Postpartum Resumption of Ovarian Activity and Uterine Involution Monitored by Ultrasonography in Holstein Cows

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ABSTRACT. Postpartum resumption of ovarian activity in 40 Holstein cows was monitored by ultrasonography twice weekly until artificial insemination. The accuracy of ultrasonography for assessments of ovarian structures was examined by comparing results of *in vivo* ultrasonography with macroscopic findings of the same ovaries after slaughter. Correlation coefficients were 0.71 and 0.85 for number of follicles 10–14 mm and ≥15 mm, and 0.99 for diameters of the largest follicle. Follicular profiles prior to first ovulation were characterized by single dominant follicle (DF≥10 mm) in 25 cows, two in 10, three in 4, and four in one, respectively. However, after first ovulation, two waves of DF prevailed. The total number of DF (7.2) or time of ovulation (3.6) before conception was positively correlated with postpartum intervals to conception (74.0 days). Profiles of the volume of corpus luteum estimated by ultrasonography paralleled with the variations of plasma progesterone levels. The volume of corpus luteum and the peak progesterone level were smaller after the first ovulation as compared with after the second or third ovulations. In the ultrasound images of uterus, two elliptical lines indicated cross section of endometrium and stratum vascularis. Uterine involution assessed by reaching the nadir of endometrium was completed by 41.5 days postpartum. Results indicated that the number of DF before the first ovulation and the volume of corpus luteum after the ovulation were smaller compared with those of the second and third ovulations.—KEY WORDS: cattle, dominant follicle, involution of uterus, ovarian activity, ultrasonography.

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In the earlier studies by Pierson and Ginther [10, 11] and Reeves *et al.* [13], ultrasonography has been used to monitor normal and pathological conditions of the bovine reproductive organs, and was evaluated to be useful in the dairy herd management [2, 6, 12]. However, ultrasonography has mainly been used to determine the normal estrous cycle and to diagnose early pregnancy in cows [2, 10–13]. Early re-establishment of cyclic ovarian function and post parturient recovery of the uterus are essential to keep a 365-day calving interval. However, numerous factors, such as abnormal pregnancy, dystocia, retained placenta, uterine infection, nutritional deficiencies etc. may affect subsequent reproductive performances in cows [5, 9, 18]. Since ultrasonographic information about bovine reproductive organs in the early postpartum period has been limited [7, 8, 15, 16], sequential postpartum ovarian activities and morphological process of uterine involution have not been clearly understood.

The objective of this study is to evaluate the accuracy of diagnostic ultrasonography for assessment of ovarian structures and to determine the resumption of ovarian activity as well as the progress of uterine involution in the early postpartum period using ultrasonography in Holstein cows.

MATERIALS AND METHODS

Animals: Forty Holstein cows were fed with wilted timothy silage and concentrates under controlled nutrition based on the Japanese feeding standard for dairy cattle [1]. No chronic illness and serious infertility problems were there in these cows. The number of parity was 2 to 7 in this herd. Their parturitions were normal. They were in good body conditions, being free from major periparturient diseases. The herd averages of milk and butter fat were 7,491 and 275 kg, respectively. The cows conceived within 100 days postpartum, averaging 74.0 ± 13.9 (s.d.) days by 1.45 ± 1.0 artificial inseminations.

Ultrasonography: After seven days postpartum, reproductive organs of cows were examined twice weekly by the same veterinarian using a linear array ultrasound scanner equipped with two kinds of transducer probe, a 7.5 MHz and a 5 MHz (SSD-630, Aloka, Tokyo, Japan). Each organ was scanned on several directions, and a video-tape recordings were made to be precisely reviewed.

In order to define the ultrasound images of the ovary and uterus *in situ*, four non-pregnant, multiparous cows (> 100 days postpartum) with clinically normal reproductive organs were examined ultrasonically immediately before slaughter, and the measurement was compared with the same organs after slaughter. Statistical correlations were run between the measurements derived from the ultrasonic images and the actual measurements of structures of the excised organs.

Follicles ≥ 2 mm were detectable and a dominant follicle (DF) was defined as the largest follicle in an ovary ≥ 10 mm in diameter in the absence of other large follicles [14]. In addition, ultrasonic cross-sectional image of a corpus luteum was frozen at its maximum, and the spheroidal area was calculated by the scanner. As the corpus luteum was assumed to be spherical in shape, the volume of corpus luteum was estimated by multiplying 4/3 × maximum spheroidal area × (height of corpus luteum/2 + width of corpus luteum/2)/2. Luteinized portion of the

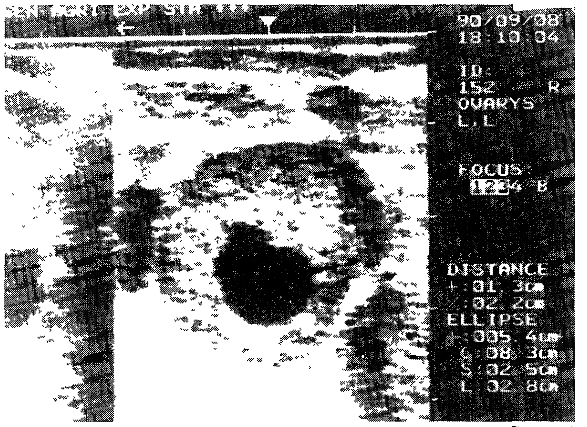

Fig. 1. Ultrasound image of corpus luteum *in situ*. Central cavity is fluid-filled and appears black on the ultrasound image. The luteal tissue is distinguished by a distinct border and different echo texture from stroma. Boundaries of the luteal tissue are demarcated by + marks.

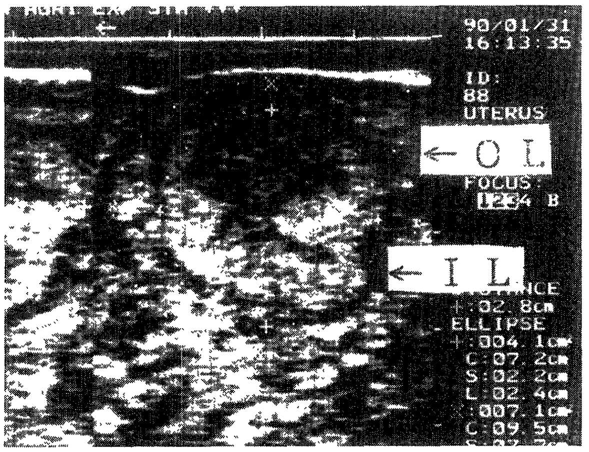

Fig. 2. Cross sectional images of the one uterine horn *in situ*. Two elliptical lines are recognized. Outer line (OL) demarcated by × marks represents the stratum vasculare. Inner line (IL) demarcated by + marks represents the endometrium.

corpus luteum (luteal tissue volume) was estimated by subtracting central luteal cavity (Fig. 1) from total corpus luteum as described by Kastelic *et al.* [6]. In the ultrasound images of uterus *in situ*, two elliptical lines indicated the cross section of the endometrium and stratum vascularis (Fig. 2). Involutional changes of uterine cross sectional area of the endometrium were monitored and those of post gravid and non-gravid horns were compared. The completion of postpartum uterine involution was defined when the cross sectional area reached the minimum value.

Prior to each ultrasound examination, a blood sample was collected from the coccygeal vein or artery into a heparinized vacuum tube. After centrifugation, plasma samples were stored at −20°C and later on assayed for progesterone concentration using a solid phase 96-well microtitre plate enzyme immunoassay kit (Ovucheck, Cambridge Life Sciences plc, England).

Cows were checked for estrus twice daily with an aid of the Heat Mount Detector device stuck on the rump. All cows were inseminated at the first estrus occurring after 45 days postpartum. The day of conception was defined as the day of final insemination. Resumption of ovarian activity was evaluated by the postpartum interval, i.e., from calving to detection of first DF and ovulation as well as the number of DF before first ovulation. Number of DF and ovulation before conception were correlated with the postpartum interval to conception, and were expressed by linear correlation coefficients. Student's *t*-test was used for the statistical significance of differences between means [17].

RESULTS

The ultrasound measurements underestimated the number of follicles 2–4 mm by approximately two follicles, although there was no statistical significance (Table 1). Correlation coefficients were 0.71 and 0.85 for number of follicles 10–14 mm and ≥ 15 mm, and 0.99 for diameters of the largest follicle.

As for the number of DF identified before first ovulation, twenty five cows ovulated with the first DF, whereas two DF in 10 cows, three DF in 4 cows and four DF in one cow, respectively (Table 2). In 32 cows the first DF was detected in the ovary contralateral to the previous pregnancy, while in only 7 cows DF was identified ipsilaterally. Simultaneous growing and succeeding ovulation of DF in both side ovaries were observed in one cow. Postpartum interval to first ovulation was highly correlated with number of DF prior to first ovulation (r=0.886, P<0.01), and averaged 21.2 ± 9.6 days (range 10–55 days). Fewer DF were detected before the first ovulation than before the second and third postpartum ovulations (1.5 vs. 2.1 and 2.2, P<0.01, Table 3). The mean number of DF and time of ovulation, and postpartum intervals in day to conception were 7.2 ± 1.6, 3.6 ± 0.7 and 74.0 ± 13.9, respectively. When the postpartum interval to conception was correlated with the number of DF and with the time of ovulation before conception, linear correlation coefficients were high, $r^2=0.677$ (P<0.01) and $r^2 = 0.403$ (P<0.01), respectively.

Table 1. Mean number of follicles for the various diameter categories and mean diameter of the largest follicle, and correlation coefficients as determined by *in vivo* ultrasonography and slicing at slaughter (values are mean±s.d.)

Diameter of follicles	Technique *In vivo*	Technique Slicing	Correlation coefficient
Number of follicles			
2– 4 mm	14.8±2.2	16.3±3.3	0.57
5– 9 mm	4.0±0.7	3.8±0.8	0.43
10–14 mm	1.0±1.0	1.0±0.7	0.71
15– mm	1.3±0.8	1.0±0.7	0.85
Diameter of largest follicle (mm)	16.8±3.2	16.3±6.1	0.99

Table 2. Number of dominant follicles before first ovulation, and the interval from calving to the detection of first dominant follicle and first ovulation (values are mean±s.d.)

No. of dominant follicles before ovulation	No. of cows	Postpartum interval to Detection of first dominant follicle	Postpartum interval to First ovulation
	(head)	(days)	(days)
1	25	7.9±3.0	16.0±3.9
2	10	7.3±1.3	24.2±5.0
3	4	11.3±6.8	37.5±4.2
4	1	17	55
Total	40	8.3±3.7	21.2±9.6

Table 3. Number of dominant follicles and duration of ovulatory intervals in 40 postpartum dairy cows (values are mean±s.d.)

Interval[a)]	No. of dominant follicles	Duration
		(days)
Calving to first ovulation (40)	1.5±0.8	21.2±9.6
First to second ovulation (40)	2.1±0.6[c)]	21.7±7.3
Second to third ovulation (38)[b)]	2.2±0.4[c)]	20.7±4.6

a) Values in parentheses indicate the number of animals.
b) Two cows conceived after second ovulation by artificial insemination.
c) Statistically significant when compared with the interval from calving to first ovulation (P<0.01).

Table 4. Maximum volume of corpus luteum and plasma level of progesterone after first, second and third ovulation monitored twice weekly by ultrasonography (values are mean±s.d.)

Corpus luteum after ovulation[a)]	Volume of corpus luteum	Plasma progesterone
	(cm^3)	(*ng*/m*l*)
After first ovulation (40)[b)]	7.76±2.73	5.9±2.0
After second ovulation (40)	8.78±3.68	6.8±1.7
After third ovulation (38)	10.11±3.34[c)]	7.6±1.5

a) Values in parentheses indicate the number of animals.
b) A twin ovulation occurred in one cow at the first ovulation. In this case, the volume of each corpus luteum was combined and assumed to be single corpus luteum.
c) Statistically significant when compared with after first ovulation (P<0.05).

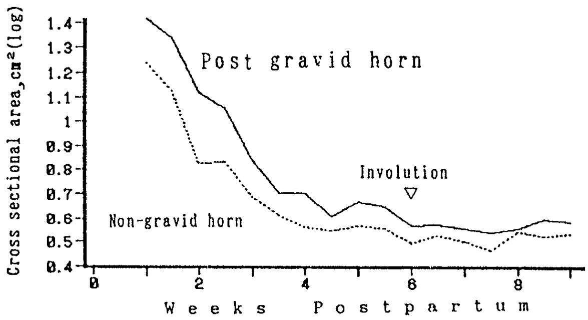

Fig. 3. Average figures of cross sectional area of uterine endometrium in post gravid and non-gravid horns. Involutional changes of uterus in 40 cows were monitored twice weekly.

The central fluid-filled cavity in the corpus luteum was identified in most cows during the growing period of luteal tissue area. In accordance with the volume of the corpus luteum, plasma progesterone level increased from 3 to 6 days after ovulation. On the day of ovulation, the plasma progesterone level in cows was below the sensitivity of the assay (<0.5 *ng*/m*l*), while the corpus luteum was still detectable by ultrasonography with the cross sectional area of 1.50 to 2.00 cm^2 and with estimated volume of 1.38 to 2.13 cm^3. Estimated volume of the corpus luteum after the third ovulation was significantly larger than that of the corpus luteum observed after the first ovulation (P<0.05, Table 4).

It was rather difficult to scan the uteri of six cows within 10 days postpartum due to their broader extension. However, the large lumen and the snowy appearance of lochia were visualized until approximately 18 days postpartum. At these stages, the whole uterine horn seemed to be edematous and less echogenic by ultrasound scanning. As the uterine involution progressed, the ultrasound image of uterine horn became more echogenic. Average figures of cross sectional area in post gravid and non-gravid uterine horns on successive days postpartum are presented by logarithm in Fig. 3. The mean cross sectional area in both uterine horns decreased rapidly during the first three weeks and then gradually involute to reach the nadir on 41.5 ± 5.8 days (range 30–54 days). This stage is defined as the completion of postpartum uterine involution, characterized by a resumed position within the pelvic cavity, and a contracted form with symmetrical diameter of uterine horn by rectal palpation. Once the uterine horn morphologically involute, the endometrial area fluctuated through the estrous cycle.

DISCUSSION

Ultrasonography proved to be useful in examining the ovarian activities by the evaluation of postpartum folliculogenesis, the number of DF before the ovulation and the volume of corpus luteum developed after the ovulation. Higher correlation coefficients between *in vivo* ultrasonography and the excised ovaries at slaughter indicated that diagnostic ultrasonography is an extremely accurate method for identifying and measuring follicles in cows.

The number of follicles showed mostly singular follicular wave prior to the first ovulation as compared with bimodal or trimodal waves prior to subsequent ovulations. Savio *et al.* [16] have described that cows with short estrous cycles developed only one DF and ovulated (5/5 cows), while those with long estrous cycles had either 2 (3/8 cows), 3 (4/8 cows) or 4 (1/8 cows) DF and cows with normal cycles, had mainly 2 DF. In accordance with Savio *et al.* [16], the present results indicated that the number of DF prior to first ovulation was mainly one (25/40 cows) or two (10/40 cows) and was highly correlated with postpartum intervals to the first ovulation. It is clear from foregoing results that the prolonged interval to first ovulation was due to failure of ovulation rather than failure of the development of DF. After the first ovulation, however, the mean DF was two or three during ovulatory intervals. Dufour and Roy [4] examined ovaries in a slaughter house and reported that postpartum folliculogenesis was suppressed in the ovary ipsilateral to the previous gravid horn. In the present study, the first DF was significantly identified in the ovary contralateral to the previous gravid horn (32/39 cases, one cow had a twin ovulation). Although the twice weekly intervals of ultrasonography were not sufficient enough to follow reliably the follicular turnover [15], the present results suggest that the postpartum interval to conception was highly correlated with the number of DF and the time of ovulations before conception.

After the first ovulation most corpora lutea (31/41 cases, one cow had a twin ovulation) retained central fluid-filled cavity which ceased eventually until subsequent ovulation. The presence or size of central fluid-filled cavity affected neither the plasma progesterone concentration nor the subsequent reproductive performance. The estimated volume of corpus luteum and plasma progesterone concentration increased with similar profiles during the luteal growth. However during luteal regression the estimated volume of corpus luteum decreased more slowly than the fall of plasma progesterone level, and still remained fairly large on the ovulation day. Duby *et al.* [3] reported that the corpus luteum removed after the first ovulation was smaller and contained fewer active cells than that obtained after subsequent ovulations. Likewise, in the present study a smaller volume of corpus luteum and lower plasma progesterone levels after the first ovulation compared with the findings after the second and third ovulations were observed. These results suggested that the ultrasonic assessment of the corpus luteum was a viable alternative to the determination of peripheral progesterone levels for assessment of luteal function.

Completion of uterine involution was observed by 41.5 days postpartum, which was similar to the previous study (40.0 days in [8]). It occurred close to the day of second ovulation (42.9 days). The indices of the postpartum recovery of ovarian function were evaluated by the folliculogenesis accompanied by ovulatory DF, exhibiting estrous behavior, and timely luteolysis before the next estrus [5]. DF that were developing in the presence of an active corpus luteum will undergo atresia due to absence of proper LH pulsatile pattern, and a LH surge [14]. If the newly formed corpus luteum after first ovulation persisted in the ovary beyond the length of the normal period, DF developing in the ovary would not ovulate, and also a case of uterine dysfunction could be suspected [6, 9]. In the present study, cows with longer ovulatory interval after first ovulation were inclined to have delayed uterine involution and consequently delayed conception. These results indicate that the early emergence of ovulatory DF, formation of corpus luteum and the succeeding timely luteolysis are necessary in order to trigger the ovarian estrous cycle and uterine involution in postpartum cows.

In conclusion, ultrasonic assessment of the bovine reproductive organs in postpartum period was evaluated to be effective for detecting follicular dynamics, volume of corpus luteum with fluid-filled cavity and involution process of uterine horns. The present results indicate that the number of DF before the first ovulation and the volume of corpus luteum developed after the first ovulation were smaller compared with those of the second and third ovulations.

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