

## JSAR Innovative Technology Award

# Formation of Follicular Cysts in Cattle and Therapeutic Effects of Controlled Internal Drug Release

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**Abstract.** Follicular cysts in cattle result from excessive growth of the dominant follicle without ovulation and still constitute a major reproductive disorder in this species. One key hormonal characteristic of cows with follicular cysts is the lack of an LH surge, although they have increased plasma estradiol concentrations. Another is a relatively high level of pulsatile secretion of LH that promotes continued growth of the dominant follicle. These LH characteristics seem to result from a functional abnormality in the feedback regulation of LH secretion by estradiol. Treatment with controlled internal drug release devices that increase circulating progesterone levels is effective in resolving follicular cystic conditions by 1) lowering pulsatile LH secretion and 2) restoring the ability of the hypothalamo-pituitary axis to generate an LH surge in response to an increase in circulating estradiol.

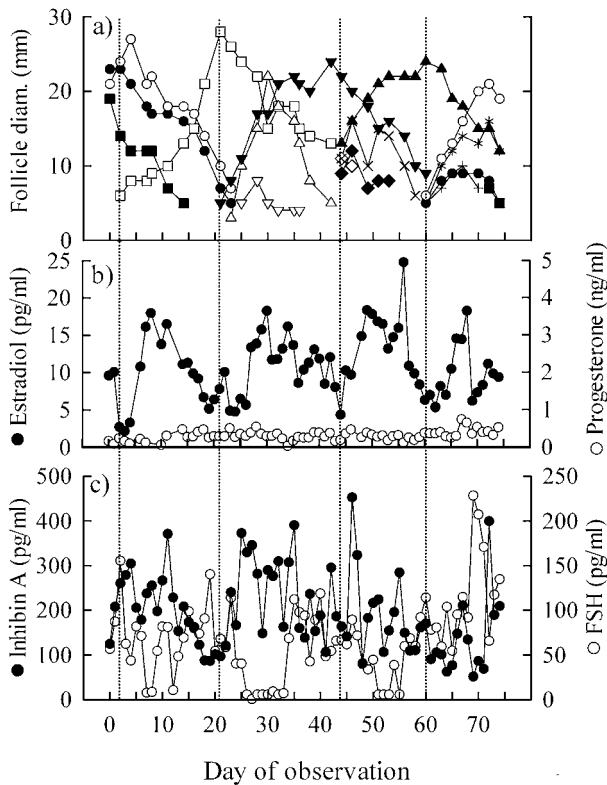
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Follicular cysts in cattle are characterized by the persistence of anovulatory follicular structures in the absence of corpora lutea, with interruption of normal estrous cycles [1-4]. In many countries, including Japan, follicular cysts affect a high proportion of dairy cows and cause economic losses to the dairy industry by extending the interval from calving to conception. The occurrence of follicular cysts in embryo donor herds is also becoming a new problem that will reduce the efficacy of embryo transfer, since the presence of persistent dominant follicles is likely to reduce the number of transferable embryos obtained from donors [5]. Epidemical studies indicate that the development of follicular cysts is associated with many factors, including periparturient stress, aging, nutritional

inadequacies, and genetic predisposition [1]; however, the mechanisms of development of these cysts have not been fully elucidated.

To date, various treatments for follicular cysts have been investigated [6]. Injection of gonadotropin-releasing hormone (GnRH) or its analogues is aimed at inducing ovulation and/or luteinization of cystic follicles and restoring normal estrous cycles, and it is now the most popular treatment for follicular cysts. On the other hand, reduction of LH pulse frequency by progesterone administration should lead to recovery from the follicular cystic condition, because prolonged growth of the dominant follicle is promoted by an LH pulse frequency greater than that observed during the mid-luteal phase [7, 8].

In the first part of this paper, we discuss the mechanisms leading to follicular cyst development, and especially the relationships between abnormalities in LH secretion and formation of

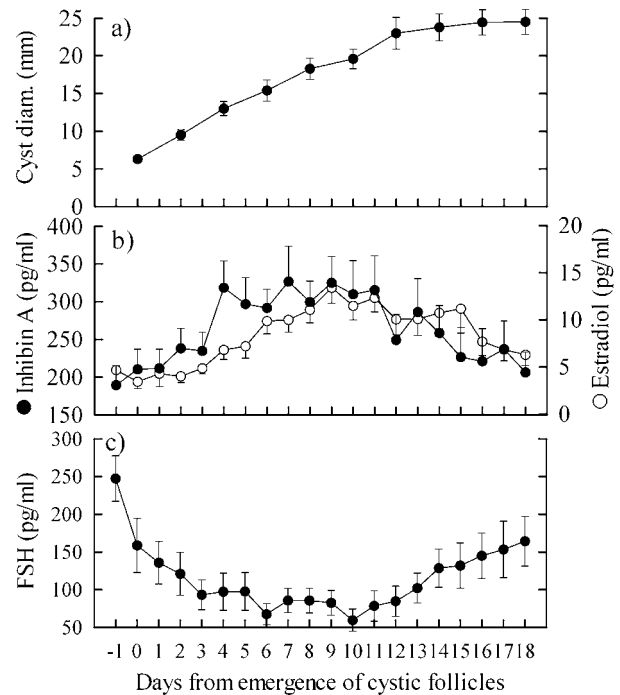


**Fig. 1.** Representative examples of a) follicular wave pattern in a cow with cystic follicles, together with changes in plasma concentrations of b) estradiol (●) and progesterone (○) and c) inhibin A (●) and FSH (○). Observation was started 10 days after detection of estrus without ovulation (Day 0=beginning of observation). Dashed vertical lines indicate detection of follicle emergence. Modified from Todoroki *et al.* [12].

cystic follicles. Secondly, we describe the therapeutic effects of progesterone-releasing controlled internal drug release (CIDR) devices on LH secretion and follicular development in cows with follicular cysts. Lastly, we evaluate the efficacy of CIDR treatment when CIDR is applied clinically to a donor beef herd.

### Growth of Cystic Follicles and Hormonal Profiles in Cows with Follicular Cysts

Ultrasonographic examination of cystic ovaries clearly reveals that waves of development of follicular cysts are replaced by different waves by a turnover process, and that the interwave interval is much longer than in normal cows [9, 10]. We investigated the precise relationships among the

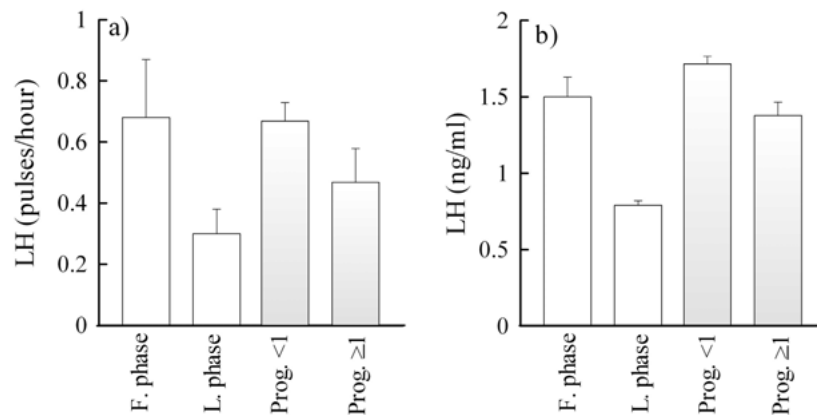


**Fig. 2.** Development of a) cystic follicles, and changes in plasma concentrations of b) inhibin A (●) and estradiol (○) and c) FSH in cows with follicular cysts. Values are mean  $\pm$  SEM ( $n=18$  waves from 5 cows that had multiple waves with cysts). Data are aligned relative to emergence of cystic follicles (Day 0=emergence of cystic follicles). Modified from Todoroki *et al.* [12].

turnover of follicular growth and plasma levels of inhibin A, gonadotropins, and steroid hormones in cows with follicular cysts [11, 12].

### Follicular development and profile of plasma inhibin A, steroid hormones and FSH

Figure 1 is a representative example of the follicular wave pattern and hormonal profiles in a cow with cystic follicles. Multiple follicular waves, with follicles 20 to 30 mm in maximum size, emerged at 15- to 25-day intervals over a period of 70 days (Fig. 1a). No corpora lutea were observed in the ovaries throughout the observation period, suggesting the lack of an LH surge and ovulation. Increases in plasma concentrations of estradiol and inhibin A were noted after emergence of each follicular wave (Figs. 1b and c). In contrast, a transient increase in plasma FSH was noted before



**Fig. 3.** a) Pulse frequencies and b) mean concentrations of LH in cows with follicular cysts, classified by plasma progesterone levels lower than 1 ng/mL (Prog.<1) or higher than 1 ng/mL (Prog.≥1). Values are means ± SEM for intensive sampling points (n=22 points under Prog.<1 or n=4 points under Prog.≥1). LH values in the follicular phase (F. phase) and luteal phase (L. phase) obtained from 5 normal cyclic cows are also presented. Modified from Todoroki *et al.* [12].

emergence of each wave (Fig. 1c). Concentrations of plasma progesterone ranged around about 0.5 ng/mL throughout the observation. Figure 2 shows the relationships among the development of cystic follicles and concentrations of inhibin A, estradiol, and FSH around the time of follicle emergence. Concentrations of inhibin A increased coincident with the appearance of cystic follicles and remained high in the follicular growth phase until 11 days after follicle emergence; thereafter they gradually decreased (Figs. 2a and b). The profile of plasma estradiol was similar to that of inhibin A (Fig. 2b). Plasma FSH levels were inversely correlated with plasma inhibin A and estradiol levels (Fig. 2c).

#### LH secretion

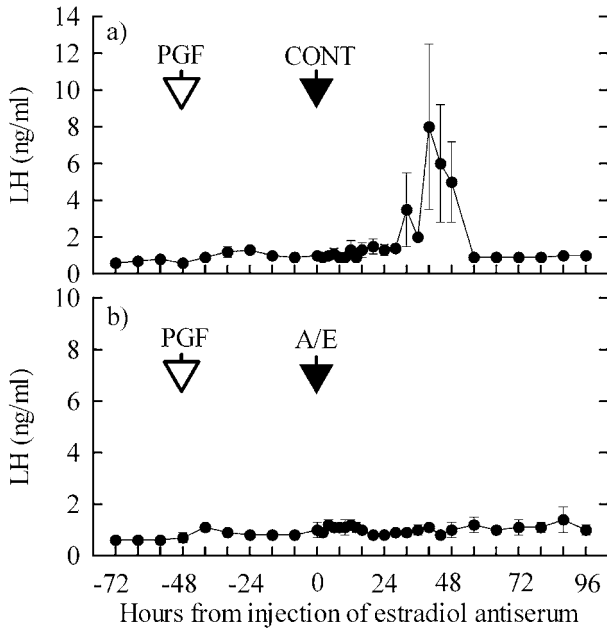
Because plasma progesterone levels above 1 ng/mL are considered to suggest luteinization of follicular walls, data on LH secretion, obtained from each cystic cow, were arranged according to progesterone levels (progesterone < 1 or progesterone ≥1 ng/mL) (Fig. 3). When the plasma progesterone level was lower than 1 ng/mL, the LH pulse frequency and mean concentrations of LH in cystic cows were comparable to the values in the follicular phase of normal cyclic cows. When the plasma progesterone level was greater than 1 ng/mL, the LH pulse frequency and mean concentration of LH in cystic cows were greater

than the values in the normal luteal phase.

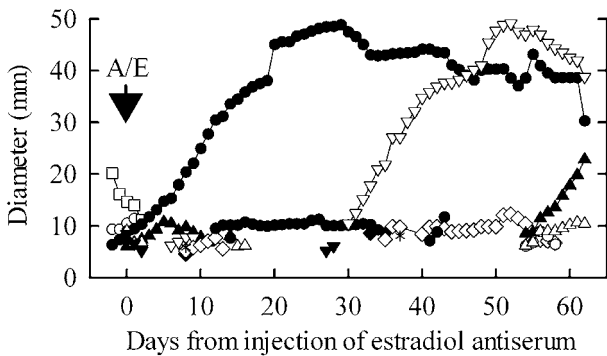
The above results indicate that pulsatile secretion of LH in cystic cows is, at least, higher than the luteal-phase levels in normal cows. The capacity to secrete inhibin A, as well as estradiol, is maintained in cystic follicles, the growth of which is extended by this relatively high level of LH secretion.

#### Induction of Follicular Cyst Formation by Perturbation of Estradiol-feedback Control of LH Secretion

Cows with follicular cysts have waves of follicular growth associated with increased estradiol secretion [9–13], but lack a preovulatory LH surge during the growth of anovulatory follicles [13]. Exogenous injection of estradiol often fails to induce an LH surge in cows with naturally occurring or induced follicular cysts [14–18], whereas injection of GnRH can induce the release of LH [19–21]. Taken together, these findings strongly suggest that an important physiological change in cystic cows is the lack of an LH surge owing to a functional abnormality in the estradiol-feedback regulation of LH secretion. Therefore, we passively immunized cows against endogenous estradiol to create a condition in which estradiol-feedback regulation was impaired [22].



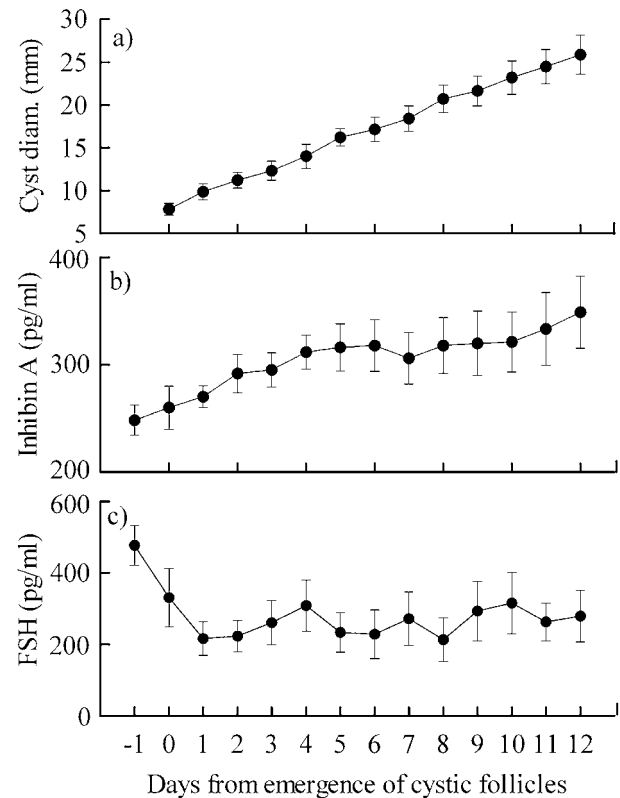
**Fig. 4.** Changes in plasma concentrations of LH during the periovulatory period in cows that received a single i.v. injection of a) control serum (CONT) or b) estradiol antiserum (A/E) 48 h after the first injection of PGF<sub>2α</sub> (PGF). The LH profile is aligned relative to injection of sera. Values are means  $\pm$  SEM (n=5). Modified from Kaneko *et al.* [22].



**Fig. 5.** Representative patterns of follicular development following injection of estradiol antiserum (A/E; day 0=injection of estradiol antiserum). Open squares signify corpora lutea, and the other symbols signify follicles. Modified from Kaneko *et al.* [22].

#### *Effects of estradiol immunization on the onset of an LH surge and follicular development*

Estradiol antiserum raised in a castrated goat [23] or castrated goat serum (control serum) was given to normal cows 48 h after injection of prostaglandin F<sub>2α</sub>. In the control cows, a preovulatory LH surge



**Fig. 6.** a) Development of cystic follicles, and changes in plasma concentrations of b) inhibin A and c) FSH in estradiol-immunized cows. Values are means  $\pm$  SEM (n=14 waves from 5 estradiol-immunized cows). Data are aligned to waves of emergence of cystic follicles (Day 0=follicular emergence). Modified from Kaneko *et al.* [22].

was detected between 24 h and 48 h after injection of control serum (Fig. 4a). In contrast, estradiol-immunized cows had no LH surge (Fig. 4b). The dominant follicles that had emerged before estradiol immunization did not ovulate, but persisted and enlarged (Fig. 5). The estradiol-immunized cows then had multiple follicular waves, with maximum follicular sizes of 20 to 45 mm, at 10- to 30- day intervals, over a period of more than 50 days. No corpora lutea were observed in the ovaries during this period.

Figure 6 shows the relationships among development of cystic follicles and concentrations of inhibin A and FSH around the time of follicle emergence. In association with emergence and growth of cystic follicles (Fig. 6a), concentrations of inhibin A increased and remained high (Fig. 6b). In contrast, plasma FSH levels remained low during cyst growth (Fig. 6c).

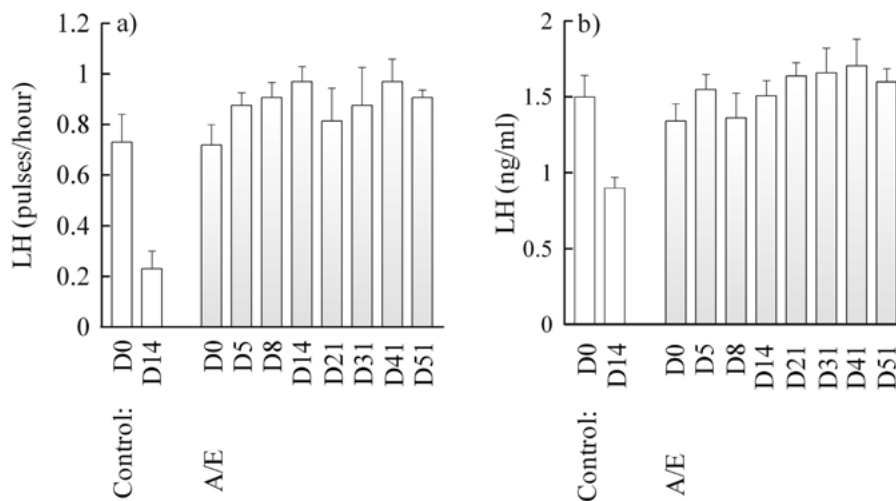


Fig. 7. a) Pulse frequencies and b) mean concentrations of LH in cows after injection of estradiol antiserum. Values are means  $\pm$  SEM ( $n=5$  estradiol-immunized cows). Day 0 (D0) indicates the day of injection of control serum or estradiol antiserum (A/E). Control D0 corresponds to the normal follicular phase, and D14 corresponds to the normal mid-luteal phase. Modified from Kaneko *et al.* [22].

#### *Pulsatile LH secretion after estradiol immunization*

The LH pulse frequency and mean concentrations of LH after estradiol immunization were higher than the values in the luteal phase of control cows (Fig. 7).

Immunoneutralization of estradiol resulted in inhibition of the LH surge. The hormonal profiles and characteristics of follicular development after estradiol immunization resembled those seen with spontaneously occurring follicular cysts. These findings provide evidence that lack of LH surge owing to dysfunction in the estradiol-mediated positive-feedback regulation of LH secretion can trigger the formation of follicular cysts.

#### **Model for Mechanism of Development of Follicular Cysts**

We proposed a hypothesis for the regulatory mechanisms producing follicular cysts (Fig. 8). Our results [11, 12, 22] and those of many other studies [7, 8, 13–18] suggest that the key physiological change in cystic cows is an abnormality in LH secretion. Under normal conditions, a sufficient amount of estradiol induces an LH surge and the dominant follicle ovulates. However, owing to a functional abnormality in the estradiol-feedback

regulation of LH secretion, cystic cows lack an LH surge. Because of the lack of negative feedback effects of progesterone in such anovulatory situations, the pulsatile secretion of LH is higher than the level observed in the normal luteal phase. The relatively high LH pulses promote continued excessive growth of dominant follicles [7, 8]. During this prolonged growth of follicles, production of estradiol, as well as of inhibin A, is sustained for longer than with a normal dominant follicle. A combination of inhibin A [23–26] and estradiol [27] establishes long-term dominance of the cystic follicle by the suppression of FSH secretion. When the cystic follicle regresses and loses its ability to produce inhibin A and estradiol, increased FSH secretion induces a new follicular wave. However, the newly emerged dominant follicle also becomes cystic unless the functional abnormality in the hypothalamus is resolved.

#### **Effects of CIDR Devices on LH Secretion and Follicular Development in Cows with Follicular Cysts**

Although pulsatile secretion of LH in cystic cows probably results from a lack of inhibition by progesterone in an anovulatory situation [22], pulsatile secretion of LH at a level greater than that

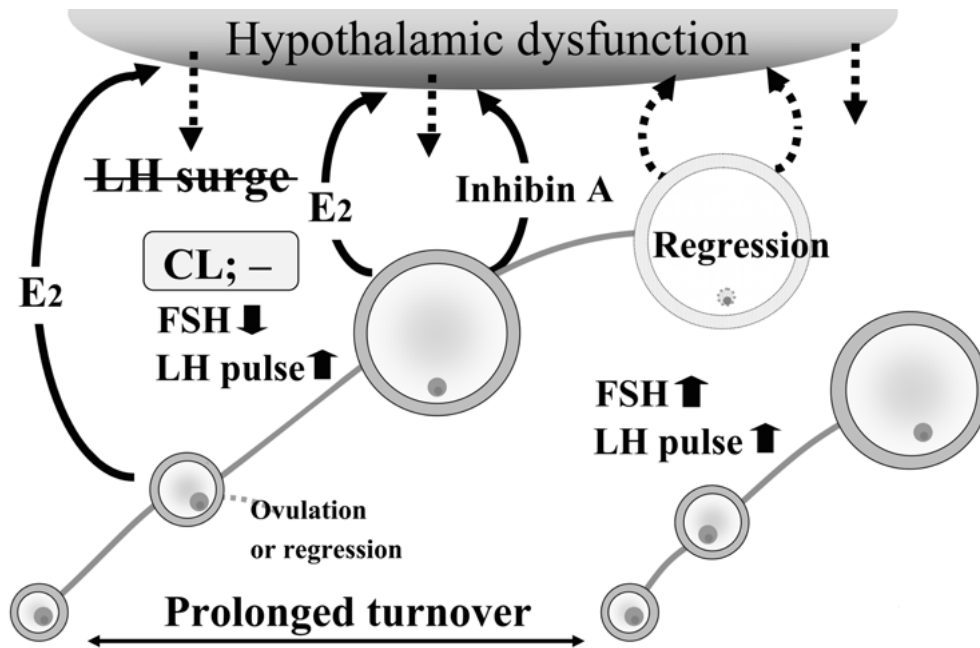


Fig. 8. Model of the mechanisms producing follicular cysts. A functional abnormality in estradiol-feedback regulation of LH secretion is likely to be involved in the lack of the LH surge and thereby initiate follicular cysts. CL, corpus luteum.

found in the normal luteal phase is important for continued growth of follicles [7, 8]. Conversely, suppression of the LH pulses in cystic cows to normal luteal-phase levels should result in cessation of the excessive growth of follicles. Therefore, we investigated the effects of CIDR devices containing progesterone on endocrine profiles and follicular growth in cows with follicular cysts [28].

#### *Effects of CIDR on initially existing cysts*

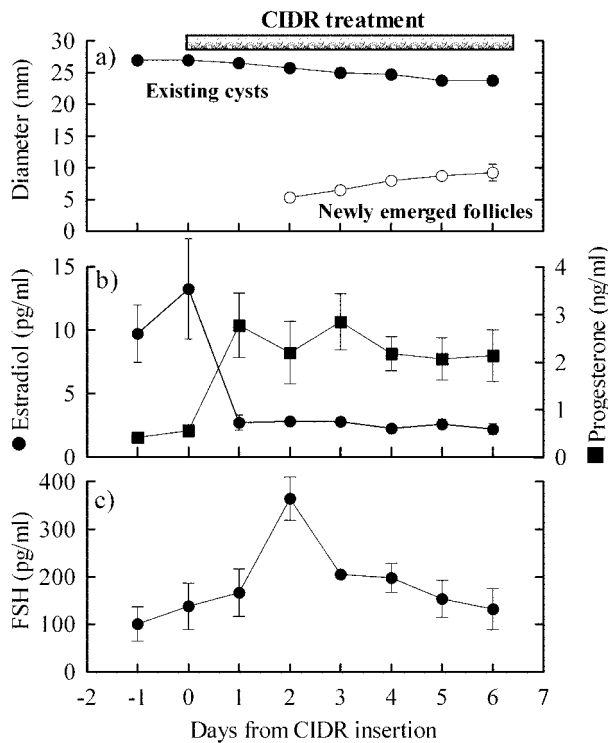
A single CIDR device (type B) containing 1.9 g progesterone was inserted into each of the cows with follicular cysts and left in place for 14 days (CIDR group). Control cows each received a CIDR device containing no progesterone for 14 days (Blank group).

Cows that initially had estrogen-active cysts showed a rapid decline in plasma estradiol levels in association with a rapid increase in progesterone levels within 1 day after insertion of the CIDR device (Figs. 9a, b). The cows then produced a rise in plasma FSH (Fig. 9c) and initiated new follicular waves within 3 days after CIDR insertion (Fig. 9a). However, CIDR had no effects in cows with estrogen-inactive cysts; emergence of the first new

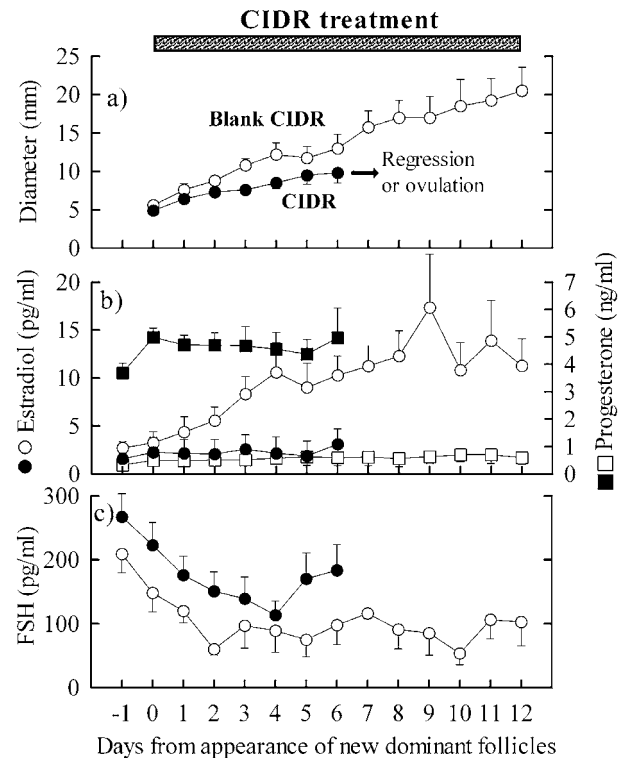
follicular waves occurred 3 to 8 days after CIDR insertion (data not shown).

#### *Effects of CIDR on newly emerged follicles*

In all cows of the CIDR group, two (occasionally three) follicular waves with a normal-sized dominant follicle (7 to 10 mm in diameter) emerged during CIDR device placement (Fig. 10a). The first new waves were replaced by different waves, and the inter-wave interval was about 7 days. The dominant follicles in those waves that appeared close to CIDR removal released ova between 2 and 4 days after CIDR removal. However, in the Blank group, new dominant follicles persisted and enlarged to over 20 mm in diameter (Fig. 10a). Concentrations of plasma estradiol showed no clear increase during CIDR placement (Fig. 10b), but increased in association with growth of dominant ovulatory follicles after CIDR removal (data not shown). In contrast, in cows that received blank CIDR devices, estradiol levels continued to rise in association with growth of the persistent dominant follicles (Fig. 10b). Plasma FSH levels were suppressed for much longer in cows that received blank CIDR devices than in cows treated by CIDR (Fig. 10c).



**Fig. 9.** a) Development and regression of follicles and changes in plasma concentrations of b) estradiol (●) and progesterone (■) and c) FSH (●) during the first 7 days after CIDR insertion in 4 cows with estrogenic follicular cysts. Data are clustered around the beginning of CIDR treatment (Day 0). Values are means  $\pm$  SEM. Modified from Todoroki *et al.* [28].



**Fig. 10.** a) Development and regression of newly emerged follicles and changes in plasma concentrations of b) estradiol (○, ●) and progesterone (□, ■) and c) FSH (○, ●) in cows with follicular cysts receiving a blank CIDR device (open marks) or a CIDR device (closed marks). Data are clustered around emergence of the dominant follicles (Day 0). Values are means  $\pm$  SEM. Modified from Todoroki *et al.* [28].

#### Pulsatile LH secretion after CIDR insertion

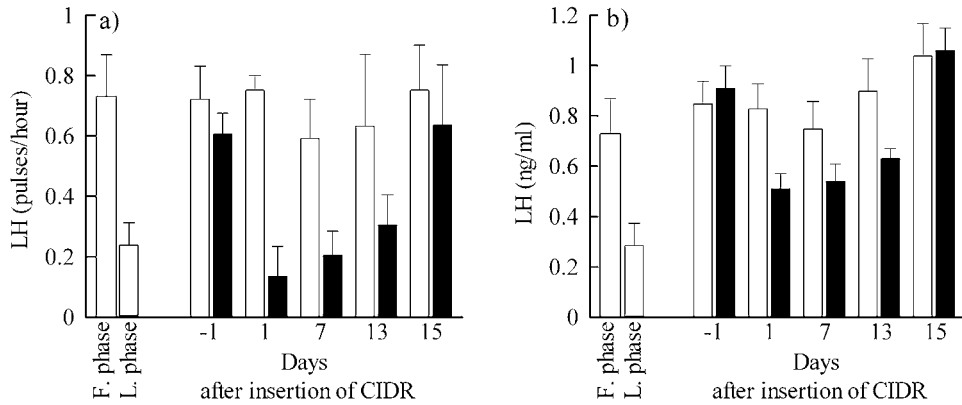
Pulse frequencies and mean concentrations of LH in cows in the Blank group were as high as those seen in the follicular phase of normal cows (Fig. 11). However, treatment with a CIDR device lowered both parameters, and the values corresponded to those in the luteal phase of normal cows.

The mechanism of action of progesterone-releasing intravaginal devices such as CIDR on follicular cysts is presumably as follows (Fig. 12). The cystic follicle inhibits emergence of a new follicular wave for a long period by maintaining increased production of inhibin A [11, 12] and estradiol [9, 10–13]. CIDR increases circulating progesterone to levels comparable to those observed during the normal luteal phase and induces atresia of cystic follicles by lowering the

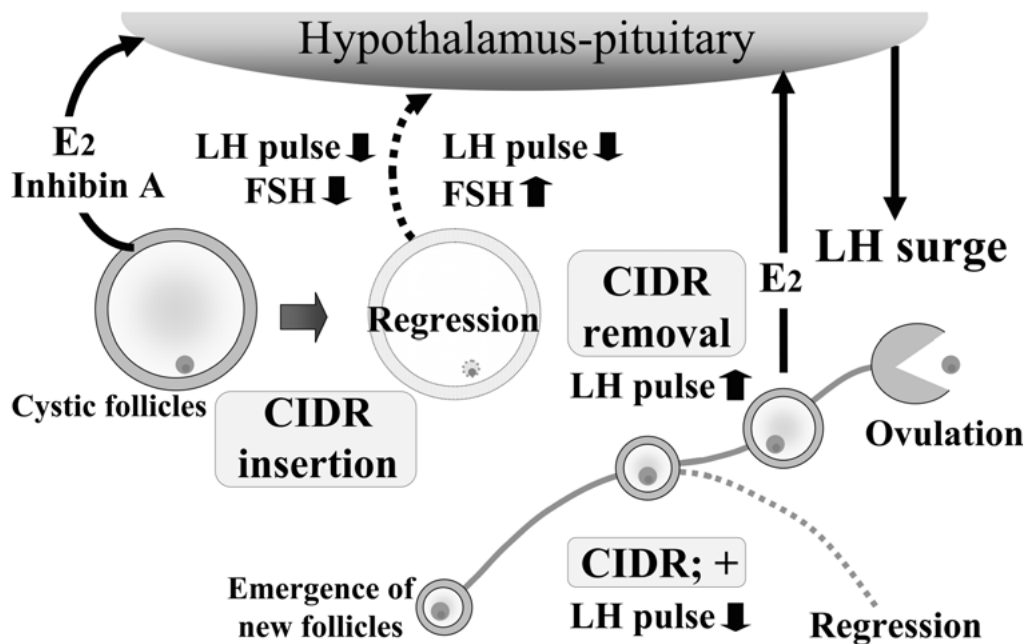
LH pulse frequency [28, 29]. Relief from the inhibitory effects of the cystic follicles results in the emergence of a new follicular wave. CIDR also prevents excessive growth of the newly emerged dominant follicles and produces turnover of follicles at normal intervals by suppression of LH secretion. Moreover, CIDR is likely to be able to restore the ability of the hypothalamo-pituitary axis to generate an LH surge in response to an increase in circulating estradiol [17, 18], and release of the ovum by the newly developed dominant follicle is induced by CIDR removal.

#### Efficacy of CIDR in Treating Follicular Cysts in An Embryo Donor Beef Herd

Insertion of a CIDR device or a progesterone-



**Fig. 11.** a) Pulse frequencies and b) mean concentrations of LH in cows with follicular cysts during treatment with a blank CIDR device (open bars) or a loaded CIDR device (closed bar) for 14 days. Data were obtained from 4 cows of the Blank group and 5 cows of the CIDR group. LH values in the follicular phase (F. phase) and luteal phase (L. phase) obtained from 5 normal cyclic cows are also presented. Modified from Todoroki *et al.* [28].



**Fig. 12.** Mechanisms of action of CIDR on follicular cysts. CIDR induces atresia of cystic follicles and prevents excessive growth of the subsequently emerged dominant follicles by lowering the LH pulse frequency. CIDR is likely to restore the ability of the hypothalamo-pituitary axis to generate an LH surge.

releasing intravaginal device (PRID) can reduce LH pulse frequency and induce atresia of cystic follicles [28, 29] and results in resumption of normal estrous cycles [28–30]. First, we applied a CIDR device clinically to embryo donors affected by follicular cysts and monitored the progress of the disease in

these animals. Second, we investigated the efficacy of CIDR in lowering the occurrence of follicular cysts when CIDR was used for estrous synchronization after embryo recovery in the same donor herd [31].



**Table 1.** Recovery from follicular cysts after insertion of CIDR devices

Year	No. of donors in the herd	No. (%) of donors with follicular cysts	No. of recovered cases*	No. of recurrent cases after initial recovery**
1997	64	15 (23)	12	3
1998	66	13 (20)***	6	7

\*Cysts did not recur after repeated embryo recovery.

\*\*Cysts recurred after embryo recovery, although ovulation and formation of corpora lutea had occurred after removal of the CIDR.

\*\*\*Includes the three donors in which follicular cysts recurred after initial recovery.

Reproduced from Todoroki *et al.* [31].

**Table 2.** Incidence of follicular cysts in the donor herd when a CIDR device was used for estrous synchronization after embryo recovery

Year	No. of donors in the herd	No. of donors with histories of follicular cysts	No. of donors with follicular cysts
1999	61	22	2
2000	54*	17	0

\*Includes two donors in which follicular cysts recurred in 1999. The two donors again received CIDR treatment and showed initial recovery, then were subjected to embryo recovery. Reproduced from Todoroki *et al.* [31].

#### *Effect of CIDR on embryo donors with follicular cysts*

Superovulation of beef cows was induced by three successive subcutaneous injections of porcine FSH over 3 days and two injections of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) analogue 48 h after the first FSH injection. Embryos were recovered from the donors 7 days after the PGF<sub>2α</sub>-induced estrus, and then the cows immediately received two i.m. injections of PGF<sub>2α</sub> for synchronization of estrus.

Follicular cysts were recorded in 28 cows between 1997 and 1998, and these animals were treated with CIDR devices for 14 days. All cows had ovulation with estrous behavior between 2 and 10 days ( $5.0 \pm 0.6$  days, n=28) after removal of the CIDR device and subsequently formed corpora lutea (Table 1). In 18 (64%) of the 28 cows that showed initial recovery, follicular cysts did not recur after repeated embryo recovery. However, in the remaining 10 (36%), cysts recurred after the next embryo recovery.

#### *Efficacy of CIDR in preventing follicular cysts in the embryo donor beef herd*

Instead of using the PGF<sub>2α</sub> that we had used for estrus synchronization after embryo recovery between 1997 and 1998, we used a CIDR device for the same purpose in the same herd from 1999 to 2000 and recorded the incidence of follicular cysts.

Immediately after embryo recovery, a single CIDR device was inserted into each cow and left for 14 days.

When CIDR was used for synchronization of estrus after embryo recovery, follicular cysts were detected in 3% of 61 cows in 1999 (Table 2). In 2000, no cysts were recorded. The number of cows in the herd with histories of follicular cysts was 22 in 1999 and 17 in 2000. The time interval between CIDR removal and detection of estrous behavior was  $2.9 \pm 0.5$  days (n=61) in 1999 and  $2.6 \pm 0.9$  days (n=54) in 2000.

CIDR treatment thus proved effective in resolving follicular cysts in the embryo donor beef herd and enabled the re-use of donors affected by follicular cysts. CIDR is also likely to be efficacious in lowering the occurrence of follicular cysts in donor herds when it is used for estrous synchronization after embryo recovery.

### Unanswered Questions

ACTH injection inhibits the LH surge by maintaining plasma progesterone at a sub-luteal level for several days after luteolysis and thus induces the formation of persistent follicles [32, 33].

ACTH-induced progesterone secretion originates from the adrenal gland [34], suggesting that stress can cause follicular cyst development. Our series of experiments demonstrated that perturbation in estradiol-feedback control of the LH surge induced the formation of follicular cysts. It is not yet clear whether several neurotransmitters, such as opioid peptides, mediate between stress and deficiency in estradiol-feedback regulation of LH.

In addition, intravaginal devices that release progesterone, such as CIDR or PRID devices, seem to have some healing effects on the functions of the hypothalamo-pituitary axis in cows with follicular cysts [28–30]. However, the mechanisms of progesterone-mediated correction of the functional

abnormality in the hypothalamus remain unclear.

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