This is to certify that the

dissertation entitled

BASAL AND ESTRADIOL-INDUCED RELEASE OF GONADOTROPINS IN DAIRY COWS WITH NATURALLY-OCCURRING OR ARTIFICIALLY-INDUCED OVARIAN CYSTS

presented by

KENT ROSWELL REFSAL

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Animal Science

R Nachreiner
Major professor

Date May 2, 1986

BASAL AND ESTRADIOL-INDUCED RELEASE OF GONADOTROPINS IN DAIRY COWS WITH NATURALLY-OCCURRING OR ARTIFICIALLY-INDUCED OVARIAN CYSTS

By

Kent Roswell Refsal

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Animal Science

1986

ABSTRACT

BASAL AND ESTRADIOL-INDUCED RELEASE OF
GONADOTROPINS IN DAIRY COWS WITH NATURALLY-OCCURRING
OR ARTIFICIALLY-INDUCED OVARIAN CYSTS

By

Kent Roswell Refsal

Two experiments were done to characterize gonadotropin release in dairy cows having ovarian cysts. In the first experiment, cows with naturally-occurring ovarian cysts were sampled to determine 1) relationships between endogenous circulating estradiol or progesterone concentrations and basal gonadotropin release and 2) the ability of the same cows to release LH and FSH after exogenous estradiol administration. Cows with high endogenous estradiol had the highest basal concentrations of LH, but there were no other significant relationships between frequency and amplitude of pulsatile gonadotropin release and endogenous sex steroid concentrations. After exogenous estradiol, a preovulatory surge of gonadotropins occured in only one of twelve cows. All cows released LH and FSH after being given exogenous GnRH.

In the second experiment, adult nonlactating dairy cows in late diestrus were given exogenous estradiol or repeated injections of ACTH to induce formation of ovarian cysts. Changes in serum LH after exogenous estradiol suggested that ovarian cysts may be formed if the preovulatory surge of LH occurs prematurely in relation to luteal regression. Changes in LH in ACTH-treated cows suggested that ovarian

cysts may also be formed when there is inhibition of the preovulatory surge of LH after luteal regression. Profiles of gonadotropin release in cows with artificially-induced ovarian cysts were similar to those of cows with the naturally-occurring condition. It was concluded from these experiments that 1) asynchrony or lack of a preovulatory surge of LH can cause formation of ovarian cysts in dairy cows, 2) the cystic condition may be maintained by refractoriness of the hypothalamo-pituitary axis to the positive feedback effects of estradiol on release of gonadotropins, and 3) dairy cows with artificially-induced ovarian cysts appear to be a suitable endocrine model for the naturally-occurring condition.

ACKNOWLEDGEMENTS

This dissertation could not have been a reality without the assistance of many people. My pursuit of an academic career in veterinary medicine was inspired by the example of Dr. Brad Seguin and the faculty of the University of Minnesota Theriogenology Section. The much appreciated efforts of Drs. E. Mather, H. Hafs, and M. Hogberg made it possible for my program to be administered in the Department of Animal Science.

I extend many thanks to the members of my graduate committee, Drs. Clyde Anderson, W. Richard Dukelow, Ed Mather, and Ray Nachreiner, for counsel and positive support throughout my stay at Michigan State. Special thanks to Clyde Anderson for sharing his expertise on statistical analysis and computers. Special thanks also to my major professor, Ray Nachreiner, for his great patience and confidence through times of frustration in my program.

The cooperation, hospitality, and camaraderie extended by the Reproductive Physiology Group is greatly appreciated, notably the consul of Drs. Ed Convey and Roy Fogwell. Thanks also to the assistance from the Staff of the Endocrine Diagnostic Laboratory. Dr. Julio Jarrin-Maldonado and his family were of great assistance in collection of the data.

Lastly, I thank my parents and especially my wife, Judy, for their concern and support throughout my program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATION

cm	centimeter(s)
OVX	ovariectomized
LH	luteinizing hormone
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
CL	corpus luteum
P_4	progesterone
E_2	estradiol-17b
pg	picogram(s)
ml	milliliter(s)
ng	nanogram(s)
hr	hour(s)
min	minute(s)
mm	millimeter(s)
HCG	human chorionic gonadotropin
C	degrees centigrade
g	gravitational force
mg	milligram(s)
ug	microgram(s)
M	molar concentration
SD	standard deviation
r	replicate(s)
p	probability
SEM	standard error of the mean
ACTH	adrenocorticotropin
PGF_{2a}	prostaglandin F_{2alpha}
IM	intramuscular
$E_2 10$	10 mg estradiol-17b-treatment
IU	international unit(s)
RIA	radioimmunoassay

INTRODUCTION

Ovarian cysts are one of the most commonly diagnosed infertility problems in dairy cattle, detected in 5-20% of reproductive cycles. The condition is characterized by the presence of an ovarian follicular structure(s) usually 2 cm or more in diameter that may persist for days to weeks with the absence of a corpus luteum. In affected cows, reproductive efficiency is reduced primarily by an increase in the number of days open, despite therapeutic measures.

Clinical diagnosis is based on identifying the presence of an ovarian cyst. The diagnostician usually has no further insight to the variability in histological anatomy and endocrine changes found with this condition. The cystic condition is not static, as affected cows may spontaneously resume estrous cycles or an ovarian cyst may regress with concomitant formation of another.

Approximately 50% or more of ovarian cysts are detected within the first 60 days postpartum, the time when estrous cycles are reinitiated. Clinical and experimental observations suggest that a critical period for cyst formation occurs between luteal regression and ovulation in cycling cows. Hypotheses concerning a lack of or asynchrony of gonadotropin release accompanying follicular growth have been proposed as causes of ovarian cysts. At present, data on basal gonadotropin secretion in cows with ovarian cysts is limited.

The theme of this dissertation was to characterize gonadotropin secretion in dairy cows with ovarian cysts. Two interrelated experiments were conducted. The objectives of the first experiment were to

determine relationships between basal sex steroid concentrations and gonadotropin release in cows with naturally-occurring ovarian cysts and assess gonadotropin response to estradiol challenge in the same animals. The second experiment also had two objectives. The first was to determine if ovarian cysts could be artificially induced in dairy cows with exogenous estradiol or ACTH treatment and record accompanying changes in LH, estradiol, and progesterone. The second objective was to characterize relationships between sex steroid concentrations and gonadotropin release in cows with artificially-induced ovarian cysts.

REVIEW OF LITERATURE

Ovarian cysts have been recognized as a cause of infertility in dairy cattle for over 50 years. Several extensive reviews have been written on the topic (Roberts, 1971; Garverick, 1982; Eyestone and Ax, 1984). This review focuses on endocrine changes associated with this condition and discusses the clinical significance of ovarian cysts in bovine reproduction. First, however, a review of gonadotropin and sex steroid release throughout the estrous cycle and postpartum period of cattle is warranted.

Interaction of the Hypothalamo-Pituitary-Ovarian Axis in the Control of Gonadotropin Release Throughout the Estrous Cycle

In ovariectomized (OVX) cows and ewes, LH (Forrest et al., 1980; Goodman and Karsch, 1980; Convey et al., 1983) and FSH (Convey et al., 1983) are released in pulsatile fashion with a frequency of approximately 1 pulse/hour. This release of LH occurs synchronously with, and as a result of, pulsatile release of hypothalamic gonadotropin releasing hormone (GnRH) into hypophyseal portal vessels (Clarke and Cummins, 1982; Levine et al., 1982). The frequency of GnRH release by hypothalamic neurons has been depicted as controlled by a neural LH pulse generator (Karsch, 1984).

Pulsatile release of gonadotropins occurs throughout the estrous cycle, with changes in both amplitude and frequency at different stages of the cycle. Questions regarding the relationship of circulating

ovarian steroids to gonadotropin release have been addressed via replacement of ovarian steroids in OVX females and in *in vitro* pituitary culture. In general, specific, homologous radioimmunoassays for bovine and ovine LH are readily available and results among laboratories have been in agreement. There has been more heterogeneity in regard to FSH radioimmunoassays used (Goodman et al., 1981; Bolt and Rollins, 1983) and as a result, pulsatile release of FSH has not been as well characterized.

Hansel and Convey (1983) recently reviewed the physiology of the estrous cycle of cattle and sheep. For discussion of endocrine changes, they divided the cycle into 3 periods; the luteal phase and before and after the preovulatory surge of gonadotropins. These divisions will be used in discussion of temporal changes in sex steroid and gonadotropin concentrations and mechanisms of control. For this discussion, day 0 of the estrous cycle will be the day of the preovulatory surge of gonadotropins.

Luteal Phase. After ovulation, proliferation of luteinized thecal and granulosa cells results in formation of the corpus luteum (CL). Accompanying CL growth is an increase in serum progesterone (P_4) that peaks (2 to 10 ng/ml) at about day 10 and remains elevated for the next 8-9 days in cows (Robinson, 1977). Estradiol-17b (E_2) remains at basal concentrations (less than 5 pg/ml) throughout the luteal phase with sporadic increases early in diestrus (Shemesh et al., 1972; Dobson and Dean, 1974) presumed to accompany growth and regression of E_2-active ovarian follicles (Ireland and Roche, 1983a).

During the luteal phase of the estrous cycle, LH is released in pulsatile fashion in cows. Pulses of LH with an amplitude of 1-6 ng/ml are released every 4-6 hours (Rahe et al., 1980; Walters et al., 1984). Recently, smaller pulses of LH (less than 1 ng/ml) were detected between the larger amplitude pulses in diestrous cows (Walters et al., 1984). FSH is also released in pulsatile manner in cows, with uniform pulses (15-20 ng/ml) occurring synchronously with both large and small pulses of LH (Walters et al., 1984).

There is a synergism of ovarian products that control gonadotropin release in the luteal phase. When OVX ewes were given implants containing P_4, frequency of pulsatile LH release was reduced (1/hr to 1/6 hr), but with no change in amplitude (Goodman and Karsch, 1980). When OVX ewes were chronically exposed to luteal concentrations of E_2 (3-5 pg/ml), the frequency of LH pulses was unchanged (1/hr), but amplitude was reduced (Goodman and Karsch, 1980). However, both P_4 and E_2 replacement in OVX ewes was needed to mimic average basal concentrations of LH in diestrous ewes (Karsch et al., 1980).

Specific effects of replacement of luteal concentrations of P_4 or E_2 on pulsatile release of FSH have not been examined. However, combined P_4 and E_2 replacement did not suppress basal FSH concentrations in OVX ewes to values seen in diestrous ewes (Goodman et al., 1981). A protein in ovarian follicular fluid, inhibin, may also play a role in regulation of FSH release. Administration of charcoal-extracted bovine follicular fluid suppressed FSH but not LH in OVX heifers (Ireland et al., 1983; Kiracofe et al., 1983).

Pre-Surge Period. On day 18-20 of the bovine estrous cycle, luteal regression is accompanied by a rapid decline in serum P_4 (Robinson, 1977). With removal of P_4 influence, the frequency of LH and FSH pulses increases to 1/40-60 minutes (Rahe et al., 1980; Walters and Schallenberger, 1984). There is also a progressive increase in serum E_2, reaching a peak of 10 pg/ml in cattle just prior to the preovulatory surge of gonadotropins (Chenault et al., 1975; Walters and Schallenberger, 1984). This increase in E_2 is mainly from one ovary (Ireland et al., 1984) and likely to be from a single, large estrogen-active ovarian follicle (Dieleman et al., 1983; Ireland and Roche, 1983b). The largest ovarian follicle at this time is the preovulatory follicle (Dufour et al., 1972). Pulsatile release of E_2 was detected in caudal vena cava blood in cows, occurring synchronously with pulsatile release of LH detected in jugular samples (Walters and Schallenberger, 1984).

The preovulatory surge of LH (Chenault et al., 1975; Walters and Schallenberger, 1984) and FSH (Walters and Schallenberger, 1984) begins approximately 36-48 hours after luteal regression has completed. This LH and FSH surge lasts for 6-10 hours and represents the summation of frequent pulses (1/20-30 minutes) that are also increased in amplitude (Rahe et al., 1980; Walters and Schallenberger, 1984). Serum E_2 decreases rapidly during the preovulatory surge of gonadotropins (Chenault et al., 1975; Walters and Schallenberger, 1984).

Both a decrease in P_4 and an increase in E_2 are necessary prerequisites for the preovulatory surge of gonadotropins in cycling cows or

ewes. Frequency of LH pulses increased after ovariectomy of diestrous ewes, but E_2 replacement was required to mimic the increased rate of pulsatile release of LH after luteal regression in cycling ewes (Karsch et al., 1983). The effect of E_2 in initiating preovulatory surges of gonadotropins appears to be threefold. Initially, E_2 reduces the sensitivity of the anterior pituitary to release LH in response to GnRH (Kesner et al., 1981; Clarke and Cummins, 1985). At the same time, however, E_2 is synergistic with GnRH in priming the gonadotrophs for enhanced release of LH to subsequent GnRH stimulus (Padmanabhan and Convey, 1981). Lastly, E_2 exposure sets a neural timing mechanism that schedules release of frequent pulses of hypothalamic GnRH and results in the preovulatory surge of gonadotropins (Kesner et al., 1981; Clarke and Cummins, 1985). The preovulatory surge of LH is terminated by pituitary refractoriness to GnRH (Kesner et al., 1981).

P_4 blocks the priming effect of E_2 on gonadotrophs (Padmanabhan and Convey, 1981). Administration of exogeneous E_2 did not result in a preovulatory surge of LH in diestrous cows (Short et al., 1979; Zaied et al., 1981; Stevenson et al., 1983). In OVX cows, exogenous P_4 blocked the preovulatory surge of LH after replacement of physiological concentrations of E_2 (Kesner et al., 1982), but did not prevent surges of LH after pharmacological doses of E_2 (Hausler and Malven, 1976; Short et al., 1979).

Post-Surge Period. P_4 and E_2 are both low for 2-3 days after the pre-ovulatory surge of gonadotropins (Chenault et al., 1975; Walters and Schallenberger, 1984). At this time, there is divergence in LH and FSH

release, where basal LH is lower after the surge than before and basal FSH is higher after the surge than before (Goodman et al., 1981; Roche and Ireland, 1981). Pulsatile release of LH was nondetectable for 6-12 hours post-surge, but FSH pulses were present (Walters and Schallenberger, 1984). The appearance of frequent, low-amplitude pulses characterize the return of episodic release of LH (Rahe et al., 1980; Walters and Schallenberger, 1984).

A secondary rise of FSH occurs about 12-24 hours after the preovulatory surge of gonadotropins (Pant et al., 1977; Ireland and Roche, 1983; Walters and Schallenberger, 1984). This secondary rise of FSH is hypothesized to result from a reduction in both E_2 and inhibin influence after ovulation (Padmanabhan et al., 1984). The second rise of FSH may also have a role in recruitment of the large, estrogen-active ovarian follicle that is present early in the luteal phase of the estrous cycle (Ireland and Roche, 1983a).

Changes in Gonadotropins and Sex Steroids in the Postpartum Period of Cattle

Gonadotropins. In nonsuckled dairy cows, serum LH concentrations are low ($\leq$ 1 ng/ml) at and shortly after parturition and increase during the first 1 to 3 weeks postpartum (Echternkamp and Hansel, 1973; Carruthers and Hafs, 1980; Peters et al., 1981). This increase of LH is the net result of initiation of pulsatile release of LH (Carruthers and Hafs, 1980; Peters et al., 1981; Gitlin et al., 1983; Karg and Schallenberger, 1983).

In contrast to LH, marked fluctuation of FSH was present before 1 week postpartum in dairy cows sampled at 6 hour intervals (Schams et al., 1978). Basal FSH increased in the first 5 days postpartum (Dobson, 1978; Webb et al., 1980) with no further increases throughout the next two weeks (Carruthers and Hafs, 1980; Webb et al., 1981). Pulsatile release of FSH was present on day 4 postpartum in dairy cows (every 2 hours) with no further increase in amplitude or frequency at one week later (Karg and Schallenberger, 1983). Thus, it appears that cows regain the ability to secrete FSH in pulsatile manner earlier in the postpartum period than LH.

The disparity in the resumption of LH and FSH release in postpartum cows was evident in other experiments. When exogenous GnRH was given in the first and second weeks postpartum, LH release was higher in the second week (Foster et al., 1980; Gitlin et al., 1983) while FSH release did not differ between weeks but tended to be higher in the first (Foster et al., 1980; Peters and Lamming, 1984a). The post-castration rise of LH was slower in cows ovariectomized on day 4 postpartum than in cows ovariectomized on day 4 of the estrous cycle, but there was no difference between groups in the response of FSH (Schallenberger and Peterson, 1982). When suckled beef cows were ovariectomized on day 7 postpartum, the castration response in gonadotropin release was more evident for FSH than LH by day 14 postpartum (Convey et al., 1983). At present, it is not known whether the effect of postpartum interval on the castration response was due to differences in relative sensitivity to endogenous GnRH release, or whether pulsatile release of FSH is not dependent on pulsatile release of hypothalamic GnRH.

Estradiol and Progesterone. Serum E_2 is elevated at parturition in cows and decreases rapidly within 2 to 4 days postpartum (Echternkamp and Hansel, 1973; Kesler et al., 1978b; Sasser et al., 1979; Humphrey et al., 1983). Serum E_2 remains low thereafter, increasing shortly before the first postpartum ovulation (Henricks et al., 1972; Echternkamp and Hansel, 1973; Kesler et al., 1979b; Humphrey et al., 1983).

Although large ovarian follicles are present in the first week postpartum, exposure to pulsatile release of gonadotropins appears to be necessary to increase E_2 concentrations in follicular fluid (Spicer, 1984). Small repeated doses of exogenous GnRH increased E_2 in ovarian follicular fluid of 21-day postpartum beef cows with a numerical, but not significant, increase in serum E_2 over controls (Spicer, 1984). Serum E_2 was doubled (3-4 to 6-7 pg/ml) after small doses of GnRH were repeated in dairy cows at day 8 postpartum (Peters and Lamming, 1984a).

P_4 in peripheral serum is low at calving (Echternkamp and Hansel, 1973; Kesler et al., 1978b). Measurement of P_4 in milk has been used as a means of monitoring luteal activity in dairy cows. In Europe and the United States, 80% or more of milked dairy cows had sustained increases of P_4 prior to day 30 postpartum (Mather et al., 1978; Schams et al., 1978; van de Weil et al., 1979; Webb et al., 1980; Ball, 1982; Caudle et al., 1982). The first increase of P_4 was of shorter duration (8-12 days) and lower in magnitude than succeeding luteal phases in 50-70% of cows sampled (Mather et al., 1978; Schams et al., 1978; van de Wiel et al., 1979; Webb et al., 1980; Caudle et al., 1982). Peters and Lamming (1984b) detected small ovarian structures (5 mm in diameter) by palpation per rectum at the time of the shortened P_4 increases and speculated

the presence of small anovulatory luteinized structures as described by Berardinelli et al., (1979) in pubertal heifers. The first P_4 increases in 10-15% of cows sampled were longer (> 20 days) than a normal luteal phase and the first luteal phase was considered of normal length (14-20 days) in the remaining cows (Mather et al., 1978; Schams et al., 1978; van de Wiel et al., 1979). Significant peaks of LH preceeded about half of the first postpartum increases of P_4, but preovulatory surges were detected prior to all second cycles (Schams et al., 1978).

The hypothalamo-pituitary axis did not have the ability to release LH in response to administration of exogenous E_2 shortly after parturition (Karg and Schallenberger, 1983). Although criteria regarding what constituted an LH response varied among researchers, it was apparent that dairy cows regain the ability to release LH in the magnitude of a preovulatory surge after exogenous E_2 between days 10 and 30 postpartum (Zaied et al., 1981; Karg and Schallenberger, 1983; Stevenson et al., 1983). In cows releasing LH after exogenous E_2, the variation in magnitude of LH release and the interval from E_2 treatment to peak LH tended to be inversely related to postpartum interval (Zaied et al., 1981; Stevenson et al., 1983). E_2 treatment did not result in LH release in postpartum cows when endogenous P_4 concentrations were indicative of luteal activity (Zaied et al., 1981; Stevenson et al., 1983). The positive feedback response of FSH release after exogenous E_2 was seen as early as day 5 postpartum (Karg and Schallenberger, 1983).

In postpartum cows, it thus appears that several endocrine events must take place for the initiation of estrous cycles. The hypothalamo-pituitary axis must regain both the ability to release basal LH and FSH in

a pulsatile manner and the ability to respond to the positive feedback effect of E_2 on gonadotropin release. Also, ovarian follicles must acquire the ability to produce E_2.

Ovarian Cysts in Bovine Reproduction

Clinical Definition. Ovarian cysts, in bovine reproduction, represent a condition characterized by the development and persistence of an anovulatory ovarian follicular structure(s) (usually > 2-2.5 cm in diameter), absence of a CL and cessation of estrous cycles (Roberts, 1971; Seguin, 1975). Diagnosis of ovarian cysts is usually based on the results of a single ovarian palpation per rectum, with subjective classification that cystic structures with a thick-walled consistency are likely to be lined with luteal tissue (Zemjanis, 1970; Roberts, 1971).

The presence of an ovarian cyst reflects an observation of a pathophysiological process that is not well defined. Cows with ovarian cysts may spontaneously resume estrous cycles, but with great variation in the persistence of the cystic condition (Whitmore et al., 1974). Regression of an ovarian cyst may also be accompanied by formation of another (Kesler et al., 1980). Profiles of ovarian steroids in peripheral serum of cows with ovarian cysts reflect the range of values seen at any stage of the estrous cycle, but with unpredictable changes within cows across time (Kesler et al., 1980; Roy et al., 1985).

Cows with ovarian cysts show considerable variation in estrous behavior. Most cows with ovarian cysts are anestrous (47-82% of cases) with the remaining cows showing intermittent or frequent estrous activity (nymphomania) (Bierschwal, 1966; Morrow et al., 1966; Nessan et al., 1977; Bostedt et al., 1979; Leslie and Bosu, 1983). Nessan and King (1981)

reported no difference in serum E_2 or testosterone in cows with ovarian cysts showing nymphomania versus anestrous behavior. Saumande et al., (1979) reported that 65% of cows showing nymphomania had serum E_2 concentrations similar to or above values seen for proestrous cows.

Etiology, Incidence and Predisposing Factors. As detected by palpation per rectum, ovarian cysts have been reported to occur in 5-30% of postpartum intervals in herds composed predominantly of Holstein cows (Bierschwal, 1966; Morrow et al., 1966; Whitmore et al., 1971; Britt et al., 1977; Kirk et al., 1982; Bargai, 1982; Peralta et al., 1982; Smith et al., 1985a,b). Ovarian cysts may be detected at any time, but 50-70% were diagnosed within the first 60 days postpartum (Morrow et al., 1966; Whitmore et al., 1971; Kirk et al., 1982). The incidence is higher in multiparous than in primiparous cows (Seguin, 1975; Marcek et al., 1985; Smith et al., 1985a,b; Roberts, 1971). The condition occasionally occurs in dairy heifers and beef animals (Roberts, 1971). In one study, the incidence of ovarian cysts was threefold higher in cows with other concurrent postpartum disorders such as metritis, retained placenta, delayed uterine involution after dystocia, ketosis, clinical mastitis, etc. (Morrow et al., 1966). If left untreated, 50% or more of cows found to have ovarian cysts in the early postpartum period (< 60 days) spontaneously resumed estrous cycles (Morrow et al., 1966; Whitmore et al., 1974). Rates of spontaneous recovery are lower and the intervals from diagnosis to recovery are more unpredictable when ovarian cysts are diagnosed later in the postpartum period (Whitmore et al., 1974; Kesler and Garverick, 1982).

Other factors have been associated with the formation of ovarian cysts in cattle, but cause-and-effect relationships have not been well established. Such factors included seasonality (higher incidence in fall and winter), nutrition and high milk production (see Seguin, 1975; Kesler and Garverick, 1982 for review). Heredity has also been shown to influence the incidence of ovarian cysts in dairy cattle (Casida and Chapman, 1951; Bane, 1964; Kirk et al., 1982).

The cause of ovarian cyst formation is unknown, but has long been speculated to be a lack of LH release at estrus. Cows with ovarian cysts did not have degranulation of anterior pituitary basophils after estrus when compared to cows that ovulated (Jubb and McEntee, 1955). The LH content of pituitary glands collected post-estrus was higher in cows with ovarian cysts than in cows that ovulated (Yamauchi et al., 1954; Donaldson and Hansel, 1968). In the first 30 days postpartum, formation of ovarian cysts was associated with a lack of LH release in response to an increase of endogenous E_2 concentrations (Kesler et al., 1979a). In cycling cows, clinical observation and results from experiments to induce ovarian cysts indicated that the period between luteal regression and ovulation was a critical time for ovarian cyst formation (Roberts, 1971; Wiltbank et al., 1961; Liptrap and McNally, 1976; Nadaraja and Hansel, 1976). Nadaraja and Hansel (1976) speculated that formation of ovarian cysts after exogenous E_2 could be caused by release of LH that was premature in relation to CL regression.

Anatomy and Steroids in Fluid of Ovarian Cysts. Most anatomical descriptions of ovarian cysts are based on data collected by abattoir survey, thus clinical history and therapeutic measures are often

unknown. There is considerable variation in the histological anatomy of bovine ovarian cysts. The granulosa layer may be very prominent (> 20 cell layers), present but in varying stages of degeneration, or completely absent (Yamauchi and Inui, 1954; Short, 1962; Al-Dahash and David, 1977b; Leidl et al., 1979; Brown et al., 1982). The thecal layer also varied in thickness and extent of degenerative changes (Yamauchi and Inui, 1954; Al-Dahash and David, 1977b; Leidl et al., 1979; Brown et al., 1982).

Evidence of luteinization may also be present. Grossly visible luteal tissue may completely line the wall of an ovarian cyst, or may appear as a crescent-shaped layer at the base of the cyst as seen on cut surface (Roberts, 1971; Al-Dahash and David, 1977a). Luteinization of the theca may also be histologically visible as generalized in distribution or in small, focal patches (Al-Dahash and David, 1977b). In general, granulosa cells were absent when there was histological evidence of luteinization in thecal layers (Yamauchi and Inui, 1954; Short, 1962; Al-Dahash and David, 1977b; Leidl et al., 1979). The basement membrane was absent in nearly all ovarian cysts examined (Al-Dahash and David, 1977b).

Steroiogenic potential of ovarian cysts appears related to the viability of granulosa cells or luteal tissue. Yamauchi and Inui (1954) observed marked estrogen bioactivity in antral fluid only from ovarian cysts with a prominent granulosa layer. E_2 concentrations were highest in antral fluid of ovarian cysts with an intact granulosa layer (Leidl et al., 1979; Bamburg et al., 1981). When the granulosa layer was degenerate or absent, steroidogenesis appeared to be interrupted at the step

for 17-alpha-hydroxylation of P_4, thus preventing accumulation of androgens in ovarian cyst fluid (Short, 1962; Choi et al., 1982). P_4 concentrations in cyst fluid were highest when the cyst wall contained luteinized tissue (Leidl et al., 1979; Bamburg et al., 1981).

Peripheral Sex Steroid and Gonadotropin Concentrations. Most endocrine data from cows with ovarian cysts was obtained from single samples collected at the time of diagnosis, usually just prior to treatment. P_4 in serum or milk can range from being nondetectable to values similar to those of diestrous cows (Kittock et al., 1974; Seguin, 1975; Nakao et al., 1976; Dobson et al., 1977; Nessan et al., 1977; Saumaude et al., 1979; Leslie and Bosu, 1983; Vasquez et al., 1984; Marcek et al., 1985), with great variation in the distribution of low and high values among reports. Serial sampling of cows with ovarian cysts showed that increases of P_4 may occur as short-interval peaks of less than 2 weeks duration, or may be similar in length to a normal luteal phase (Kesler et al., 1980; Gunzler and Schallenberger, 1980; Roy et al., 1985). Sources of P_4 in these instances could include luteinized ovarian cysts or an undiagnosed CL (Al-Dahash and David, 1977a; Gunzler and Schallenberger, 1980). Recent endocrine and gross anatomical data from an individual cow with an ovarian cyst raises the possibility of anovulatory luteal structures as being the source of short-duration P_4 peaks (Roy et al., 1985).

E_2 concentrations in peripheral serum of cows with ovarian cysts may also span the range of values seen throughout the estrous cycle (Seguin et al., 1975; Dobson et al., 1977; Kesler et al., 1981) with one

report suggesting that some individuals with ovarian cysts may have circulating E_2 well above proestrual concentrations (Saumande et al., 1979). Changes in serum E_2 across time have not been as well defined as for P_4 in cows with ovarian cysts, but it is apparent that some cows can maintain high concentrations of E_2 for at least a month (Lunaas et al., 1974; Kesler et al., 1980). In general, cows with ovarian cysts and high endogenous E_2 have low P_4 values (Kittock et al., 1974; Dobson et al., 1977; Leidl et al., 1979; Saumande et al., 1979). There has been no evidence for increased testosterone in peripheral sera of cows with ovarian cysts when compared to values in cycling cows (Kesler et al., 1979; Nessan and King, 1981).

Basal gonadotropin secretion has not been well characterized in cows with ovarian cysts. LH concentrations in single samples collected from cows with ovarian cysts were within the range of values in cycling cows, excluding the preovulatory surge of LH (Seguin et al., 1976; Dobson et al., 1977; Zaied et al., 1981). Kesler et al. (1980) measured LH in samples collected at three-day intervals for a month in cows with ovarian cysts and saw sufficient variation within cows to suggest that pulsatile release may be present. Serum FSH concentrations in single samples from cows with ovarian cysts have been reported in one study, and were also within ranges seen for cycling cows (Dobson et al., 1977).

Zaied et al. (1981) gave exogenous E_2 to cows with ovarian cysts to test the ability of the hypothalamo-pituitary axis to release LH. LH release did not occur in postpartum cows or cows with ovarian cysts when serum P_4 was above 1.0 ng/ml. When endogenous P_4 was low, 3 of 5 cows

with ovarian cysts released LH, but the response was more variable and delayed when compared to LH release by 4 of 4 anestrous cows at 30-40 days postpartum.

Therapy of Ovarian Cysts. The objective of therapy of ovarian cysts in cattle is to initiate fertile estrous cycles. Manual rupture of the cystic structure offered little if any improvement over rates of spontaneous recovery (Roberts, 1971; Vasquez et al., 1984). Casida et al. (1974) gave ovine pituitary extract to cows with ovarian cysts and were the first to report success with a preparation of LH bioactivity. At present, perhaps the most common treatment of ovarian cysts is administration of either human chorionic gonadotropin (HCG) or GnRH. The therapeutic effect of exogenous GnRH results from the release of endogenous LH in cows with ovarian cysts (Kittock et al., 1973; Garverick et al., 1975; Seguin et al., 1976; Kesler et al., 1979b).

A positive response to HCG or GnRH therapy occurred either with luteinization of the cystic structure(s) or ovulation of an ovarian follicle with subsequent CL development (Yamauchi, 1955; Seguin, 1975; Kesler et al., 1981; Tanabe and Brofee, 1982). This response was accompanied by an increase in serum P_4 ($\geq$ 2.0 ng/ml) by 2 to 4 weeks post-treatment (Kittock et al., 1974; Seguin et al., 1976; Kesler et al., 1979b; Nakao et al., 1983). In cows with ovarian cysts and high endogenous P_4, spontaneous luteolysis may be responsible for return to estrus and not the effects of exogenous gonadotropin therapy (Gunzler and Schallenberger, 1980; Marcek et al., 1985). In field trials, less than 30% of untreated cows with ovarian cysts resumed estrous cycles within

30 days of diagnosis (Bierschwal et al., 1975; Seguin et al., 1976; Stolla et al., 1980; Vasquez et al., 1984). HCG or GnRH treatment resulted in resumption of estrous cycles within 30 days in 70 to 100% of cows with ovarian cysts (Bierschwal et al., 1975; Elmore et al., 1975; Garverick et al., 1976; Seguin et al., 1976; Whitmore et al., 1979; Stolla et al., 1980; Kudlac et al., 1984; Marcek et al., 1985). In the latter-cited field trials, conception rates after HCG or GnRH ranged from 40 to 80% of treated cows with ovarian cysts, with average intervals from treatment to conception ranging from 30 to 87 days. Stolla et al. (1980) reported that conception rates were reduced in cows diagnosed and treated for ovarian cysts after 12 weeks postpartum.

Leslie and Bosu (1983) used a prostaglandin analogue as an initial treatment for ovarian cysts and evaluated the response with respect to pretreatment P_4 concentrations. Seventy-seven percent of cows with P_4 above 1.0 ng/ml were in estrus within 7 days posttreatment with an 80% conception rate reported over the first two subsequent estrous periods. Only 8.3% of cows with low P_4 values at the time of prostaglandin treatment conceived within 6 weeks posttreatment. At present, the main limitation of using prostaglandins as a treatment for ovarian cysts was that palpation per rectum did not provide a means of accurate identification of cows that would be likely to respond (Leslie and Bosu, 1983; Nakao et al., 1983; Marcek et al., 1985).

EXPERIMENT I

Gonadotropin Secretion in Dairy Cows with Ovarian Cysts: Pulsatile Release Patterns and Response to Estradiol Challenge

BACKGROUND TO EXPERIMENT I

Interactions within the hypothalamo-pituitary-ovarian axis are important in the regulation of estrous cycles in domestic animals (Hansel and Convey, 1983). In ovariectomized (OVX) cows (Forrest et al. 1980) and ewes (Goodman and Karsch, 1980), there is pulsatile release of LH, resulting from secretion of hypothalmic gonadotropin releasing hormone (GnRH) (Levine et al., 1982). Replacement of luteal concentrations of either progesterone (3-5 ng/ml) or estradiol-17b (2-3 pg/ml) modified the frequency and/or amplitude of episodic pulses of LH in OVX ewes. Progesterone (P_4) decreased frequency but didn't alter amplitude, and estradiol-17b (E_2) decreased amplitude without changing frequency (Goodman and Karsch, 1980). Similar temporal relationships of LH secretion are seen in bovine estrous cycles. Episodic pulses of LH during diestrus were of lower frequency and higher amplitude than during the transitions from luteal regression to estrus or from the preovulatory LH peak to corpus luteum maturation (Rahe et al., 1980; Walters et al., 1984; Schallenberger et al., 1984; Walters and Schallenberger, 1984).

The preovulatory surge of LH is also controlled by ovarian steroids. Exogenous E_2 initiated a preovulatory-like release of LH in prepubertal heifers (Saba et al., 1976; Schillo et al., 1983), OVX cows (Short et al., 1979; Kesner et al., 1981), and 30-40 day postpartum dairy cows that had not resumed estrous cycles (Zaied et al., 1981). This positive feedback effect, seen when P_4 is low, is thought to be due to both an increase in GnRH release and a direct effect on pituitary responsiveness to GnRH, as seen *in vitro* (Padmanabhan and Convey, 1981) and supported by data obtained *in vivo* (Kesner et al., 1981).

Ovarian cysts are one of the most commonly diagnosed infertility problems in dairy cows, reported to occur in 5-30% of postpartum intervals in dairy cows (Morrow et al., 1966; Whitmore et al., 1971; Britt et al., 1977, Bargai et al., 1982; Peralta et al., 1983; Smith et al., 1985a,b). The condition has been defined as the persistence of an anovulatory ovarian follicular structure in the absence of a CL (Roberts, 1971; Seguin et al., 1976), with interruption of estrous cycles. The cystic condition is not static however, as affected cows may spontaneously resume estrous cycles or cystic structures may regress with concomittant formation of another (Morrow et al., 1966; Whitmore et al., 1974; Kesler et al., 1980). Concentrations of E_2 and P_4 in peripheral serum span the range of values seen throughout bovine estrous cycles (Seguin et al., 1976; Dobson et al., 1977; Saumande et al., 1979). Measurement of ovarian steroids across time show that changes within and among affected cows are extremely variable (Lunaas et al., 1974; Gunzler and Schallenberger, 1980; Kesler et al., 1980; Roy et al., 1985).

Inadequate release of LH at estrus has been hypothesized as cause of ovarian cyst formation (Jubb and McEntee, 1955; Donaldson and Hansel, 1968). Kesler et al. (1979a) measured LH, E_2 and P_4 in early postpartum dairy cows and observed a lack of LH release in response to an increase in endogenous E_2 in cows that formed ovarian cysts. Administration of exogenous E_2 after 30 days postpartum resulted in release of LH in 3 of 5 cows with ovarian cysts that was delayed when compared to the response of 4 of 4 cows without ovarian cysts (Zaied et al., 1981).

Information on the nature of pulsatile gonadotropin secretion in dairy cows with ovarian cysts may provide insight into the apparent relative lack or delay of LH release after increase of endogenous or exogenous E_2. At present, pulsatile release of gonadotropins has not been characterized in cows with ovarian cysts. An experiment was conducted to address two objectives. The first was to determine whether gonadotropins are released in episodic patterns in cows with ovarian cysts and identify relationships, if any, between peripheral serum sex steroid concentrations and gonadotropin secretion. The second objective was to assess gonadotropin release after exogenous E_2 in the same cows.

MATERIALS AND METHODS FOR EXPERIMENT I

Animals. Seven Holstein cows (3-10 years old) culled from dairy herds due to infertility resulting from ovarian cysts were referred by veterinary practitioners. The postpartum interval of these cows ranged from 7 to 24 months. Milking was discontinued at the farm of origin. The cows were brought to the Michigan State University Veterinary Clinical Center. Three other Holstein and two Guernsey adult nonlactating cows used at the Veterinary Clinical Center for teaching purposes were found to have ovarian cysts and made available for study. The first cow was admitted during August, 1980 and the last in November, 1982. Animals were held in individual tie stalls and no observations of estrous behavior were made. The cows were fed brome grass-alfalfa hay twice daily, given trace mineral salt twice weekly and had fresh water ad libidum.

Ovarian cysts were defined as follicular structures of at least 2.5 cm diameter, persisting for at least 10 days in the absence of a corpus luteum (Roberts, 1971). Ovarian palpation per rectum and measurement of coccygeal venous serum P_4 at 3 to 7 day intervals were used to monitor the presence of ovarian cysts and rule out the occurrence of estrous cycles. This monitoring period ranged from 7 to 21 days.

Blood Sampling and Hormonal Treatments. An indwelling jugular catheter was inserted in each cow on the day before experimental sampling. Catheter patency was maintained with heparinized saline.

Blood samples were collected and treatments administered by the following schedule: On Day 1, starting at 0800 hr, blood (10 ml) was collected at 15 minute intervals for 4 hours, with an additional 10 ml drawn at each hourly interval. Immediately after the last 15-minute sample at 1200 hr, 1.0 mg estradiol was given intramuscularly. The first two cows sampled received estradiol in the form of estradiol-17b-cyclopentylproprionate (ECP®, Upjohn Co., Kalamazoo, MI). The other 10 cows received 1.0 mg E_2 dissolved in 3 ml safflower oil (Sigma Chemical Co., St. Louis, MO), a preparation shown to initiate predictable LH surges in OVX cows (Kesner et al., 1981). After E_2 challenge, blood (20 ml) was collected at 3 hr intervals for 30 hr (until 1800 hr, Day 2) to determine whether or not preovulatory-like surges of gonadotropins occurred. Immediately after the 1800 hr, Day 2 sample was taken, all cows received a subcutaneous injection of 100 ug GnRH (Sigma Chemical Co.) dissolved in 2 ml of 0.15M NaCl. Blood (10 ml) was collected at 30 min intervals for 2 hr. Because the results of the E_2 challenge would not be known until after hormonal assay, GnRH was given to show whether releasable pituitary gonadotropins were present in the advent of no gonadotropin release after exogenous E_2.

After collection, blood was allowed to clot for 4-8 hr at 20 C and then centrifuged at 1000g for 15 min. Serum was aspirated and stored at -20 C until thawed for assay.

Hormone Assays. Validated radioimmunoassays were used to quantify LH (Convey et al., 1976) and FSH (Carruthers et al., 1980) in all sera collected. E_2 (Appendix), P_4 and testosterone (Appendix) were measured in the first and last hours of the 15 min interval collection period.

Because stress-associated cortisol elevation had been shown to suppress pulsatile LH in OVX beef cows (Echternkamp, 1984) cortisol (Roth et al., 1983) was measured in all samples drawn at 15 min intervals.

Progesterone Radioimmunoassay. Serum P_4 concentrations were measured by radioimmunoassay, using a commercially available kit (Coat-a-Count® No Extraction Progesterone, Diagnostic Products Corporation, Los Angeles, CA). Preliminary validation data showed that dilution curves of unextracted bovine sera did not parallel binding inhibition curves made with P_4 standards in human plasma supplied by the manufacturer.

To remove binding differences probably caused by species' differences in serum proteins, P_4 was removed from serum by solvent extraction and new standards were made. One ml of serum was extracted twice with 6 ml petroleum ether (Mallinckrodt, Inc., Paris, KY). Each extraction step included mixing by horizontal shaking for 10 min, centrifugation at 520g for 5 min, freezing of the aqueous layer, and decanting of the solvent layer into 16 x 100 mm glass culture tubes. The petroleum ether was then evaporated under vacuum in a prewarmed (40°C) vortex evaporator (Haake-Buchler Instruments Inc., Saddle Brook, NJ). One ml of phosphate buffered saline-0.25% gelatin (PBSG) was added to each tube after solvent evaporation and the tubes were incubated in a 37 C water bath for 1 hr. Eighty-six percent of ^3H-P_4 added to bovine sera (New England Nuclear, Boston, MA) was recovered with the extraction and reconstitution steps. Standards were prepared by dissolving P_4 (Sigma Chemical Co., St. Louis, MO) in methanol at a concentration of 1 mg/ml and then further dissolving aliquots of this solution in PBSG to a final

concentration of 20 ng/ml. Final PBSG dilutions used in standard curves were 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, 10.0 and 20 ng/ml.

Assays were run in 12 x 75 mm anti-progesterone antibody-coated polypropylene tubes supplied by the manufacturer. Total count tubes received 1.0 ml of ^{125}I-progesterone, also supplied by the manufacturer (approximately 40-50 x 10^3 cpm). One hundred ul of the appropriate standard or sample was mixed with 1.0 ml of tracer in the antibody-coated tubes. A 12-20 hr room temperature (20°C) incubation was allowed for antigen-antibody equilibration. The contents of all except total count tubes were poured off and the tubes allowed to stand inverted for 5-10 min to maximize drainage. The tubes were placed in a gamma radiation counter (Micromedic, Horsham, PA) and antibody-bound counts were determined. Standard curves were calculated with a log-logit transformation, with mean $\pm$ SD regression coefficient of -0.899 $\pm$ 0.054 and y-intercept value of 0.964 $\pm$ 0.216 for 6 assays. In the absence of unlabeled hormone, 58.8 $\pm$ 2.9% of ^{125}I-P_4 was bound to antibody. Antibody specificity data against 15 other related steroids were provided by the manufacturer, and the highest cross reactivity at 50% decrease of total binding was 2.4% for 11-desoxycortisol. Extrapolation of the standard curve at two standard deviations below the mean total binding value indicated an assay sensitivity of 25 pg/tube. Intra- and interassay coefficients of variation were 8.1 and 11.9%, respectively. When 0.25 or 1.25 ng P_4 were added to bovine serum, 98.5 and 103% were measured in the assay, respectively. Dilution curves of extracted bovine sera paralleled displacement curves of progesterone in PBSG.

Definition of Variables and Statistical Analyses. The 12 cows were retrospectively classified by basal serum E_2 and P_4 concentrations into estrogenic ($\geq$ 10 pg/ml E_2 and < 0.5 ng/ml P_4, Group 1), low steroid (< 10 pg/ml E_2 and < 0.5 ng/ml P_4, Group 2), and luteinized (< 10 pg/ml E_2 and $\geq$ 0.5 ng/ml P_4, Group 3) groups. These classifications were based on correlations of histological anatomy of ovarian cysts with steroid content in cyst fluid (Leidl et al., 1979; Choi et al., 1982).

The 95% confidence intervals of the mean LH or FSH concentrations in a bovine serum pool were calculated (r=17). In 15 min samples, LH or FSH peaks were defined as values exceeding the previous or second previous sample by the 95% confidence limit of the assay (Convey et al., 1983). The critical increases in this study were 1.0 ng/ml for LH and 10 ng/ml for FSH. A nadir was the lowest point between 2 peaks. A gonadotropin baseline was the mean of all points within one standard deviation of the mean of the nadirs. Average overall LH, FSH or cortisol was the mean of all 15 min samples from an individual cow. A preovulatory surge of LH was thought to have occurred post E_2 when a peak above 10 ng/ml was flanked by values showing ascending and descending trends (Swanson and Hafs, 1971). Similar temporal changes were used to identify a surge of FSH, with expected increases of at least 50 ng/ml above basal values.

Basal concentrations of sex steroid and gonadotropin and cortisol variables described above were contrasted among the three groups by one-way analysis of variance with differences identified with Scheffe's interval (Gill, 1978). Chi-square analysis was used to determine

whether the synchrony of pulsatile secretion of LH and FSH differed among groups. Differences among groups in LH or FSH after exogenous E_2 or GnRH were tested with split-plot analysis of variance.

RESULTS OF EXPERIMENT I

Ovarian Changes and Serum P_4 Prior to Intensive Sampling. Ovarian cysts ranged in size from 2.5 to 5.0 cm in diameter. In the pre-sampling period for monitoring the cystic condition, two cows showed disappearance of a cystic structure accompanied by development of another on the contralateral ovary. Subsequently, one of these cows was assigned to Group 1, the other to Group 2. Cystic structures were initially detected on both ovaries of a third cow later assigned to Group 2, with one ovarian cyst disappearing during the documentation period. The three cows had each been examined 4 times throughout a 14 day period. No palpable ovarian changes were detected in the other 9 cows.

There were no P_4 concentrations above 1.0 ng/ml throughout the documentation period. Only one cow (later assigned to Group 3) had P_4 values consistently between 0.5 ng/ml and 1.0 ng/ml. The mean $\pm$ SD pre-sampling. P_4 concentrations for cows subsequently assigned to Groups 1, 2, or 3 were 0.06 $\pm$ 0.05 (r=14), 0.30 $\pm$ 0.30 (r=10), and 0.5 $\pm$ 0.3 (r=4), respectively.

Basal Steroids and Gonadotropins. Classification Groups 1, 2, and 3 contained 6, 4, and 2 cows, respectively. Steroid and gonadotropin means for each group on the day of frequent blood collection are shown in Table 1. As expected by classification criteria, basal serum E_2 was higher ($p = 0.002$) in Group 1 than in the other groups and serum P_4 was higher ($p=0.001$) in Group 3 cows than in the other groups. Baseline and average overall LH concentrations were higher ($p < 0.05$) in Group 1 than

in Groups 2 and 3. There were no differences among groups in testosterone, cortisol, or other gonadotropin variables.

TABLE 1. Basal serum sex steroid, gonadotropin, and cortisol data ($\bar{X} \pm$ SD) from dairy cows with naturally-occurring cystic ovarian follicles.[a] Cows were assigned to groups based on estradiol and progesterone concentrations.

	Ovarian Cyst Classification		
Variable	Group 1	Group 2	Group 3
Estradiol-17b (pg/ml)	18.5 ± 6.6[b]	3.5 ± 1.5	3.5 ± 0.5
Progesterone (ng/ml)	0.03 ± 0.08	0.18 ± 0.17	0.70 ± 0.14[b]
Testosterone (ng/ml)	0.12 ± 0.06	0.10 ± 0.0	0.13 ± 0.05
Overall LH (ng/ml)	2.4 ± 0.2[c]	1.7 ± 0.5	1.7 ± 0.3
No. LH peaks/4hr	2.2 ± 1.5	2.3 ± 1.7	1.5 ± 0.7
LH peak amplitude (ng/ml)	1.4 ± 1.0	1.4 ± 1.1	1.4 ± 0.5
Baseline LH (ng/ml)	1.9 ± .4[c]	1.3 ± 0.3	1.2 ± 0.4
Overall FSH (ng/ml)	74 ± 27	60 ± 18	78 ± 6
No. FSH peaks/4hr	1.8 ± 1.2	2.5 ± 1.7	1.5 ± 0.7
FSH peak amplitude (ng/ml)	14.5 ± 8.8	14.7 ± 10.3	23.8 ± 6.7
Baseline FSH (ng/ml)	69 ± 27	54 ± 14	71 ± 5.6
Overall Cortisol (ng/ml)	14.1 ± 8.3	15.8 ± 4.6	21.3 ± 6.4

[a]Note that the precision of estimation is variable among hormone assays
[b]Differs from other values in a row by $p \leq 0.002$
[c]Differs from other values in a row by $p < 0.05$

There was considerable variation in frequency of LH and FSH pulsatile release within groups, probably accounting for the lack of differences among groups. The frequency of LH or FSH pulses/4hr ranged from 0 to 4 in Groups 1 and 2 and from 1 to 2 in Group 3. Gonadotropin release patterns of six cows are shown on Figure 1. These individuals were selected as examples of low and high pulse numbers from Groups 1 and 2 and one example from Group 3.

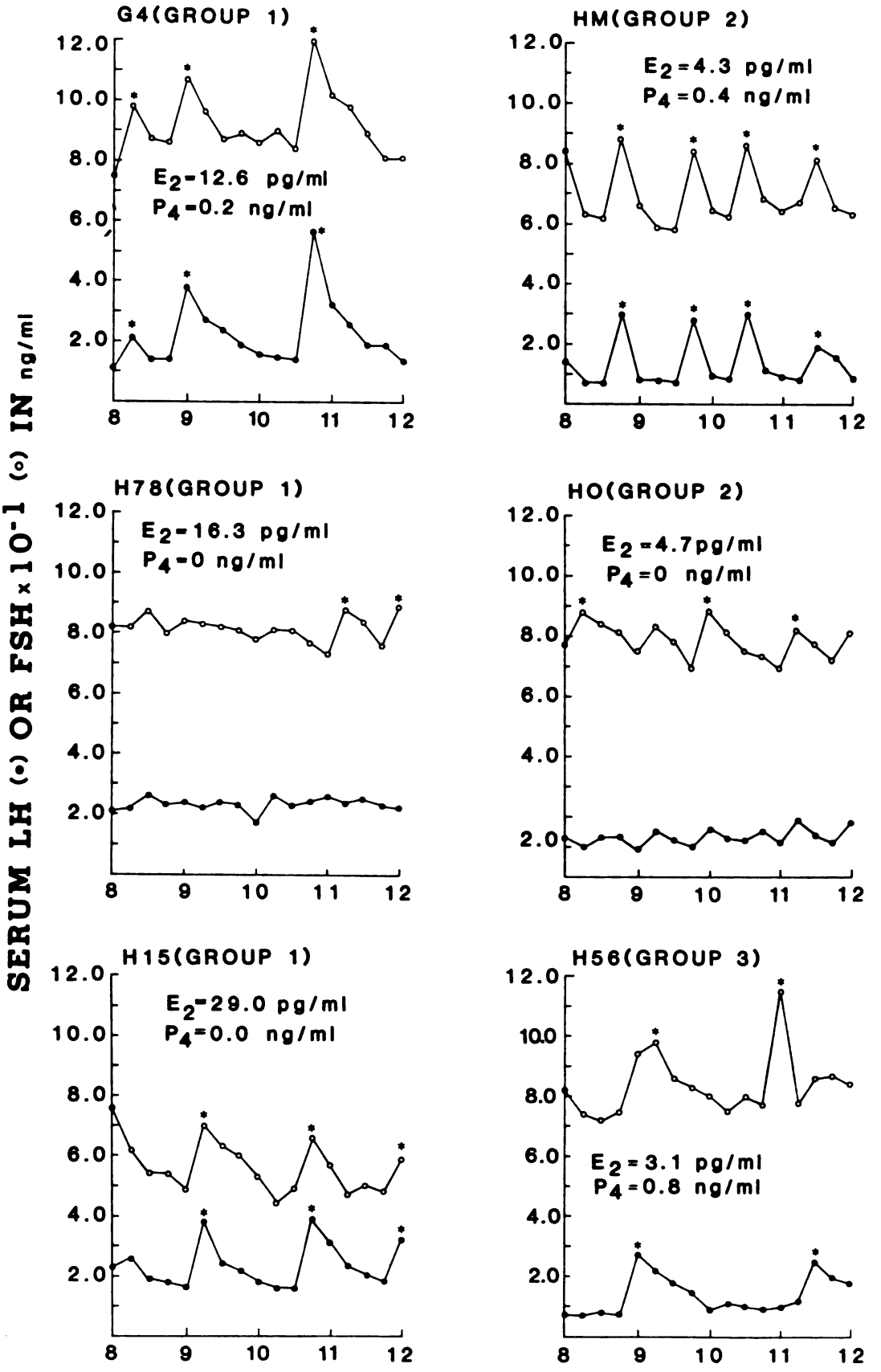
Seventy-two percent of all LH pulses were accompanied by concomitant FSH pulses and 75% of FSH pulses were accompanied by LH pulses. This relationship of synchronous versus asynchronous pulses did not differ among groups ($p > 0.3$) for LH or FSH. An additional 16% and 21% of gonadotropin pulses occurred when cows had only LH or only FSH pulses during the 4 hr sampling period, respectively.

Gonadotropin Response to Exogenous E_2. The ability to detect differences among groups in gonadotropin secretion after exogenous E_2 was confounded with the E_2 preparations used. Use of ECP was discontinued because of lack of data on the ability of ECP to initiate preovulatory surges in cows. There were no differences among groups in LH or FSH release after exogenous E_2 ($p > 0.05$). Gonadotropin secretion in response to exogenous E_2 was then assessed on a within-cow basis, by identification of LH or FSH values after E_2 treatment that were more than three standard deviations above the basal overall LH or FSH mean for each individual. Changes in LH and FSH after exogenous E_2 for each cow are shown on Figures 2 and 3, respectively.

The two cows that received ECP were from Group 1. Neither cow had post treatment LH or FSH values that were considered as outliers from pre-treatment basal LH or FSH values.

Ten cows received E_2 in safflower oil. Four of these cows were from Group 1, with no change in LH or FSH after treatment. Four were from Group 2, of which 2 individuals showed increases in gonadotropins after E_2. One individual, Cow HM, showed the only preovulatory-like LH surge recorded in the experiment, with increased LH values at 15, 18,

Figure 1. Pulsatile release of LH and FSH in adult dairy cows with ovarian cysts (H = Holstein, G = Guernsey). LH or FSH pulses are identified by an *. Corresponding serum concentrations of estradiol-17b (E_2) and progesterone (P_4) are also listed.


G4(GROUP 1)
E2=12.6 pg/ml
P4=0.2 ng/ml
HM(GROUP 2)
E2=4.3 pg/ml
P4=0.4 ng/ml
H78(GROUP 1)
E2=16.3 pg/ml
P4=0 ng/ml
HO(GROUP 2)
E2=4.7pg/ml
P4=0 ng/ml
H15(GROUP 1)
E2=29.0 pg/ml
P4=0.0 ng/ml
H56(GROUP 3)
E2=3.1 pg/ml
P4=0.8 ng/ml
SERUM LH (•) OR FSH x 10-1 (○) IN ng/ml
TIME OF DAY (AM)

and 21 hr post-E_2 that peaked above 20 ng/ml (see Figure 2). FSH values from HM were also markedly elevated at 15, 18, and 21 hr post-E_2 (see Figure 3). Prior to E_2 treatment, this cow had four synchronous episodic pulses of LH and FSH/4 hr and was one of the animals that had a changeover of cystic structures detected by palpation per rectum. Cow HO, another member of Group 2, had a transient increase in LH at 21 through 30 hr post E_2, with values ranging from 3.0 to 5.7 ng/ml (see Figure 2). This cow had 0 and 3 episodic pulses of LH and FSH respectively, prior to exogenous E_2, and was the individual showing disappearance of an ovarian cyst prior to sampling. One of the two Group 3 members also showed transient increase in LH. Cow HS had increased LH values from 18 through 30 hr post E_2 with values ranging from 3.0 to 6.3 ng/ml (see Figure 2). This cow had one pulse/4 hr of LH and FSH.

Gonadotropin Response to Exogenous GnRH. All cows released both LH and FSH after GnRH, with no differences observed among Groups ($p > 0.05$). Mean values of LH and FSH for each sample taken after GnRH administration are shown on Figure 4. The cow having the preovulatory LH surge after exogenous E_2 had the lowest LH values for post GnRH samples, with concentrations of 1.0, 5.9, 7.9 and 9.7 ng/ml at 0.5, 1.0, 1.5 and 2.0 hr after GnRH, respectively. Three of these values were below and one just at the 99% confidence interval for the mean LH value for each time period. Corresponding FSH values for the same cow were also below 99% confidence intervals of mean response after GnRH, with concentrations

Figure 2. Serum LH after exogenous estradiol-17b in 12 dairy cows with ovarian cysts. Prior to treatment, $\bar{X} \pm$ SD endogenous estradiol-17b for Groups 1, 2 and 3 were 18.6 ± 6.6, 3.5 ± 1.5 and 3.5 ± 0.5 pg/ml, respectively. Also prior to treatment, $\bar{X}$ ± SD serum progesterone for Groups 1, 2 and 3 were 0.03 ± 0.08, 0.18 ± 0.17, and 0.70 ± 0.14 ng/ml, respectively. Values marked with an * are 3 SD above the mean of 17 samples collected at 15 min intervals from -4 to 0 hr within each individual (data not shown).

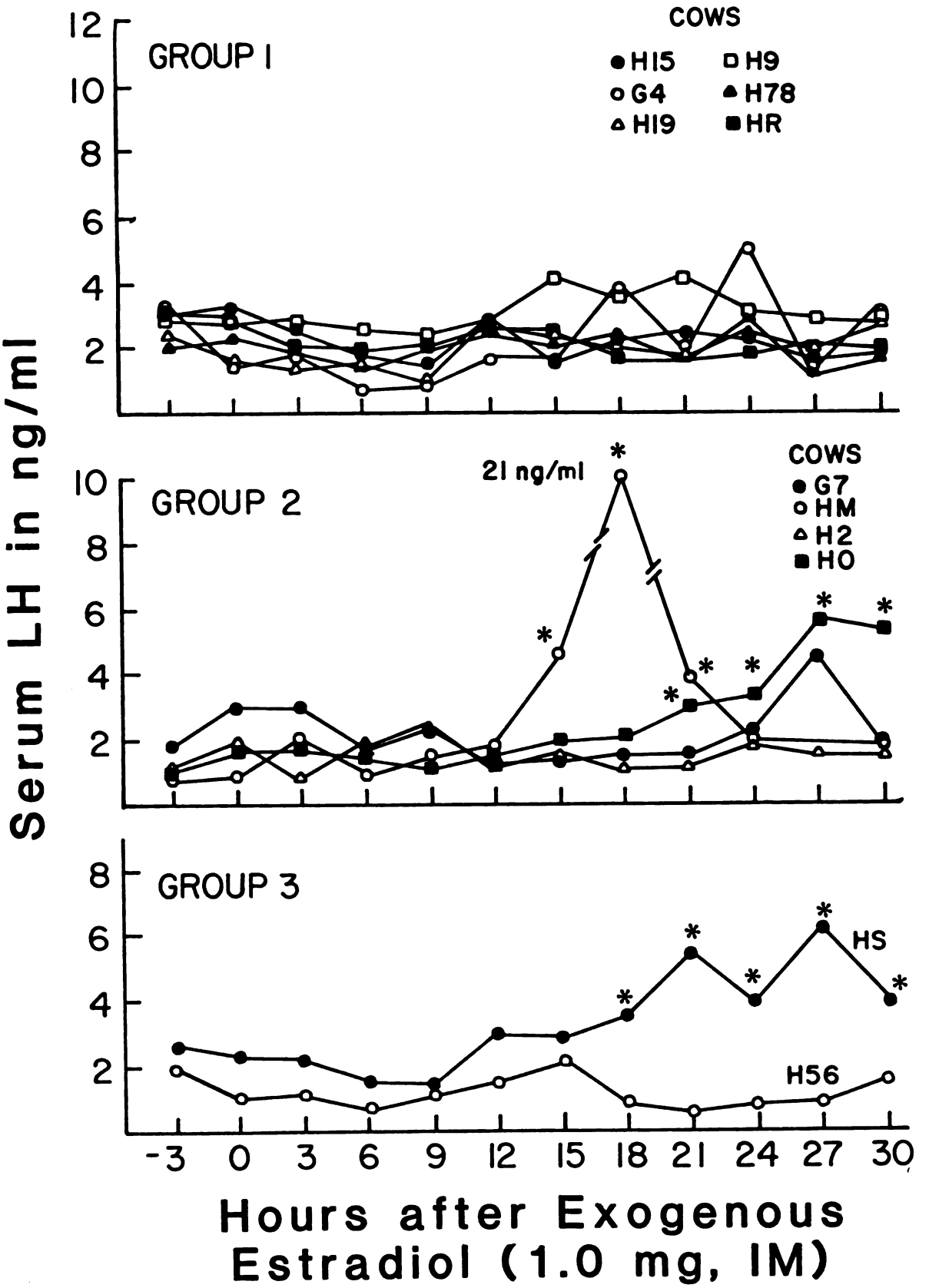
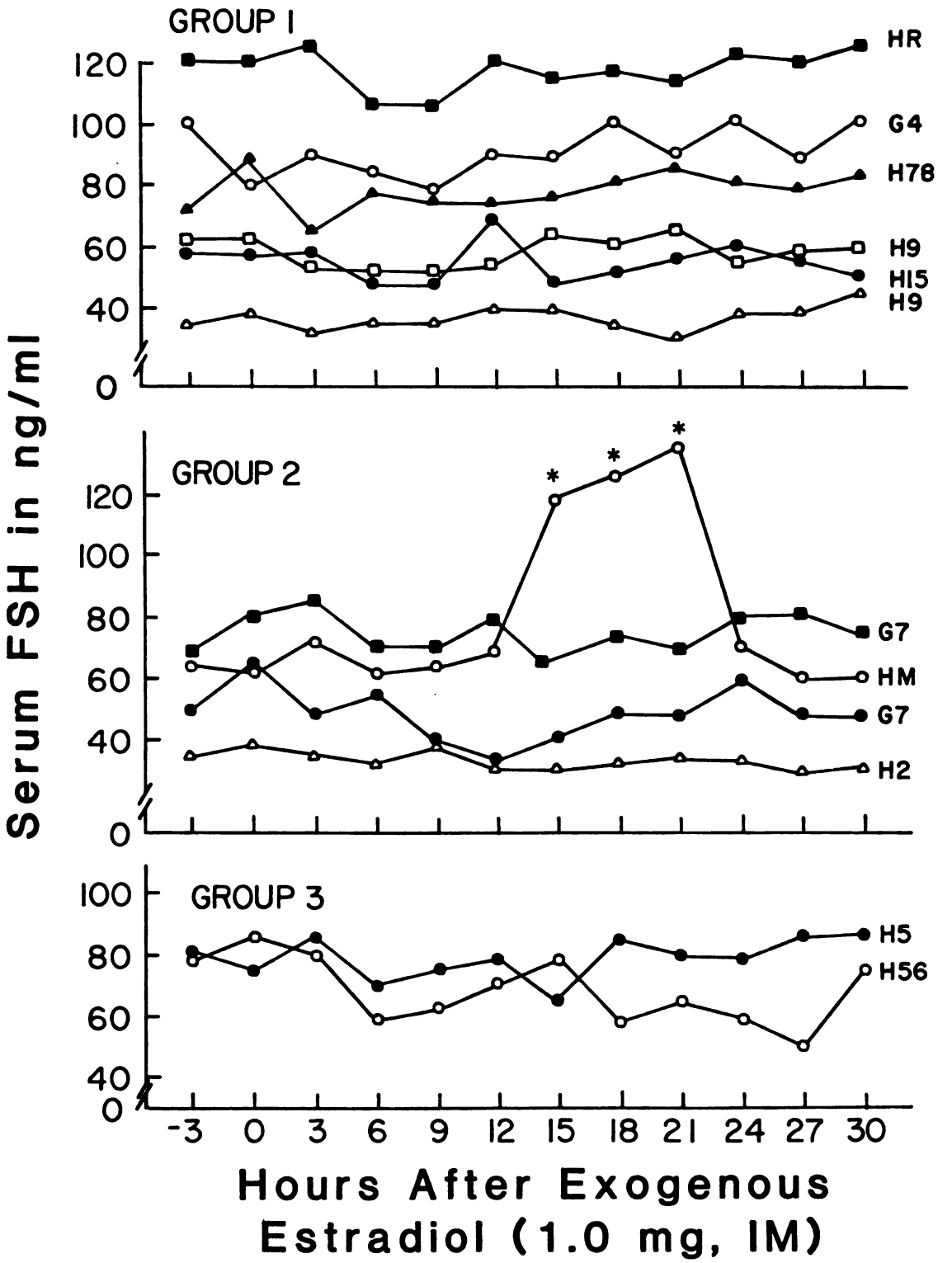
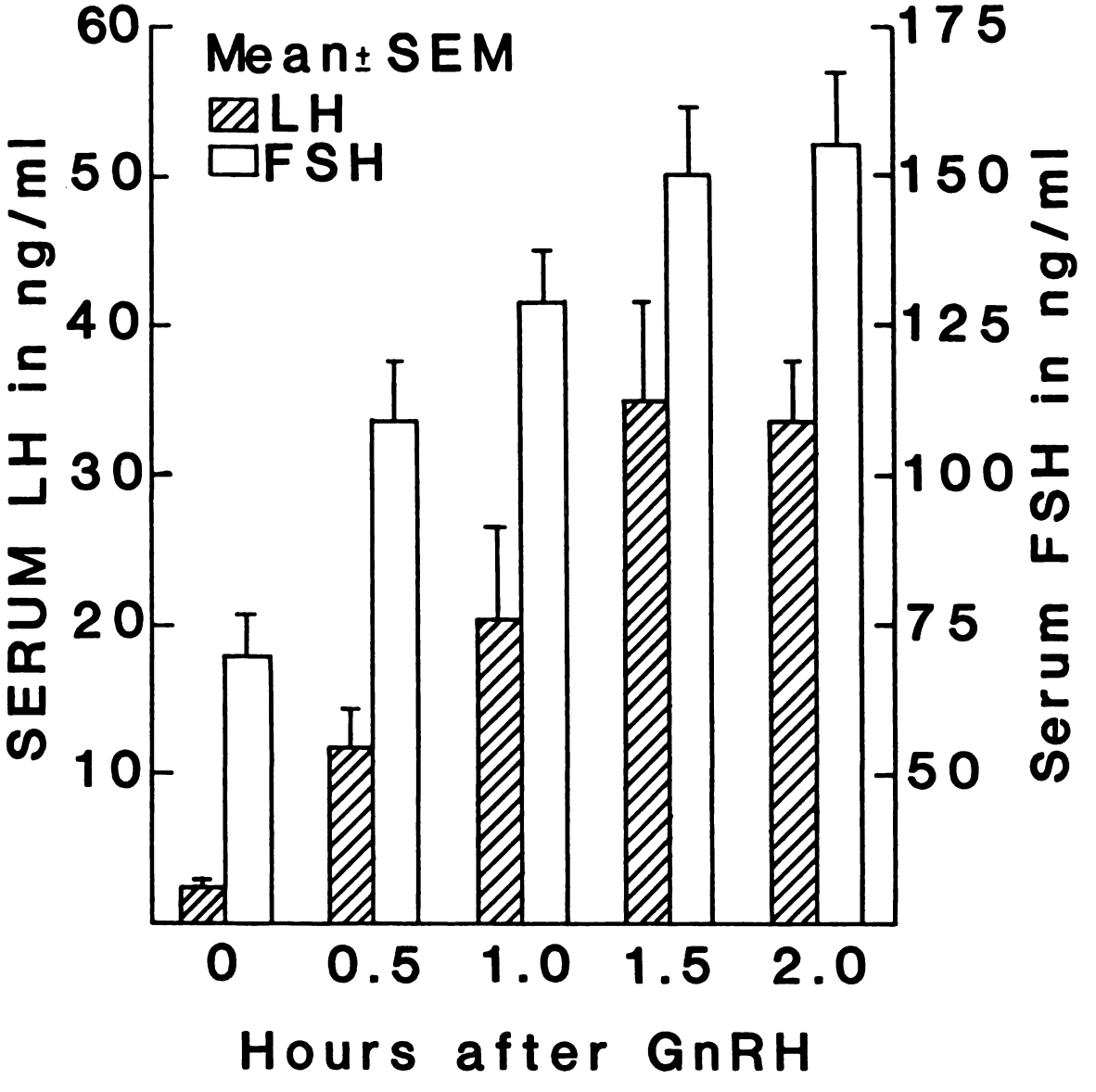

GROUP 1
COWS
H15
H9
G4
H78
H19
HR
GROUP 2
21 ng/ml
COWS
G7
HM
H2
HO
GROUP 3
HS
H56
Serum LH in ng/ml
Hours after Exogenous Estradiol (1.0 mg, IM)

Figure 3. Serum FSH after exogenous estradiol-17b in 12 dairy cows with ovarian cysts. Prior to treatment, X ± SD endogenous estradiol-17b for Groups 1, 2 and 3 were 18.6 ± 6.6, 3.5 ± 1.5 and 3.5 ± 0.5 pg/ml, respectively. Also prior to treatment, X ± SD serum progesterone for Groups 1, 2 and 3 were 0.03 ± 0.08, 0.18 ± 0.17, and 0.70 ± 0.14 ng/ml, respectively. Values marked with an * are 3 SD above the mean of 17 samples collected at 15 min intervals from -4 to 0 hr within each individual (data not shown).


GROUP 1
HR
G4
H78
H9
HI5
H9
120
100
80
60
40
0
GROUP 2
*
*
*
G7
HM
G7
H2
120
100
80
60
40
0
GROUP 3
H5
H56
100
80
60
40
0
-3
0
3
6
9
12
15
18
21
24
27
30
Serum FSH in ng/ml
Hours After Exogenous
Estradiol (1.0 mg, IM)

of 59, 92, 105, and 116 ng/ml measured at 0.5, 1.0, 1.5, and 2.0 hr post GnRH, respectively.

Figure 4. Mean ± SEM serum LH and FSH in 12 dairy cows with naturally-occurring ovarian cysts after administration of 100 ug GnRH in 0.15M NaCl. GnRH was given 30 hr after administration of 1.0 mg estradiol-17b.


Mean± SEM
LH
FSH
SERUM LH in ng/ml
Serum FSH in ng/ml
60
50
40
30
20
10
175
150
125
100
75
50
0
0.5
1.0
1.5
2.0
Hours after GnRH

DISCUSSION OF EXPERIMENT I

In an abbatoir survey, 30% of bovine reproductive tracts with ovarian cysts also had a corpus luteum (Al-Dahash and David, 1977a). These may have represented situations where cystic structures remained after a cow spontaneously resumed estrous cycles or responded to gonadotropin therapy. P_4 concentrations recorded in the present study indicate that the occurrence of undetected corpora lutea was unlikely.

Classification of ovarian cysts was based on the assumption that high E_2 concentrations were accompanied by active granulosa tissue and P_4 was positively related to the extent of thecal lutenization. In this study, the two cows classified as having luteal tissue (Group 3) had circulating P_4 concentration between 0.5 and 1.0 ng/ml. Others (Leslie and Bosu, 1983; Nakao et al., 1983) did not consider ovarian cysts as having significant luteal activity until serum or skim milk concentrations of P_4 were above 1.0 ng/ml. Within the capabilities of the present assay used, further work is necessary to determine minimal P_4 concentrations that would be considered as having P_4-mediated influence on gonadotropin release.

Correlation of histological anatomy of ovarian cysts and sex steroid content in cyst fluid showed that when granulosa cells were present, E_2 usually accumulated. If not, steroidogenesis was interrupted at the conversion of P_4 to 17-alpha-hydroxyprogesterone (Choi et al., 1982/1983). Androgens of thecal origin thus should not accumulate. This is supported by the lack of differences in testosterone among

groups in this study and other reports showing no difference in serum testosterone between cycling cows and cows with ovarian cysts (Kesler et al., 1979; Nessan and King, 1981).

Our data depict circulating sex steroids in one discrete point in time. Kesler et al. (1980) showed great variation in E_2 and P_4 among and within untreated cystic cows in a 30 day period. Inspection of their hormone data across time suggested that significant E_2 concentrations (> 6 pg/ml) were maintained in some cows while increases in P_4 (> 1.0 ng/ml) were much shorter-lived. Further work is needed to determine how long the high circulating E_2 concentrations seen in Group 1 cows could be maintained. The follicular basement membrane was absent in almost all ovarian cysts examined histologically (Al-Dahash and David, 1977b) and this may allow for prolonged granulosa cell viability.

Peripheral sex steroid concentrations measured in this study resemble values seen in the periovulatory period of the bovine estrous cycle. Apart from the preovulatory surge, LH secretion at that time was characterized as having frequent, low amplitude pulsatile patterns (Rahe et al., 1980; Schallenberger et al., 1984; Walters and Schallenberger, 1984). A similar secretion pattern was seen in postpartum cows just prior to the initiation of estrous cycles (Gitlin et al., 1983). LH release in many of the 12 cows sampled in this study qualitatively resembled these patterns. Sampling intervals of 4-5 min were necessary to delineate small amplitude, short-lived LH pulses in ewes (Karsh et al., 1983) and cows (Walters et al., 1984) and therefore the 15 min interval employed in this study may not have detected small pulses in

some individuals. This may have contributed to the elevated baseline LH in Group 1 cows.

Episodic FSH secretion patterns have not been well characterized in cattle. This is partly due to controversy among assay results regarding relationships between LH and FSH release (Bolt and Rollins, 1983). Within the confines of our ability to detect pulsatile release of gonadotropins, release of LH and FSH generally occurred simultaneously. This is in agreement with data from cycling cows (Schallenberger et al., 1984; Walters et al., 1984; Walters and Schallenberger, 1984). There was considerable variation among the cows with ovarian cysts in baseline FSH, but the range of values was not different than those seen in postpartum or cycling cows (Carruthers et al., 1980; Schallenberger et al., 1984; Walters et al., 1984; Walters and Schallenberger, 1984). Two members of Group 1 had FSH values consistently above 90 ng/ml, which could also be consistent with the elevated values seen in OVX heifers (Ireland et al., 1983). There was no evidence for stress-related increase of cortisol causing suppression of gonadotropin release.

Simultaneous collection of jugular and caudal vena cava (Walters and Schallenberger, 1984) or utero-ovarian venous blood (Ireland et al., 1984) has provided information on interactions in gonadotropin and E_2 secretion. In proestrus, pulsatile release of E_2 was synchronous with pulsatile release of LH (Walters and Schallenberger, 1984). The cows in Group 1 had high serum E_2, low serum P_4, and LH or FSH pulse frequencies ranging from 0 to 4/4 hr. Further work is needed to first

confirm whether the ovarian cyst is the site of E_2 production and if so, whether E_2 secretion is independent of pulsatile gonadotropin release.

None of the Group 1 cows released LH or FSH above basal concentrations after exogenous E_2. These results suggest that the high endogenous E_2 concentrations may have provided negative feedback on hypothalamic centers involved in the positive feedback response to E_2. In rats, 2-hydroxyestrone, an E_2 metabolite, may interfere with the interaction of catecholaminergic and GnRH-producing neurons and prevent release of the preovulatory surge of LH (Okatani and Fishman, 1984). A similar mechanism may be in effect in the Group 1 cows.

Gonadotropin release after exogenous E_2 was extremely varied in the cows in Groups 2 and 3. The responses could be classified as no increase, transient release (LH increase, but less than 7 ng/ml), or a preovulatory-like surge of LH. A similar variety of responses was seen after E_2 was given to postpartum dairy cows, where the interval to LH peak and magnitude of peak LH were inversely and positively related to postpartum interval, respectively (Karg and Schallenberger, 1983; Stevenson et al., 1983). P_4 inhibits the positive feedback effect of E_2 on LH release, so lack of or a transient release of LH after exogenous E_2 would be an expected response in Group 3 cows (Zaied et al., 1981; Kesner et al, 1982). If the formation or maintenance of ovarian cysts is caused by a reduced sensitivity to the positive feedback effect of E_2, then the graded increases in LH could be indicative of stages of recovery of hypothalamo-pituitary sensitivity to E_2. Turnover of cystic

structures was detected in the present study, in support of data from Kesler et al. (1980).

All cows released both LH and FSH after exogenous GnRH, as expected from the results of other studies (Kittok et al., 1973; Seguin et al., 1976; Kesler et al., 1979c). The uniformly low LH response by the cow having the post E_2 surge of gonadotropins is suggestive of depletion of releasable pituitary gonadotropin (Convey et al., 1977) and/or refractoriness to GnRH (Kesner et al., 1981; Walters and Schallenberger, 1984). Although the exogenous GnRH was in essence a treatment for ovarian cysts, the cows were not monitored for the return of estrous cycles since the potential influence of exogenous E_2 was not controlled.

In summary, cows with ovarian cysts were grouped according to E_2 and P_4 profiles. The classification groups represented individuals with high endogenous E_2 (Group 1), low E_2 and P_4 (Group 2), and P_4 concentrations suggestive of luteal activity (Group 3). The frequency and amplitude of pulsatile LH or FSH release were variable, with no characteristics unique to any group. Baseline LH was higher in Group 1 cows ($p < 0.05$). Given the present knowledge on pulsatile release of LH and FSH, patterns seen in the cows with ovarian cysts were similar to what would be expected in postpartum or cycling cows, apart from the preovulatory surge of gonadotropins.

When given exogenous E_2, none of the six cows in Group 1 responded with an increase in LH and FSH over basal concentrations. But all members of the group released LH and FSH after GnRH. This suggested that the hypothalamic centers involved in initiation of the preovulatory

gonadotropin surges were either not responsive to or under inhibition by the high endogenous E_2 concentrations.

Of the four cows in Group 2, one had a preovulatory gonadotropin surge, one had transient release of LH, and the others showed no increase in baseline gonadotropin concentrations after exogenous E_2. This group, with its low endogenous E_2 and P_4 concentrations were expected to be the best candidates to show a positive feedback response to exogenous E_2. The lack of a preovulatory surge of gonadotropins in 3 of the 4 cows supports an earlier hypothesis of a reduced hypothalamic sensitivity to E_2 having a role in ovarian cyst formation (Kesler et al., 1979[a]; Kesler et al., 1980; Zaied et al., 1981). As previously discussed, the endogenous P_4 concentrations in Group 3 cows may have been sufficient to suppress the positive feedback response to exogenous E_2.

Because the incidence of ovarian cysts in dairy cows is highest in the first 60 days postpartum, the cows in this study represent a less common subgroup at six months or more postpartum. Further work is needed to determine if similar relationships between sex steroid production and gonadotropin release are seen in early postpartum cows with ovarian cysts. Future therapeutic studies should attempt to classify ovarian cysts by E_2 and P_4 profiles and address efficacy of treatment for each category.

EXPERIMENT II

Formation of Ovarian Cysts in Dairy Cows After Exogenous Estradiol or ACTH and Endocrine Profiles in Cows With Experimentally-Induced Ovarian Cysts

BACKGROUND TO EXPERIMENT II

Steroidogenic capabilities of bovine ovarian cysts are probably determined by several factors. The development and viability of granulosa or luteinized thecal tissue are directly related to concentrations of estradiol-17b (E_2) or progesterone (P_4) in cyst fluid, respectively (Choi et al., 1982/1983). Episodic release of LH and FSH varies greatly among cows with ovarian cysts (see Experiment 1) and the contribution of pulsatile gonadotropin release toward maintenance of steroidogenesis by ovarian cysts is unknown. A recent review (Eyestone and Ax, 1984) emphasized that most endocrine data from cows with ovarian cysts was collected at unknown intervals after cyst formation. It is not known if the morphological or endocrine variation seen with ovarian cysts represent stages of a single or multiple pathophysiologic mechanisms.

Ovarian cysts have been induced in cattle, intentionally or not, by a variety of treatments given in late diestrus or proestrus. Such treatments have included exogenous E_2 (Wiltbank et al., 1961; Nadaraja and Hansel, 1976), exogenous ACTH (Liptrap and McNally, 1976), or antiserum against bovine LH (Nadaraja and Hansel, 1976). Exogenous E_2 given concurrently with manual CL enucleation resulted in formation of ovarian cysts lined with grossly-visible luteal tissue (Whitmore et al., 1972). In one study where ovarian cysts were formed after exogenous E_2, changes in peripheral serum E_2 and P_4 were extremely variable among affected heifers (Nadaraja and Hansel, 1976). After exogenous ACTH, cyst formation was characterized by a rise of serum E_2 to proestrual

concentrations that were then maintained for several days until ovulation after spontaneous cyst regression or treatment with human chorionic gonadotropin (Liptrap and McNally, 1976).

The mechanisms whereby ovarian cysts are formed after exogenous E_2 or ACTH are unknown. Nadaraja and Hansel (1976) speculated that a pharmacologic dose of E_2 would cause luteal regression with a premature surge of LH that could alter follicular growth or maturation. ACTH treatment blocked the preovulatory surge of LH in synchronized heifers (Stroebel and Moberg, 1982), probably through a cortisol-mediated inhibition of LH release and increased P_4 production by the adrenals (Stoebel and Moberg, 1982; Li and Wagner, 1983; Echternkamp, 1984; Watson and Munro, 1984).

An experiment was conducted with two objectives. The first was to induce ovarian cysts in dairy cows with exogenous E_2 or ACTH and measure LH and sex steroid changes accompanying cyst formation. The second was to characterize basal gonadotropin release and the response to exogenous E_2 in cows with artificially-induced ovarian cysts.

MATERIALS AND METHODS FOR EXPERIMENT II

I. Induction of Ovarian Cysts

Animals and Treatments. Fifteen adult (14 Holstein and 1 Guernsey) parous cows were brought to the Michigan State University Veterinary Clinical Center in August, 1980. The cows were purchased at an auction market and age, parity, and previous reproductive history were unknown. Criteria for selection included complete uterine involution and evidence of estrous cyclicity as determined by palpation per rectum.

The cows were kept in tie stalls throughout the course of the experiment. Water was provided ad libidum. Bromegrass-alfalfa hay was fed twice daily and trace-mineral salt was given to each cow twice weekly.

On Day -28 of the study, 7 cows were diagnosed by palpation per rectum as being in diestrus and were given 25 mg prostaglandin F_{2a} (PGF_{2a}, Lutalyse, Upjohn, Kalamazoo, MI) intramuscularly (IM). On Day -18, all cows were diagnosed as having a corpus luteum (CL) and were given 35 mg PGF_{2a}, IM. The intent of this synchronization schedule was to have all cows in mid- to late diestrus at the time of treatment.

On Day -1, an indwelling jugular cannula was inserted in each cow. Cannulae were kept patent with heparinized saline. Rectal examination results indicated that 3 cows had begun CL regression and were in proestrus or estrus at this time. These individuals were blocked among each of three treatment groups. The other cows were randomly assigned to the 3 treatment groups: 1) controls, receiving an IM injection of safflower oil, 2) 10 mg E_2 ($E_2$10) in safflower oil (Sigma Chemical Co., St. Louis, MO), 3) 100 IU ACTH gel (Adrenomone^R, Burns-Biotech, Omaha, NE) given subcutaneously and repeated at 12 hr intervals for 10 consecutive days. Oil, $E_2$10 and the first ACTH injection were given at 12 noon on Day 0.

Blood Collection and Rectal Examination Schedules. Twenty ml of blood was drawn just prior to treatment on Day 0. Ten or 20 ml blood samples were then collected at 4 hr intervals from Day 0 through 12 noon on Day 8. Blood was allowed to clot for 4-8 hr at 20 C and then kept at

4 C for 12-16 hr before centrifugation at 1000 g for 15 min. Sera were poured into 12 x 75 mm glass tubes and stored at -20 C before assay.

Reproductive tracts were examined by palpation per rectum (Zemjanis, 1970) on Day -1 and then daily from Days 2 through 20 of the experiment. Description of ovarian structures and uterine consistency were recorded.

Hormone Assays. Hormones were quantified by radioimmunoassay techniques. LH (Convey et al., 1976) was measured in all samples collected at 4 hr intervals. E_2 (Appendix) was measured at 8 hr intervals from Days 0 through 8. Serum P_4 (see EXPERIMENT I) was measured at 8 hr intervals from Days 0 through 4 and then once daily from Days 5 through 8. Cortisol (Roth et al., 1983) was measured at 4 hr intervals for the first 36 hr of the experiment and then at 4 hr intervals throughout Days 3 and 5.

Definition of LH Release and Statistical Analysis. A preovulatory surge of LH was defined as a peak of at least 10 ng/ml magnitude lasting for 2 or more consecutive 4 hr interval samples. Cortisol changes among treatment groups were analyzed by split-plot analysis of variance with differences among groups identified with Scheffe's interval (Gill, 1978).

II. Endocrine Profiles of Cows With Experimentally-Induced Ovarian Cysts

Blood Sampling Schedule. On Day 15 of the cyst induction experiment, cows that were identified as having ovarian cysts were submitted to the sampling and treatment regimen described in EXPERIMENT I. In

short, blood samples were collected at 15 min intervals from 8 am through 12 noon. One mg E_2 in safflower oil was then injected IM and additional blood samples were drawn every 3 hr for 30 hr to determine if a preovulatory surge of gonadotropins occurred.

Hormone Assays. LH and FSH (Carruthers et al., 1980) were measured in all samples collected. E_2 and P_4 were measured in the first and last hours of the 15 min sampling period.

Definitions of Gonadotropin Release. In 15-min samples, pulsatile release of LH or FSH was identified as values exceeding the previous or second previous sample by the 95% confidence interval of the assay (Convey et al., 1983). These intervals were 1.0 ng/ml for LH and 10 ng/ml for FSH. Average overall LH or FSH was the mean of all samples drawn at 15 min intervals.

Criteria for identification of a preovulatory surge of LH were described in Materials and Methods for cyst induction. A preovulatory surge of FSH was defined as an increase of more than 50 ng/ml above average FSH, lasting for at least two consecutive 3 hr interval samples. Evidence for an increase in basal LH or FSH secretion was assessed on a within-cow basis, by identification of values more than three standard deviations above the average overall mean.

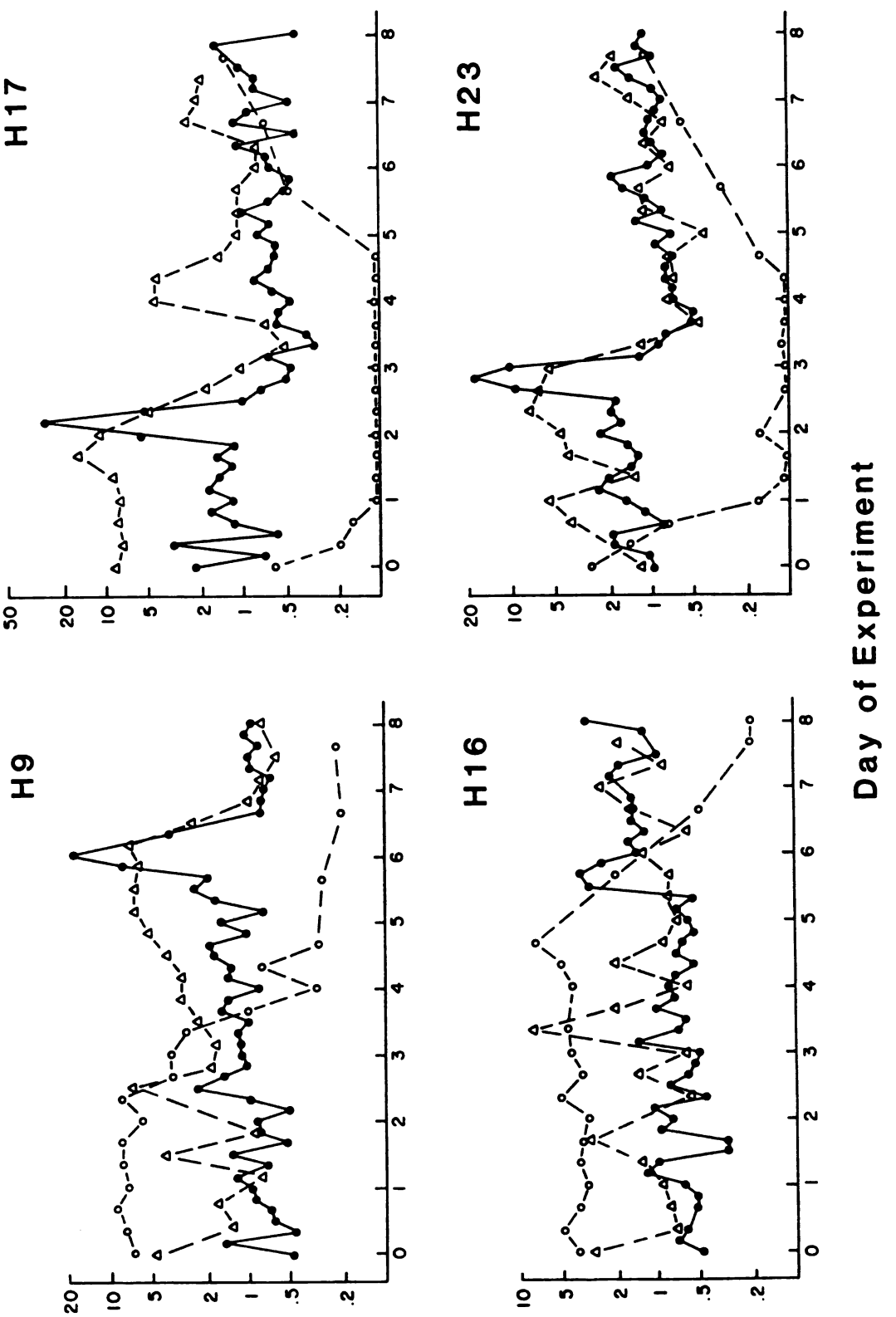
RESULTS OF EXPERIMENT II

I. Induction of Ovarian Cysts

Control Group. All control cows showed palpation and hormonal changes indicative of CL regression, ovulation, and new CL formation. Ovarian cysts were not diagnosed at any time. Despite prior estrus synchronization measures, naturally-occurring estrus was estimated to occur as early as Day -3 and as late as Day 9 in control animals. As a result, preovulatory surges of LH were detected in 3 of 5 individuals. Changes in serum LH, P_4, and E_2 for the control cows that underwent luteal regression after Day 0 of the experiment are shown on Figure 5. LH surges were preceeded by a decline in serum P_4, with concentrations remaining below 0.3 ng/ml for 20-24 hr before the surge of LH. The three preovulatory surges of LH ranged from 18.5 to 28.5 ng/ml in magnitude and were of at least 8-10 hr duration. Serum E_2 increased in a 24 hr period prior to the LH surge, with peak values ranging from 7 to 16 pg/ml. Serum E_2 decreased abruptly during the LH surges, to concentrations below 2 pg/ml. The LH surges occurred early enough in the sampling period in two individuals to record the rise in P_4 accompanying CL growth. Serum P_4 was above 0.8 ng/ml by 4-5 days after the LH surge in these individuals.

Serum P_4 increased progressively from 0.1 to 1.5 ng/ml from Days 0 to 3 in the cow that ovulated prior to Day -1 (data not shown). Serum P_4 remained between 1.7 to 2.9 ng/ml from Days 3 through 8 in this individual. Palpation results indicated that this latter cow was returning to estrus on Day 19 and 20 of the experiment.

Figure 5. Temporal changes in serum LH, progesterone and estradiol-17b in adult Holstein cows given 3.0 ml safflower seed oil on Day 0. All cows showed changes of the reproductive tract, as detected by palpation per rectum, indicative of spontaneous luteal regression and ovulation.


H9
H16
H17
H23
Serum LH (•—•) or Progesterone (○—○) in ng/ml
or Estradiol (▲–▲) in pg/ml
Day of Experiment

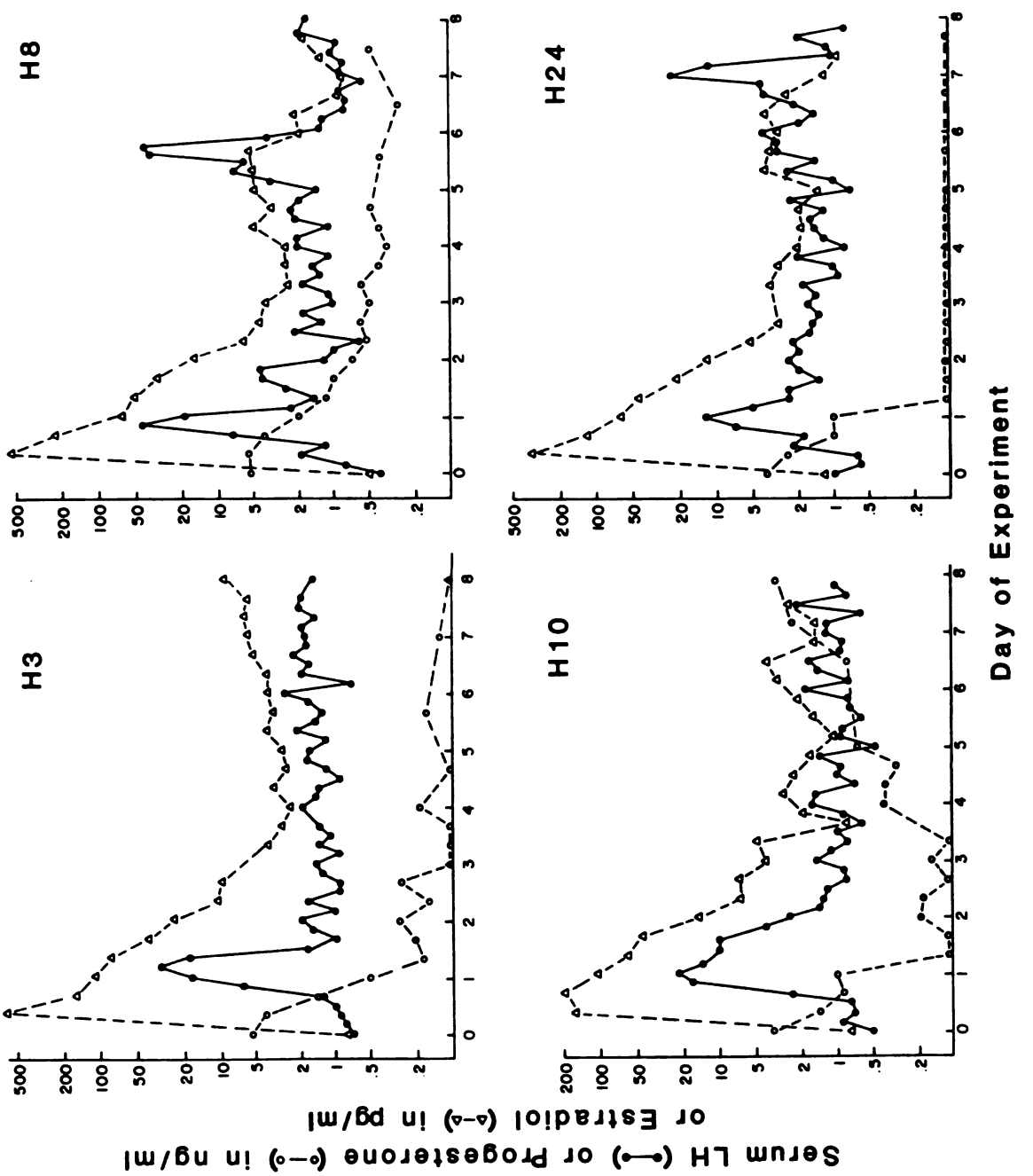
$E_2$10 Treated Group. Peak serum E_2 concentrations ranged from 200-600 pg/ml at 8-16 hr post injection and then progressively declined to values below 3 pg/ml by Day 3 (see Figure 6). Marked vulvar swelling and hyperemia was noted for a 1-2 day period in all cows given $E_2$10.

Four cows had a palpable CL with serum P_4 ranging from 3.5 to 5.4 ng/ml just prior to treatment. Serum P_4 declined rapidly after $E_2$10 treatment, with all cows showing palpation changes indicative of luteal regression on Day 2. As P_4 was decreasing, surges of LH were recorded in these four cows, beginning at 16-20 hr post-injection (Figure 6). These surges of LH were of 12-24 hr duration with peak values ranging from 12 to 44 ng/ml.

Ovarian cysts were diagnosed in 2 cows after $E_2$10-induced luteolysis. In cow H3, the ovaries remained inactive after CL regression until a 10mm diameter follicle was identified on the left ovary on Day 7. This follicle increased to 25 mm in diameter by the next day and was diagnosed as an ovarian cyst. An increase in serum E_2 accompanied development of the cyst, with a concentration of 9 pg/ml measured on Day 8 (Figure 6). Cow H10 was diagnosed as having a 35mm diameter follicular cyst on the right ovary on Day 7. This structure was thought to originate from a 15mm follicle first detected on Day 5. An increase of serum P_4 accompanied formation of this ovarian cyst, with concentrations above 2 ng/ml by Day 7 (Figure 6).

Two other cows with $E_2$10-induced luteal regression did not form ovarian cysts. However, neither animal had palpable evidence of CL growth or an increase in P_4 by Day 8. Instead, one cow had a spontaneous

Figure 6. Temporal changes in serum LH, progesterone and estradiol-17b in adult dairy cows given 10 mg estradiol-17b in safflower seed oil on Day 0. Luteal regression, as determined by ovarian palpation per rectum, occurred in all cows shortly after treatment. An ovarian cyst was diagnosed in each of cows H3 and H10 on Day 7. Cows H8 and H24 did not form ovarian cysts, but ovarian structures were not identified by palpation until detection of luteal tissue on Days 12-13.


H3
H8
H10
H24
Serum LH (•—•) or Progesterone (○—○) in ng/ml
or Estradiol (▵–▵) in pg/ml
Day of Experiment

preovulatory surge of LH occurring on Day 5, the other on Day 8, (Figure 6). These surges were of 8-12 hr duration and reached peak values of 22 and 42 ng/ml. Maximum serum E_2 values were 4-5 pg/ml just prior to the LH surges, with a decline to 1 pg/ml occurring while the surges were in progress (Figure 6). Moderate uterine tone was detected on the day of the LH surge for both cows, but ovarian follicles of preovulatory size were not detected. Both cows showed evidence of CL growth as detected by palpation, by Days 12 or 13 of the experiment.

One additional cow ovulated prior to $E_2$10 injection. Palpation observations and serum P_4 changes were indicative of CL growth (not shown). This cow showed palpation changes characteristic of CL regression and estrus on Days 18-20.

ACTH-Treated Group. Mean $\pm$ SEM concentrations of cortisol in serum samples collected on Days 1, 3 and 5 of the experiment are shown on Figure 7. Cortisol was higher in ACTH-treated cows at all times sampled except 12 and 24 hr after the start of the experiment ($p < 0.05$). Cortisol means from control and $E_2$10-treated cows did not differ.

Four of 5 cows had a palpable CL with serum P_4 concentrations ranging from 1.9 to 5.4 ng/ml just prior to the start of ACTH treatment. Ovarian cysts formed in 2 of these individuals. These cows (H12 and H22) had palpation evidence of CL regression on Days 2 and 3, respectively. CL regression was followed by the appearance of a single 20-25mm diameter follicular cyst one day later in each animal. CL regression was not accompanied by a typical decline in serum P_4, where

Figure 7. Changes in serum cortisol in nonlactating adult dairy cows after administration of oil (controls) or estradiol-17b at 0 hr, or 100 IU ACTH gel given at 12 hr intervals. ACTH treatment was continued throughout the periods where cortisol was not measured. Means marked with * are higher than other means within the same time ($p < 0.05$).

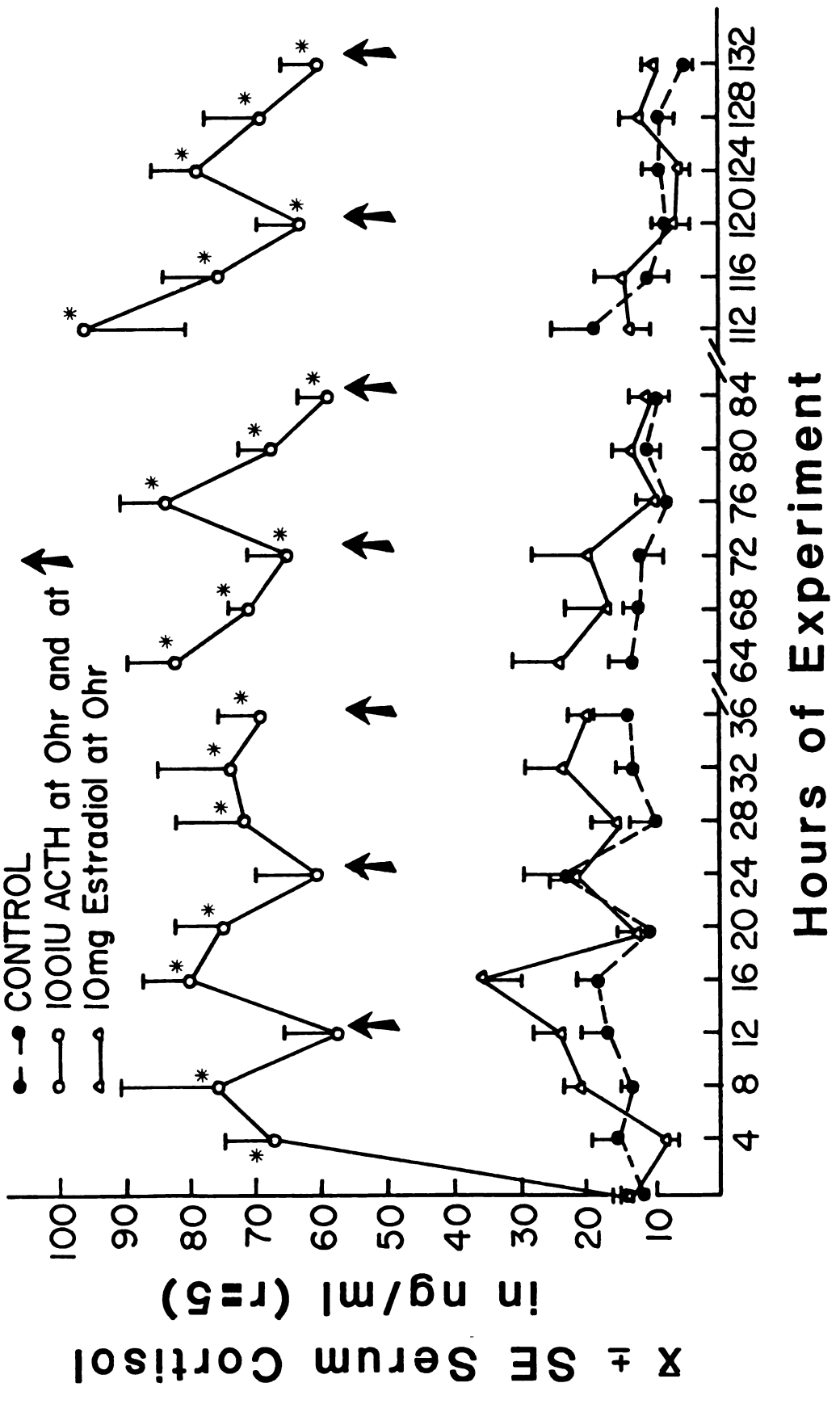
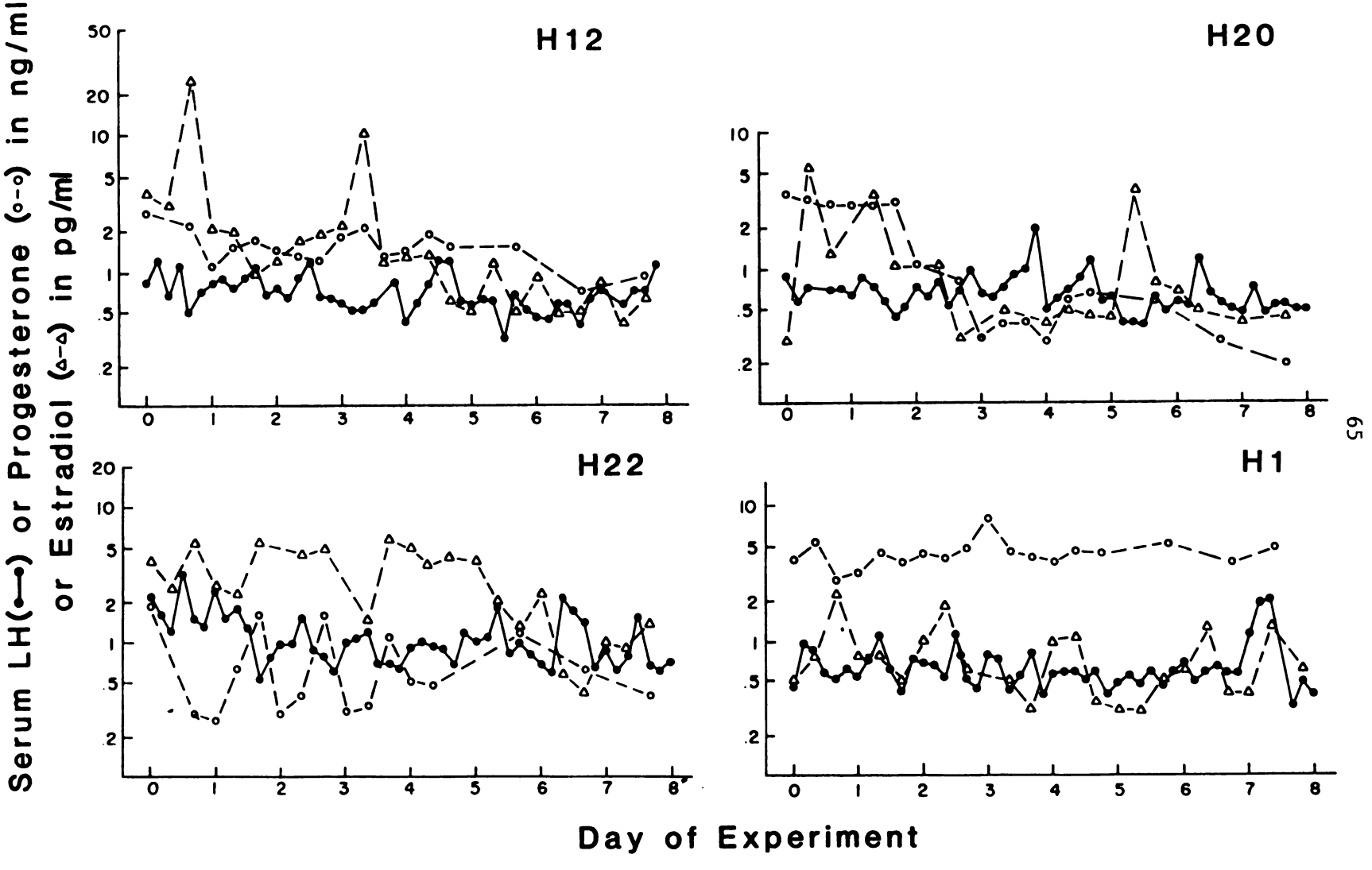

CONTROL
100IU ACTH at 0hr and at
10mg Estradiol at 0hr
X + SE Serum Cortisol
in ng/ml (r=5)
100
90
80
70
60
50
40
30
20
10
4 8 12 16 20 24 28 32 36 64 68 72 76 80 84 112 116 120 124 128 132
Hours of Experiment

Figure 8. Temporal changes in serum LH, estradiol and progesterone in nonlactating adult Holstein cows given 100 IU ACTH gel at 0 hr and repeated at 12 hr intervals for 10 days. Cows H12 and H22 each formed an ovarian cyst, first diagnosed by palpation per rectum on Days 2 and 3, respectively. Cow H20 underwent luteal regression and became anestrus. Cow H1 did not undergo luteal regression during the sampling period.


H12
H20
H22
H1
Serum LH(●—●) or Progesterone (o-o) in ng/ml
or Estradiol (Δ-Δ) in pg/ml
Day of Experiment

concentrations remained between 0.7-2 ng/ml in cow H12 and 0.3-1.5 ng/ml in Cow H22 just before and after cyst diagnosis (Figure 8). Cow H12 had 2 short-lived peaks of serum E_2 above 10 pg/ml prior to cyst formation, while transient increases of serum E_2 did not rise above 6 pg/ml in cow H22 (Figure 8). Preovulatory surges of LH did not occur.

The two other cows with a CL present at the onset of ACTH treatment did not form ovarian cysts, but had altered estrous cycles. Cow H1 did not show palpation evidence of CL regression until Day 20. Serum P_4 remained above 2.0 ng/ml in all sera assayed and an LH surge was not observed. Palpation results showed that CL regression had occurred after the Day -18 PGF_{2a} treatment in this cow and post-estrual bleeding was observed on Day -15. Thus, Cow H1 apparently had an interestrous interval of approximately 35 days. Cow H20 showed palpation changes and a decrease in P_4 on Days 2-3 that were indicative of CL regression (Figure 8). By Day 6, this cow had clinical mastitis and maintained a rectal temperature above 40 C for several days despite systemic antibiotic therapy. After CL regression, there was no surge of LH and the ovaries remained inactive through the course of the experiment.

The remaining cow that received ACTH was thought to have ovulated on Days 0 or 1. This cow had palpation changes and an increase in serum P_4 (not shown) indicative of CL growth. This individual subsequently had palpation characteristics of CL regression and estrus on Days 19-20.

II. Gross Ovarian Changes and Endocrine Profiles of Cows with Induced Ovarian Cysts

Ovarian Structures. On Day 15 of the experiment, ovarian cysts that were initially formed after $E_2$10 or ACTH treatment were still

present as determined by palpation per rectum. Since the time of cyst diagnosis, no changes in ovarian structures were detected in Cow H10. A second 25 mm follicular structure was detected on the ovary ipsilateral to the original ovarian cyst on Days 9 and 14 in cows H22 and H3, respectively. The two ovarian cysts were still present by Day 15 in H22, but the second cystic structure had disappeared one day later in H3. An additional 10-15 mm follicle was detected on each of Days 8 and 14 in Cow 12 and both follicles were still present on Day 15.

Basal Gonadotropin and Sex Steroid Secretion and Response to Exogenous E_2. Serum E_2 and P_4 concentrations and patterns of basal LH and FSH release are shown on Figure 9. Sex steroid concentrations, average overall LH and FSH and numbers of episodic LH and FSH pulses/4 hr are also summarized on Table 2. Although there were few animals and considerable variation, there was suggestion of some relationships between E_2, P_4 and LH release. Basal E_2 and P_4 concentrations appeared inversely related. There also appeared to be an inverse relationship between both numbers of episodic pulses of LH and overall average serum LH with serum concentrations of P_4.

The increases in serum E_2 or P_4 that were associated with cyst formation in Cows H3 and H10, respectively, were still evident on Day 15. Serum E_2 in Cow H3 was 18.6 pg/ml, a concentration similar to those seen in control cows prior to preovulatory surges of LH. Pulsatile release of LH and FSH occurred in a synchronous manner in H3. Sex steroid concentrations in H10 were indicative of the presence of functional luteal

Table 2.--Serum sex steroid and gonadotropin release in 4 adult dairy cows with experimentally-induced ovarian cysts.

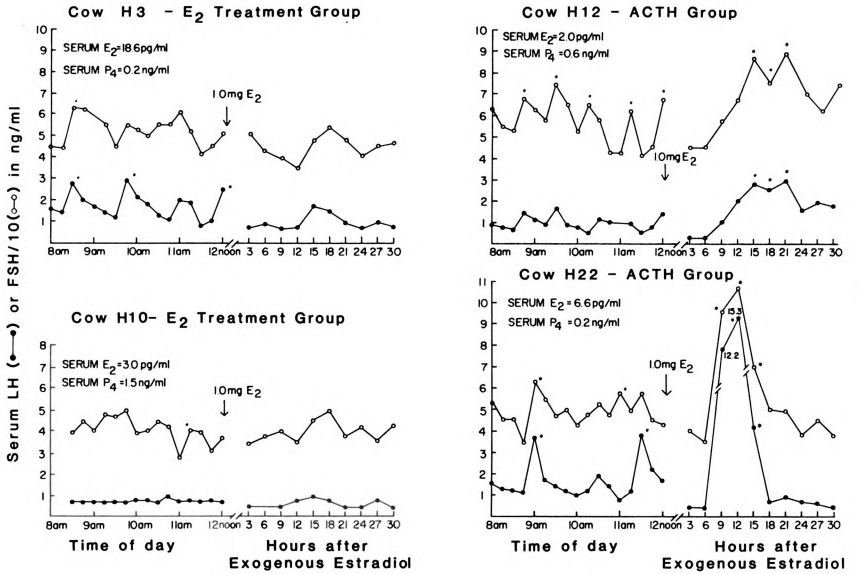
Item	Individual Cow[a] H3	H10	H12	H22
Serum Estradiol (pg/ml)	18.6	3.0	2.0	6.6
Serum Progesterone (ng/ml)	0.2	1.5	0.6	0.2
LH pulses/4 hr	3	0	0	2
$\bar{X} \pm$ SD Average LH (ng/ml)	1.7±0.6	0.7±0.1	1.0±0.3	1.7±0.9
FSH pulses/4 hr	1	1	5	2
$\bar{X} \pm$ SD Average FSH (ng/ml)	52±7	40±6	58±10	49±7

[a]From samples drawn 7-12 days after ovarian cyst formation.

tissue, with no LH pulses and one FSH pulse/4 hr. Neither H3 or H10 had increased LH or FSH after exogenous E_2 (Figure 9).

Both cows forming ovarian cysts after ACTH treatment also showed evidence of ovarian steroidogenesis. P_4 concentrations in Cow H12 suggested the presence of some active luteal tissue (0.6 ng/ml). This individual had 5 pulses of FSH in the 4 hr sampling period, but no pulses of LH. After 1.0 mg E_2 challenge, Cow H12 had an increase in serum LH and FSH over basal values at 15-21 hr, but not of the magnitude of preovulatory surges. At the time of sampling, Cow H22 had evidence of significant E_2 production (7 pg/ml), with pulsatile release of both LH and FSH were seen in 15 min samples. Cow H22 had a preovulatory surge-like release of both LH and FSH at 9-15 hr after exogenous E_2 (Figure 9).

Figure 9. Pulsatile release of LH and FSH and response to exogenous estradiol (E_2) in 4 adult cows with experimentally-induced ovarian cysts. Corresponding basal E_2 and progesterone (P_4) concentrations are also listed. LH or FSH pulses are marked with an *. Post-E_2 increases of LH or FSH above basal values are marked with a *.


Cow H3 - E_2 Treatment Group
SERUM E_2=18.6pg/ml
SERUM P_4=0.2ng/ml
1.0mg E_2
Cow H12 - ACTH Group
SERUM E_2=2.0pg/ml
SERUM P_4=0.6ng/ml
1.0mg E_2
Cow H10- E_2 Treatment Group
SERUM E_2=3.0pg/ml
SERUM P_4=1.5ng/ml
1.0mg E_2
Cow H22 - ACTH Group
SERUM E_2=6.6pg/ml
SERUM P_4=0.2ng/ml
15.3
12.2
1.0mg E_2
8am 9am 10am 11am 12noon 3 6 9 12 15 18 21 24 27 30
Serum LH (•—•) or FSH/10(○–○) in ng/ml
Time of day
Hours after Exogenous Estradiol

DISCUSSION OF EXPERIMENT II

Temporal changes in serum LH, E_2, and P_4 concentrations occurring with CL regression and the preovulatory surge of LH in control cows were similar to previous description of normal bovine estrous cycles (Hansel and Convey, 1983). Control cows also showed palpable changes in ovarian structures and uterine consistency that were indicative of normal CL regression, ovulation, and subsequent CL development (Zemjanis, 1970).

Three cows were blocked among the treatment groups because they had either just ovulated or begun estrus prior to the initiation of respective treatments. None of these cows formed ovarian cysts. Palpation data indicated that all of these three cows had a normal interestrous interval over the course of the experiment. Wiltbank et al. (1961) reported a shortened interestrous interval in 6 of 10 cycling heifers when given exogenous E_2 on days 3 or 4 of the estrous cycle. Heifers receiving continuous infusion of ACTH, beginning on day 2 of the estrous cycle had no preovulatory surges of LH 18-20 days later (Li and Wagner, 1983). When given early in the estrous cycle, the ability of exogenous E_2 or ACTH to alter estrous cycle length may depend on the day of cycle when treated or duration and dosage of treatment regimen.

Four cows had a CL present at the time of $E_2$10 treatment. With pre-treatment estrous synchronization procedures, these animals would have been in mid- to late diestrus. Ten mg E_2 was a pharmacological dose and its effects appeared to be both luteolytic and directly stimulatory to LH release. The interval from $E_2$10 to the onset of the surge

of LH was 16-20 hr, an interval similar to the positive feedback response of LH after exogenous E_2 was given to OVX cows (Short et al., 1973; Kesner et al., 1981). However in the present study, these induced surges of LH occurred before luteal regression was complete. These were likely to be dose-related effects of E_2, since LH release did not occur in diestrous cows after 1 or 2 mg exogenous E_2 (Short et al., 1979; Zaied et al., 1981).

Luteal regression and LH release after $E_2$10 treatment apparently did not directly result in ovulation. Instead, 2 cows each either formed an ovarian cyst or had a spontaneous preovulatory surge of LH 5-8 days later. At present, it is not known what factors may have determined whether an ovarian cyst formed or a preovulatory surge of LH occurred.

Although the number of cows in this study was small, the percentage forming ovarian cysts after E_2 treatment (50%) was between the previously reported 44% (Whitmore et al., 1972) and 70% (Wiltbank et al., 1961) occurrence rates. Nadaraja and Hansel (1976) described formation of ovarian cysts in 5 heifers given exogenous E_2 on day 15 of the estrous cycle, but it was not clear if that was the total number treated.

The diversity of sex steroid changes accompanying ovarian cyst formation after $E_2$10 treatment suggest the possibility of different pathophysiologic mechanisms. The lack of a preovulatory surge of LH in response to the rise in serum E_2 (Cow H3) supports a previous hypothesis of reduced hypothalamic sensitivity to the positive feedback effects of

E_2 on LH release (Kesler et al., 1979b; Kesler and Garverick, 1982). Marked release of LH occurred before CL regression was complete, as proposed by Nadaraja and Hansel (1976). This might result in premature luteinization of a growing follicle and account for the rise in serum P_4 that accompanied cyst development in Cow H10.

At one week after ovarian cyst formation, the endocrine profile of Cow H3 was characterized as having high endogenous E_2, episodic basal release of LH and FSH, and no increase in gonadotropin after exogenous E_2. Those traits of gonadotropin release closely resembled LH and FSH secretion characteristics of the cows with naturally-occurring ovarian cysts and high endogenous E_2 that were sampled in EXPERIMENT I. If the elevated E_2 concentrations remained stable between Days 8-15 of the experiment in Cow H3, the lack of gonadotropin release after 1.0 mg E_2 may be due to negative feedback of the hypothalamo-pituitary axis from chronic E_2 exposure.

In Cow H10, the increase in serum P_4 that occurred with cyst formation would also be consistent with normal CL growth (Hansel and Convey, 1983). Whitmore et al. (1972) showed that the weight of luteal tissue present in an ovarian cyst could equal the weight of a normal CL. Such ovarian cysts could theoretically be susceptible to natural luteolytic mechanisms and spontaneous recovery. The low number of episodic LH or FSH pulses/4 hr (Rahe et al., 1980; Walters et al., 1984) and lack of gonadotropin release after 1.0 mg E_2 were expected results for a cow having functional luteal tissue (Short et al., 1979; Zaied et al., 1981).

Serum cortisol values after exogenous ACTH treatment were similar to those previously reported where preovulatory surges of LH (Stoebel and Moberg, 1981; Li and Wagner, 1983) or pulsatile LH release (Echternkamp, 1984) were suppressed in cattle. Also, serum cortisol means in control or $E_2$10-treated cows were similar to previous reports of normal cows (Roth et al., 1983; Li and Wagner 1983; Thompsen et al., 1984).

Liptrap and McNally (1976) did not report percentages of cows forming ovarian cysts after ACTH treatment, so it is not known if the variety of responses seen in the present study were encountered. Three cows underwent luteal regression after ACTH treatments were begun. No preovulatory surges of LH were detected. It was likely that the persistent fever resulted in one cow becoming anestrus. The appearance of ovarian cysts shortly after luteal regression in the other two cows suggested that these follicles may have been recruited as preovulatory follicles. Formation of these cysts was accompanied by periodic fluctuations of serum E_2 and P_4, not the sustained increases in plasma E_2 as reported by Liptrap and McNally (1976). In the present study, the relative contribution of the adrenal and ovary as sources of circulating P_4 was unknown.

At best, serum E_2 and P_4 changes accompanying and following ovarian cyst formation after ACTH treatment could be called transient in nature. Based on previously discussed reports (Stroebel and Moberg, 1982; Li and Wagner, 1983), it was expected that preovulatory surges of LH would be suppressed by ACTH-mediated cortisol release. In both cows that formed

ovarian cysts after ACTH-treatment, ovarian follicles newly identified after Day 9 of the experiment may have been the source of circulating E_2 and P_4 measured on Day 15. The presence of pulsatile gonadotropin release and increase in gonadotropins after exogenous E_2 suggested that these cows may have been in the process of reinitiating estrous cycles.

One cow given ACTH had a prolonged estrous cycle. Administration of a synthetic glucocortacoid on day 10 of the cycle prolonged luteal function for 10 days in heifers (Kanchev et al., 1976). However, luteal regression was not prevented in heifers receiving exogenous ACTH infusion beginning on day 16 of the cycle (Li and Wagner, 1983).

Morrow et al. (1966) reported a higher incidence of ovarian cysts in cows with clinical problems such as metritis, milk fever, ketosis, or clinical mastitis in the early postpartum period. Further work is needed to determine whether metabolic and environmental stresses could interfere with LH release and result in the formation of ovarian cysts, especially in the early postpartum period.

In summary, ovarian cysts formed in cows undergoing luteal regression after receiving $E_2$10 or ACTH (4 of 6 cases, excluding the individual who became anestrus). Spontaneous surges of LH did not occur at the time of cyst formation. However, increases in E_2 and P_4 accompanying cyst formation after $E_2$10 treatment suggested the presence of gonadotropin stimulus. The relative lack of E_2 or P_4 release in cows forming ovarian cysts while receiving ACTH was suggestive of suppression of gonadotropin release. Thus, ovarian cysts may be formed when either significant gonadotropin release occurs, but is asynchronous

with luteal regression and follicular maturation or gonadotropin release is inhibited.

After cyst formation, a variety of sex steroid and gonadotropin secretion traits were seen in the 4 cows with artificially-induced ovarian cysts. In the respective cows with highest endogenous E_2 or P_4 concentrations (H3 and H10), the lack of gonadotropin release after exogenous E_2 suggested that the positive feedback response of the hypothalamo-pituitary axis can be inhibited not only by P_4, but E_2. The appearance of additional ovarian follicles, presence of pulsatile basal LH and/or FSH release, and gonadotropin release after exogenous E_2 in cows H12 and H22 may have represented transition toward resumption of estrous cycles.

Further work is needed to determine whether these artificially-induced ovarian cysts are appropriate models for the naturally-occurring condition. Information needed would include diagnostic confirmation by direct visualization of ovarian structures, monitoring endogenous endocrine changes over a longer observation period, and histological evaluation of artificially-induced ovarian cysts.

GENERAL DISCUSSION

The overall theme of this research was to study relationships between circulating sex steroid concentrations and gonadotropin release in dairy cows with ovarian cysts. This was done in cows with naturally-occurring or artificially-induced ovarian cysts. Gonadotropin secretion was measured in terms of characteristics of basal release and the ability of the hypothalamo-pituitary axis to release gonadotropins in response to exogenous E_2. Assessment of basal gonadotropin secretion was based on reports by others of characteristics of gonadotropin changes throughout estrous cycles, the postpartum period, and after ovariectomy. Assessment of gonadotropin release after exogenous E_2 was based on the documented positive feedback response in ovariectomized cattle, prepubertal heifers, and dairy cows that are at least two weeks postpartum, but without a functional CL (Saba et al., 1976; Short et al., 1979; Zaied et al., 1981; Kesner et al., 1982; Schillo et al., 1983; Stevenson et al., 1983). The basic question was whether gonadotropin release in cows with ovarian cysts was similar to that of cows with comparable sex steroid concentrations in other normal physiological states.

All ovarian cysts diagnosed in the present experiments were of a thin-walled, follicular consistency (Roberts, 1971). No differences were noted in naturally-occurring or artificially-induced cystic structures. Physical characteristics of the ovarian cysts and uterine consistency, as detected by palpation <u>per rectum</u>, did not provide insight as to the endocrine status of the cows that were studied. There was

considerable variation in E_2 and P_4 concentrations, but the cows with ovarian cysts could be grouped into three basic categories. These categories reflected physiological states where cows were under high E_2 influence (proestrus-like), low steroid influence (early postpartum or after preovulatory surges of gonadotropins), or under luteal influence (presence of a growing, mature, or regressing CL).

Seven cows with ovarian cysts (6 naturally-occurring, 1 artificially-induced) had basal E_2 concentrations (10-30 pg/ml) that were at or above peak proestrual values in control cows in EXPERIMENT II (7-16 pg/ml). In proestrous cows, pulsatile gonadotropin release was present at a frequency of approximately 1/hr (Rahe et al., 1980; Walters and Schallenberger, 1984). Thus, cows with ovarian cysts with 2 or more LH pulses/4 hr are probably not divergent from expected results in cycling cows (Figure 1). Some individuals with high endogenous E_2 had no obvious pulsatile gonadotropin release (See Figure 1) and further documentation would be needed to determine if this may be of special clinical significance in regard to prognosis for recovery.

None of the 7 high E_2 cows with ovarian cysts had gonadotropin surges after exogenous E_2. This result is similar to the loss of the positive feedback release of LH that occurred in old female rats that were in a constant-estrus state, an anovulatory condition with high endogenous E_2 (Lu et al., 1980). In OVX rats, replacement of E_2 for 10 or 17 days resulted in a progressive inhibition of LH surges that was thought to be mediated via alterations of hypothalamic norepinephrine and serotonin metabolism (Walker, 1983). In the present experiments,

the duration of exposure to high endogenous E_2 in cows with naturally-occurring cysts was unknown. The cow with the experimentally-induced ovarian cyst in this category (Cow H3, Figures 6 and 9) could possibly have had E_2 concentrations at or above 10 pg/ml for a week. It is not known if experimental E_2 replacement will result in inhibition of the positive feedback response in cattle.

Five cows with ovarian cysts (4 naturally-occurring, 1 artificially-induced) had serum steroid concentrations that suggested a lesser likelihood of being under E_2 ($\leq$ 7 pg/ml) or P_4 ($\leq$ 0.5 ng/ml) influence. These individuals also had extensive variation in pulsatile gonadotropin release (Figures 1 and 9), however patterns were similar to those of postpartum cows, or cycling cows after the preovulatory surge of gonadotropins (Rahe et al., 1980; Peters et al., 1981; Gitlin et al., 1983; Karg and Schallenberger, 1983; Walters and Schallenberger, 1984). Gonadotropin release after exogenous E_2 was not consistent in this group, where two individuals had preovulatory surges, one showed a significant rise in LH above baseline, and the remaining two showed no increase in LH or FSH (Figures 2, 3, and 9). The two individuals having the preovulatory surges (Cows HM and H22) both had evidence of ovarian follicular growth when monitored by palpation per rectum, and both had pulsatile release of LH and FSH (Figures 1 and 9) prior to E_2 challenge. The variety of gonadotropin responses after exogenous E_2 in this group were suggestive of those of early postpartum cows prior to initiation of estrous cycles (Zaied et al., 1981, Karg and Schallenberger, 1983; Stevenson et al., 1983).

Four cows with ovarian cysts (2 naturally-occurring and 2 artificially induced) had evidence of functional luteal tissue. The individuals (Cow H56, Figures 1-3; Cow H10, Figure 6 and 9) with the highest P_4 concentrations had basal gonadotropin secretion that was similar in character to diestrous cows (Rahe et al., 1980; Walters and Schallenberger, 1984). These cows also showed no increase in LH or FSH after exogenous E_2, an expected response in cows with functional luteal tissue (Short et al., 1979; Zaied et al., 1981; Stevenson et al., 1983). The remaining cows (HS, Figures 2 and 3; H12, Figure 9) had slightly lower serum P_4 values and more variable patterns of pulsatile gonadotropin release. After exogenous E_2, one individual showed an increase in LH over baseline (Figure 2) and the other had an increase in both LH and FSH (Figure 9). It is possible that the endogenous P_4 concentrations in these latter individuals may have negatively influenced E_2-induced gonadotropin release.

The range of basal FSH concentrations in cows with naturally-occurring ovarian cysts was considerable (35-120 ng/ml, Figure 3), while concentrations were more uniform among cows with artificially-induced ovarian cysts (40-60 ng/ml, Table 2). Leidl et al. (1983) reported a lack of inhibin activity in bovine ovarian cysts, where injection of ovarian cyst fluid did not depress FSH in cows but injection of bovine ovarian follicular fluid resulted in a significant decrease of FSH. Perhaps the high basal FSH concentrations in some cows with ovarian cysts are the result of a lack of development of other ovarian follicles.

In EXPERIMENT II, 10 mg E_2 administration resulted in consistent luteolysis and LH release in four cows, but only two formed ovarian cysts. One explanation for this result may be the status of the ovarian follicle population at the time of E_2 treatment, where cows that formed ovarian cysts had follicles that were affected by the LH surge. Convey et al. (1976) gave exogenous GnRH at selected intervals (10, 30 and 50 hr) after prostaglandin treatment in Holstein heifers. Heifers that received GnRH at 30 hr did not show estrus and 1 of 5 formed an ovarian cyst. Those results suggested that the timing of GnRH therapy may have resulted in premature luteinization of ovarian follicles and may have predisposed formation of the ovarian cyst. Further work needs to be done to determine if there is a critical day of the estrous cycle effect or ovarian follicular status that will result in a predictable high incidence of ovarian cyst formation after exogenous E_2.

Liptrap and McNally (1976) did not measure LH in cows forming ovarian cysts after ACTH administration, but reported significant increases in E_2 that accompanied and were maintained after cyst formation. Similar increases in E_2 were not seen with ovarian cyst formation after ACTH in EXPERIMENT II, where preovulatory surges of LH were not detected. Recently, Peters and Liptrap (1985) induced formation of ovarian cysts in sows with ACTH given in late-diestrus and proestrus. They reported no rise of E_2 when gonadotropin surges were completely inhibited, but increased E_2 with partial suppression of gonadotropin surges and ovarian cyst formation. Perhaps the extent of suppression of

LH release may account for the differences in E_2 changes seen in the results of Liptrap and McNally (1976) and EXPERIMENT II.

Echternkamp (1984) showed that stress in the form of acute restraint in previously unhandled beef cows resulted in increases in serum cortisol comparable to concentrations seen in ACTH-treated cows in EXPERIMENT II. Relocation of a dairy herd resulted in approximate doubling (9 to 20 ng/ml) of cortisol for 2-3 days, but it is questionable whether that would constitute sufficient stress to inhibit preovulatory surges of gonadotropins. The more frequent occurrence of ovarian cysts in cows with other early postpartum metabolic or disease problems (Morrow et al., 1966) may reflect instances of stress-related suppression of LH release.

Although direct contrasts were not made, it appeared that relationships between sex steroid profiles and gonadotropin release was similar in cows with naturally-occurring and artificially-induced ovarian cysts. This suggests that experimentally-induced ovarian cysts are a reasonable endocrine model for the naturally-occurring condition. Use of such a model would provide a means for further characterization of the endocrinology of ovarian cysts. This information could have clinical application in prevention or improvement of therapeutic measures for ovarian cysts in cattle.

SUMMARY AND CONCLUSIONS

In EXPERIMENT I, 12 adult dairy cows with naturally-occurring ovarian cysts were grouped according to endogenous serum concentrations of E_2 and P_4. Assignment to groups reflected endocrine states of high E_2 influence, low E_2 and P_4 influence, and luteal influence. The frequency of pulsatile release of gonadotropins varied considerably within high E_2 and low steroid groups ranging from 0 to 4 per 4 hr. The frequency of pulsatile release of gonadotropins in cows with luteal influence tended to be more consistent (1 to 2 per 4 hr). Overall, there were no differences among groups in frequency of pulsatile LH or FSH release, baseline FSH, serum testosterone or serum cortisol. Baseline LH was higher in the high E_2 group. Pulsatile release of gonadotropins in cows with ovarian cysts was variable, but characteristics were similar in general to patterns reported by others in postpartum or cycling cows of corresponding E_2 and P_4 status. Only 1 of 12 cows with naturally-occurring ovarian cysts had a preovulatory surge-like release of gonadotropins after exogenous E_2 challenge. The low incidence of preovulatory surges after exogenous E_2, especially in cows in high E_2 and low steroid groups, suggested a refractoriness of the hypothalamo-pituitary axis to the positive feedback effects of E_2.

In EXPERIMENT II, non-lactating dairy cows in mid- to late-diestrus were given a placebo, 10 mg E_2, or repeated injections of 100 IU ACTH to induce formation of ovarian cysts. Serum cortisol was higher in ACTH-treated cows than in cows receiving the other treatments. All cows

given placebo treatment underwent spontaneous luteal regression and ovulation. Administration of 10 mg E_2 initiated synchronous luteal regression with preovulatory surges of LH occurring before luteal regression was complete. Two of 4 cows with E_2-induced luteal regression formed ovarian cysts, with one showing a progressive increase of endogenous E_2 accompanying cyst formation and the other showing a progressive increase of P_4. These results suggested that formation of ovarian cysts after exogenous E_2 occurred with gonadotropin-supported ovarian steroidogenesis.

Ovarian cysts were formed in 2 of 3 cows that underwent spontaneous luteal regression while receiving ACTH. The cow not forming an ovarian cyst became anestrus. Preovulatory surges of LH did not occur after luteal regression. Changes in serum E_2 or P_4 in cows forming ovarian cysts after ACTH were transient in nature. These results indicated that ovarian cyst formation may occur when the preovulatory surge of LH is suppressed.

Serum E_2 and P_4 concentrations in the 4 cows with artificially-induced ovarian cysts indicated the presence of examples to fit each of the high E_2, low steroid, and luteal-influence categories used to group cows with naturally-occurring ovarian cysts. Characteristics of both pulsatile basal gonadotropin release and gonadotropin release after 1.0 mg E_2 in cows with artificially-induced ovarian cysts resembled those of cows with naturally-occurring ovarian cysts. Cows with artificially-induced ovarian cysts appear to be a suitable endocrine model for the naturally-occurring condition.

APPENDIX

Validation of Commercially-Available Radioimmunoassay Kits for Measuring Estradiol-17b and Testosterone in Bovine, Canine and Equine Serum

APPENDIX

Validation of Commercially-Available Radioimmunoassay Kits for Measuring Estradiol-17b and Testosterone in Bovine, Canine and Equine Serum

INTRODUCTION

The widespread application of radioimmunoassay (RIA) techniques has prompted the development and commercial marketing of RIA kits. Several classes of hormones, such as thyroid or adrenal and sex steroids are structurally similar among mammalian species, thus these commercial RIA kits may provide a readily-available source of assay materials for the researcher or diagnostician in animal science or veterinary medicine.

Midgley et al. (1969) and Hafs et al. (1977) recommended that criteria for RIA performance include data on assay specificity, sensitivity, accuracy, precision, and parallelism. In the adaptation of a human diagnostic RIA kit for measurement of the structurally similar hormone in another species, the criteria of sensitivity and parallelism should receive particular attention. For instance, animal samples may not be accurately quantified if the normal physiological concentrations do not fall in the effective range of standard curves designed to optimally measure concentrations in human samples (Reimers et al., 1981). In cases where hormones are measured in serum, the equilibrium of the antibody-radioligand reaction may be affected by interactions with endogenous serum proteins (Malvano, 1983). These non-specific effects may differ where standards are prepared in the serum of one species and unknowns are from a different species (Gostein and VanHaelst, 1973).

Estradiol-17b and testosterone have been measured by RIA in a variety of biological fluids and variation due to reproductive status or pathology

within a species may be as diverse as variation among species. The purpose of this paper is to present assay validation data for the measurement of estradiol-17b and testosterone in bovine, canine, and equine serum using commercially-available RIA kits.

MATERIALS AND METHODS

RIA kits for the measurement of estradiol-17b or testosterone in diethylether-extracted serum were obtained from Serono Laboratories, 11 Brooks Drive, Braintree, MA (ESTRADIOL ^{125}I Kit and TESTO® RIA Kit, respectively). Each kit contained a phosphate buffer (pH=7.3) with 0.1% Na azide (PB), standards, antibody, and ^{125}I-labeled radioligand (tracer). These reagents were in lyophilized form and were reconstituted with double-distilled water, as per manufacturer's instructions. A solution of 20% w/v polyethylene glycol (PEG) for precipitation of antibody-bound tracer was also provided in each kit.

Extraction Procedure - The serum extraction method was similar for both assays. Serum or reagent volumes for the estradiol validation will be noted in the text, and the appropriate volumes used in the testosterone extraction will be noted in brackets. Two [0.5] ml of double distilled water was extracted to determine background (extraction blank). Three-tenths to 3 ml [.3 to 1.5 ml] of serum was pipetted into 16x125 mm pyrex screw-topped culture tubes (Corning Glass Works, Corning, NY). Extraction tubes used in the estradiol RIA had been baked at 400 C for 4 hours and cooled to room temperature prior to extraction, in an attempt to remove residual contamination that would increase assay background (England et al., 1974). Ten [5] ml of freshly-opened anhydrous

diethylether (Mallinckrodt, Inc. Paris, KY) was added to each tube and the tubes capped with teflon-lined screw caps. Use of freshly-opened ether was critical to the estradiol assay. Extraction blank values from ether that had been exposed to air for at least 12 hr ranged from 20-30 pg/ml, whereas extraction blanks from freshly-opened ether were below 0.5 pg/ml. The tubes were laid horizontally in a shaker (Eberbach Corp., Ann Arbor, MI) and the contents mixed by shaking for 30 min. The extraction tubes were stood upright and the aqueous and organic layers were allowed a 10 min period to fully separate. The caps were removed and the aqueous layer was aspirated off the bottom of each tube with a glass Pasteur pipette. One hundred ul of 0.1N NaOH was pipetted into each tube, the caps replaced, and the shaking step was repeated for 10 min. The caps were removed and 100 ul of 0.1N glacial acetic acid was added to each tube followed by a final 10 min shaking period. This washing procedure was done to remove neutral lipids in the solvent phase via saponification with a basic solution and pH readjustment with the addition of acid. The tubes were placed in a -20 C freezer until the aqueous portion was frozen. The ether fraction was then decanted into 16x100 mm glass tubes. These latter tubes had also been baked prior to use in the estradiol RIA. The tubes containing the solvent fraction were placed in a 40 C warmed vortex-evaporator (Haake Buchler Inc, Saddle Brook, NJ) and the ether evaporated under vacuum. One ml of PB was added to each dried tube and the tubes were placed in a 37 C water bath for 1 hr for dissolution of the extracted hormone. The contents of each warmed tube was then mixed by vortexing for 15 sec. Aliquots of reconstituted samples were then added to assay tubes. Extraction

efficiency was assessed as the percentage of ^{3}H-estradiol-17a (Carruthers and Hafs, 1980) or ^{3}H-testosterone (both from New England Nuclear, Boston, MA) present in the reconstituted sample versus the amount added to serum prior to extraction. Tritiated hormone was added to 1, 2 or 3 ml of serum prior to extraction for the estradiol assay or to 0.5 or 1.5 ml serum prior to extraction for the testosterone assay. Effects of species and serum volume on extraction efficiency were tested by two-way analysis of variance.

Assay Procedure - In an attempt to improve the estradiol RIA sensitivity and decrease expenses for a large number of research samples, the reconstituted estradiol tracer and antibody were further diluted in half with PB. Reagent dilution resulted in a shift of the standard curve with a tendency for the assay to be more sensitive (see Table 3). When bovine sera were assayed with diluted and undiluted reagents, there was virtually no difference in results (Table 3). Thus, further estradiol validation data was obtained with the additional reagent dilution. The estradiol standard curves were modified with the addition of 1.0 and 2.5 pg/ml to the 0, 5, 10, 25, 50, 100 and 200 pg/ml standards supplied by the manufacturer. The 1.0 and 2.5 pg/ml standards were prepared with appropriate combinations of 0 and 5 or 10 pg/ml standards, respectively.

Effects of further dilution of testosterone tracer and antibody have not been investigated. The testosterone standard curve was not modified; the 0, 0.05, 0.1, 0.2, 0.5, 1.5, 3.0 and 6.0 ng/ml standards supplied with the kit were all included in the assay.

The following assay procedures were identical for both estradiol and testosterone RIA. Reagents were pipetted into 12x75 mm polystyrene

culture tubes (Walter Starstedt, Princeton, NJ). Total count tubes contained 100 ul tracer only. Non-specific binding tubes received 200 ul

Table 3.--Influence of 50% dilution of both antibody and radioligand on standard curves and serum values in a commercial radioimmunoassay for measurement of estradiol-17b.

	Estradiol Concentrations (pg/ml, r=2) Extrapolated From Standard Curves			
	Level of binding inhibition			
Reagents	90%	75%	50%	35%
Undiluted	4.3	14.0	45.6	89.3
Diluted[a]	2.2	8.0	29.0	60.3

	Serum Estradiol Measured (pg/ml)				
	Bovine Serum Samples (r=2)				
Reagents	1	2	3	4	5
Undiluted	18.8	3.0	12.1	20.4	11.0
Diluted	19.5	2.8	11.9	21.4	13.6

[a]Reconstituted antibody and radioligand were further diluted in assay buffer.

of 0 standard and 100 ul of tracer. The remaining assay tubes received 100 ul of the appropriate standard or sample, 100 ul of respective antibody and 100 ul of tracer. The contents of the tubes were mixed by vortexing for 5 sec. The assay tubes were covered and an 8-16 hour incuba-

tion period at room temperature (20 C) was allowed for equilibration of the antigen-antibody reaction. One ml of the 4 C PEG solution was added to all tubes except total count tubes. After PEG addition, the contents of the assay tubes were mixed by vortexing for 5-10 seconds and the tubes were kept at room temperature for 20 minutes for precipitation of protein (antibody-bound fraction). The tubes were then centrifugated at 1500 g at 4 C for 20 minutes. The supernatant was poured from all except total count tubes. The inverted tubes were allowed to stand for 5-10 minutes on absorbant paper for further drainage of supernatant. The tracer remaining in each tube was determined with a 1-2 minute counting time on a gamma-counter (Micromedic, Horsham, PA). Standard curves were calculated by fitting a linear regression of the log of the standard with the logit of the % radioactive counts bound to antibody. Concentrations of unknowns were extrapolated from standard curves.

Assessment of Validation Criteria - Specificity data was provided by the manufacturer, expressed as percent relative crossreactivity at 50% supression of total binding. Assay sensitivity was the estimated hormone concentration corresponding to the binding percentages at the 95 and 99% confidence intervals (CI) below the mean specific binding at the 0 standard. Accuracy was estimated by measurement of known amounts of hormone added to serum samples prior to ether extraction.

Parallelism was assessed by subjective comparison of standard curves with binding inhibition curves obtained with extraction of different volumes of the same serum sample. Precision was estimated by determining intra- and interassay coefficients of variation for each species.

RESULTS

Extraction efficiency results for estradiol and testosterone are shown on Tables 4 and 5, respectively. The species from which serum was obtained did not affect recovery of either ^{3}H-estradiol-17a or ^{3}H-testosterone. Less ^{3}H-estradiol-17a was recovered after extraction of 3 ml serum (86.4%) than from 1 ml serum (101.1%) with 10 ml ether ($p < 0.05$). There was no difference in ^{3}H-testosterone recovery after extraction of 0.5 or 1.5 ml serum with 5 ml ether.

Table 4.--Effects of species and serum volume on recovery of ^{3}H-estradiol-17a after extraction with 10 ml freshly-opened diethyl ether. Values are expressed as % of 2334 cpm.

Species of Origin	1.0 ml	2.0 ml	3.0 ml	Overall species mean
Canine	109	88	81	92.7
Equine	102	93	92	95.7
Bovine	92	93	86	90.3
Overall volume mean	101.0[a]	91.3	86.3[a]	92.9

[a]values in a row differ at $p < 0.05$

Table 5.--Effects of species and serum volume on recovery ^{3}H-testosterone after extraction with 5 ml of diethyl ether. Values are expressed as % of 8275 cpm.

	Volume of serum extracted (r = 3)		
Species of Origin	0.5 ml	1.5 ml	Overall species mean
Canine	84	80	82.0
Equine	86	83	84.5
Bovine	84	82	83.0
Overall volume mean	84.3	81.6	83.0

Specificity data provided by the manufacturer for the relative crossreaction of various steroids is shown on Table 6.

For estradiol, the corresponding hormone concentrations at 95% and 99% CI from the mean total specific binding were 1.7 and 2.8 pg/tube, respectively. For the testosterone RIA, 0.02 and 0.04 ng/tube were the respective values at the 95% and 99% CI from the mean total specific binding. These values are estimates of assay sensitivity.

Accuracy of measuring different amounts of exogenous estradiol or testosterone added to serum are shown on Tables 7 and 8, respectively. When corrected for extraction efficiency, 98% and 107% of exogenous estradiol and testosterone was measured, respectively.

TABLE 6. **Percent crossreactivity at 50% of total binding capacity of various steroids with antibody from commercially-available estradiol and testosterone radioimmunoassays.[a]**

	% RELATIVE CROSSREACTIVITY	
Compound	Estradiol antibody	Testosterone antibody
Estradiol 17b	100	.058
Estrone	1.3	.013
Testosterone	0.016	100
Cortisol	0.006	.006
Aldosterone	<0.0009	NG
Androstenedione	0.0001	NG
Progesterone	0.0005	.031
17-Hydroxyprogesterone	<0.0001	NG
Desoxycorticosterone	0.0001	NG
Dehydroepiandrosterone	<0.0001	NG
Pregnenolone	0.0006	NG
Dihydrotestosterone	NG	22.5
Estriol	NG	.063

[a] Values were obtained from kit instructions supplied by Serono Laboratories, Braintree, MA.

NG = Not given

Table 7.--Accuracy of measuring known amounts of estradiol-17b when added to serum prior to ether extraction. Values are expressed as [amount measured/amount added] x 100% (r = 2).

	Estradiol added (pg/tube)				
Species Serum	2.5	5.0	10.0	20.0	Species Avg.
Bovine	80	81	87	121	92.3
Canine	74	122	91	110	99.3
Equine	132	107	89	88	104.0
Estradiol Dose Avg.	95.3	103.3	89.0	106.3	98.4

Table 8.--Accuracy of measuring known amounts of testosterone when added to serum prior to ether extraction. Values are expressed as [amount measured/amount added] x 100% (r = 2).

	Testosterone added (ng/tube)			
Species Serum	.05	.15	.60	Species Avg.
Bovine	80	107	87	91.3
Canine	140	107	112	119.7
Equine	120	107	103	110.0
Testosterone Dose Average	113.3	107	100.7	107

Table 9.--Precision of estradiol and testosterone radioimmunoassays for bovine, canine and equine serum.

Hormone Assayed	Species	$\bar{X}$ Conc. of Sample	Coefficients of Variation (%) interassay (no. assays)	Coefficients of Variation (%) intraassay (r=5)
Estradiol	Bovine	9.0 pg/ml	20.4 (14)	9.2
	Canine	3.1 pg/ml	12.6 (3)	13.4
	Equine	3.5 pg/ml	7.4 (3)	17.1
Testosterone	Bovine	8.6 ng/ml	14.4 (16)	3.9
	Canine	0.56 ng/ml	27.7 (13)	4.5
	Equine	0.50 ng/ml	14.1 (4)	11.4

Figure 10. Inhibition curves for estradiol standard solutions and samples reconstituted after diethylether extraction of different volumes of bovine, canine and equine serum pools.

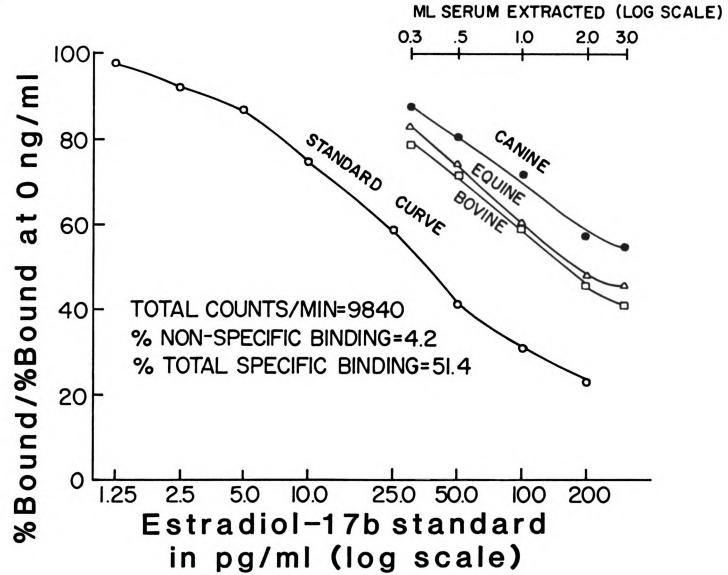
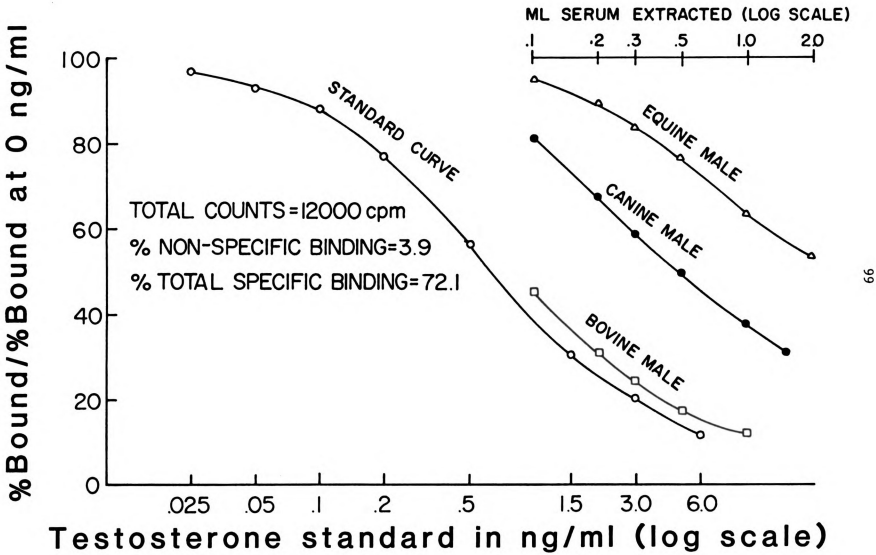

ML SERUM EXTRACTED (LOG SCALE)
0.3
.5
1.0
2.0
3.0
CANINE
EQUINE
BOVINE
STANDARD CURVE
TOTAL COUNTS/MIN=9840
% NON-SPECIFIC BINDING=4.2
% TOTAL SPECIFIC BINDING=51.4
%Bound/%Bound at 0 ng/ml
100
80
60
40
20
0
1.25
2.5
5.0
10.0
25.0
50.0
100
200
Estradiol-17b standard
in pg/ml (log scale)

Figure 11. Inhibition curves for testosterone standard solutions and samples reconstituted after diethylether extraction of different volumes of bovine, canine and equine serum pools.


ML SERUM EXTRACTED (LOG SCALE)
.1
.2
.3
.5
1.0
2.0
100
80
60
40
20
0
%Bound/%Bound at 0 ng/ml
STANDARD CURVE
EQUINE MALE
CANINE MALE
BOVINE MALE
TOTAL COUNTS=12000 cpm
% NON-SPECIFIC BINDING=3.9
% TOTAL SPECIFIC BINDING=72.1
.025
.05
.1
.2
.5
1.5
3.0
6.0
Testosterone standard in ng/ml (log scale)

Binding inhibition curves for standards and extraction of different volumes of the same serum sample are shown on Figures 10 (estradiol) and 11 (testosterone). For each hormone, inhibition curves of bovine, canine and equine sera were parallel to standard curves.

The mean concentration and intra- and interassay coefficients of variation for bovine, canine and equine control sera are shown in Table 9 for estradiol and testosterone assay.

DISCUSSION

Validation data have been presented to demonstrate the performance of commercial RIA kits for measuring estradiol and testosterone in bovine, canine, and equine serum. Since both hormones are removed from serum by diethylether extraction, use of the kits could be adapted for serum measurements in other species or from other biological fluids.

Perhaps the main limitation of the estradiol RIA kit is in sensitivity. However, extraction of 2 or more ml of serum would permit measurement of previously reported estradiol concentrations that occur throughout bovine (Chenault et al., 1975; Walters and Schallenberger, 1984), canine (Chakraborty et al., 1980; Wildt et al., 1981) and equine (Noden et al., 1978) estrous cycles, especially changes within an individual across time.

The testosterone RIA also showed acceptable performance in meeting validation criteria. Without modification, this kit provides a means of measuring peripheral serum testosterone concentrations in intact bovine (McCarthy et al., 1979) canine (DePalatis et al., 1978), and equine (Cox and Williams, 1975; Ganjam, 1979) males. Changes of testosterone during the canine periovulatory period (Olson et al., 1984) could also be

quantified with the testosterone RIA described herein. Serum testosterone concentrations in cows (Kesler, et al., 1979; Nessan and King, 1981) are near the sensitivity of the assay.

Our validation data provide evidence that these estradiol and testosterone RIA kits can be used to satisfactorily measure the respective hormones in peripheral bovine, canine, and equine sera. Use of these kits offers an alternative to duplication of other protocols where assay reagents may not be readily available. Each laboratory must determine optimal volumes of sample and ether for extraction to help insure that the results obtained will allow investigators to adequately address experimental objectives.

REFERENCES

Al-Dahash, S.Y.A. and David, J.S.E. 1977a. Anatomical features of cystic ovaries in cattle found during an abattoir survey. Vet. Rec. 101:320.

Al-Dahash, S.Y.A. and David, J.S.E. 1977b. Histological examination of ovaries and uteri from cows with cystic ovaries. Vet. Rec. 101: 342.

Ball, P.J.H. 1982. Milk progesterone profiles in relation to dairy herd fertility. Br. Vet. J. 138:546.

Bamburg, E., Choi, H.S., Mostl, E., Klaring, W.J. and Stockl, W. 1981. Steroidhormongehalt and aromatasenaktivitat in ovarialzysten des rindes. Zbl. Vet. Med. 28:366.

Bane, A. 1964. Fertility and reproductive disorders in Swedish cattle. Br. Vet. J. 120:431.

Bargai, U. 1982. A ten year survey of cystic ovary cows in an intensive dairy herd. Proc. 12th World Cong. on Diseases of Cattle. Sept. 7-10, Amsterdam, Netherlands, p. 601.

Berardinelli, J.G., Dailey, R.A., Butcher, R.L. and Inskeep, E.K. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276.

Bierschwal, C.J. 1966. A clinical study of cystic conditions in the bovine ovary. J. Am. Vet. Med. Assn. 149:1591.

Bierschwal, C.J., Garverick, H.A., Martin, C.E., Youngquist, R.S., Cantley, T.C., and Brown, M.D. 1975. Clinical response of dairy cows with ovarian cysts to GnRH. J. Anim. Sci. 41:1660.

Bolt, D.J. and Rollins, R. 1983. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone. J. Anim. Sci. 56:146.

Bostedt, H., Stolla, R., Hundschell adn Leidl, W. 1979. Zur ovarialzyste des Rindes II. Klinische und hormonanalytische befunde. Berl. Munch. Tierarztl. Wschr. 92:506.

Britt, J.H., Harrison, D.S. and Morrow, D.A. 1977. Frequency of ovarian follicular cysts, reasons for culling, and fertility in Holstein-Freisian cows given gonadotropin-releasing hormone at two weeks after parturition. Am. J. Vet. Res. 83:749.

Brown, E.M., Elmore, R.G., Garverick, H.A. and Kesler, D.J. 1982. Gonadotropin-releasing hormone treatment of dairy cows with ovarian cysts II. histology of ovarian cyst walls. Theriogenology 17:689.

Carruthers, T.D. and Hafs, H.D. 1980. Suckling and four times daily milking: Influence on ovulation, estrus and serum luteinizing hormone, glucocortiacoids and prolactin in postpartum Holsteins. J. Anim. Sci. 50:919.

Casida, L.E. and Chapman, A.B. 1951. Factors affecting the incidence of cystic ovaries in a herd of Holstein cows. J. Dairy Sci. 34:1200.

Casida, L.E., McShan, W.H., and Meyer, R.K. 1944. Effects of an unfractionated pituitary extract upon cystic ovaries and nymphomania in cows. J. Anim. Sci. 3:273.

Caudle, A.B., Thompson, F.N., Purswell, B., Sharlin, J.S., Brooks, P.M. and Smith, C.K. 1982. Effect of monitoring corpus luteum function on days open. J. Dairy Sci. 66:638.

Chakraborty, P.K., Panko, W.B. and Fletcher, W.S. 1980. Serum hormone concentrations and their relationships to sexual behavior at the first and second estrous cycles of the laborador bitch. Biol. Reprod. 22:227.

Chenault, J.R., Thatcher, W.W., Kalra, P.S., Abrams, R.M. and Wilcox, C.J. 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:709.

Choi, H.S., Mostl, E. and Bamburg, E. 1982. Progesterone, 17a-hydroxyprogesterone, androgens and oestrogens in bovine ovarian cysts. Anim. Reprod. Sci. 5:175.

Clarke, I.J. and Cummins, J.T. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endo. 111:1737.

Clarke, I.J. and Cummins, J.T. 1985. Increased gonadotropin-releasing hormone pulse frequency associated with estrogen-induced luteinizing hormone surges in ovariectomized ewes. Endo. 116:2376.

Convey, E.M., Beal, W.E., Seguin, B.E., Tannen, K.J. and Lin, Y.C. 1976. Gonadotropin releasing hormone induced luteinizing hormone release after prostaglandin F_2 in heifers. Proc. Soc. Exptl. Biol. Med. 151:84.

Convey, E.M., Tucker, H.A. and Short, R.E. 1983. Acute effect of suckling on gonadotropin, prolactin and glucocortacoid concentrations in serum of intact and ovariectomized beef cows. Theriogenology 20:661.

Cox, J.E. and Williams, J.H. 1975. Some aspects of the reproductive endocrinology of the stallion and cryptorchid. J. Reprod. Fert. 23, Suppl. 1:75.

DePalatis, L., Moore, J. and Falvo, R.E. 1978. Plasma concentrations of testosterone and LH in the male dog. J. Reprod. Fert. 52:201.

Dieleman, S.J., Kruip, Th.A.M., Fontijne, P., deJong, W.H.R. and vander Weyden, G.C. 1983. Changes in oestradiol, progesterone and testosterone concentrations in follicular fluid and in the micromorphology of preovulatory bovine follicles relative to the peak of luteinizing hormone. J. Endo. 97:31.

Dobson, H. 1978. Radioimmunoassay of FSH in the plasma of post-partum dairy cows. J. Reprod. Fert. 52:45.

Dobson, H. and Dean, P.D.G. 1974. Radioimmunoassay of oestrone, estradiol-17b in bovine plasma during the oestrus cycle and last stages of pregnancy. J. Endo. 61:479.

Dobson, H., Rankin, J.E.F. and Ward, W.R. 1977. Bovine cystic ovarian disease: plasma hormone concentrations and treatment. Vet. Rec. 101:459.

Donaldson, L.E. and Hansel, W. 1968. Cystic corpora lutea and normal and cystic Graffian follicles in the cow. Aust. Vet. J. 44:304.

Dufour, J., Whitmore, H.L., Ginther, O.J. and Casida, L.E. 1972. Identification of the ovulating follicle by its size on different days of the estrous cycle in heifers. J. Anim. Sci. 34:85.

Echternkamp, S.E. 1984. Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 22:305.

Echternkamp, S.E. and Hansel, W. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Anim. Sci. 37:1362.

Elmore, R.G., Bierschwal, C.J., Youngquist, R.S., Cantley, T.C., Kesler, D.J. and Garverick, H.A. 1975. Clinical responses of dairy cows with ovarian cysts following treatment with 10,000 IU HCG or 100 mcg GnRH. Vet. Med./Sm. Anim. Clin. 70:1346.

England, B.G., Niswender, G.D. and Midgley, Jr., A.R. 1974. Radioimmunoassay of estradiol-17 beta without chromatography. J. Clin. Endo. Metab. 38:42.

Eyestone, W.H. and Ax, R.L. 1984. A review of ovarian follicular cysts in cows with comparisons to the condition in women, rats, and rabbits. Theriogenology 22:109.

Forrest, D.W., Fleeger, J.L., Long, C.R., Sorenson, A.M., Jr., and Harms, P.G. 1980. Effect of exogenous prolactin on peripheral luteinizing hormone levels in ovariectomized cows. Biol. Reprod. 22: 197.

Foster, J.P., Lamming, G.E. and Peters, A.R. 1980. Short-term relationships between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of GnRH injection. J. Reprod. Fert. 59:321.

Ganjam, V.K. 1979. Episodic nature of the ^{4}-ene and ^{5}-ene steroid ogenic pathways and their relationship to the adreno-gonadal axis in stallions. J. Reprod. Fert. 27, Suppl. 1:67.

Garverick, H.A., Kesler, D.J., Cantley, T.C., Elmore, R.G., Youngquist, R.S., Bierschwal, C.J. 1976. Hormone response of dairy cows with ovarian cysts after treatment with HCG or GnRH. Theriogenology 6:43.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. 1. Iowa State Univ. Press, Ames, Iowa. pp. 177-178.

Gitlin, S.A., Forrest, D.W. and Harms, P.G. 1983. Bovine pituitary release of luteinizing hormone during gestation and early postpartum. J. Anim. Sci. 57 (Suppl. 1):339.

Golstein, J. and VanHaelst, L. 1973. Influence of thyrotropin-free serum on the radioimmunoassay of human thyrotropin. Clinica Chimica Acta 49:141.

Goodman, R.L. and Karsch, F.J. 1980. Pulsatile secretion of luteinizing hormone: differential suppression by ovarian steroids. Endo. 107:1286.

Goodman, R.L., Pickover, S.M. and Karsch, F.J. 1981. Ovarian feedback control of follicle-stimulating hormone in the ewe: evidence for selective suppression. Endo. 108:772.

Gunzler, O. and Schallenberger, E. 1980. The treatments of ovarian cysts in cattle with prostaglandins - possibilities and limitations. Acta Vet. Scand. Suppl. 77:327.

Hansel, W. and Convey, E.M. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57 (Suppl. 1):404.

Hausler, C.L. and Malven, P.V. 1976. Interaction of progesterone, GnRH and estradiol in the control of LH release in castrate heifers. J. Anim. Sci. 42:1239.

Henricks, D.M., Dickey, J.F., Hill, J.R. and Johnston, W.E. 1972. Plasma estrogen and progesterone levels after mating and during late pregnancy and postpartum in cows. Endo. 90:1336.

Humphrey, W.D., Kaltenbach, C.C., Dunn, T.G., Kortnik, D.R. and Niswander, G.D. 1983. Characterization of hormonal patterns in the beef cow during postpartum anestrus. J. Anim. Sci. 56:445.

Ireland, J.J., Curato, A.D. and Wilson, J. 1983. Effect of charcoal-treated bovine follicular fluid on secretion of LH and FSH in ovariectomized heifers. J. Anim. Sci. 57:1512.

Ireland, J.J., Fogwell, R.F., Oxender, W.D., Ames, K. and Cowley, J.L. 1984. Production of estradiol by each ovary during the estrous cycle of cows. J. Anim. Sci. 59:764.

Ireland, J.J. and Roche, J.R. 1983a. Development of non-ovulatory antral follicles in heifers. Changes in steroids in follicular fluids and receptors for gonadotropins. Endo. 112:150.

Ireland, J.J. and Roche, J.F. 1983b. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J. Anim. Sci. 57:157.

Jubb, K.V. and McEntee, K. 1955. Observations on the bovine pituitary gland. II. Architecture and cytology with special reference to basophil cell function. Cornell Vet. 45:593.

Karg, H. and Schallenberger, E. 1982. Regulation der ovariellen steroidhormonesekretion post partum. Wien. tierarztl. Mschr. 70:238.

Karsch, F.J. 1984. Neuroendocrine control of LH secretion during the estrous cycle of the ewe. Proc. Tenth Intl. Cong. on Anim. Reprod. and A.I., Vol. 4, I-10.

Karsch, F.J., Foster, D.L., Bittman, E.L. and Goodman, R.L. 1983. A role for estradiol in enhancing luteinizing hormone pulse frequency during the follicular phase of the estrous cycle of sheep. Endo. 113:1333.

Karsch, F.J., Legan, S.J., Ryan, K.D. and Foster, D.L. 1980. Importance of estradiol and progesterone in regulating LH secretion and estrous behavior during the sheep estrous cycle. Biol. Reprod. 23:404.

Kesler, D.J., Elmore, R.G., Brown, E.M. and Garverick, H.A. 1981. Gonadotropin releasing hormone treatment of dairy cows with ovarian cysts. I. Gross ovarian morphology and endocrinology. Theriogenology 16:207.

Kesler, D.J. and Garverick, H.A. 1982. Ovarian cysts in dairy cattle: a review. J. Anim. Sci. 55:1147.

Kesler, D.J., Garverick, H.A., Bierschwal, C.J., Elmore, R.S. and Youngquist, R.S. 1979a. Reproductive hormones associated with normal and abnormal changes in ovarian follicles in postpartum dairy cows. J. Dairy Sci. 62:1290.

Kesler, D.J., Garverick, H.A., Caudle, A.B., Bierschwal, C.J., Elmore, R.G. and Youngquist, R.S. 1979b. Testosterone concentrations in plasma of dairy cows with ovarian cysts. J. Dairy Sci. 62:1825.

Kesler, D.J., Garverick, H.A., Caudle, A.B., Elmore, R.G., Youngquist, R.S. and Bierschwal, C.J. 1980. Reproductive hormone and ovarian changes in cows with ovarian cysts. J. Dairy Sci. 63:166.

Kesler, D.J., Garverick, H.A., Elmore, R.G., Youngquist, R.S. and Bierschwal, C.J. 1979c. Reproductive hormones associated with the ovarian cyst response to GnRH. Theriogenology 12:109.

Kesler, D.J., Garverick, H.A., Youngquist, R.S. Elmore, R.G. and Bierschwal, C.J. 1978. Ovarian and endocrine responses and reproductive performance following GnRH treatment in early postpartum dairy cows. Theriogenology 9:363.

Kesner, J.S., Convey, E.M. and Anderson, C.R. 1981. Evidence that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release. Endo. 108:1386.

Kesner, J.S., Padmanabhan, V. and Convey, E.M. 1982. Estradiol produces and progesterone inhibits the preovulatory surges of luteinizing hormone and follicle-stimulating hormone in heifers. Biol. Reprod. 26:571.

Kiracofe, G.H., Ramirez-Godinez, J.A., McGowan, R.D. and Bolt, D.J. 1983. Reduction of serum FSH in ovariectomized heifers with bovine follicular fluid. J. Anim. Sci. 57 (Suppl. 1):350.

Kirk, J.H., Huffman, E.M. and Lane, M. 1982. Bovine cystic ovary disease: hereditary relationships and case study. J. Am. Vet. Assn. 181:474.

Kittock, R.J., Britt, J.H. and Convey, E.M. 1973. Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cysts. J. Anim. Sci. 37:985.

Kittock, R.J., Britt, J.H. and Edgerton, L.A. 1974. Serum steroids after gonadotropin treatment in cows with ovarian follicular cysts. Am. J. Vet. Res. 35:1576.

Kudlac, E., Vrtel, M., Vlcek, Z. and Vinkler, A. 1984. Therapeutic methods and their effect on ovarian cysts on milk cows. Vlaams. Dier. Tijds. 53:159.

Leidl, W., Braun, U., Okuda, K., Stolla, R. and Schams, D. 1983. Klassifizierung der ovarzysten des rindes. Wien. tierarztl. Mschr. 70: 208.

Leidl, W., Stolla, R., Handscell, C. and Bostedt, H. 1979. Zur ovarialzyste des rindes. 1. Klassiferizierung und diagnose. Berl. Munch. Tierarztl. Wschr. 92:369.

Leslie, K.E. and Bosu, W.T.K. 1983. Plasma progesterone concentrations in dairy cows with cystic ovaries and clinical responses following treatment with fenprostalene. Can. Vet. J. 24:352.

Levine, J.E., Kwok-Yuen, F. Pau, Ramirez, V.D. and Jackson, G.L. 1982. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. Endo. 111:1449.

Li, P.S. and Wagner, W.C. 1983. Effects of hyperadrenal states on luteinizing hormone in cattle. Biol. Reprod. 29:11.

Liptrap, R.M. and McNally, P.J. 1976. Steroid concentrations in cows with corticotropin-induced cystic ovarian follicles and the effect of prostaglandin F_2 and indomethacin given by intrauterine injection. Am. J. Vet. Res. 37:369.

Lu, J.K.H., Damassa, D.A., Gilman, D.P., Judd, H.L. and Sawyer, C.H. 1980. Differential patterns of gonadotropin responses to ovarian steroids and to LH-releasing hormone between constant-estrous and pseudopregnant states in aging rats. Biol. Reprod. 23:345.

Lunaas, T., Refsdal, A.O. and Garm, O. 1974. Urinary excretion of oestrone and oestradiol-17a in cows with cystic ovaries. Acta Endo. 75:350.

Malvano, R. 1983. Radioimmunoassay of steroids. In, Principles of Competitive Protein-Binding Assays, 2nd Ed., W.D. Odell and P. Franchimont, eds., John Wiley and Sons, New York, NY, p. 187-191.

Marcek, J.M., Appell, L.H., Hoffman, C.C., Moredick, P.T. and Swanson, L.V. 1985. Effect of supplemental B-carotene on incidence and responsiveness of ovarian cysts to hormone treatment. J. Dairy Sci. 68:71.

Mather, E.C., Camper, P.M., Vahdat, F., Whitmore, H.L. and Gustafsson, B.G. 1978. Assessment of ovarian activity in the postpartum dairy cow by use of a milk progesterone assay. Theriogenology 10:119.

McCarthy, M.S., Hafs, H.D. and Convey, E.M. 1979. Serum hormone patterns associated with growth and sexual development in bulls. J. Anim. Sci. 49:1012.

Midgley, A.R., Jr., Niswender, G.D. and Rebar, R.W. Principles for the assessment of the reliability of radioimmunoassay methods (precision, accuracy, sensitivity, specificity). In, 1st Karolinska Symposia on Research Methods in Reproductive Endocrinology, E. Diczfalusy, ed., Sept. 23-25, 1969, Stockholm, Sweden, pp. 163-180.

Morrow, D.A., Roberts, S.J., McEntee, K. and Gray, H.G. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. J. Am. Vet. Med. Assn. 149:1596.

Nadaraja, R. and Hansel, W. 1976. Hormonal changes associated with experimentally produced cystic ovaries in the cow. J. Reprod. Fert. 47:203.

Nakao, T. 1976. The ovarian condition diagnosed per rectum and its relations to serum concentrations of progesterone and estradiol 17-B and prognosis in cows with cystic ovaries. Jap. J. Anim. Reprod. 21:147.

Nakao, T., Sugihashi, A., Saga, N., Tsunoda, N. and Kawata, K. 1983. Use of milk progesterone enzyme immunoassay for differential diagnoses of follicular cyst, luteal cyst, and cystic corpus luteum in cows. Am. J. Vet. Res. 44:888.

Nessan, G.K. and King, G.J. 1981. Relationship of peripheral estrogens and testosterone to sexual behavior in normal and cystic cows. Can. Vet. J. 22:9.

Nessan, G.K., King, G.J., McKay, G.W., Thomson, J.D. and Bertrand, W. 1977. Treatment of cystic ovarian degeneration in dairy cows with gonadotrophic releasing hormone or human chorionic gonadotrophic hormone. Can. Vet. J. 18:33.

Nodan, P.A., Oxender, W.D. and Hafs, H.D. 1978. Plasma luteinizing hormone, progestogens, and estrogens in mares during gestation, parturition, and first postpartum estrus (foal estrus). Am. J. Vet. Res. 39:1964.

Okatani, Y. and Fishman, J. 1984. Suppression of the preovulatory luteinizing hormone surge in the rat by 2-hydroxyestrone: relationship to endogenous estradiol levels. Endo. 115:1082.

Olson, P.N., Bowen, R.A., Behrendt, M.S., Olson, J.D. and Nett, T.M. 1984. Concentrations of testosterone in canine serum during late anestrus, proestrus, estrus and early diestrus. Am. J. Vet. Res. 45:145.

Padmanabhan, V. and Convey, E.M. 1981. Progesterone inhibits the ability of estradiol to increase basal and luteinizing hormone-releasing hormone-induced luteinizing hormone release from bovine pituitary cells in culture: neither progesterone nor estradiol affects follicle-stimulating hormone release. Endo. 109:1091.

Padmanabhan, V., Convey, E.M., Roche, J.F. and Ireland, J.J. 1984. Changes in inhibin-like bioactivity in ovulatory and atretic follicles and utero-ovarian venous blood after prostaglandin-induced luteolysis in heifers. Endo. 115:1332.

Pant, H.C., Hopkinson, C.R.N. and Fitzpatrick, R.J. 1977. Concentrations of oestradiol-17b, progesterone, luteinizing hormone and follicle-stimulating hormone in the jugular venous plasma of ewes during the oestrous cycle. J. Endo. 73:247.

Peralta, R.U., Ax, R.L. and Elford, W.G. 1982. Incidence of cystic ovaries in Wisconsin dairy herds. J. Dairy Sci. 62 (Suppl. 1): 182 (Abst.).

Peters, A.R. and Lamming, G.E. 1984a. Endocrine changes in the post-partum period. Proc. Tenth Intl. Cong. Anim. Reprod. and A.I., June 10-14, Urbana-Champaign, Vol. IV.

Peters, A.R. and Lamming, G.E. 1984b. Reproductive activity of the cow in the postpartum period. II. Endocrine patterns and induction of ovulation. Br. Vet. J. 140:269.

Peters, A.R., Lamming, G.E. and Fisher, M.W. 1981. A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fert. 62:567.

Peters, A.T. and Liptrap, R.M. 1985. Plasma gonadotrophin levels in sows with experimental cystic ovarian follicles. Br. Vet. J. 141:288.

Rahe, C.H., Owens, R.E., Fleeger, J.L., Newton, H.J. and Harms, P.G. 1980. Pattern of luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endo. 107:498.

Reimers, T.J., Cowan, R.G., Davidson, H.P. and Colby, E.D. 1981. Validation of radioimmunoassays for triiodothyronine, thyroxine, and hydrocortisone (cortisol) in canine, feline, and equine sera. Am. J. Vet. Res. 42:2016.

Roberts, S.L. 1971. Veterinary Obstetrics and Genital Diseases. Published by the author, Ithaca, NY. Lithographed by Edwards Brothers, Inc., Ann Arbor, MI, p. 422-423.

Robinson, T.J. 1977. Reproduction in cattle. In, Reproduction in Domestic Animals, H.H. Cole and P.T. Cupps, eds., Academic Press, New York, NY, 440.

Roche, J.F. and Ireland, J.J. 1981. The differential effect of progesterone on concentrations of luteinizing hormone in heifers. Endo. 108:568.

Roth, J.A., Kaeberle, M.L., Appell, L.H. and Nachreiner, R.F. 1983. Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. Am. J. Vet. Res. 44:247.

Roy, J.H.B., Perfitt, M.W., Glencross, R.G. and Turvey, A. 1985. Performance and progesterone and oestradiol-17b concentrations in fat-free milk of a cow with a cystic ovarian follicle. Vet. Rec. 116:370.

Saba, N., Symons, A.M., Cunningham, N.F. and Boarer, C.D. 1976. The acute effect of oestrogen injection on plasma LH in freemartin heifers. J. Reprod. Fert. 48:317.

Sasser, R.G., Falk, D.E. and Ross, R.H. 1979. Estrogen in plasma of parturient paretic and normal cows. J. Dairy Sci. 62:551.

Saumande, J., LeCoustumier, J. and Marais, C. 1979. Oestradiol-17B and progesterone in nymphomaniac cows. Theriogenology 12:27.

Schallenberger, E. and Peterson, A.J. 1982. Effect of ovariectomy on tonic gonadotrophin secretion in cyclic and postpartum dairy cows. J. Reprod. Fert. 64:47.

Schams, D., Schallenberger, E., Menzer, Ch., Stangl, J., Zottmeier, K., Hoffmann, B. and Karg, H. 1978. Profiles of LH, FSH and progesterone in postpartum dairy cows and their relationship to the commencement of cyclic functions. Theriogenology 10:453.

Schillo, K.K., Dierschke, D.J. and Hauser, E.R. 1983. Estrogen-induced release of luteinizing hormone in prepubertal and postpubertal heifers. Theriogenology 19:727.

Seguin, B.E. 1975. Endocrine status of cows with ovarian cystic degeneration and therapeutic efficacy of gonadotropin releasing hormone and human chorionic gonadotropin in affected cows. Ph.D. Dissertation, Michigan State University, East Lansing, MI.

Seguin, B.E., Convey, E.M. and Oxender, W.D. 1976. Effect of gonadotropin-releasing hormone and human chorionic gonadotropin on cows with ovarian follicular cysts. Am. J. Vet. Res. 37:153.

Shemesh, M., Ayalon, N. and Lindner, H.R. 1972. Oestradiol levels in the peripheral blood of cows during the oestrous cycle. J. Endo. 55:73.

Short, R.V. 1962. Steroid concentrations in normal follicular fluid and ovarian cyst fluid from cows. J. Reprod. Fert. 4:27.

Short, R.E., Randel, R.D., Staigmiller, R.B. and Bellows, R.A. 1979. Factors affecting estrogen-induced LH release in the cow. Biol. Reprod. 21:683.

Smith, R.D., Erb, H.N. and Oltenacu, P.A. 1985a. Relationships between reproductive disorders and their effects on reproductive performance in primiparous Holstein cows. J. Dairy Sci. 68 (Suppl. 1): 198 (abst.).

Smith, R.D., Erb, H.N. and Oltenacu, P.A. 1985b. The relationship between health and reproductive disorders and their effects on the reproductive performance and culling in multiparous Holstein cows. J. Dairy Sci. 68 (Suppl. 1):197 (abst.).

Spicer, L.J. 1984. Control of ovarian follicular growth and steroidogenesis during postpartum anestrus in beef cows. Ph.D. Dissertation, Michigan State University, East Lansing, MI.

Stevenson, J.S., Spire, M.F. and Britt, J.H. 1983. Influence of the ovary on estradiol-induced luteinizing hormone release in postpartum milked and suckled Holstein cows. J. Anim. Sci. 57:692.

Stoebel, D.P. and Moberg, G.P. 1982. Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior of cows. J. Dairy Sci. 65:1016.

Stolla, R., Bostedt, H., Wendt, V. and Leidl, W. 1980. Zur ovarialzyste des rindes. III. Vergleichende wertung von therapievrefahren. Berl. Munch. Tierarztl. Wschr. 93:4.

Swanson, L.V. and Hafs, H.D. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038.

Tanabe, T.Y. and Brofee, R.D. 1982. Treatment of cystic ovarian follicles in dairy cows with chorionic gonadotropin. Theriogenology 18:497.

van de Wiel, D.F.M., Kalis, C.H.J. and Nasir Hussain Shah, S. 1979. Combined use of milk progesterone profiles, clinical examination and oestrus observation for the study of fertility in the post-partum period of dairy cows. Br. Vet. J. 135:568.

Varner, M.A., Johnson, B.H., Britt, J.H., McDaniel, B.T. and Mochrie, R.D. 1983. Influence of herd relocation on production and endocrine traits of dairy cows. J. Dairy Sci. 66:466.

Vasquez, L.A., Whitmore, H.L., Brodie, B.O., Kesler, D.J. and Brown, M.D. 1984. Comparison of GnRH treatment and manual rupture of ovarian cysts in dairy cattle. Proc. Tenth Intl. Congress of Anim. Reprod. and A.I., Vol. 3, abst. 476.

Walker, R.F. 1983. Serotonin - reinstatement of luteinizing hormone surges after loss of positive feedback in ovariectomized rats bearing subcutaneous capsules containing oestrogen. J. Endo. 98:7.

Walters, D.L., Schams, D. and Schallenberger, E. 1984. Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrus cycle in the cow. J. Reprod. Fert. 71:479.

Walters, D.L. and Schallenberger, E. 1984a. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the periovulatory phase of the oestrus cycle in the cow. J. Reprod. Fert. 71:503.

Walters, D.L., Short, R.E., Convey, E.M., Staigmiller, R.B., Dunn, T.G. and Kaltenbach, C.C. 1982. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small dose injections of GnRH. Biol. Reprod. 26:655.

Watson, E.D. and Munro, C.D. 1984. Adrenal progesterone production in the cow. Br. Vet. J. 140:300.

Webb, R., Lamming, G.E., Haynes, N.B. and Foxcroft, G.R. 1980. Plasma progesterone and gonadotrophin concentrations and ovarian activity in postpartum dairy cows. J. Reprod. Fert. 59:133.

Whitmore, H.L., Ginther, O.J. and Casida, L.E. 1972. Corpus luteum removal and steroid treatment of heifers in four consecutive estrous cycles: effects on frequency of cystic ovaries and expression of estrus. Am. J. Vet. Res. 33:519.

Whitmore, H.L., Tyler, W.J. and Casida, L.E. 1974. Incidence of cystic ovaries in Holstein-Friesian cows. J. Am. Vet. Med. Assn. 165:693.

Wildt, D.E., Seager, S.W.J. and Chakraborty, P.K. 1981. Behavioral ovarian and endocrine relationships in the pubertal bitch. J. Anim. Sci. 53:182.

Wiltbank, J.N., Ingalls, J.E. and Rowden, W.W. 1981. Effects of various forms and levels of estrogens alone or in combination with gonadotrophins on the estrous cycle of beef heifers. J. Anim. Sci. 20:341.

Yamauchi, M. 1955. Studies on the ovarian cyst in the cow. IV. The course of recovery from ovarian follicle cyst by the chorionic gonadotrophin therapy. Jap. J. Vet. Sci. 17:54.

Yamauchi, M., Ashida, K. and Inui, S. 1954. Studies on the ovarian cyst in the cow. III. Endocrinological studies on the cause of the ovarian cyst. Jap. J. Vet. Sci. 16:65.

Yamauchi, M. and Inui, S. 1954. Studies on the ovarian cyst in the cow II. Endocrinological and histological studies on the correlation between the ovarian cyst and the symptom. Jap. J. Vet. Sci. 16:27.

Zaied, A.A., Garverick, H.A., Kesler, D.J., Bierschwal, C.J., Elmore, R.G., and Youngquist, R.S. 1981. Luteinizing hormone response to estradiol benzoate in cows postpartum and cows with ovarian cysts. Theriogenology 16:349.

Zemjanis, R. 1970. Diagnostic and Therapeutic Techniques in Animal Reproduction, 2nd Ed. Williams and Wilkins Co., Baltimore, MD 47-78.