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ENDOCRINE RESPONSES AFTER GONADOTROPIN OR GONADOTROPIN RELEASING HORMONE IN COWS WITH OVARIAN FOLLICULAR CYSTS

Thesis for the Degree of M. S.

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ROGER JOHN KITTOK

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ABSTRACT

ENDOCRINE RESPONSES AFTER GONADOTROPIN OR GONADOTROPIN RELEASING HORMONE IN COWS WITH OVARIAN FOLLICULAR CYSTS

By

Roger John Kittok

In a survey designed to monitor serum hormone changes during several phases of reproduction, blood was collected from 91 lactating Holstein cows on days 0 (estrus), 2, 4, 7 and 11 during postpartum estrous cycles. In six cows, ovarian follicular cysts were detected ($\bar{x}$ = 116 days of lactation, range 37 to 167 days), and blood was taken on days 0, 2, 4, 7 and 11 after injection of 10,000 IU human chorionic gonadotropin (HCG) and during post-treatment estrous cycles. Before and after the cystic period, estrous cycles were classified according to length, normal (18 to 23 days) or abnormal (3 to 15 days). Steroids were quantified by radioimmunoassay (estradiol and estrone) or competitive protein binding assay (glucocorticoids and progesterone). During normal length cycles in cows with follicular cysts, serum estradiol on days 2 to 7 (11 ± 2 pg/ml) was less than on respective days of abnormal length cycles (16 ± 1 pg/ml; $P<0.025$) in these same animals.

Serum progesterone in cows which subsequently became cystic increased ($P<0.005$) from 0.1 ± 0.1 to 1.2 ± 0.3 ng/ml between days 0 to 2 and days 4 to 11 of normal length cycles. After injection of HCG in cows with follicular cysts, progesterone increased ($P<0.05$) from 0.8 ± 0.4 ng/ml on days 0 to 2 to 2.8 ± 1.0 ng/ml on days 4 to 11. In these cows, estrous cycles with normal length were typified by higher ($P<0.01$) progesterone (1.2 ± 0.3 ng/ml) than cycles with abnormal length (0.1 ± 0.1 ng/ml) on days 4 to 11. Corticosterone, cortisol and estrone did not differ between normal and abnormal estrous cycles or after HCG treatment. These data suggest HCG increased serum progesterone but did not influence estrogens or glucocorticoids.

Luteinizing hormone (LH) and progesterone were assayed in blood sera collected before and after gonadotropin releasing hormone (GnRH) administration (iv) to nine Holstein cows, five of which had ovarian follicular cysts. The four remaining cows were treated on day 10, 11 or 13 of the estrous cycle. GnRH was given in three doses of 100 μg each, at 120 min. intervals. Serum LH (ng/ml) in cows with ovarian follicular cysts was 1.2 ± 0.3, 23.8 ± 7.5, 14.3 ± 2.8 and 4.7 ± 1.2 at 0, 120, 240 and 360 min. after GnRH administration, respectively, compared to concentrations of 0.5 ± 0.1, 5.9 ± 0.9, 7.2 ± 1.2 and 2.9 ± 0.5, respectively, for luteal phase cows ($P<0.01$). Peak LH occurred at 170 min.

(33.9 ± 6.6 ng/ml) in cystic cows and 145 min. (17.3 ± 2.4 ng/ml) in luteal cows. Change in LH concentration after each injection of GnRH was greater ($P<0.01$) in cows with follicular cysts. Serum progesterone (ng/ml) increased from 0.9 ± 0.4 at 0 min. to 2.0 ± 1.2 at 20 min. in cystic cows, but this response was due to increased serum progesterone in two animals. Progesterone increased ($P<0.05$) from 5.9 ± 0.8 at 0 min. to 10.3 ± 0.9 and 8.2 ± 1.0 at 260 and 360 min., respectively, in luteal phase cows. Serum progesterone was 3.6 ± 1.4 on day 11 after GnRH administration in cows with ovarian follicular cysts although only one cow had a palable corpus luteum. All of the cows with follicular cysts exhibited estrus 20 to 24 days after GnRH treatment. Serum estradiol and estrone (pg/ml) prior to GnRH treatment for cows with follicular cysts averaged 9.4 ± 3.1 and 3.8 ± 0.5, respectively, which was not different from means for cows treated during the luteal phase of the estrous cycle (8.8 ± 0.7 and 3.0 ± 0.4, respectively).

Administration of repeated does of GnRH produced an LH response similar to the preovulatory surge in the bovine and initiated estrous cycles in cows with ovarian follicular cysts.

ENDOCRINE RESPONSES AFTER GONADOTROPIN OR GONADOTROPIN RELEASING HORMONE IN COWS WITH OVARIAN FOLLICULAR CYSTS

By

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INTRODUCTION

Endocrine changes during puberty, estrous cycles, gestation and parturition have been studied in the bovine female. Thus, some understanding of the processes involved in reproduction has arisen. However, such information during aberrant physiological states is not complete. Ovarian follicular cysts cause a significant decrease in reproductive efficiency in dairy cattle and the etiology of the condition is lacking. Parameters other than hormonal changes have been studied in cows with ovarian follicular cysts and the conclusions are quite variable. Information is limited on serum hormone concentrations in cows with ovarian follicular cysts and although human chorionic gonadotropin (HCG) is an accepted treatment for follicular cysts, there are no reports of serum hormone levels in cows after treatment.

Synthesis of gonadotropin releasing hormone (GnRH) made it available in quantities that allow study of endocrine responses elicited from domestic animals, including the bovine. While cattle of many different physiological states have been subjected to the releasing hormone, responses obtained from cows with aberrant ovarian function have not been reported.

Specifically, the objectives of this work were, (1) to quantify serum steroid hormones in cows before ovarian follicular cysts were detected and after treatment with HCG and (2) to quantify serum luteinizing hormone and progesterone responses in cows with ovarian follicular cysts after administration of gonadotropin releasing hormone.

REVIEW OF THE LITERATURE

Although many reports have dealt with ovarian follicular cysts, conflicting observations and lack of research in some critical areas prevent concise summarization of the etiology of this condition. Thus, the cause of this aberrant ovarian function is not fully understood. Perhaps confusion concerning the definition of follicular cysts or existence of differing types of cysts may account for the lack of knowledge concerning the disorder.

Definition of Ovarian Follicular Cysts

Large Ovarian Follicules (>2.0 cm) were classified ovarian follicular cysts (Albrechtsen, 1917). Garm (1949) used persistent follicules and lack of luteal tissue as criteria. To be considered an ovarian follicular cyst, Casida, McShan and Meyer (1944) specified a follicle must be larger than 2.5 cm in the absence of palpable luteal tissue. Others specified both size of follicle and length of time palpable luteal tissue was absent from the ovary: >1.0 cm for more than 7 days (Johnson, Legates and Ulberg, 1966); >1.5 cm for more than 7 days (Johnson and Ulberg, 1967); >2.0 cm for more than 14 days (Marion and Gier, 1968); and >2.5 cm for more than 10 days (Morrow *et al.*, 1966).

Roberts (1955) and Henricson (1957) diagnosed animals with ovarian follicular cysts after irregular estrous cycles or anestrous was observed, but criteria used was not given. Follicles maintained over 14 days in the absence of palpable luteal tissue, failure to ovulate or undectable luteal tissue were conditions used by Callahan *et al*. (1971).

Incidence of Ovarian Follicular Cysts

Several researchers have suggested that ovarian follicular cysts occur in 10 to 15 percent of the dairy cattle studied. Wiltbank, Tyler and Casida (1953) observed 31 cases of follicular cysts out of 274 postpartum reproductive periods (11.3%) and Morrow *et al*. (1966) observed 357 postpartum cycles with an incidence of follicular cysts of 12.3 percent. In a much smaller survey, Callahan *et al*. (1971) found 10 out of 65 postpartum cows with follicular cysts (15.4%).

Incidence of follicular cysts during the first postpartum estrous cycle was 22.3 percent compared with 6.2 percent during the second cycle ($P<0.005$) (Morrow *et al*., 1966). Wiltbank *et al*. (1953) indicated 12.0 percent of cows had ovarian follicular cysts during 15 to 74 days postpartum, but only 4.6 percent were observed to be cystic from 75 to 300 days ($P<0.05$). Of 29 follicular cysts that occurred 11 to 78 days postpartum, 24 (83%) were observed 11 to 35 days postpartum (Marion and Gier, 1968).

The incidence of ovarian follicular cysts in heifers is low, 4.6 percent (Wiltbank et al., 1953). Roberts (1955) reported that out of 352 cases treated for ovarian follicular cysts, only 3 were heifers.

Incidence of ovarian follicular cysts increased with number of times milked per day (Casida and Chapman, 1951). In cows not milked, mostly heifers, 3.4 percent were observed to have ovarian follicular cysts compared with 6.8 percent for animals milked 2 times per day and 10.6 percent in cows milked more than 2 times per day.

Wiltbank et al. (1953) observed 72.6 percent of the annual total of follicular cysts in November and Roberts (1955) treated 48.4 percent of the annual total during the period of December to February. Cows calving from August to December had a 13.4 percent incidence of ovarian follicular cysts compared with 8.6 percent of those calving from January to July (Marion and Gier, 1968). In addition, Morrow et al. (1966) also commented that follicular cysts occurred more frequently from November to January.

Possible Causes of Ovarian Follicular Cysts

Albrechtsen (1917) considered that formation and maintenance of follicular cysts were related to infection of the uterus at the last parturition. Although later research (Garm, 1949) refutes this possible cause, Morrow et al.

(1966) reported that the incidence of follicular cysts in cows normal at calving was 8.3 percent compared with 23.4 percent in cows with abnormal calvings. Cows, abnormal at the last calving, had been afflicted with one or more of a variety of periparturient diseases, such as metritis, milk fever, mastitis or ketosis, or had suffered from abortion, dystocia or retained placenta.

Others (Garm, 1949; Henricson, 1957) concluded that follicular cysts involved genetic predisposition and stress of heavy lactation provoked its incidence. Genetic predisposition is possible, Casida and Chapman (1951), from records in a single Holstein herd, computed a coefficient of heritability of becoming cystic sometime in life of 0.43. Although observing a small number of animals, Wiltbank et al. (1953) reported that line breeding increases the incidence ($P<0.01$) of ovarian follicular cysts.

Callahan et al. (1971) reported that cows destined to have ovarian follicular cysts exhibit earlier follicular growth (15 ± 1.2 vs 20 ± 2.3 days), delayed ovulation (29 ± 3.9 vs 17 ± 1.2 days), delayed corpus luteum formation (39 ± 3.7 vs 25 ± 1.5 days) and earlier first estrus after parturition (25 ± 3.0 vs 34 ± 3.3 days) compared with normal cows. However, Morrow et al. (1966) found the interval between parturition and first estrus longer ($P<0.01$) than in normal cows (37 vs 20 days).

Enzymatic and Steroidogenic Activity within the Ovarian Follicular Cyst

Nakama (1969) reported that luteinized portions of follicular cysts have enzyme activities similar to luteal cells of functional corpora lutea.

Steroid producing cells in follicular cysts degenerate (Short, 1962); cyst fluid contains large amounts of progesterone but little or no detectable androstenedione, 17β-estradiol, estrone or 17α-progesterone. Short (1962) suggested progesterone was produced by luteal tissue within the cyst and estrogens were produced in the ovary, but not within the follicular cyst.

By histological examination, anovulatory follicles after superovulation with gonadotropins are similar to spontaneous ovarian follicular cysts (Archbald *et al.*, 1973).

Hormone Data and Pituitary Cytology in Cows with Ovarian Follicular Cysts

In addition to the above mentioned data on steroid hormone concentrations in cyst fluid (Short, 1962), Erb *et al.* (1971) have measured urinary estrogens, and serum glucocorticoids, progesterone and luteinizing hormone postpartum in cows which developed ovarian follicular cysts. No differences were observed in estrogens and progesterone from days 0 to 2 to day 7 of estrous cycles which ocurred before day 60 postpartum. And levels of these hormones were not different from normal cows on respective days. Garverick et al. (1973) measured serum estrogen, progesterone and luteinizing hormone on days 5, 6, 7, 8, 9, 11 and 13 following parturition. Levels of these hormones in cows destined to have ovarian follicular cysts did not differ significantly from normal cows.

In cows induced to retain follicles with multiple injections of estrogen and progesterone (Erb et al., 1973), plasma estrogen and progesterone were higher and luteinizing hormone lower during the period prior to cyst detection than the period in which cysts were present. After the ovarian follicular cysts were detected, treatment with human chorionic gonadotropin increased progesterone.

Anterior pituitary acidophils in cows with follicular cysts have increased granulation, with the nucleus being pycnotic (Jubb and McEntee, 1955). Both of the above changes are indicative of inactivity. Jubb and McEntee (1955) also found the basophils of these animals had increased activity. This suggests hyperproduction or failure to release gonadotropins. The hypothesis of failure to release gonadotropins is supported by the report of increased pituitary gonadotropin content in animals that retained ovarian follicles after oxytocin treatment (Donaldson and Hansel, 1968).

In cows with ovarian follicular cysts that were exhibiting signs of nymphomania, 200 IU ACTH increased serum corticosterone, cortisol and progesterone while estradiol and LH decreased (Troconiz et al., 1973). In these same animals, dexamethasone administered three days later resulted in decreased serum corticosterone, cortisol, progesterone, estradiol and LH. Three of five animals ovulated following dexamethasone treatment.

Relationship Between Milk Production and Ovarian Follicular Cysts

Relationship between milk production and incidence of ovarian follicular cysts is not clear. Johnson et al. (1966) reported cows with ovarian follicular cysts produced more ($P<0.01$) than herdmates during 305 days of lactation (DHIA, 2X, adjusted to mature equivalent and for days open). On the basis of previous lactations, it was concluded that cows produced more because follicular cysts were present rather than high production inducing cysts. Marion and Gier (1968) observed 22.6 percent of cows producing over 30 kg of milk per day had ovarian follicular cysts, significantly more ($P<0.001$) than animals producing 22 to 30 kg per day (9.9%). In addition, Henricson (1957) reported a positive correlation between high milk production and incidence of follicular cysts. However, others have found no such relationship (Casida and Chapman, 1951; Wiltbank et al., 1953; Morrow et al., 1966).

Treatment and Spontaneous Recovery of Cows with Ovarian Follicular Cysts

As a means of treatment, rupture of the follicular cyst has been used and Albrechtsen (1917) reported 65.9 percent conception using this method.

Using unfractionated pituitary extracts, Casida et al. (1944) treated 96 cows and observed corpora lutea in 74 (77.1%) and normal estrus in 71.8 percent within 31 days.

Human chorionic gonadotropin (HCG) has been administered to cows with follicular cysts. Injected intravenously 5,000 IU produced a 88.2 percent recovery rate in 51 cases (Roberts, 1955). Roberts (1957), using 500 to 1,000 IU HCG injected directly into the cyst, observed 83.3 percent recovery. And of the 23 cystic animals subjected to 10,000 IU HCG, half intravenously and half intramuscularly, 85.7 percent recovered (Morrow et al., 1966). An intravenous treatment of 3,000 IU HCG and 100 mg progesterone, administered to 110 cows with follicular cysts effected 83.6 percent recovery (Trainin and Alder, 1967).

Others have administered progesterone as treatment for follicular cysts (Johnson and Ulberg, 1967). Progesterone was injected daily for 14 days in doses of 50 and 100 mg and resulted in normal estrus within a few days post-treatment in 61.5 and 62.5 percent of the animals, respectively.

Four animals (13.3%) conceived out of 30 control cows with ovarian follicular cysts (Johnson and Ulberg, 1967). Morrow *et al.* (1966) treated animals only if ovarian cysts persisted after 60 days postpartum; 48.0 percent of cystic cows recovered without treatment before 60 days postpartum.

Induction of Ovarian Follicular Cysts

In the bovine, retention of ovarian follicles has been induced by various treatments. Foote *et al.* (1959) removed corpora lutea from 21 heifers in two consecutive cycles, and induced 3 cysts after the first removal and 9 after the second. Anovulatory follicles occur after oxytocin treatment on days 4 or 7 of the cycle (Donaldson and Hansel, 1968). Four heifers, treated 2 times each with 250 or 500 mg of 17β-estradiol after corpus luteum removal on day 13 of the cycle resulted in 4 follicular cysts (Whitmore, Ginther and Casida, 1972). Erb *et al.* (1973) induced cystic follicles in 7 of 8 cows receiving twice daily injections of progesterone (0.25 mg/day/kg) and 17β-estradiol (0.1 mg/day/kg) for 7 days beginning on day 3 of the estrous cycle.

Gonadotropin Releasing Hormone in the Bovine

The recent synthesis of gonadotropin releasing hormone (GnRH) (Matsuo *et al.*, 1971) has allowed its use for study of endocrine responses. In the bovine, responses have been elicited from mature bulls (Golter et al., 1972; Zolman, Convey and Hafs, 1972; Zolman and Convey, 1973), pubertal bulls (Mongkongpunya et al., 1973) and cycling heifers (Kaltenbach et al., 1974; Peck et al., 1973; Zolman et al., 1973). And ovulation has been observed in response to releasing hormone when administered after withdrawal of a synchronizing dose of progesterone (Mauer and Rippel, 1972; Kaltenbach et al., 1974).

METHODS AND MATERIALS

Experiment 1

This study was part of a larger survey (Edgerton and Hafs, 1973) designed to monitor serum hormone changes during several phases of reproduction in dairy cattle. During the survey, which involved twice weekly rectal palpations of 91 cows after parturition, six lactating Holstein cows ($\bar{x}$=116 days of lactation, range of 37 to 167 days) retained at least one ovarian follicle (>2.5 cm in diameter) for at least 14 days in the absence of a palpable corpus luteum; then, 10,000 IU human chorionic gonadotropin (HCG) (Follutein, E.R. Squibb and Sons, Inc.) was administered, half intravenously and half intramuscularly. Blood was collected via jugular puncture during estrous cycles before the ovarian follicular cysts developed (available from the survey of Edgerton and Hafs, 1973), during the period following treatment with HCG and during post-treatment estrous cycles. Estrous cycles before follicular cysts were detected and after the condition was no longer present were classified as normal length (18 to 23 days) or abnormal length (3 to 15 days). Of the six cows with follicular cysts, one animal did not have an estrous cycle of normal length, another animal was represented twice in the abnormal cycles and all are represented once in the period following HCG. For all periods, blood was taken on days 0 (estrus or day of HCG administration), 2, 4, 7 and 11. In addition, blood was collected on day 0 and 7 of normal estrous cycles in four contemporary non-cystic herdmates. All animals were observed twice daily for signs of estrus.

After elution of serum extracts by column chromatography (Sephadex LH-20), appropriate fractions were subjected to radioimmunoassay for estradiol and estrone (Wettemann *et al.*, 1972) and to competitive protein binding assay for corticosterone, cortisol and progesterone (Swanson, Hafs and Morrow, 1972).

Statistical differences in serum hormone concentrations in these animals were determined by t-test if two means had homogenous variances or approximate t-test if variances were heterogenous (Sokal and Rohlf, 1969).

Experiment 2

Gonadotropin releasing hormone[1] (GnRH) was administered via jugular cannula (three doses of 100 μg each at 120 min. intervals) to each of nine lactating Holstein cows ($\bar{x}$=76 days of lactation, range of 41 to 104 days). Five cows were included because they had ovarian follicular

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[1]GnRH was generously supplied by Dr. R.H. Rippel, Abbott Laboratories, North Chicago, Illinois.

cysts; as in the first experiment, each animal had at least one ovarian follicle larger than 2.5 cm which was retained in one location for at least 14 days in the absence of a palpable corpus luteum. GnRH was administered after these criteria were satisfied. Prior to treatment, all cows with ovarian follicular cysts were anestrus. The remaining four control cows showed normal length estrous cycles and were treated on day 10 (2 animals), 11 or 13 of the estrous cycle. Blood was collected to monitor serum hormone levels more frequently immediately after GnRH administration and less frequently as time from administration increased (Figure 1). All animals were observed twice daily for estrus and ovarian activity was monitored twice weekly by rectal palpation.

All samples were assayed for serum luteinizing hormone (LH) by double antibody radioimmunoassay[2] as described by Oxender, Hafs and Edgerton (1972). Pretreatment serum estradiol and estrone were determined by radioimmunoassay (Wettemann _et_ _al_., 1972). Serum progesterone in selected samples was quantified by radioimmunoassay as described below.

Serum (0.5 ml) was placed in a 15 ml screw cap culture tube and 3,000 dpm of ^{3}H-1,2-progesterone (New England Nuclear; 34 c/mM; repurified by column chromatography) added. After allowing labeled and endogenous

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[2]NIH-LH-B5 standard was supplied by the National Institutes of Health, Bethesda, Maryland. Purified bovine LH (LER-1072-2) for iodination was supplied by Dr. L.E. Reichert, Emery University, Atlanta, GA.

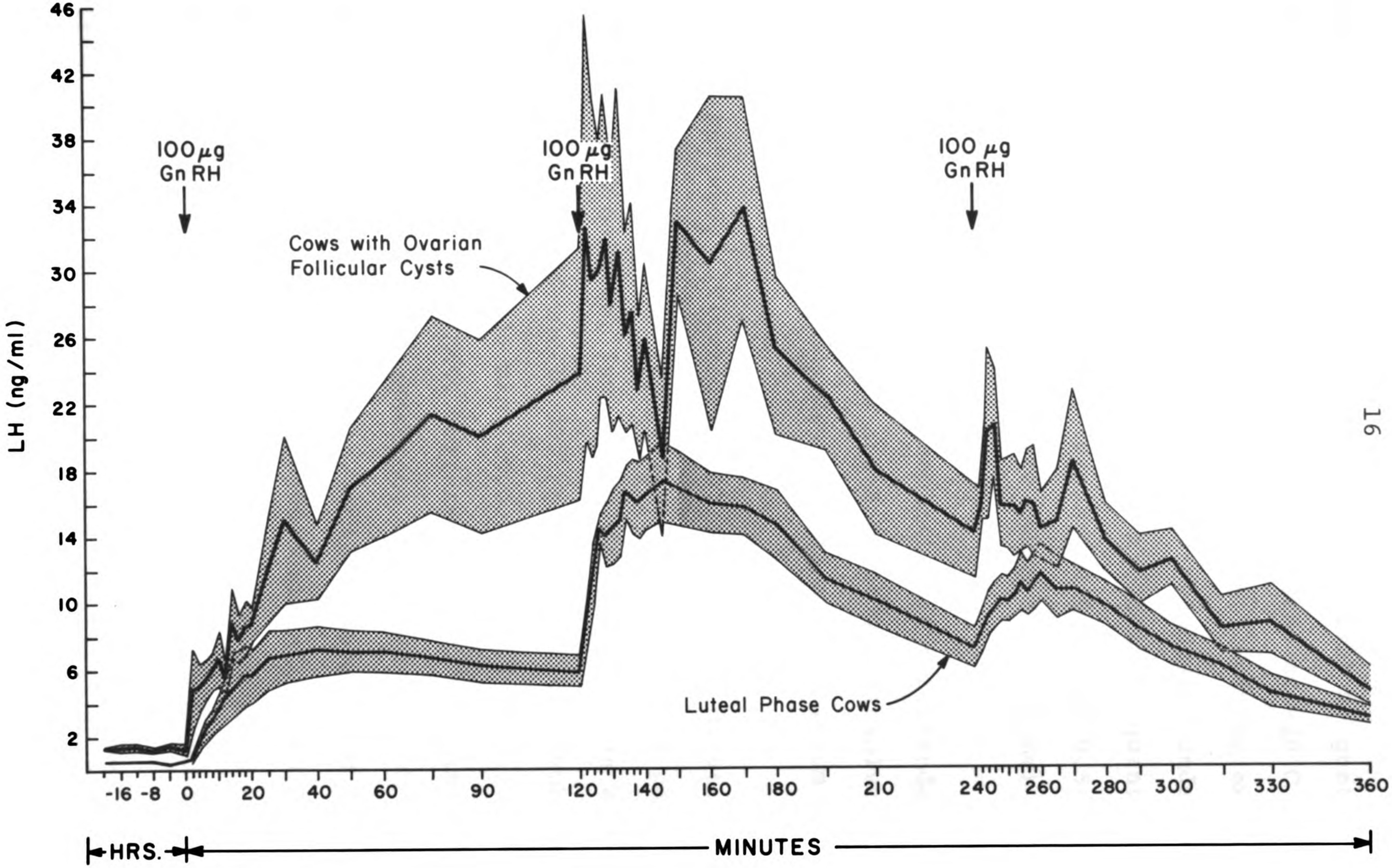

Figure 1.--Serum LH after GnRH in cows with ovarian follicular cysts or corpora lutea.

steroids to equilibrate for 30 min., serum was extracted with 10 ml of nanograde benzene:hexane (1:2) by gentle inversion for 20 min. Samples were stored at -20 C for at least 1 hr. to freeze the aqueous phase. Solvent extracts were decanted into conical tubes and evaporated under nitrogen to approximately 2.5 ml. A 0.5 ml aliquant was taken to measure procedural losses and 0.25 and 0.5 ml aliquants were placed in culture tubes (12 x 75 mm) for radioimmunoassay.

Progesterone (Sigma Chemical Co.) for standards (0, 0.1, 0.5, 1.0, 1.5, 2.0 and 5.0 ng) was dissolved in absolute ethanol. Standards and aliquants of serum extracts were dried under nitrogen.

Antibody[3] (Niswender, 1973) (0.2 ml), diluted 1:3,000 in 0.1 M phosphate buffered saline containing 0.1 percent gelatin (Knox Gelatin, Inc., Johnstown, N.Y.), was added to each tube, mixed for 10 sec. and allowed to incubate at room temperature for 30 min. Two hundred μl of 0.1 percent gelatin in 0.1 M phosphate buffered saline, containing 45,000 dpm of ^{3}H-1,2-progesterone was added to each tube. The tubes were mixed for 5 sec. and incubated at 4 C for 12 to 18 hr. One ml of 0.025 percent dextran 150 and 0.25 percent carbon decolorizing neutral norit in glass disstilled water was added to each tube to separate bound and free hormone.

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[3]Anti-progesterone, generously supplied by Dr. G.D. Niswender, Department of Physiology, Colorado State University, Fort Collins. The rabbit antiserum (#869) was prepared against 6β-succinyl progesterone conjugated to bovine serum albumin.

The contents were mixed, incubated for 10 min. in an ice bath and then centrifuged at 2,500 g for 10 min. at 5 C. A 0.5 ml aliquant of the supernatant was diluted with scintillation fluid (Bray, 1960) for quantification of radioactivity in a liquid scintillation spectrometer.

Significant differences in serum hormone concentrations were determined by t-test if two means had homogenous variances or approximate t-test if variances were heterogenous (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

Experiment 1

Since previous workers have demonstrated that during normal estrous cycles estrogen and glucocorticoids are high at estrus and lower thereafter and progesterone is low at estrus but higher thereafter (Swanson *et al.*, 1972; Wettemann *et al.*, 1972), estrogen and glucocorticoid data for days 0 or 2 to 7 and progesterone data for days 0 to 2 or 4 to 11 were pooled for statistical comparisons.

Serum estradiol was lower on days 2 to 7 of normal length estrous cycles (11 ± 2 pg/ml) in cows with follicular cysts than on respective days of both abnormal length cycles (16 ± 1 pg/ml; $P<0.025$; Table 1) or after HCG (15 ± 3 pg/ml; $P<0.10$) in these same animals. On day 0 cows with follicular cysts on day 0 of abnormal length cycles (16 ± 2 pg/ml). In addition, serum estradiol on day 7 in non-cystic herdmates (4 ± 1 pg/ml; $P<0.05$) was lower than in cows with ovarian follicular cysts on days 2 to 7 during normal length cycles, abnormal length cycles and following HCG. This estrogen data supports the work of Erb *et al.* (1971) in which no differences were observed in excretion of 17α-estradiol, 17β-estiadiol or estrone between day 0 to 2 and day 7 of the estrous sycle in cows with ovarian follicular cysts; however, it was also reported that excretion of these estrogens did not differ from normal cows on respective days.

During normal length estrous cycles in cows with follicular cysts, serum progesterone increased ($P<0.005$) from 0.1 ± 0.1 ng/ml on days 0 to 2 to 1.2 ± 0.3 ng/ml on days 4 to 11 (Table 1). Following HCG, serum progesterone in cows with follicular cysts increased ($P<0.05$) from 0.8 ± 0.4 ng/ml on days 0 to 2 to 2.8 ± 1.0 ng/ml on days 4 to 11. Serum progesterone was greater ($P<0.005$) from days 4 to 11 during normal length estrous cycles (1.2 ± 0.3 ng/ml) than abnormal length cycles (0.1 ± 0.1 ng/ml) in cows with follicular cysts. Others (Erb _et al_., 1971) have shown no increase in serum progesterone during the first 7 days following estrus in cows with ovarian cysts.

Equal replications of serum estrone observations were not available due to limited amounts of serum and unsuccessful attempts to assay the hormone.

Corticosterone, cortisol and estrone were not significantly affected by day of estrous cycle or administration of HCG (Table 1).

Experiment 2

At time 0, prior to GnRH administration, cows with ovarian follicular cysts had higher serum LH ($P<0.05$) than luteal phase cows (1.2 ± 0.3 vs 0.5 ± 0.1 ng/ml, respectively; Figure 1).

TABLE 1.--Serum steroids during estrous cycles and after HCG in cows with ovarian follicular cysts and during estrous cycles in non-cystic herdmates.

Hormone	Days of Estrous Cycle or After HCG	Cows with ovarian follicular cysts: Normal Length Cycle[b]	Cows with ovarian follicular cysts: Abnormal Length Cycle[b]	Cows with ovarian follicular cysts: After HCG	Non-Cystic Herdmates[a]
		(pg/ml)			
Estradiol	0	14±2 (5)[c]	16±2 (7)	13±4 (6)	9±3 (4)
Estradiol	2-7	11±2 (14)	16±1 (23)	15±3 (21)	4±1 (4)
Estrone	0	13 (2)	13±6 (4)	15±6 (5)	5±1 (4)
Estrone	2-7	10±4 (3)	14±2 (14)	16±4 (11)	9±3 (4)
		(ng/ml)			
Progesterone	0-2	0.1±0.1 (10)	0.1±0.1 (17)	0.8±0.4 (12)	0.4±0.4 (4)
Progesterone	4-11	1.2±0.3 (11)	0.1±0.1 (16)	2.8±1.0 (19)	5.7±3.4 (4)
Corticosterone	0	0.2±0.1 (5)	0.2±0.1 (8)	0.2±0.1 (7)	- - -
Corticosterone	2-7	0.2±0.1 (15)	0.2±0.1 (22)	0.2±0.1 (22)	- - -
Cortisol	0	3.2±0.2 (5)	8.1±3.4 (8)	3.6±1.3 (7)	- - -
Cortisol	2-7	3.7±0.9 (15)	4.8±1.0 (22)	4.7±0.7 (22)	- - -

[a] For non-cystic herdmates, samples were obtained on day 0 or 7 only.

[b] Normal length cycles, 18 to 23 days; abnormal length cycles, 3 to 15 days.

[c] Means ± S.E.; number of parenthesis is number of observations.

Average serum LH response after each dose of GnRH for cows with ovarian follicular cysts and cows during luteal phase of the estrous cycle are depicted in Figure 1. Pituitary LH release from cows with ovarian follicular cysts appeared more sporadic, especially following the second dose of GnRH (note standard errors in Figure 1). In contrast, luteal phase cows has a more uniform LH response. The change in serum LH concentration (ΔLH) after each dose of GnRH indicates that cows with cystic follicles were more responsive than cows during luteal phase of the estrous cycle (Table 2). Thus $^{\Delta}1$, $^{\Delta}2$, $^{\Delta}3$ and $^{\Delta}$greatest of the cystic cows were significantly greater ($P<0.05$) than respective values for luteal phase cows.

Time to peak serum LH response of individual doses of GnRH and time to peak of greatest LH response did not differ between cows with ovarian follicular cysts and cows treated during the luteal phase of the estrous cycle (Table 2).

Peak serum LH in cows with cystic follicles and luteal cows occurred after the second dose of GnRH. In anestrous ewes (Reeves _et al_., 1972), peak LH response after the first 200 μg intramuscular dose of LH-releasing hormone (LH-RH) was not different from the response of a second 200 μg dose administered 4 hr. later. However, 16 intramuscular injections of LH-RH (administered at 6 hr. intervals; 25 μg/injection) to prepubertal gilts (Chakraborty _et al_., 1972)

TABLE 2.--Serum LH changes after GnRH in cows with follicular cysts or corpora lutea.

	Δ_1	Δ_2	Δ_3	$\Delta_{Greatest}$
	(ng/ml)			
Cystic	27±7	24±6	11±3	46±11
Luteal	7±2	12±2	5±1	18±2
	Time to Peak[a] (min.)			
Cystic	81±15	148±8	256±6	148±8
Luteal	70±20	138±2	261±3	138±2

Δ1, 2, 3 --Change from immediate pre-injection level of LH to peak response after each injection of 100 µg of GnRH. Individual peak responses averaged to obtain ΔLH.

ΔGreatest--Change from time 0 to highest LH response, regardless of time 0 of injections.

[a]Time to peak of ΔLH figured from time 0 using individual animal peak responses. GnRH administered at time 0, 120 and 240 min.

produced LH responses from the first and second injections that were significantly greater than subsequent injections, but there was no difference in average pituitary LH content or concentration between treated and control gilts slaughtered 24 hr. later. The ability of the pituitary to release additional LH after three injections of GnRH may diminish, however, data obtained in this study after only three doses cannot be conclusive.

Serum progesterone concentration before and after GnRH in cows with ovarian follicular cysts is shown in Table 3. Average serum progesterone increased during the first 20 min. following GnRH administration, but this increase is attributable to two cows while the other three exhibited no change in serum progesterone. Cows in the luteal phase of the estrous cycle exhibited significant increases ($P<0.05$) at 260 and 360 min. compared to preinjection levels (Table 3).

Acute changes in serum progesterone reflect the luteotropic capacity of LH in the bovine (Carlson, Norimoto and Hansel, 1971). Individual differences in amount and activity of luteal tissue present in follicles of cows with ovarian follicular cysts may account for between animal variation in acute progesterone responses to GnRH. In luteal phase cows, presence of a functional corpus luteum, and thus more luteal tissue, would permit the increase in serum progesterone observed.

Although a palpable corpus luteum was detected in only one of five cows with ovarian follicular cysts 10 days after treatment, serum progesterone for 3 weeks following treatment (Figure 2) appear similar to that of the normal bovine estrous cycle (Wettemann _et al_., 1972). All of the cows diagnosed cystic exhibited standing estrus 20 to 24 days after treatment with GnRH. Serum progesterone concentration of luteal phase cows during the remaining days of the cycle and cycle lengths are shown in Table 4.

TABLE 3.--Serum progesterone after GnRH in normal luteal phase cows and cows with ovarian follicular cysts.

Time	Cows with follicular cysts	Luteal Cows
	(ng/ml)	
-24 hr.	0.6±0.3	5.9±0.7
-12 hr.	1.3±0.6	4.9±0.8
0	0.9±0.4	5.9±0.8
+20 min.	2.0±1.2	6.1±0.8
+60 min.	---	6.4±0.9
+120 min.	1.4±1.0	7.0±0.3
+140 min.	---	7.7±1.3
+240 min.	1.7±1.3	7.9±1.0
+260 min.	---	10.3±0.9
+360 min.	0.4±0.1	8.2±1.0

TABLE 4.--Serum progesterone in luteal phase cows after GnRH administration.

Day of Cycle	Cow number			
	847[b]	1037[a]	1048[a]	1118[c]
	(ng/ml)			
11	---	4.0	4.6	---
15	9.0	9.8	6.5	4.8
18	8.5	7.6	0.5	6.7
19	5.8	10.7	0.6	6.1
20	4.0	6.9	0.4	3.5
21	1.8	7.9		0.4
22	0.5	6.9		0.5
23	0.3	1.2		0.4
24	0.3			
25	0.3[d]			

[a]Treated on day 10 of the estrous cycle.

[b]Treated on day 11 of the estrous cycle.

[c]Treated on day 13 of the estrous cycle.

[d]Underlined value is on the day of estrus.

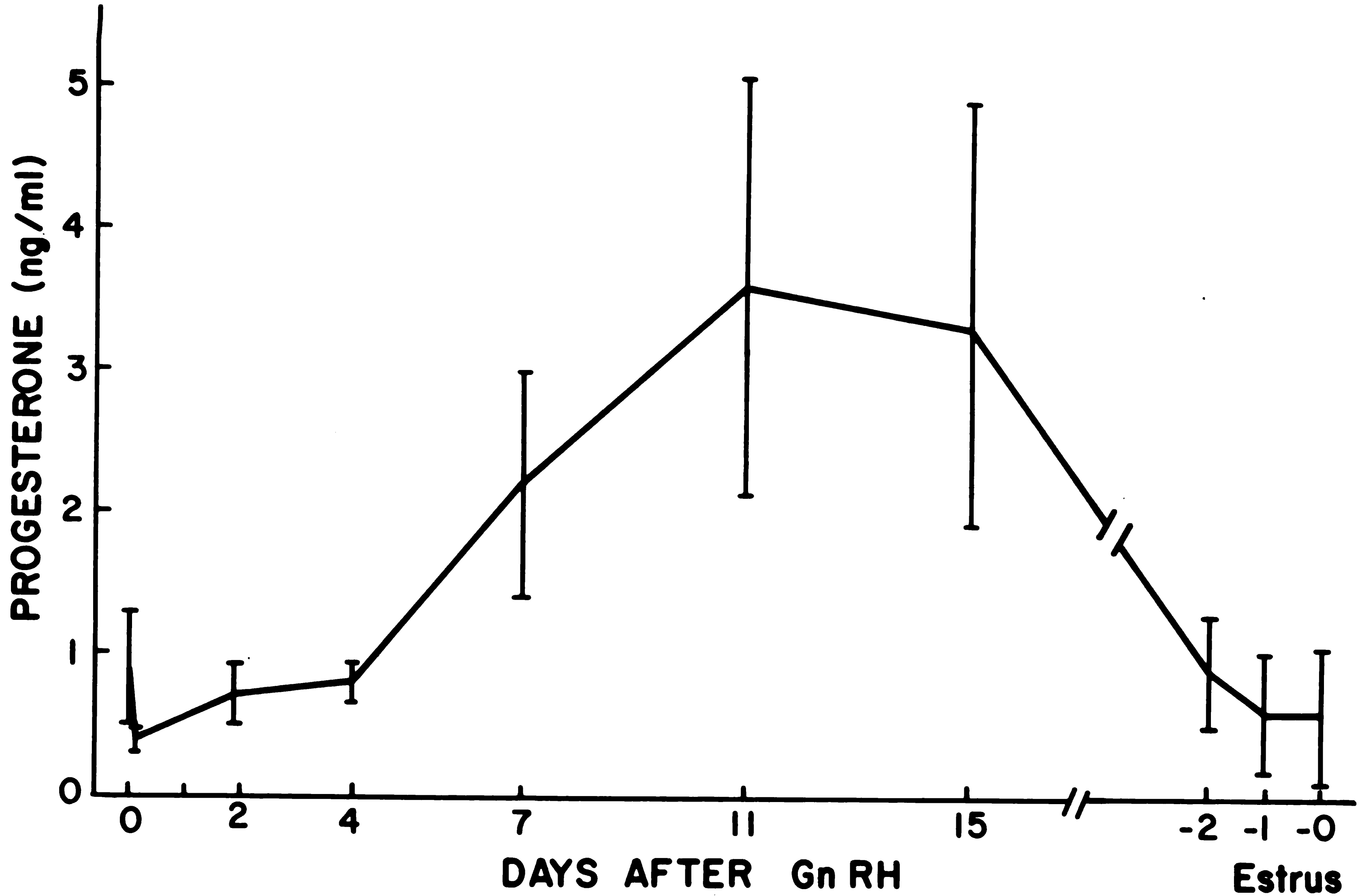

Figure 2.--Serum progesterone after GnRH in cows with ovarian follicular cysts.

Pretreatment serum estradiol and estrone concentration in cows with follicular cysts were 9.4 ± 3.1 and 3.8 ± 0.5 pg/ml, respectively, and did not differ ($P<0.05$) from that of luteal phase cows (8.8 ± 0.7 and 3.0 ± 0.4 pg/ml), respectively. Endogenous ovarian steroid changes, as affected by the estrous cycle (Reeves, Arimura and Schally, 1971a) and exogenous steroid changes, such as pretreatment with estrogen (Reeves _et al_., 1971b), can alter pituitary responsiveness to LH-releasing hormone in sheep. However, infused exogenous progesterone in anestrous ewes does not affect LH response to GnRH (Cumming _et al_., 1972). Differences observed in LH response to GnRH in this study may be due to differences in preinjection progesterone concentration or other differences in pituitary responsiveness associated with the cystic condition.

Serum estrogen concentrations in cows with ovarian follicular cysts in this study did not differ from concentrations in luteal phase cows. This is not in agreement with the results of the first study, in which serum estrogens were higher in cows with follicular cysts than in normal animals in luteal phase of the estrous cycle. Perhaps lack of agreement lies in differences of days postpartum (116 and 76 days in experiment 1 and 2, respectively) and thus possible differences in type of follicular cysts of the two groups of animals.

The interval from GnRH administration to subsequent estrus in cows with follicular cysts suggests that luteal tissue, presumably in the follicular cyst, assumed the role of a functional corpus luteum in the four cows in which a palpable corpus luteum was not detected. It may be assumed that increased serum progesterone levels were in response to the increase in acute serum LH after GnRH. Heifers subjected to 100 or 250 μg of GnRH intramuscularly after estrus synchronization with progesterone (Mauer and Ripple, 1972) exhibited a LH response sufficient to cause ovulation without behavioral estrus. Kaltenbach et al. (1974) observed similar results with 250 μg of GnRH intracarotidly after estrous synchronization. Zolman et al. (1973) failed to elicit a LH response of that magnitude from heifers on day 15 and 20 of the estrous cycle, when doses of 5, 40 and 320 μg of GnRH were administered intravenously in a single injection. Perhaps repeated or prolonged exposure to gonadotropin releasing hormone may be required to produce or mimic the preovulatory LH surge in the bovine. Repeated doses of GnRH initiate estrous cycles when administered to cattle with ovarian follicular cysts.

SUMMARY AND CONCLUSIONS

Blood was collected from six lactating Holstein cows on days 0 (estrus or day of HCG administration), 2, 4, 7 and 11 during estrous cycles before ovarian follicular cysts were detected, after administration of HCG and during post-treatment estrous cycles. Estrous cycles, before and after the cystic period, were classified according to length as normal (18 to 23 days) or abnormal (3 to 15 days). During normal length cycles, estradiol was lower on days 2 to 7 than on respective days during abnormal length cycles ($P<0.05$). Progesterone increased ($P<0.05$) from days 0 to 3 to days 4 to 11 following HCG. On days 4 to 11, progesterone was higher ($P<0.01$) during normal length cycles than on these same days during abnormal length cycles. No differences were observed in estrogens or glucocorticoids within normal and abnormal estrous cycles or after HCG treatment. These data suggest human chorionic gonadotropin increased serum progesterone but did not influence estrogens or glucocorticoids.

Gonadotropin releasing hormone was administered to five cows with ovarian follicular cysts and four cows during the luteal phase of the estrous cycle. Three doses of GnRH, 100 μg each, were given at 120 min. intervals. Luteinizing hormone peaked at 170 min. (33.9 ± 6.6 ng/ml) in cows with follicular cysts and 145 min. (17.3 ± 2.4 ng/ml) in cows during luteal phase of the cycle. Cows with follicular cysts had a larger ($P<0.01$) change in LH concentration after each administration of GnRH than luteal cows. Progesterone in luteal phase cows at 260 and 360 min. after GnRH was higher ($P<0.05$) than at 0 min. Only one cystic cow had a palpable corpus luteum on day 11 after treatment, but average serum progesterone was typical of the bovine estrous cycle. After GnRH treatment, all cows with follicular cysts exhibited estrus within 20 to 24 days. Serum estradiol and estrone prior to GnRH in cows with follicular cysts did not differ from pretreatment levels of luteal phase cows. Repeated doses of GnRH produced a LH response similar to the preovulatory surge in the bovine and estrous cycles were initiated in cows with ovarian follicular cysts.

Further study of the endocrine state of the dairy cow that is destined to have ovarian follicular cysts is required if hormone values and cause of the condition are to be related. High estrogen during days 0 to 7 of abnormal and normal length cycles of animals destined to have ovarian follicular cysts is an effect rather than cause. Serum LH should be evaluated because cows with follicular cysts have a higher LH baseline ($P<0.05$) compared with luteal phase cows, in experiment 2.

Perhaps the greater serum LH response to GnRH in cows with ovarian follicular cysts may be accounted for by greater pituitary sensitivity to releasing hormone or larger releasible stores of pituitary LH.

Pituitary sensitivity may be affected by high estrogens in cows with ovarian follicular cysts, although at time of treatment in experiment 2, estrogen did not differ from cows during the luteal phase of the cycle. This does not preclude the possibility of high estrogen prior to initiation of follicular cysts. Also low serum progesterone may increase pituitary sensitivity. Increased pituitary sensitivity would cause release of more LH in response to a given amount of endogenous releasing hormone and raise baseline levels of serum LH. Estrogen and progesterone may also be active at the hypothalamus and affect biosynthesis and release of gonadotropin releasing hormone.

Hypothalamic dysfuction could increase endogenous releasing hormone available to the pituitary. Increased and sporadic releases of endogenous GnRH may result in pituitary refractoriness and prevent sufficient stimulus to effect a subsequent serum LH surge required for ovulation.

It is the opinion of the author that the hypothalamus is at fault. In cows predisposed to have ovarian follicular cysts, a hypothalamic dysfunction results in erratic release of gonadotropin releasing hormone and subsequent pituitary LH release that is also erratic. High levels of serum LH affect follicular growth, ovulation and steroid biosynthesis. The steroids act back to potentiate the ovarian follicular cyst.

An alternative opinion would be that there is normal hypothalamic function and the pituitary is more sensitive to the releasing hormone; more pituitary LH is released. High serum LH would have the same effects as mentioned above.

Further study is warranted to gain more complete information concerning ovarian follicular cysts in cattle and resolve the etiology of the condition.

BIBLIOGRAPHY

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Albrechtsen, J. 1917. Sterility of Cattle and Methods of Treatment. Cornell Vet. 7:57.

Archbald, L.F., R.H. Schultz, M.L. Fahning, H.J. Kurtz and R. Zemjanis. 1973. Sequential morphologic study of the ovaries of heifers injected with exogenous gonadotropins. Am. J. Vet. Res. 34:21.

Bray, G. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1:279.

Callahan, C.J., R.E. Erb, A.H. Surve and R.D. Randel. 1971. Variables influencing ovarian cycles in postpartum dairy cows. J. Anim. Sci. 33:1053.

Carlson, J.C., K. Norimoto and W. Hansel. 1971. Effects of LH on peripheral progesterone concentrations in intact and hysterectomized heifers. Endocrinol. 89: 1530.

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

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

Chakraborty, P.K., J.J. Reeves, A. Arimura and A.V. Schally, 1973. Serum LH levels in prepubertal female pigs chronically treated with synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH). Endocrinol. 92:55.

Cumming, I.A., J.M. Buckmaster, J.C. Cerini, M.E. Cerini, W.A. Chamley, J.K. Findlay and J.R. Goding. 1972. Effect of progesterone on the release of luteinizing hormone induced by synthetic gonadotropin releasing factor in the ewe. Neuroendocrinol. 10:338.

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

Edgerton, L.A. and H.D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoids and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

Erb, R.E., E.L. Monk, C.J. Callahan and T.A. Mollett. 1973. Endocrinology of induced follicular cysts. J. Anim. Sci. 37:310. (Abstr.).

Erb, R.E., A.H. Surve, C.J. Callahan, R.D. Randel and H.A. Garverick. 1971. Reproductive steroids in the bovine. VII. Changes postpartum. J. Anim. Sci. 33:1060.

Foote, W.D., R.G. Zimbelman, R.G. Loy and L.E. Casida. 1959. Endocrine activity of corpora lutea from first-service and repeat-breeder dairy heifers. J. Dairy Sci. 42:1944.

Garm, O. 1949. Investigation of cystic ovarian degeneration in the cow, with special regard to etiology and pathogenesis. Cornell Vet. 39:39.

Garverick, H.A., W.E. Brady, E.C. Mather, C.J. Biershwal and R.E. Erb. 1973. Postpartum plasma hormone levels in dairy cows. J. Anim. Sci. 37:311. (Abstr.).

Golter, T.D., J.J. Reeves, C.C. O'Mary, A. Arimura and A.V. Schally. 1973. Serum LH levels in bulls treated with synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH). J. Anim. Sci. 37:123.

Henricson, B. 1957. Genetical and statistical investigations into so-called cystic ovaries in cattle. Acta Agr. Scand. 7:4.

Johnson, A.D., J.E. Legates and L.C. Ulberg. 1966. Relationship between follicular cysts and milk production. J. Dairy Sci. 49:865.

Johnson, A.D. and L.C. Ulberg. 1967. Influence of exogenous progesterone on follicular cysts in dairy cattle. J. Dairy Sci. 50:758.

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

Kaltenbach, C.C., T.G. Dunn, T.E. Kiser, L.R. Corah, A.M. Akbar and G.D. Niswender. 1974. Release of FSH and LH in beef heifers by synthetic gonadotropin releasing hormone. J. Anim. Sci. 38:357.

Marion, G.B. and H.T. Gier. 1968. Factors affecting bovine ovarian activity after parturition. J. Anim. Sci. 27:1621.

Matsuo. H., A. Arimura, R.M.G. Nair and A.V. Schally. 1971. Synthesis of the LH and FSH-releasing hormone by the solid phase method. Biochem. Biophys. Res. Comm. 45:822.

Mauer, R.E. and R.H. Rippel. 1972. Response of cattle to synthetic gonadotropin releasing hormone. J. Anim. Sci. 35:249. (Abstr.).

Mongkonpunya, K., H.D. Hafs, W.D. Oxender and E.M. Convey. 1973. LH and testosterone after GnRH in pubertal bulls. J. Anim. Sci. 37:321. (Abstr.).

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

Nakama, S. 1969. Enzyne histochemistry of the bovine ovaries in normal and cystic conditions. Acta Histochem. 32:157.

Niswender, G.D. 1973. Influence of the site of conjugation on the specificity of antibodies to progesterone. Steroids 22:413.

Oxender, W.D., H.D. Hafs and L.A. Engerton. 1972. Serum growth hormone, LH and prolactin in the pregnant cow. J. Anim. Sci. 35:51.

Peck, C.C., J.R. Reel, W.C. Dermody and R.W. Coppock. 1973. Release of luteinizing hormone (LH) in the heifer during late luteal phase by intravenous and intrauterine synthetic luteinizing hormone releasing factor (LRF). J. Anim. Sci. 37:300 (Abstr.).

Reeves, J.J., A. Arimura and A.V. Schally. 1971a. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32:123.

Reeves, J.J., A. Arimura and A.V. Schally. 1971b. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pre-treated with estradiol benzoate. Biol. Reprod. 4:88.

Reeves, J.J., A. Arimura, A.V. Schally, C.L. Kragt, T.W. Beck and J.M. Casey. 1972. Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anestrous ewes. J. Anim. Sci. 35:84.

Roberts, S.J. 1955. Clinical observations on cystic ovaries in dairy cattle. Cornell Vet. 45:497.

Roberts, S.J. 1957. A preliminary report on the treatment of cystic ovaries in dairy cattle by the injection of gonadotropic hormones directly into the follicular cyst. J. Amer. Vet. Med. Assoc. 131:510.

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

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Company, San Francisco.

Swanson, L.V., H.D. Hafs and D.A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoid from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 43:284.

Trainin, D. and J.H. Adler. 1962. The treatment of ovarian cysts in cattle by the intravenous injection of gonadotropin and progesterone. Refuah. Vet. 19:109.

Troconiz, J.F., W.W. Thatcher, P.S. Kalra and C.J. Wilcox. 1973. Adrenal effects of hormonal status of cystic cows. J. Anim. Sci. 37:331 (Abstr.).

Wettemann, R.P., H.D. Hafs, L.A. Edgerton and L.V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020.

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