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Changes in the ovary and the peripheral concentrations of sex hormones after the aspiration of follicular fluid from the spontaneous follicular cysts of dairy cows

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Abstract. The aim of the present study was to clarify the ovarian and hormonal dynamics after the aspiration of follicular fluid in cows with follicular cysts. Follicular fluid was aspirated from the follicular cysts and follicles that were fated to become cystic follicles and other coexisting normal follicles, respectively, in lactating cows (n = 3). After the aspiration procedure, new follicles developed and reached a diameter of 25 mm without ovulation within 13–19 days. The plasma concentrations of inhibin decreased and follicle-stimulating hormone increased rapidly after the aspiration procedure, and subsequently increased and decreased, respectively, as a new follicle grew. No luteal structures developed after the aspiration procedure, and the animals' plasma progesterone levels remained low. The present study indicates that the cystic follicles are never luteinized by the aspiration of follicular fluid, and consequently, new follicular cysts are observed to repeatedly develop.
Key words: Follicular cyst, Follicular fluid aspiration, Ovarian and pituitary hormones, Ovarian ultrasonography
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Ovarian cysts (OC) are a pathological condition observed in cows in which a mature follicle continues to grow to an abnormal size (usually > 25 mm in diameter); however, ovulation does not occur, and the unovulated abnormally large follicles (cystic follicles) remain in the ovary for > 10 days [1]. OCs are subdivided into follicular cysts that do not exhibit cystic follicular wall luteinization and luteal cysts and those that do [1]. In cows with follicular cysts, the blood estrogen level increases to a sufficiently high level to induce a luteinizing hormone (LH) surge for ovulation when the growing follicle reaches maturity; however, no LH surge occurs [2–4]. The lifespan of the cystic follicles is approximately 20 days. Following the regression of the cystic follicle, new follicles start to develop and continue to grow to maturity, and a new cystic follicle forms, which also does not ovulate. This process is called cystic follicle turnover [2, 3, 5–7]. The turnover mechanism has been demonstrated in previous studies [2, 3, 5].

In veterinary practice, the most frequently encountered pathological ovarian conditions in cows are an abnormally sized structure that is larger than an ovulatory follicle and a normal functional corpus luteum (CL). These abnormally sized structures are mostly caused by ovarian cysts or cystic CLs (CCLs) [1]. A CCL is a CL in which a fluid-containing central cavity > 7–10 mm in diameter develops [1]. In several cases, the CCLs have diameters of approximately 30 mm or more, although in our experience, they can be approximately 50–70 mm in diameter in some cases. Currently, it is postulated that CCL is regarded in several cases as a physiological condition of CL [8], although CCL has been considered to be a cause of infertility in cows [1]. Clinically, it is easy to differentiate CCL from OC in the case of CCL with typical ovulation papilla or corpus luteum projection. However, in cases in which ovulation had not been confirmed and the ovarian large-sized structure had a fluid-filled cavity, unclear or no ovulation papilla, and no obvious luteal tissue layer around the cavity, the veterinarians would suffer due to the difficulty of accurately diagnosing CCL or OC. In such cases, it may be diagnostically useful to aspirate the fluid from the central cavity because the functional CL filled with luteal tissue generally is observed to heal within a few days in the case of CCL [9, 10], whereas in the case of follicular cysts, it is suspected that luteal tissue would not develop after the aspiration of the fluid from the central cavity because ovarian cysts do not induce an LH surge. However, the changes in the ovarian structures and the associated changes in sex hormone levels that occur after the aspiration of follicular fluid from follicular cysts have not been investigated. In this study, the changes in aspirated cystic follicles, ovarian structures, and blood ovarian and pituitary hormone levels were investigated after the aspiration of follicular fluid from spontaneous follicular cysts in cows to demonstrate the diagnostic usefulness of the aspiration of follicular fluid from cystic follicles to differentiate between the cystic follicles and CCL.

The number of fluid-aspirated cystic follicles and growing follicles and the number of new follicles and cystic follicles that subsequently developed after the aspiration procedure are shown in Table 1. The changes in the mean diameters of the largest ones of cystic follicles and coexisting normal follicles before and after the aspiration procedure in the three cows are shown in Fig. 1. No luteinization was observed after the aspiration of any of the cystic follicles or follicles in any of the cows. After the aspiration procedure, 2–5 follicles were observed to start growing and reached 8 mm in diameter within 3–4 days (Fig. 1 and Table 1). It was observed that 2–3 of the follicles grew to 15 mm in diameter within 7–9 days after the aspiration procedure. These newly developing follicles continued to grow and reached ≥ 20 mm in diameter by day 11–14 and ≥ 25 mm in diameter, i.e., the size of a

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Table 1. Follicular cysts and follicles from which fluid was aspirated and the subsequent development of new follicles and cystic follicles

Cow No.	Length of observation period (days)	Number of aspirated cystic follicles and [follicles]		Newly developed follicles after aspiration						
				Day *d) on which new follicle (≥ 8 mm) was detected	Diameter of newly developed follicles / cystic follicles					
					≥ 15 mm		≥ 25 mm		Maximum	
		Regressing *a)	Growing *b)		Number	Day	Number	Day	Size (mm)	Day
20	50	2	1	3	2	9	1	19	33	27
22	35	2	1	4	2	7	1	13	25	13
25	42	1	[3] *c)	4	3	7	1	18	30	23

*a) Follicle decreasing in diameter. *b) Follicle increasing in diameter. *c) Growing follicle with diameter of 20 mm. *d) Days after the Day 0 when follicular fluids were aspirated.

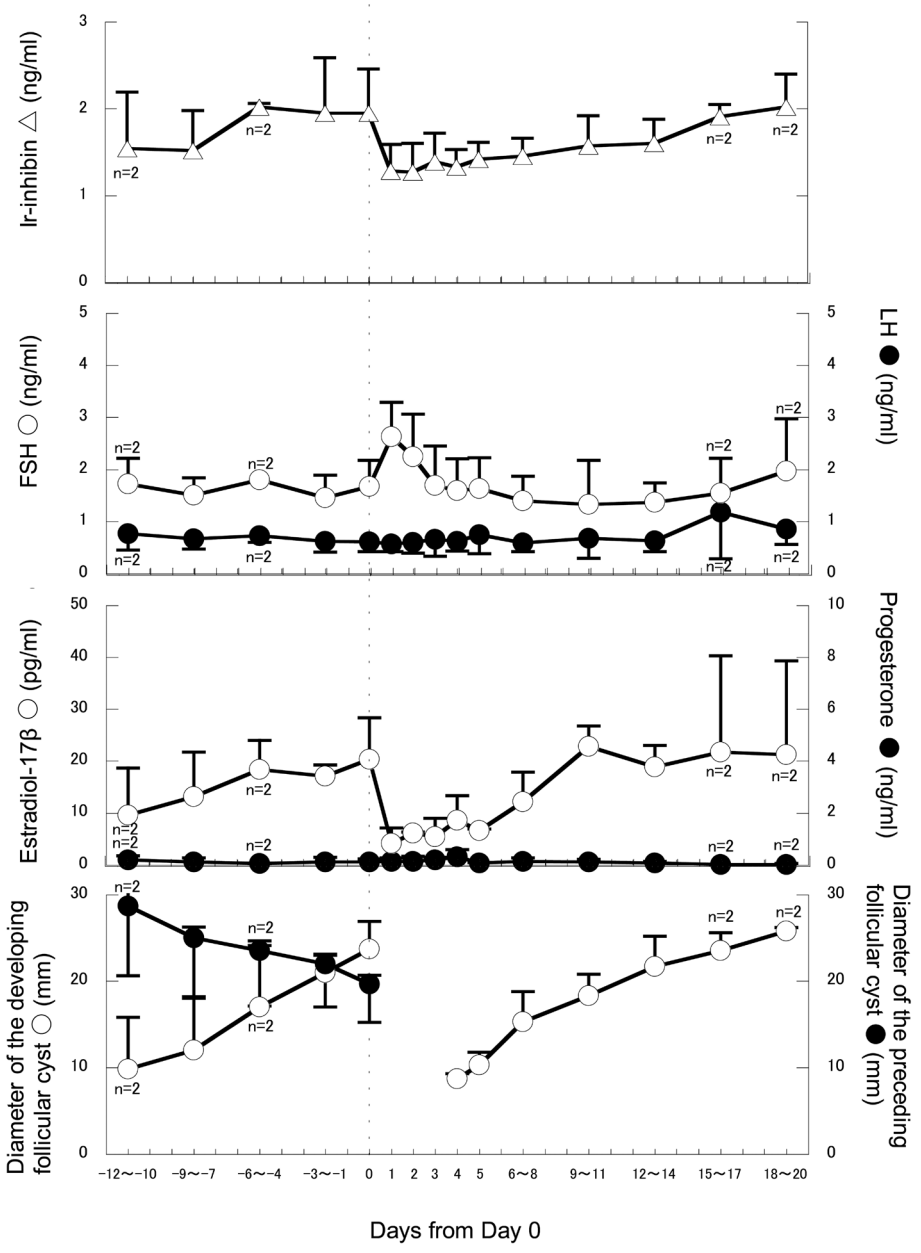

Fig. 1. Changes in the mean diameters of atrophying cystic follicles and developing cystic follicles and the peripheral concentrations of progesterone, estradiol-17β, LH, FSH, and ir-inhibin seen prior to and after the follicular fluid aspiration procedure in the 3 cows. Data obtained before and after day 0 are shown at 3-day intervals, except during the first 5 days after the aspiration procedure when data were obtained every day. In cases where multiple values of data were obtained during the same 3 days interval, the mean value was utilized. The maximum diameters of the preceding largest cystic follicle and the largest developing follicle were used as the representative values for each cow. The hormone concentrations are shown as the mean ± standard deviation. Day 0: The day of the follicular fluid aspiration procedure. n = 2: Indicates that 2 sets of data were utilized.

Table 2. The mean plasma hormone levels before and after the aspiration of fluid from ovarian follicular cysts and follicles

Hormones	Before aspiration [a)]	After aspiration [a)]	P-values before and after aspiration
	Mean ± SD of averages from Day –6 to Day 0	Mean ± SD of averages from Day 1 to Day 3	
Estradiol-17β (pg/ml)	19.07 ± 13.23 (n = 3)	5.24 ± 0.70 (n = 3)	0.105
Ir-inhibin (ng/ml)	1.89 ± 0.51 (n = 3)	1.31 ± 0.30 (n = 3)	< 0.05
FSH (ng/ml)	1.53 ± 0.41 (n = 3)	2.19 ± 0.65 (n = 3)	< 0.05
LH (ng/ml)	0.62 ± 0.19 (n = 3)	0.61 ± 0.22 (n = 3)	0.308
Progesterone (ng/ml)	0.07 ± 0.06 (n = 3)	0.16 ± 0.13 (n = 3)	0.12

[a)] Day 0 was the day of aspiration.

cystic follicle, by day 13–19 in all the three cows. The cystic follicles reached their maximum sizes (33 mm, 25 mm, and 30 mm in diameter) on days 27, 13, and 23 after the aspiration procedure, respectively.

In all the cows, the mean plasma progesterone (P_4) concentration remained at a low level (≤ 0.6 ng/ml); additionally, the mean plasma LH concentration fluctuated in the range of 0.37–1.17 ng/ml both before and after the aspiration procedure (Fig. 1). The plasma hormone levels observed during the 6-day period between day –6 and day 0 (before the aspiration procedure) were compared with those levels observed during the 3-day period from day 1 to day 3 (after the aspiration procedure) (Table 2). The mean plasma immunoreactive inhibin (ir-inhibin) concentrations decreased abruptly after the aspiration procedure ($P < 0.05$) and subsequently gradually increased as the new follicles and cystic follicles developed. The mean plasma follicle-stimulating hormone (FSH) concentration increased rapidly after the aspiration procedure and remained significantly high ($P < 0.05$) for 1–2 days, before returning to a low level.

In the present study, it was revealed that no luteinization or luteal tissue formation occurred in the cystic follicles or coexisting normal-sized follicles after fluid aspiration. It was also shown that a new set of follicles developed after the aspiration procedure and continued to grow without ovulation and subsequently became cystic follicles again. It was previously demonstrated that in the cases of CCL, the enriched CL was remodeled after the aspiration of the central fluid or the finger rupture of the structure [9, 10]. This difference in the cystic follicles and CCLs is very important and useful for clinically differentiating the two structures. The current study clearly demonstrated that follicular fluid aspiration from cystic follicles had no beneficial effect on luteinization or luteal tissue formation in ovarian follicular cysts.

To the best of our knowledge, none of the previous studies have examined changes in the peripheral concentrations of P_4, estradiol-17β (E_2), inhibin, LH, and FSH that occur after fluid aspiration from cystic follicles and the coexisting normal-sized follicles in cows with ovarian follicular cysts. Similar to the findings of the present study, Todoroki *et al*. [11] reported a marked reduction in the peripheral concentrations of E_2 and ir-inhibin and marked increases in the peripheral concentrations of LH and FSH were observed after the aspiration of fluid from follicles in cyclic cows; subsequently, a cohort of small follicles started to grow. It is known that inhibin secreted by the follicles controls the secretion of FSH in cows [12–14], goats [15, 16], and mares [17, 18]. In the present study, the plasma concentrations of ir-inhibin decreased and the plasma concentration of FSH increased markedly after the aspiration of the follicular fluid. It was suspected that the follicular production of inhibin was disrupted by the aspiration of the follicular fluid, and consequently, the plasma concentration of FSH increased due to the absence of negative feedback mechanisms involving ir-inhibin.

Several studies have reported on follicle growth after increases in FSH concentration [7, 12, 16, 19–23], the continuous growth of follicles in response to LH stimulation [24], and the reduction in the FSH levels induced by an increased secretion of E_2 and inhibin from growing follicles [12, 16, 25–28]. Ginther *et al*. [21, 22] and Kaneko *et al*. [12] reported in detail regarding the follicle selection that occurred in a cohort of small follicles, which started to grow after an increase in the FSH level, i.e., follicle deviation; finally, one follicle was selected as the dominant follicle, and the others remained as subordinate follicles. The changes in the peripheral concentrations of inhibin and FSH and new follicular development observed after the aspiration of fluid from the cystic follicles and coexisting normal-sized follicles in the present study agreed with the findings of the above-mentioned studies.

When the blood concentration of P_4 decreases to a low level (≤ 1 ng/ml) and the blood concentration of E_2 increases to a high level, as occurs in the estrous phase, an LH surge is usually generated by the positive feedback effects of E_2 in cyclic cows. In contrast, it was reported that ovulation did not occur because no LH surge was generated in cows with ovarian follicular cysts [3, 4, 7, 11, 29]. Based on these and the present results, it is suspected that the aspiration of follicular fluid from a cystic follicle results in artificial turnover of the cystic follicle; however, it has little therapeutic effect on the associated endocrine disturbance.

In conclusion, the aspiration of fluid from cystic follicles and coexisting normal-sized follicles did not induce luteinization or luteal tissue formation in the aspirated structures. After the aspiration procedure, the plasma levels of ir-inhibin decreased markedly, and the plasma levels of FSH increased. The plasma levels of LH and P_4 did not change significantly. A cohort of newly developed follicles appeared 2–3 days after the aspiration procedure, and one of them continued to develop and matured. The mature follicle continued to grow and reached ≥ 25 mm in diameter, i.e., cystic follicles, forming an ovarian follicular cyst again. Therefore, referring to previous evidence regarding CCL, the aspiration of the central fluid from cystic structures, such as follicular cysts and CCL is postulated as an effective method for the clinical differential diagnosis of cystic structures.

Methods

In this study, three lactating Holstein cows from dairy farms in Chiba Prefecture, Japan, were selected. They were subjected to critical diagnostic examinations for follicular cysts at 3–4 day intervals for 2–3 weeks, which were based on the confirmation of the presence of cystic follicular structures measuring >25 mm

in diameter that did not ovulate and the absence of CL formation, according to rectal palpation and ultrasonographic examinations [25, 26]. None of the three cows were treated with any hormone preparations for > 30 days before the start of the experiment. The three cows were consequently confirmed to have ovarian follicular cysts based on their low plasma P_4 concentrations (< 1 ng/ml) during the experimental period. On the day of aspiration of follicular fluid (Day 0), the ages of Cow Nos. 20, 22, and 25 were 5, 5, and 4 (mean 4.7 ± 0.6 (standard deviation)) years; their parities were 2, 3, and 2 (mean 2.3 ± 0.6); their postpartum periods were 575, 184, and 175 (mean 311.3 ± 228.4) days; the number of inseminations they received since their last parturition were 9, 2, and 2 (mean 4.3 ± 4.0), and their body weights, as estimated based on their chest girth, were 789, 664, and 685 (mean 712.7 ± 66.9) kg, respectively. All experimental procedures were approved by the University Committee for the Use and Care of Animals, Tokyo University of Agriculture and Technology (No. 25-36).

The ovarian structures were examined by rectal palpation and ultrasonography every 2 or 3 days for 59–95 days during the experimental period to observe the growth or regression of each structure. Additionally, the cows were subjected to follicular fluid aspiration, as described below, and subsequently were examined for ovarian changes and blood samples were collected every day for a period of 5 days. A 5-MHz transducer and linear-array ultrasound scanner (SSD-210DXII, Aloka, Tokyo, Japan) was used for the examinations. The follicles were depicted as echo-free round areas and the major and minor axes of the follicles were measured on the largest images of the follicles using the virtual calipers of the scanner. The follicle diameters were calculated by averaging the major and minor axes [27]. At the same time as the ovarian examinations, peripheral blood samples were collected in heparin tubes from the tail vein by venipuncture, and the plasma was separated out within 30 min of the sample collection by centrifuging the samples at 3,000 rpm for 30 min. The plasma samples were frozen and kept at –20ºC until the hormone assay.

Two cows (Nos. 20 and 22) underwent follicular fluid aspiration from one growing cystic follicle when they reached ≥ 25 mm in diameter, two regressing cystic follicles, and one coexisting normally sized follicle. In the other cow (No. 25), follicular fluid was aspirated from three growing follicles when they reached a diameter of 20 mm and one regressing cystic follicle. The follicular fluid aspiration was carried out 18–22 days after the start of routine examination of ovarian structures. The follicular fluid was aspirated using an ovary syringe (Kaneda type). The syringe was inserted aseptically into the vagina using a colposcope; subsequently, the ovary was held by hand using rectal palpation, the needle of the syringe was inserted into the follicular cavity, and the follicular fluid was aspirated as calmly as possible. After the aspiration procedure, an ultrasonographic examination was performed to confirm that the follicular fluid had been removed, i.e., it was confirmed that no follicular fluid or follicles ≥ 5 mm in diameter remained (Supplementary Fig. 1). The day of the follicular fluid aspiration procedure was designated as day 0.

The plasma levels of P_4 and E_2 were measured in duplicate by subjecting aliquots of plasma to a specific radioimmunoassay (RIA), as described previously [28]. The sensitivity of the P_4 assay was 54.7 pg/tube; additionally, the intra- and inter-assay coefficients of variation were 10.0% and 10.2%, respectively. The sensitivity of the E_2 assay was 1.2 pg/tube, and the intra- and inter-assay coefficients of variation were 5.7% and 1.4%, respectively. The plasma concentration of ir-inhibin was measured in duplicate using a double-antibody RIA, as described by Hamada *et al.* [29]. The sensitivity of the inhibin assay was 9.6 ng/ml, and the intra-assay coefficient of variation was 10.0%. The plasma concentrations of LH and FSH were measured in duplicate using a double-antibody RIA [30]. The sensitivity of the LH assay was 0.7 ng/ml, and the intra-assay coefficient of variation was 35.2%. The sensitivity of the FSH assay was 0.59 ng/ml, and the intra-assay coefficient of variation was 7.1%. The ir-inhibin, LH, and FSH were assayed at one time, respectively.

The significance of the differences was determined using a two-sided paired *t*-test. In this case, the average values were calculated for approximately 6 days prior to (day –6 to day 0) and 3 days after the aspiration (day 1 to day 3) in individual cows. Additionally, the test was carried out utilizing these pre- and post- individual average values of each hormone. The statistical software (Yanai H, Statcel -The useful addin forms on Excel- 2nd ed. Tokorozawa: OMS, 2004; Japan) was used. The differences were considered significant at $P < 0.05$.

Conflict of interests: The authors have nothing to declare.

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References

1. **Roberts JS.** Infertility in the cow. *In:* Roberts JS (ed.), Veterinary obstetrics and genital diseases (Theriogenology). 3rd ed., Michigan: Edwards Brothers, Inc.; 1986: 434–580.
2. **Kamomae H, Iwamura S, Yosioka K, Taniguti T, Satou M, Tanaka S, Nagasawa S, Siono H, Takahasi Y, Oohasi T, Motoi Y, Hiramatu M.** Clinico-endocrinological biochemical and histopathological analyses on ovarian cyst in cow. *Tohoku J Vet Clin* 1995; **18**: 55–64. (In Japanese). [CrossRef]
3. **Yoshioka K, Iwamura S, Kamomae H.** Ultrasonic observations on the turnover of ovarian follicular cysts and associated changes of plasma LH, FSH, progesterone and oestradiol-17 β in cows. *Res Vet Sci* 1996; **61**: 240–244. [Medline] [CrossRef]
4. **Yoshioka K, Iwamura S, Kamomae H.** Changes of ovarian structures, plasma LH, FSH, progesterone and estradiol-17 β in a cow with ovarian cysts showing spontaneous recovery and relapse. *J Vet Med Sci* 1998; **60**: 257–260. [Medline] [CrossRef]
5. **Kengaku K, Tanaka T, Kamomae H.** Changes in the peripheral concentrations of inhibin, follicle-stimulating hormone, luteinizing hormone, progesterone and estradiol-17β during turnover of cystic follicles in dairy cows with spontaneous follicular cysts. *J Reprod Dev* 2007; **53**: 987–993. [Medline] [CrossRef]
6. **Cook DL, Smith CA, Parfet JR, Youngquist RS, Brown EM, Garverick HA.** Fate and turnover rate of ovarian follicular cysts in dairy cattle. *J Reprod Fertil* 1990; **90**: 37–46. [Medline] [CrossRef]
7. **Hamilton SA, Garverick HA, Keisler DH, Xu ZZ, Loos K, Youngquist RS, Salfen BE.** Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. *Biol Reprod* 1995; **53**: 890–898. [Medline] [CrossRef]
8. **Noakes D.** Endogenous and exogenous control of ovarian cyclicity. In: Noakes DE, Parkinson TJ, England GCW (eds.), Veterinary reproduction and obstetrics. 9th ed. Edinburgh: Saunders; 2009: 3–58.
9. **Kaneda Y, Matsuda K.** Treatment for the cystic corpus luteum and its influence on fertility in the dairy cow. *Jpn J Anim Reprod* 1970; **15**: 134–138. (In Japanese with English summary). [CrossRef]
10. **Kaneda Y, Domeki I, Nakahara T.** Effects of removal of cystic fluid from cystic corpus luteum on luteinization and conception rate in dairy heifers. *Jpn J Anim Reprod* 1980; **26**: 37–42. (In Japanese with English summary). [CrossRef]
11. **Todoroki J, Yamakuchi H, Mizoshita K, Kubota N, Tabara N, Noguchi J, Kikuchi K, Watanabe G, Taya K, Kaneko H.** Restoring ovulation in beef donor cows with ovarian cysts by progesterone-releasing intravaginal silastic devices. *Theriogenology* 2001; **55**: 1919–1932. [Medline] [CrossRef]
12. **Kaneko H, Kishi H, Watanabe G, Taya K, Sasamoto S, Hasegawa Y.** Changes in plasma concentration of immunoreactive inhibin, estradiol and FSH associated with follicular waves during the estrous cycle of the cow. *J Reprod Dev* 1995; **41**: 311–320.

[CrossRef]
13. **Akagi S, Kaneko H, Nakanishi Y, Takedomi T, Watanabe G, Taya K.** Ovarian response and FSH profile in cows following injection of various doses of inhibin antiserum. *J Vet Med Sci* 1997; **59**: 1129–1135. [Medline] [CrossRef]
14. **Bleach ECL, Glencross RG, Feist SA, Groome NP, Knight PG.** Plasma inhibin A in heifers: relationship with follicle dynamics, gonadotropins, and steroids during the estrous cycle and after treatment with bovine follicular fluid. *Biol Reprod* 2001; **64**: 743–752. [Medline] [CrossRef]
15. **Medan MS, Watanabe G, Sasaki K, Nagura Y, Sakaime H, Fujita M, Sharawy S, Taya K.** Effects of passive immunization of goats against inhibin on follicular development, hormone profile and ovulation rate. *Reproduction* 2003; **125**: 751–757. [Medline] [CrossRef]
16. **Medan MS, Watanabe G, Sasaki K, Sharawy S, Groome NP, Taya K.** Ovarian dynamics and their associations with peripheral concentrations of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. *Biol Reprod* 2003; **69**: 57–63. [Medline] [CrossRef]
17. **Nagamine N, Nambo Y, Nagata S, Nagaoka K, Tsunoda N, Taniyama H, Tanaka Y, Tohei A, Watanabe G, Taya K.** Inhibin secretion in the mare: localization of inhibin α, betaA, and betaB subunits in the ovary. *Biol Reprod* 1998; **59**: 1392–1398. [Medline] [CrossRef]
18. **Nagaoka K, Arai KY, Nagamine N, Nambo Y, Nagata S, Tanaka Y, Shimbo H, Taniyama H, Tsunoda N, Yamanouchi K, Hondo E, Yamada J, Watanabe G, Taya K.** Expression of inhibin/activin subunits, aromatase and 3β-hydroxysteroid dehydrogenase in various sized ovarian antral follicles of the mare. *J Equine Sci* 2001; **12**: 77–83. [CrossRef]
19. **Bergfeld EGM, Kojima FN, Cupp AS, Wehrman ME, Peters KE, Mariscal V, Sanchez T, Kinder JE.** Changing dose of progesterone results in sudden changes in frequency of luteinizing hormone pulses and secretion of 17 β-estradiol in bovine females. *Biol Reprod* 1996; **54**: 546–553. [Medline] [CrossRef]
20. **Bodensteiner KJ, Kot K, Wiltbank MC, Ginther OJ.** Synchronization of emergence of follicular waves in cattle. *Theriogenology* 1996; **45**: 1115–1128. [Medline] [CrossRef]
21. **Ginther OJ, Kot K, Wiltbank MC.** Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrous cycle in ewes. *Theriogenology* 1995; **43**: 689–703. [Medline] [CrossRef]
22. **Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K.** Selection of the dominant follicle in cattle. *Biol Reprod* 1996; **55**: 1187–1194. [Medline] [CrossRef]
23. **Medan MS, Watanabe G, Sasaki K, Groome NP, Sharawy S, Taya K.** Follicular and hormonal dynamics during the estrous cycle in goats. *J Reprod Dev* 2005; **51**: 455–463. [Medline] [CrossRef]
24. **Stock AE, Fortune JE.** Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. *Endocrinology* 1993; **132**: 1108–1114. [Medline] [CrossRef]
25. **Youngquist RS.** Cystic follicular degeneration in the cow. In: Morrow DA (ed.), Current Therapy in Theriogenology 2. Philadelphia: W. B. Saunders Company; 1986: 243–246.
26. **Garverick HA.** Ovarian follicular cysts in dairy cows. *J Dairy Sci* 1997; **80**: 995–1004. [Medline] [CrossRef]
27. **Pierson RA, Ginther OJ.** Ultrasonic imaging of the ovaries and uterus in cattle. *Theriogenology* 1988; **29**: 21–37. [CrossRef]
28. **Taya K, Watanabe G, Sasamoto S.** Radioimmunoassay for progesterone, testosterone and estradiol-17β using ^{125}I-iodohistamine radioligands. *Jpn J Anim Reprod* 1985; **31**: 186–197. (In Japanese). [CrossRef]
29. **Hamada T, Watanabe G, Kokuho T, Taya K, Sasamoto S, Hasegawa Y, Miyamoto K, Igarashi M.** Radioimmunoassay of inhibin in various mammals. *J Endocrinol* 1989; **122**: 697–704. [Medline] [CrossRef]
30. **Suganuma C, Kuroiwa T, Tanaka T, Kamomae H.** Changes in the ovarian dynamics and endocrine profiles in goats treated with a progesterone antagonist during the early luteal phase of the estrous cycle. *Anim Reprod Sci* 2007; **101**: 285–294. [Medline] [CrossRef]