CONTROL OF OVARIAN FOLLICULAR GROWTH AND STEROIDOGENESIS DURING POSTPARTUM ANESTRUS IN BEEF COWS

By

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ABSTRACT

CONTROL OF OVARIAN FOLLICULAR GROWTH
AND STEROIDOGENESIS DURING POSTPARTUM
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Two studies were conducted to examine follicular growth, selected steroid hormones in follicular fluid and serum, follicular gonadotropin receptors, and secretion of various pituitary hormones prior to first postpartum ovulation in suckled beef cows.

In Experiment 1, amplitude of LH pulses increased between days 7 and 14 after parturition. Frequency of LH pulses, and baseline and overall concentrations of LH did not change from days 7 through 42-56. Progesterone concentrations in large follicles increased between days 7 and 14 and remained elevated through days 42-56. Estradiol concentrations in large follicles ($\geq$8 mm) increased 4-fold between days 14 and 28. Numbers of gonadotropin binding sites did not change and hence, was not associated with increased estradiol in follicular fluid. Concentrations of FSH, PRL, estradiol, progesterone and cortisol in serum, and androstenedione in follicular fluid did not change with time postpartum. In addition, no change in numbers of gonadotropin binding sites or steroids in small and medium follicles were observed. Numbers of medium follicles increased 2-fold between days 7 and 14, and again between days 28 and 42-56.

Perhaps these increases in numbers of medium follicles provides a larger pool of follicles from which ovulatory follicles can be selected prior to first postpartum ovulation.

In Experiment 2, LHRH priming induced LH and FSH pulses but because amplitude of induced pulses were lower than endogenous pulses, no change in overall LH and only slight increases in overall FSH concentrations were observed. LHRH priming did not alter numbers of small, medium and large follicles or size of the two largest follicles. Estradiol concentrations in large follicles were unchanged after 48 h of LHRH priming but increased 2.3-fold after 96 h. Coincident with increased estradiol production by large follicles was increased numbers of LH but not FSH binding sites. Progesterone concentrations in large follicles were 4-fold greater in LHRH- than in saline-treated cows at 48 h. At 96 h, progesterone in follicular fluid was 2-fold greater in saline- than in LHRH-treated cows.

A major hormonal change that occurs before the first postpartum ovulation is increased estradiol production by large ovarian follicles. Also, increased progesterone concentrations preceded the increase in estradiol. This may indicate that steroidogenic capabilities of large follicles undergo sequential transformation prior to the first postpartum ovulation. Increased frequency or amplitude of LH pulses were associated with the shifts in estradiol and progesterone production by large follicles. Increased capacity of large follicles to produce estradiol may be essential for onset of the first postpartum ovulation in suckled beef cattle.

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INTRODUCTION

Interval to first postpartum ovulation affects calving intervals. Within a given breed, several factors influence this interval to first ovulation including: demands of lactation (Baker, 1969; Moller, 1970; Edgerton, 1980; Donkin, 1980c; Hanson et al., 1982; Fonseca et al., 1983), uterine and cervical involution (Moller, 1970; Kiracofe, 1980; Donkin, 1980c; Oltenacu et al., 1983), nutrition (Baker, 1969; Moller, 1970; Dunn and Kaltenbach, 1980; Hanson et al., 1982; Rutter and Randel, 1984), season and stress (Moller, 1970; Christenson, 1980; Donkin, 1980c; Hanson and Hauser, 1983; Lewis et al., 1984), and diseases (Moller, 1970; Donkin, 1980c). It is reasonable to assume that for estrus and ovulation to occur after parturition, endocrine changes such as the pattern of synthesis and release of pituitary and ovarian hormones that typifies a normal estrous cycle must be reinitiated. Factors such as lactational demands and nutrition may directly or indirectly affect such endocrine changes (Baker, 1969; Wettemann, 1980; Stevenson and Britt, 1980). Understanding the precise endocrine changes that occur prior to the first postpartum ovulation could eventually lead to development of manipulative techniques hastening first postpartum ovulation, thereby reducing the calving interval and subsequently increasing the number of calves produced during a cow's lifetime.

General changes in levels of pituitary and ovarian hormones in the blood of cows from parturition to first ovulation are known (Wettemann, 1980; Lamming et al., 1982). Specifically, it appears that the pituitary gland is capable of releasing preovulatory-like surges of LH and FSH long before the first postpartum ovulation occurs, and therefore, the anterior pituitary gland is not a limiting factor for return to estrous cyclicity. Recently, the ability of the hypothalamus to release sufficient amounts of LHRH has been suggested to be the limiting factor for return to estrous cyclicity postpartum (Cox and Britt, 1982; Walters et al., 1982c). Another possibility is that the ability of ovarian follicles to function normally and secrete estrogens is the limiting factor for return to estrous cyclicity after parturition. However, little is known about changes in follicular growth and follicular steroidogenesis during the anovulatory period in postpartum beef cows. The overall objective of this dissertation, therefore, was to study control of ovarian folliculogenesis and follicular steroidogenesis during the postpartum anovulatory period in beef cows. Spontaneous changes in follicular growth, follicular steroid production, follicular gonadotropin receptor concentrations, and coincident secretion of various pituitary hormones prior to the first postpartum ovulation were characterized in suckled beef cows. Additionally, in an attempt to mimic preovulatory changes in gonadotropins, LHRH was administered in multiple, low-dose injections to suckled, anovulatory postpartum beef cows. The subsequent changes in ovarian follicular growth and steroid production, follicular gonadotropin receptor concentrations, and coincident secretion of pituitary gonadotropins were characterized.

REVIEW OF LITERATURE

A. Hormonal Profiles in Serum of Cattle Prior to First Postpartum Ovulation

1. Luteinizing Hormone (LH)

Since ovarian function is associated with concentrations of LH in blood, this review will describe the changes in concentrations of LH that occur in blood of cattle during the postpartum interval.

Dairy Cattle.--In dairy cattle that are milked twice a day (calves removed at parturition), peripheral plasma levels of LH increase during the first 1 to 3 weeks postpartum (Erb et al., 1971; Echternkamp and Hansel, 1973; Ingalls et al., 1973; Kesler et al., 1977; Fernandes et al., 1978; Goodale et al., 1978; Schallenberger et al., 1978; Webb et al., 1980; Peters et al., 1981; Bolt and Rollins, 1983) and remain at the higher levels for the remaining postpartum anovulatory period (Erb et al., 1971; Fernandes et al., 1978; Schallenberger et al., 1978). This increase in overall concentrations of LH in peripheral plasma is probably due to increased pulsatile release of LH (Peters et al., 1981; Convey et al., 1983) which in turn may be due to an acceleration of pulsatile release of LHRH from the hypothalamus (Schallenberger and Peterson, 1982). These increases in pulsatile release of LH in serum also occur prior to the preovulatory LH surge in cycling cattle (Rahe et al., 1980). During the postpartum anovulatory period LH concentrations after LHRH treatment are greater

after 14 days postpartum than during days 3 to 10 (Kesler et al., 1977; Fernandes et al., 1978; Schallenberger et al., 1978; Azzazi et al., 1983). Thus, this increased ability of the pituitary to secrete LH in dairy cows occurs well before the first postpartum ovulation (Casida, 1968; Baker, 1969; Wettemann, 1980; Donkin, 1980a), indicating LH secretion may not be the limiting factor for return to cyclic ovarian activity.

Beef Cattle.--In suckled beef cows, concentrations of LH in peripheral plasma do not increase to maximal levels until after the fourth week after parturition (Peters et al., 1981; Williams et al., 1982; Humphrey et al., 1983; Convey et al., 1983). Concentrations of LH in plasma after LHRH treatment are greater 20 days after parturition than during days 3 to 20 (Webb et al., 1977; Williams et al., 1982; Gauthier and Mauleon, 1983). As with dairy cows, it appears that this increase in the ability of the anterior pituitary gland to secrete LH in beef cows occurs well before the first spontaneous postpartum ovulation (Casida, 1968; Baker, 1969; Wettemann, 1980; Donkin, 1980a). Again, increased pulsatile release of LHRH may be involved (Walters et al., 1982b; Humphrey et al., 1983).

2. Follicle-Stimulating Hormone (FSH)

Since ovarian function is also associated with concentrations of FSH in the blood, this review will describe changes in FSH that occur in the blood during the postpartum interval in cattle.

In the few studies reported, concentrations of FSH in peripheral plasma do not appear to change in dairy or beef cattle

during the first 14 to 50 days after parturition (Schallenberger et al., 1978; Williams et al., 1982; Bolt and Rollins, 1983; Convey et al., 1983). In addition, FSH concentrations after LHRH treatment are greater 10 days after parturition than during days 3 to 5 in both beef and dairy cattle (Schallenberger et al., 1978; Williams et al., 1982) suggesting that normal secretory activity of FSH returns in cows earlier than that of LH. These results also suggest that gonadotropin secretion in cattle *per se* may not be the limiting factor for return to cyclic ovarian activity after parturition.

B. Ovarian Folliculogenesis

1. Definition of Folliculogenesis

Follicular growth during the postpartum anestrous period in cattle has not been well characterized. Since it is likely that follicular growth from parturition to the first ovulation may follow similar trends to follicular growth seen during repetitive estrous cycles, this review will describe ovarian follicular growth first during repetitive estrous cycles and then during postpartum anestrus in cattle.

Folliculogenesis may be defined as formation of Graafian (mature, preovulatory) follicles from a pool of primordial (non-growing, preantral) follicles. In the cow, the pool of primordial follicles remains stable (average 133,000 follicles) from birth until about the fourth year of life, but primordial follicle numbers subsequently decline until approximately 3000 remain in ovaries of cows 15 to 20 years of age (Erickson, 1966). Erickson (1966) separated growing follicles into two groups: 1) preantral and 2) antral. Both types

of growing follicles increase coincidently in number from birth to about 70 days of age. Numbers of preantral follicles remain constant (200 to 250 per pair of ovaries) in cows until 4 years of age and then decline coincident with the decline in primordial follicles until fewer than 50% of maximal numbers remain in ovaries from cows 15 to 20 years of age. In contrast, antral follicle numbers are constant (30 to 60 per pair of ovaries) in cows up to 10 years of age declining to less than 50% of maximal numbers at 15 to 20 years of age (Erickson, 1966). This age-dependent exhaustion of the primordial pool of follicles in other species has been reviewed elsewhere (Byokov, 1978; Richards, 1980). The dynamics of antral follicular growth during estrous cycles in rats and mice has been achieved through radiolabeling techniques and extensive morphological analysis (Peters, 1978; Richards, 1980). Since these radiolabeling and morphometric techniques require large sample sizes, measuring the dynamics of growth of antral follicles within an estrous cycle of a cow would be difficult to accomplish. Therefore, in large mammals such as cattle, researchers have relied on crude measurements of follicular growth, such as numbers of various size follicles and mean sizes of various types of follicles. The numbers of antral follicles within any particular size category or stage of growth are related to: 1) The rate of entry of growing, preantral follicles into the pool of antral follicles within a size category, 2) The rate of growth of these antral follicles into a larger size category, and 3) The rate of loss (atresia) of these follicles from a large size category into a smaller size category. If these three rates were constant, then

the number of follicles of all sizes would remain constant throughout the estrous cycle. However, there is significant variation in numbers of various size antral follicles during the bovine estrous cycle (Rajakoski, 1960; Marion et al., 1968; Swanson et al., 1972; Schams et al., 1977; Ireland et al., 1979; Matton et al., 1981; Merz et al., 1981; Staigmiller and England, 1982). Therefore, these changes in numbers of follicles within different size categories during an estrous cycle could be a result of any combination of the three factors mentioned above. With this in mind, a brief review of attempts to measure growth of antral follicles in cattle will be presented.

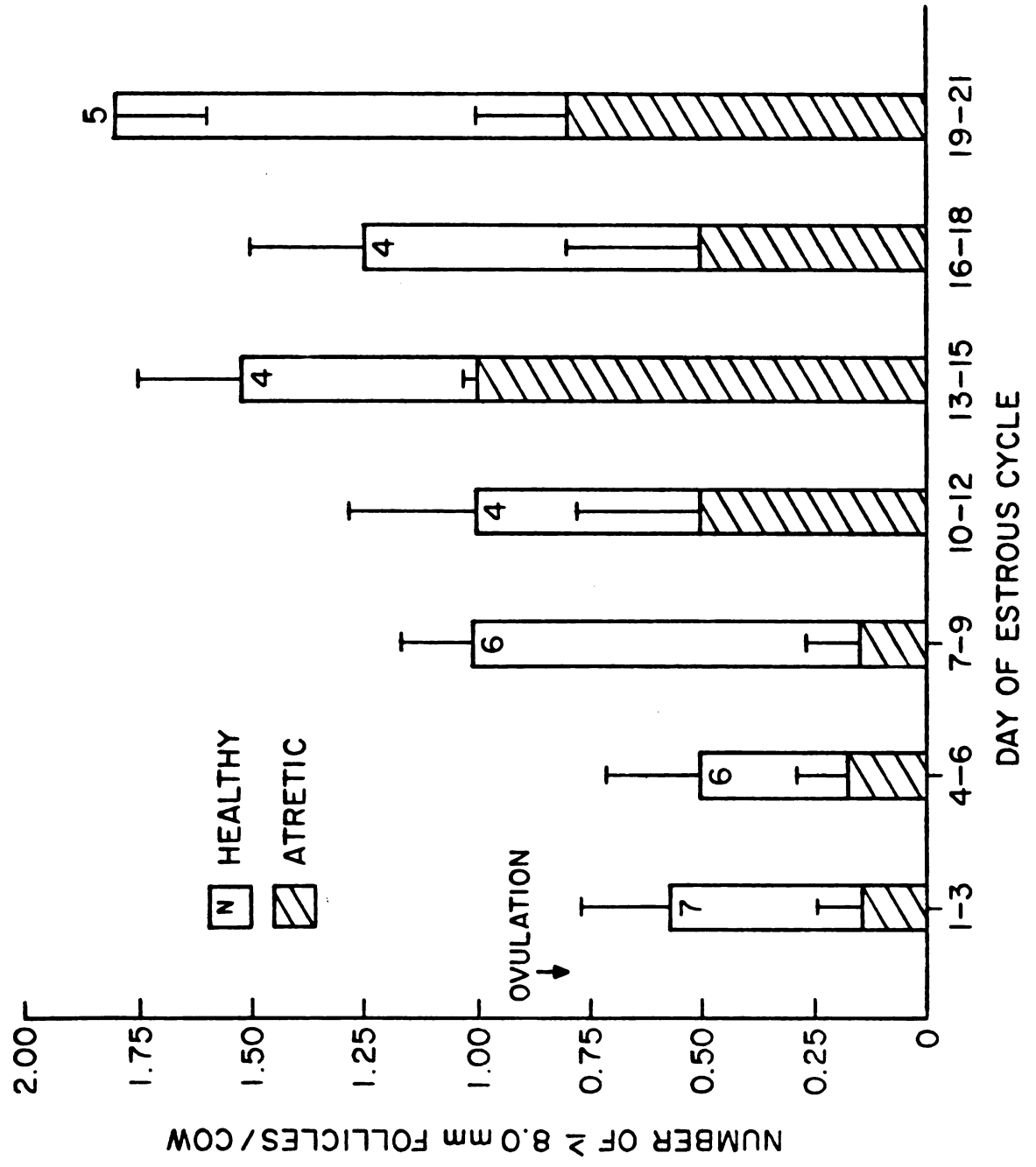
2. During Repetitive Estrous Cycles

Few studies have been conducted to characterize ovarian follicular development during the bovine estrous cycle, most likely due to the limitations mentioned previously. The first and most extensive study conducted was published by Rajakoski (1960), who proposed that two "waves" of follicular growth exist during the estrous cycle. However, on 9 of the 21 days of the cycle there was only one cow examined per day. On three of the remaining 12 days three cows were examined per day. Two cows per day were examined on the remaining days. Moreover, no statistical analysis was conducted on the data. A qualitative analysis of across-day trends of follicular growth by Rajakoski was done in spite of the fact that 6-fold differences in the numbers of follicles 5 mm or greater in diameter existed between cows within days. This large between-animal variation confounded the across-day trends observed since only one

animal per day was inspected on 9 of the 21 days. If one were to group the data into seven groups of four to seven cows per group, each encompassing a 3-day interval, only one "wave" of large antral follicular growth would be seen (Figure 1). From Rajakoski (1960) it can also be determined that there is an increase in the rate of atresia of large antral follicles between 7 and 15 days of the estrous cycle (Figure 1). This increase in rate of atresia of large antral follicles has been verified (Choudary et al., 1968; Merz et al., 1981). An increase in the total number of large antral follicles from about day 7 to about day 12 in the bovine estrous cycle has also been observed (Swanson et al., 1972; Schams et al., 1977; Merz et al., 1981) along with a decrease in number of smaller antral follicles (Matton et al., 1981). Therefore, the available evidence suggests that there is an increase in the rate of growth of small antral follicles into larger antral follicles as the estrous cycle progresses toward ovulation.

Measuring the size of the one or two largest follicles on each ovary is another way to measure follicular growth. In all (Donaldson and Hansel, 1968; Hackett and Hafs, 1969; England et al., 1973; Matton et al., 1981) but one (Merz et al., 1981) study, it was found that the diameter of the largest follicle present on the ovary increases between days 1 and 20 of the bovine estrous cycle (day 0 = day of estrus). In addition, Ireland and Roche (1983b) found that the diameter and volume of large estrogen-active follicles increases from day 17 through the end of the LH surge (approximately day 21). These data suggest that in addition to transformation of small antral follicles into

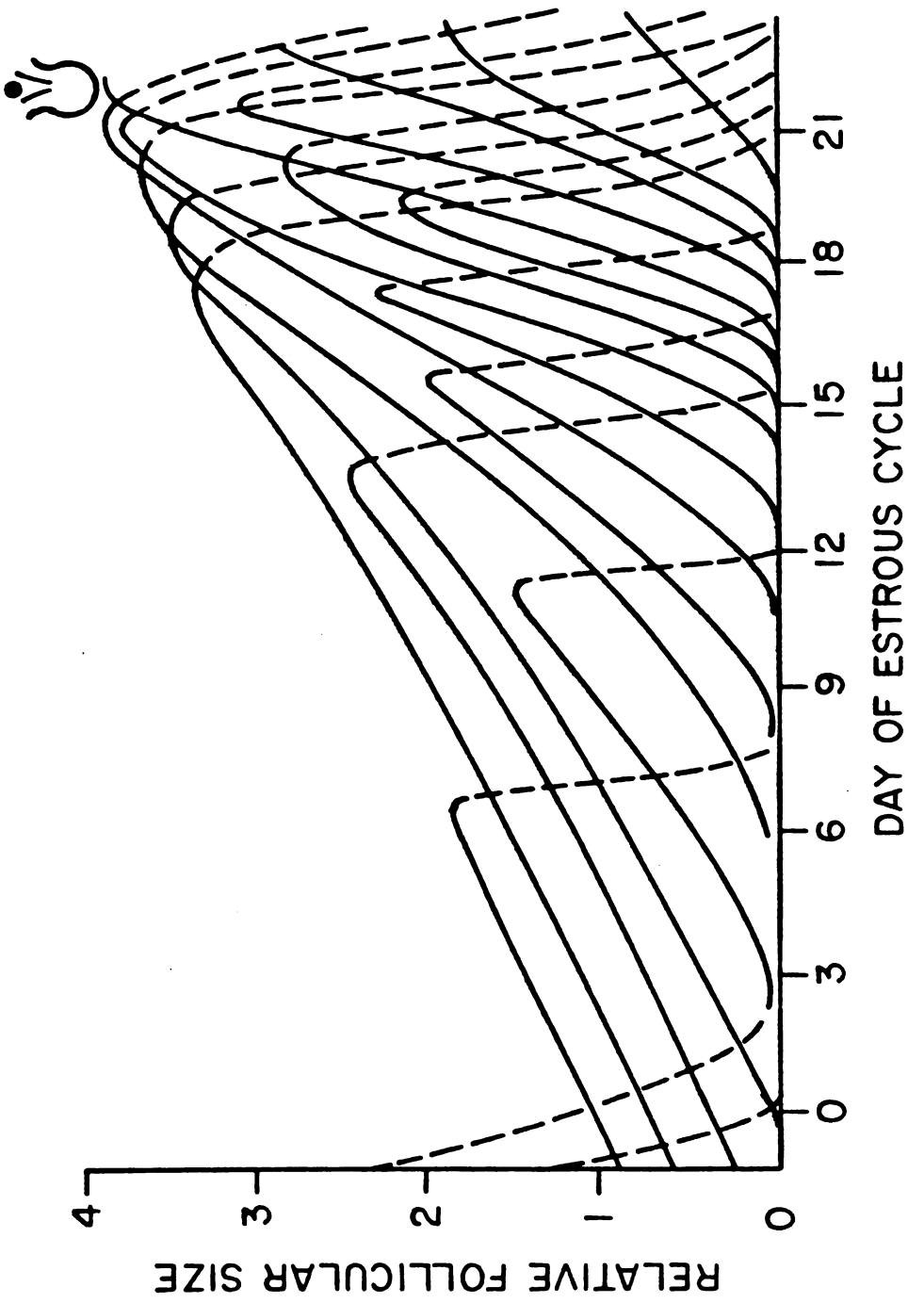
Figure 1.--Numbers of healthy and atretic follicles 8.0 mm or greater quantified at various days of the bovine estrous cycle (data revised from Rajakoski, 1960). N = number of cows.


NUMBER OF ≥ 8.0 mm FOLLICLES/COW
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0
OVULATION
HEALTHY
ATRETIC
N
7
6
6
4
4
4
5
1-3
4-6
7-9
10-12
13-15
16-18
19-21
DAY OF ESTROUS CYCLE

larger ones, the largest antral follicle present on the ovary grows as estrus approaches. However, the probability that the largest follicle present is the one that finally ovulates increases only at day 18 of the estrous cycle (Dufour et al., 1972).

Recent work by Matton et al. (1981) indicates that follicular growth and replacement (turnover) of follicles greater than 6 mm in diameter are more rapid after day 13 than before day 8 of the bovine estrous cycle. Moreover, growth of medium-sized follicles (3 to 6 mm in diameter) occurs only when the largest follicle is rapidly turning over. With the evidence presented above, a schematic for follicular growth can be devised (Figure 2). Each sigmoidal line depicts the path individual follicles follow during growth (solid line) and atresia (dotted line). The slope (growth rate) and height (follicular size) of the growth lines increase as the cycle advances. Thus, as the number of both solid and dotted lines increase, follicular turnover increases. The precise time at which an individual follicle enters the growing phase, and the number of estrous cycles an individual antral follicle is present before undergoing atresia is still unknown in the cow. Recent preliminary data presented by Lussier et al. (1983) indicates that a period of two estrous cycles is required for a follicle to grow from preantral size to an antral size of 8.5 mm. None the less, it can be hypothesized (Figure 2) that the total number of follicles within a size category, including atretic follicles (obtained by the number of sigmoidal lines that can be intersected by a given vertical line), increases as time of ovulation approaches. The rate at which follicles enter, grow, and become atretic accelerates

Figure 2.--Schematic diagram of large antral follicular growth and atresia during the bovine estrous cycle. The y-axis depicts relative changes in follicular size, and the x-axis depicts day of the estrous cycle. Solid lines represent growth paths of follicles and dotted lines represent atresia paths of follicles. Slopes of the solid lines depicts relative rate of follicular growth. The number of sigmoidal lines indicate relative number of follicles.


RELATIVE FOLLICULAR SIZE
0
1
2
3
4
0
3
6
9
12
15
18
21
DAY OF ESTROUS CYCLE

as ovulation approaches. This acceleration does not appear to occur in rats or mice (Richards, 1980). The process whereby selection of the follicle that finally ovulates is unknown.

3. During Postpartum Anestrus

The meager amount of information available on ovarian antral follicular growth in cattle during postpartum anestrus suggests that ovarian follicular growth increases dramatically after the first week after parturition (Saiduddin et al., 1968; Wagner and Hansel, 1969; Kesler et al., 1980), and that large antral follicles (10 mm in diameter and greater) may be present within 5 weeks prior to the first postpartum estrus (Wiltbank et al., 1964). Thus, large antral follicles are present during postpartum anestrus, but for some reason do not ovulate shortly after they appear. Since gonadotropin secretion is restored by this time, perhaps the follicles are unable to produce enough estrogen to stimulate an ovulatory surge of LH. The results of the studies cited above are based on observations of only the largest follicles present in ovaries or follicles greater than 5 mm in diameter, thus quantitative dynamics of ovarian follicle growth in cattle during postpartum anestrus remains unknown. Recently, data presented by Dufour and Roy (1983) indicates that the percent of non-atretic follicles 1.58 mm and greater does not change with time after parturition in ovaries of dairy cows.

C. Ovarian Follicular Steroidogenesis

1. Introduction

The ovaries of cattle are the major source of serum estradiol, androgens and progesterone (Kanchev et al., 1976). Consequently, serum concentrations of these steroids are a measure of follicular and ovarian function. Since concentrations of these steroids in blood during postpartum anestrus are not well characterized, understanding trends of ovarian steroid secretion into the blood during repetitive estrous cycles may help to explain follicular and ovarian function after parturition. Thus, this review will describe changes in concentrations of estradiol, androgens, and progesterone in serum during repetitive estrous cycles and then, in a later section, during postpartum anestrus in cattle.

2. During Repetitive Estrous Cycles

Serum Steroids

Estradiol. At the time of estrus in cattle, concentrations of estradiol in blood increase 2- to 6-fold above mid-luteal levels (Henricks et al., 1971; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Dobson and Dean, 1974; Smith et al., 1975; Bartol et al., 1981; Kotwica and Williams, 1982). During the remainder of the estrous cycle several researchers reported that concentrations of estradiol in blood remain relatively constant (Wettemann et al., 1972; Mason et al., 1972; Echternkamp and Hansel, 1973; Kotwica and Williams, 1982). However, several other studies report a 1.5- to 4-fold rise in concentrations of estradiol in blood between days 3 and 11 (Henricks et al., 1972; Shemesh et al., 1972; Glencross et al., 1973; Peterson et al., 1975;

Smith et al., 1975; Kanchev et al., 1976; Lukazewska and Hansel, 1980), but in only two of these seven studies (Henricks et al., 1972; Glencross et al., 1973) was this rise statistically significant. Perhaps a secondary rise in serum estradiol does not occur in all cows. This possibility is supported by findings of Echternkamp and Hansel (1973) in which only 20% of the cows exhibited two estradiol peaks during the estrous cycle.

Androgens. The few studies in which serum androgens have been measured during the bovine estrous cycle indicate that concentrations of androgens in serum vary tremendously throughout the estrous cycle but there is no consistent or predictable pattern (Shemesh and Hansel, 1974; Kanchev et al., 1976; Kanchev and Dobson, 1976; Kotwica and Williams, 1982; Wise et al., 1982). In some of these studies (Shemesh and Hansel, 1974; Kanchev and Dobson, 1976; Kotwica and Williams, 1982) increases in concentrations of peripheral testosterone and estradiol occurred coincidently. Since androgens are precursors of estrogens, changes in concentrations of androgens in serum may reflect periods of changing follicular function during the estrous cycle.

Progesterone. Concentrations of progesterone are greatest (2 to 10 ng/ml) during days 8 to 18 of the estrous cycle (Wettemann et al., 1972; Glencross et al., 1973; Kanchev et al., 1976; Schams et al., 1977; Kotwica and Williams, 1982). Between days 20 and 3, when the corpus luteum has regressed, concentrations of progesterone are very low (<1 ng/ml) in serum (Wettemann et al., 1972; Glencross et al., 1973; Kanchev et al., 1976; Schams et al., 1977; Spicer et al., 1981; Kotwica and Williams, 1982). These results indicate that the

corpus luteum is the primary source of serum progesterone, and therefore, any change in follicular secretion of progesterone during the luteal phase will most likely be overshadowed.

Follicular Fluid Steroids.--In addition to concentrations of steroids in serum, follicular steroidogenic capability may be ascertained by quantifying concentrations of steroids in fluid. There is strong evidence to indicate a high positive correlation between *in vitro* follicular cell steroid production and the concentration of steroids in follicular fluid (Channing, 1980; Hillier et al., 1981; Bieszczad et al., 1982). Since changes in concentrations of steroids in follicular fluid during postpartum anestrus have not been characterized, understanding trends in follicular fluid concentrations during repetitive estrous cycles may provide a greater understanding of ovarian follicular function during postpartum anestrus. Thus, this review will describe changes in concentrations of estradiol, androgens and progesterone in follicular fluid during repetitive estrous cycles and then, in a later section, during postpartum anestrus in cattle.

Estradiol. Lunaas (1964) was first to observe that total ovarian estradiol concentrations were low on days 7, 14 and 21 of the estrous cycle of cattle but increased nearly 10-fold during the preovulatory period on day 0. In comparison, pooled follicular fluid from several follicles within three follicular size catagories contained concentrations of estrogen that were highly variable within a given stage of the estrous cycle (Ireland et al., 1979). In addition, individual follicles collected on various days of the estrous cycle

have highly variable concentrations of estradiol in follicular fluid (with at least one follicle capable of producing high amounts of estradiol during most days of the cycle) (Bartol et al., 1981; Merz et al., 1981; Ireland and Roche, 1983a). Perhaps this highly variable production of estradiol accounts for the inconsistent pattern of concentrations of estradiol in blood previously reported during days 1 to 17 of the cycle. In contrast to most of the estrous cycle, there appears to be consistent short-term changes in concentrations of estradiol in follicular fluid during the periovulatory period. Prior to and during the preovulatory LH surge, concentrations of estradiol in follicular fluid of large follicles (>6 mm) are high and then decline after the LH surge (Ireland and Roche, 1982; 1983b; Staigmiller and England, 1982; Dieleman et al., 1983a,b) coincident with changes in concentrations of estradiol in peripheral serum.

Androgens. Little emphasis has been placed on the temporal patterns of follicular fluid concentrations of androgens during the estrous cycle, even though androgens are present in bovine follicles in nanogram per ml concentrations (Short, 1972) and serve as precursors for follicular estrogen production (Richards, 1980; Hillier, 1981). Recently, it was found that concentrations of androgens in follicular fluid did not change in large follicles (>6 mm) collected at 2-day intervals from heifers between days 3 and 13 of the estrous cycle (Ireland and Roche, 1983a), indicating that the low follicular estradiol secretion observed during most of the estrous cycle is not due to a lack of an aromatizable precursor. However, concentrations of androgens in follicular fluid increased in large antral follicles

(Dieleman et al., 1983a,b; Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982) just prior to and during the LH surge in cattle but decreased after the LH surges. This was coincident with the decline in concentrations of estradiol and the increase in concentrations of progesterone in follicular fluid of these same follicles. Whether the decrease in estradiol observed after the LH surge is due to the reduction in aromatizable androgen or a result of a shift in the steroidogenic pathway toward luteinization remains to be determined.

Progesterone. The presence of large quantities of progesterone in bovine follicular fluid has been well established (Short, 1972; Ireland et al., 1979). It is also well known that intrafollicular progesterone serves as a precursor to androgen and, subsequently, estrogen production of follicles.

The steroidogenic capability of individual bovine follicles to produce progesterone at various stages of the estrous cycle, as measured by follicular fluid progesterone concentrations, appears to increase only near the time of ovulation (Ireland and Roche, 1982, 1983a,b; Staigmiller and England, 1982; Dieleman et al., 1983a). Perhaps this increase in intrafollicular progesterone is a result of preovulatory luteinization of granulosal cells as observed in other species (Channing, 1980). Certainly the decline in intrafollicular concentrations of estradiol seen prior to ovulation (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Dieleman et al., 1983a) is not a result of declining production of progesterone.

3. During Postpartum Anestrus

Serum Steroids

Estradiol. In cattle, serum concentrations of estradiol decrease sharply at parturition reaching basal levels within 2 to 6 days (Henricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974; Sasser et al., 1979; Humphrey et al., 1983) and then increase just before the first postpartum estrus (Henricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974; Corah et al., 1974; Rawlings et al., 1980; Williams and Ray, 1980; Chang et al., 1981; Humphrey et al., 1983). This estrual increase is similar in duration and magnitude to that observed during repetitive estrous cycles. Whether ovarian follicles have this ability to secrete estrogen well before the first postpartum ovulation is unknown.

Androgens. Serum androgens have not been quantified during the postpartum anestrus period.

Progesterone. Concentrations of progesterone in serum are low (<1.0 ng/ml) at parturition due to the preparturient regression of the corpus luteum of pregnancy. Concentrations of progesterone remain low in cows until initiation of estrous cycles (Robertson, 1972; Wettemann, 1980; Rawlings et al., 1980; Humphrey et al., 1983). In 40 to 70% of cows examined, a small progesterone peak (<2 ng/ml) occurs 1 to 6 days prior to the first postpartum estrus (Robertson, 1972; Wettemann, 1980; Rawlings et al., 1980; Humphrey et al., 1983). This increase in concentration of progesterone in peripheral plasma, which precedes the first postpartum estrus, is most likely due to formation of a transitory corpus luteum (Donaldson et al., 1970) or luteinization of

some small follicles (Berardinelli et al., 1979). However, these structures are unable to maintain normal luteal phase progesterone secretion (Kesler et al., 1981). The cause of the shortened life span of these corpora lutea (or luteinized follicles) is unknown.

Follicular Fluid Steroids.--Few data are available on follicular fluid steroid concentrations collected during postpartum anestrus. In one of the two studies conducted, no difference in in vitro production of estradiol, progesterone, testosterone or androstenedione by antral follicles of various sizes (1 to 15 mm) was found between days 25 to 30 postcalving and days 10 to 16 of the estrous cycle (Weiss et al., 1981). In another study (Walters et al., 1982a), total ovarian follicular fluid content of estradiol and progesterone on day 25 postpartum was not different between weaned and suckled cows, even though the weaned cows were approaching first estrus (Walters et al., 1982c). Since there is large variation between concentrations of steroids in follicular fluid of individual follicles within a cow (Merz et al., 1981; Bellin and Ax, 1984) it is not surprising that differences in follicular fluid steroid levels were not observed by Weiss et al. (1981) or Walters et al. (1982a) who obtained data from pooled follicles. Thus, at present not enough data are available to determine how and when follicular function changes during the postpartum period. Obtaining this information is important since functional capabilities of ovaries may be limiting the return to postpartum estrous cyclicity.

D. Ovarian Responsiveness to Exogenous Gonadotropins After Parturition

Although estradiol secretion (Echternkamp, 1978; Wettemann et al., 1982) and ovulation (Casida et al., 1943; Echternkamp, 1978; Foote et al., 1966; Oxenreider, 1968; Rovira et al., 1978) can be induced with exogenous gonadotropins in lactating beef cows prior to 45 days postpartum, it appears that the gonadotropin-induced corpus luteum has a shorter lifespan in as high as 75% of the animals (Echternkamp, 1978; Foote et al., 1966; Wettemann et al., 1982; Troxel et al., 1983). Echternkamp (1978) also observed minimal increases in plasma estradiol concentrations to the gonadotropic stimulus in some cows. Thus, the data summarized above suggests that stage of follicular growth or differentiation in many postpartum cows may not be conducive to normal luteal development, even though follicles are responsive to gonadotropins. Perhaps these follicles lacked their potential maturity (i.e., did not have a complete complement of gonadotropin receptors and/or follicular cells).

E. Binding of Gonadotropins to Ovarian Follicles

The concept that the responsiveness of follicles depends not only on changes in concentrations of gonadotropins in serum but also on changes in the concentration of hormone binding sites (or receptors) in cellular membranes of follicles has gained considerable attention during the past few years. Since changes in follicular function may be associated with changes in numbers of follicular gonadotropin binding sites, this review will describe changes in the cellular concentrations of ovarian LH and FSH binding sites, first during repetitive estrous

cycles, and secondly during postpartum anestrus. Since very little work on receptors for gonadotropins in follicles has been done in cattle or sheep, research done in other mammals will be included.

1. During Repetitive Estrous Cycles

Luteinizing Hormone Receptors.--Between diestrus and proestrus the concentrations of LH receptors in granulosal cells increase in pigs (Stouffer et al., 1976) and rats (Cheng, 1976; Uilenbroek and Richards, 1979). Similarly, the concentration of LH receptors in granulosal cells from large follicles increases dramatically between onset of the preovulatory LH surge and peak of the LH surge, and subsequently declines just prior to ovulation in sheep (Webb and England, 1982a,b) and cattle (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Staigmiller et al., 1982; Walters et al., 1982c). Together these results suggest that final preovulatory maturation of ovarian follicles is associated with increased numbers of LH receptors in granulosal cells and hence increased sensitivity of granulosal cells to concentrations of LH in serum.

The concentration of LH receptors in granulosal cells removed from large follicles is low on days 3, 5, 11 and 13 of the estrous cycle in heifers (Ireland and Roche, 1983a). On day 7, however, concentrations of LH receptors in granulosal cells are 2- to 4-fold higher than on any of the other days measured. Ireland and Roche (1983a) speculated that this rise in numbers of LH receptors on day 7 is indicative of development of potential ovulatory follicles that subsequently undergo atresia after day 7.

Numbers of LH receptors in thecal tissue appear to follow similar changes, although not as dramatic, to changes observed in the granulosal cells of the same follicles in rats (Uilenbrock and Richards, 1979), ewes (Webb and England, 1982a,b) and heifers (Ireland and Roche, 1982, 1983a,b; Staigmiller et al., 1982).

Follicle-Stimulating Hormone (FSH) Receptors.--Cheng (1976) found that levels of FSH receptors (binding sites) in rat ovarian homogenates increased nearly 4-fold between diestrus and proestrus, only to decrease between proestrus and estrus, and again between metestrus and diestrus. However, the binding data in this study were expressed on a tissue weight basis and may not reflect changes per cell. Indeed, when granulosal cells were isolated from rat ovaries and binding data expressed per μg of DNA, no change in concentrations of FSH receptors was noted throughout the rat estrous cycle (Uilenbroek and Richards, 1979).

In cattle, concentrations of FSH receptors of granulosal cells removed from large follicles before the preovulatory gonadotropin surges (Ireland and Roche, 1982, 1983b; Staigmiller et al., 1982; Walters et al., 1982c) and from days 3 to 13 of the estrous cycle (Ireland and Roche, 1983a) did not change. Together these results suggest that final preovulatory maturation of ovarian follicles is not associated with a change in concentrations of FSH receptors (and hence sensitivity to FSH) in granulosal cells.

Compared with granulosal cells, concentrations of FSH receptors in thecal tissue is very low (Uilenbroek and Richards, 1979; Richards and Kersey, 1979). This low binding of FSH to thecal tissue probably

reflects contamination of the thecal component with granulosal cells (Richards, 1980).

2. During Postpartum Anestrus

Gonadotropin Receptors.--To my knowledge, there have been no longitudinally designed studies which characterize changes of either LH or FSH receptors in ovarian follicles during the postpartum anestrous period of any mammalian species. Walters et al. (1982a,b) found that numbers of LH receptors in pooled follicular homogenates were significantly higher on day 25 after parturition in weaned than in suckled cows. Since weaned cows were approaching first estrus (Walters et al., 1982c), this may indicate that receptors for LH increase in follicles prior to first postpartum ovulation. This increase in numbers of LH receptors is also observed as ovulation approaches in cyclic heifers (Ireland and Roche, 1982; Staigmiller and England, 1982; Staigmiller et al., 1982; Walters et al., 1982c). Number of follicular FSH receptors did not differ between weaned and suckled cows (Walters et al., 1982c). Similarly, there is no change in number of FSH receptors during the estrous cycle of cattle (Ireland and Roche, 1982, 1983a,b).

F. Induction of Folliculogenesis Using LHRH

1. Primates

Recently, follicular maturation, increased estradiol secretion and/or ovulation have been induced in hypogonadotropic women with primary and secondary amenorrhea (Crowley and McArthur, 1980; Leyendecker et al., 1980; Shoemaker et al., 1981; Skarin et al., 1982)

using multiple pulsatile, 1 to 20 μg dose LHRH treatments. These doses of LHRH must be administered at short intervals (60 to 120 min apart) to produce optimal gonadotropin secretion and for a period of at least 1 to 3 weeks to achieve ovulation. Studies in which less than 1 μg doses of LHRH have been given to amenorrheic women, estradiol secretion (and presumably follicular maturation) has been induced within 3 to 5 days, but these low doses failed to induce ovulation within 3 weeks (Marshall and Kelch, 1979; Reid et al., 1981; Valk et al., 1981; Marshall et al., 1983). Presumably, ovarian stimulation is due to increased pulsatile secretion of LH and FSH observed in response to pulsatile administration of a constant dose of LHRH (Marshall et al., 1983). Exactly how these changes in gonadotropin secretion alter follicular growth and steroidogenesis is unknown. None the less, these results indicate that low dose LHRH treatment may provide a useful means by which ovulation can be induced in other mammals in similar anovulatory states such as cattle during postpartum anestrus.

2. Anestrus Cows

An increase in the frequency of pulsatile LH release into the peripheral circulation has been observed just before the preovulatory surge of LH in cycling cows whose calves were weaned (Walters et al., 1982a; Faltys et al., 1983). In an attempt to mimic this increase in pulsatile LH release and thereby induce ovulation, intermittent small doses (500 ng every 2 h) of LHRH injected for 4 days to anestrous suckled (one calf) beef cows have successfully induced ovulation in 73% of cows within 11 days (Short et al., 1982; Walters et al., 1982c). Higher dose-injections of LHRH (5 μg every 2 h) administered for a

shorter period (2 days) in beef cows also induced ovulations in 4 out of 5 cows within 8 days (Riley et al., 1981). Whether this 5 µg dose of LHRH was the cause for the hastened time (8 vs. 11 days) of ovulation, as compared with the previous study in which a 500 ng dose of LHRH was used, remains to be determined. Ovulation has also been induced using multiple intermittent small doses (<2.5 µg every 2 h) for 2 to 8 days in anestrous ewes (McLeod et al., 1982a,b; Wright et al., 1984) and sows (Cox and Britt, 1982).

EXPERIMENT 1

A. Background and Objectives

Spontaneous changes in ovarian follicular growth, follicular steroid production, and follicular gonadotropin receptor concentration in cattle during postpartum anestrus remains unknown (Wettemann, 1980). Understanding these endocrine changes that occur prior to the first postpartum ovulation could eventually lead to development of manipulative techniques hastening first postpartum ovulation, thereby reducing and controlling the calving interval such that beef cattle production becomes more efficient.

Therefore, the objective of Experiment 1 was to characterize the change(s) that occur in follicular growth, concentrations of follicular fluid steroids, gonadotropin receptor concentrations, and coincident secretion of various pituitary hormones prior to the first postpartum ovulation in suckled beef cows.

B. Materials and Methods

1. Animals and Design

Thirty-two beef heifers (primarily Hereford x Angus) were bred by artificial insemination between September 22 and October 16 after estrus was synchronized with prostaglandin $F_{2\alpha}$. At the time of parturition each primiparous cow (400 to 600 kg body weight) was assigned randomly to one of five groups (6 or 8 cows/group) to be slaughtered between July 18 and September 10, on either day 7, 14,

28, 42 or 56 after parturition. Each cow was suckled by one calf until time of slaughter. All cattle were "halter broken" by placing halters on cows daily for at least 1 week prior to slaughter. In addition, all cows were acclimated to the bleeding pen at least 4 days before blood collection. Neither mean birth weights of the calves (27 kg) nor mean gestation lengths (278 days) were different among groups (P>.10, Table 1). Prior to calving and up to time of slaughter, cows were housed in a barn with one open side, assigned to one of six straw-bedded pens, and fed 10 kg dry matter of a supplemented 84:16 corn silage:high moisture corn ration (69% TDN and 11% protein).

2. Serum, Follicular Fluid and Tissue Collection Procedures

Two days before the designated date of slaughter, each animal was fitted with a jugular cannula. Between 0800 and 1400 h 1 day

TABLE 1.--Calf Weight and Gestation Length of Animals in Experiment 1.

Variable			
Day after parturition slaughtered	N	Calf weight at birth (kg)	Gestation length (days)
7.0±.0[a]	6	26.8±1.4	277±2
14.3±.2	6	26.0±1.7	279±2
28.2±.2	6	24.1±0.4	277±1
42.1±.6	8	27.3±1.5	280±1
56.0±.6	6	29.1±1.8	275±1

[a]Means ± SEM.

later, blood samples were collected at 10-min intervals for determination of LH, FSH, PRL, cortisol, progesterone and estradiol concentrations in serum. During this period of sampling blood, cows were loosely restrained with halters and calves were present with the cows. Also, each suckling event was recorded during the 6-h period of blood collection. A wall with a two-way mirror separated the cow and calf from the person taking blood samples. Starting at 21 days after parturition, blood samples were collected via jugular venipuncture every 3 days until time of slaughter. These samples were assayed for progesterone and used to approximate time of first ovulation in each cow.

After each period of collection, whole blood was stored at 4°C for 2 to 4 h. Blood was incubated at 37°C for 2 h and then stored at 4°C for an additional 20 to 24 h to allow blood to clot. Blood samples were centrifuged at 1500 x g at 4°C for 15 min; sera were decanted and frozen at -20°C until radioimmunoassays were conducted.

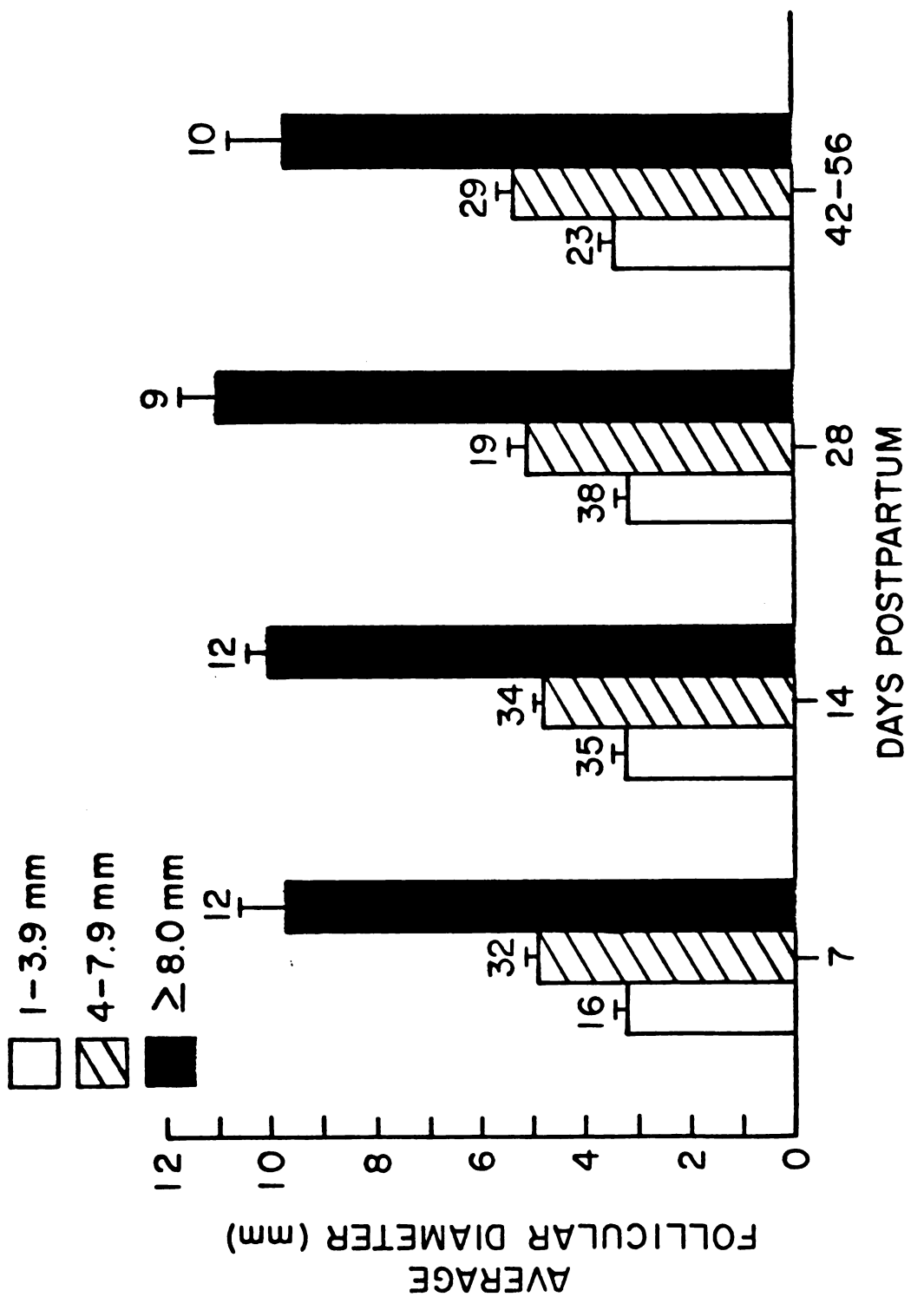
Animals were slaughtered between 0700 and 0900 h the day after collection of blood. Within 30 min of slaughter, ovaries and uteri were removed. Total uterine weights, uterine horn diameters and cervical diameters were recorded. Excised ovaries were weighed and placed on ice in phosphate buffered saline (PBS, pH = 7.4). The number and size of all follicles one mm or greater in diameter on the surface of each ovary were recorded. Ten to 15 individual follicles from each pair of ovaries were then dissected free of ovarian stromal tissue, weighed, diameters determined with a vernier caliper, and follicular fluid removed and volumes recorded. Care was taken so that the two

largest follicles including all follicles 8 mm or larger in diameter were removed from each pair of ovaries. In addition, several follicles 1.0 to 7.9 mm in diameter were randomly selected and removed. The volume of follicular fluid was estimated by aspirating the fluid into a 0.1 ml-graduated 1-ml syringe for follicles greater than 7 mm in diameter and into 1.0 µl-graduated Hamilton syringes for follicles 7 mm or less in diameter. All dissected follicles were classified into three arbitrary sizes: small (1.0 to 3.9 mm), medium (4.0 to 7.9 mm), and large ($\geq$8.0 mm). Mean size and number of follicles dissected from each pair of ovaries in each size category was similar across days (Figure 3). Corpora lutea of previous pregnancy and any newly formed corpora lutea were also dissected free of ovarian stromal tissue and weighed. Granulosal and thecal layers were removed and separated via blunt dissection from follicles 8 mm and greater in diameter, quickly frozen and stored in PBS-20% glycerol (V/V) at -70°C until used. Whole follicles less than 8 mm were diced and frozen as above. All follicular tissue was frozen within 3 to 5 h after ovaries were removed from the cows.

3. Radioimmunoassay of Serum Hormones

Protein Hormones.--Concentrations of LH, FSH and PRL in serum were measured by double-antibody radioimmunoassays (Convey et al., 1976; Carruthers et al., 1980; Koprowski and Tucker, 1971; respectively).

Figure 3.--Average diameter and number of follicles (shown above each bar) in each size category dissected from ovaries on various days after parturition. Small = 1-3.9 mm; medium = 4-7.9 mm; large = ≥8.0 mm.


AVERAGE
FOLLICULAR DIAMETER (mm)
0
2
4
6
8
10
12
1-3.9 mm
4-7.9 mm
≥8.0 mm
12
32
16
7
12
34
35
14
9
19
38
28
6
29
23
42-56
10
DAYS POSTPARTUM

Steroid Hormones.--Selected samples were analyzed for concentrations of serum progesterone using a dextran-charcoal radioimmunoassay (Convey et al., 1977) and serum cortisol by a newly validated assay (Purchas et al., 1984). Concentrations of estradiol in serum were quantified using methods of Carruthers and Hafs (1980). Concentrations of LH, PRL, estradiol, cortisol and progesterone in serum of these cows were determined in a cooperative effort with Leung (1984).

4. Radioimmunoassay of Follicular Fluid Hormones

Concentrations of estradiol, progesterone and androstenedione were measured in follicular fluid using dextran-charcoal radioimmunoassays as previously described (Ireland and Roche, 1982). A brief description of the assay for each steroid is given in Table 2. Cross-reactions of other steroids in the estradiol, progesterone and androstenedione assays have been previously reported (Oxender et al., 1977; Convey et al., 1977; Mongkonpunya et al., 1975, respectively). In addition, a pool of follicular fluid at 1:1 or 1:50 dilution (diluted in PBS-.1% gelatin[1]) was used to demonstrate parallelism for each assay (Figure 4a,b,c). In all cases parallelism between standards and unknowns was verified according to Rodbard (1974).

Concentrations of PRL in follicular fluid were determined as described for serum (Koprowski and Tucker, 1971). A brief description of the assay for PRL is given in Table 3. In addition, a pool of

[1]Knox Gelatine Inc., Johnstown, NY.

TABLE 2.--Description of Each Steroid Radioimmunoassay Used for Follicular Fluid Samples.

Criterion	Description		
	Estradiol assay	Progesterone assay	Androstenedione assay
Antisera	Rabbit-MSU #74[1]	Rabbit-MSU #74[2]	Rabbit-GDN #866[3]
Antisera dilution (200 µl)	1:40,000	1:2,000	1:2,000
CPM ^{3}H-steroid	5,000	10,000	5,000
% Total ^{3}H-steroid bound	34-45	33-50	35-50
% Non-specific binding	18-20	2-4	3-4
Sensitivity[4]	4.0 pg	40.0 pg	30.0 pg
Coefficient of variation (%, n=14)			
Inter-assay	20.3	11.2	10.5
Intra-assay	12.9	7.2	9.8
Volume of sample (µl)			
1:50	10-50	50	100
1:100	20-100	20-100	---
Recovery (%)[5]	100	100	100

[1]Rabbit antiserum against estradiol-6-oxim-human serum albumin.

[2]Rabbit antiserum against progesterone-20-oxim-human serum albumin.

[3]Rabbit antiserum against androstenedione supplied by Dr. G. D. Niswender, Colorado State University.

[4]Sensitivity defined as the amount of hormone required to displace ^{3}H-Hormone binding equivalent to two S.D. from zero.

[5]No extractions = 100%.

Figure 4.--A. Inhibition of ^{3}H-estradiol binding by estradiol and unextracted bovine follicular fluid (1:50).
B. Inhibition of ^{3}H-progesterone binding by progesterone and unextracted bovine follicular fluid (1:50).
C. Inhibition of ^{3}H-androstenedione binding by androstenedione and unextracted bovine follicular fluid (1:50 and 1:1). In all cases, the radioactivity is expressed as a percentage of the ^{3}H-steroid bound to the antibody in control tubes containing buffer and is the mean of two or three observations.

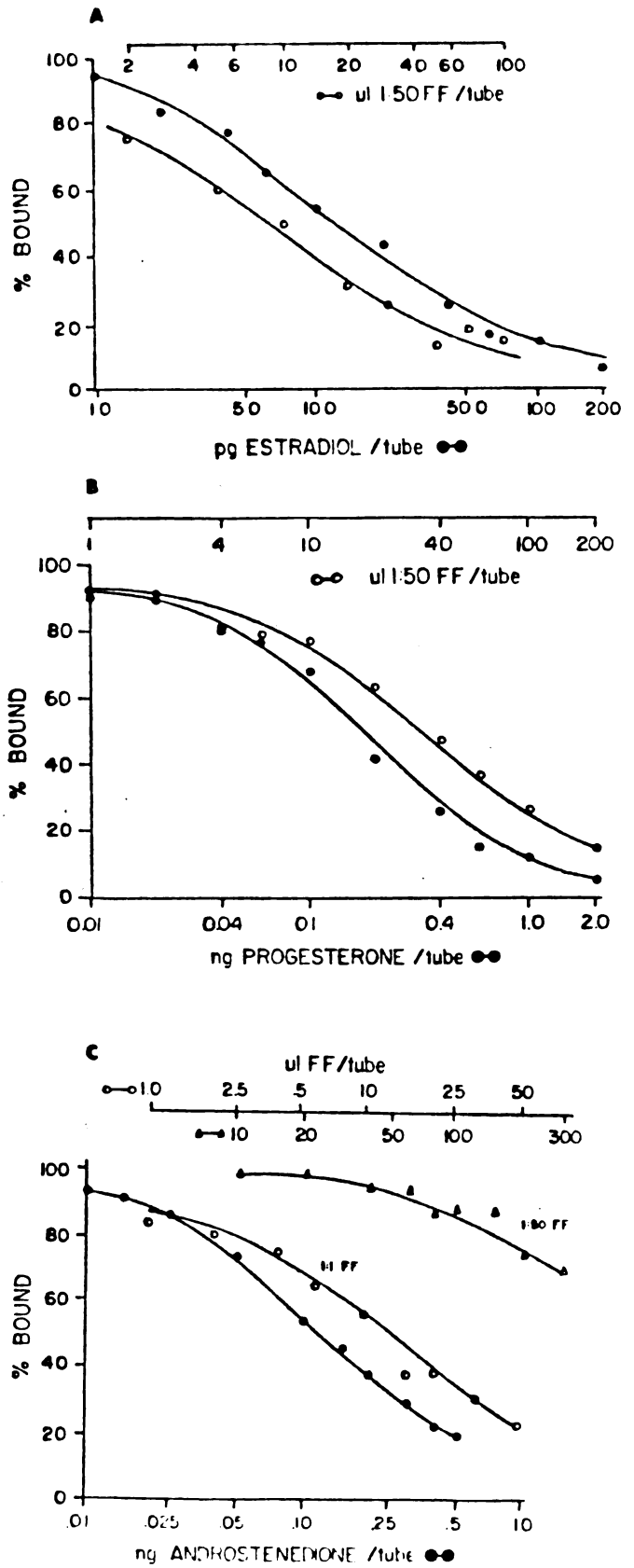

A
2 4 6 10 20 40 60 100
ul 1:50 FF /tube
100
80
60
40
20
0
% BOUND
10 50 100 500 100 200
pg ESTRADIOL /tube
B
1 4 10 40 100 200
ul 1:50 FF /tube
100
80
60
40
20
0
% BOUND
0.01 0.04 01 0.4 1.0 2.0
ng PROGESTERONE /tube
C
ul FF/tube
1.0 2.5 .5 10 25 50
10 20 50 100 300
100
80
60
40
20
0
% BOUND
.01 .025 .05 .10 .25 .5 10
ng ANDROSTENEDIONE /tube

TABLE 3.--Description of Radioimmunoassay Used for Quantifying Prolactin (PRL) in Follicular Fluid.

Criterion		Description
Antisera	1st:	Guinea pig anti-bovine PRL
	2nd:	Sheep anti-guinea pig gamma globulin
Antisera dilution	1st:	1:30,000
	2nd:	1:5
Hormone for iodination		NIH-PRL-B4
CPM ^{125}I-PRL added		20,000
% Total ^{125}I-PRL bound		38.1
% Non-specific binding		1.4
Sensitivity		0.8 ng
Coefficient of variation Intra-assay (%)		8.8
Volume of sample (undiluted, µl)		20 to 100

undiluted follicular fluid was used to demonstrate parallelism with serum and NIH-PRL-B4 (Figure 5).

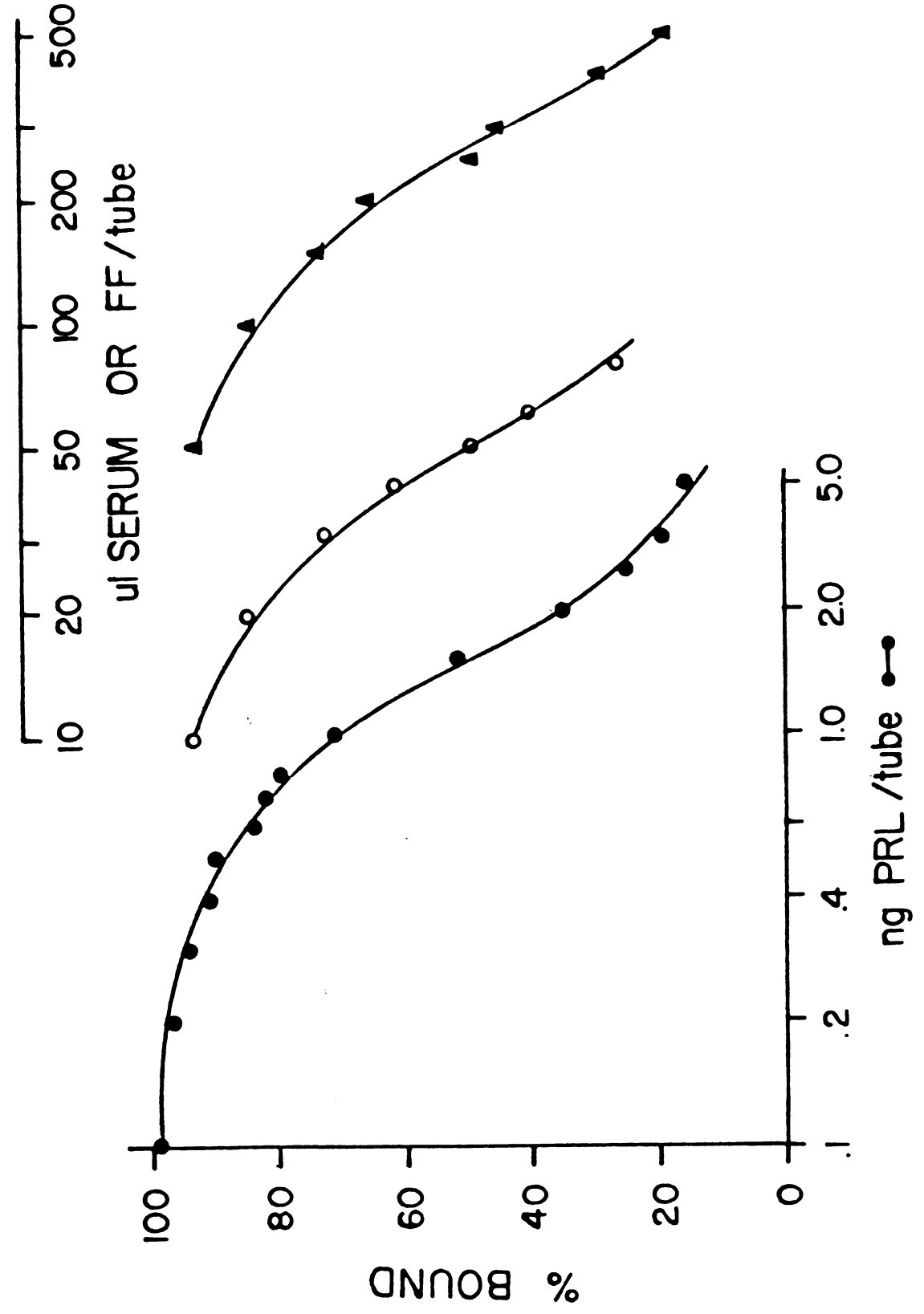
5. Binding Assays of Gonadotropins in Follicular Tissue

Iodination Procedure.--Five µg of highly purified hCG (CR-119, 11,600 IU/mg) and 10 µg of ovine FSH (Siaram-oFSH-s1390; 110X NIH-FSH-S10) were radioiodinated using chloramine-T methods previously described (Spicer et al., 1981; Tonetta et al., 1984). Determinations of specific activity and maximum bindability are described elsewhere (Spicer et al., 1981; Ireland and Roche, 1982; Tonetta et al., 1984) but were routinely 20 to 50 cpm/pg and 15 to 40%, respectively.

Validation of Binding Assays

Tissue preparation. For validation of the binding assays, a pool of ovarian follicles from non-pregnant cattle was used. Fresh ovaries were collected at a local slaughter house and stored on ice until individual follicles 4 mm in diameter and greater were dissected from the ovaries, as much excess stromal tissue as possible was removed, diameters recorded and fluid removed. Follicles were then homogenized for 5 to 10 sec in an automatic blender (Waring Products Corp., NY) at top speed. Ice cold PBS-20% glycerol (V/V, PBSG) was added prior to homogenization at approximately twice the volume of tissue. The homogenate was then filtered through two layers of cheesecloth and rinsed two times in PBSG. After each rinse, the homogenate was centrifuged at 2200 x g for 10 min at 4°C. The final precipitated homogenate was resuspended in PBSG, dispensed in 2 ml

Figure 5.--Inhibition of ^{125}I-bPRL by bPRL (●-●), bovine serum (○-○) and bovine follicular fluid (▲-▲). The radioactivity is expressed as a percentage of the ^{125}I-bPRL bound to the antibody in control tubes containing buffer and is the mean of two or three observations.


% BOUND
100
80
60
40
20
0
.1
.2
.4
1.0
2.0
5.0
ng PRL /tube
10
20
50
100
200
500
ul SERUM OR FF/tube

aliquots, frozen, and stored at -70°C. The filtered homogenate was thawed on day of use and rinsed three times with 1 ml of PBS (pH 7.4) as above. Unless stated otherwise, the follicular homogenate was used at a final concentration of 1 mg wet weight/5 µl PBS.

General binding assay procedure. Binding assays were conducted in polypropylene tubes (12x75 mm) precoated with PBS-5% BSA to reduce nonspecific absorption of ^{125}I-labeled hormone to tubes during incubation. For all validation assays, aliquots of 5 to 20 mg of follicular homogenate (4.7±.5 µg DNA/mg and 19.7±1.0 µg protein/mg) were incubated with various amounts of ^{125}I-labeled hormones per tube in a shaker at 25°C for 24 h (interval determined from time-study below) in the presence or absence of unlabeled bovine LH (1000-fold excess; 20 µg NIH-LH-B10) for ^{125}I-hCG or ovine FSH (100-fold excess; 10 µg Abbott oFSH-S90390) for ^{125}I-oFSH. The final incubation volume was 250 µl unless stated otherwise. At the end of the incubation, 2 ml cold PBS was added to each tube and the contents were centrifuged at 2200 x g for 15 min at 4°C. The supernatant was discarded, the pellet resuspended in 2 ml cold PBS and the contents recentrifuged as above. The supernatant was again discarded, and the precipitate counted in an automated gamma counter (counting efficiency 84%).

Time-temperature dependence. To determine the interval required for binding to reach equilibrium, bovine follicular homogenates were incubated with ^{125}I-oFSH at 25°C or 4°C for times ranging from 1 h to 30 h. Two concentrations of ^{125}I-oFSH (20,000 cpm/tube or 200,000 cpm/tube) were used which represented the range of ^{125}I-labeled hormone used in Scatchard analysis. The specific

binding of ^{125}I-oFSH to follicular tissue was time- and temperature-dependent (Figure 6). Equilibrium was obtained at 20 h with both amounts of ^{125}I-hCG and ^{125}I-oFSH at 25°C. Specific binding was low and inconsistent between 4 and 30 h at 4°C. Similarly, equilibrium of binding of ^{125}I-hCG to bovine follicular homogenates was obtained after 24 h of incubation at 25°C (Ireland and Roche, 1982).

Saturation analysis. The amount of ^{125}I-labeled hormones needed to saturate all gonadotropin binding sites in 10 mg (19.7±1.0 µg protein/mg) of follicular homogenate (average amount of tissue used in experiments) were determined by incubating this tissue with increasing amounts of ^{125}I-labeled hormone (20,000 to 600,000 cpm/tube) in the presence or absence of excess unlabeled hormones. Saturation of presumed LH binding sites by ^{125}I-hCG is shown in Figure 7A and saturation of presumed FSH binding sites by ^{125}I-oFSH is shown in Figure 7B. These data were used to construct Scatchard plots (Scatchard, 1949) to estimate equilibrium association constants (K_a) and numbers of unoccupied hormone binding sites (R_o) present in follicular homogenates (Figure 8).

Specific binding of ^{125}I-labeled hormones to follicular homogenates were saturable phenomena (Figure 7). Scatchard analysis of these data using 10 mg of tissue (19.7±1.0 µg protein/mg) revealed that hormone binding (Figure 8) was of high affinity (K_a = 4.40±0.18 X 10^{10} M^{-1} for hCG and K_a = 4.48±.21 X 10^{10} M^{-1} for oFSH) and low capacity (R_o = 4.14 X 10^{-12} M for hCG and R_o = 6.43 X 10^{-12} M for

Figure 6.--Specific binding of ^{125}I-oFSH (250,000 cpm) to bovine follicular homogenates (5 mg) as a function of time at either 25°C (●-●) or 4°C (0-0). Each point is the mean of two observations.

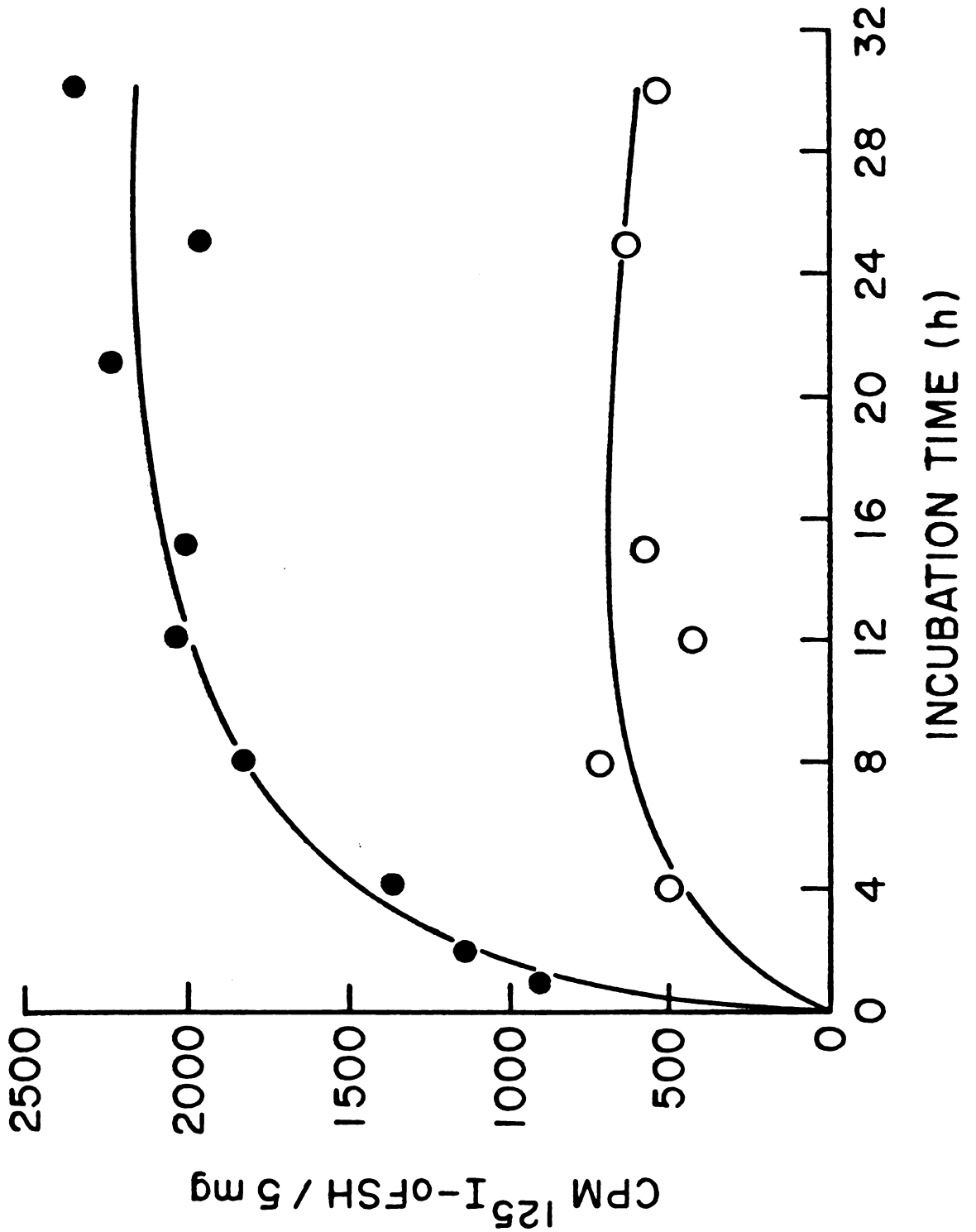

CPM ^{125}I-oFSH / 5 mg
2500
2000
1500
1000
500
0
0
4
8
12
16
20
24
28
32
INCUBATION TIME (h)

Figure 7.--Specific binding of ^{125}I-hCG (A) and ^{125}I-oFSH (B) to two different amounts of bovine follicular homogenates as a function of ^{125}I-labeled gonadotropin concentration. Each point is the mean of two observations.

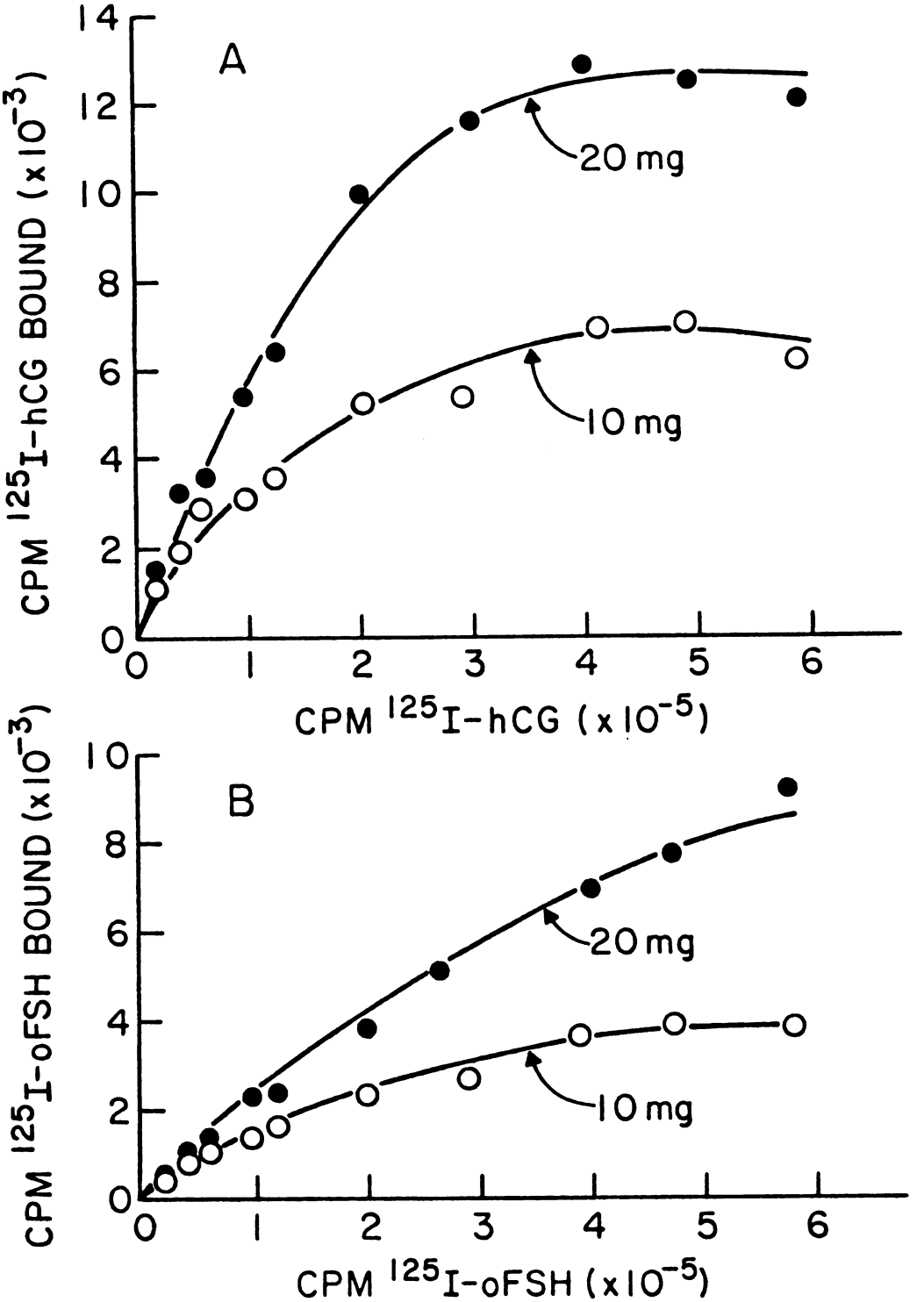
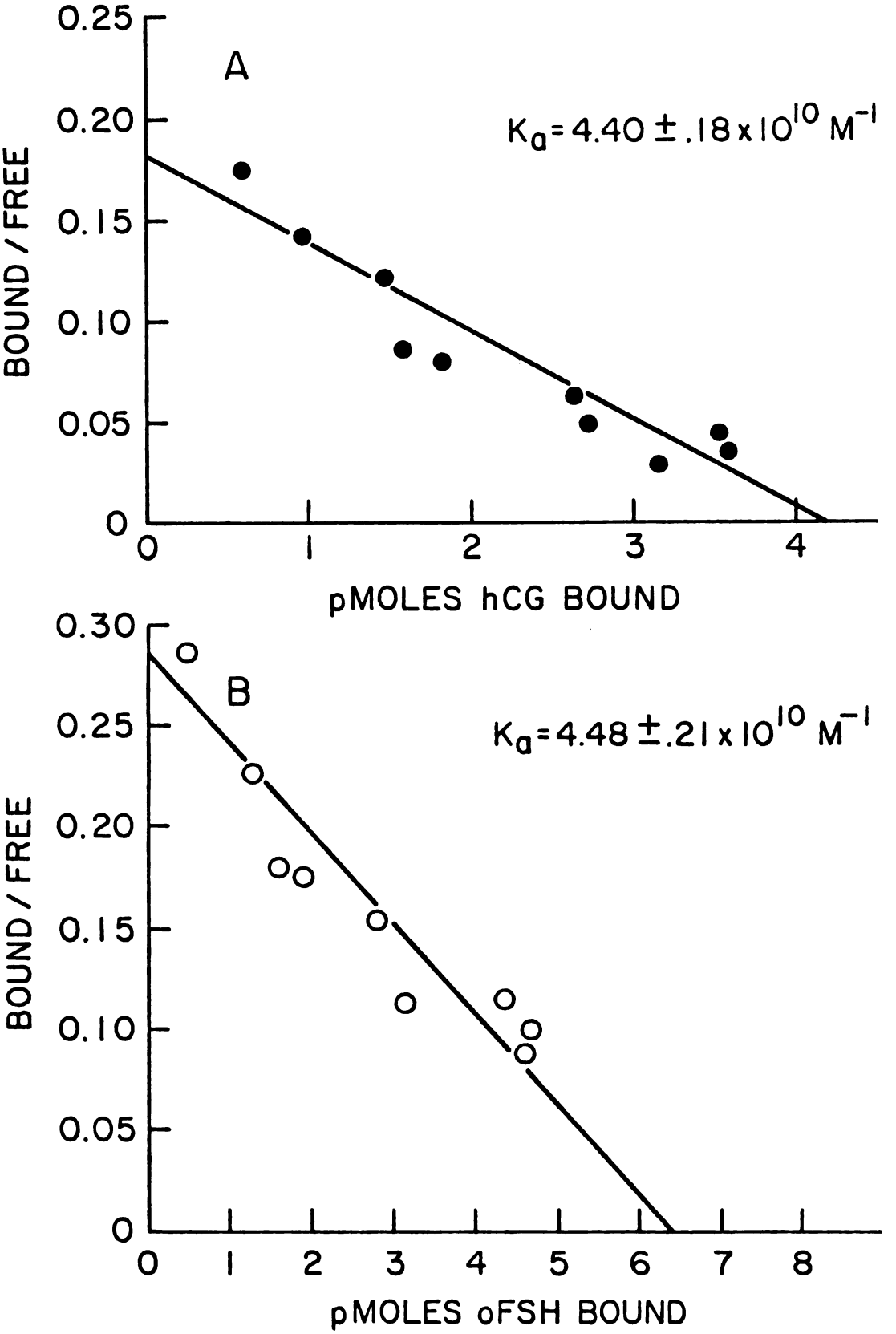

A
CPM ^{125}I-hCG BOUND ($\times 10^{-3}$)
CPM ^{125}I-hCG ($\times 10^{-5}$)
20 mg
10 mg
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
B
CPM ^{125}I-oFSH BOUND ($\times 10^{-3}$)
CPM ^{125}I-oFSH ($\times 10^{-5}$)
20 mg
10 mg
0
2
4
6
8
10
0
1
2
3
4
5
6

Figure 8.--Estimation of the number of hCG/LH (A) and FSH (B) receptor sites and association constants in 10 mg of bovine follicular homogenate. The follicular homogenates were incubated with ^{125}I-labeled gonadotropins for 24 h at 25°C and were subjected to Scatchard analysis.

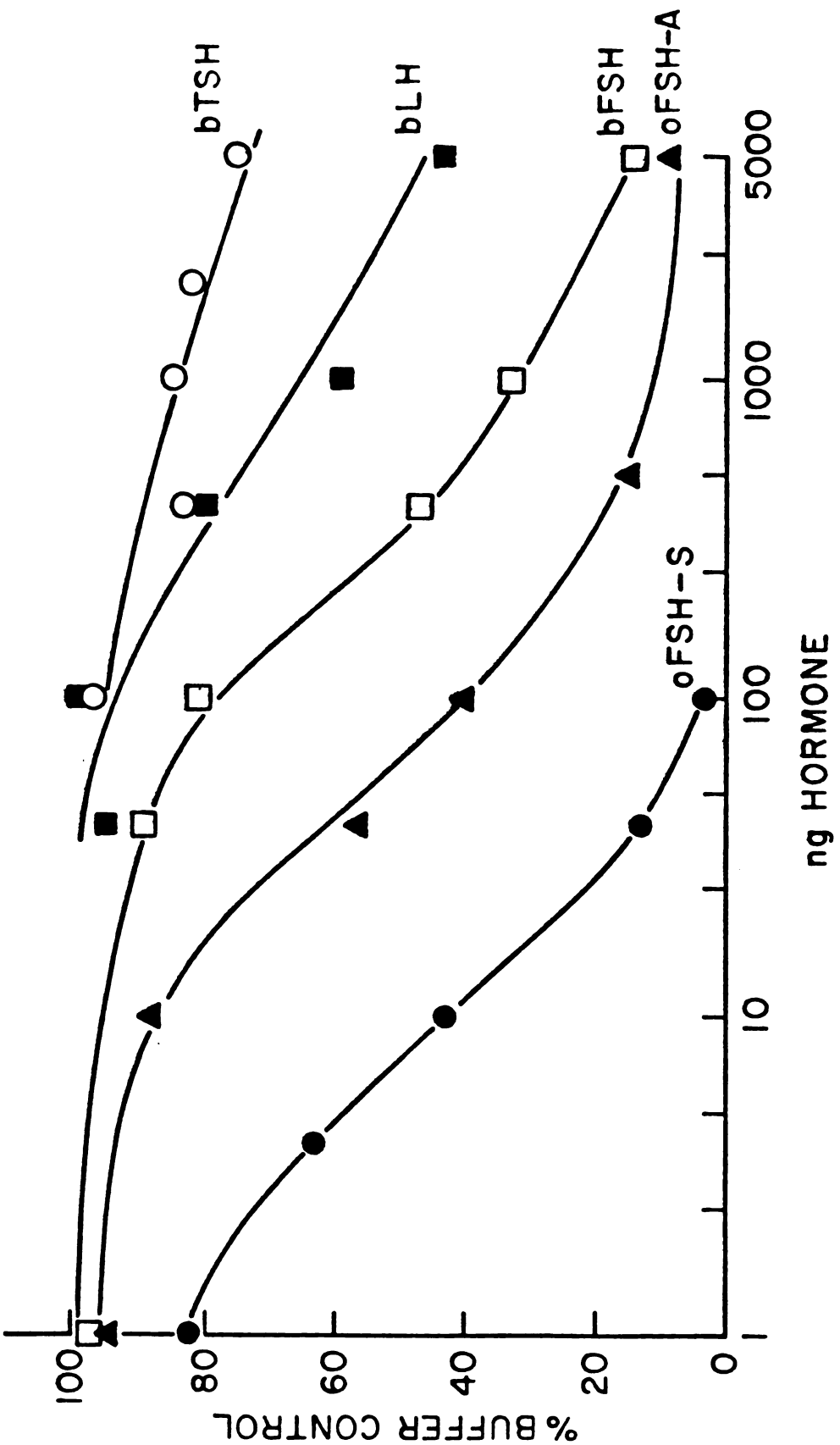

A
$K_a = 4.40 \pm .18 \times 10^{10}\ M^{-1}$
BOUND / FREE
0.25
0.20
0.15
0.10
0.05
0
0
1
2
3
4
pMOLES hCG BOUND
B
$K_a = 4.48 \pm .21 \times 10^{10}\ M^{-1}$
BOUND / FREE
0.30
0.25
0.20
0.15
0.10
0.05
0
0
1
2
3
4
5
6
7
8
pMOLES oFSH BOUND

oFSH). The bound/free ratios of labeled hormone were corrected for maximum bindability.

Hormone specificity. Hormone specificity of ^{125}I-hCG binding to bovine follicular homogenates has been reported previously (Ireland and Roche, 1982). Specificity of ^{125}I-oFSH binding was determined by increasing amounts of various non-radioactive hormones (NIH-LH-B10, NIH-FSH-B1, Siaram-oFSH-S1390, Abbott-oFSH-S90390; NIH-TSH-B6) with a constant amount of radiolabeled oFSH (250,000 cpm/tube) and follicular homogenates (10 mg/tube). The hormone specificity of ^{125}I-oFSH binding to follicular homogenates is shown in Figure 9; bovine FSH (NIH-FSH-B1) completely inhibited binding of the ^{125}I-oFSH, but the efficacy was not as high as it was for unlabeled oFSH. There was slight competition between oFSH and bovine LH or TSH.

Binding Assays for Experimental Tissues.--Specific binding of ^{125}I-hCG and ^{125}I-oFSH was quantified in granulosal cells and thecal homogenates of follicles 8 mm or greater. LH/hCG and FSH binding was measured in homogenates of whole follicles 1 to 7.9 mm in diameter. On the day of the binding assay, granulosal cells from follicles 8 mm or greater were thawed and washed twice in PBS, centrifuging at 1500 x g for 10 min after each wash. The theca from follicles 8 mm and greater and whole follicles 4.0 to 7.9 mm in diameter were thawed and homogenized separately over ice using a Teflon to glass homogenizer. For follicles 8 mm and greater, each thecal homogenate was filtered through two layers of cheesecloth and rinsed twice in PBS, centrifuging at 1500 x g for 10 min after each wash. Homogenates of whole follicles

Figure 9.--Hormonal specificity of binding of ^{125}I-oFSH to bovine follicular homogenates (10 mg). Competition for binding of ^{125}I-oFSH to follicular homogenates with increasing amounts of different unlabeled hormones added. Approximately 250,000 cpm of ^{125}I-oFSH were added per tube. bTSH = bovine thyroid-stimulating hormone (NIH-TSH-B6); bLH = bovine luteinizing hormone (NIH-LH-B10); bFSH = bovine follicle-stimulating hormone (NIH-FSH-B1); oFSH-A = Abbott ovine FSH-S90390; oFSH-S = ovine FSH-Siaram-S1390. Each point is the mean of two observations.


bTSH
bLH
bFSH
oFSH-A
oFSH-S
% BUFFER CONTROL
100
80
60
40
20
0
0
10
100
1000
5000
ng HORMONE

4.0 to 7.9 mm in diameter were not filtered but rinsed twice in PBS, centrifuging at 1500 x g for 10 min after each wash. Whole follicles 1.0 to 3.9 mm in diameter from each cow were pooled, homogenized and prepared as described for 4.0 to 7.9 mm follicles. Final pellets of granulosal cells, thecal or whole follicular homogenates were resuspended in PBS to a concentration of 1 mg per 10 µl PBS. Amounts of DNA in each preparation was quantified by the method of Burton (1956). Inter- and intra-assay coefficients of variation for DNA assays were 9.3% and 4.1%, respectively.

To determine specific binding of ^{125}I-labeled hormone in granulosal, thecal and follicular homogenates, 30- to 150-µl aliquots of each preparation (3 to 15 mg homogenate) were incubated with saturating amounts of ^{125}I-hCG or ^{125}I-oFSH (~400,000 cpm/tube) in a shaker at 25°C for 24 h in the presence or absence of excess unlabeled bovine LH or ovine FSH (40 µg, NIH-LH-B10 or 20 µg Abbott-oFSH-S90390). The final incubation volume was 250 µl. At the end of the incubation period, samples were rinsed twice with 2 ml PBS and the amount of specific binding of each ^{125}I-labeled gonadotropin was quantified as described in assay validation. Intra-assay coefficient of variation averaged 5.2±1.2% for ^{125}I-hCG and 5.6±1.9% for ^{125}I-oFSH. Inter-assay coefficient of variation determined from four assays were 14.6% for ^{125}I-hCG and 13.1% for ^{125}I-oFSH.

6. Statistical Analyses

Concentrations of LH, FSH and PRL in serum of individual animals were evaluated by an objective method to determine variables

of hormone secretion (frequency of secretory pulses, height, overall mean, baseline and amplitude) using the following criteria: 1) A pulse was defined as any value that exceeded an adjacent value by one 95% confidence interval of pooled control serum values. The concentration of gonadotropins in the pooled serum was within the range of values found in the experimental samples. 2) Pulse height was the maximal concentration within a detected pulse. 3) Overall mean was the mean of all samples taken. 4) Baseline was defined as the mean of all samples that were equal to nadirs ± assay sensitivity. 5) Amplitude was equal to pulse height minus baseline.

A one-way analysis of variance with "days after parturition" as the main effect was used to test the effect of time after parturition on uterine measurements and weights of ovaries and corpora lutea in both acyclic and cyclic cows. Follicular size and numbers, serum hormones, follicular fluid hormones, and follicular gonadotropin binding were analyzed in a similar fashion except that all data from cyclic cows were removed from analysis. Follicular fluid steroid and gonadotropin binding data from the three follicular size categories were analyzed separately within each size category. Mean differences among means were determined using Bonferroni's t test (Gill, 1978) and Fisher's protected LSD mean test (Ott, 1977) where appropriate. In addition, Chi square analysis was used to test the proportions of large follicles appearing on the ovary opposite the corpus luteum of pregnancy in each group of cows. Any data showing heterogeneous variance were analyzed after using the transformation natural log (X+1).

C. Results

1. Ovarian and Uterine Weights

Right and left ovarian weights were not different from each other ($P>.10$) and did not change from days 7 to 42 after parturition (Table 4). However, ovarian weights increased ($P<.05$) between days 42 and 56, coinciding with an increase in weight and number of newly formed corpora lutea (Table 4). If weight of luteal structures were subtracted from total ovarian weight, no significant change after parturition was noted (Table 4).

No cows had initiated estrous cyclicity (as determined by presence of newly formed corpora lutea and increased concentration of progesterone in serum) at days 7 or 14 after parturition. However, the percentages of cows that had initiated estrous cycles increased to 83.0% by day 56 (Table 4). Therefore, data from anovulatory cows on days 42 and 56 were combined, and will subsequently be identified and presented as a group days 42-56 (n=4). Data from cyclic cows were not included in analysis of variance.

Uterine involution was complete by day 28, since diameters of both the pregnant and nonpregnant horns, and total uterine weight did not decline further after day 28 ($P>.10$, Figure 10). Significant declines ($P<.01$) in uterine horn diameter and uterine weight occurred between days 7 and 14, and again between days 14 and 28. Coincident with the declines in uterine size between days 7 and 28 were significant declines ($P<.01$) in weight of the regressing corpora lutea of pregnancy and diameter of the cervix (Figure 10).

TABLE 4.--Ovarian and Corpora Lutea (CL) Weights in Both Acyclic and Cyclic Cows from 7 to 56 Days After Parturition.[a]

Days after parturition	N	Weight (g) Right ovary	Left ovary	Total ovarian weight[e]	New CL[b]	(n)	% Cycling
7	6	4.5±.9[c]	4.7± .7[c]	8.3±1.0	--	0	0.0
14	6	4.4±.5[c]	4.3± .3[c]	8.1±0.6	--	0	0.0
28	6	4.5±.5[c]	4.5± .3[c]	8.2±0.7	1.4	1	16.7
42	8	4.8±.4[c]	4.9±1.6[c]	8.7±0.5	1.6±.1	5	62.5
56	6	6.4±.9[c]	8.0± .9[d]	11.2±1.0	3.4±.7	5	83.3

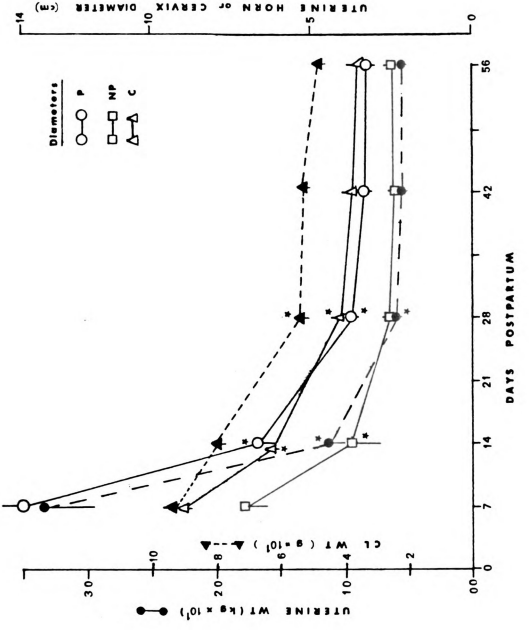
[a]Values are means ± SEM; N = total number of cows; n = number of cows with newly formed CL.

[b]Five of eleven ovulations occurred opposite the gravid uterine horn.

[c,d]Means with different superscripts differ ($P<.05$).

[e]Weight of all luteal structures subtracted from total ovarian weights.

Figure 10.--Weights of corpora lutea of pregnancy and uteri, and diameter of previously pregnant (P) and previously non-pregnant (NP) uterine horns, and cervix (C) from 7 to 56 days after parturition in acyclic and cyclic cows. Values are means ± SEM of 6 or 8 cows. Means marked with an asterisk differ ($P<.01$) from previous mean.


UTERINE WT (kg x 10¹)
CL WT (g x 10¹)
UTERINE HORN OF CERVIX DIAMETER (cm)
DAYS POSTPARTUM
Diameters
P
NP
C

2. Follicular Growth

Numbers of small (1.0 to 3.9 mm) and large (≥8.0 mm) follicles on the surface of each pair of ovaries did not change from days 7 to 42-56 ($P>.05$; Figure 11). However, numbers of medium follicles (4.0 to 7.9 mm) increased ($P<.05$) from 4.7±.9 follicles per pair of ovaries on day 7 to 8.2±1.4 on day 14 after parturition (Figure 11). A further increase (2.2-fold) in number of medium follicles was observed between days 28 and 42-56 ($P<.01$). This resulted in a 2-fold increase in total number of follicles per pair of ovaries from days 7 to 42-56 (21 vs 42 follicles per pair of ovaries; Table 9).

Diameters of the largest and second largest follicles were unchanged between days 7 and 42-56 in acyclic cows (Table 5). Diameters of these follicles were similar to those in cycling cows on days 42 and 56. In addition, in acyclic cows there was a higher proportion of largest follicles on the ovary opposite the ovary containing the previous corpus luteum (CL) of pregnancy ($P<.05$; Table 5). Ten of twelve follicles with concentrations of estradiol greater than progesterone were also opposite to the previous CL of pregnancy ($P<.05$; data not shown). However, the proportion of ovaries which contained the CL of pregnancy and second largest follicle was not different ($P>.05$) than the proportion of ovaries containing the second largest follicle which were opposite the CL. In 5 of 11 cycling cows (45%), the first postpartum ovulation occurred on the ovary opposite the ovary with the previous CL of pregnancy which was not different from the expected proportion of ovulations in the right and left ovaries ($P>.10$).

Figure 11.--Numbers of various sized follicles per pair of ovaries in acyclic cows from days 7 to 42-56 after parturition. Values are means ± SEM. Numbers in parenthesis are number of cows.

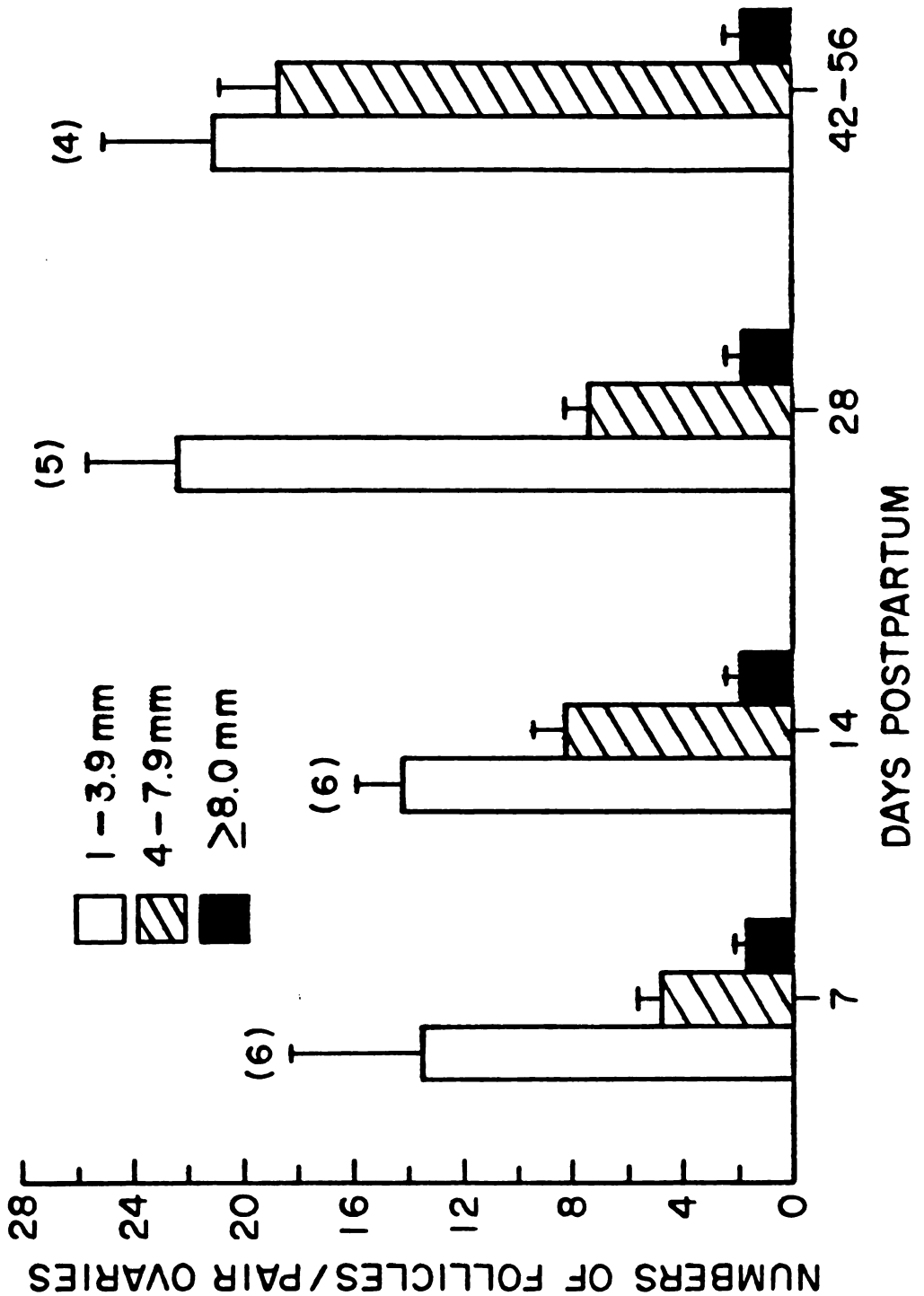

NUMBERS OF FOLLICLES/PAIR OVARIES
28
24
20
16
12
8
4
0
(6)
(6)
(5)
(4)
1–3.9mm
4–7.9mm
≥8.0mm
7
14
28
42–56
DAYS POSTPARTUM

TABLE 5.--Diameters of First and Second Largest Follicles, and the Proportions of the First and Second Largest Follicles Opposite Ovaries Containing the Previous Corpora Lutea (CL) of Pregnancy in Acyclic Cows.[a]

Days after parturition	N	Follicular Diameter (mm)		% Largest opposite CL	% Second largest opposite CL
		Largest	Second largest		
7	6	10.2±.9	8.0± .6	66.7	33.3
14	6	10.9±.5	7.6± .7	83.3	50.0
28	5	11.6±.4	7.0±1.1	100.0	60.0
42-56	4	10.6±.9	9.4±1.2	75.0	50.0
Overall $\bar{X}$		10.8±.3	8.0± .5	81.0[b]	47.6

[a]Means ± SEM.

[b]$P<.05$ as determined by Chi-square analysis.

Follicular diameter and follicular fluid volume were significantly correlated (r=.875, P<.001) with each other (Figure 12A). However, logarithmic transformation (log 10) of both diameter and volume resulted in a better linear relationship (r=.983, P<.001, Figure 12B).

3. Suckling

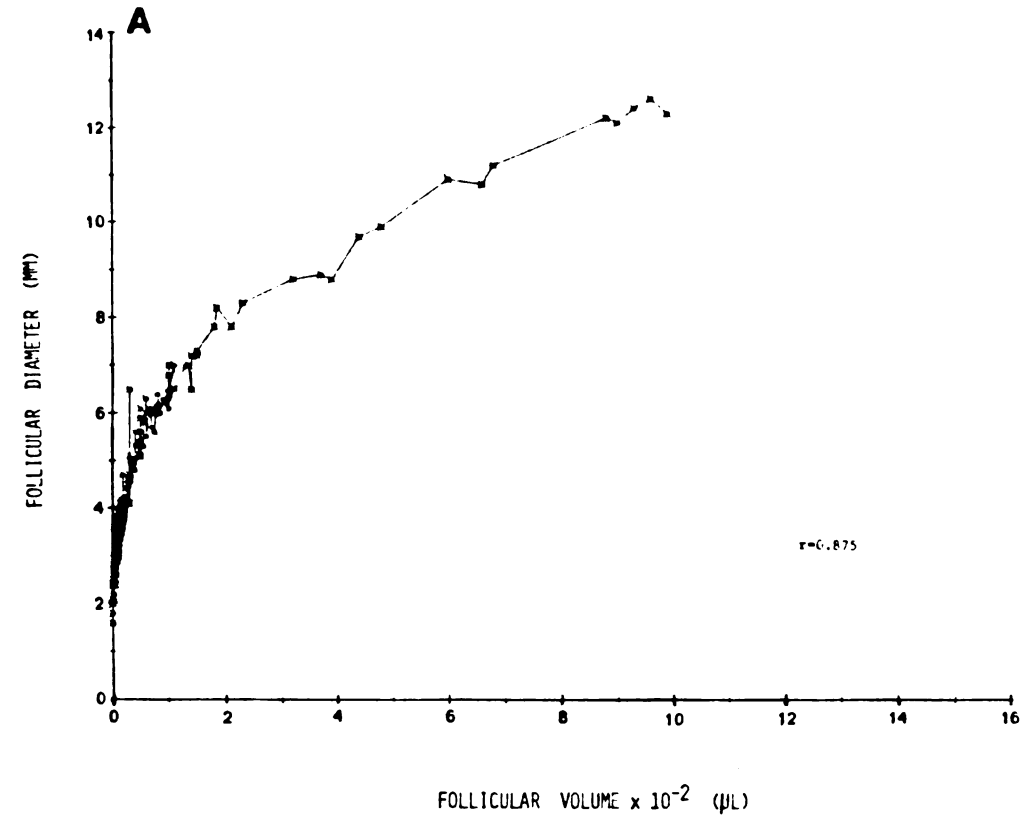
During the postpartum period studied, there was no change in the frequency or duration of suckling events during the 6-h bleeding periods (P>.05, Table 6). Mean (±SEM) frequency (number/6 h) of suckling events was 2.4±.5 with a duration of 8.2±.9 min. Total time spent suckling per 6 h was about 19.7 min or 3.3 min/h. Data from both acyclic and cyclic cows are included in Table 6.

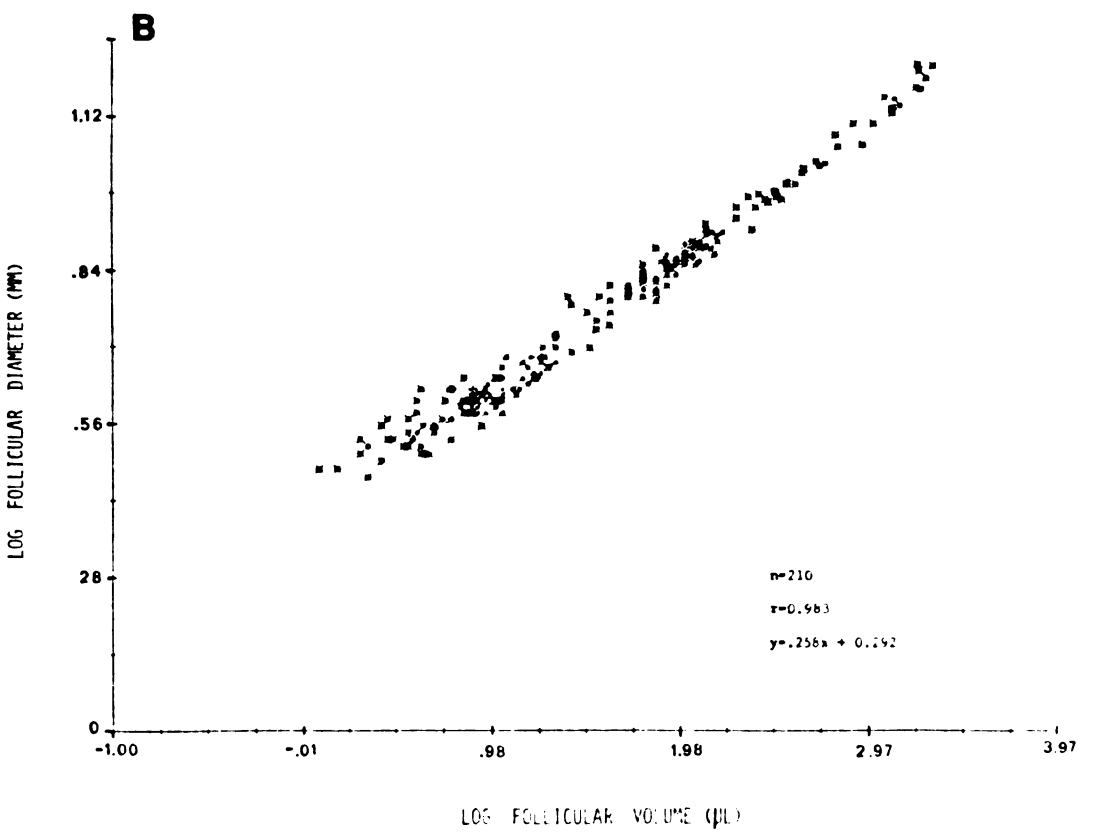
TABLE 6.--Frequency and Duration of Suckling Events from 7 to 56 Days After Parturition in Acyclic and Cyclic Beef Cows.

Days after parturition	N	Frequency (events/6 h)[a]	Duration (min)[a]
7	6	2.6±.4	8.0± .7
14	6	2.7±.5	7.4± .8
28	6	1.7±.5	8.4±1.0
42	8	2.5±.7	9.6± .9
56	6	2.7±.5	7.6± .9
Overall $\bar{x}$		2.4±.2	8.2± .4

[a]Values are means ± SEM.

Figure 12.--Relationship between follicular diameter and follicular fluid volume of 210 follicles dissected from 21 pairs of ovaries. A, simple correlation coefficient of actual data, r=0.875 (P<.001). B, simple correlation coefficient of $\log_{10}$ versus $\log_{10}$ plot, r=0.983 (P<.001).


A
FOLLICULAR DIAMETER (MM)
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
r=0.875
FOLLICULAR VOLUME x 10-2 (µL)


B
LOG FOLLICULAR DIAMETER (MM)
1.12
.84
.56
28
0
-1.00
-.01
.98
1.98
2.97
3.97
n=210
r=0.983
y=.256x + 0.292
LOG FOLLICULAR VOLUME (µL)

4. Serum Hormones[2]

Progesterone.--Concentrations of progesterone in serum reported in Figure 13A were measured in samples collected at 0800 and 1400 h during the blood sampling period. Concentrations of progesterone in serum remained between .3 to .5 ng/ml from days 7 to 42 after parturition (Figure 13A) in acyclic cows, with the exception that in two of four acyclic cows slaughtered on days 42-56, small transitory rises in serum progesterone (1.7±.3 ng/ml occurring 7±1 days prior to slaughter) were observed (data not shown). Only luteal tissue from the previous pregnancy was found in ovarian tissue at time of slaughter. Similarly, 4 of 11 cyclic cows showed small transitory rises (2.3±.3 ng/ml) occurring 5.8±1.1 days prior to the next increase in progesterone greater than 1 ng/ml. The remaining 7 of 11 cows that had ovulated by time of slaughter were still early in their first estrous cycle and thus we were unable to assess whether small transient peaks of progesterone would have occurred.

Cyclic cows had higher ($P<.05$) concentrations of progesterone in serum than acyclic cows when slaughtered on day 42. As determined by the first sample with concentrations of progesterone in serum greater than 1 ng/ml, the cyclic cows (n=11) first ovulated 37.6±1.4 days after parturition (Table 7).

Estradiol.--Concentrations of estradiol in serum were determined in a composite of seven hourly samples obtained from

---

[2]Serum hormone data for LH, PRL, total glucocorticoids, estradiol and progesterone has been reported elsewhere (Leung, 1984).

Figure 13.--Mean concentrations of progesterone (A) and estradiol (B) in serum of acyclic and cyclic cows from 7 to 56 days after parturition. Values are means ± SEM.

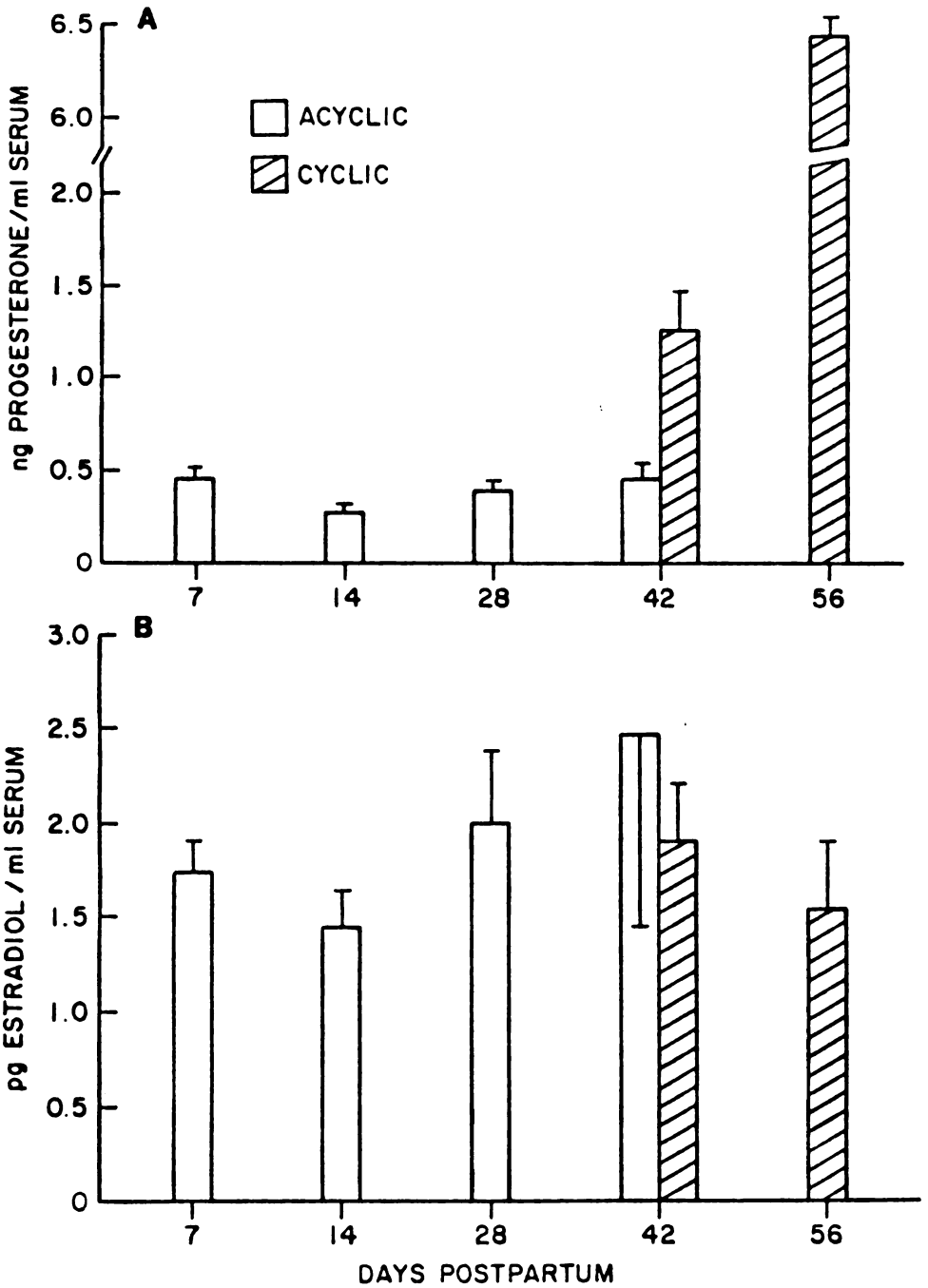

A
6.5
6.0
2.0
1.5
1.0
0.5
0
ng PROGESTERONE / ml SERUM
ACYCLIC
CYCLIC
7
14
28
42
56
B
3.0
2.5
2.0
1.5
1.0
0.5
0
pg ESTRADIOL / ml SERUM
7
14
28
42
56
DAYS POSTPARTUM

TABLE 7.--Distribution of Cyclic Cows and Mean Day (± SEM) of First Ovulation After Parturition.

	Days after parturition					
	7	14	28	42	56	$\bar{X}$
Number of cows	0	0	1	5	5	11
Day of first ovulation[a]	-	-	27	38.2±1.9	39.2±1.2	37.6±1.4

[a]As approximated by first serum sample containing >1.0 ng/ml of progesterone.

each cow during the blood sampling period. Concentrations of estradiol in serum of acyclic cows did not change between days 7 and 42 (P>.10; Figure 13B) or differ between acyclic and cyclic cows. Although mean concentrations of estradiol in serum of acyclic cows were not significantly different across days, mean concentrations on days 28 and 42-56 (2.0 to 2.5 pg/ml) were 40% greater than on days 7 and 14 (1.4 to 1.7 pg/ml).

Cortisol.--Concentrations of cortisol in serum were assayed in samples obtained at 0800, 1000, 1200 and 1400 h during the blood sampling period. Concentrations of cortisol in serum of acyclic cows did not change between days 7 and 42 or differ between acyclic and cyclic cows (P>.10; Figure 14).

Prolactin.--Overall mean, baseline, mean amplitude, height and frequency of secretory pulses of prolactin (PRL) in serum of acyclic cows did not change between days 7 and 28 (Table 8). Note

Figure 14.--Mean concentrations of cortisol in serum of acyclic and cyclic cows from 7 to 56 days after parturition. Values are means ± SEM.

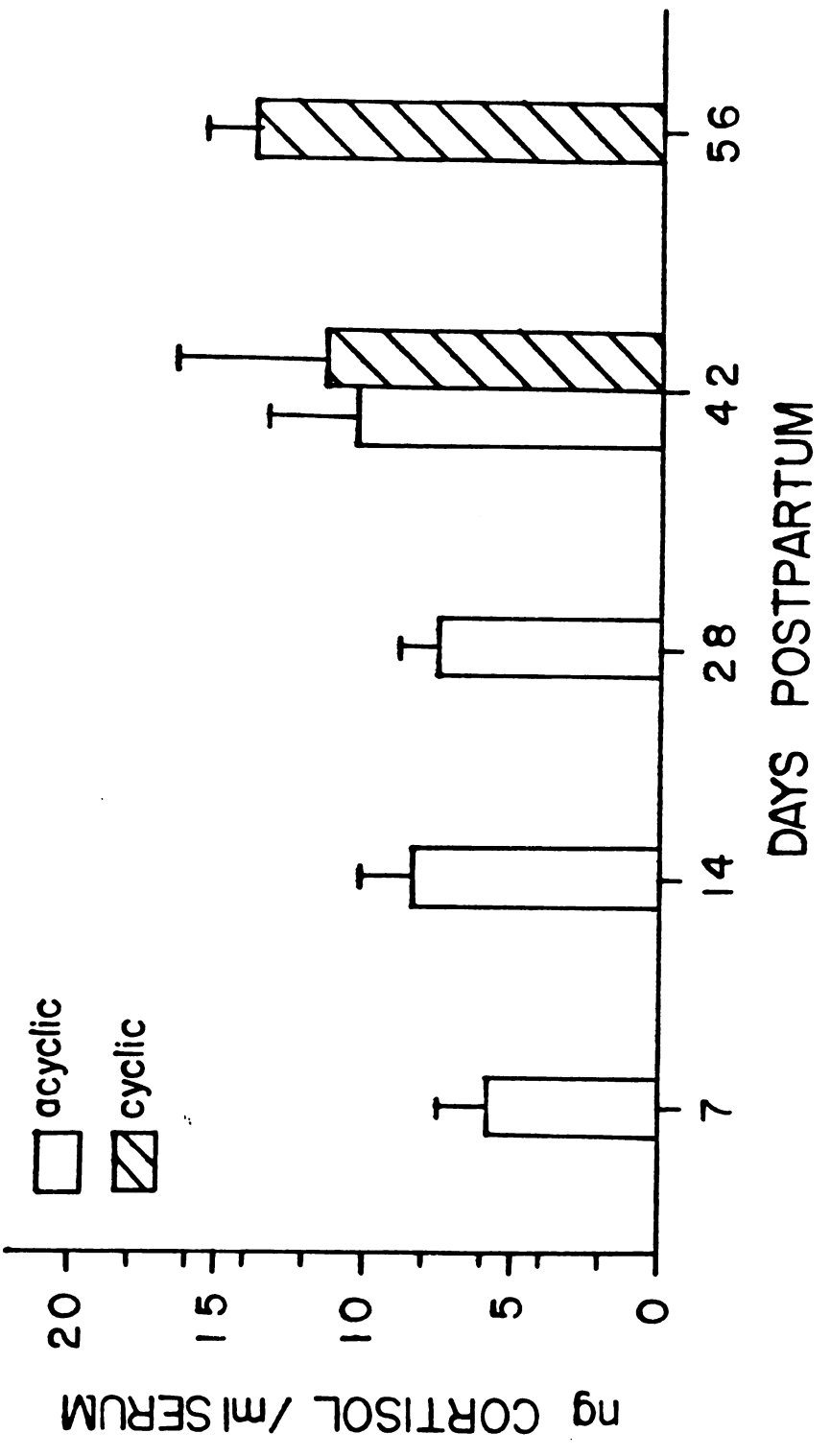

ng CORTISOL /ml SERUM
0
5
10
15
20
acyclic
cyclic
7
14
28
42
56
DAYS POSTPARTUM

TABLE 8.--Mean Overall Concentrations of Prolactin (PRL) in Serum, Frequency of Pulses of PRL in Serum and Mean Calendar Date of Bleed.[a]

Days after parturition	N	Overall PRL (ng/ml)	Frequency of PRL pulses (number/4 h)	Mean date of bleed
7	6	21.0±2.2[b,c]	1.2±.5	7-24
14	6	23.9±4.7[b]	1.8±.6	8-1
28	5	24.5±3.0[b]	1.6±.2	8-9
42-56	4	16.7±1.4[c]	0.5±.3	8-27

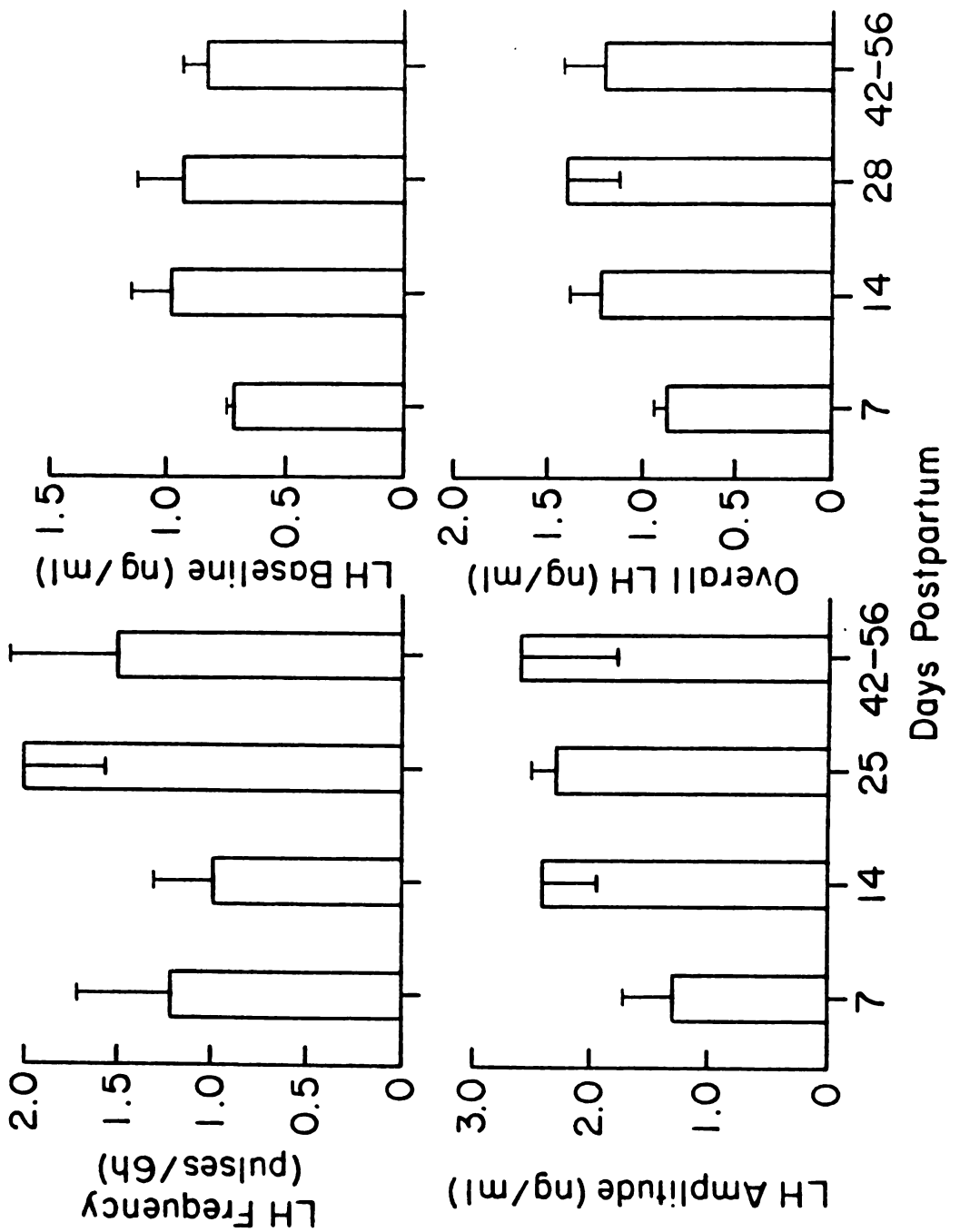
[a]Means ± SEM.

[b,c]Means with different superscripts tended to differ ($P<.10$).

that when the cows were bled during the shorter daylength and cooler temperatures of late August, concentrations of PRL tended ($P<.10$) to be lower than in samples taken during late July and early August.

Luteinizing Hormone (LH).--There was no significant effect of time after parturition on frequency of LH pulses, overall mean of LH in serum, or baseline mean of LH ($P>.10$; Figure 15). However, there was a tendency ($P<.10$) for the amplitude (peak height minus baseline) of LH pulses to increase with time after parturition. The temporal pattern of LH secretion in serum from one representative cow of each group of acyclic cows is presented in Figure 16A. Each representative cow was selected based on having a secretory profile closest to the average of the group.

Figure 15.--Variables of LH secretion in acyclic cows between days 7 and 42-56 after parturition. Values are means ± SEM.


LH Frequency (pulses/6h)
2.0
1.5
1.0
0.5
0
LH Baseline (ng/ml)
1.5
1.0
0.5
0
LH Amplitude (ng/ml)
3.0
2.0
1.0
0
Overall LH (ng/ml)
2.0
1.5
1.0
0.5
0
7
14
25
28
42–56
Days Postpartum

Follicle-Stimulating Hormone (FSH).--Concentrations of FSH in serum were unchanged after parturition; overall mean concentrations (± SEM) for days 7, 14, 28 and 42-56 were 61.3±6.6, 69.9±4.8, 72.8±4.7 and 65.0±11.3, respectively. The temporal pattern of FSH secretion in serum from one representative cow of each group of acyclic cows is presented in Figure 16B. Each representative cow was selected based on having a secretory profile closest to the average of the group.

Serum FSH to LH ratio did not change between days 7 and 42-56 after parturition in acyclic cows. Mean ratios (± SEM) were 70.4±7.7, 62.3±9.3, 56.7±8.3 and 62.6±16.9 for days 7, 14, 28 and 42-56, respectively.

5. Follicular Fluid Hormones

Progesterone.--Concentrations of progesterone in follicular fluid of small (1.0-3.9 mm) and medium (4.0-7.9 mm) follicles did not change between days 7 and 42-56 ($P>.10$; Figures 17 and 18). However, concentrations of progesterone in follicular fluid of large follicles (Figure 19) increased ($P<.05$) between days 7 (80±11 ng/ml) and 14 (273±92 ng/ml), but decreased ($P<.05$) by day 28 (123±19 ng/ml). On days 42-56, concentrations of progesterone (188±59 ng/ml) were similar to concentrations on day 28 in large follicles (Figure 19).

Androstenedione.--Concentrations of androstenedione in follicular fluid of large and medium follicles did not change ($P>.10$) between days 7 and 42-56 (Figures 18 and 19). However, concentrations

Figure 16.--Temporal concentrations of LH (A) and FSH (B) observed in one representative cow from each group of acyclic cows.

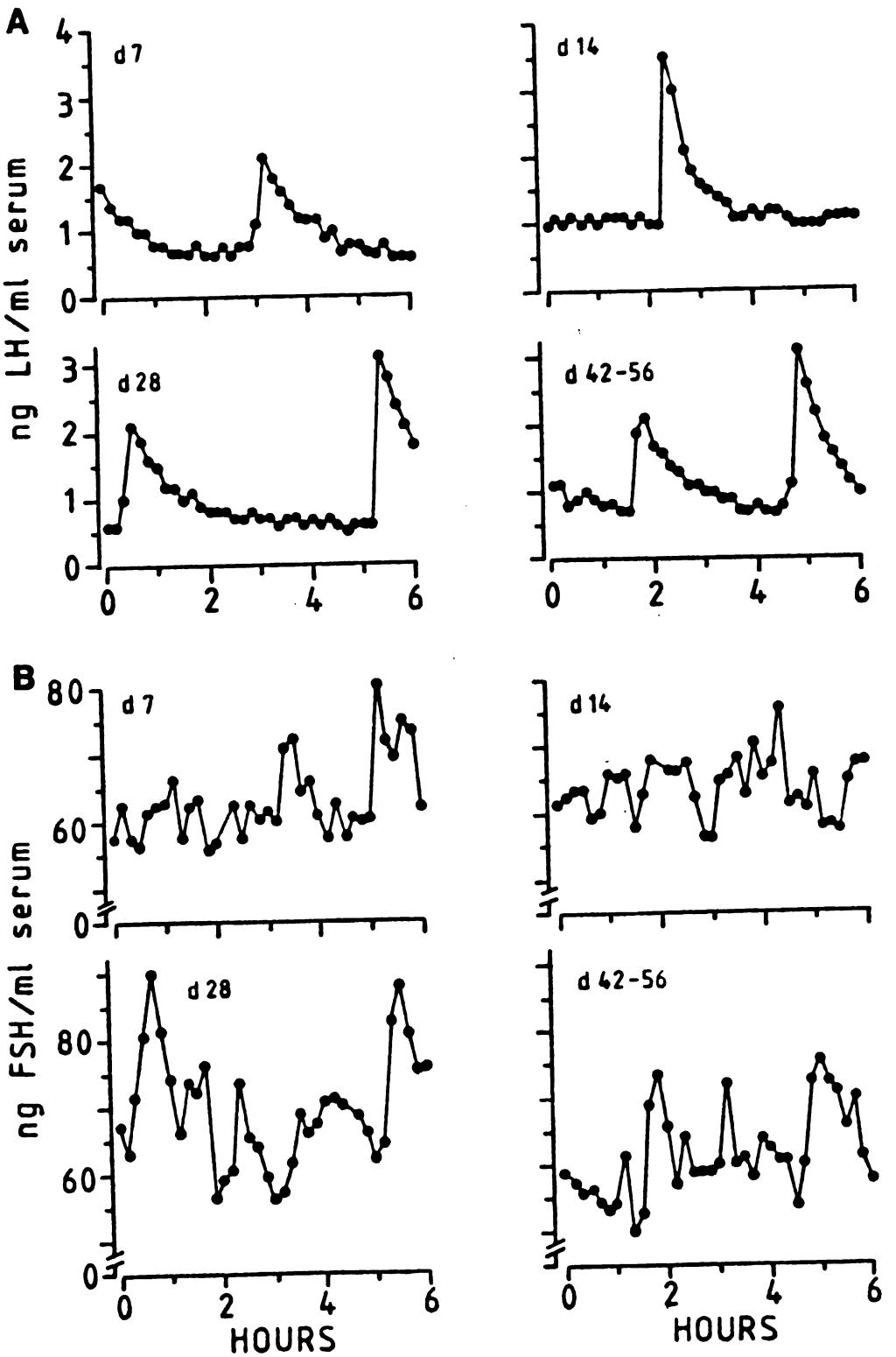

A
B
d 7
d 14
d 28
d 42-56
ng LH/ml serum
ng FSH/ml serum
HOURS
HOURS

Figure 17.--Concentrations of steroids in follicular fluid of small follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM. P = progesterone; E = estradiol.

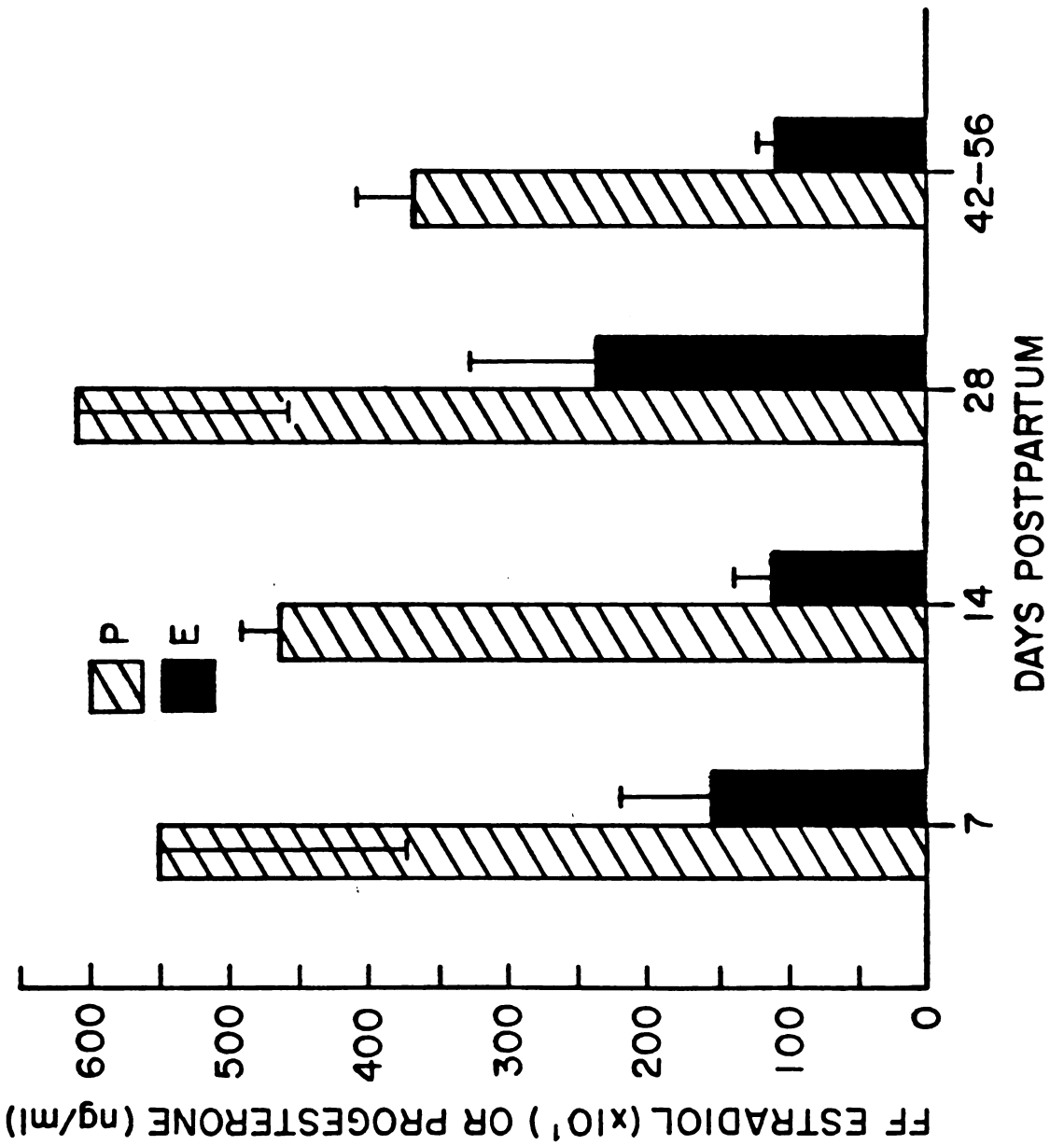

FF ESTRADIOL (x10¹) OR PROGESTERONE (ng/ml)
0
100
200
300
400
500
600
P
E
7
14
28
42–56
DAYS POSTPARTUM

Figure 18.--Concentrations of steroids in follicular fluid of medium follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM. A = androstenedione; P = progesterone; E = estradiol.

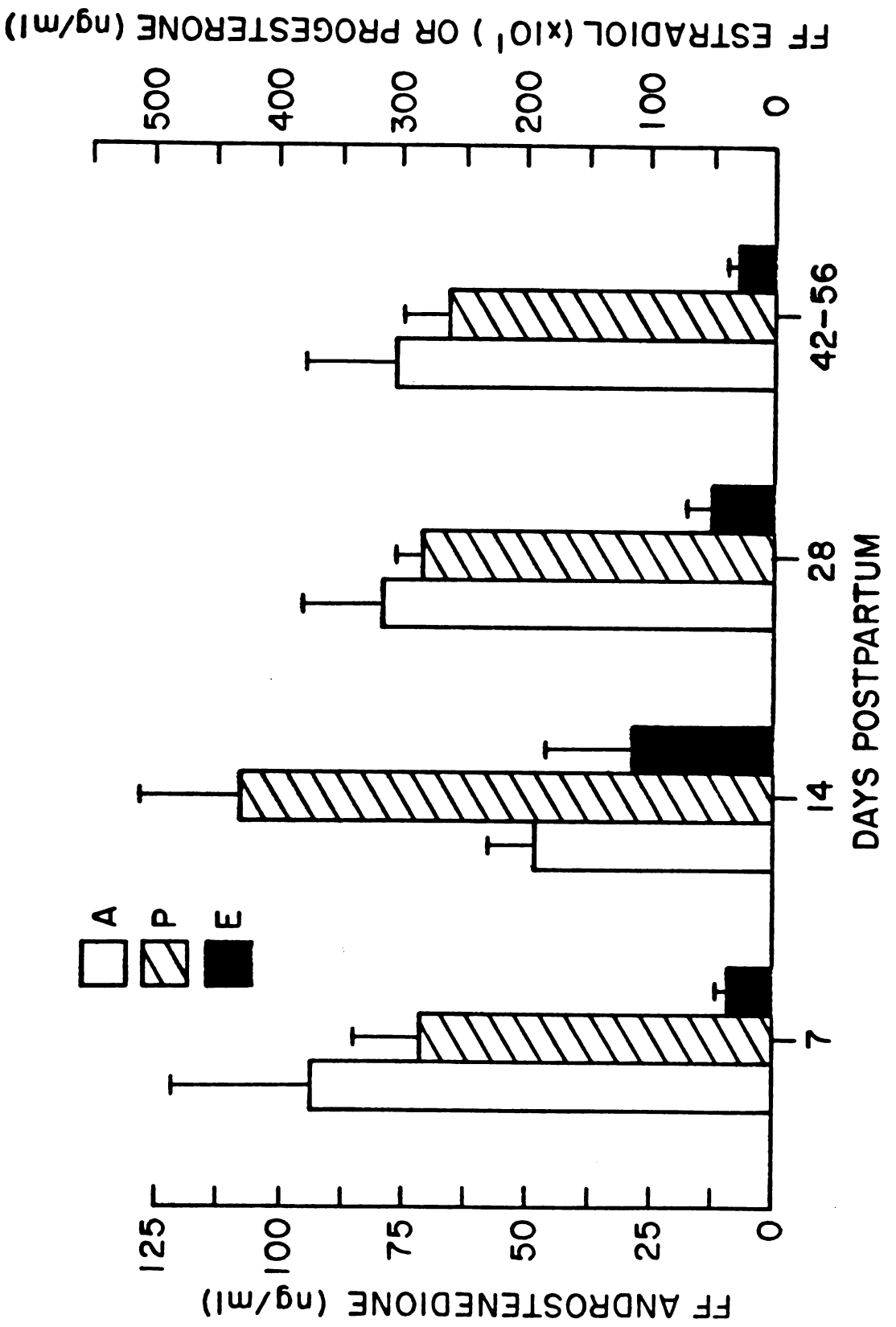
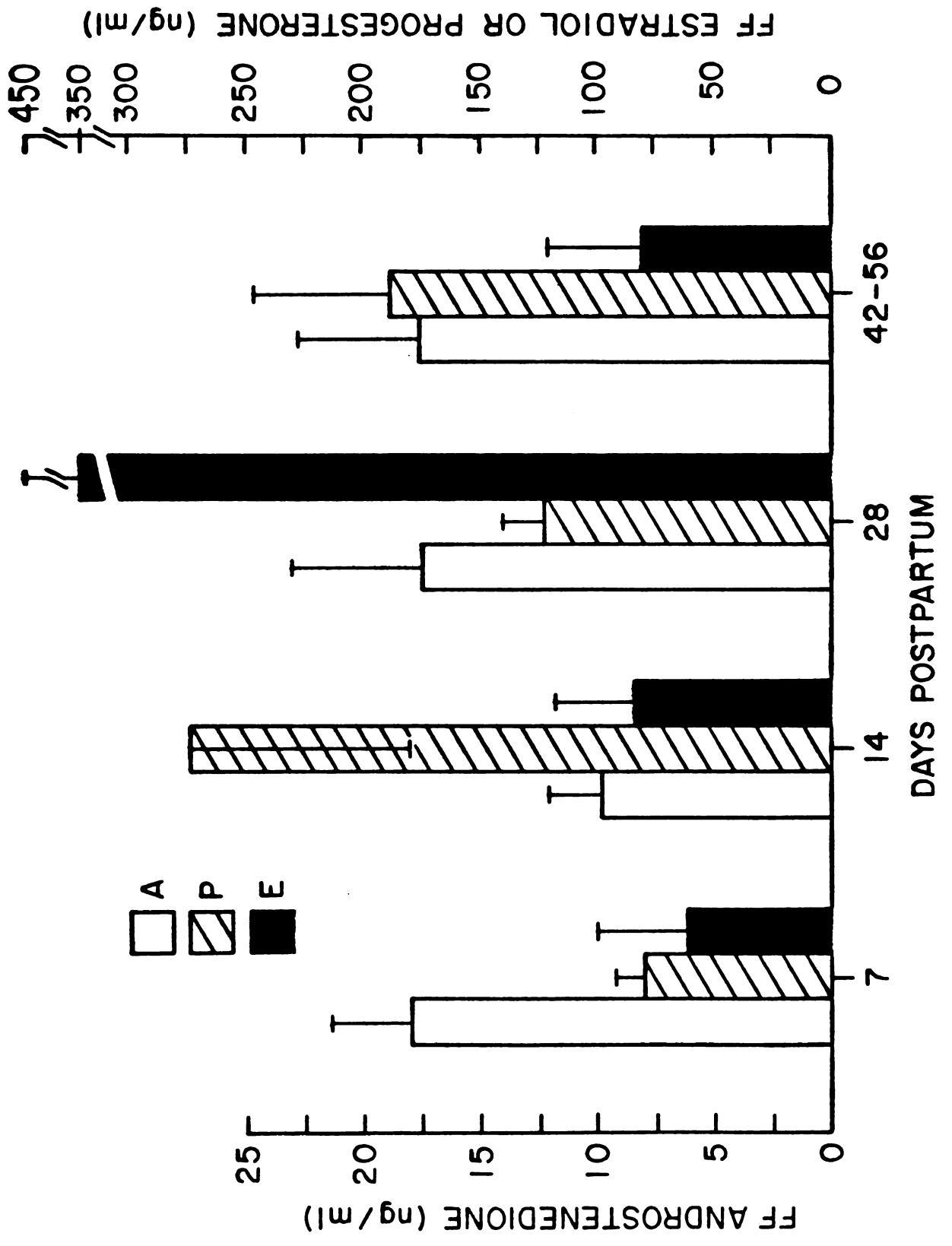

FF ANDROSTENEDIONE (ng/ml)
125
100
75
50
25
0
A
P
E
7
14
28
42-56
DAYS POSTPARTUM
FF ESTRADIOL (x10¹) OR PROGESTERONE (ng/ml)
500
400
300
200
100
0

Figure 19.--Concentrations of steroids in follicular fluid of large follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM. A = androstenedione; P = progesterone; E = estradiol.


FF ESTRADIOL OR PROGESTERONE (ng/ml)
450
350
300
250
200
150
100
50
0
42-56
28
14
7
DAYS POSTPARTUM
A
P
E
FF ANDROSTENEDIONE (ng/ml)
25
20
15
10
5
0

of androstenedione in follicular fluid were 2- to 4-fold greater in medium follicles than in large follicles (Figures 18 and 19).

Estradiol.--Concentrations of estradiol in follicular fluid of small and medium follicles did not change between days 7 and 42-56 ($P>.10$; Figures 17 and 18). However, concentrations of estradiol in large follicles increased ($P<.05$) between days 14 (85±33 ng/ml) and 28 (359±108 ng/ml), but decreased ($P<.05$) to 80±40 ng/ml on days 42-56. Although concentrations of estradiol in serum did not significantly change with days after parturition, estradiol concentrations in serum were positively correlated ($r=.53$, $P<.01$) with average concentrations of estradiol in follicular fluid of the large follicles removed from the animals less than 24 h later.

Total ovarian estradiol content (ng/pair of ovaries; calculated using average follicular numbers and volumes, and estradiol concentrations of each size category of follicles) increased ($P<.05$) from 63 on day 7 to 98 on day 14, and to 581 on day 28 (Table 9). Total ovarian estradiol content was 84 ng on days 42-56. Large follicles contributed 94 to 99% of the estimated total ovarian estradiol content (Table 9). Medium and small follicles as groups each contributed .2 to 3.2% of the total.

Since ratios of steroids in follicular fluid have been used to access atretic state of follicles (Ireland and Roche, 1982; Westergaard et al., 1982; Bellin and Ax, 1984), ratios of progesterone to androstenedione, androstenedione to estradiol and progesterone to estradiol were calculated for medium and large follicles (Table 10).

TABLE 9.--Estimation of Total Content of Estradiol in Ovaries of Cattle on Days 7 to 42-56 After Parturition Using Average Numbers, Volume and Estradiol Concentration in Small, Medium and Large Follicles.[a]

Group[b]	Average numbers per pair of ovaries	Average volume/ follicle (µl)	Average FF estradiol (ng/ml)	Total estradiol (ng)	% of total
Day 7					
Small	14	6.7	16	1.5	2.4
Medium	5	35.0	4	0.7	1.1
Large	2	493.0	62	61.1	96.5
Total	21			63.3	100.0
Day 14					
Small	14	7.6	11	1.2	1.2
Medium	8	32.0	12	3	3.1
Large	2	555.0	85	94.4	95.7
Total	24			98.6	100.0
Day 20					
Small	22	6.7	24	3.5	0.6
Medium	7	27.0	5	0.9	0.2
Large	2	803.0	359	576.5	99.2
Total	31			581.0	100.0
Days 42-56					
Small	21	8.5	11	1.9	2.4
Medium	19	47.0	3	2.7	3.2
Large	2	493.0	80	78.9	94.4
Total	42			83.5	100.0

[a]Volume was estimated from average diameter using equation in Figure 12.

[b]Small = 1-3.9 mm, Medium = 4-7.9 mm, Large = $\geq$8 mm.

TABLE 10.--Ratios of Steroids in Follicular Fluid of Small, Medium and Large Follicles at Various Days After Parturition in Acyclic Cows.

Group[b]	N	Ratio[a] P:A[c]	A:E[d]	P:E[e]
Day 7				
Small	16	---	---	58±9
Medium	30	4±1	104±31	295±89
Large	12	5±1	6±3	20±13
Day 14				
Small	35	---	---	129±35
Medium	34	23±9	104±45	785±258
Large	12	44±16	13±9	272±118
Day 28				
Small	38	---	---	72±15
Medium	19	11±5	110±36	660±203
Large	9	19±11	2±1	50±46
Days 42-56				
Small	23	---	---	123±33
Medium	29	19±8	89±34	545±220
Large	9	22±8	14±6	207±78

[a] P = progesterone, A = androstenedione, E = estradiol; Means ± SEM.

[b] Small = 1-3.9 mm, Medium = 4-7.9 mm, Large = $\geq$8 mm.

[c] Means on day 7 differ from means on days 14, 28 and 42-56 ($P<.05$).

[d] Means of medium follicles differ from means of large follicles on all days ($P<.05$).

[e] Means of medium follicles differ from means of small and large follicles on all days ($P<.05$) but means of large and small follicles do not differ.

Only ratios of progesterone to estradiol could be calculated for small follicles. Ratios of progesterone to androstenedione were significantly lower on day 7 than on days 14, 28 or 42-56 after parturition in both medium and large follicles. Progesterone to androstenedione ratios were similar between medium and large follicles. Androstenedione to estradiol ratios did not change with days after parturition in either large or medium follicles and were 6- to 55-fold greater ($P<.05$) in medium follicles as compared with large follicles. Ratios of progesterone to estradiol did not change with days after parturition in any size follicle. Average progesterone to estradiol ratios varied the least in small follicles (58 to 123) and varied the most in large follicles (20 to 272). These ratios did not differ between small and large follicles. Medium follicles consistently had the highest progesterone to estradiol ratios (295 to 785) which differed significantly from small and large follicles (Table 9).

Prolactin.--Concentrations of prolactin (PRL) were only measured in follicular fluid of large follicles and did not change ($P>.10$) between days 7 and 28 after parturition but decreased ($P<.05$) between days 28 and 42-56 (Table 11). This trend was similar to that seen in serum (Table 8). However, concentrations of PRL in follicular fluid were 110% to 169% greater than concentrations in serum samples that were collected the previous day (Table 11).

TABLE 11.--Concentrations of Prolactin (PRL) in Follicular Fluid (FF) of Large Follicles in Acyclic Cows.

Variable	Days after parturition			
	7	14	28	42-56
FF PRL	39.3±5.2[a]	31.3±2.7[a]	41.4±4.5[a]	17.1±3.3[b]
% of sera concentrations	164±17	127±22	169±14	110±33

[a,b]Superscripts with different letters differ ($P<.05$).

6. Gonadotropin Binding in Follicular Tissue

Large Follicles.--Specific binding of ^{125}I-hCG to thecal-enriched homogenates of large follicles did not change between days 7 and 42-56 ($P>.10$; Figure 20) after parturition. However, specific binding of ^{125}I-hCG to granulosal cells increased 1.5- to 1.7-fold ($P<.10$) between days 7 and 14 (Figure 20). In contrast, specific binding of ^{125}I-oFSH decreased 30% ($P<.10$) between days 7 and 14 after parturition but by days 42-56 binding was similar to levels on day 7 (Figure 20).

Medium Follicles.--Specific binding of ^{125}I-hCG or ^{125}I-oFSH to whole follicular homogenates of medium follicles did not change between days 7 and 42-56 ($P>.10$; Figure 21).

Small Follicles.--Specific binding of ^{125}I-oFSH to pooled follicular homogenates of small follicles did not change between days 7 and 42-56 ($P>.10$; Figure 22). In contrast, specific binding

Figure 20.--Specific binding of ^{125}I-hCG to thecal homogenates and granulosal cells and specific binding of ^{125}I-oFSH to granulosal cells of large follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM.

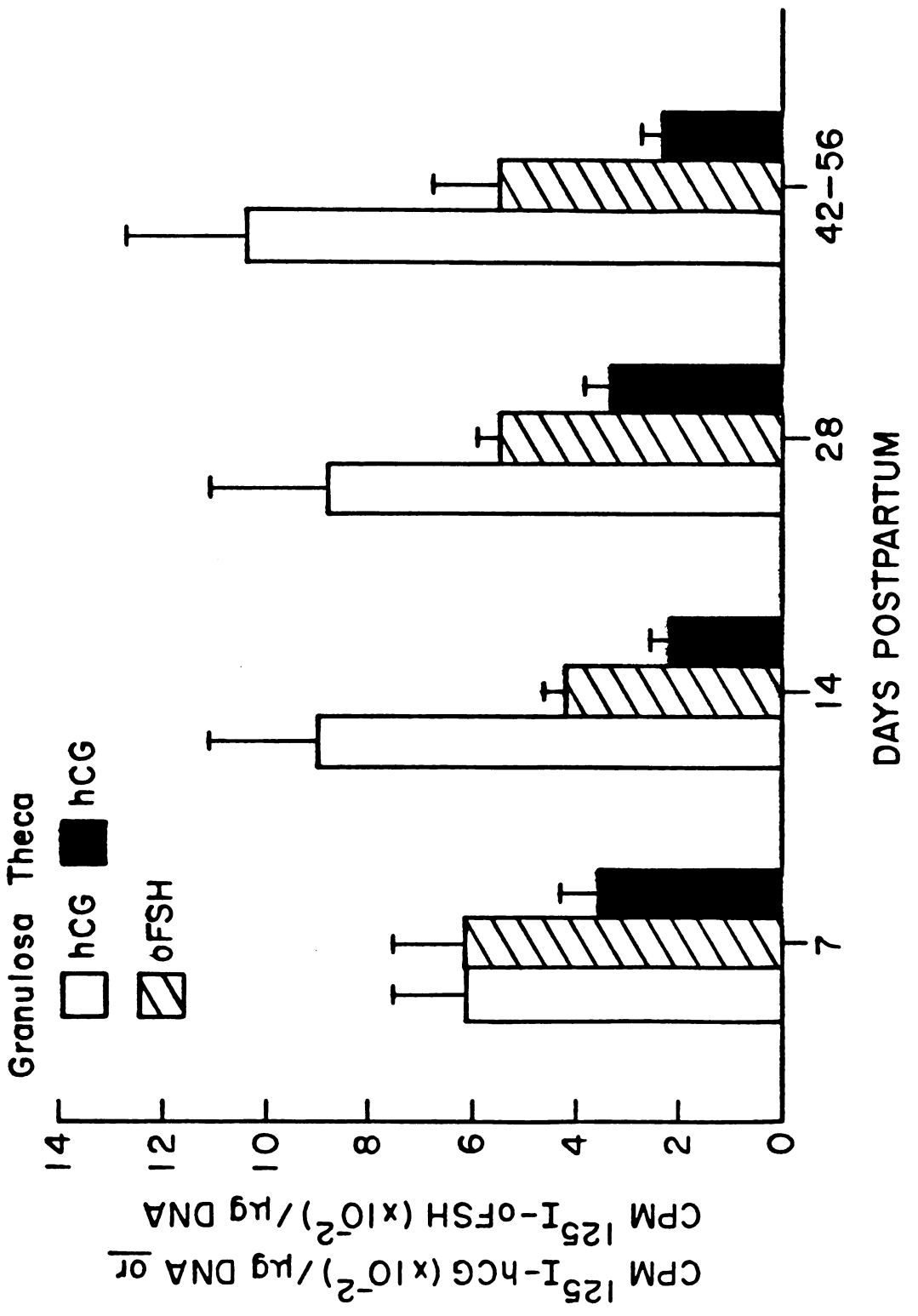

Granulosa Theca
hCG
hCG
oFSH
CPM 125I-hCG (x10-2)/µg DNA or
CPM 125I-oFSH (x10-2)/µg DNA
0
2
4
6
8
10
12
14
7
14
28
42-56
DAYS POSTPARTUM

Figure 21.--Specific binding of ^{125}I-hCG and ^{125}I-oFSH to whole follicular homogenates of medium follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM.

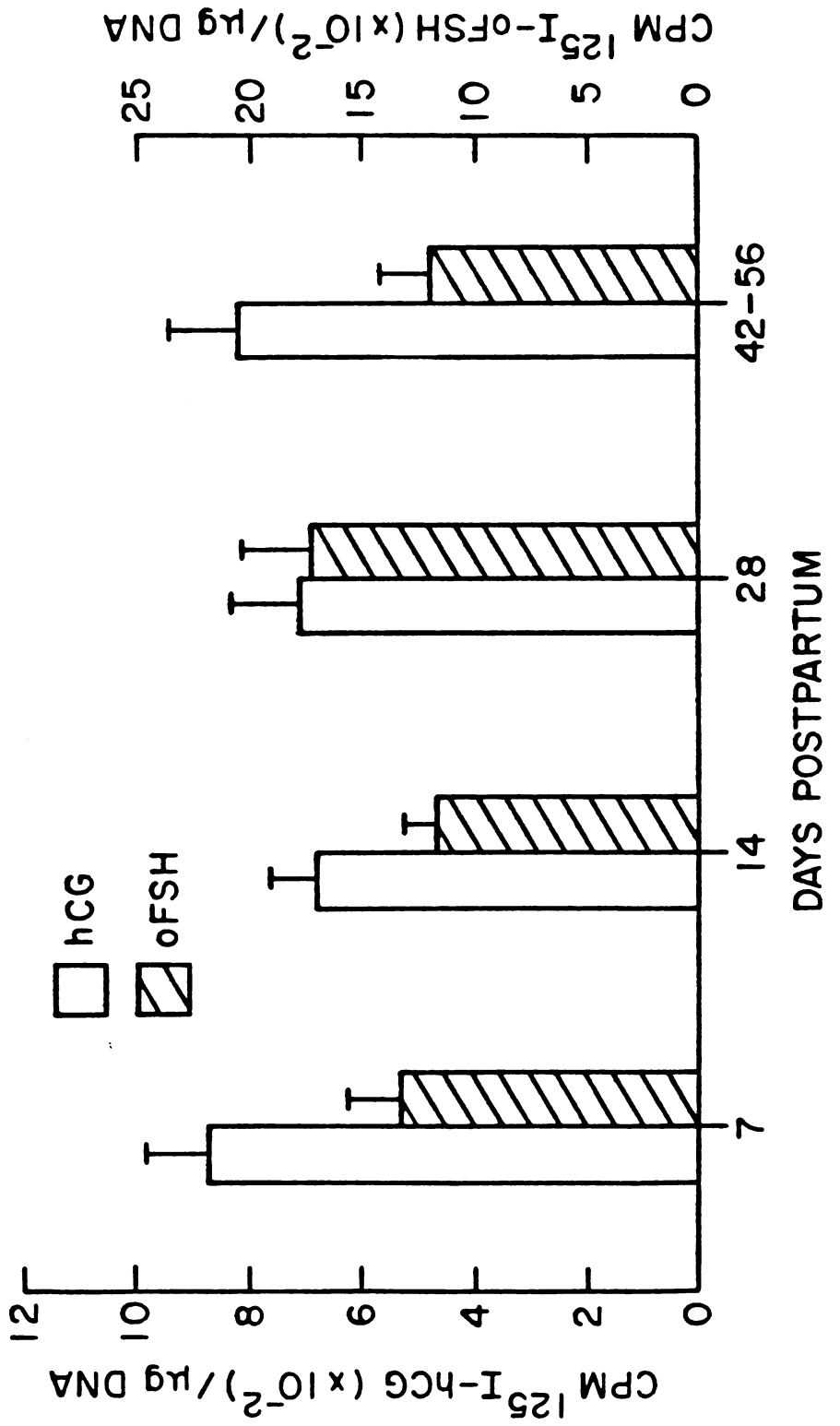
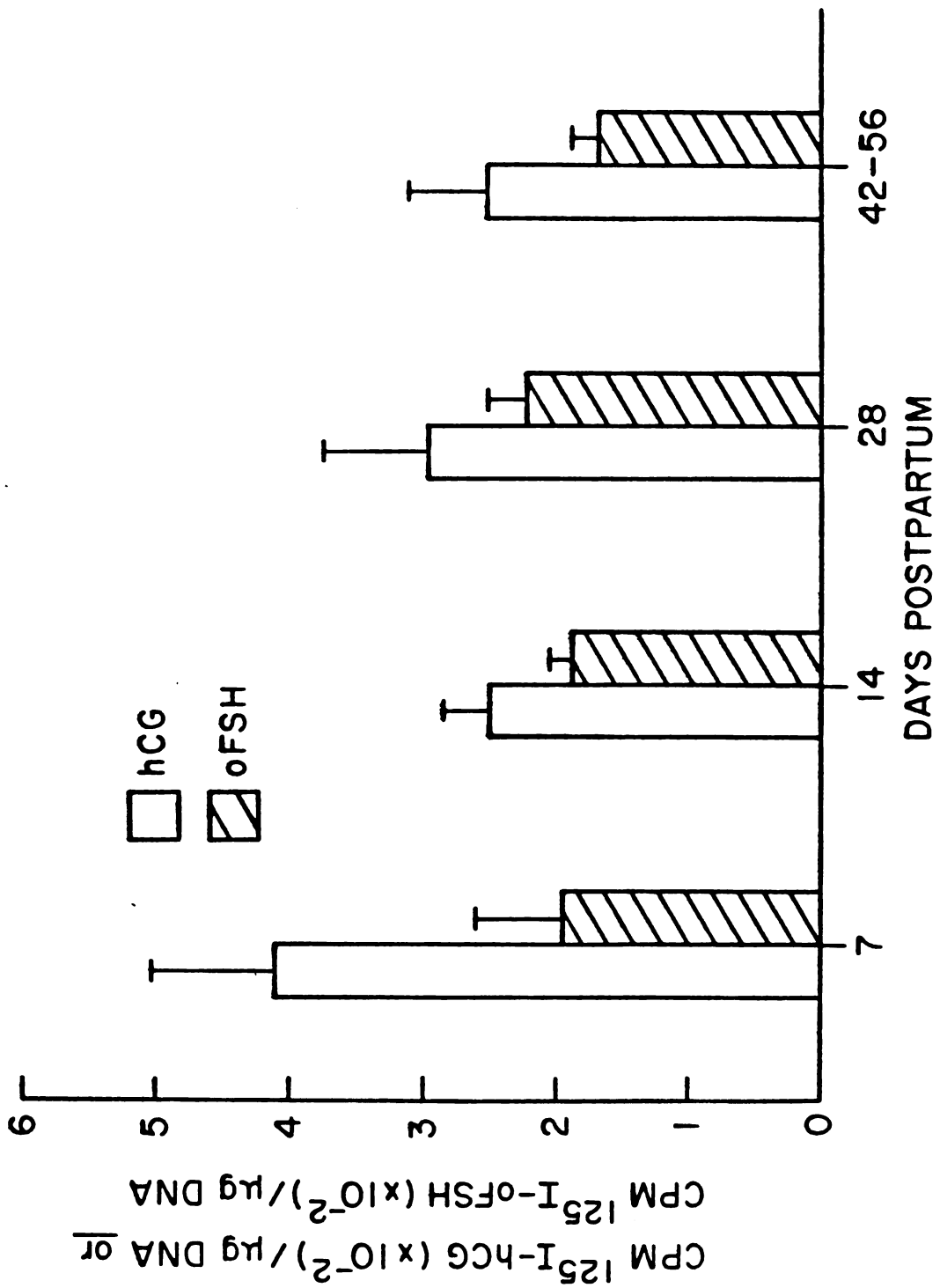

CPM ^{125}I-hCG (x 10^{-2})/µg DNA
0
2
4
6
8
10
12
CPM ^{125}I-oFSH (x 10^{-2})/µg DNA
0
5
10
15
20
25
hCG
oFSH
7
14
28
42-56
DAYS POSTPARTUM

Figure 22.--Specific binding of ^{125}I-hCG and ^{125}I-oFSH to pooled follicular homogenates of small follicles between days 7 and 42-56 after parturition in acyclic cows. See Figure 3 for the numbers and average size of follicles at each day after parturition. Values are means ± SEM.


CPM ^{125}I-hCG ($\times 10^{-2}$)/μg DNA or
CPM ^{125}I-oFSH ($\times 10^{-2}$)/μg DNA
0
1
2
3
4
5
6
hCG
oFSH
7
14
28
42-56
DAYS POSTPARTUM

of ^{125}I-hCG decreased 55% (P<.10) between days 7 and 14 after parturition and remained lower than day 7 values through days 42-56 (Figure 22).

7. Correlations Among Ovarian Follicular Variables

Large Follicles.--Correlation coefficients were calculated among follicular diameter, concentrations of estradiol, progesterone and androstenedione in follicular fluid, and specific binding of gonadotropins to thecal and granulosal cells. Only significant correlation coefficients are included in Table 12. Follicular diameter was positively correlated with estradiol concentrations and LH binding to granulosal cells. Follicular diameter was negatively correlated with FSH binding to granulosal cells (r=-.28).

TABLE 12.--Correlation Coefficients Among Follicular Diameter, Concentrations of Estradiol (E), Progesterone (P) and Androstenedione (A) in Follicular Fluid, LH and FSH Binding Sites in Thecal (T) and Granulosal Cells (GC) of Large Follicles.[a]

Variable	E	LH binding to GC	FSH binding to GC	LH binding to T
Diameter	.36[b]	.41[b]	-.27[c]	--
A	.27[c]	--	--	.49[b]
P	--	.45[b]	--	--
LH binding sites to GC	--	--	-.27[c]	--

[a] n = 57.

[b] P<.001.

[c] P<.060.

Androstenedione concentrations were positively correlated to estradiol concentrations and LH binding to thecal homogenates (r=.27 and r=.49, respectively). LH binding to granulosal cells was positively correlated with progesterone concentrations (r=.45) but negatively correlated to FSH binding to granulosal cells (r=-.27).

Medium Follicles.--Correlation coefficients were calculated among follicular diameter, concentrations of estradiol, progesterone and androstenedione in follicular fluid, and specific binding of gonadotropins to whole follicular homogenates. Only significant correlation coefficients are included in Table 13. Follicular diameter was negatively correlated with androstenedione concentrations and both LH and FSH binding sites. Specific binding of LH was also negatively correlated with estradiol concentrations (r=-.18) and positively correlated with androstenedione concentrations (r=.20) and specific binding of FSH (r=.56). Concentrations of progesterone in follicular fluid of medium follicles were not significantly associated with any follicular variable measured.

D. Discussion

Usually, the uterus is considered to be involuted after parturition when it has returned to its normal, non-pregnant shape, tone and diameter. The average interval from parturition until completion of uterine involution ranges from 25 to 50 days in cattle (Casida, 1968; Moller, 1970). Uterine involution in the present study occurred by day 28 after parturition and may be at the earlier end of the range reported in the literature, since all cattle used in the

TABLE 13.--Correlation Coefficients Among Follicular Diameter, Concentrations of Estradiol (E) and Androstenedione (A) in Follicular Fluid, and LH and FSH Binding Sites in Whole Follicular Homogenates of Medium Follicles.[a]

Variable	LH binding sites	FSH binding sites	A	E
Diameter	-.49[b]	-.50[b]	-.21[c]	--
LH binding sites	--	.56[b]	-.20[c]	-.17[c]

[a] n = 183.

[b] P<.001.

[c] P<.020.

present study were primiparous. Uterine involution appears to occur about 1 week sooner in primiparous cows than pluriparous cows (Moller, 1970; Foote, 1971).

Concentrations of progesterone in serum decline rapidly to basal levels during the first 2 to 6 days after parturition and remain at these levels until 1 to 6 days before the first postpartum estrus (Wettemann, 1980; Rawlings et al., 1980; Humphrey et al., 1983). At this time, small transitory peaks of progesterone (<2 ng/ml) occur in 40 to 70% of cattle examined (Wettemann, 1980; Rawlings et al., 1980; Humphrey et al., 1983). In my study at least 4 of 11 cows exhibited similar transitory progesterone peaks. This increase in concentration of progesterone in peripheral serum, which precedes the first postpartum estrus, is most likely due to formation of a transitory corpus luteum (Donaldson et al., 1970) or luteinization of small follicles

(Berardinelli et al., 1979). However, these structures are unable to maintain normal luteal phase progesterone secretion (Kesler et al., 1981). The cause of the shortened life span of these corpora lutea (or luteinized follicles) is unknown.

Duration and frequency of suckling events did not change between days 7 and 42-56 after parturition. This is in agreement with Gimenez et al. (1980) in which suckling duration (measured every 4 to 16 days for 10 h per day) did not significantly change between days 28 and 80 after parturition in beef cows. Average time spent suckling per h in my study was 3.3 min/h, similar to durations of 2.5 to 5.0 min/h reported by Gimenez et al. (1980). Thus, suckling intensity per se does not appear to be associated with return to ovulatory cyclicity in postpartum cattle. In contrast, suckling intensity in rats and monkeys lowers ovarian responsiveness to exogenous gonadotropins (Maneckjee et al., 1977). Furthermore, a decline in suckling intensity in rats is associated with initiation of postpartum estrous cycles (Taya and Greenwald, 1982). This decline in suckling intensity in rats is dependent on age of pups rather than days after parturition (Swanson and Campbell, 1981; Blake et al., 1984).

Hormones released in association with suckling, such as glucocorticoids or PRL, are potential inhibitors of postpartum ovulations in cattle (Wettemann, 1980). However, no significant change in concentrations of cortisol in serum from parturition to first ovulation in beef cows were observed in previous studies (Dunlap et al., 1981; Convey et al., 1983; Humphrey et al., 1983) or in the present study.

These data suggest that concentrations of cortisol in serum *per se* are not associated with postpartum anestrus. However, studies mentioned above measured total glucocorticoids in serum. Since glucocorticoids in blood are bound to serum proteins, it is possible that unbound or "free" glucocorticoids mediate inhibition of the reproductive system. In support for such a suggestion, Barnett and Star (1981) found that ewes with two suckling lambs, which have longer anestrous periods (Edgerton, 1980) had higher free glucocorticoids than ewes suckling only one lamb. To establish a relationship between free glucocorticoids and reestablishment of postpartum estrous cycles in cattle will require further study.

In addition, concentrations of PRL in serum do not change with time after parturition (Humphrey et al., 1983) or affect the postpartum anestrous interval in cattle (Williams and Ray, 1980). Similarly, I found no change in concentrations of PRL in serum or follicular fluid between days 7 and 28 after parturition, a time in which dramatic shifts in steroid production of large follicles were observed. Therefore, these data suggest that concentrations of PRL in serum *per se* are not associated with reestablishment of postpartum estrous cycles in cattle. Moreover, PRL release induced by suckling increases between days 14 and 42 after parturition in beef cattle (Convey et al., 1983), suggesting that increased PRL due to suckling does not inhibit return to postpartum estrous cyclicity as hypothesized in rats (Selmanoff and Selmanoff, 1983).

Concentrations of PRL in fluid of large follicles were 1.1- to 1.7-fold greater than concentrations in serum. Since PRL secretion

increases in cattle under stress (Tucker, 1971), perhaps stress associated with slaughter of cattle in the present study accounts for higher PRL concentrations found in follicular fluid.

Follicular growth during postpartum anestrus has not been investigated in beef cows. A few studies utilizing dairy cows, which ovulate on the average 3 to 4 weeks earlier (14 to 45 days after parturition) than beef cows, have found that follicles 5 mm and greater appear between days 7 and 14 after parturition (Saiduddin et al., 1968; Wagner and Hansel, 1969; Kesler et al., 1979). In the present study, follicles 8 mm and greater were present on ovaries by day 7 after parturition with no subsequent change in average size of these follicles. Thus, results of all studies indicate that appearance of large follicles occurs long before the first postpartum ovulation in cattle.

Changes in numbers of various sized follicles with time after parturition has not been reported for either dairy or beef cattle. In the present study, slight increases in numbers of small follicles and significant increases in numbers of medium follicles were observed between days 7 and 42-56 after parturition. Thus, we observed that total number of follicles 1.0 mm and greater increased linearly from 21 to 42 follicles per pair of ovaries. Lack of an increase in numbers of small follicles should be interpreted carefully, since many of these small follicles could be imbedded in the ovary and would not have been counted. Perhaps this increase in numbers of small and medium follicles provides an increased pool of antral follicles from which ovulatory follicles can be selected. Whether this larger pool of

follicles is necessary for reinitiation of ovulatory cycles after parturition in cattle is unknown.

In the present study, diameters of the largest follicle dissected from each pair of ovaries did not change with time after parturition in acyclic beef cows. These diameters are similar to diameters of the largest follicle reported by Wagner and Hansel (1969) in acyclic, milked dairy cows on days 7 and 14 after parturition. However, these diameters are 2 to 8 mm smaller than diameters of the largest follicles removed from ovaries of cattle during estrus (Donaldson and Hansel, 1968; Marion et al., 1968; Merz et al., 1981; Staigmiller et al., 1982). This suggests final preovulatory growth of follicles is inhibited during postpartum anestrus.

In dairy cattle, the majority (>80%) of ovulations that occur before day 20 after parturition are on the ovary contralateral to the ovary containing the previous CL of pregnancy (Saiduddin et al., 1967; Foote and Peterson, 1968; Marion and Gier, 1968). However, if ovulation occurred after day 20, less than 60% of the newly formed CL were opposite the CL of pregnancy (Saiduddin et al., 1967; Foote and Peterson, 1968; Marion and Gier, 1968), indicating that in early postpartum stages there is a block to ovulation on the ovary containing the previous CL of pregnancy. Similarly, I observed that the presence of the previous CL of pregnancy or side of the gravid uterine horn inhibited growth of the largest follicles on the ipsilateral ovary from days 7 through 42-56. In addition, 83% of these largest follicles that had concentrations of estradiol greater than progesterone (ratio >1) were opposite the ovary containing the previous CL of

pregnancy from days 7 to 42-56. Collectively, these data suggest that in addition to growth of follicles, function of the largest follicle located on the ovary containing the CL of pregnancy is inhibited by some carryover effect of the previous pregnancy. Moreover, this inhibitory effect is present in all anestrous cattle, whether slaughtered before or after day 28, suggesting that this inhibitory effect of the previous pregnancy on follicular growth and function can be present in both early and late postpartum stages at least in cattle that remain anestrus. Since ovulation occurred in 10 of 14 cows slaughtered after day 28 in the present study, the four anestrous cows slaughtered on days 42-56 were beyond the average interval to first ovulation. But once a cow ovulated, there was no effect of the previous CL of pregnancy on the location of the new ovulation. Thus, it is possible that this inhibitory effect of the previous pregnancy is still present in the four anestrous cows.

Concentrations of estradiol in serum decline rapidly to basal levels during the first 2 to 6 days after parturition and remain at these levels until 2 or 3 days before the first postpartum estrus in suckling beef cows (Wettemann, 1980; Rawlings et al., 1980; Humphrey et al., 1983). In agreement with these findings, I found no significant change in estradiol concentrations in serum between days 7 and 42-56 postpartum. Furthermore, Rawlings et al. (1980) found no correlation between concentrations of estradiol in plasma and growth of ovarian follicles in postpartum beef cattle. Similarly, we found no significant correlation between numbers of small, medium or large follicles and serum estradiol within individual cows. Therefore, no

clear association exists between concentrations of estradiol in serum or plasma and return to ovulatory cycles after parturition in suckled beef cattle.

Results of this study provide evidence that the large follicles contribute the majority of ovarian content of estradiol in cattle and that estradiol concentrations in these large follicles correlate significantly (r=.53) with serum concentrations of estradiol. Similarly, in both sheep (McNatty, 1982) and monkeys (Marut et al., 1983) high correlations were found between concentrations of estradiol in large follicles and serum from ovarian venous blood draining the ovary containing the large follicles. Concentrations of estradiol in follicular fluid reflect capacity of a follicle to produce estradiol in vitro (McNatty, 1976; Channing, 1980; England et al., 1981; Hillier et al., 1981; Bieszczad et al., 1982). Thus, strong evidence exists to support the suggestion that changes in concentrations of estradiol and perhaps other steroids in follicular fluid of individual follicles reflect alterations in the capacity of a follicle to synthesize and secrete estradiol. It is important to point out that, although concentrations of estradiol in follicular fluid may reflect synthetic and secretory capacity of a follicle, estradiol produced and secreted by the follicle is diluted in the general circulation. Thus, very dramatic changes in estradiol production within a follicle may be seen as only small changes in the peripheral circulation or no change at all.

Frequency of LH pulses during postpartum anestrus in beef cattle (expressed as pulses/6 h) ranges between 1.2 to 2.2 (Convey

et al., 1983; Humphrey et al., 1983). These LH pulse frequencies are similar to frequencies observed in the present study. Moreover, Convey et al. (1983) observed no significant change in the frequency of LH pulses between days 14 and 42 (2 to 6 weeks before first ovulation) and these findings were confirmed by my study. However, Humphrey et al. (1983) reported frequencies of LH pulses increase over the 6-week period preceding the first postpartum estrus. One obvious difference exists among these studies. Data presented by Convey et al. (1983) and data in my study were expressed as days after parturition, and serum was collected in both studies before the first postpartum ovulation. In contrast, Humphrey et al. (1983) expressed their data as weeks before first estrus. Numerous reports indicate the majority of first ovulations occur without coincident estrus (for review see Wettemann, 1980; Donkin, 1980b). Therefore, it is likely that data from cows already exhibiting ovarian cyclicity were included in results reported by Humphrey et al. (1983). Inclusion of such cows would inflate frequency estimates since frequencies of LH pulses increase from 1 or 2 pulses per 6 h to 5 to 7 pulses per 6 h 2 to 3 days prior to ovulation in both cyclic cattle (Rahe et al., 1980) and in nonsuckled postpartum beef cows (Walters et al., 1982a). Nonetheless, in previous studies (Convey et al., 1983; Humphrey et al., 1983) an increase in amplitude of LH pulses at least 3 weeks before first estrus (or ovulation) was observed. I also observed an increase in LH pulse amplitude 3 to 4 weeks before first ovulation (between days 7 and 14 after parturition). Together these data support the suggestion that amplitude of the LH pulses increases gradually several weeks before

the first postpartum ovulation. Furthermore, this increase in LH secretion with time after parturition is in addition to acute preovulatory changes in LH secretion observed in cattle (Rahe et al., 1980).

Although the secretory pattern of LH may change between days 7 and 14 after parturition (3 to 4 weeks before first ovulation), overall concentrations of LH in serum did not change significantly during this period in the present study. Other reports indicate overall concentrations of LH increase in serum within the first 3 to 10 days after parturition in dairy and beef cattle (Erb et al., 1971; Ingalls et al., 1973; Kesler et al., 1977; Fernandes et al., 1978; Williams et al., 1982; Peters et al., 1981), which may be due to the appearance of LH pulses (Walters et al., 1982a) after parturition. The low LH pulse frequency and amplitude during late gestation in cattle (Little et al., 1982) may be due to high progesterone and estrogen concentrations in serum (Goodman and Karsch, 1980). Perhaps with parturition, the hypothalamus and/or pituitary gland recovers from negative feedback of estradiol and progesterone, resulting in increased frequency of LH pulses (Lamming et al., 1982).

Secretory patterns of FSH in serum do not change with time after parturition in dairy or beef cattle during the first 30 to 50 days (Schallenberger et al., 1978; Williams et al., 1982; Convey et al., 1983). These findings were confirmed in the present study since no change in secretory patterns of FSH was observed between days 7 and 42-56 after parturition. Collectively, these results suggest that FSH may be of little importance in the reinitiation of

ovulatory cycles after parturition or alternatively, that a constant secretory profile of FSH is sufficient to allow the reinitiation of ovulatory cycles after parturition in cattle.

My study is the first to characterize changes in concentrations of steroids in follicular fluid of individual follicles during postpartum anestrus in any mammalian species. Previously, Walters et al. (1982a) found total ovarian follicular fluid content of estradiol and progesterone on day 25 after parturition was not different between nonsuckled and suckled beef cows, even though nonsuckled cows were closely approaching first estrus (Walters et al., 1982c). However, since there is large variation between concentrations of steroids in follicular fluid of individual follicles within a cow (Merz et al., 1981) it is reasonable that no differences in concentrations of steroid hormones in follicular fluid were observed by Walters et al. (1982a) who obtained data from pooled follicles. Indeed, data collected from individual follicles in the present study suggests that significant shifts in progesterone and estradiol production by large follicles occurs during the postpartum anovulatory period in cattle. Progesterone and estradiol concentrations in large follicles are nearly equal on day 7 but by day 14 progesterone was the predominant steroid found in follicular fluid. Subsequently, estradiol became the predominant steroid in follicular fluid on day 28. The importance of these shifts in steroid production is uncertain since no change was observed in estradiol concentration in serum. Perhaps these changes in steroid production are necessary for local intraovarian changes observed, such as increased numbers of medium

follicles, which may be essential for reinitiation of postpartum ovulatory cycles in cattle. Alternatively, since blood samples were collected 18 to 20 h after follicular fluid, changes in steroid production by large follicles could have occurred after blood was collected and thus gone undetected.

On days 42-56 after parturition, progesterone was again the predominant steroid in follicular fluid. This shift from estradiol dominance to progesterone dominance was unexpected. One possible explanation for this phenomenon could be that rates of turnover and atresia of follicles is higher on days 42-56 than day 28, since it is presumed that cows in days 42-56 group are closer to time of first ovulation than cows in the day 28 group. Both turnover (Matton et al., 1981) and atresia (Rajakoski, 1960) of large follicles increase as ovulation approaches. Thus, the increase in concentrations of progesterone in follicular fluid may reflect increased rate of atresia, since high progesterone in follicular fluid is associated with atresia in bovine follicles (Bellin and Ax, 1984). In addition, numbers of medium-sized follicles increase as rate of turnover of large-sized follicles increase (Matton et al., 1981). Thus, increased numbers of medium follicles observed on days 42-56 may reflect increased turnover of large follicles.

These dramatic shifts in steroid production by large follicles were not associated with changes in numbers of gonadotropin binding sites in thecal or granulosal cells, since numbers of gonadotropin binding sites remained relatively constant over time after parturition. However, one exception exists. Concentrations of progesterone in

follicular fluid of large follicles were significantly correlated (r=.45) with LH binding sites in granulosal cells. This association exists because both progesterone concentrations and LH binding sites were lower in follicles on day 7 than on days 14, 28 or 42-56. In contrast, Ireland and Roche (1983a) observed a decrease in LH binding sites in granulosal cells of large follicles ($\geq$6 mm) coincident with an increase in progesterone concentrations between days 3 and 13 of the estrous cycle in beef heifers. Only around the time of the LH surge in cattle does LH binding increase coincident with progesterone in follicular fluid (Ireland and Roche, 1982; 1983b). More often, increasing estradiol concentrations in follicular fluid were associated with increased LH binding to granulosal cells (Ireland and Roche, 1982; 1983a,b; Webb and England, 1982). I found no significant correlation between estradiol in follicular fluid and LH binding sites in granulosal cells of large follicles. This suggests that the control mechanism for follicular steroidogenesis in anestrous cattle may be different than in cyclic cattle. Perhaps a transformation in the functional control of follicular steroidogenesis must occur before ovarian cyclicity resumes after parturition.

A significant increase in the proportion of cattle with one large estrogen-active follicle (estradiol > progesterone in follicular fluid; Ireland and Roche, 1982) was also observed between days 7 and 28 after parturition. This may be responsible for coincident increases in average estradiol concentrations observed in follicular fluid of large follicles. Since high concentrations of estradiol have been associated with healthy, non-atretic follicles (Kruip and Dieleman,

1982; Ireland and Roche, 1982; 1983a,b; Bellin and Ax, 1984), perhaps the increase in proportion of estrogen-active follicles reflects an increase in numbers of large, non-atretic follicles.

The hormonal requirements for increased estradiol production by large follicles observed on day 28 are not known, but increased amplitude of LH pulses at day 14 could play a dominant role in follicular estradiol production as it does in vitro (Peluso et al., 1984a,b). Previously, estradiol concentrations in large follicles were reported to increase coincident with overall mean concentrations of LH in serum 1 to 2 days before the LH surge in cyclic beef heifers (Ireland and Roche, 1982). This suggests that follicular response to gonadotropin changes in serum is more sluggish in anestrous cattle than cyclic cattle.

Although significant increases in numbers of medium follicles occurred with time after parturition, function (as assessed by follicular fluid steroids and gonadotropin binding sites) of these same sized follicles was unchanged between days 7 and 42-56. In contrast, LH binding sites in small follicles decreased between days 7 and 14 with no corresponding change in concentrations of either estradiol or progesterone in follicular fluid. These results suggest a differential control mechanism may exist for steroid production by small and medium bovine follicles.

EXPERIMENT 2

A. Background and Objectives

Previously, low-dose injections of LHRH (500 ng/2 h) given for 4 days in anestrous, suckled beef cows have been shown to induce LH pulses and hasten time to first ovulation (Walters et al., 1982c). The mechanism whereby the LHRH-induced LH pulses stimulate ovarian activity and ovulation remains unknown. Therefore, the objective of Experiment 2 was to characterize the change(s) that occur within the ovary in response to increased frequency of LH pulses in acyclic postpartum beef cows using low-dose injections of LHRH to induce LH pulses. The variables examined were: 1) serum concentrations of gonadotropins and steroids, 2) ovarian follicular inventory and growth, 3) ovarian follicular fluid steroids, and ovarian follicular gonadotropin receptor concentrations.

B. Materials and Methods

1. Animals and Design

Forty anestrous pluriparous beef cows (27 Angus and 13 Hereford) averaging 6 to 7 yr of age and 400 to 500 kg body weight, were allotted to one of five treatment groups beginning on September 14 at USDA-MARC, Clay Center, Nebraska. Two days prior to start of the treatments each week, 10 or 15 cattle were brought in from the range, placed in a holding pen, assigned to one of the five treatment groups, and fed 11 kg dry matter of a supplemented 50:50 corn silage:alfalfa haylage

ration (60% TDN and 12% protein on a dry matter basis). Throughout the treatment period each cow was loosely restrained in stanchion stalls in an unheated enclosed barn and had her suckling calf present. Treatments were begun on day 21.2±.4 after parturition and were: 1) No injections, n=8; 2) Injections of saline for 48 h, n=7; 3) Injections of saline for 96 h, n=7; 4) Injections of LHRH for 48 h, n=9; and 5) Injections of LHRH for 96 h, n=9. Saline (0.9% NaCl, 5 ml) or LHRH[3] (500 ng/5 ml saline) was injected i.v. at 2-h intervals via a jugular cannula. Two h after the last injection, all cows were ovariectomized (ovex) by inserting a serrated spay sissors through the dorsal wall of the vagina. Cows that received no injections were the first group of cattle ovex each week and were ovex at the end of a 6-h bleeding period (see below).

2. Serum, Follicular Fluid and Tissue Collection Procedures

Blood samples (20 ml) were collected via jugular cannula at 15-min intervals for 6 h before and 3 h after ovex. Thus, cattle that received injections were bled during the same time the last three injections were given. Samples were taken at 30-min intervals for an additional 3 h after ovex. Blood samples were stored for 12 to 20 h at 25°C and centrifuged at 1500 x g at 4°C for 15 min; sera were decanted and cooled at 4°C for an additional 2 to 10 h, frozen and stored at -20°C until assayed for LH and FSH as described in Experiment 1. Selected serum samples were analyzed for progesterone

---

[3]Beckman, Palo Alto, CA.

as described in Experiment 1 and for total serum glucocorticoids by competitive protein binding assay (Smith et al., 1973). Concentrations of estradiol in selected serum samples were quantified using an estradiol ^{125}I-RIA Kit (Serono Laboratories, Braintree, MA).

Immediately after ovex, ovaries were placed on ice, a follicular inventory was conducted and follicular fluid and tissue were collected as described in Experiment 1. Mean size and number of follicles dissected from each pair of ovaries in each size category was similar across time and treatment groups (Table 14). Concentrations of estradiol, progesterone and androstenedione were measured in follicular fluid as described in Experiment 1. Gonadotropin binding sites were measured in follicular tissue as described in Experiment 1.

3. Statistical Analyses

For analyses, hormone data were divided into two periods: 1) 6 h before ovex; and 2) 3 h after ovex. Concentrations of progesterone and cortisol in serum were measured twice, 3 h apart, in each period. Concentrations of estradiol in serum were measured twice, 3 h apart, only in Period 1. Means of each variable for serum LH and FSH (as determined in Experiment 1), and mean concentrations of each steroid within a period were subjected to a factorial analysis of variance with "LHRH" and "Interval of Injection" as main effects and interactions. In a separate analysis, "Period" was also included as a main effect to test the effect of ovariectomy on the various hormones. Differences in follicular size and numbers, follicular gonadotropin receptors and follicular fluid hormones were determined

TABLE 14.--Average Diameter and Number of Follicles Dissected from Each Pair of Ovaries in Each Size Category after Either LHRH (L) or Saline (S) Injections for 48 or 96 h.[a]

	Size					
	1.0-3.9 mm (small)		4.0-7.9 mm (medium)		≥8.0 mm (large)	
Group	n[b,c]	Average diameter[c]	n[b,c]	Average diameter[c]	n[b,c]	Average diameter[c]
NI	6.1±1.2	3.4±.1	3.9±1.3	4.9±.2	1.5±.3	11.7±0.9
48S	5.4±1.0	3.2±.1	4.4±0.9	5.1±.2	1.6±.2	9.8±0.4
48L	4.8±0.7	3.2±.1	5.1±0.4	4.8±.2	1.8±.3	10.9±0.7
96S	4.3±1.2	3.2±.2	6.7±1.1	5.4±.3	1.3±.5	11.8±1.2
96L	5.8±0.9	3.2±.1	3.7±1.0	5.4±.3	1.3±.2	11.9±0.6

[a]Mean ± SEM; NI = No injections.

[b]n = number of follicles per cow.

[c]No significant time or treatment effect (P>.10).

by a 2X2 factorial analysis of variance with "LHRH" and "Interval of Injection" as main effects and interactions. Steroid and receptor data from follicles 1.0-3.9, 4.0-7.9 and ≥8.0 mm in diameter were analyzed separately within each size. Any data showing heterogeneous variance were statistically analyzed after using the transformation natural log (X+1). Mean differences were determined using Bonferroni's t test (Gill, 1978) and Fisher's protected LSD mean test (Ott, 1977) where appropriate.

C. Results

1. Follicular Growth

Diameter of the largest or second largest follicle was not affected by either 48 or 96 h of LHRH priming, nor were numbers of follicles within the small (1.0 to 3.9 mm), medium (4.0 to 7.9 mm) or large (≥8.0 mm) categories affected by LHRH priming (Table 15). Numbers of follicles per pair of ovaries (±SEM) in the small, medium and large categories were 24.7±3.9, 11.1±2.5 and 1.8±.4 for saline-treated groups, and 20.4±4.1, 7.0±1.6 and 1.4±.4 for LHRH-treated groups, respectively. Average diameters for first and second largest follicles were 11.2±.8 and 7.5±.6 mm, respectively, for controls, and 11.9±.5 and 7.3±.6, respectively, for LHRH-treated groups (Table 15).

Follicular diameter and follicular fluid volume of 243 follicles were significantly correlated ($r=.868$, $P<.001$) with each other (Figure 23A). Furthermore, logarithmic transformation (log 10) of both diameter and volume resulted in a better linear relationship ($r=0.985$, $P<.001$, Figure 23B).

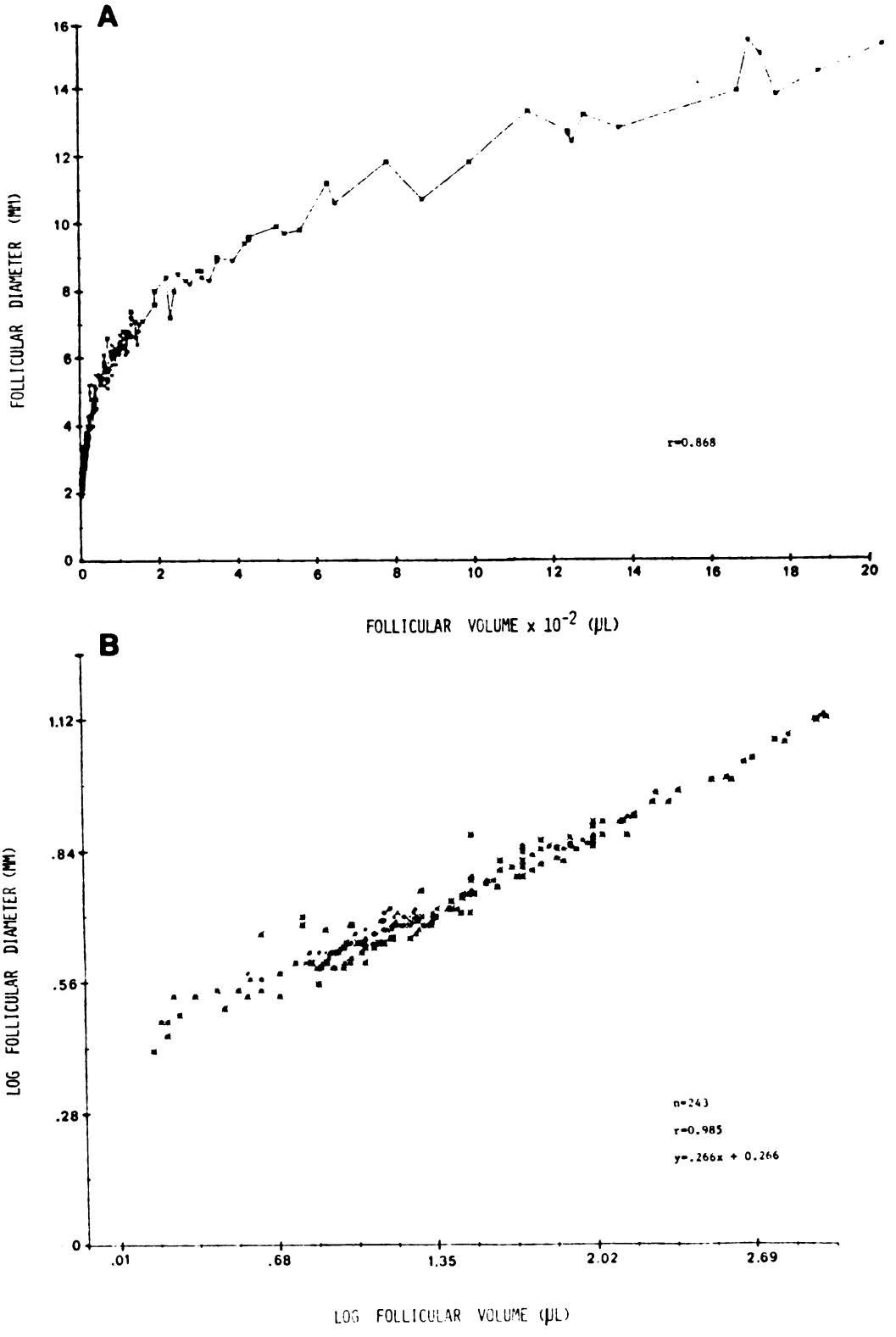
TABLE 15.--Numbers of Small, Medium and Large Antral Follicles Per Pair of Ovaries, and Diameters of First and Second Largest Follicles in Anestrous, Postpartum Beef Cows Injected for 48 or 96 h With Either LHRH (L) or Saline (S).[a]

	Numbers of follicles/pair of ovaries[b]			Diameter (mm)[b]	
Group	Small	Medium	Large	First largest	Second largest
NI	21.9±4.0	5.1±2.0	1.50±.42	12.7±.8	6.5±.8
48 S	26.1±5.5	9.3±1.5	1.43±.38	10.6±.7	7.5±.7
48 L	16.4±2.2	8.2±1.6	1.60±.42	11.5±.5	7.6±.6
96 S	23.3±2.4	12.8±3.5	1.99±.42	11.7±.9	7.6±.4
96 L	25.3±5.9	6.2±1.5	1.33±.44	12.4±.5	7.0±.6

[a]Means ± SEM; NI = no injections.

[b]No significant differences (P>.10).

Figure 23.--Relationship between follicular diameter and follicular fluid volume of 243 follicles dissected from 32 pair of ovaries. A, simple correlation coefficient of actual data, r=.868. B, simple correlation coefficient of $\log_{10}$ versus $\log_{10}$ plot, r=.985.


A
FOLLICULAR DIAMETER (MM)
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
r=0.868
FOLLICULAR VOLUME x 10^{-2} (µL)
B
LOG FOLLICULAR DIAMETER (MM)
1.12
.84
.56
.28
0
.01
.68
1.35
2.02
2.69
n=243
r=0.985
y=.266x + 0.266
LOG FOLLICULAR VOLUME (µL)

2. Serum Hormones

Progesterone.--Mean concentrations (±SEM) of progesterone in serum before ovex were .42±.04 ng/ml for LHRH-treated groups (n=18) and .42±.03 ng/ml for control cows (n=14). Concentrations of progesterone in serum were higher ($P<.05$) in cows receiving no injections (NI) before ovex than in either saline- or LHRH-treated groups. After ovex, concentrations of progesterone in serum increased ($P<.05$) above pre-ovex concentrations (Figure 24) in only the NI group (.58±0.08 vs 1.12±.40 ng/ml).

Estradiol.--Concentrations of estradiol in serum were not significantly affected by 48 or 96 h of LHRH priming but tended ($P<.20$) to be higher after 96 h of either saline or LHRH injections as compared with 48 h of injections (Figure 25). Although not significantly different, mean concentrations of estradiol were 15 to 20% greater after LHRH priming than after saline injections. Concentrations of estradiol were not measured after ovex.

Cortisol.--Concentrations of cortisol in serum were not different between saline- and LHRH-treated groups either before or after ovex (Figure 26). However, concentrations of cortisol in serum increased ($P<.05$) after ovex in both saline- and LHRH-treated groups, from 16.6±2.9 ng/ml to 35.9±5.5 ng/ml. In addition, concentrations of cortisol in serum were higher in the NI group than in saline- and LHRH-treated groups, both before and after ovex. Mean concentrations for uninjected controls were 64.6±12.8 ng/ml before ovex and 76.5±16.3 ng/ml after ovex (Figure 26).

Figure 24.--Concentrations of progesterone in serum before and after ovariectomy (OVEX). Values are means ± SEM. Arrow indicates time of ovex. 48- and 96-h LHRH treatment group means (▨) were combined as were the saline treatment group means (▭). Uninjected controls (▭).

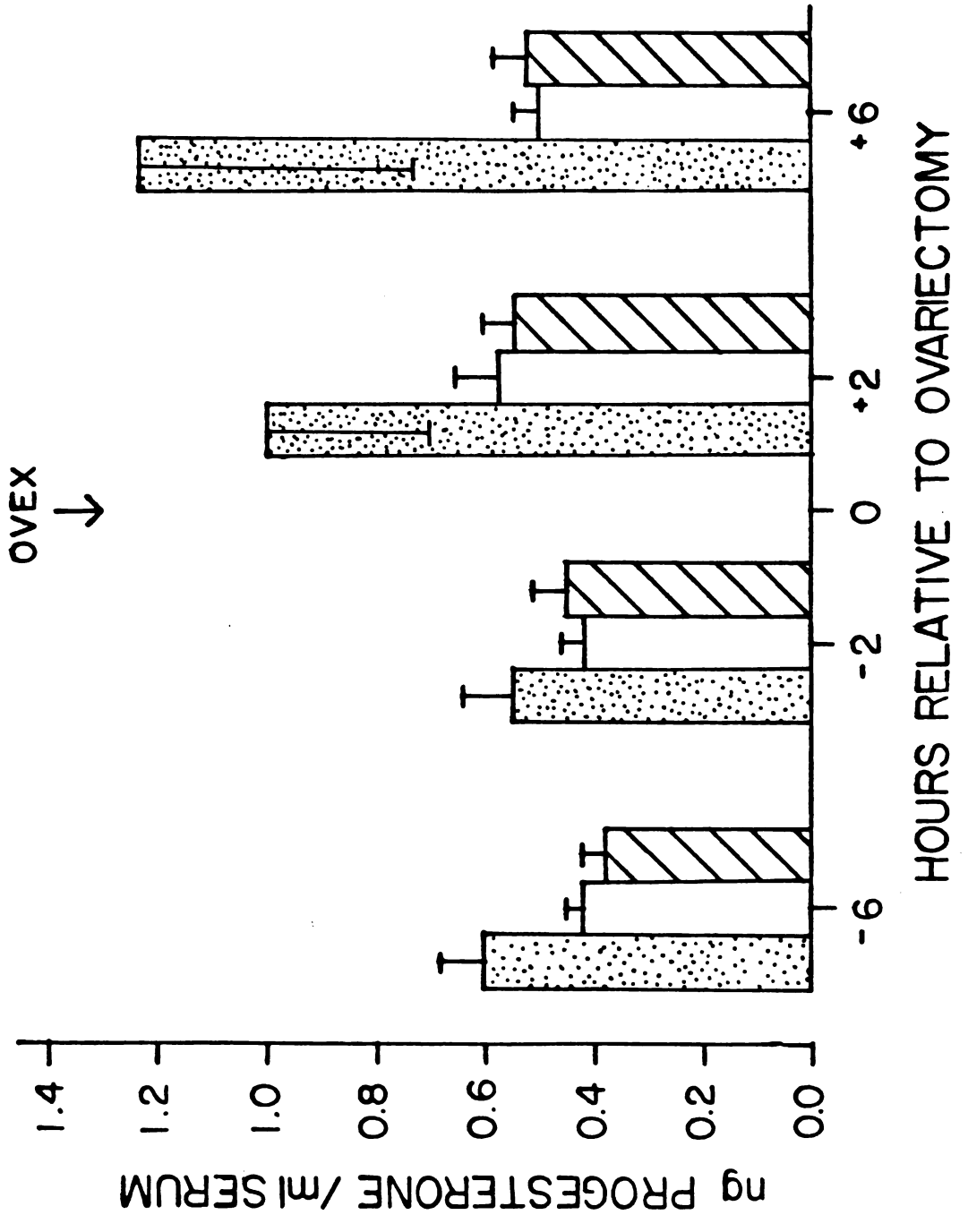

ng PROGESTERONE /ml SERUM
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
OVEX
-6
-2
0
+2
+6
HOURS RELATIVE TO OVARIECTOMY

Figure 25.--Concentrations of estradiol in serum before ovariectomy in cows injected for either 48 or 96 h with saline (□) or LHRH (▨). Values are means ± SEM of samples collected 2 and 4 h before ovariectomy.

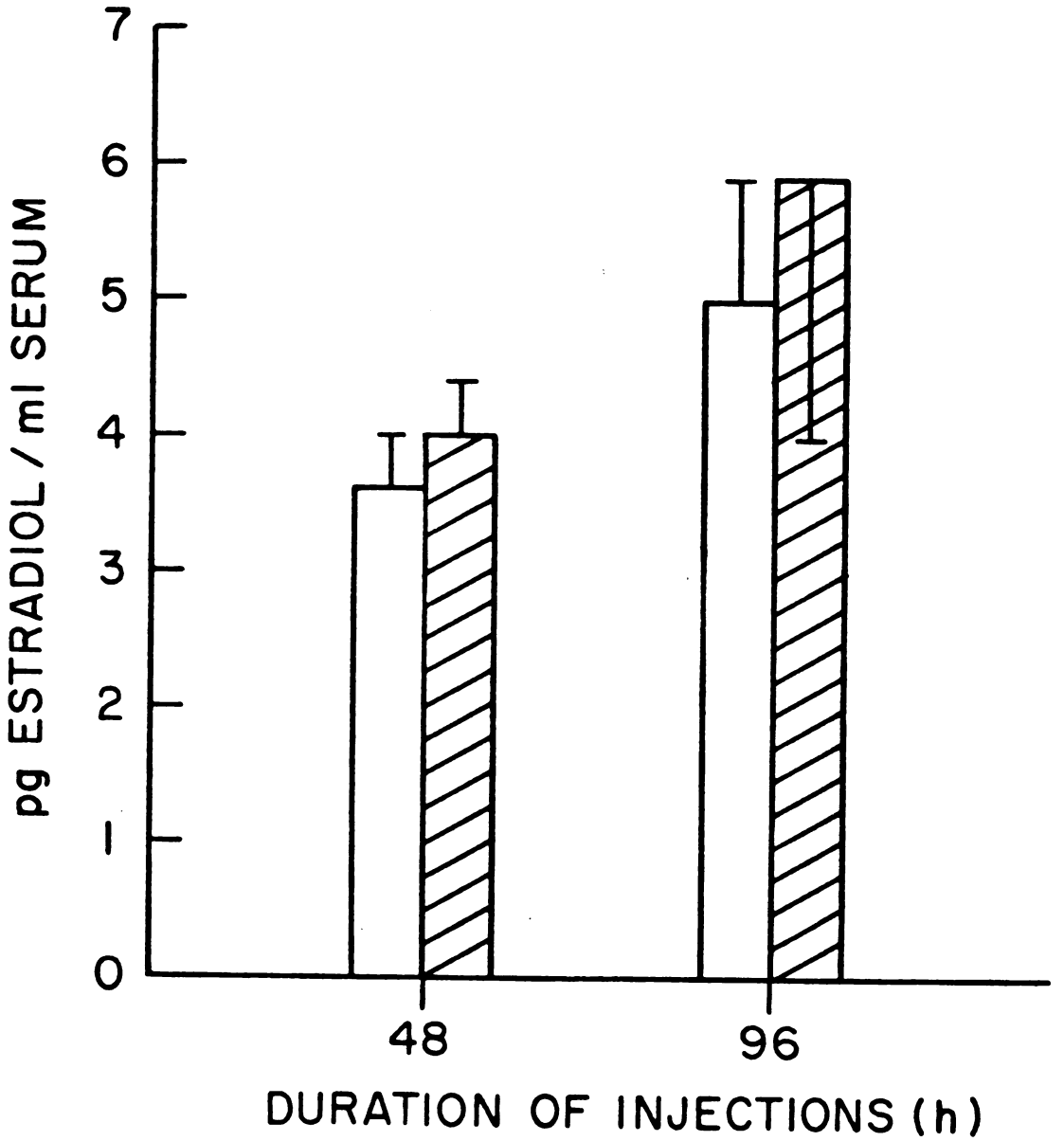
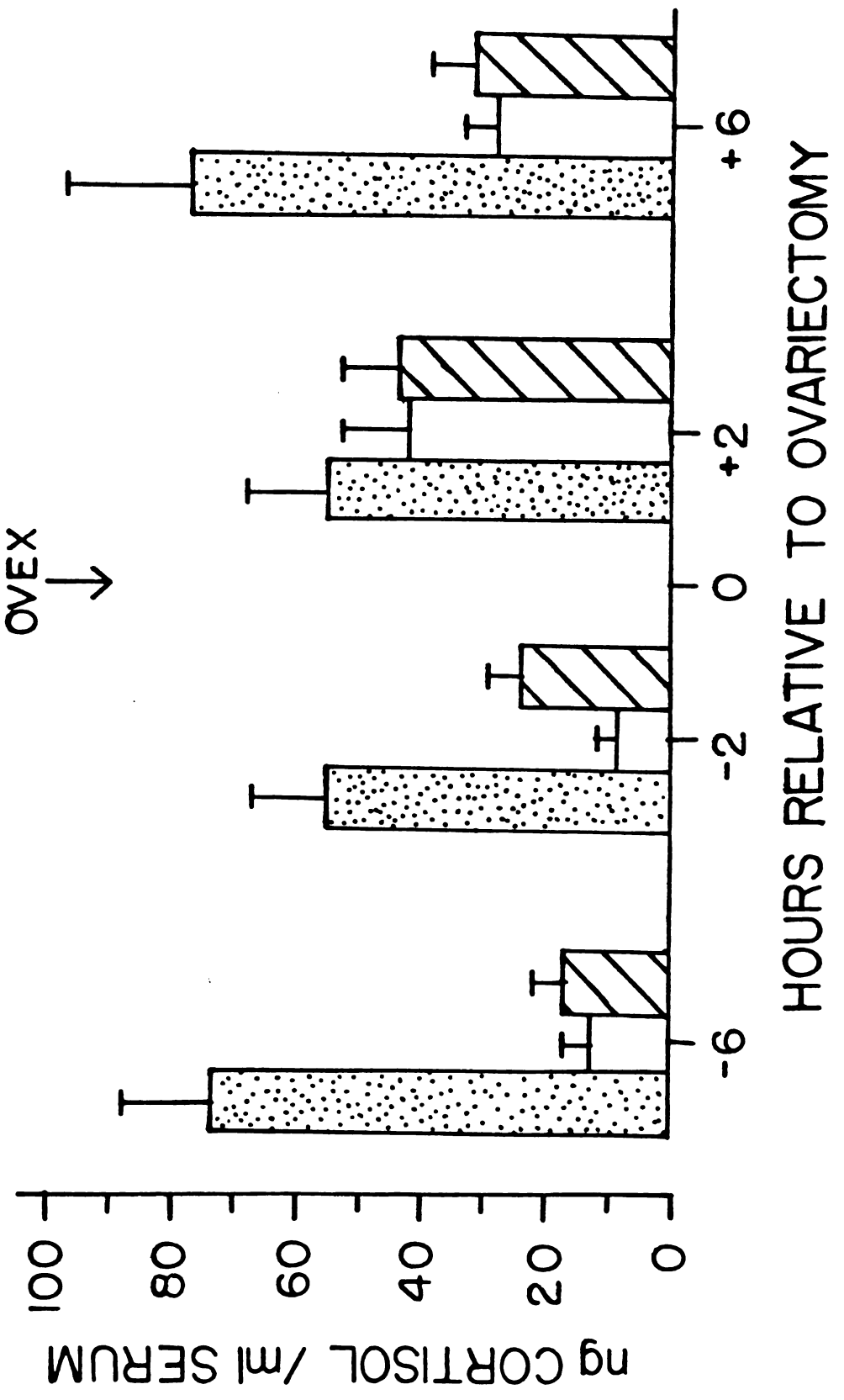

7
6
5
4
3
2
1
0
pg ESTRADIOL / ml SERUM
48
96
DURATION OF INJECTIONS (h)

Figure 26.--Concentrations of cortisol in serum before and after ovariectomy (OVEX). Values are means ± SEM. Arrow indicates time of ovex. 48- and 96-h LHRH treatment group means (▨) were combined as were the saline treatment group means (□). Uninjected controls (▩).


ng CORTISOL /ml SERUM
100
80
60
40
20
0
OVEX
-6
-2
0
+2
+6
HOURS RELATIVE TO OVARIECTOMY

Luteinizing Hormone (LH).--LHRH priming for 48 or 96 h did not affect (P>.10) mean baseline concentrations of LH in serum but increased (P<.01) frequency of pulses of LH nearly 2-fold from 1.57 per 6 h in controls to 2.94 in LHRH-treated groups (Table 16). Although frequency of LH pulses increased, height and amplitude (height minus baseline) of LH pulses decreased (P<.01) after LHRH priming which resulted in no change in overall mean concentration of LH in serum. Average amplitude of LH pulses was 2.56 ng/ml in saline-treated cows and 1.13 ng/ml in LHRH-treated cows (Table 16). LHRH priming did not affect (P>.05) LH secretion 0 to 6 h after ovex (Table 17).

Frequency of LH pulses was 73% lower (P<.05) after ovex (.43 pulse/6 h) than before ovex (1.57 pulses/6 h) in all saline-control groups except the NI group (Table 17). Before ovex, animals in the NI group had lower (P<.05) frequency, height and amplitude (height minus baseline) of LH pulses, and lower mean baseline and overall LH than any of the other groups (Table 16). The pulsatile pattern of LH release before ovex for one representative cow from either saline- or LHRH-treated groups is depicted in Figure 27. Each representative cow was selected based on having a secretory profile closest to the average of the group.

Follicle-Stimulating Hormone (FSH).--Frequency of FSH pulses significantly increased only after 96 h of LHRH priming (Table 18). However, LHRH priming for 48 or 96 h had no effect (P>.10) on the height of FSH pulses. In contrast, mean baseline of FSH in serum

TABLE 16.--Variables of LH Secretion in Anestrous Cattle During the Last 6 h of Either LHRH (L) or Saline (S) Injections for 48 or 96 h.[a]

Group	N	Frequency (number/6 h)[b]	Baseline (ng/ml)	Overall (ng/ml)	Amplitude (ng/ml) (n)[b]	Height (ng/ml)[b]
NI	8	.25±.16[c]	.32±.07	.34±.06[c]	.44±.04 (2)[c]	.60± .09[c]
48 S	7	1.43±.30[d]	.53±.10	.94±.18[d,e]	2.42±.54 (6)[d]	2.86± .61[d]
48 L	9	3.00±.00[e]	.44±.06	.72±.08[d]	.81±.11 (9)[e]	1.25± .14[c]
96 S	7	1.71±.68[d]	.65±.19	1.04±.25[d,e]	2.69±.98 (5)[d]	3.38±1.22[d]
96 L	9	2.89±.11[e]	.59±.07	1.06±.13[e]	1.45±.36 (9)[e]	2.05± .36[c,d]

[a]Mean ± SEM; N = number of animals; n = number of peaks; NI = no injections.

[b]Significant LHRH effect (P<.01).

[c,d,e]Means with different superscripts differ (P<.05).

TABLE 17.--Variables of LH Secretion During the 6-h Period Immediately Following Ovariectomy in Anestrous Cattle Previously Injected for 48 or 96 h With Either LHRH (L) or Saline (S).[a]

Group	N	Frequency (number/6 h)[b]	Baseline (ng/ml)[c]	Overall (ng/ml)[c]	Amplitude (ng/ml)	(n)[b]	Height (ng/ml)
NI	8	.00±.00	.28±.06	.28±.06	---	(0)	---
48 S	7	.00±.00[d]	.54±.10	.54±.10	---	(0)	---
48 L	9	.00±.00[d]	.34±.06	.34±.06	---	(0)	---
96 S	7	.86±.59[d]	.66±.20	.73±.21	.82±.19	(2)	1.50±.52
96 L	9	.11±.11[d]	.45±.08	.46±.08	.73	(1)	1.47

[a]Mean ± SEM; N = number of animals; n = number of peaks; NI = no injections.

[b]Time effect approached significance (P<.10).

[c]LHRH effect approached significance (P<.10).

[d]Frequencies were significantly lower (P<.05) than frequencies before ovariectomy (Table 16).

Figure 27.--Temporal patterns of LH in serum from representative cows during 6 h before ovariectomy selected from either saline- or LHRH-treated groups. Arrows indicate time of injections and asterisks indicate defined pulses.

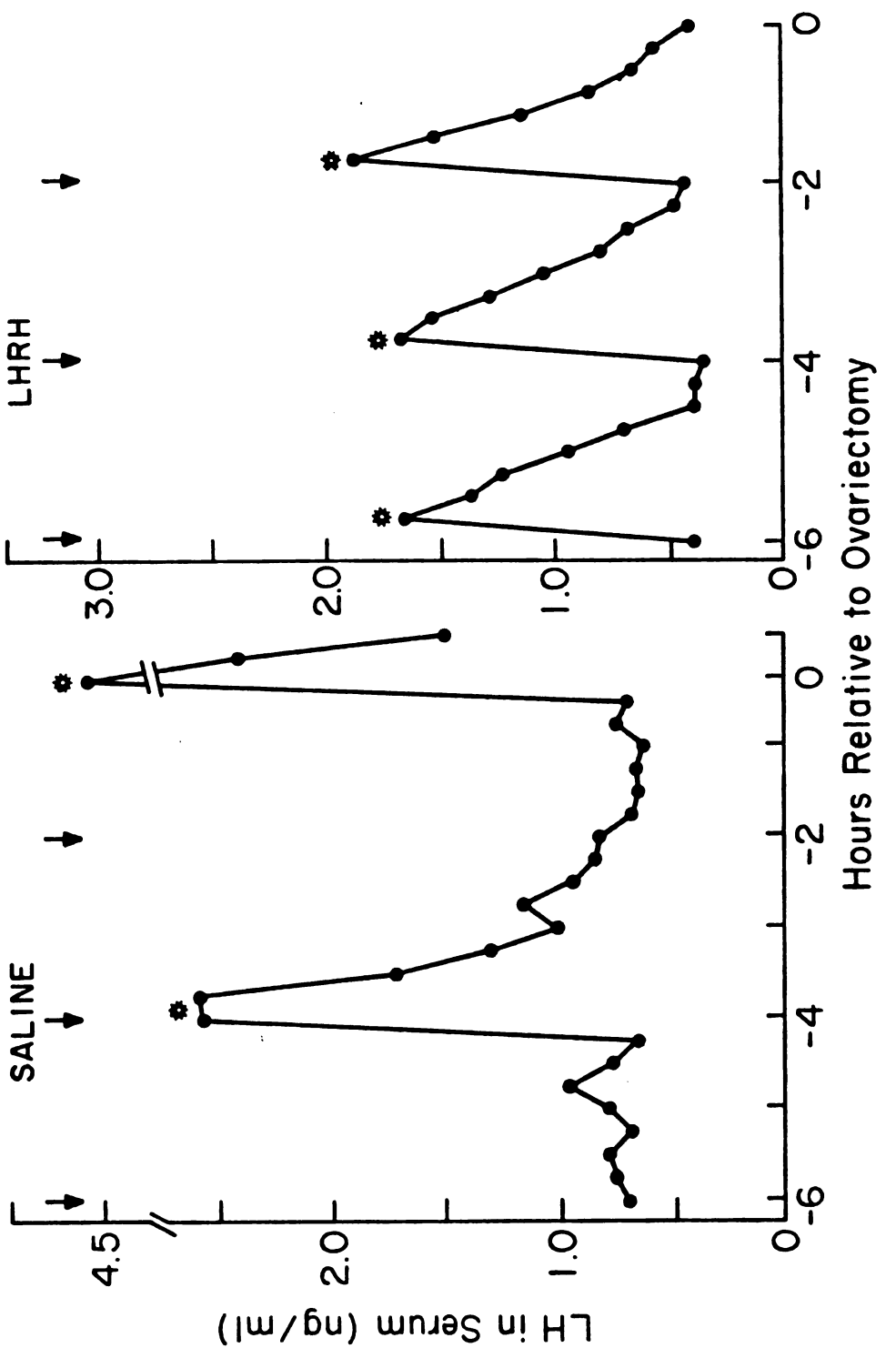

SALINE
LHRH
LH in Serum (ng/ml)
4.5
2.0
1.0
3.0
2.0
1.0
0
-2
-4
-6
Hours Relative to Ovariectomy

TABLE 18.--Variables of FSH Secretion in Anestrous Cattle During the Last 6 h of Either LHRH (L) or Saline (S) Injections for 48 or 96 h.[a]

Group	N	Frequency (number/6 h)[b]	Baseline (ng/ml)[b]	Overall (ng/ml)[f]	Amplitude (ng/ml)[b] (n)	Height (ng/ml)
NI	8	.88±.48[c]	66.3±8.6[c]	67.8±9.4	20.9±3.7[c,d] (7)	83.1±18.9
48 S	7	1.57±.29[c]	42.6±2.9[d]	48.9±3.5	27.9±2.4[d,e] (11)	69.6± 4.3
48 L	9	1.89±.31[c,d]	55.5±5.5[e]	59.2±5.5	19.0±2.2[c] (17)	74.2± 5.4
96 S	7	1.43±.65[c]	45.6±4.4[d,e]	50.9±3.9	32.4±4.6[e] (10)	75.2± 5.1
96 L	9	2.56±.38[d]	53.3±4.6[d,e]	59.0±4.8	21.6±2.6[c,d] (23)	75.0± 5.6

[a]Mean ± SEM; N = number of animals; n = number of peaks; NI = no injections.

[b]Significant LHRH effect ($P<.05$).

[c,d,e]Means with different superscripts differ ($P<.05$).

[f]LHRH effect approached significance ($P<.10$).

increased (P<.05) 30% after 48 h of LHRH priming but increased only 17% (P>.05) after 96 h of LHRH priming. Thus, FSH pulse amplitude (height minus baseline) decreased (P<.05) 32 to 34% after 48 and 96 h of LHRH priming (Table 18). Together, these changes in FSH secretion induced by LHRH priming resulted in only a slight (P<.10) increase in overall concentrations of FSH during the 6-h sampling period. Overall concentrations of FSH were 16 to 21% greater in LHRH-treated than saline-treated cows. The pulsatile pattern of FSH release before ovex for one representative cow from either saline- or LHRH-treated groups is depicted in Figure 28. Each representative cow was selected based on having a secretory profile closest to the average of the group.

LHRH priming had no affect (P>.05) on secretion of FSH after ovex (Table 19). In saline-control groups, frequency of FSH pulses was 86% lower (P<.05) after ovex than before ovex (.21 pulses 16 h vs 1.50 pulses/6 h). Animals in the NI group had higher (P<.05) mean baseline and overall concentrations of FSH in serum than in saline-treated groups before ovex (Table 18).

3. Follicular Fluid Hormones

Progesterone.--Concentrations of progesterone in follicular fluid of small follicles were not affected (P>.10) by either 48 or 96 h of LHRH priming (Figure 29). In medium follicles, concentrations of progesterone in follicular fluid was unchanged after 48 h of LHRH priming but increased 1.5-fold (P<.05) after 96 h of LHRH priming (Figure 30A). Concentrations of progesterone in large follicles were

Figure 28.--Temporal patterns of FSH in serum from representative cows during 6 h before ovariectomy selected from either saline- or LHRH-treated groups. Arrows indicate time of injections and asterisks indicate defined pulses.

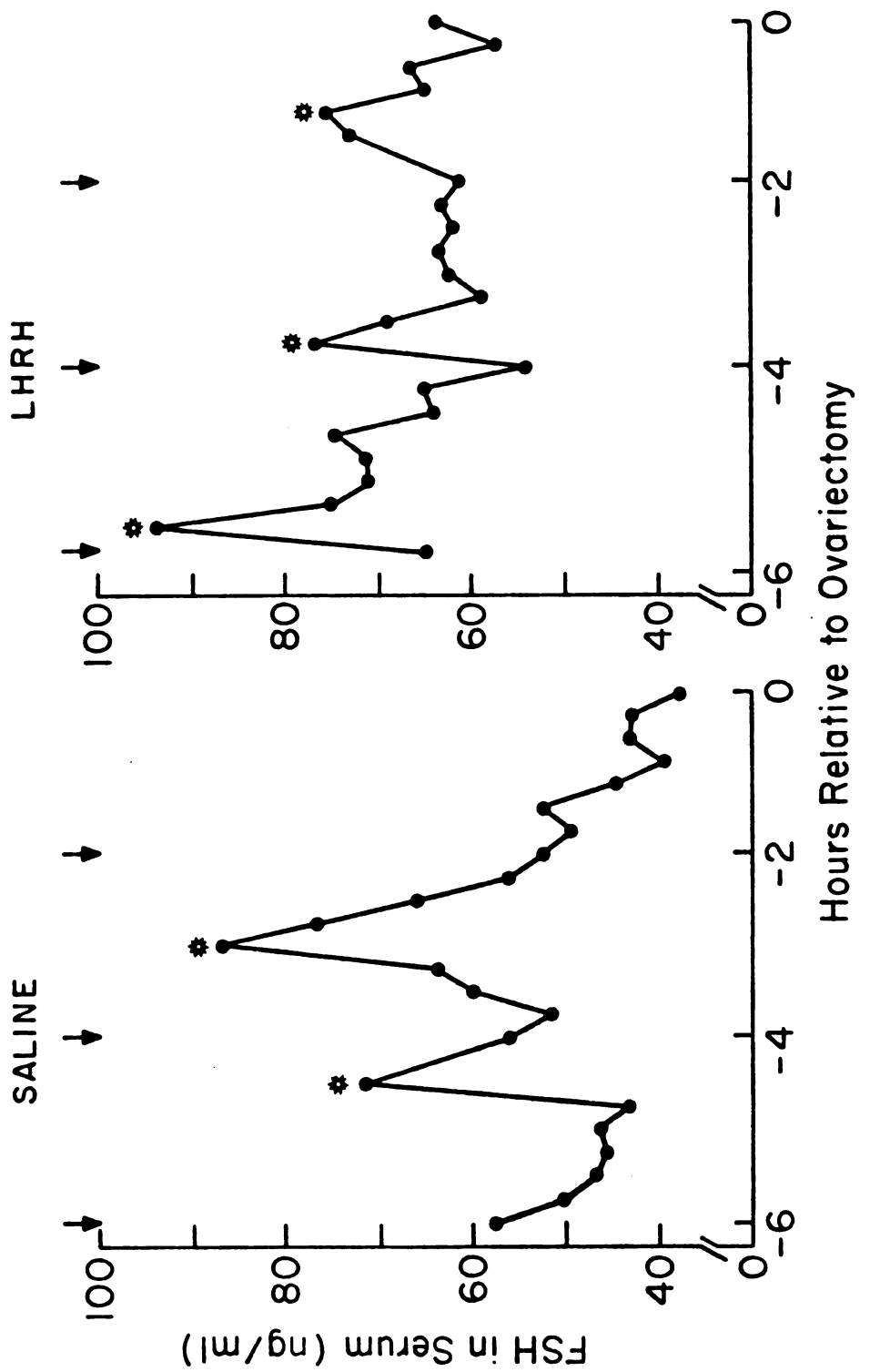

SALINE
LHRH
FSH in Serum (ng/ml)
100
80
60
40
0
-2
-4
-6
Hours Relative to Ovariectomy

TABLE 19.--Variables of FSH Secretion During the 6-h Period Immediately Following Ovariectomy in Anestrous Cattle Previously Injected for 48 or 96 h With Either LHRH (L) or Saline (S).[a]

Group	N	Frequency (number/6 h)	Baseline (ng/ml)	Overall (ng/ml)	Amplitude (ng/ml) (n)	Height (ng/ml)
NI	8	.25±.25	65.4±8.1	66.7±9.2	28.8±12.9 (2)	144.8±12.9
48 S	7	.43±.20[b]	40.8±2.6	42.4±2.2	27.8± 6.3 (3)	64.5± 7.3
48 L	9	.44±.18[b]	54.6±5.3	56.4±5.5	21.8± 3.8 (4)	70.2±10.1
96 S	7	.00±.00[b]	47.7±4.1	47.7±4.1	--- (0)	---
96 L	9	.25±.16[b]	50.2±4.4	50.9±4.3	21.4±10.6 (2)	76.0± 0.6

[a]Mean ± SEM; N = number of animals; n = number of peaks; NI = no injections.

[b]Frequencies were significantly lower ($P<.05$) than frequencies before ovariectomy (Table 18).

Figure 29.--Concentrations of progesterone (P) and estradiol (E) in follicular fluid of small follicles (1.0 to 3.9 mm) after 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy.

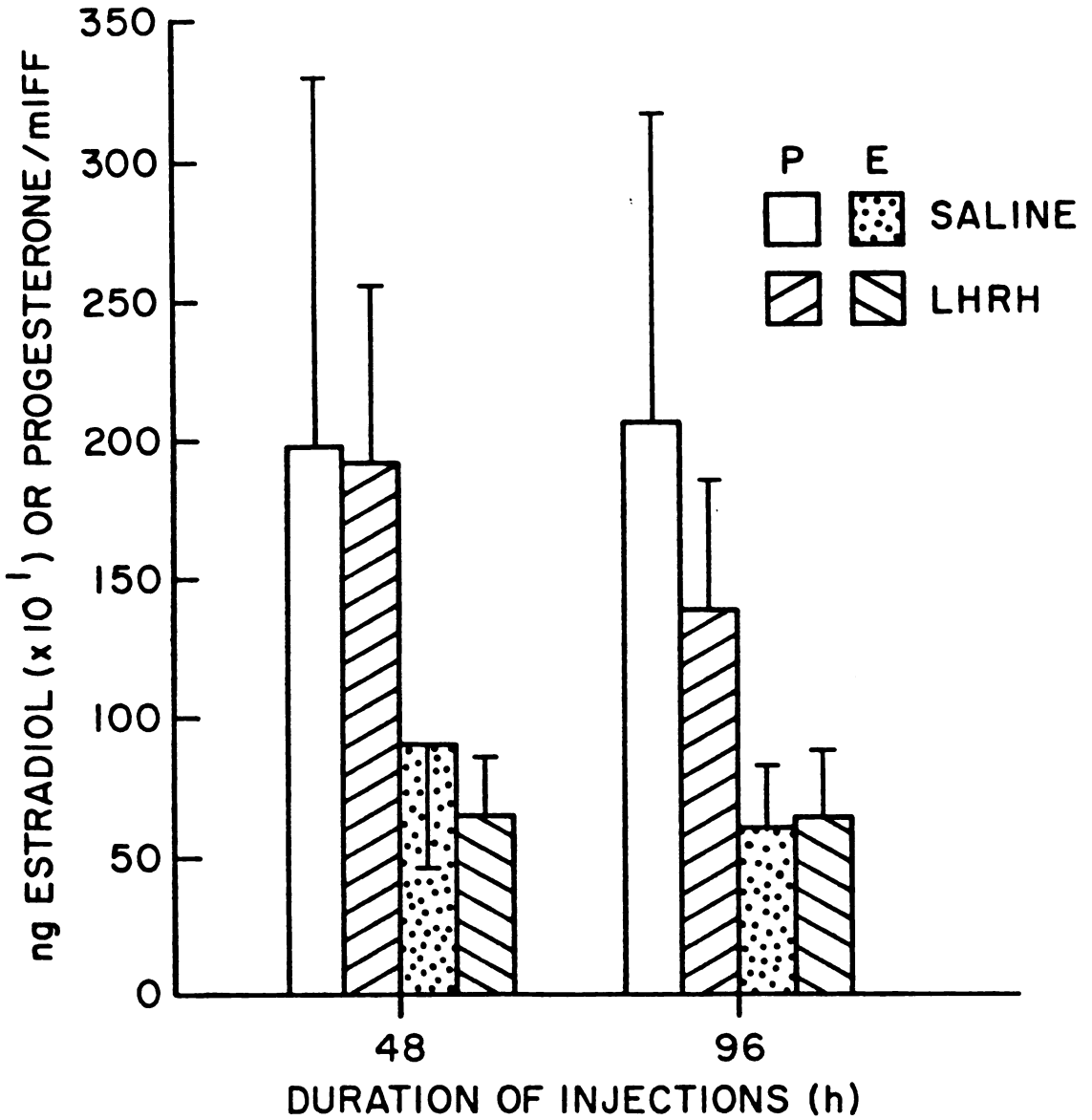
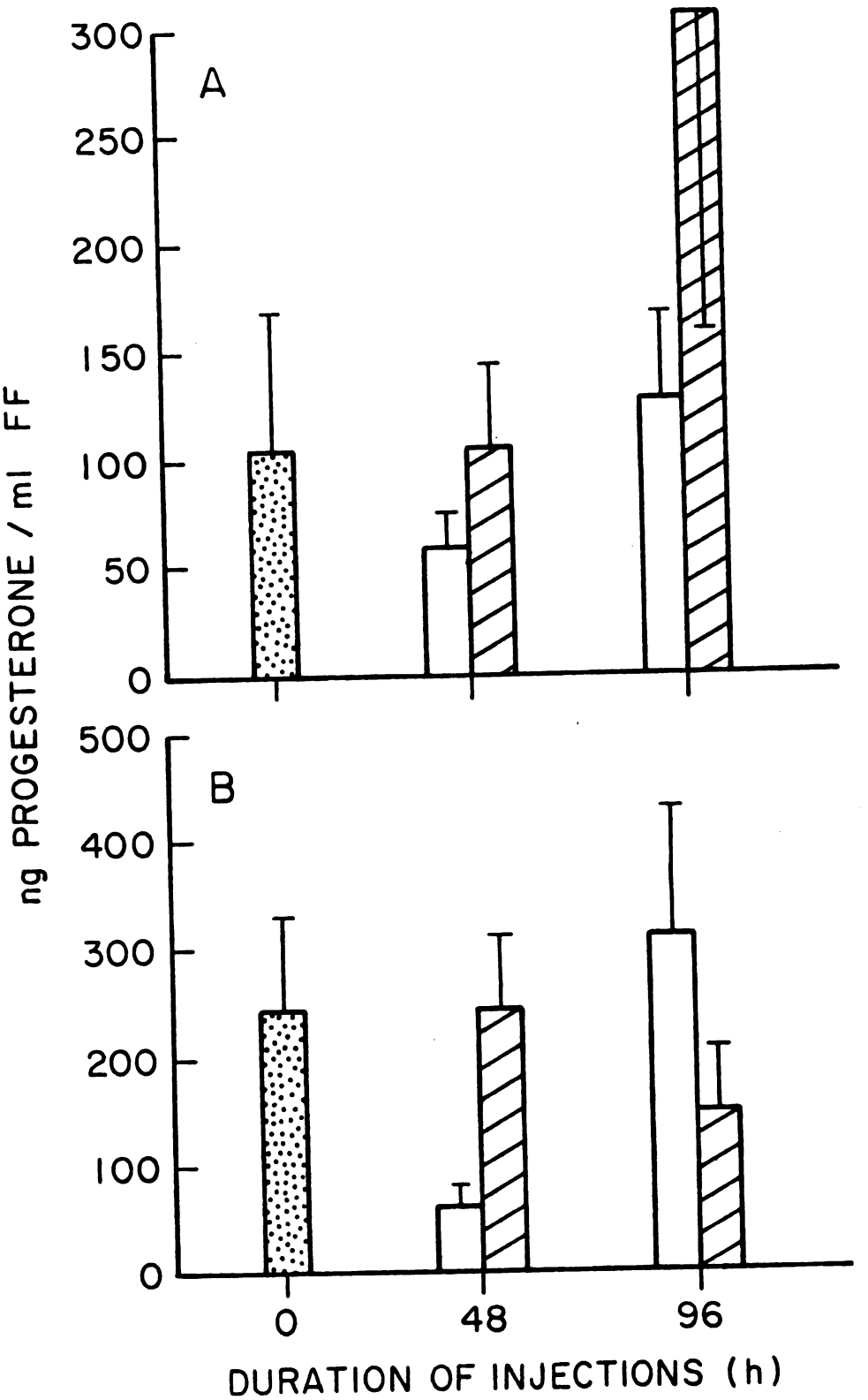

350
300
250
200
150
100
50
0
ng ESTRADIOL (x 10 ⁻¹) OR PROGESTERONE/mlFF
P
E
SALINE
LHRH
48
96
DURATION OF INJECTIONS (h)

Figure 30.--Concentrations of progesterone in follicular fluid of medium (A) and large (B) follicles after 0, 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls ( ); saline ( ); LHRH ( ).


A
B
300
250
200
150
100
50
0
500
400
300
200
100
0
ng PROGESTERONE / ml FF
0
48
96
DURATION OF INJECTIONS (h)

4-fold higher ($P<.05$) in LHRH-treated cows than saline-treated cows after 48 h of injections. Subsequently, concentrations of progesterone in large follicles increased 5-fold ($P<.05$) between 48 and 96 h in saline-treated groups but decreased slightly ($P>.10$) between 48 and 96 h in LHRH-treated groups such that saline-treated cattle had 2-fold greater progesterone concentrations in follicular fluid of large follicles at 96 h than LHRH-treated cows (Figure 30B).

Androstenedione.--LHRH priming for 48 or 96 h did not affect ($P>.10$) concentrations of androstenedione in follicular fluid of medium or large follicles (Figure 31). In addition, concentrations of androstenedione were nearly 2-fold greater in medium follicles (25.6±5.2 ng/ml) than in large follicles (13.0±2.3 ng/ml). Androstenedione was not measured in small follicles due to limited sample volume.

Estradiol.--LHRH priming for 48 or 96 h did not affect ($P>.10$) concentrations of estradiol in follicular fluid of small or medium follicles (Figures 29 and 32A). In contrast, concentrations of estradiol in large follicles increased 2.3-fold ($P<.05$) between 48 and 96 h in LHRH-treated animals, whereas concentrations of estradiol in large follicles decreased 74% in saline-treated animals during the same interval (Figure 32B). Concentrations of estradiol in follicular fluid of these large follicles were 84±34 ng/ml and 60±21 ng/ml at 48 h, and 22±14 ng/ml and 141±34 ng/ml at 96 h for saline- and LHRH-treated groups, respectively. In addition, mean concentrations of estradiol were similar between small (6.3±0.6 ng/ml) and medium

Figure 31.--Concentrations and androstenedione in follicular fluid of medium (A) and large (B) follicles after 0, 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls (); saline (); LHRH ().

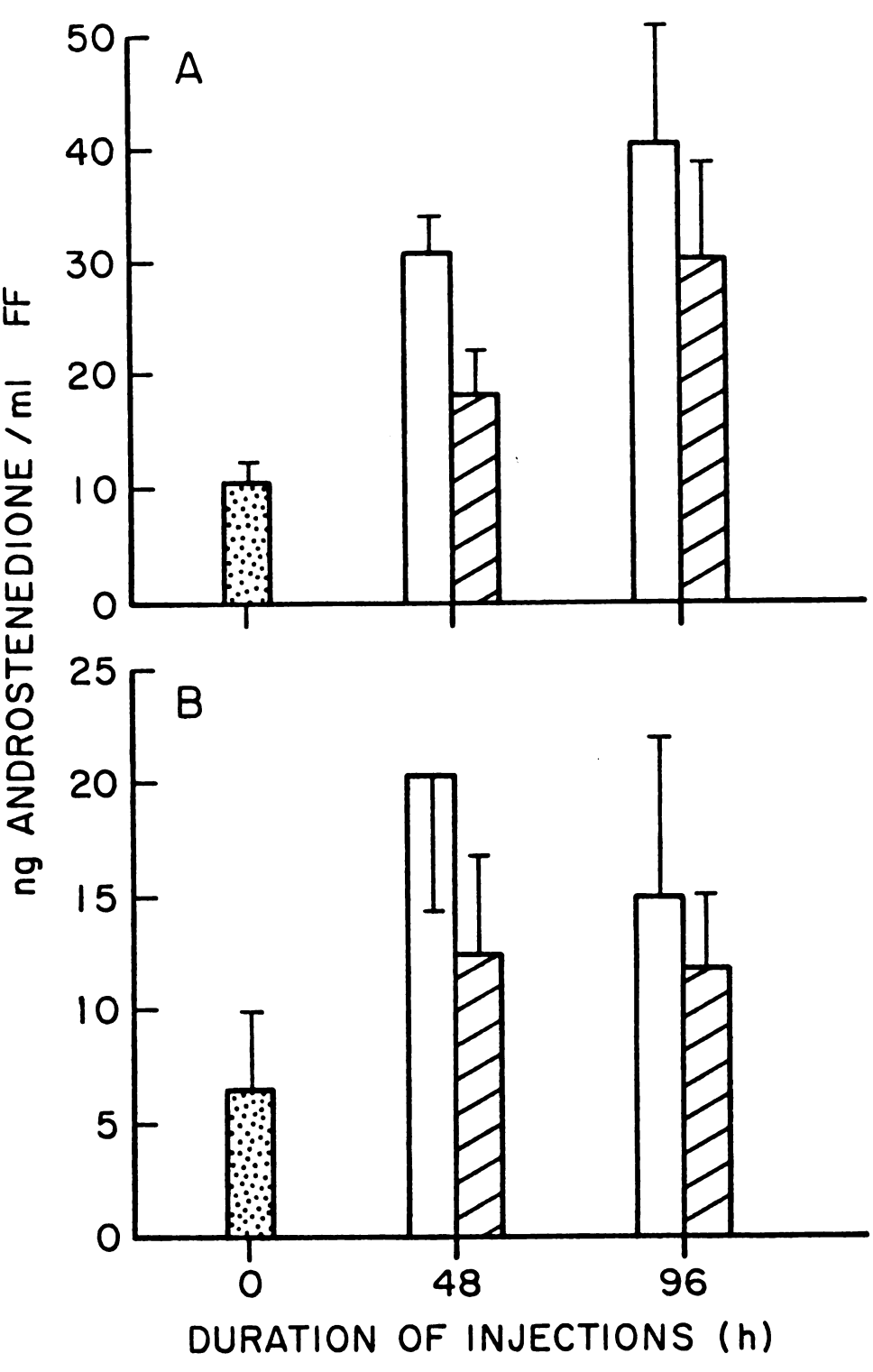
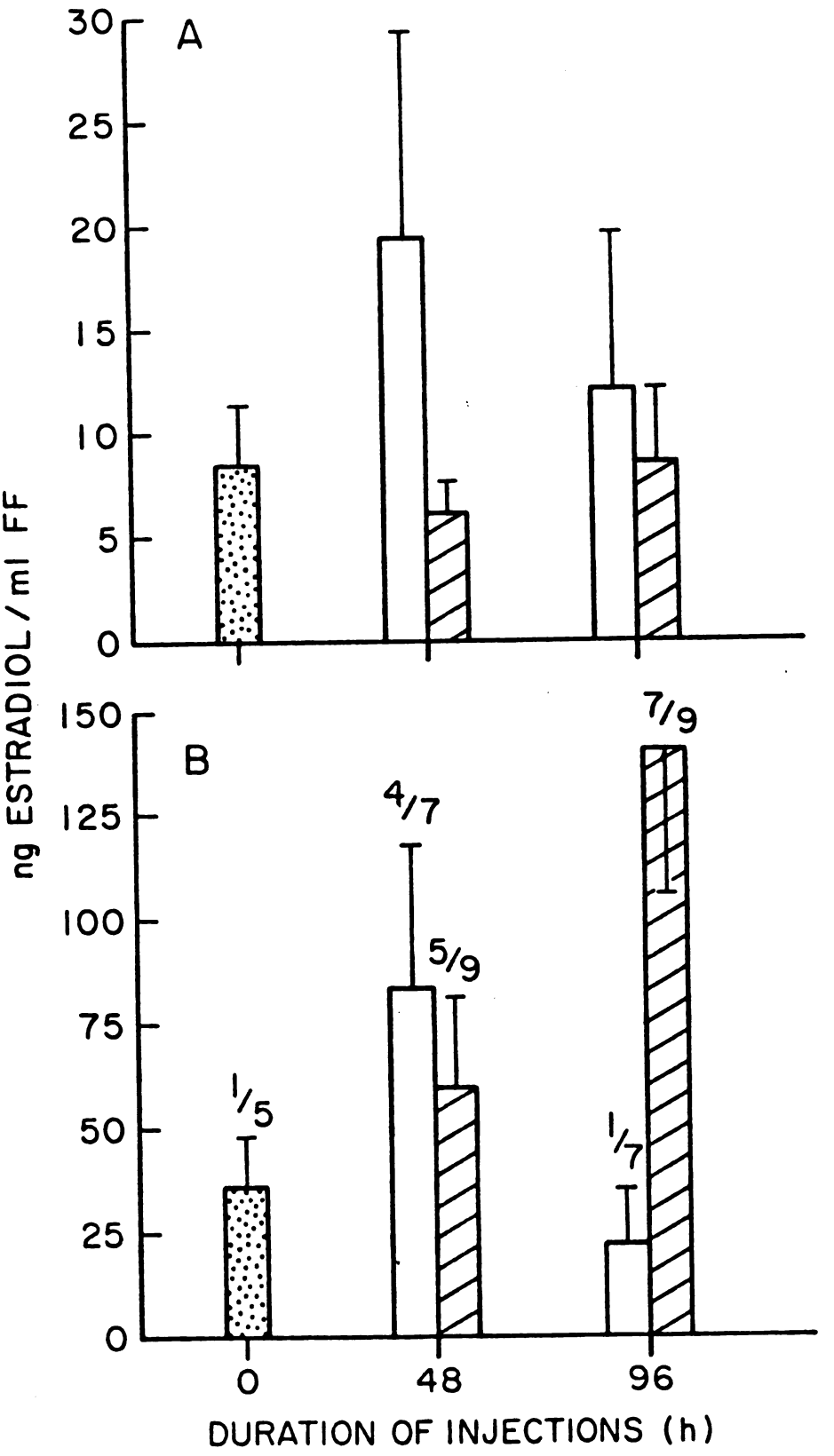

A
B
50
40
30
20
10
0
25
20
15
10
5
0
0
48
96
ng ANDROSTENEDIONE / ml FF
DURATION OF INJECTIONS (h)

Figure 32.--Concentrations of estradiol in follicular fluid of medium (A) and large (B) follicles after 0, 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls (); saline (); LHRH (). Numbers above bars are numbers of cows with at least one large estrogen-active (estradiol > progesterone in follicular fluid) follicle per total numbers of cows.


A
B
30
25
20
15
10
5
0
150
125
100
75
50
25
0
1/5
4/7
5/9
1/7
7/9
0
48
96
ng ESTRADIOL / ml FF
DURATION OF INJECTIONS (h)

(10.9±2.3 ng/ml) follicles. Large follicles contained 6- to 11-fold greater concentrations of estradiol (68.0±21.1 ng/ml) than small or medium follicles.

Although concentrations of estradiol in serum did not change significantly with hours of injections of LHRH, estradiol concentrations in serum were positively correlated ($r=.588$, $P<.001$) with average concentrations of estradiol in follicular fluid of large follicles removed from the same animals less than 4 h later.

Since ratios of steroids in follicular fluid have been used to access atretic state of follicles (Ireland and Roche, 1982; Westergaad et al., 1982; Bellin and Ax, 1984), ratios of progesterone to androstenedione, androstenedione to estradiol, and progesterone to estradiol were calculated for medium and large follicles (Table 20). Only ratios of progesterone to estradiol could be calculated for small follicles. Ratios of progesterone to androstenedione in medium or large follicles were not affected by LHRH priming but were higher in large follicles than in medium follicles after 48 h of LHRH priming and 96 h of saline injections. Androstenedione to estradiol ratios were not affected by LHRH priming and were similar between medium and large follicles. Ratios of progesterone to estradiol were not affected by LHRH priming in either small or medium follicles. Ratios of progesterone to estradiol were also similar in medium and small follicles. In large follicles, ratios of progesterone to estradiol were higher after 48 h of LHRH priming but lower after 96 h of LHRH priming as compared with saline-injected cows at each time (Table 20).

TABLE 20.--Ratios of Steroids in Follicular Fluid of Small, Medium and Large Follicles in Anestrous, Postpartum Beef Cows Injected for 48 or 96 h with Either LHRH or Saline.

Group[b]	N	Ratio[a] P:A	A:E	P:E
48-Saline				
Small	38	---	---	849±752[c]
Medium	30	3±2[c]	34±15	122±68[c]
Large	11	6±2[c]	7±5	23±13[d]
48-LHRH				
Small	44	---	---	167±47[c]
Medium	50	7±2[c]	21±5	82±20[c]
Large	16	102±42[d]	19±13	152±74[c]
96-Saline				
Small	30	---	---	92±27[c]
Medium	42	9±3[c]	32±8	81±21[c]
Large	10	159±77[d]	8±6	296±129[d]
96-LHRH				
Small	50	---	---	200±109[c]
Medium	31	13±6[c]	15±4	83±30[c]
Large	12	37±21[c,d]	6±4	11±9[d]

[a]P = progesterone, A = androstenedione, E = estradiol; Means ± SEM.

[b]Small = 1-3.9 mm, medium = 4-7.9 mm, large = ≥8 mm.

[c,d]Means within a column with different superscripts differ ($P<.05$).

4. Gonadotropin Binding in Follicular Tissue

Large Follicles.--Specific binding of ^{125}I-hCG to thecal-enriched homogenates and granulosal cells was not different between saline- and LHRH-treated groups at 48 h (Figures 33 and 34A). However, specific binding of ^{125}I-hCG to both thecal homogenates and granulosal cells increased after 96 h of LHRH priming but decreased after 96 h of saline injections. Thus, specific binding of ^{125}I-hCG to thecal homogenates and granulosal cells was 4.6-fold and 3.7-fold greater, respectively, in LHRH-treated animals than in saline-treated cows at 96 h (Figures 33 and 34A). In addition, thecal LH binding, on the average, was 30% of granulosal LH binding.

Specific binding of ^{125}I-oFSH to granulosal cells was 2.0-fold greater ($P<.10$) in saline-treated animals than LHRH-treated animals at 48 h (Figure 34B). However, specific binding of ^{125}I-oFSH to granulosal cells was similar between saline- and LHRH-treated groups after 96 h of injections.

Medium Follicles.--LHRH priming for 48 or 96 h did not affect ($P>.10$) specific binding of ^{125}I-hCG and ^{125}I-oFSH to whole follicular homogenates of medium follicles (Figure 35), although there was a tendency for numbers of LH and FSH binding sites at 96 h to be higher ($P<.20$) in LHRH-treated than in saline-treated cows.

Small Follicles.--LHRH priming for 48 or 96 h did not affect specific binding of ^{125}I-hCG or ^{125}I-oFSH to pooled follicular homogenates of small follicles (Figure 36).

Figure 33.--Specific binding of ^{125}I-hCG to thecal homogenates of large follicles after 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls ( ); saline ( ); LHRH ( ).

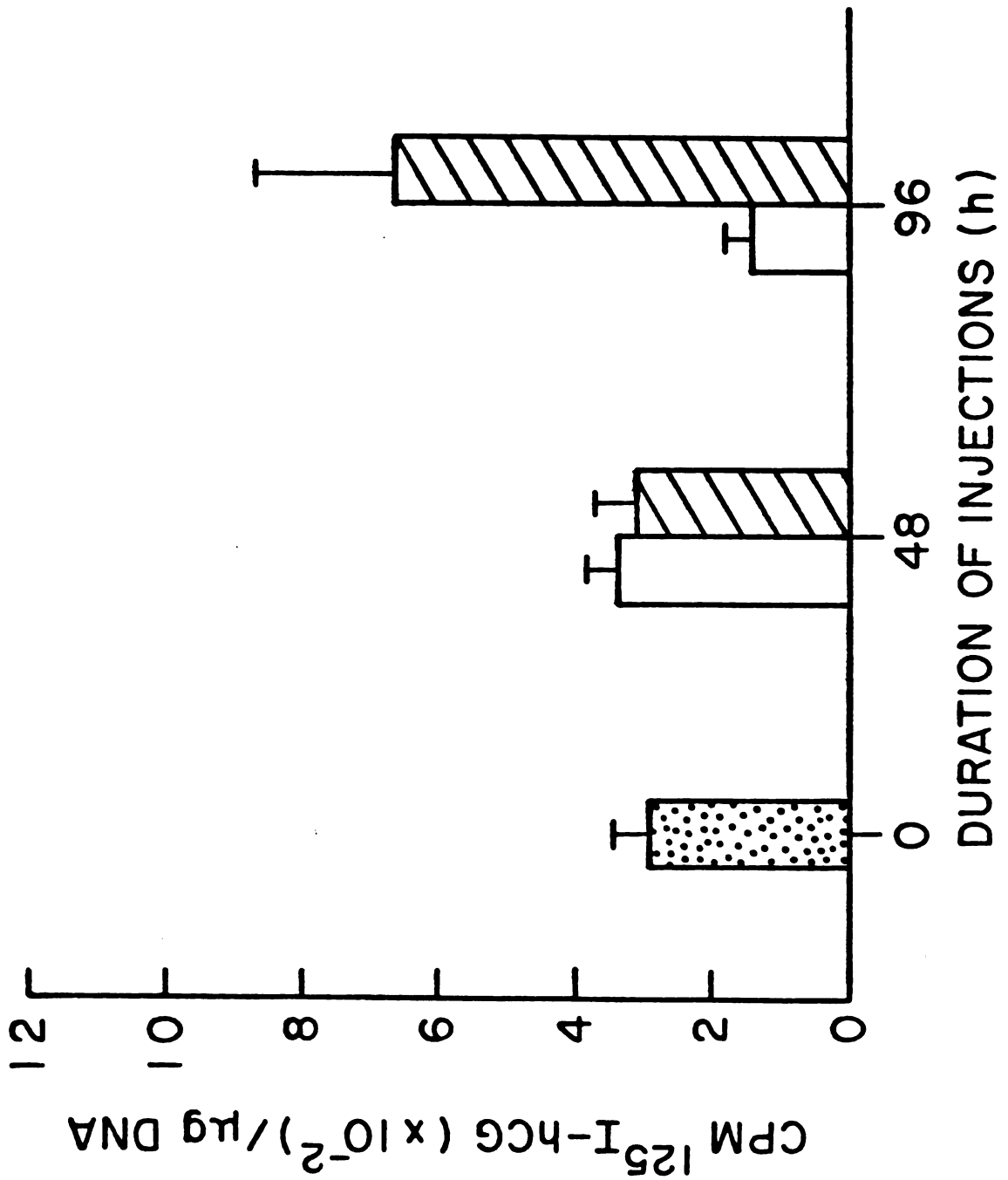

CPM ^{125}I-hCG ($\times 10^{-2}$)/μg DNA
0
2
4
6
8
10
12
DURATION OF INJECTIONS (h)
0
48
96

Figure 34.--Specific binding of ^{125}I-hCG (A) and ^{125}I-oFSH (B) to granulosal cells of large follicles after 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls ( ); saline ( ); LHRH ( ).

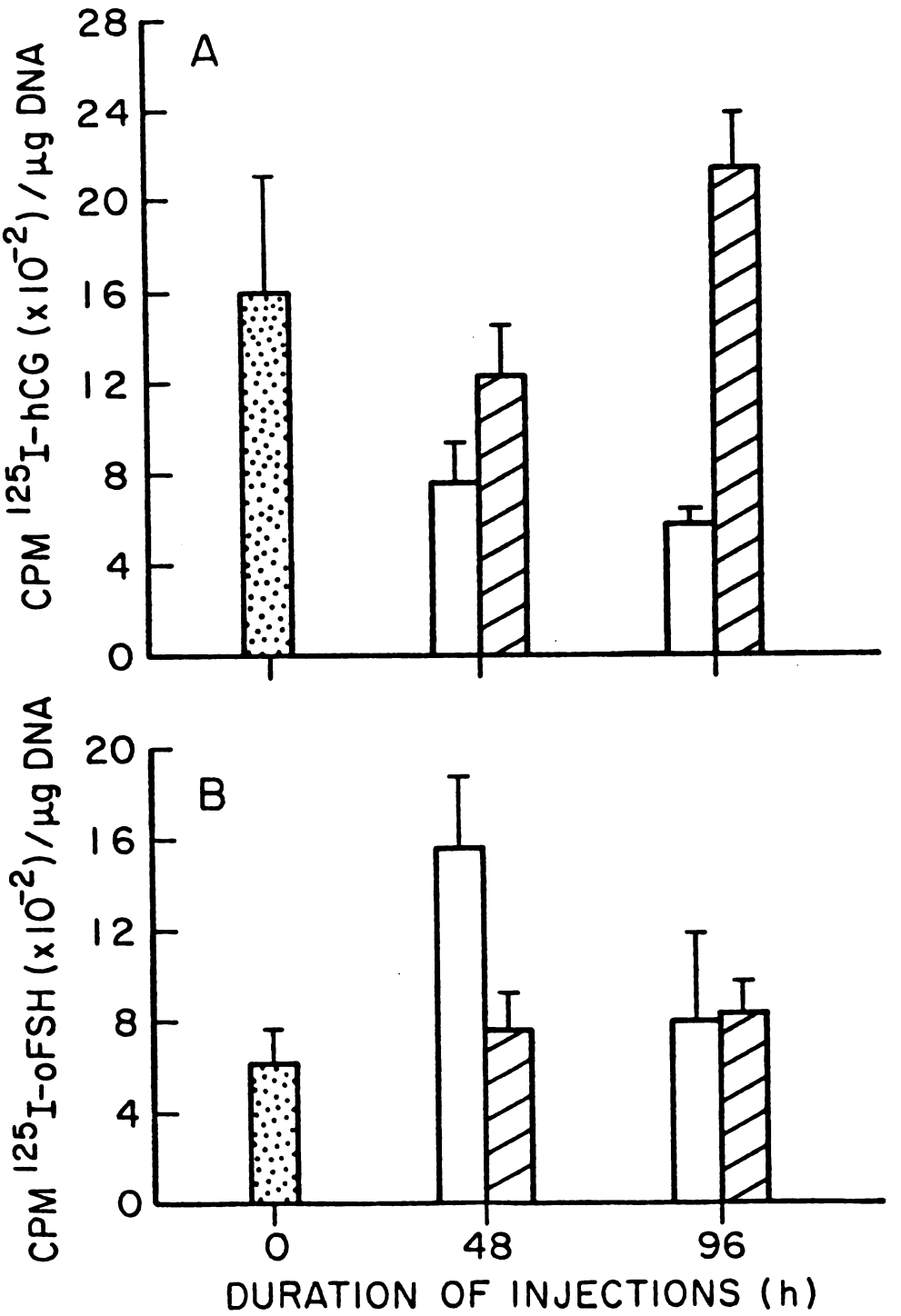

A
B
28
24
20
16
12
8
4
0
20
16
12
8
4
0
0
48
96
CPM ^{125}I-hCG (x10^{-2})/µg DNA
CPM ^{125}I-oFSH (x10^{-2})/µg DNA
DURATION OF INJECTIONS (h)

Figure 35.--Specific binding of ^{125}I-hCG (A) and ^{125}I-oFSH (B) to whole follicular homogenates of medium follicles after 48 and 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Uninjected controls ( ); saline ( ); LHRH ( ).

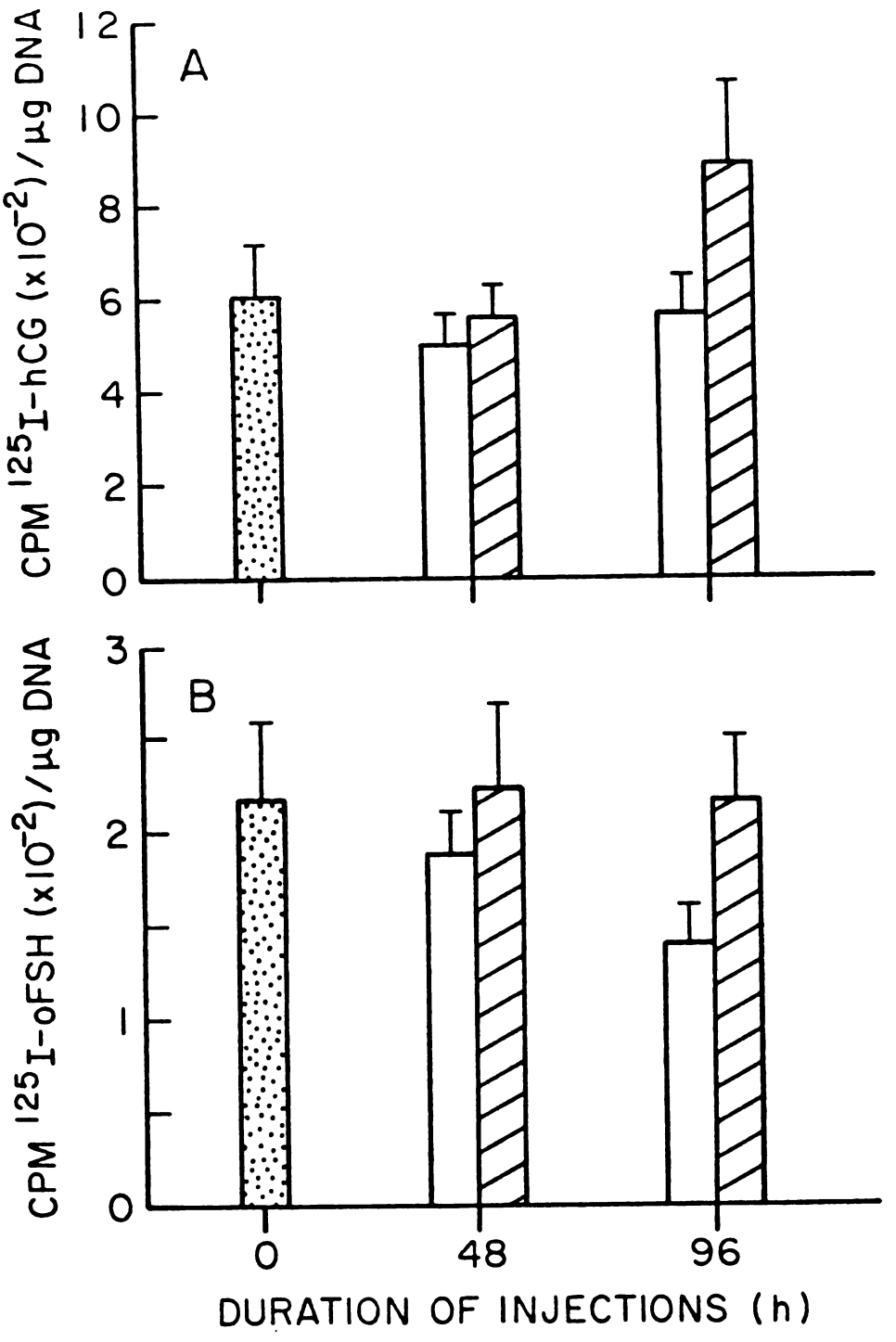
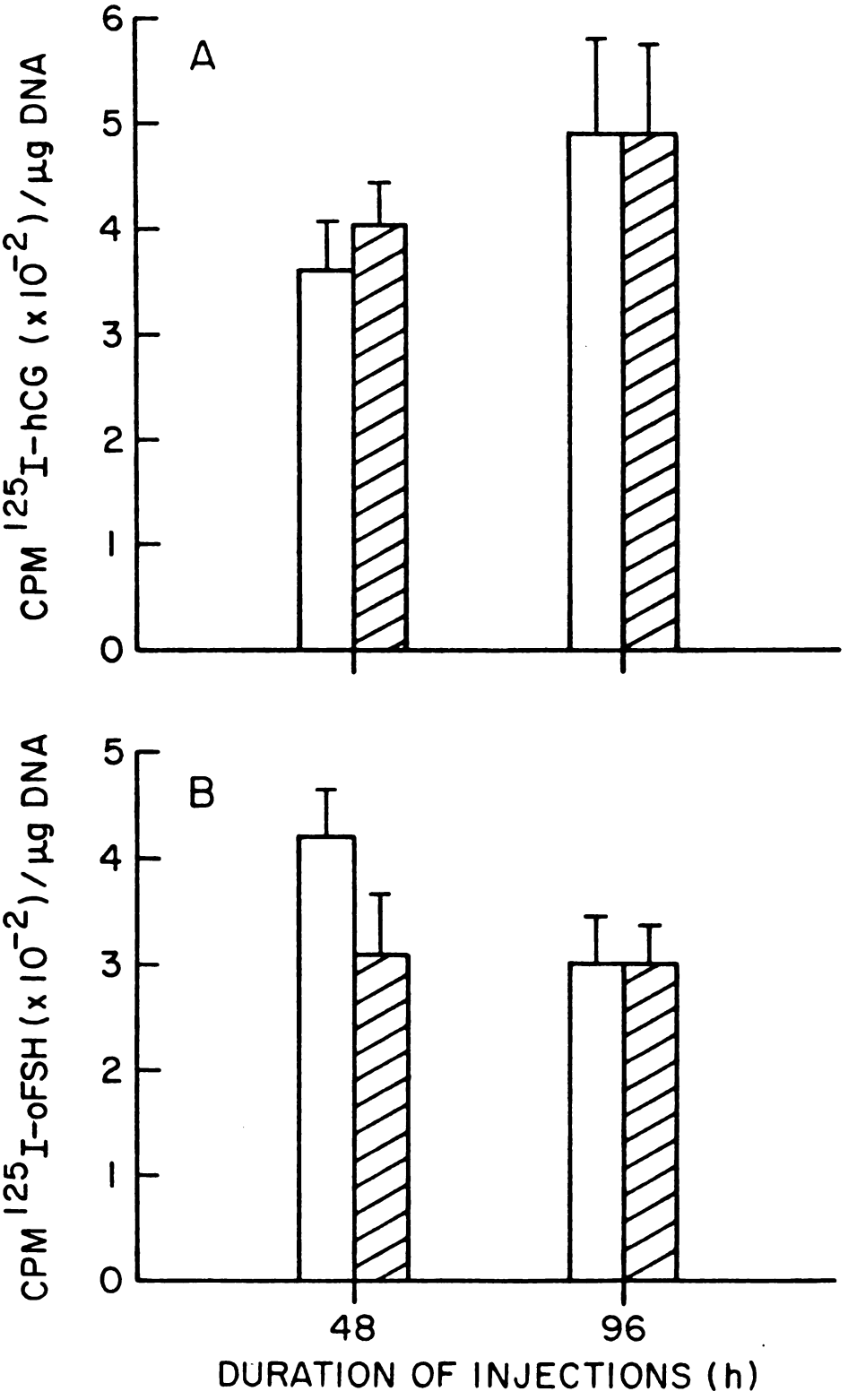

A
B
CPM ^{125}I-hCG (x10^{-2})/µg DNA
CPM ^{125}I-oFSH (x10^{-2})/µg DNA
0
2
4
6
8
10
12
1
3
0
48
96
DURATION OF INJECTIONS (h)

Figure 36.--Specific binding of ^{125}I-hCG (A) and ^{125}I-oFSH (B) to whole pooled follicular homogenates of small follicles after 48 or 96 h of either LHRH or saline injections. See Table 14 for the numbers and average size of follicles at each time of ovariectomy. Saline (□); LHRH (▨).


A
B
CPM ^{125}I-hCG (x 10^{-2})/µg DNA
CPM ^{125}I-oFSH (x 10^{-2})/µg DNA
0
1
2
3
4
5
6
48
96
DURATION OF INJECTIONS (h)

5. Correlations Among Ovarian Follicular Variables

Large Follicles.--Correlation coefficients were calculated among follicular diameter, concentrations of estradiol, progesterone and androstenedione in follicular fluid, and specific binding of gonadotropins to thecal and granulosal cells. Only significant correlation coefficients are included in Table 21. Concentrations of progesterone in follicular fluid were positively correlated with LH binding to granulosal cells but negatively correlated with LH binding to theca, FSH binding to granulosal cells and estradiol concentrations in follicular fluid (Table 21). Specific binding of FSH to granulosal cells was positively correlated with estradiol concentrations (r=.28) and LH binding to thecal homogenates (r=.33). Concentrations of androstenedione in follicular fluid of large follicles were not significantly associated with any follicular variable measured.

Medium Follicles.--Correlation coefficients were calculated among follicular diameter, concentrations of estradiol, progesterone and androstenedione in follicular fluid, and specific binding of gonadotropins to whole follicular homogenates. Only significant correlation coefficients are included in Table 22. Follicular diameter was positively correlated with estradiol concentrations and negatively correlated with both LH and FSH binding sites. Specific binding of LH was also correlated with estradiol concentrations (r=-.17) and FSH binding sites (r=.59). Concentrations of androstenedione or progesterone in follicular fluid of medium follicles were not significantly associated with any follicular variable measured.

TABLE 21.--Correlation Coefficients Among Follicular Diameter, Concentrations of Estradiol (E) and Progesterone (P) in Follicular Fluid, LH and FSH Binding Sites in Thecal (T) and Granulosal Cells (GC) of Large Follicles.[a]

Variable	FSH binding to GC	LH binding to GC	LH binding to T	E
P	-.44[b]	.38[b]	-.28[c]	-.32[c]
FSH binding to GC	---	---	.33[c]	.28[c]

[a] n = 58.

[b] P<.005.

[c] P<.050.

TABLE 22.--Correlation Coefficients Among Follicular Diameter, Concentrations of Estradiol (E) in Follicular Fluid, and LH and FSH Binding Sites in Whole Follicular Homogenates of Medium Follicles.[a]

Variable	LH binding sites	FSH binding sites	E
Diameter	-.40[b]	-.27[b]	.33[b]
LH binding sites	---	.59[b]	-.17[c]

[a] n = 184.

[b] P<.001.

[c] P<.050.

D. Discussion

Concentrations of progesterone in serum are low (.3 to .5 ng/ml) during postpartum anestrus in cattle (Wettemann, 1980; Experiment 1); a result confirmed in cows of the present study injected with saline or LHRH. In contrast, concentrations of progesterone in serum of uninjected controls were elevated. Stress-induced increase in adrenal progesterone may have contributed to the greater concentration of progesterone in these uninjected controls. This suggestion is supported by two observations: 1) progesterone increased slightly in all cattle after ovaries were removed, but significant increments occurred only in uninjected cows; 2) cortisol, an adrenal steroid, was dramatically higher in uninjected cows as compared with saline- or LHRH-treated cows before ovex. The cause for the presumptively greater stress may be due to the fact that cattle in the uninjected group were acclimated to the barn for only 2 days before blood samples were collected, whereas injected cattle had 2 to 4 additional days of acclimation before blood samples were collected. For the first day or two after relocation, cattle experience social disruption concurrent with an increase in concentration of corticosteroids in serum (Varner et al., 1983). Therefore, data from this uninjected control group will not be discussed further.

It has been suggested that an increase in release of LH from 1 or 2 pulses per 6 h to 3 to 8 pulses per 6 h is a prerequisite for onset of the first postpartum ovulation in suckled beef (Humphrey et al., 1983) and dairy (Peters et al., 1981) cows. Therefore, low-dose injections of LHRH at a frequency of three injections per

6 h have been used to mimic these preovulatory changes in LH secretion and to stimulate ovulation and ovarian cycles in anestrous, suckled beef cows (Riley et al., 1981; Short et al., 1982; Walters et al., 1982c). Furthermore, it has been suggested that suckling prolongs the postpartum interval by reducing the frequency of pulsatile LHRH releases from the hypothalamus since weaning calves from anestrous, suckled beef cows increases the frequency of LH pulses and hastens ovulation (Walters et al., 1982b). However, to my knowledge endocrine changes that occur within the ovary in response to these increased pulses of LH were unknown at the beginning of my research program. Thus, characterization of intraovarian events leading to ovulation was the objective of the present study.

Walters et al. (1982c) induced ovulation in 8 of 11 suckled beef cows within 8 days with injections of 500 ng of LHRH every 2 h for 96 h. However, none of the 9 cows injected with LHRH in the present study ovulated during the 4-day treatment period. Whether cows in my study would have ovulated within 8 days is unknown since they were ovex at 96 h. Walters et al. (1982c) also observed preovulatory LH surges in 8 of 11 cows to occur within the 4-day injection period. Whether preovulatory LH surges occurred in cattle of the present study cannot be determined since blood samples were taken only during one 6-h sampling period.

Frequency of gonadotropin pulses during postpartum anestrus in beef cattle range from 1.2 to 2.2 pulses per 6 h for LH (Walters et al., 1982c; Convey et al., 1983; Humphrey et al., 1983; Experiment 1) and from 1.2 to 2.8 per 6 h for FSH (Walters et al., 1982c; Convey

et al., 1983; Experiment 1). LHRH injections given at 2-h intervals nearly doubled these frequencies in the present study and in a previous report (Walters et al., 1982c). Since increased frequencies of gonadotropin pulses occurred in the present study coincident with decreased amplitude of gonadotropin pulses, overall LH concentrations did not change and overall FSH concentrations increased only slightly. Previously, identical LHRH treatment regimens increased frequency of both LH and FSH pulses without changing LH or FSH pulse height (Walters et al., 1982c). Overall concentrations and pulse amplitudes of LH or FSH were not reported by Walters et al. (1982c). Why amplitude of gonadotropin pulses decreased after LHRH injections in the present study is unknown. Perhaps the amount of gonadotropin available to be released from the gonadotrophs (i.e., releasable pools) is limited (Pickering and Fink, 1979; Evans et al., 1983).

Increased estradiol secretion can be induced with low-dose injections of LHRH in anestrous cows (Walters et al., 1982c) and sows (Cox and Britt, 1982). In the present study, however, concentrations of estradiol in serum were not significantly affected by LHRH treatment. In these previous studies (Cox and Britt, 1982; Walters et al., 1982c), blood samples were collected during the period immediately before time of preovulatory LH surges, a period during which estradiol concentrations are normally maximal. Thus, it appears that dramatic increases in estradiol production by large follicles occurs without significant changes in concentrations of estradiol in serum as observed in Experiment 1. Although estradiol concentrations in fluid of individual follicles reflect the capacity of follicles to

synthesize and secrete estradiol (McNatty et al., 1976; Channing, 1980; England et al., 1981; Hillier et al., 1981; Bieszczad et al., 1982; McNatty, 1982), increased estradiol production by large follicles may be seen in peripheral circulation as only small increases in serum. In fact, after 96 h of injections, LHRH-treated cows had 18% greater concentration of estradiol in serum than saline-treated cows. This may explain why correlations existed between estradiol in serum and follicular fluid. Perhaps changes in estradiol production are necessary for local intraovarian changes observed, such as increased numbers of gonadotropin binding sites.

Changes in numbers of different sized follicles were unaltered by increased frequency of gonadotropin pulses in serum. Similarly, size of the two largest follicles was unchanged within 4 days of LHRH priming. Together these data suggest that follicular growth is not affected by LHRH-induced changes in gonadotropin secretion at least within a 4-day treatment period. However, lack of an increase in numbers of small follicles should be interpreted carefully, since many of these small follicles could be imbedded in the ovary and would not have been counted.

Numbers of follicles on the surface of ovaries collected between days 20 and 30 after parturition in the present experiment were similar to numbers reported on days 28 to 42-56 after parturition in Experiment 1. Size of the two largest follicles in the present experiment were also similar to sizes reported on days 28 to 42-56 after parturition (Experiment 1) but diameters of the largest follicle were smaller than diameters reported for the largest follicle in cattle

during estrus (Donaldson and Hansel, 1968; Marion et al., 1968; Merz et al., 1981; Staigmiller et al., 1982). Thus, it appears that in the present experiment final preovulatory development of the largest follicle was not completed.

My study is the first to characterize changes in concentrations of steroids in follicular fluid of individual follicles in response to low-dose injections of LHRH in any mammalian species. Previously, Walters et al. (1982a) found total ovarian follicular fluid content of estradiol and progesterone on day 25 after parturition was not different between nonsuckled and suckled beef cows, even though nonsuckled cows were closer to first estrus than were suckled cows (Walters et al., 1982c). However, since there is large variation between concentrations of steroids in follicular fluid of individual follicles within a cow (Merz et al., 1981) it is not surprising that differences in pooled follicular fluid steroid levels were not observed by Walters et al. (1982a). Indeed, data collected from individual follicles in the present study suggests that increased estradiol production by large follicles and increased progesterone production by medium follicles are induced by LHRH priming in anestrous cattle. Moreover, these shifts in follicular steroid production may be essential for intraovarian changes to occur prior to ovulation, such as increased number of LH binding sites in large follicles, in response to LHRH priming in anestrous cattle.

The increase in mean estradiol in follicular fluid of large follicles in LHRH-treated cows was associated with increases in mean numbers of LH binding sites in both thecal and granulosal cells.

However, calculated across individual follicles, LH binding in large follicles was not significantly correlated with follicular fluid estradiol concentrations, but LH binding was positively correlated with follicular fluid progesterone. These findings are similar to results in large follicles in Experiment 1. In addition, low progesterone production at 48 h in saline-injected controls was associated with high FSH binding to granulosal cells. Thus, FSH binding was positively correlated with follicular fluid estradiol and negatively correlated with follicular fluid progesterone. These correlations were not observed in Experiment 1. Moreover, Ireland and Roche (1982, 1983a,b) observed that low concentrations of progesterone and high concentrations of estradiol were associated with greater FSH binding to granulosal cells and greater LH binding to thecal and granulosal cells of large ($\geq$6 mm) follicles during the estrous cycle of beef heifers. Only around the time of the preovulatory gonadotropin surges does LH binding increase and FSH binding decrease coincident with an increase in progesterone concentrations in follicular fluid of these large follicles (Ireland and Roche, 1982; 1983b). Collectively, these data suggest that changes in function of follicles of postpartum cattle induced by LHRH injections resemble changes in follicular function prior to ovulation in beef heifers.

Whether the increase in estradiol in follicular fluid of large follicles is a result of or a cause of the increase in LH binding sites in thecal and granulosal cells cannot be determined from my study. In vivo studies have shown that estradiol in the presence of FSH or FSH plus LH can increase LH and FSH binding sites in rat

granulosal cells collected from antral follicles (Richards et al., 1978; Richards, 1980). Perhaps estradiol or LH in the presence of FSH induce the changes in LH binding sites observed in the present study.

In medium-sized follicles, high progesterone concentrations at 96 h in LHRH-treated cows were associated with slightly higher LH and FSH binding sites than observed in saline-injected cows. However, no significant correlation among gonadotropin binding sites and concentrations of progesterone in follicular fluid was observed. These results suggest that identical hormonal stimuli results in a very different change in intrafollicular steroid concentrations in medium versus large follicles and provides further evidence that steroidogenesis in medium and large follicles is under different control mechanisms as suggested in Experiment 1.

A significant increase in the proportion of cattle with one large estrogen-active follicle (estradiol > progesterone in follicular fluid; Ireland and Roche, 1982) was observed between 48 and 96 h of LHRH priming and may be responsible for coincident increases in average estradiol concentrations observed in follicular fluid of large follicles. Since high concentrations of estradiol and low concentrations of progesterone have been associated with non-atretic, healthy bovine follicles (Ireland and Roche, 1982, 1983a,b; Bellin and Ax, 1984) perhaps this increase in estrogen-active follicles reflects an increase in number of healthy (non-atretic) follicles after 96 h of LHRH priming. Whether this increase in estrogen-active follicles is due to development of new follicles or is due to a change in

function of pre-existing large follicles cannot be determined from this study. However, the following evidence supports the former of these two suggestions. Firstly, coincident with the increase in large estrogen-active follicles there was an increase in the concentration of progesterone in medium follicles, indicative of atresia (Bellin and Ax, 1984). Perhaps some of these medium follicles with high progesterone concentrations at 96 h were originally in the large follicle category 48 h earlier. Secondly, concentrations of progesterone in large follicles were higher in LHRH-treated than saline-treated cows at 48 h. Although the proportion of estrogen-active follicles present at 48 h was similar in LHRH- and saline-treated cows, perhaps more of these large follicles in LHRH-treated cows were beginning the process of atresia. For these events to occur, follicular turnover would have to be greater in LHRH- than saline-treated cattle. Whether this occurs or not is unknown. However, turnover of large follicles increases during the follicular phase (after day 13) of the bovine estrous cycle (Matton et al., 1981) a period during which estrogen-active and estrogen-inactive follicles exist in equal numbers (Ireland and Roche, 1983b).

Alternatively, the increase in progesterone seen at 48 h in large follicles and 96 h in medium follicles of LHRH-treated cows may be due to stimulation of progesterone synthesis by LHRH priming. Since increased progesterone concentrations in follicular fluid of large follicles preceded increased estradiol concentrations in both experiments, transformation of steroidogenic capabilities of follicles (e.g., increased aromatase) may have to occur prior to increased

estradiol secretion. My studies were not designed to distinguish between these possibilities.

GENERAL DISCUSSION

The overall objective of this dissertation was to study control of ovarian follicular growth and function during the postpartum anovulatory period in beef cows and associate these changes with postpartum anestrus. Based on results of my experiments and previous studies, it can be concluded that factors not associated with prolonged anestrus in suckled beef cattle include suckling intensity, concentrations of cortisol, PRL and FSH in serum, and concentrations of androstenedione in follicular fluid. The major hormonal change observed in these studies was the pronounced increase in intrafollicular concentrations of estradiol in large antral follicles. Increased estradiol production by large follicles may be an important factor in resumption of ovulatory cycles after parturition, since estradiol is directly involved in follicular growth and differentiation (Richards, 1980), oocyte maturation (Fukui et al., 1982; Bar-Ami et al., 1983), pulsatile LH secretion (Karsch et al., 1983) and initiation of preovulatory surges of gonadotropins (Kesner et al., 1982a,b). Changes in episodic LH pulses preceded these changes in follicular estradiol production by 2 weeks in Experiment 1 and by 2 days in Experiment 2. The hormonal stimuli for increased estradiol production during postpartum anestrus are not known, but increased amplitude of LH pulses (in the presence of FSH) stimulates estradiol production in vitro (Peluso et al., 1984a,b). Furthermore, increased

frequency of LH pulses can stimulate estradiol secretion rate in vivo (McNeilly et al., 1982). Increased frequency or amplitude of LH pulses long before the first postpartum ovulation, therefore, could be a major signal which impels the ovary into ovulatory cycles.

Another pronounced change observed in Experiment 1 was an increase in numbers of medium follicles. This increase in the pool of medium sized follicles may be a prerequisite for resumption of ovulatory cycles after parturition. Factors regulating growth of these medium follicles are unknown. Since estradiol has been shown to stimulate follicular growth in rats (Richards, 1980), perhaps an increase in estradiol concentrations of large follicles is an important intraovarian factor in stimulating growth of medium follicles in postpartum cattle.

Although LH (and FSH) is released in a pulsatile pattern, the physiological importance of this temporal release is unknown. Peluso et al. (1984a,b) hypothesized that both amplitude and rate of change of LH stimuli may be important factors controlling ovarian secretory responses. Furthermore, repeated exposure to small doses of LH has a priming effect on ovarian (Hillensjo et al., 1983) and testicular tissues (Powell et al., 1981) which results in a potentiation of the response to a second stimulus of LH. Perhaps this episodic release is necessary to overcome ovarian desensitization (or loss of hormone responsiveness) which can occur after prolonged exposure to LH (Conti et al., 1976; Jonassen and Richards, 1980; Zor et al., 1984).

The causes for increases in amplitude (or frequency) of LH pulses are not known, but they may be related to functional

transformation of the hypothalamus with time after parturition. Since increased amplitude of LH pulses occurred before measurable changes in follicular estradiol production, hypothalamic transformation (i.e., increased secretion of LHRH) presumably occurs independent of estradiol. Similarly, it is unlikely that progesterone is involved since no detectable change in progesterone was observed in serum. Perhaps hypothalamic transformation after parturition involves a decrease in sensitivity of the brain to negative feedback by ovarian steroids. This transformation has been suggested to occur after weaning calves from suckled beef cattle (Acosta et al., 1983).

Suckling delays onset of postpartum estrus and ovulation in lactating cattle (Wettemann, 1980; LaVoie et al., 1981) via some inhibitory mechanism. Presumably, suckled postpartum cows "escape" from this inhibitory influence of suckling which then leads to their first postpartum ovulation (Walters et al., 1982c) as in rats (Selmanoff and Selmanoff, 1983). When and how this occurs is unknown. Perhaps the subtle increase in pulsatile secretion of LH observed between days 7 and 14 after parturition is one of the initial endocrine changes indicative of this "escape" from inhibition of suckling.

In both experiments, increases in concentrations of estradiol in follicular fluid of large follicles does not appear to be dependent on changes in numbers of FSH binding sites. However, only in Experiment 1 was it apparent that estradiol production by large follicles was independent of numbers of LH binding sites. This suggests that

a change in responsiveness of large follicles with time after parturition is not dependent on a change in gonadotropin binding sites.

In contrast, numbers of LH binding sites (and hence responsiveness to LH) may have been a limiting factor for estradiol production by large follicles in Experiment 2. The cause for this increased capacity of follicles to bind LH is unknown but similar results have been observed during preovulatory follicular development in cattle (Ireland and Roche, 1982; 1983b; Staigmiller and England, 1982; Walters et al., 1982c), sheep (Webb and England, 1982a,b), pigs (Stouffer et al., 1976) and rats (Uilenbroek and Richards, 1979).

Concentrations of FSH in serum did not change with time after parturition. Thus, if FSH is involved in the reinitiation of ovulatory cycles, as it appears to be in humans (Zarate and Canales, 1983), a constant secretory pattern of FSH in serum probably plays a permissive role.

Concentrations of FSH (but not LH) in serum increased with LHRH priming prior to an increase in concentrations of estradiol in follicular fluid observed in Experiment 2. Both LH and FSH pulse frequency increased. Thus, determining whether LH or FSH separately, or in combination caused the increase in estradiol production by large follicles cannot be determined from Experiment 2. To ascertain the precise requirements and interrelationships of LH and FSH in stimulating follicular estradiol production prior to the first postpartum ovulation will require further study. Nonetheless, considering data from both experiments, it may be concluded that

estradiol production by large antral follicles is stimulated under various profiles of gonadotropin secretion during postpartum anestrus. Furthermore, this increase in concentration of estradiol in large follicles apparently precedes postpartum ovulatory events. Therefore, based on results of these experiments I hypothesize that increased capacity of large follicles to produce estradiol is essential for onset of the first postpartum ovulation in cattle.

SUMMARY AND CONCLUSIONS

The studies presented in this dissertation examined follicular growth, selected steroid hormones in follicular fluid and serum, follicular gonadotropin receptors, and coincident secretion of various pituitary hormones prior to the first postpartum ovulation in suckled beef cattle under two experimental settings.

In Experiment 1, amplitude of LH pulses increased between days 7 and 14 after parturition. However, frequency of LH pulses, and baseline and overall concentrations of LH did not change from days 7 through 42-56. Concentrations of progesterone in large follicles ($\geq$8 mm) increased between days 7 and 14 and remained elevated through days 42-56. Concentrations of estradiol in large follicles increased 4-fold between days 14 and 28. Numbers of LH and FSH binding sites did not change and hence, were not associated with increased estradiol in follicular fluid. I suggested that increased estradiol production by large follicles may be an important factor in resumption of ovulatory cycles after parturition.

Numbers of medium follicles increased 2-fold between days 7 and 14, and again between days 28 and 42-56. Perhaps these increases in numbers of medium follicles provides a larger pool of follicles from which ovulatory follicles can be selected prior to first postpartum ovulation.

Concentrations of FSH, PRL, estradiol, progesterone and cortisol in serum, and androstenedione in follicular fluid did not change with time after parturition. In addition, no change in numbers of gonadotropin binding sites or steroid concentrations in small and medium follicles were observed. Thus, these factors were probably not associated with prolonged anestrus in suckled beef cattle.

In Experiment 2, LHRH priming induced a 2-fold increase in LH and FSH pulses but because amplitude of induced pulses were lower than endogenous pulses, no change in overall LH and only slight increases in overall FSH concentrations were observed. LHRH priming did not alter numbers of small, medium and large follicles or size of the two largest follicles.

Concentrations of estradiol in large follicles were unchanged after 48 h of LHRH priming but increased 2.3-fold after 96 h. Coincident with increased estradiol production by large follicles was increased numbers of LH binding sites but not FSH binding sites. Thus, increased frequency of LH and FSH pulses preceded both increased estradiol in follicular fluid and LH binding.

Concentrations of progesterone in large follicles were 4-fold greater in LHRH- than in saline-treated cows at 48 h. At 96 h, progesterone in follicular fluid was 2-fold greater in saline- than in LHRH-treated cows. Thus, increased progesterone production preceded estradiol production in large follicles.

In summary, increased amplitude or frequency of LH pulses in serum and coincident increases in progesterone concentrations in large follicles are the first hormonal changes observed in

anestrous cattle. These changes in serum LH and follicular fluid progesterone are followed by increased estradiol concentrations in large follicles. These results suggest that increased amplitude or frequency of LH pulses may be a major factor stimulating shifts in the steroidogenic capabilities of large follicles in postpartum acyclic cattle. Perhaps this increased capacity of large follicles to produce estradiol is essential for onset of the first postpartum ovulation in suckled beef cattle.

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