ウシの妊娠中期に出現する卵胞と血中性ステロイドホルモンの関係

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Relationship between ovarian Follicles and Peripheral Levels of Sex Steroid Hormones during Early Midpregnancy in Cows

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ABSTRACT. A transrectal real-time ultrasound scanner was used to study the structures of the ovaries during early midpregnancy in cows. The serum concentrations of sex steroids between cows with large and small or no follicles in the ovaries were not significantly different. The presence of large follicles in the ovaries was not consistently associated with an increase in serum estrogen levels and did not have a luteolytic effect on the corpus luteum of pregnancy. The changes in the serum concentration of sex steroids before and after the puncture of large follicles in the ovaries of 4 pregnant cows were also examined, together with six steroids concentration in the follicular fluid. The findings suggest that although large follicles are present in the ovaries of cows during midpregnancy, they do not significantly contribute to peripheral levels of sex steroids.—KEY WORDS: follicle, pregnancy, sex steroids, ultrasonic scanning.

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The ovaries of the new born calves are in the surprisingly advanced stage of development with visible follicles present. A prepuberal heifer has follicles as large as 12 mm in diameter but it is not until proestrus or estrus that a follicle makes a pre-ovulatory growth when it may reach a diameter of 16 to 19 mm [11]. Follicles below 10 mm in diameter are always present even in pregnant cows [21]. It is also well-documented that cows may show estrous behavior associated with follicular activities in the ovaries during pregnancy especially in the first 3 months [1, 3]. However, the role of these follicles is not yet clearly understood during midpregnancy. Follicles are sometimes present in the case of abortion due to progesterone deficiency occurring prior to 100 days of pregnency [13]. It has been considered that a large follicle present may raise estrogen levels, lead to involution of the corpus luteum and terminate pregnancy [15]. The objectives of the present study are: a) to correlate the presence of follicles in the ovary with serum concentration of sex steroids and b) to determine if the presence of large follicles in the ovary has luteolytic effect on the corpus luteum (CL) at midpregnancy.

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MATERIALS AND METHODS

Animals: A total of 12 pregnant Holstein-Friesian cows/heifers from the herd of Obihiro University Dairy Farm were used. They were divided into 3 groups (4 animal each) as shown in Table 1. The ovaries of Group I and II animals were subjected to the ultrasonic scanning every 4 days. The overies of Group III animals were examined at the start of the experiment, 4 days later, and before the puncture and aspiration of follicular fluid from the large follicles. Thereafter, and 2 more times at 3 days intervals the ultrasonic scanning was also performed.

Equipment and recording method: The

Table 1. Diameter of the follicle, location of the follicle and the corpus luteum in the ovary

Group	Cow No.	Age (months)	Stage of pregnancy (days)	Follicular diameter (range in mm)		Location	
						Follicle	Corpus luteum
I[a)]	1	21	90–102	0–10		left	right
	2	87	90–102	9–10		left	right
	3	55	90–102	0– 8		right	right
	4	45	90–102	9–11		left	left
II[b)]	5	21	90–102	15–15		left	right
	6	33	90–102	15–15		right	left
	7	67	90–102	12–16		left	right
	8	82	90–102	13–14		left	right
				Before puncture	*After puncture*		
III[c)]	9	36	90–102	12–16	0–0	left	right
	10	57	90–102	15–16	0–0	left	right
	11[d)]	65	90–102	14–18	0–0	left	right
	12[d)]	82	96–108	12–16	0–0	left	right

a) Cows with small or no follicle (smaller than 12 mm in diameter).
b) Cows with large follicle (12 mm or more in diameter).
c) Cows with large follicle which is punctured later.
d) More than one large follicle in the same ovary.

real-time scanner (Aloka SSD-21 DX, Aloka Co., Tokyo) was used with a 5.0 MHz transducer. The transducer was introduced into the rectum and directed towards the ovary. The folliclar size was measured by freezing an appropriate image on the display unit with an aid of a built-in caliper. Photographic recordings were made using Polaroid 667 film and a Polaroid camera (Polaroid Corp., Massachusetts).

Hormone assay: Approximately 20 m*l* of blood was collected from the coccygeal vein. The serum was separated by centrifugation at 3000 rpm for 10 minutes and then stored at −20°C until it could be assayed. Blood was collected at the start of the experiment, then every other day. After the puncture of the large follicles in Group III animals, blood collections were done daily for 6 days. The concentration of estrone, estradiol-17β and progesterone in the serum was determined by respective radioimmunoassays reported by Makino [10]. Follicular fluid was collected with a needle introduced into the peritoneal cavity via the dorsal fornix of the vagina in a manner reported by Short [17]. The fluid was then placed in a freezer at −20°C until assayed. The assay procedure was essentially similar to that described for radioimmunoassay of serum steroids.

Statistical analysis: The data were analyzed using Student's t-test.

RESULTS

Ultrasonography of the ovary: The corpus luteum of pregnancy was found in the right ovary in 10 of the 12 animals used in this study. Large follicles were often located in the opposite ovary containing the CL as shown in Table 1. Follicles ranging between 8 and 11 mm in diameter were occasionally observed in Group I animals. In Group II animals, follicles ranging between 12 and 16 mm in diameter were continuously observed throughout the examination period. One or more follicles were found in the same ovary in 2 of the 4 pregnant cows

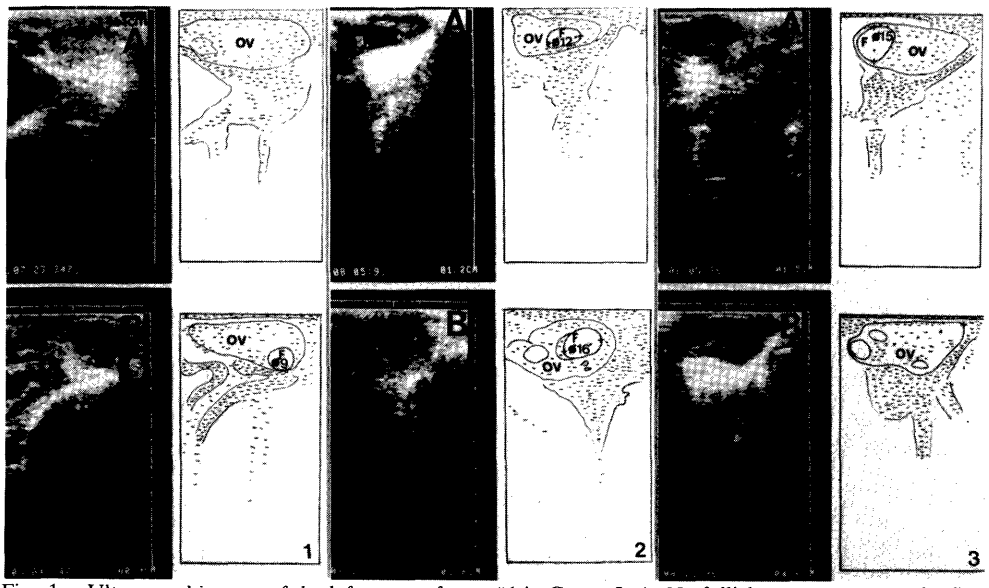

Fig. 1. Ultrasound images of the left ovary of cow #1 in Group I. A: No follicle was present on the first day of examination. B: A small follicle (9 mm) was present on the last day of examination. OV: Ovary. F: Follicle. ϕ: Diameter (mm).

Fig. 2. Ultrasound images of the left ovary of cow #7 in Group II. A: A large follicle (12 mm) was present on the first day of examination. B: The same follicle but had increased in size (16 mm) was present on the last day of examination. OV: Ovary. F: Follicle. ϕ: Diameter (mm).

Fig. 3. Ultrasound images of the left ovary of cow #10 in Group III. A: A large follicle (15 mm) was present on the first day of examination. B: The left ovary 6 days after the puncture of the large follicle. No follicle was present. OV: Ovary. F: Follicle. ϕ: Diameter (mm).

Table 2. Concentrations of sex steroid hormones in the serum (mean±S.D) during midpregnancy

Sex steroid hormone	Group	Number of animals	Days of pregnancy						
			90	92	94	96	98	100	102
Progesterone (ng/m*l*)	I	4	6.6±2.9	5.7±3.4	6.4±1.5	6.4± 2.5	6.2±1.6	6.1± 3.7	8.3± 3.1
	II	4	5.4±3.3	7.2±2.8	6.6±3.4	3.5± 2.5	5.1±3.4	6.2± 3.6	7.3± 3.2
Estrone (pg/m*l*)	I	4	17.4±6.7	13.9±6.6	14.3±4.6	20.0±10.5	17.9±7.1	20.9±10.0	22.2±10.6
	II	4	13.1±2.8	13.6±3.7	14.1±2.6	14.8± 1.6	13.2±1.8	15.7± 2.8	15.9± 1.3
Estradiol-17β (pg/m*l*)	I	4	7.4±3.1	8.4±4.2	9.1±4.6	9.9± 4.6	9.0±4.8	10.5± 2.9	10.8± 5.1
	II	4	10.7±4.8	10.6±5.3	8.1±4.1	11.4± 2.6	7.6±4.3	5.9± 1.7	5.9± 3.5

of Group III, and the largest follicle having a diameter of 18 mm. In each case, the diameter of the largest follicle was recorded (Figs. 1–3).

Serum steroid concentration in Group I and II animals: Changes in serum progesterone, estrone and estradiol-17β from 90 to 102 days of pregnancy are indicated in Table 2. The mean progesterone concentration ranged from 5.7±3.4 to 8.3±3.1 ng/m*l* in Group I and from 3.5±2.5 to 7.3±3.2 ng/m*l* in Group II animals. No significant difference ($P>0.05$) was observed between the 2 groups and the progesterone level in the serum was within the normal range for pregnant cows. The mean serum estrone

Table 3. Concentration of sex steroid hormones in the serum (mean±S.D) before and after puncture of large follicles in Group III animals

	Cow No.	Before puncture	After puncture
Progestrone (ng/m*l*)	9	6.5± 1.7	6.5±2.5
	10	7.4± 3.2	7.9±2.6
	11	5.7± 2.2	6.8±3.1
	12	7.6± 3.3	4.1±2.3
Estron (pg/m*l*)	9	12.4± 2.1	20.2±2.9
	10	10.5± 3.6	10.6±7.6
	11	7.5± 2.7	15.6±8.6
	12	17.9±10.2	22.9±4.5
Estradiol-17β (pg/m*l*)	9	8.4± 2.1	5.2±3.6
	10	4.4± 2.6	5.5±3.4
	11	16.1± 2.6	13.7±2.7
	12	4.4± 2.6	5.5±3.4

Table 4. Concentration of sex steroid hormones in the follicular fluid (mean±S.D) of Group III cows

Number of animals	Progesterone (ng/m*l*)	Estrone (ng/m*l*)	Estradiol-17β (ng/m*l*)
4	59.2±31.4	2.6±1.3	15.5±11.2

concentration ranged from 13.9±6.6 to 22.2±10.6 pg/m*l* in Group I and 13.1±2.8 to 15.9±1.3 pg/m*l* in Group II animals with no significant difference ($P>0.05$) between the 2 groups. The mean serum estradiol-17β concentration was lower than that of estrone and ranged from 7.4±3.1 to 10.8±5.1 pg/m*l* in Group I and 5.9±1.7 to 11.4±2.6 in Group II animals with no significant difference ($P>0.05$) between the 2 groups.

Serum steroid concentration in Group III animals: The mean serum sex steroid hormone concentration before and after the puncture of the large follicles in the ovaries are shown in Table 3. The aspiration of the follicular fluid did not cause a significant change ($P>0.05$) in the serum progesterone and estradiol-17β concentrations. The mean concentration of estrone before the puncture was lower than that after the puncture in all the 4 pregnant cows, but the difference was not significant.

Steroid concentrations in the follicular fluid of Group III animals: Progesterone was found to be the major sex steroid present in the follicular fluid and estradiol-17β, the second highest. Estrone was also present but in much smaller amounts (Table 4).

DISCUSSION

The CL of pregnancy resided in the right ovary in 10 of the 12 cows and this agrees with the previous findings [5, 8] that the right ovary is more active than the left one. The percentage (83%) observed in this study is still higher than that (60%) reported [5, 8]. Serum levels of progesterone in Group I and II animals were generally comparable to those reported by other authors [14, 16] and fell within a range capable to maintain pregnancy. No significant difference in the serum progesterone levels nor the CL images was found between the 2 groups. These findings suggest that the presence of large follicles in the ovary during midpregnancy does not cause regression of the CL.

Significant difference between the concentration of estrogen in the serum of

Group I and II animals was not observed. Nor did puncture of the large follicle and aspiration of the follicular fluid cause a significant change in the serum estrone and estradiol-17β. The mean serum concentration of estrone rose after the puncture. The above findings suggest that there are other sources of estrogens, particularly estrone during midpregnancy aside from the follicles examined. It has been considered that the stromal tissue of the ovary being distinct from the follicle and the CL may possess steroidogenic properties [4]. Other authors [9, 12, 19] suggested that the main estrogen during pregnancy was derived from extraovarian sources, notably from the placenta.

Although it has been clearly demonstrated that exogenous estrogens, particularly estrone and estradiol-17β are luteolytic in the cow [2, 7], the findings in this study suggest that endogenous levels of estrogens are non-luteolytic. It appears on the other hand that a certain amount of estrogen of placental or ovarian origin is necessary during pregnancy.

In cycling cows, estradiol-17β is produced by the theca interna cells of the growing follicle under the influence of FSH and LH [18]. Estradiol-17β induces the typical changes in the tubular portion of the genital tract during proestrus and estrus together with estrous behavior by its action on the central nervous system. During pregnancy, especially in the first 3 months, cows may show estrous behavior due to the presence of functional follicles in the ovary. However, during midpregnancy, the role of these follicles is not yet well understood. Estrous behavior was not observed in our present study even when a large follicle was found in the ovary. The follicular fluid from 4 pregnant cows contained progesterone as a dominant steroid hormone, while estrone and estradiol-17β were low. According to Short [18] the follicular fluid from estrous cows contains high estradiol-17β and low progesterone and the fluid from follicular cysts, high progesterone and low estrogens. The latter constituent resembles the result of our study in pregnant cows. The follicles found in pregnant cows at midpregnancy may be undergoing cystic or atretic changes. Atretic or degenerating follicles like follicular cysts contain low estrogen and high progesterone due to impaired ability to convert progesterone into strone and estradiol-17β [6, 18, 20]. This could explain why the follicular fluid samples in this study contained high progesterone and low estrogen. Histological study is now under way to ascertain if follicles in the ovary of pregnant cows are atretic or under degeneration.

Although high progesterone concentration was observed in the follicular fluid samples of the 4 pregnant cows, it is doubtful whether a certain percentage of this hormone would be transferred into the circulation. Puncture of the large follicles did not significantly influence the concentration of progesterone in the serum. Most of peripheral progesterone necessary for the maintenance of pregnancy must be derived from the CL.

The physiological role of the large follicles in the ovary of cows during midpregnancy is still to be defined. However, the results of the present study suggest that they do not significantly contribute to the concentration of sex steroid hormones in the serum.

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要　約

ウシの妊娠中期に出現する卵胞と血中性ステロイドホルモンの関係：RAYOS, A. A., 宮澤清志・奥田　潔（帯広畜産大学家畜臨床繁殖学教室）——超音波断層診断装置を用いて，妊娠牛を卵胞直径が12mm 以下の群，および12mm 以上で卵胞を穿刺破砕した群としない群の3群に分け，血中性ステロイドホルモン値の変化を観察した。小卵胞群と大卵胞群の血中性ステロイドホルモン値に差は見られず，また，卵胞穿刺破砕の前後において血中性ステロイドホルモン値に変動はなかった。穿刺破砕卵胞では卵胞液中プロジェステロン値が高値を示した。以上の結果から，妊娠中期に出現する卵胞は機能的に発情期卵胞と異なり，血中性ステロイドホルモン値に影響を及ぼさないことが判明した。