a dose of estrogen which failed to decrease serum LH concentrations immediately after ovariectomy in monkeys was effective in these same monkeys 14 months later.

The increase in serum LH 18.5 h after administration of estradiol was similar in both magnitude and duration to the LH surge which occurs before ovulation in heifers (Niswender et al., 1969; Henricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). Since exogenous estradiol induces an LH release in ovariectomized heifers and endogenous serum estrogen concentrations normally increase markedly prior to the preovulatory LH surge in

heifers (Wettemann et al., 1972; Chenault et al., 1975), rising serum estrogen concentration is probably the stimulus causing preovulatory LH release in cattle. These data, however, do not indicate whether estradiol is acting at the hypothalamus, pituitary, or at both loci.

Experiment IV: Estradiol and Gonadotropin Releasing Hormone Induced Luteinizing Hormone Release

The LH release in heifers after a single injection of GnRH has a much shorter duration (2 to 4 h; Zolman et al., 1974; Kinder et al., 1975) than both the preovulatory (Chenault et al., 1975) and the estradiol-induced LH surges (8 to 10 h). This discrepancy may be due to: 1) estradiol causing a prolonged release of LH-RH, 2) estradiol prolonging the pituitary response to a single or multiple bolus type release of LH-RH, 3) estradiol decreasing the metabolic clearance rate of LH or LH-RH, or 4) any combination of the above. Several workers have reported increased LH release when GnRH was given during the estrogenic phase of reproductive cycles in ewes (Reeves et al., 1971a), rats (Cooper et al., 1974a; Zeballos and McCann, 1975) and women (Thomas et al., 1973). Estrogen administration also augments GnRH-induced LH release in castrate ewes (Reeves et al., 1971b) and rats (Arimuar and Schally, 1971).

In cattle, serum estradiol and estrone concentrations at the time GnRH was given was positively correlated with magnitude of change in serum LH concentrations after GnRH (Zolman et al., 1974). Estradiol also augments the LH response to GnRH *in vitro* when heifers serving as pituitary donors were treated with estradiol 18 h prior to slaughter (Hobson and Hansel, 1974). The present experiment

was designed to monitor the pituitary response to GnRH with time after estradiol exposure. One injection of GnRH was administered to each ovariectomized heifer 48 h prior to estrogen treatment. The LH response to this GnRH injection was to be used as a check for any pre-estrogen differences in the pituitary response to GnRH. Gonadotropin-releasing hormone was then given at a time after estradiol when serum LH concentrations are decreased (6 h) or at a time after estradiol when serum LH concentrations are increasing but before the major part of the LH surge (12 h).

When 40 μg of GnRH was given to ovariectomized heifers before and after administration of estradiol implants, several changes were noted in the serum LH response. The average serum LH concentration after a standard GnRH challenge was greater ($P<0.05$) 6 h after estradiol relative to the comparable average 48 h before estradiol (Figure 12). Although there was no difference ($P>0.05$) in maximum serum LH concentrations induced by GnRH 48 h before or 6 h after estradiol, increased serum LH concentrations were prolonged by estradiol as evidenced by a time by treatment interaction ($P<0.01$). In the second group, where GnRH was given 48 h before and 12 h after estradiol, average serum LH concentration after GnRH was again greater ($P<0.01$) after, as opposed to before, estradiol exposure (Figure 13). Peak LH concentration for this group of heifers averaged 81 ng/ml before and 93 ng/ml after estradiol, but this difference was not significant ($P>0.05$). There was, however, a time by treatment interaction ($P<0.01$) which suggests that the increased average serum LH concentrations at 12 h after estrogen was a function of an increased duration of the serum LH increase.

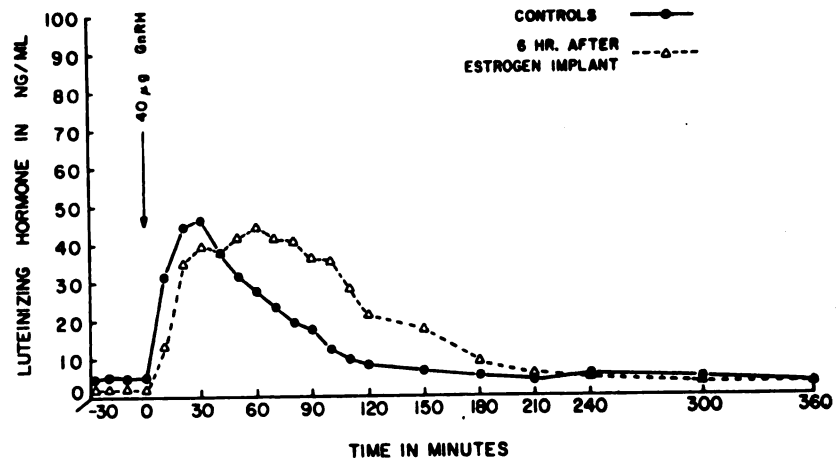

Figure 12. Serum luteinizing hormone concentrations in ovariectomized heifers given 40 µg GnRH 2 days before and 6 h after estradiol implants.

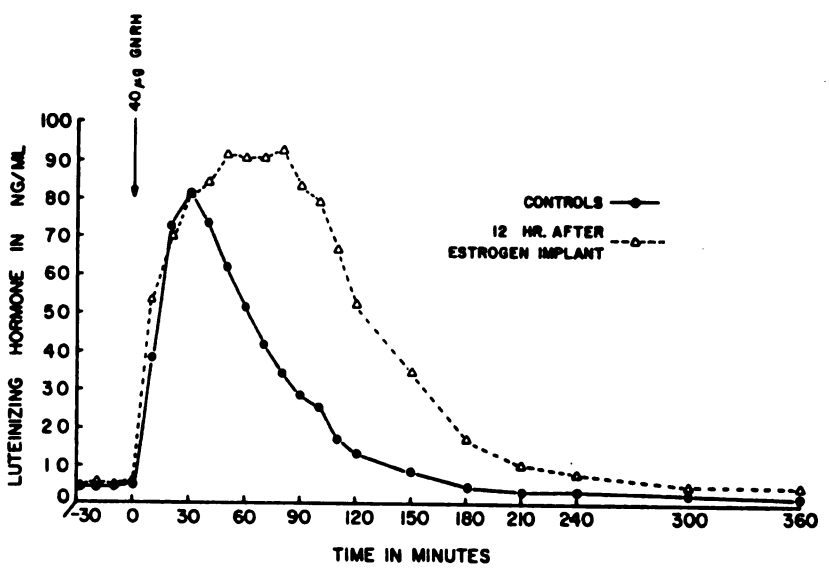

Figure 13. Serum luteinizing hormone concentrations in ovariectomized heifers given 40 µg GnRH 2 days before and 12 h after estradiol implants.

Although the two groups of heifers showed a large difference in average peak serum LH concentration to GnRH before estradiol (Figures 12 and 13; 46 vs 81 ng/ml), average serum LH concentrations were not different ($P>0.05$) between these two LH responses. Nevertheless, individual heifer LH responses to GnRH were adjusted for pretreatment differences. The LH concentration of each serum sample collected after the first GnRH injection (pre-estradiol) was subtracted from the LH concentration of a corresponding serum sample taken after the second GnRH injection (post-estradiol). Comparison of corrected average serum LH concentrations after GnRH given 6 or 12 h after estradiol revealed a greater ($P<0.01$) increase in serum LH release at 12 h than at 6 h after estradiol.

If one considers the overall effects of estradiol and GnRH treatment on serum LH concentration in these heifers, several phenomena are evident. Serum LH concentrations were decreased ($P<0.01$) for 6 h after estradiol treatment (Figure 14) for both groups of heifers. Heifers given GnRH at 6 h after estradiol showed a sharp increase ($P<0.01$) in serum LH concentration 10 min later which lasted 5 hours. Serum LH concentrations in these same heifers increased ($P<0.01$) again between 16 and 24 h after estradiol treatment (Figure 14). In heifers given GnRH 12 h after estradiol, serum LH concentrations increased ($P<0.01$) within 20 min and remained greater than baseline for 5 hours. Blood samples collected for an additional 24 h (60 h after estradiol) from these heifers revealed no additional increases in serum LH concentrations (Figure 14).

The present experiment demonstrates increased LH response to GnRH *in vivo* 6 and 12 h after heifers were exposed to estrogen. An

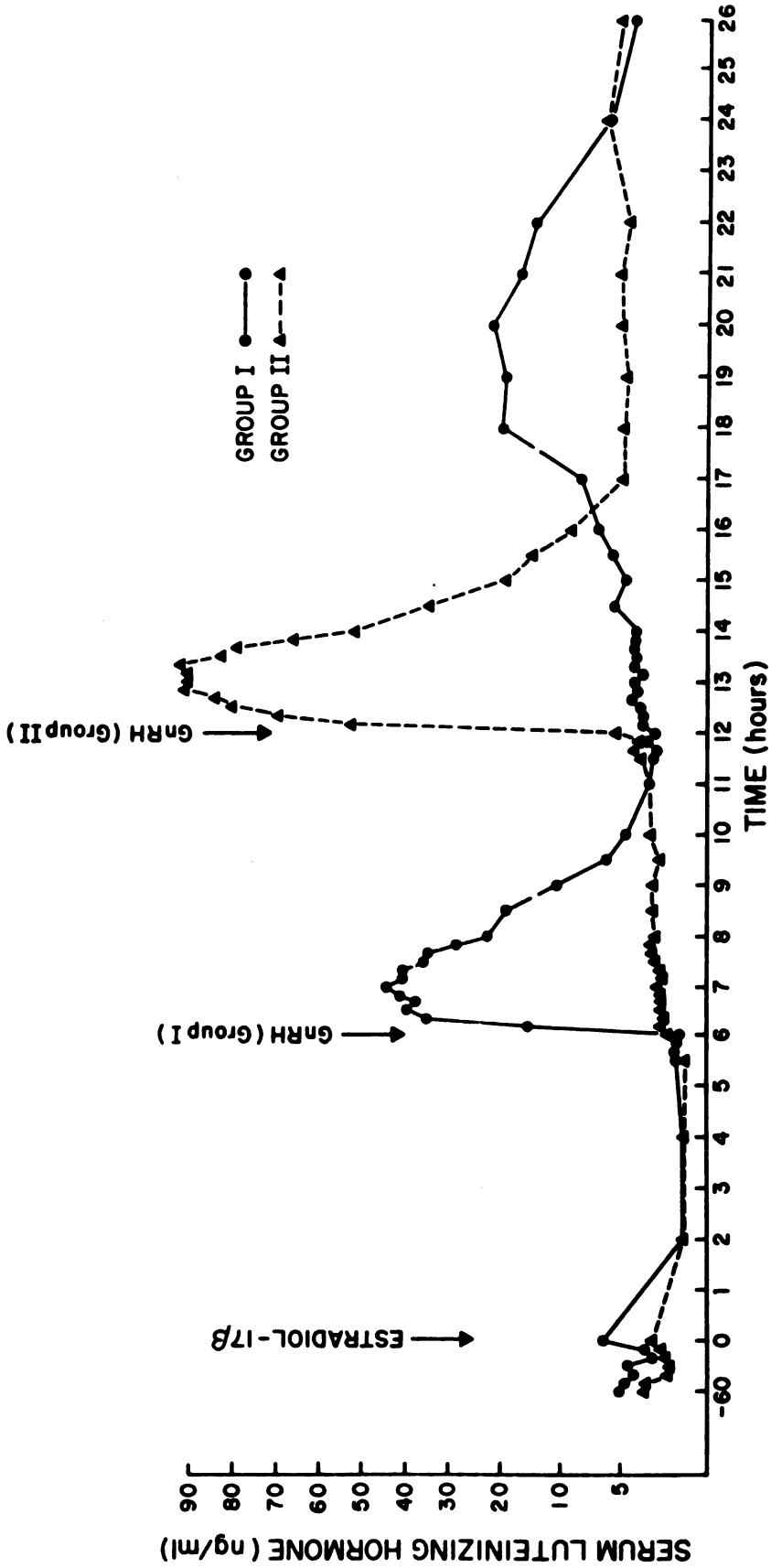

Figure 14. Serum luteinizing hormone concentrations in ovariectomized heifers given 40 µg GnRH 6 (Group I) or 12 (Group II) h after estradiol implants.

increase in duration of the increase in serum LH concentrations occurred rather than an increase in peak concentration. These results may have been due to a longer half-life of LH in the sera of estradiol-treated heifers. Jaffe and Keye (1975) reported that disappearance of LH following a GnRH injection was slower in women given estradiol over several days than in those given no estradiol. In addition, if GnRH clearance from serum was delayed by estrogen treatment, a longer LH response might be expected. There is, however, evidence to refute any change in clearance rate of either GnRH or gonadotropins which may be attributed to increased serum estrogen. Akbar et al. (1974) reported no change in metabolic clearance rates of gonadotropins in anestrous, estrous and diestrous ewes, and Nett et al. (1974) reported no change in serum GnRH concentrations in anestrous ewes treated with estradiol.

Alternatively, increased affinity and/or numbers of GnRH receptor sites on pituitary cells could account for an increase in the duration of increased serum LH concentrations. Spona (1973) demonstrated two receptors for GnRH in rat pituitaries. One was a low affinity, high capacity receptor present in pituitary cells from intact and ovariectomized rats. The other was a high affinity, low capacity receptor which was lost by 30 days after ovariectomy but could be reinduced by estrogen administration (Spona, 1974). In addition, Park et al. (1975) reported maximum binding of ^{125}I-LH-releasing hormone to rat pituitaries on the afternoon of proestrus. Therefore, the induction of a high affinity GnRH receptor by estrogen may be a part of the mechanism by which sustained LH releases are realized in estrogen-treated animals.

Another possibility is that estrogen increases the releasable pool of LH in the pituitary. Although there is no direct evidence to support this view, Roche et al. (1970) reported that pituitary LH concentrations increase from 573 to 998 μg/g during proestrus in ewes.

The surge of LH which normally occurs at 12 to 20 h after estradiol was completely inhibited when GnRH was injected at 12 h after estradiol treatment. However, this increase in serum LH was only partially inhibited when GnRH was given 6 h after estradiol. Several factors must be considered in order to explain this phenomenon. First, the increase in serum LH concentrations after GnRH at 6 or 12 h were of equal duration but neither lasted as long as an estrogen-induced LH release. This would indicate that the estrogen-induced LH releasing stimuli were not superimposed on the 12-h GnRH-induced LH release. Serum LH concentrations were measured until 60 h after estrogen treatment and no increase in LH concentrations occurred in addition to that induced by GnRH. Apparently, the estrogen-induced signal for LH release was completely inhibited and not just delayed. If, in fact, estrogen-induced LH is inhibited by GnRH treatment, there are several possible mechanisms by which this may be accomplished. The GnRH itself or resulting increased serum LH concentrations may prevent a LH-releasing signal from being released from the hypothalamus. However, if an LH-releasing signal was released from the hypothalamus at 12 to 20 h after estrogen, it may have been too soon after the 12-h GnRH injection for regeneration of releasable LH stores or restimulation of GnRH receptor sites. In support of the latter hypothesis, work in ewes (Rippel et al., 1974), gilts

(Chakraborty et al., 1973) and cows (Kinder et al., 1975) demonstrate repeated injections of GnRH produce progressively lower serum LH responses.

Experiment V: Serum Luteinizing Hormone Concentration after Abortion and Ovariectomy in Heifers

Short et al. (1973) reported that serum LH concentrations increased continually for 30 days after ovariectomy in heifers. However, in experiment I reported herein, serum LH concentrations stabilized by 7 days after ovariectomy in heifers not given exogenous steroids. Additionally, serum LH concentrations oscillated continuously at approximately 45-min intervals in heifers ovariectomized 1.5 months (experiment II). How soon after ovariectomy serum LH concentrations begin to oscillate is unknown. The present experiment was designed to evaluate serum LH concentrations shortly after ovariectomy in heifers. The sampling schedule used in this experiment was chosen as one which would allow an accurate estimate of mean serum LH concentrations and variance associated with that mean for each heifer. Additionally, frequent blood samples would provide information as to the pattern of LH secretion immediately after ovariectomy.

The endocrine environment at the time of ovariectomy may influence post-ovariectomy changes in serum LH concentrations. Therefore, the opportunity to ovariectomize heifers immediately after abortion of a 120-day-old fetus allowed us to monitor post-ovariectomy serum LH concentrations in heifers, all of which had similar endocrine environments at the time of ovariectomy. These

heifers were all assumed to be under a sustained progesterone environment with low serum estrogen and LH concentrations.

Blood samples taken at 10-min intervals for 70 min revealed low (<2 ng/ml) stable concentrations of LH in sera of heifers 120 days pregnant (Figure 15; Table 3). Analysis of LH concentrations in blood samples taken according to this same schedule at 12 h after ovariectomy showed 8 of 12 heifers exhibiting fluctuating serum LH concentrations. These fluctuations in serum LH concentration became more pronounced by 4 days after ovariectomy and all 12 heifers exhibited fluctuations in serum LH concentrations 8 days after ovariectomy (Table 3).

Table 3. Mean serum LH concentrations and average within heifer variance

Days relative to ovariectomy	Mean LH (ng/ml)	Average LH variance [(ng/ml)^2]
-.25	.62^a	.0136^c
.25	.91^a	.0319^c
.5	1.04^a	.1406^d
1.0	1.72^a	.7373^{d,e}
2.0	.86^a	.2277^{d,e}
4.0	1.39^a	.4786^{d,e}
8.0	2.88^b	1.8124^e

^{a,b}Means which have different superscripts are significantly different at the 1% level.

^{c,d,e}Variances which have different superscripts resulted in a significant F max ratio at the 5% level.

Figure 15. Serum luteinizing hormone concentrations before and after abortion and ovariectomy of 6 of the 12 heifers studied. Ten minute samples were taken for 70 min before ovariectomy and again .25, .5, 1, 2, 4 and 8 days after ovariectomy.

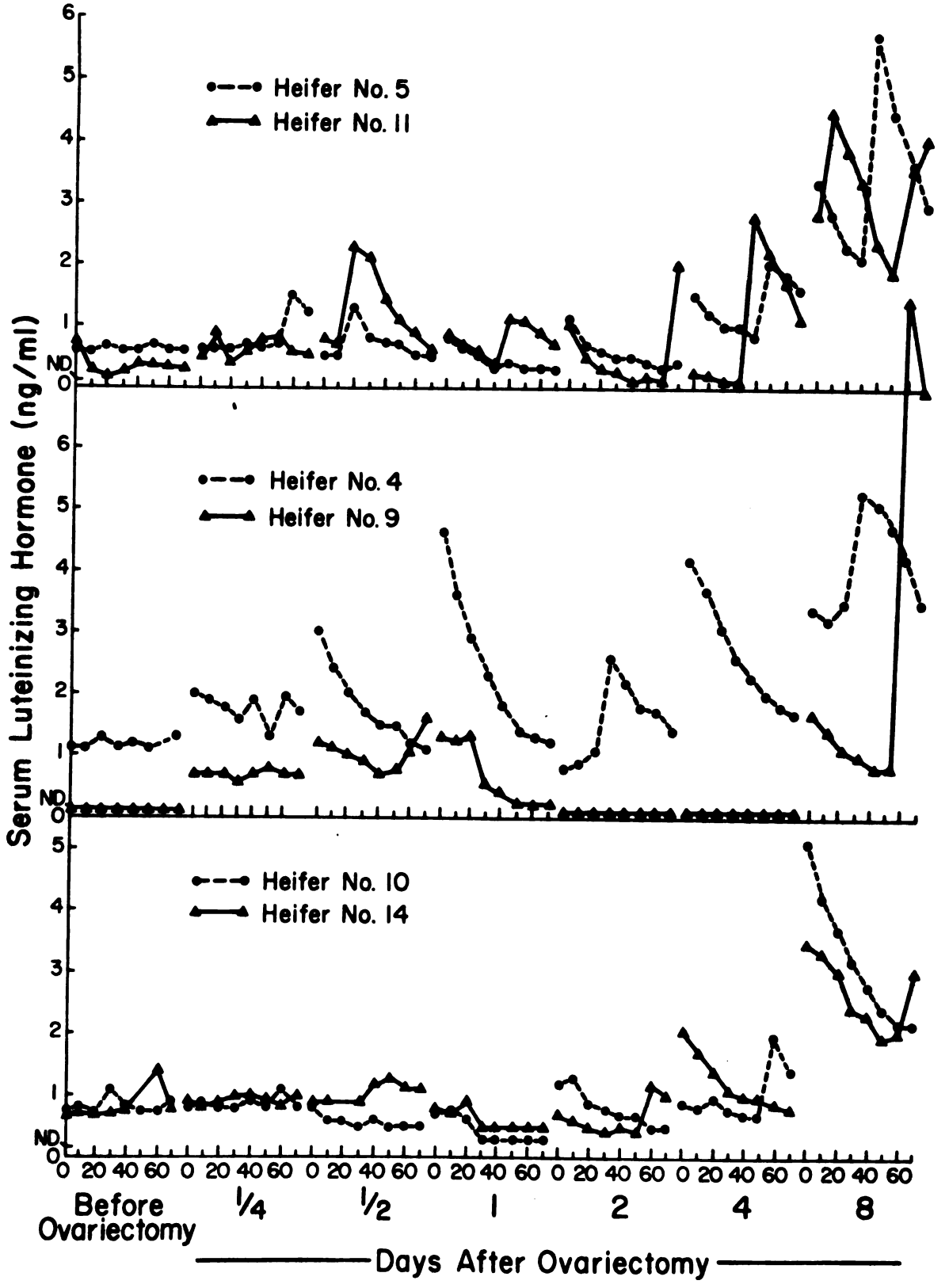
Heifer No. 5
Heifer No. 11
Heifer No. 4
Heifer No. 9
Heifer No. 10
Heifer No. 14
Serum Luteinizing Hormone (ng/ml)
ND
0 20 40 60 0 20 40 60 0 20 40 60 0 20 40 60 0 20 40 60 0 20 40 60 0 20 40 60
Before Ovariectomy
1/4
1/2
1
2
4
8
Days After Ovariectomy

Figure 15

The sampling schedule used in this experiment provided an estimate of average serum LH concentrations and variation about the mean. Average serum LH concentrations increased ($P<.01$) 8 days after ovariectomy; however, the average variance about that mean increased ($P<.05$) between .25 and .5 days and .5 and 8 days after ovariectomy (Table 3). The 70-min sampling periods, however, were not of sufficient duration to directly monitor frequency of serum LH fluctuations in individual heifers (Figure 15). However, animal replication supplied some insight into this matter. If it is assumed that blood samples taken at 10-min intervals for 70 min constitute a brief observation of a continuously changing phenomenon and the same phenomenon is occurring in each heifer, then the frequency with which an event is observed in these sampling periods should be proportional to the rate of occurrence of that event. Therefore, the number of heifers exhibiting fluctuations in serum LH concentrations in one sampling period can be used as a measure of the frequency of serum LH fluctuations at that post-ovariectomy period. Results indicate that oscillations in serum LH concentrations in heifers are sporadic or intermittent immediately after ovariectomy and occurrence of these oscillations become more frequent with increasing time after ovariectomy. Changes in serum LH concentrations after ovariectomy have not been monitored adequately to determine exactly when these oscillations become continuous, but the present data suggest that they begin between 4 and 8 days after ovariectomy.

Experiment VI: Progesterone Effect on Estradiol Induced Changes in Serum Luteinizing Hormone Concentrations

Estradiol does not induce LH release in the presence of progesterone in monkeys (Dierschke et al., 1973), ewes (Scaramuzzi et al., 1971) and rats (Davidser et al., 1970). Therefore, progesterone is considered antagonistic to estradiol-induced LH release. In the present experiment, progesterone pessaries were inserted before, during or after administration of estradiol implants.

Serum Steroids

Serum concentrations of progesterone and estradiol were quantified to assess the ability of the pessaries and implants to obtain physiological serum concentrations of these steroids. Serum progesterone concentrations in heifers given pessaries are shown in Figures 16, 17 and 18. Serum progesterone increased ($P<0.01$) within 1 h to between 4 and 6 ng/ml. In all heifers serum progesterone remained at these concentrations until the pessaries were removed, at which time serum progesterone rapidly (within 2 h) decreased to pretreatment concentrations (.2 ng/ml). Serum progesterone concentrations in heifers bearing progesterone pessaries in this experiment were similar to those achieved in experiment I. These serum progesterone concentrations are similar to those observed in diestrous heifers (Wettemann et al., 1972).

Serum estradiol concentrations were increased ($P<0.01$) at 30 min after implant administration to greater than 30 pg/ml but decreased in all groups by 6 h (Figure 19). Subsequently, serum estradiol concentrations decreased slowly to approximately 15 pg/ml at 24 h where it remained throughout the 72-h sampling period.

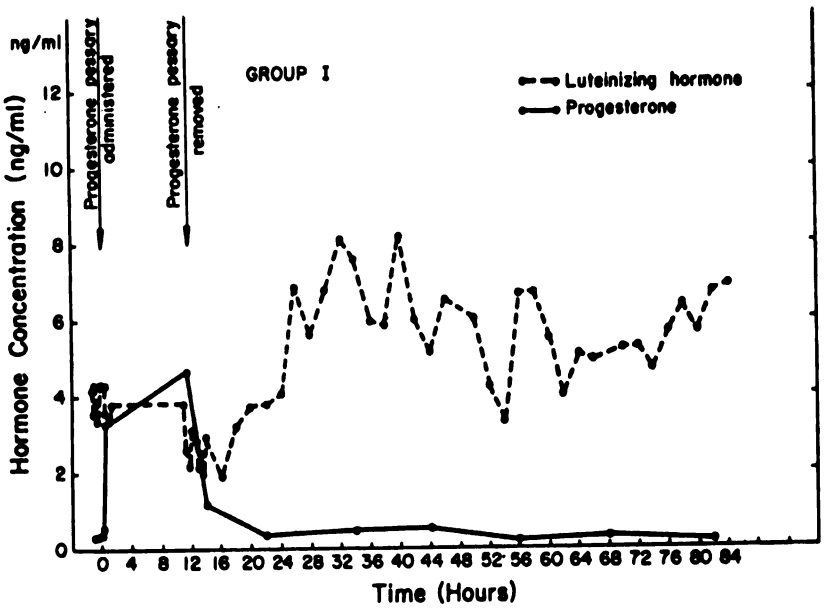

Figure 16. Serum LH and progesterone concentrations in ovariectomized heifers treated with progesterone pessaries for 12 h and then estradiol implants from hour 12 to 80.

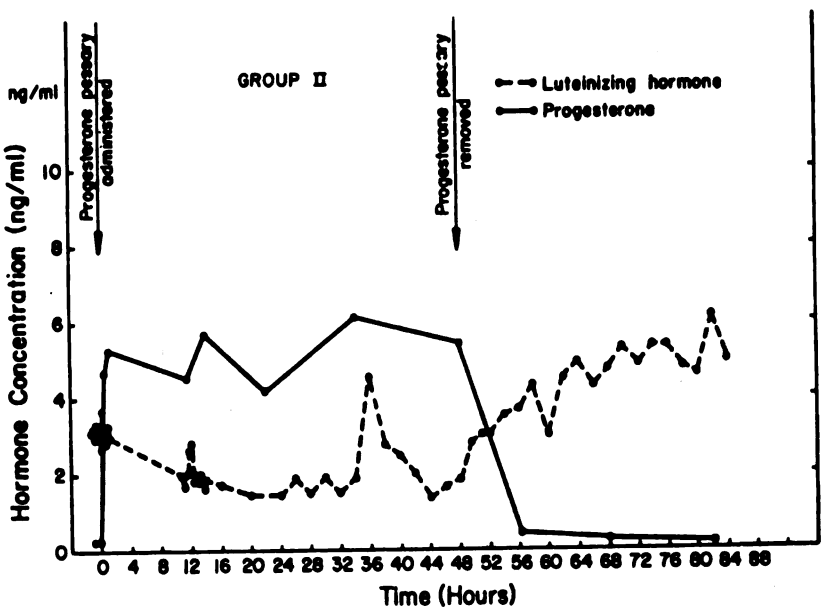

Figure 17. Serum LH and progesterone concentrations in ovariectomized heifers treated with progesterone pessaries from hour 0 to 48 and estradiol implants from hour 12 to 80.

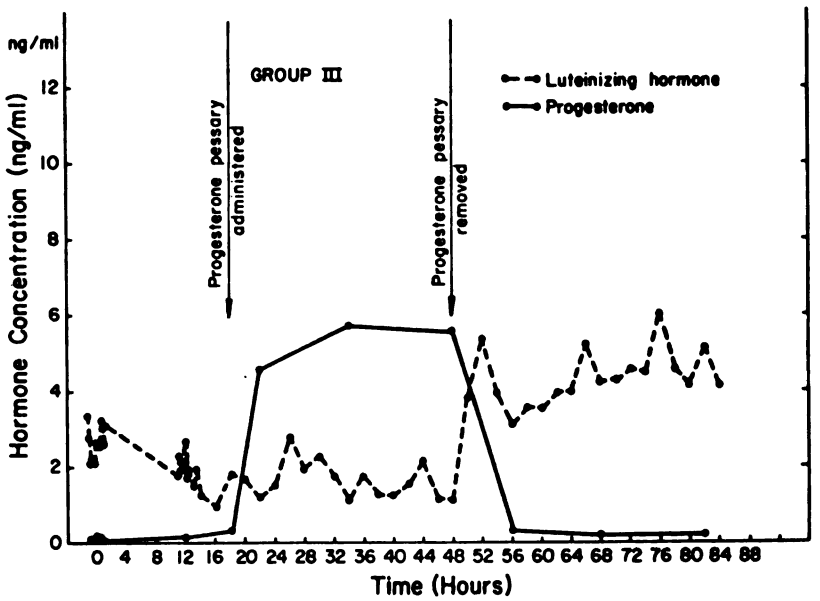

Figure 18. Serum LH and progesterone concentrations in ovariectomized heifers treated with estradiol implants from hour 12 to 82 and progesterone from hour 18 to 48.

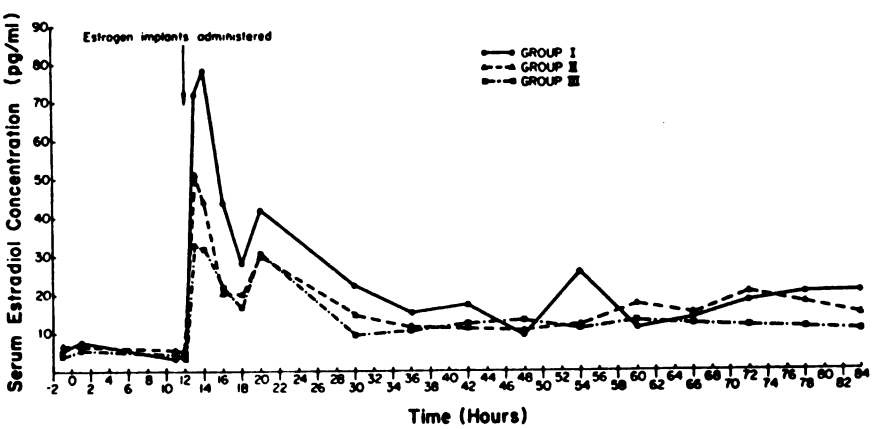

Figure 19. Serum estradiol concentrations in ovariectomized heifers treated with estradiol implants from hour 12 to 82. Group I was given progesterone pessaries from hour 0 to 12. Group II was given progesterone pessaries from hour 0 to 48. Group III was given progesterone pessaries from hour 18 to 48.

Serum estradiol concentrations were unaffected (P>0.05) by progesterone treatment.

Maximum serum estradiol concentrations immediately after administration of four estradiol implants was less than those observed in experiment III (169 pg/ml; Figure 10, vs. 80 pg/ml; Figure 19). In a recent experiment (Beck and Convey, unpublished data), average serum estradiol concentrations in heifers increased to a maximum of 50 pg/ml after administration of four estradiol implants which had been used in previous experiments. Therefore, the lower serum estradiol concentrations in the present experiment were probably due to the use of estradiol implants which had been used previously.

Serum Luteinizing Hormone

There was no difference (P>0.05) in mean serum LH concentrations between treatment groups for the first 20 h of the experiment (8 h after estradiol; Figures 16, 17 and 18). However, serum LH concentrations increased (P<0.01) from 1.9 ng/ml at 16 h to 6.9 ng/ml at 26 h (14 h after estradiol) in heifers which had progesterone pessaries removed prior to estradiol treatment (Group I; Figure 16). Serum LH continued to increase to 8.2 ng/ml at 32 h (28 h after estradiol) and then decreased to 3.3 ng/ml at 54 h (42 h after estradiol). After hour 54 serum LH concentrations again increased throughout the sampling period (84 h).

When progesterone pessaries were removed from heifers in the other two groups at 48 h, LH increased so that serum concentrations were indistinguishable (P>0.05) from those of the heifers in Group I after hour 56 (44 h after estrogen).

Serum LH concentrations were increased 20 h after estradiol in heifers which had progesterone pessaries removed prior to estradiol treatment. The duration of this increase in serum LH concentrations was similar to that reported in estrous heifers (Chenault et al., 1975) and the increase in serum LH concentrations after estradiol in experiment III reported herein (8-10 h). However, the maximum average serum LH concentration achieved after estradiol in the present experiment (8.2 ng/ml) was considerably less than that achieved after estradiol in experiment III (57 ng/ml). In a recent experiment, serum LH increased to an average peak concentration of 17.2 ng/ml when ovariectomized heifers were treated with four used estradiol implants (Beck and Convey, unpublished data). Possibly the lower initial serum estradiol concentrations in the latter experiments resulted in a lower magnitude of peak serum LH concentrations.

Another possible explanation for the increase in serum LH concentration after estradiol in heifers from Group I was that serum LH secretion was being suppressed by progesterone and estradiol and when the progesterone was removed serum LH concentrations increased because the pituitary was being released from negative feedback. This appears to be a valid explanation for the increased serum LH concentrations after pessary removal in the second and third groups of heifers (Figures 17 and 18) because serum progesterone concentrations decreased and serum LH concentrations increased within 2 h after the pessary was removed. However, in Group I (Figure 16) serum LH concentration did not increase until 14 h after progesterone

pessary removal. In addition, the timing and duration of the increased serum LH concentrations in Group I heifers is similar to those observed after estradiol treatment alone.

Therefore, it is my opinion that four estradiol implants given after progesterone withdrawal in this experiment was the causative factor in the increased serum LH concentrations 14 to 42 h after estradiol treatment.

Four estradiol implants did not produce increases in serum LH concentrations if serum progesterone concentrations were between 2 and 4 ng/ml at the time of estradiol treatment (Group II). This is in agreement with results reported by Hobson and Hansel (1972c); LH release induced by 2 or 4 mg estradiol in heifers was inhibited when 100 or 1000 mg of progesterone were given simultaneously. However, Short et al. (1973) reported that 25 mg injections of progesterone given at -12, 0, 12 and 24 h relative to estradiol (10 mg) did not prevent the estradiol induced LH release in heifers. The reason for the differences in these two latter reports may be the relative proportions of estradiol and progesterone administered.

Progesterone exposure from 6 to 36 h after estradiol treatment (Group III) also prevented the estradiol-induced increase in serum LH concentrations. This indicates that while estradiol stimulates a mechanism which results in a surge release of LH, progesterone is able to intervene as late as 6 h after estrogen exposure and prevent the LH release from the pituitary. In the monkey progesterone administration 12 h after an injection of estradiol benzoate was able to block any subsequent increases in serum LH concentrations (Dierschke et al., 1973).

Progesterone also blocks the GnRH induced LH release in ewes and rats (Debeljuk et al., 1972). In addition, *in vitro* experiments ascribe negative effects of progesterone directly on pituitary LH secretion (Schally et al., 1973; Tang and Spies, 1975). This indicates a direct action of progesterone on the pituitary to prevent the estradiol-induced LH release. However, Cumming et al. (1972) could not prevent the GnRH induced LH release in ewes with infusion of physiological amounts of progesterone and Zolman et al. (1974) found no correlation between endogenous progesterone concentrations and serum LH responses after GnRH. Therefore, the possibility that progesterone also acts at some other site cannot be ruled out.

In this experiment, the negative effects of estradiol and progesterone on serum LH concentrations were not easily discernible. Progesterone or estradiol alone did not decrease serum LH concentrations in these ovariectomized heifers. However, when progesterone was withdrawn and estradiol implants left in place, serum LH concentration increased. These results reaffirm the requirements for both estradiol and progesterone in the control of baseline serum LH concentrations.

GENERAL DISCUSSION

Steroid replacement methods used herein successfully replaced steroids at a steady state in ovariectomized heifers. Thus, polydimethylsiloxane implants and pessaries maintained serum estradiol and progesterone concentrations in ovariectomized heifers at or above those found in intact heifers during an estrous cycle.

Physiologically, these experiments demonstrate several aspects of hypothalamic-pituitary-gonadal interactions. Progesterone synergizes with estradiol to maintain low constant serum LH concentrations in ovariectomized heifers. This result is consistent with that reported by Hobson and Hansel (1972b) where estradiol alone did not prevent a post-castration increase in serum LH concentrations in heifers. Additionally, experiments in monkeys demonstrated a synergistic effect of estradiol and progesterone in maintenance of low constant serum LH concentrations (Karsch et al., 1973c).

Serum LH concentrations decreased initially after estradiol treatment of heifers ovariectomized 1.5 months. However, this decrease is only temporary and occurs when serum estradiol concentrations are extremely high. Reports demonstrating a decrease in serum LH concentrations after long term exposure (2 to 3 weeks) to estradiol in the ewe (Brown et al., 1972) are probably not of physiological significance, especially since ewes have a 16-day estrous cycle. Therefore, the combination of progesterone and estradiol

still seems the most plausible explanation as to the ovarian constituents controlling basal LH secretion.

Serum LH concentration in these ovariectomized heifers oscillated similarly to those of ovariectomized ewes and monkeys. However, oscillations appeared sporadic immediately after ovariectomy and only became continuous 8 days later. Bhattachyarya et al. (1972) interrupted the circhoral pulsatile discharges of LH in ovariectomized monkeys with single injections of chlorpromazine, haloperidol, phentolamine or phenoxybenzamine. Therefore, it appears that the pulsatile discharges of LH in ovariectomized animals are the consequence of intermittent dopaminergic and/or non-adrenergic signals to LH-RH secreting cells of the hypothalamus resulting in a rhythmic release of LH-RH. Blake and Sawyer (1974) could not disrupt the regular fluctuating plasma LH concentration in rats by deafferentation of the medial basal hypothalamus. Therefore, the signal for rhythmic fluctuations in serum LH concentrations probably arises within the medial basal hypothalamic region. Thus, it would appear that the estrogen and progesterone suppression of serum LH is an effect on the medial basal hypothalamus to prevent discharges of LH releasing hormone as well as progesterone block at the pituitary level.

Goding et al. (1969) demonstrated that estradiol initiates an LH surge but the effect of estradiol was complete after 2 h in anestrous ewes. In the present studies, estradiol initially inhibited LH release through the mechanisms described earlier, but at the same time probably set into motion a mechanism which resulted in a surge release of LH 18 to 20 h later. The large surge of LH

release which occurs 18 to 20 h after estradiol was prevented by GnRH administration 12 h after estradiol exposure or progesterone administration up to 6 h after estradiol treatment. Either treatment regime blocks the estradiol-induced LH surge well after the mechanism for LH release was supposedly set in motion. This is in contrast to estrogen antibodies (Ferin et al., 1969) or antiestrogen compounds (Shirley et al., 1968), which must be given concurrently with the rise in serum estradiol concentrations to block the preovulatory surge of LH. Apparently progesterone and GnRH blocked LH release by interrupting the normal sequence of events initiated by estradiol. Gonadotropin releasing hormone may act to deplete LH-RH receptors or releasable LH stores at the pituitary itself. Progesterone may act at the hypothalamus to decrease release of LH-RH and also at the pituitary to decrease the quantity of LH released by LH-RH.

The serum LH response to GnRH increases with time after estrogen exposure (i.e., 6 vs. 12 h). Therefore, the mechanism for LH release set in motion by estradiol promotes a progressive increase in pituitary responsiveness to GnRH. However, estradiol plus a single injection of GnRH did not produce an LH release of equal duration to the estradiol-induced or preovulatory LH release in heifers. Therefore, estradiol must act on the hypothalamus to produce a sustained release of LH-RH. In the rat, neural afferents from the anterior hypothalamus to the medial basal hypothalamus are necessary for an ovulatory discharge of LH but not for the postcastration rise or pulsatile discharges of plasma LH (Blake and

Sawyer, 1974). Therefore, estradiol could have a negative effect on the medial basal hypothalamic area suppressing basal LH secretion while stimulating anterior hypothalamic nuclei to produce a sustained release of LH releasing hormone resulting in the preovulatory type surge of LH.

From these data a model can be constructed to explain the interactions of ovarian steroids in control of LH release in heifers.

Estradiol and progesterone act synergistically to maintain low basal serum LH concentrations. Estradiol probably acts on the medial basal hypothalamus and progesterone on the pituitary. However, hypothalamic effects of progesterone cannot be ruled out at this point. This hypothesis is consistent with reports that progesterone inhibits LH release by low but not high doses of GnRH (Debeljuk et al., 1972). Thus, if estradiol decreases LH-RH released from the hypothalamus and progesterone suppresses the pituitary response to the decreased serum LH-RH concentration, a synergistic effect would be seen in the presence of both hormones.

The preovulatory release of LH in heifers is thought to be controlled by serum estradiol concentrations. Estradiol probably has long term positive effects on synthesis of LH and LH-RH receptors at the pituitary. In addition, estradiol must stimulate the anterior hypothalamus to cause a sustained LH-RH release. These two effects of estradiol could likely produce a surge release of LH of the magnitude and duration of that observed prior to ovulation in heifers (Chenault et al., 1975).

Gonadotropin releasing hormone stimulates LH release from bovine pituitaries *in vitro* (Zolman et al., 1973). In the present experiments, GnRH increased serum LH concentrations *in vivo* presumably by a direct effect on the pituitary. However, GnRH also decreased and precluded the estradiol-induced LH release when given 6 and 12 h after estradiol, respectively. Therefore, GnRH appears to deplete LH-RH receptors or releasable LH stores at the pituitary and thereby prevents subsequent increases in serum LH concentrations. This may account for serum LH concentrations remaining low the first 2 or 3 days after estrus when ovarian steroids are low.

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