Secretory patterns of LH and FSH during development and hypothalamic and hypophysial characteristics following development of steroid-induced ovarian follicular cysts in dairy cattle*

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Summary. Two experiments were conducted to (1) investigate developmental endocrinology of ovarian follicular cysts (cysts) in cattle and (2) evaluate effects of cysts on hypothalamic and hypophysial characteristics. Cysts were induced with oestradiol-17 β (15 mg) and progesterone (37.5 mg) dissolved in alcohol and injected s.c. twice daily for 7 days. Cysts were defined as the presence of follicular structures (which may or may not have been the same structure) of 2.0 cm in diameter or greater that were present for 10 days without ovulation and corpus luteum development.

In Exp. 1, 22 non-lactating, non-pregnant Holstein cows were allocated to 3 groups. Beginning on Day 5 (oestrus = Day 0) of the oestrous cycle, 7 cows (Controls) were treated with twice daily s.c. injections of ethanol (2 ml/injection) for 7 days. Luteolysis was then induced with PGF-2 α and blood samples were collected daily every 15 min for 6 h from the morning after the PGF-2 α injection (Day 13) until oestrus. Steroids to induce cysts were injected as previously described into the remaining cows (N = 15). Three blood samples were collected at 15-min intervals every 12 h throughout the experimental period. Additional blood samples were collected every 15 min for 6 h on a twice weekly basis. After steroid injections, follicular and luteal structures on ovaries were not detected via rectal palpation for a period of 36 ± 4 days (static phase). Then follicles developed which ovulated within 3–7 days (non-cystic; N = 7) or increased in size with follicular structures present for 10 days (cystic; N = 8). Mean ($\pm$ s.e.m.) concentrations of LH, FSH, oestradiol-17 β and progesterone in serum remained low and were not different during the static phase between cows that subsequently developed cysts or ovulated. During the follicular phase, mean serum concentration of LH (ng/ml) was higher ($P < 0.1$) in cows with cysts (2.9 ± 0.2) than in cows without cysts (1.1 ± 0.1) or control cows (1.4 ± 0.2). In addition, LH pulse frequency (pulses/6 h) and amplitude (ng/ml) were higher ($P < 0.1$) in cows with cysts (3.6 ± 0.3 and 2.2 ± 0.3 , respectively) than in non-cystic (2.3 ± 0.2 and 1.0 ± 0.2 , respectively) and control (1.8 ± 0.1 and 1.1 ± 0.2 , respectively) groups during the follicular phase. There were no differences in the FSH, oestradiol-17 β or progesterone characteristics in cows of any of the 3 groups during the follicular phase.

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In Exp. 2, 20 non-lactating, non-pregnant dairy cows were used: 15 cows received exogenous steroids as previously described. Hypothalamic and hypophyseal tissues were collected after diagnosis of cystic structures in 11 cows (cystic group). The remaining 4 cows in the steroid-treated group ovulated and were assigned to the control group in addition to 5 non-steroid treated cows. Hypothalamic and hypophyseal tissues were collected during the late-luteal phase (Days 16–18) from these control cows ($N = 9$). Anterior pituitary concentrations ($\mu\text{g/g}$) of LH (60.5 ± 11.0 , 44.6 ± 11.7), FSH (30.2 ± 4.0 , 22.1 ± 4.6) and receptors for GnRH (17.2 ± 2.2 , $23.4 \pm 2.6 \text{ M} \times 10^{-10}/\text{mg protein}$) did not differ between cows with cysts and control cows, respectively. Content of GnRH (ng) in the combined preoptic area and hypothalamus proper was higher ($P < 0.05$) in control cows (37.7 ± 6.6) than cows with cysts (18.6 ± 6.1). In the pituitary stalk median eminence, GnRH content (ng) tended to be higher ($P \geq 0.1$) in cows with cysts (38.5 ± 9.6) compared with control (21.1 ± 15.2) cows.

Secretory patterns (mean concentration, pulse frequency and amplitude) of LH were therefore increased during the follicular phase in cows which developed cysts compared to cows which subsequently ovulated. In addition, hypothalamic GnRH content, but not pituitary characteristics, appeared to be altered in cows with cysts.

Keywords: ovary; follicular cysts; dairy cattle; gonadotrophins; hypothalamus; pituitary

Introduction

Ovarian follicular cysts (cysts) are anovulatory follicular structures which usually result in an extended calving interval in dairy cattle. It has been proposed that cysts develop as a result of an endocrine imbalance involving the hypothalamic–hypophyseal–gonadal axis (for reviews, see Kesler & Garverick, 1982; Eyestone & Ax, 1984). Several specific factors such as excess follicle-stimulating hormone (FSH) and inadequate luteinizing hormone (LH) (Erb *et al.*, 1971) have been suggested as factors associated with cyst development. The pituitary gland of cows with cysts is able to respond to gonadotrophin-releasing hormone (GnRH) by releasing LH (Cantley *et al.*, 1975; Seguin *et al.*, 1976). However, the feedback regulation of the hypothalamic–hypophyseal axis to oestradiol- 17β may be altered in cystic cows. Zaied *et al.* (1981) reported that the oestradiol- 17β -induced preovulatory-like release of LH is delayed in cows with cysts compared to cows *post partum* without cysts.

Secretion of LH has been associated with GnRH secretion. The response to GnRH is also modulated by steroids (for review see, Clarke, 1987). The frequency and amplitude of GnRH has been correlated with the pattern of LH released (Levine *et al.*, 1982; Clarke & Cummins, 1982). Brown *et al.* (1986) found no difference in concentration of serum LH, pituitary LH or content of GnRH receptors in the pituitary of cows with cysts compared with cows during the follicular phase of the oestrous cycle. These observations were made for cows which did not respond to exogenous hormone treatment and had cysts for extended periods of time.

Most studies investigating the onset of cysts are limited to observations of endocrine measurements that occur after the condition has been diagnosed (Eyestone & Ax, 1984). The unpredictability of the onset of cysts has made investigation of their aetiology difficult to study. Although many factors have been associated with increased occurrence of cysts, endocrine mechanisms associated with development of cysts remain obscure (Kesler & Garverick, 1982).

Injections of oestradiol- 17β and progesterone have been used to induce cysts in cattle (Erb *et al.*, 1973; Winters *et al.*, 1986; Cook *et al.*, 1990). Clinical, endocrine and histological characteristics and development of steroid-induced cysts (Cook *et al.*, 1990) are similar to those previously observed in spontaneously occurring cysts (Kesler *et al.*, 1981; Brown *et al.*, 1982; Hernandez-Ledezma *et al.*, 1982). The level of steroid treatment is based upon the amount calculated to raise

concentrations of oestradiol-17 β and progesterone in serum similar to those observed near the end of pregnancy. Since this treatment results in a predictable onset of cysts (about 30 days after treatment), it provides a model to investigate the aetiology of cyst development in cattle. The objectives of the present study were to determine (1) secretory patterns of gonadotrophins associated with the development of cysts in dairy cattle and (2) the effect of cysts on hypothalamic and hypophyseal characteristics.

Materials and Methods

General

Non-lactating, non-pregnant Holstein cows at least 200 days after parturition that had previously displayed normal oestrous cycles were used for both studies. Cows weighed between 500 and 700 kg. Cows were kept in a dirt paddock and were fed alfalfa hay and supplemental grain to meet maintenance requirements for non-lactating cows. Oestrus was synchronized in all cows by using two injections (i.m.) of prostaglandin (PG) F-2 α (PGF-2 α ; Lutalyse, Upjohn Co., Kalamazoo, MI, USA) given 11 days apart. Before the second injection of PGF-2 α , cows were palpated *per rectum* to estimate ovarian activity, and blood samples were collected for progesterone analysis to confirm the presence of a functional corpus luteum (CL). Most of the cows were used in only one of the experiments. Exogenous steroids to induce cysts were injected at three different times since the number of cows, facilities and personnel were not sufficient to begin all treatments at one time. Observations from control cows were taken during the period when some cows were being treated with steroids.

Experiment 1

Experimental procedures. The 22 cows were allocated to 3 treatment groups. The 7 control cows received twice daily injections (s.c.) of ethanol (2 ml/injection) for 7 days, beginning on Day 5 of the oestrous cycle (Day 0 = day of oestrus). On the afternoon of Day 12, control cows were given a third injection of PGF-2 α and observed 3 times daily for oestrus. Only cows which stood when mounted by other cows were considered in oestrus. Blood samples were collected by jugular venepuncture every 15 min for 6 h daily beginning on the morning after the third PGF-2 α injection until oestrus. The experimental period for these cows was from the first injection of ethanol to detection of a CL via palpation *per rectum* and an increase in concentration of progesterone in serum following subsequent oestrus.

The remaining cows (N = 15) received twice daily injections (s.c.) of 15 mg oestradiol-17 β and 37.5 mg progesterone, dissolved in ethanol, for 7 days beginning on Day 15 after the second PGF-2 α injection (Cook *et al.*, 1990). These cows were palpated *per rectum* 3 times weekly until follicular/cyst development to estimate ovarian activity. Blood samples were collected daily by jugular venepuncture during the 7 days of steroid injections. Beginning the morning after the last steroid injection, 3 blood samples were collected at 15-min intervals every 12 h until ovulation or development of a cyst occurred. Additional blood samples were collected via indwelling jugular cannulae every 15-min for 6 h twice weekly during this period to determine secretory patterns of gonadotrophins. After collection, blood samples were placed on ice and stored for 12 h at 4°C. Serum was harvested by centrifugation of blood at 1500 g for 30 min and stored at -20°C until concentrations of reproductive hormones were measured.

Cysts, as detected by ovarian palpation *per rectum*, were defined as the development and presence of follicular structure(s) (which may or may not have been the same structure) of 2.0 cm or greater in diameter that were present for 10 days without occurrence of ovulation and CL development (Cook *et al.*, 1990). Ovulation was estimated to occur when a CL was detected following oestrus which was accompanied by a subsequent rise in serum concentration of progesterone. Of the 15-steroid-treated cows, 8 developed cysts (cystic) while the other 7 developed follicular structures which ovulated (non-cystic). None of the cows which developed cysts exhibited oestrus. All cows that ovulated exhibited oestrous behaviour.

All cows (with and without cysts) treated with exogenous steroids underwent a period during which follicular and luteal structures (static phase) were not detectable by rectal palpation of ovaries. After this phase, a period of follicular growth occurred in all cows (follicular phase) which culminated in ovulation (non-cystic) or development of cysts (cystic). The follicular phase of non-cystic cows was defined as the period when a follicle was detected on the ovary, but before oestrus and ovulation. In cows with cystic follicles, the follicular phase was the period when follicles were first detected until structures attained a size of 2.0 cm or larger in diameter. Cows not receiving exogenous steroids (controls) did not undergo a static phase. The follicular phase in the control cows was limited to blood samples taken from 12 h after the third PGF-2 α injection to oestrus.

Endocrine procedures. Concentrations of LH and FSH in serum were measured by radioimmunoassay (RIA) as described by Zaid *et al.* (1980) and Garverick *et al.* (1988), respectively. The intra- and inter-assay coefficients of variation (CV) were 11.7% and 7.9%, respectively, for LH and 9.5% and 7.9%, respectively, for FSH. Concentration of progesterone was determined by RIA, as described by Cantley *et al.* (1975). The intra- and inter-assay CVs for progesterone were 22.4% and 15.7%, respectively. Serum concentration of oestradiol-17 β was determined as

described by Cook *et al.* (1990). The intra- and inter-assay CVs for oestradiol-17 β were 2.9% and 5.6%, respectively. Sensitivities of the assays were 0.1 ng LH/tube, 2.0 ng FSH/tube, 0.05 ng progesterone/tube and 1.2 pg oestradiol-17 β /tube.

Data analyses. Daily mean concentrations of LH, FSH, oestradiol-17 β and progesterone were determined in blood samples collected during the duration of the experiment. Daily concentrations of LH and FSH in serum were calculated from the average of the 6 daily samples (06:00 and 18:00 h) except on days of intensive sampling. The first 3 serum samples from the twice weekly intensive sampling period were averaged to obtain a mean concentration for each cow. Daily concentrations of oestradiol-17 β and progesterone were determined from pools of all samples collected on that day. Pools were made from equal volumes of samples collected. Secretory patterns (frequency, amplitude and duration of pulses) of LH and FSH were evaluated in serum of samples collected on the twice weekly intensive sampling days.

Hormone pulse frequency (pulses/6 h), amplitude (ng/pulse) and duration (min/pulse) for individual cows were calculated by computer algorithms for each sampling period. The following criteria were used to define a hormone pulse: (1) an increase above the preceding nadir greater than or equal to one standard deviation of the mean concentration of hormone for each sampling period, (2) the ascending side of the peak could not contain more than one point between the nadir and peak value; and (3) the peak must be accompanied by at least two other consecutive decreasing hormone concentrations. Pulse frequency for individual cows was calculated as the sum of all pulses detected during each sampling period. Square root transformation was used before statistical analysis to achieve homogeneity of variance for number of hormone pulses. Amplitude of hormone pulses was the height from the preceding nadir to maximum height of all pulses detected in each sampling period. Duration of hormone pulses was calculated as the average interval for all peaks detected during each 6-h period. A preovulatory surge of LH was presumed to have occurred if there was a consecutive increase or decrease in concentration of LH observed in the 3 samples collected twice daily at 15-min intervals with the highest concentration exceeding 10 ng/ml.

Data were analysed using Statistical Analysis System (SAS, 1985). The endocrine data were analysed using a factorial split-plot in time analysis of variance for repeated measures (Gill & Hafs, 1971). Two separate analyses were done. In the first analysis, endocrine parameters of cystic and non-cystic groups during the static and follicular phases were compared. In the second analysis, endocrine parameters of the control, cystic and non-cystic groups were compared during the follicular phase. Treatment groups, phase, day and cows were the main effects. The effect of cow within treatment was used as the error term for testing the effect of treatment.

Experiment 2

Experimental procedures. Starting between Days 16 and 18 of the oestrous cycle, 15 cows received s.c. injections of oestradiol-17 β (15 mg) and progesterone (37.5 mg) as previously described to induce the formation of cysts (see above). After exogenous steroid treatment, cows were palpated *per rectum* 3 times weekly until diagnosis of cysts or spontaneous ovulation. Four cows in the steroid-treated group spontaneously ovulated 35–50 days following steroid treatment and were grouped with 5 additional cows which were exhibiting normal oestrous cycles to serve as the control group.

Between 35 and 50 days after steroid treatment, hypothalamic and hypophysial tissues were collected within 30 min after slaughter from cows with cystic follicles. Hypothalamic and hypophysial tissues were collected during the late-luteal phase (Days 16–18) from all control cows within 30 min following slaughter. The hypothalamic region was divided into the hypothalamus proper, preoptic-suprachiasmatic area (POA) and pituitary stalk–median eminence (SME). The hypothalamus consisted of a block of tissue limited rostrally by the optic chiasma, caudally by the mamillary bodies, laterally 3 mm from the midline and dorsally 4 mm from the base of the hypothalamus. The POA consisted of a block of tissue limited rostrally 4 mm anterior to the optic chiasma and caudally by the rostral borders of the hypothalamus. The lateral and dorsal borders extended rostrally from the corresponding borders of the hypothalamus. For collection of the SME, the pituitary gland was separated from the SME by a dorsal cut in the diaphragma sellae. The median eminence was severed from the hypothalamus, leaving it attached to the pituitary stalk.

Tissue processing and endocrine parameters. The hypothalamic regions were weighed and homogenized in methanol containing 0.1 N-HCl (1:1, v/v) to extract the neuropeptides. Particulate matter was removed by centrifugation at 1800 g for 30 min. Supernatants were evaporated under a stream of nitrogen gas and the residue was reconstituted in 1 g of tissue/ml of PBS containing 0.1% gelatin. GnRH in extracts was measured in duplicate (Nett & Adams, 1977), except that the present procedure used anti-LHRH CRR1B73 as described by Gale *et al.* (1988). Intra- and inter-assay CVs for the GnRH assay were 7.0% and 16.2%, respectively. Sensitivity of the assay was 0.5 pg/tube.

Pituitary glands were excised and the neural–intermediate lobes were immediately trimmed from the pituitary. The anterior pituitary was weighed and stored frozen until analysed for content of LH (Zaied *et al.*, 1981), FSH (Garverick *et al.*, 1988) and number of receptors for GnRH (Moss *et al.*, 1985). To measure number of receptors for GnRH, pituitaries were homogenized and membranes were isolated by centrifugation as described by Moss *et al.* (1985). The supernatant was stored frozen until determination of hormone content. To determine the numbers of GnRH receptors, membrane fragments were incubated for 6 h at 4°C with increasing concentrations of [¹²⁵I]-labelled D-Ala⁶ (monoiodo-des-Gly¹⁰, [D-Ala⁶]-LH-RH ethylamide). [¹²⁵I] was purchased from Amersham Corporation, Arlington Heights, IL, USA; and D-Ala⁶ (monoiodo-des-Gly¹⁰, [D-Ala⁶]-LH-RH ethylamide) from Sigma Chemical

Company, St Louis, MO, USA. At the end of the incubation, the membranes were centrifuged, and the amount of radioactivity remaining in the pellet was quantified. Numbers of receptors for GnRH in each pituitary were determined by methods described by Moss *et al.* (1985).

Data analyses. Data were analysed as a one way analysis of variance design (SAS, 1985). Treatment was the main effect included in the model (Steel & Torrie 1960).

Results

Experiment 1

General. Mean ($\pm$ s.e.m.) serum concentration of oestradiol-17 β on Day 7 of steroid injections was 198.1 ± 4.7 pg/ml, but had declined ($P < 0.05$) to 6.7 ± 0.3 pg/ml by 5 days after the last injection. Similarly, serum concentration of progesterone was elevated by Day 7 of steroid injections (6.7 ± 0.3 ng/ml) and declined ($P < 0.05$) to 0.4 ± 0.1 ng/ml by Day 5 after the last injection.

Following the last steroid injection, cows did not exhibit normal oestrous behaviour, and there was an absence of follicular and luteal structures for a period lasting 29–41 days (static phase; 36 ± 4 days). Low concentration (< 1.0 ng/ml) of progesterone in serum was indicative of the absence of luteal tissue. Moreover, preovulatory surges of LH were not detected during the static phase in any cows based upon the twice daily sampling regimen.

In steroid-treated cows, follicular growth (follicular phase) was detected in all cows after the static phase. Seven cows exhibited oestrous behaviour within 3–7 days after the static phase, and a preovulatory surge of LH was detected in 6 of 7 cows (non-cystic). In these cows, serum concentration of progesterone increased to > 1 ng/ml over the next 10 days and CL were detected by rectal palpation. In contrast, 8 cows did not exhibit oestrous behaviour, and ovarian follicular structures developed in the absence of a CL. Concentration of progesterone did not exceed 1 ng/ml, and a preovulatory surge of LH was not detected in any of the 8 cows (cystic).

Endocrine parameters. Mean concentrations and secretory pulse patterns (frequency, amplitude and duration) of LH and FSH in serum were not different during the static phase between cows that subsequently developed cysts and cows that ovulated (Fig. 1). As cows entered the follicular phase, daily mean concentration, pulse frequency and pulse amplitude of LH were higher ($P < 0.01$) than during the static phase (Figs 1 & 2). Differences in LH values between the static and follicular phase were largely due to changes observed in cows that developed cysts. However, differences were not observed for any FSH parameters measured.

During the follicular phase, the cows that developed cysts exhibited higher ($P < 0.01$) LH pulse frequency (3.6 ± 0.2 pulses/6 h) and amplitude (2.2 ± 0.2 ng/ml), than did cows not developing cysts (2.3 ± 0.2 pulses/6 h and 1.0 ± 0.2 ng/ml, respectively) and control cows (1.8 ± 0.1 pulses/6 h and 1.1 ± 0.2 ng/ml, respectively; Fig. 1). The higher ($P < 0.01$) LH pulse frequency and amplitude observed in cows with cysts resulted in a higher ($P < 0.01$) mean concentration of LH (2.9 ± 0.3 ng/ml) than in cows without cysts (1.1 ± 0.1 ng/ml) or control cows (1.4 ± 0.2 ng/ml) during the follicular phase (Fig. 1). Duration of LH pulses was longer ($P < 0.01$) for control cows (81.7 ± 3.5 min) compared with cows without cysts (65.7 ± 3.2 min) and cows with cysts (69.3 ± 1.5 min; Fig. 1). LH concentration increased over time in a linear fashion ($P < 0.01$) during the follicular phase in the cystic and non-cystic groups, but the increase in LH over time was greater for the cystic group (Fig. 2).

Mean daily concentration of FSH and pulse frequency, amplitude and duration of FSH were not different ($P = 0.21$; Fig. 1) among groups. During the follicular phase, control cows exhibited a similar daily concentration of FSH (8.2 ± 1.1 ng/ml) compared with non-cystic (8.7 ± 0.6 ng/ml) and cystic (7.0 ± 0.4) groups. Frequency (pulses/6 h), amplitude (ng/ml) and duration (min/pulse) of FSH pulses were similar for non-cystic (2.2 ± 0.2 , 5.1 ± 0.6 and 74.3 ± 1.5 , respectively), cystic (2.3 ± 0.1 , 2.9 ± 1.1 and 85.8 ± 3.3 , respectively) and control (2.5 ± 0.3 , 6.6 ± 2.3 and 70.5 ± 4.3 , respectively) groups during the follicular phase.

Fig. 1. Mean LH and FSH pulse parameters for cows with cystic or non-cystic follicles during the static and follicular phases. ^{A,B} Means with different superscripts differ ($P < 0.01$) between phases (static and follicular). ^{a,b} Means with different superscripts differ ($P < 0.01$) between groups within phases (cystic, non-cystic, and control).

Fig. 2. Mean daily LH concentrations in cows with cystic and non-cystic follicles after steroid treatment (static phase) and before ovulation or cyst development (mostly during the follicular phase). Cows were in the static phase during the first 24 days after steroid injections. The follicular phase was usually initiated 7–10 days before ovulation or development of a cyst.

Although fluctuations in serum concentration of progesterone were observed during the static and follicular phases there were no differences in concentration of progesterone (ng/ml) between the static and follicular phases or among cystic (0.54 ± 0.1), non-cystic (0.54 ± 0.1) or control (0.63 ± 0.1) groups during the follicular phase.

Daily fluctuations in oestradiol-17 β were also observed, but mean concentrations were not different during the static and follicular phases (6.7 ± 0.7 and 8.4 ± 0.9 pg/ml). Mean concentration (pg/ml) of oestradiol-17 β was also similar between the cystic (8.1 ± 0.9), non-cystic (7.7 ± 0.8) and control (9.1 ± 1.1) groups during the follicular phase.

Experiment 2

General. Of the 15 cows treated with exogenous steroids, 11 developed cysts which were present at slaughter. The remaining 4 cows spontaneously ovulated after steroid treatment and resumed normal oestrous cycles. Data collected from tissues of these 4 cows and from tissues collected from 5 additional cows during the luteal phase (Days 16–18) were combined as controls ($N = 9$).

Endocrine parameters. Combined weights of the hypothalamus, POA and SME collected at slaughter did not differ ($P > 0.05$) between treatment groups (Table 1). Content of GnRH in the hypothalamus + POA was higher ($P < 0.05$) in cows without cysts than cows with cysts. The percentage of GnRH in the hypothalamus + POA was higher ($P < 0.05$) in cows without cysts ($76 \pm 10\%$) than in cows with cysts ($44 \pm 9\%$). Considerable variation in the content of GnRH in the pituitary SME existed among cows, and content of GnRH tended (not significant) to be higher in the cystic group than in the non-cystic group. Total content of GnRH in the hypothalamic region (hypothalamus + POA + SME) was similar between treatment groups. Weight of the anterior pituitaries, concentrations of LH, FSH and receptors for GnRH in anterior pituitaries were not different between groups (Table 2).

Table 1. Least square means ($\pm$ s.e.m.) of content of GnRH and weight of hypothalamic tissues collected from cows ($N=11$) with exogenous steroid-induced ovarian follicular cysts and from cows ($N=9$) during the luteal phase of the oestrous cycle

	Control cows	Cows with cystic follicles
GnRH content (ng)		
Combined hypothalamus + POA	37.7 ± 6.6	$18.6 \pm 6.1^*$
Pituitary SME	21.1 ± 15.2	38.5 ± 9.6
Combined hypothalamus + POA + SME	47.0 ± 10.5	53.6 ± 9.5
Total weight (g) of hypothalamus + POA + SME	2.8 ± 0.2	2.6 ± 0.2

* $P < 0.05$ compared with value for control cows.

Table 2. Least square means ($\pm$ s.e.m.) of anterior pituitary characteristics in tissues collected from cows ($N=11$) with exogenous steroid-induced ovarian follicular cysts and from cows ($N=9$) during the luteal phase of the oestrous cycle

	Control cows	Cows with cystic follicles
LH ($\mu\text{g/g}$)	44.6 ± 11.7	60.6 ± 11.0
FSH ($\mu\text{g/g}$)	22.1 ± 4.6	30.2 ± 4.0
Receptors ($\text{M} \times 10^{-10}/\text{mg protein}$)	23.4 ± 2.6	17.2 ± 2.2
Weight (g)	2.3 ± 0.2	2.5 ± 0.2

Discussion

Several investigators have proposed that an endocrine imbalance is associated with the development of cysts in cattle. As recently reviewed there may not be a clearly definable cause of all cysts. It was

suggested that the origin may be at the hypothalamus, pituitary, ovary and(or) adrenal (Kesler & Garverick, 1982). Previous studies have examined some aspects of putative aberrations associated with cysts in cows, but most observations have been made following diagnosis of the cyst after its development (for reviews, see Kesler & Garverick, 1982; Eyestone & Ax, 1984). Experimental induction of cysts in this and other (Erb *et al.*, 1973; Winters *et al.*, 1986; Cook *et al.*, 1990) studies allows for a prediction of the time of onset of cyst development in cattle, and therefore permits study of endocrine changes that occur during follicular/cyst development.

One mechanism previously thought to be associated with development of cysts in dairy cattle was overstimulation of follicular growth by excessive stimulation by FSH (Erb *et al.*, 1971). However, mean concentration, pulse frequency, amplitude and duration of FSH in serum were not different in cows developing cysts compared with cows that ovulated. Pulsatile patterns of FSH did not reflect those of LH. In addition, there was no difference in pituitary content of FSH in cows with cysts that had been present for at least 10 days. Similar observations have been reported by Brown *et al.* (1986) for chronically long-term cows with cysts. Aberrant secretion of FSH may therefore not be associated with cyst development.

Aberrant secretory patterns of LH might be associated with development of cysts in cattle. Increased pulsatile secretion of LH and FSH increases follicular oestradiol production that eventually stimulates the preovulatory surge of LH (Murdoch, 1985). In rats, FSH and oestradiol increase follicular responsiveness to LH (Richards, 1980), and there is an increased number of unoccupied LH receptors in preovulatory follicles before the LH surge (Ireland, 1987). Subnormal peripheral concentrations of LH or decreased responsiveness of LH from the pituitary at the appropriate time have been related to development of cysts in dairy cattle (Erb *et al.*, 1971; Kesler *et al.*, 1979). However, in the present study, an increase in mean concentration, pulse frequency and amplitude of LH in serum was observed in cows which developed cysts.

High blood concentrations of LH act on the preovulatory follicle to inhibit the production of oestradiol-17 β by the granulosa to initiate premature luteinization of granulosa (Moor, 1974). The decline in oestradiol production reflects a progressive loss of steroidogenic capacity by the cells of the theca interna as well as decreased aromatase activity in the granulosa. This functional loss is accompanied by degenerative changes visible at the stigma at the light microscope level (Moor *et al.*, 1975). Perhaps associated with the induced cysts in the present study, the high peripheral concentration of LH which accompanied the early developmental stages of cyst formation initiated luteinization in the absence of follicular rupture. However, concentration of oestradiol-17 β in serum of cows developing cysts was not different from that of those which ovulated. Since the cows without cysts in this study that were given exogenous steroids exhibited LH pulse amplitudes and frequencies of LH which were similar to those of control cows developing a preovulatory follicle, the higher pulse amplitude and frequency of LH observed in cows with cysts is not likely to be due solely to an effect of the steroid treatment. It is possible that this alteration in gonadotrophin secretion is an effect (or cause) of the development of a cystic follicle rather than one that will ovulate.

Another endocrine aberration that could result in formation of cysts is the absence or mistiming of the preovulatory surge of LH. Lee *et al.* (1988) demonstrated that inhibition or delay of the preovulatory surge of LH in cattle with injections (i.v.) of progesterone was followed by development of persistent follicles. Also, Zaied *et al.* (1981) have shown that some cows with cysts release LH following administration of exogenous oestradiol benzoate, but the time from injection of oestradiol benzoate to the preovulatory surge of LH was delayed. In our study, three serum samples were obtained at 15-min intervals every 12 h. While it was possible for a preovulatory surge of LH to have occurred during this 12-h interval, surge levels (> 10 ng/ml) of LH were detected in the non-cystic and control groups while none of the cows with cysts exhibited preovulatory surge levels of LH. In this study, daily sampling was not frequent enough to characterize the intensity or timing of the preovulatory surge of LH.

Depletion or decreased stores of LH in the pituitary have also been suggested as a cause for cysts (Erb *et al.*, 1971). The early post-partum period in dairy cows, a time when pituitary content of LH (Saiddudin *et al.*, 1968; Moss *et al.*, 1985) and mRNA for the β -subunit of LH (Nett, 1987) are low compared with values in normally cycling cows, is characterized by an increased incidence of cysts. Perhaps the increased secretion of LH during cyst development observed in this study resulted in depletion of pituitary stores of LH. However, pituitary content of LH in cows which had recently developed cysts in this study (Exp. 2) or were long-term, chronically cystic (Brown *et al.*, 1986) was not depressed compared with normally cyclic cows. Similarly, concentrations of receptors for GnRH in this study were not different between cows with cysts or those exhibiting normal oestrous cycles. Furthermore, many investigators have shown that GnRH can induce release of LH in cows with cysts (for review, see Kesler & Garverick, 1982) or after 7–10 days *post partum* (Kesler *et al.*, 1977; Fernandes *et al.*, 1978).

Pituitary secretion of LH is controlled by hypothalamic GnRH and is modulated by steroids from the ovary (for review, see Nett, 1987). Aberrant release of GnRH from the hypothalamus, and subsequent induced release of gonadotrophins, might also be associated with cyst development. In Exp. 2, there was a lower content of GnRH in the hypothalamus of cows with cysts, indicating an aberration in one or more of the rates of synthesis, release, storage and/or degradation. In that same study, GnRH content in the median eminence tended (not significant) to be higher in cows with cysts. If those endocrine values were similar during cyst development, it would partly explain an increased release of LH during the follicular phase (increased GnRH in the median eminence) and lack of a preovulatory surge of LH (decreased GnRH in the hypothalamus) at the proper time.

In conclusion, secretory patterns of LH, but not FSH were associated with development of cysts in cattle (Exp. 1). During cyst development, secretion of LH was characterized by an increase in mean basal concentration, and frequency and amplitude of pulses. Aberration of secretory patterns of LH may be associated with aberrant hypothalamic (GnRH) function which is probably modulated by ovarian secretions. In Exp. 2, the anterior pituitary element of the hypothalamic–hypophysial–ovarian axis appeared to be fully competent after the development of ovarian follicular cysts. The cysts apparently inhibited the neural elements responsible for the synthesis and/or release of GnRH, and, subsequently, the secretory pattern of LH release.

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