FULL PAPER ***Theriogenology***

Dynamic Changes in Plasma Concentrations of Gonadotropins, Inhibin, Estradiol-17β and Progesterone in Cows with Ultrasound-guided Follicular Aspiration

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ABSTRACT. To elucidate the effects of ultrasound-guided transvaginal follicular aspiration, plasma concentrations of FSH, LH, inhibin, estradiol-17β and progesterone, and folliculogenesis were examined in Holstein cows. Four clinically healthy cows with regular estrous cycles were scanned by ultrasound per rectum once a week for 9 weeks before the commencement of follicular aspiration. All visible follicles were divided into 3 categories based on their sizes (2 ≤ small < 5 mm; 5 ≤ medium < 10 mm, large ≥ 10 mm). The follicular aspiration was started at random during the estrous cycle and conducted under epidural anesthesia induced with 5 m*l* of 2% lidocaine once a week for 6 weeks. The average number of total visible follicles ≥ 2 mm in diameter at 7 days after aspiration (21.7 ± 7.4, n=24) was similar to that before starting aspiration (26.7 ± 10.5, n=36). Plasma inhibin and estradiol-17β declined and fell to a trough on 1.5 days and returned to pre-aspiration values by 5 days after aspiration. Plasma concentrations of FSH increased and reached peak levels between 1 and 1.5 days after aspirations. Plasma concentrations of LH also increased and reached peak levels between 0.5 and 1.5 days after aspirations. Both plasma FSH and LH had returned to pre-aspiration levels by 5 days after aspirations. Plasma concentrations of progesterone did not change with the follicular aspiration. These results demonstrate that follicular aspiration decreases plasma concentrations of inhibin and estradiol-17β, which in turn leads to a rise in plasma concentrations of FSH and LH. It is suggested that marked increases in plasma concentrations of FSH and LH after the aspiration stimulate the development and maturation of a new cohort of follicles within one week in cows.

KEY WORDS: bovine, estradiol, follicular aspiration, gonadotropin, inhibin.

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The inability to obtain a large number of viable embryos from selected female cattle populations restricts genetic improvement. Females are born with a large store of primordial follicles in ovaries, of which a predetermined number of follicles are stimulated at a time to undergo further development [38]. If the oocytes in these follicles are normal then there is scope for increasing the reproductive potential of selected females since less than 1% of follicles reach the ovulatory stage [38]. A more homogeneous and reliable source of oocytes could be obtained by growing primordial and preantral follicles *in vitro* [32, 44]. Recent improvements in our understanding of the factors involved, together with improved culture systems, indicate that this is feasible [45].

In the past decade, remarkable progress has been made in improving the procedures for embryo production *in vitro* using oocytes obtained from ovaries of cattle at slaughterhouses [7, 11, 26, 46]. Oocytes from slaughtered animals have provided an important source of embryos for research purposes, but they have limited value in the production of embryos for use in livestock improvement programs. Considerable effort has been made by cattle breeders to get offspring from genetically superior cows. Furthermore, hormonal induction of multiple ovulation and embryo transfer are also widely used. Multiple ovulation induced by exogenous gonadotropins can be repeated in the same animal several times per year, however, there are also some disadvantages with the use of this method. For example, the ova or embryo collection can only be performed with long intervals between hormonal treatment [29], and results from different studies were not consistent due to individual animal variation in response to exogenous gonadotropins [28, 37].

Ultrasound-guided follicular aspiration has been performed in human gynecology since the 1970's [24, 25, 36, 39], but great progress have been achieved only in veterinary medical practice in the past decade [9, 22, 23, 33, 40]. The collection of bovine oocytes by follicular aspiration during the estrous cycle, followed by *in vitro* maturation, fertilization, and culturing up to the transferable stage of embryonic development, is a useful alternative way to superovulation for producing embryos. Some previous studies have reported that oocytes after aspiration developed well without exogenous gonadotropin [9, 23], and have suggested that the negative feedback effects of inhibin and estradiol-17β may be removed by follicular aspiration, which in turn causes increased secretion of gonadotropins in cattle [9]. Ultrasound scanning and ultrasound-guided follicular aspiration are quite useful to study the process of follicle selection and dominance [10, 11], which is poorly understood at present [27], however, the endocrine response following ultrasound-guided follicular aspiration has not been well documented in cows.

In the present study, ultrasound-guided transvaginal follicular aspiration was performed on Holstein cows once weekly, and plasma concentrations of FSH, LH, inhibin, estradiol-17β and progesterone before and after follicular aspiration were examined.

MATERIALS AND METHODS

Experimental design: Four clinically healthy Holstein cows between 8–10 yr of age and weighing 700–800 kg, with regular estrous cycles and non-milking and non pregnancy, were used. All cows were scanned by ultrasound per rectum once a week for 9 weeks before the commencement of follicular aspiration. Then, follicles were aspirated under epidural anesthesia induced with 5 m*l* of 2% lidocaine, once a week for 6 weeks. Blood samples (10 m*l*) were collected from the jugular vein at -12, 0, 12, 24, 36 and 120 hr after each follicular aspiration. The samples were centrifuged (2000 × g for 15 min at 4°C) and the sera were removed and stored at –20°C until assay for FSH, LH, inhibin, estradiol-17β and progesterone.

Ovarian observations and follicular aspiration: All visible follicles were divided into 3 categories based on their sizes (2 ≤ small < 5 mm; 5 ≤ medium < 10 mm, large ≥ 10 mm). Follicular aspiration was conducted as described in previous studies [21,22] with an ultrasound monitor (Aloka SSD-1200, Tokyo, Japan) and a 7.5 MHz convex array transducer attached to a specially designed puncturing device with a 17 g stainless steel needle guide. All follicles ≥ 2 mm in diameter were aspirated with a vacuum pressure (100 mm Hg) through the stainless steel needle 59 cm in length into a 50 m*l* centrifuge tube via 100 cm of teflon tubing; PBS containing 80 μg/m*l* heparin was used for rinsing the needle and tubing.

Radioimmunoassays (RIA) for FSH, LH, inhibin, estradiol-17β and progesterone: Plasma concentrations of FSH were measured by RIA as described previously [2] using anti-bovine FSH β-subunit serum (USDA-5-pool), USDA-FSH-BP3 for radioiodination, and USDA-bFSH-B1 as a reference standard. Plasma concentrations of LH were measured by RIA [6] using anti-bovine LH serum (USDA-309-684P), USDA-bLH-I-1 for radioiodination, and USDA-bLH-B1 as a reference standard. The sensitivity of the assays were 6 *p*g/tube for FSH and 0.24 *n*g/tube for LH. The intra- and inter-assay coefficients of variation were 5.3% and 9.8% for FSH and 6.5% and 11.5% for LH, respectively.

Plasma concentrations of estradiol-17β and progesterone were determined by a double antibody RIA system using ^{125}I-labeled radioligands as described previously [16, 30, 43]. Antisera against estradiol-17β (GDN 244) and progesterone (GDN 377) were kindly provided by Dr. G. D. Niswender (Animal Reproduction and Biotechnology, Colorado State University, Fort Collins, CO, U.S.A.). The sensitivity of the assays were 0.62 *p*g/tube for estradiol-17β and 2.5 *p*g/tube for progesterone. The intra- and inter-assay coefficients of variation were 6.0% and 9.8% for estradiol-17β, 3.5% and 13.4% for progesterone, respectively.

Plasma concentrations of inhibin were measured by a homologous double-antibody RIA as described previously [14] using bovine 32-kDa inhibin for radioiodination and anti-bovine inhibin serum (TNDH-1). The sensitivity was 39 *p*g/tube. The intra- and inter-assay coefficients of variation were 6.8% and 13.5% .

Statistics: All data were expressed as mean ± SEM and were analyzed by one-way ANOVA followed by Duncan's Multiple Range test. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Follicular development with follicular aspiration: The number of follicles was measured weekly using the ultrasound-guided technique 9 times before starting follicular aspiration. At seven days after follicular aspiration the procedure was repeated for another 5 weeks. The average number of total visible follicles ≥ 2 mm in diameter at 7 days after aspiration (21.7 ± 7.4, n=28) was similar to that before starting aspiration (26.7 ± 10.5, n=36). The aspiration did not significantly affect the average number of follicles of each size (small, 17.5 ± 8.9 vs. 14.6 ± 7.1; medium, 7.3 ± 4.2 vs. 5.2 ± 2.6; large, 1.9 ± 1.1 vs. 1.9 ± 1.2) (Table 1).

Plasma concentrations of FSH, LH, inhibin, estradiol-17β and progesterone with follicular aspiration: Similar changes in plasma concentrations of FSH, LH, inhibin and estradiol-17β following follicular aspirations were observed in all individual cows throughout the experimental period (Fig. 1). The changes in plasma concentrations of progesterone in experimental cows still showed cyclic fluctuation following follicular aspirations. Plasma concentrations of inhibin were 700–900 *p*g/m*l* before aspiration, and were lowest (420–680 *p*g/m*l*) at 36 hr after aspirations ($P<0.01$) (Fig. 2e). The concentrations of estradiol-17β in plasma were 6.5–10.0 *p*g/m*l* before aspiration. After aspiration they decreased and reached their troughs (2.5–4.0 *p*g/m*l*) at 24 hr after aspiration ($P<0.01$) (Fig. 2b). Plasma estradiol-17β decreased more quickly than plasma inhibin, but both inhibin and estradiol-17β returned to pre-aspiration values by 120 hr after aspiration. Plasma concentrations of FSH increased and reached peak levels (from 10.5–18.3 *n*g/m*l* to 19.0–47.5 *n*g/m*l*) between 24 hr and 36 hr after aspiration ($P<0.01$) (Fig. 2d). A negative correlation between plasma concentration of inhibin and FSH was observed after aspirations (r=–0.232, n=144, $P<0.01$). Plasma concentrations of LH also increased after aspiration, and reached peak levels (from 290–320 *p*g/m*l* to 556–806 *p*g/m*l*) between 12 hr and 48 hr after aspira-

Table 1. Size distributions of follicles in cows before and after follicular aspirations

Treatment	Number	Follicle size			Total
		Small	Medium	Large	
Before	36	17.5 ± 8.9	7.3 ± 4.2	1.9 ± 1.1	26.7 ± 10.5
After	24	14.6 ± 7.1	5.2 ± 2.6	1.9 ± 1.2	21.7 ± 7.4

Results represented as mean ± SEM in 4 cows once a week for 9 weeks before starting aspiration and at 7 days after aspirations for 6 weeks.

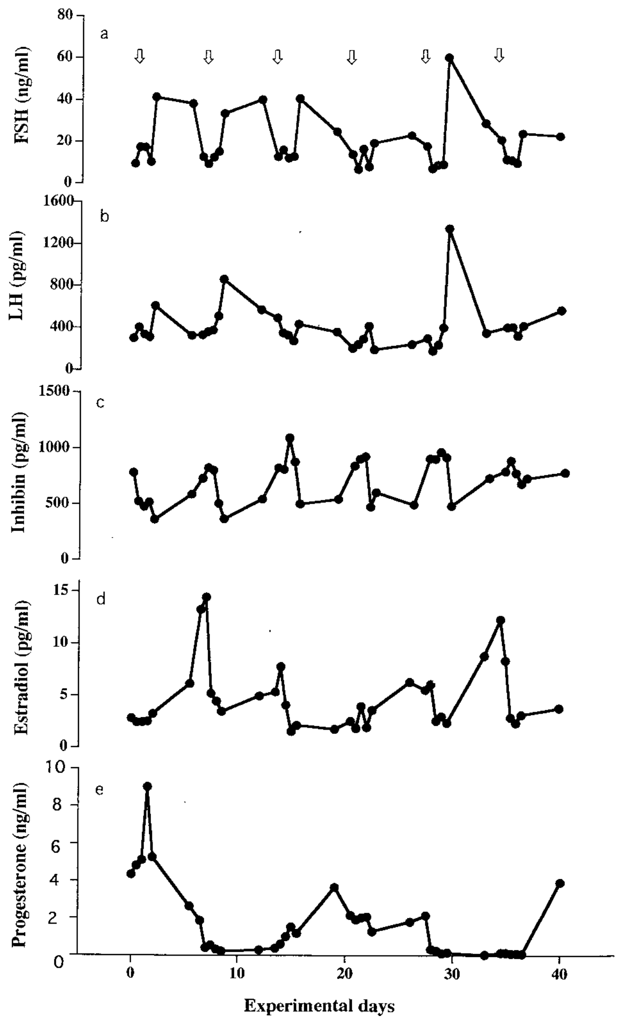

Fig. 1. Circulatory profiles of FSH (a), LH (b), inhibin (c), estradiol-17β (d; Estradiol) and progesterone (e) in a representative cow. Follicular aspirations were conducted once a week for 6 weeks throughout the experimental period. Arrows indicate the times of aspirations.

tion (P<0.05) (Fig. 2a). Both plasma FSH and LH returned to the pre-aspiration levels by 120 hr after aspiration. No statistical changes in plasma concentrations of progesterone were observed following follicular aspiration (Fig. 2e).

DISCUSSION

This is the first report to describe the dynamic changes in plasma concentrations of gonadotropins, inhibin, estradiol-17β and progesterone in cows with ultrasound-guided follicular aspiration. The results clearly demonstrate that follicular aspiration decreases plasma concentrations of inhibin and estradiol-17β, resulting in attenuation of the feedback actions of inhibin and estradiol-17β on FSH and LH secreted by the pituitary gland. Thus, the removal of inhibition by ovarian hormones results in an increase in plasma concentrations of FSH and LH. Marked increases in plasma concentrations of FSH and LH may stimulate development and maturation of a new cohort of follicles within one week in the cow after the aspiration.

In other studies, the most popular aspiration schedule used has been weekly [34] starting on Day 3 or 4 (Day 0 = estrus) of the estrous cycle, which corresponds with the emergence of the first follicular wave [1, 15]. In the present study, the average number of total follicles were not different after aspiration, though apparently all visible follicles (≥ 2.0 mm) had been aspirated from the ovaries. Both plasma concentrations of inhibin and estradiol-17β decreased after aspiration, and returned to their pre-aspiration levels by 120 hr. However, plasma estradiol-17β decreased more quickly than plasma inhibin after aspiration, this may be because of their different metabolism rates. Plasma concentrations of FSH and LH, on the other hand, increased after aspiration, and returned to their pre-aspiration levels by 120 hr. These results indicate that a new cohort of follicles had developed within one week, following the aspiration of follicles.

It has been known that inhibin is mainly secreted by granulosa cells of antral follicles in the ovary, and has the ability to suppress FSH secretion in mammals [5]. Immunoneutralization of inhibin results in increases in plasma concentrations of FSH and the rate of ovulation in cattle [12, 16–18, 42], indicating that inhibin has an important role in the regulation of FSH secretion in this species [5, 12, 15–18]. It has also been reported that a negative correlation exists between plasma concentrations of estradiol-17β and FSH during the follicular phase [3, 19, 35, 41], and that estradiol-17β has some inhibitory effects on FSH secretion in cows. However, it was reported that injections of estradiol-17β antiserum did not result in any changes in FSH secretion in cows [16]. Therefore, a marked increase in FSH secretions observed after follicular aspiration in the present study was probably due to the reduction in plasma inhibin concentrations. Ovariectomy in cattle results in an increase in frequency of release of LH pulses [4, 20]. This post-ovariectomy increase in frequency of LH pulses can be inhibited by administration of estradiol-17β [4]. Thus, the increase in plasma levels of LH following the aspiration in the present experiment may be mainly because of the removal of negative feedback of estradiol-17β on the pituitary gland. Corpora lutea usually have a definite life span in cows [8, 31], but follicular aspiration did not affect the plasma concentrations of progesterone in the present experiment, probably indicating that follicular aspiration dose not affect the function of corpora lutea.

In conclusion, the present study clearly demonstrates that follicular aspiration decreases plasma concentrations of inhibin and estradiol-17β, thereby resulting in an increase in plasma concentrations of FSH and LH. Marked increases in plasma concentrations of FSH and LH after aspiration, may stimulate the development and maturation of a new cohort of follicles within one week in cows.

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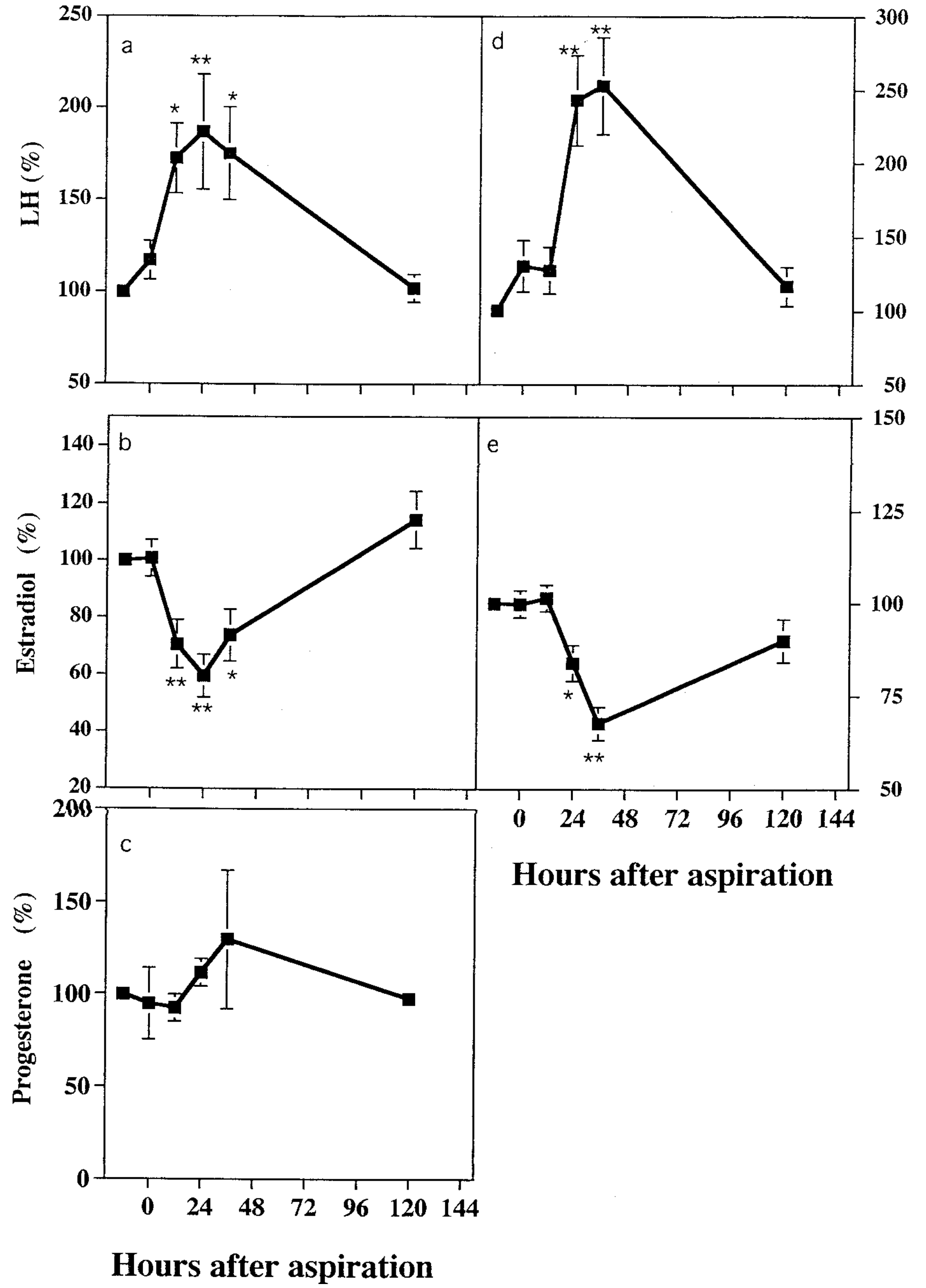

Fig. 2. The effects of follicular aspirations on plasma concentrations of LH (a), estradiol-17β (b; Estradiol), progesterone (c), FSH (d) and inhibin (e) in cows. Values are represented as mean ± SEM of 24 observations in 4 cows. In each panel, results are expressed as percentage value of time -12 hr (before follicular aspiration). *, P<0.05; **, P<0.01 compared with the value of before aspirations (Duncan's multiple-range test).

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