Periodicity of Ovarian Follicular Dynamics in Postpartum Cows Demonstrated Using Time-Series Analysis Based on the Maximum Entropy Method

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Abstract. A specific decrease in the number of ultrasonographically identified follicles (uiFOL) in Japanese Black cows at 92 to 98 days after parturition had previously been reported. In the present study, the number of uiFOL in cows was observed until 500 days postpartum by an ultrasonic wave technique. The observation results showed that there are two specific decrease periods at about 303 to 308 days and 495 to 500 days after parturition. Also when superovulation treatments were done at these periods, many transferrable embryos were recovered. Therefore, these periods well resembled the previously reported decrease of uiFOL and embryo production at 92 to 98 days. To elucidate whether or not the variation of uiFOL including the specific decrease period resulted from a phenomenon with multiple periodicity or random variation, the ovarian follicular dynamics for 14 to 500 days after parturition were analyzed using the spectral analysis method based on the maximum entropy method (MEM). The variation in the number of uiFOL was constituted of a distinct periodic structure in each cow. In addition, the frequency distribution of the values estimated by MEM spectral analysis was investigated. As a result, five peaks were identified and a peak of 0.045 corresponded to 21 days postpartum. The other three peaks except for 0.03 corresponded to multiples of 21 days. Accordingly, the frequency distribution demonstrates that ovarian follicular dynamics is based on a 21-day cycle, and that it starts from parturition day.

Key words: Periodicity of follicle, Maximum entropy method, Superovulation, Cow, Mem Calc.


The estrous cycle in the cow has a regular 21-day pattern that cycles from ovulation, development, and degeneration of the corpus luteum to subsequent ovulation. The estrous cycle of cows includes three waves (peaks) that are caused by synchronous growth of the ovarian follicular population [1]. According to this report, the periodic duration time of each wave is about seven days and the largest follicle in the third wave ovulates. It is reasonable to propose that the ovulatory cycle appears to result from intrinsic periodic growth of the ovarian follicular population, includ-
ing the presence of many non-ovulatory follicles. Meanwhile, the estrous cycle including ovulation, development and degeneration of the corpus luteum is temporarily suppressed by pregnancy and starts again from parturition. Therefore, the time of parturition (day) in the reproductive cycle is presumed to be a significant triggering mechanism responsible for the resumption of the estrous cycle. Previously it was reported that the number of ultrasonographically identified follicles (uiFOL) clearly decreases at 92 to 98 days in all cows observed 14 to 147 days after parturition [2]. Moreover, many transferrable embryos were recovered by superovulation treatment using follicle stimulating hormone (FSH) at this period in comparison with another period, 120 to 207 days after parturition. This finding led to the idea that if the decrease period is specific and if it is related to an unknown periodic or rhythmic factor, it may also occur at other times when many follicles, induced by exogenous FSH, exist.

In this study, to elucidate this idea, the number of uiFOL in Japanese Black cows were investigated using an ultrasonic wave technique at 105 to 504 days after parturition was analyzed using a new computer program for time-series analysis, called “MemCalc,” based on the maximum entropy method (MEM).

Materials and Methods

Observation of uiFOL by Ultrasoundsonography

Eighteen non-lactating Japanese Black cows, 3 to 7 years of age with body weights of 450 to 550 kg were used. They were given 2 kg/day commercial concentrate pellet feed (11.5% CP [crude protein] and 63% TDN [total digestible nutrient]) and 5 kg/day timothy hay. The cows were examined for signs of estrus twice daily throughout the experimental period.

Four cows were divided into two equal pair, and uiFOL in these cows were observed for two periods, 105 to 392 and 399 to 504 days postpartum, by ultrasonography at 7-day intervals. This observation was performed as described in a previous report [2]. Briefly, a real-time B-mode ultrasound scanner equipped with a 5 MHz linear-array intrarectal transducer (model 210 DX; Aloka Co., Japan) was used. Round or elliptical follicles with a maximum diameter of 4 mm or more, examined from the ovary from at least 3 directions and distinguishable from the ovarian parenchyma by a clear curved line, were recorded by 2 investigators.

“MemCalc” analysis

Time-series data of 4 uiFOL were observed for 105 to 392 days and for 399 to 505 days after parturition in this study. In addition, the data which were observed for 14 to 98 days in a previous report was also analyzed [2]. The analysis of these time-series data was done using a new computer program called MemCalc, based on MEM. The theoretical background and procedures of analysis in MemCalc are briefly described below.

Theoretical background: The values of observations $X(t)$ in some phenomena fluctuating irregularly with time can be separated into a systematic part and a fluctuating one:

$$X(t) = \text{systematic part} + \text{fluctuating part} \quad (1)$$

where $X(t)$ is a discrete time series at $t=k\Delta t$ ($k=1, 2, 3, \ldots, N$; $N$ is the length of the time series) and $\Delta t$ is the sampling time interval. The systematic part indicates an underlying variation of the original time series, and the fluctuating part, including undeterministic components such as noise, presents a residual time series in which the underlying part is subtracted from the original time series. A key point is the estimation of underlying variation.

The underlying variation is expressed by:

$$X_{UV}(t) = A_0 + \sum_{n=1}^{N_p} A_n \cos\{2\pi f_n(t+\theta_n)\} \quad (2)$$

where $f_n (-1/T_n; T_n : \text{its period})$, $A_n$ and $\theta_n$ are frequency, the amplitude and the phase shift of the $n$-th periodic component respectively; $A_0$ the average value of the time series and $N_p$ is the total number of dominant components. The nonlinear LSM using eq. (2) is linearized by the periods $T_n$ estimated by MEM spectral analysis.

The value of $T_n$ is determined by the positions of peaks in the MEM power spectral density (MEM-PSD) function, $P(f)$ ($f$ : frequency), which is calculated from:

$$P(f) = \frac{P_m\Delta t}{1 + \sum_{m,k} \gamma_{m,k} \exp(-i2\pi f k \Delta t)}^2 \quad (3)$$
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where the values of $P_m$ (the output power of prediction-error filter of order $m$ [$m$ is the so-called “lag”]) and $\gamma_{m,k}$ (the corresponding filter coefficient, $m=0, 1, 2, \ldots, M$ [$M$ is the optimum filter order]) are determined by solving the Yule-Walker equation using of Brug’s algorithm.

Procedures of analysis

1) Setting up time series data for analysis: Equal sampling-time intervals were chosen, lack of data was compensated for and outliers were corrected.

2) Frequency-domain analysis (MEM spectral analysis): To determine the optimum value $M$ of the lag $m$, the dependence of MEM-PSD peaks on $m$ is investigated. MEM-PSD was calculated from eq. (3) with the value $M$. The period was determined to be the reciprocal of the peak frequency and the nature of fluctuation was deduced from the overall trend of PSD.

3) Time-domain analysis (LSF analysis): Least square calculations were performed using a suitable periodic function (eq. (2)) with the MEM-estimated periods. The optimum LSF function (underlying part) was obtained by the LSM.

4) Residual time series analysis: The residual time series were obtained by subtracting the optimum LSF function from the original time series. Procedure (1) through (4) were also applied to the residual time series if necessary.

5) Prediction analysis: The underlying variation expressed by eq. (2) exactly corresponds to the predictable part (deterministic part) in Wold’s decomposition theorem [3]. Therefore, the extrapolation curve of the LSF using eq. (2) for the prediction could be used. The details of the theoretical background and procedure of analysis were described previously [4–6].

Superovulation study

In the superovulatory treatment group, 14 cows were divided into two equal groups. In Group 1, exogenous FSH administration was performed 304 to 309 days after parturition, and embryos were recovered at 315 to 320 days. In Group 2, the administration was started at 469 to 505 days after parturition, and embryos were recovered at 507 to 516 days. Superovulation was induced using 28 mg of FSH-P (FSH-P, Antrin; Denka Seiyaku Co., Japan) which was administered intramuscularly at decreasing doses twice daily at 0800 h and 1700 h over a 4-day period (5 mg × 2, 4 mg × 2, 3 mg × 2, 2 mg × 2). Luteolysis was induced by an intramuscular injection of prostaglandin $F_2\alpha$ (PGF$F_{2\alpha}$, Pronalgon F, Upjohn Co., Japan) 48 h after the first FSH-P injection. Fifty-six h after the PGF$F_{2\alpha}$ injection, all cows exhibited estrous behavior and were artificially inseminated twice at 12 h intervals with frozen-thawed semen. Seven days after the first insemination, embryos were recovered non-surgically. The embryos collected were classified according to their morphology [2].

Results

The measurement of ultrasound sonography

The uiFOL for two pairs were observed for 105 to 392 days and 399 to 504 days postpartum. In these four cows, the first estrus appeared at 44.5 ± 3.4 (±SD: standard deviation) days postpartum. The synchronous decreases in the number of uiFOL of two pairs usually were observed at 303 to 308 and 495 to 500 days after parturition in each observation (Fig. 1). The mean numbers of ovarian follicles (±SD) at 303 to 308 and 495 to 500 days postpartum were 1.5 (± 0.25) and 1.5 (± 0.25) respectively. Although there were many another
increase points in each cow, such as 168, 252, 350 and 476 days, the markedly synchronous decrease periods in each observation period were only 303 to 308 and 495 to 500 days.

The periodicity of ovarian follicular dynamics

Time-series data of 4 uiFOL which were observed in the range from 105 to 392 days and from 399 to 505 days after parturition were analyzed. Time-series data for 7 cows, which were observed from 14 to 98 days postpartum in a previous report were also analyzed [2]. Figs. 2a, b and c showed the observations of uiFOL in these cows for 14 to 98, 105 to 392 and 399 to 504 days postpartum respectively. First, the MEM power spectral densities were obtained (right side). The values of periods were determined from the locations of peaks of the power spectral densities (Frequencies: 1/days).

Fig. 2. Figs. 2 a, b and c show observations for 14 to 98, 105 to 392 and 399 to 504 days respectively. These time-series data were analyzed using the "MemCalc" program. First, the MEM power spectral densities were obtained (right). The values of periods were determined from the locations of the peaks of the power spectral densities (Frequencies: 1/days). Next, the best fitting curves (solid line; left) for the original data of ovarian follicular populations (dashed line; left) were calculated by the nonlinear LSM with the MEM-estimated periods.
Next, the best fitting curves (solid line, left side) for the observed data of uFOL, (dashed line, left side) were calculated by the nonlinear LSM with the MEM-estimated periods. The curves reproduced the observed data quite well. Accordingly, this data demonstrated that the variation of uFOL of each cow was not random, but composed of a periodic structure.

Fig. 2. (continued)
Frequency distribution of MEM power spectra

The peaks of MEM power spectra calculated in this study presented periodic structures for the ui-FOL. From these, the frequency distribution of the values of periods was obtained (Fig. 3). The data in Fig. 3 confirmed that there are five distinct peaks (these peaks are marked as (1) through (5) in Fig. 3). The first peak showed a period of 21 to 24 days, the second showed a period of 31 to 33 days, the third showed a period of 44 to 50 days, the fourth showed a period of 67 to 80 days and the fifth showed a period of 100 to 133 days postpartum. The first peak corresponded to 21 days. The third, fourth and fifth peaks corresponded to 2, 3 to 4, and 5 times the 21-day cycle, respectively. The biological significance of the second peak corresponding to the period of 31 to 33 days postpartum is not yet understood.

Superovulation treatment at specific decrease periods

In a previous study, when the superovulation treatment was performed at the decrease period, 92 to 98 days postpartum, many embryos were recovered [2]. To elucidate whether or not, in the decrease periods at 303 to 308 and 495 to 500 days, many embryos can be recovered after superovulation treatment in the same way described previously, this treatment regimen was carried out at these periods. In Group 1 (n=7), exogenous FSH administration was performed at 304 to 309 days after parturition, and embryos were recovered at 315 to 320 days. In Group 2 (n=7), FSH administration was started at 469 to 505 days after parturition, and embryos were recovered at 507 to 516 days. As a result, for Groups 1 and 2, the mean numbers of embryos (± SD) were 11.0 (± 2.9)
that of embryos was observed at any period except for a specific decrease such as 120 to 207 days postpartum [2]. Therefore, it is important for both synchronizing decreases and high embryos production to identify specific decrease period in addition to the 92 to 98 day period. In this study, although superovulation treatment was not tested during the 208 to 500 day period (except for these decrease periods), it was suggested that two periods, 303 to 308 and 495 to 500 days postpartum, were the specific decrease periods having both synchronizing decreases and high embryos production.

The “MemCalc” analysis revealed that the variation in the number of uiFOL for about 14 to 500 days postpartum in cows exists as periodic structures. Alternatively, the change in the number of uiFOL is not random, but consists of biological rhythm structures. The reason why there are marked decreases at about 90, 300 and 495 days postpartum is uncertain at present. However, it is significant that the marked decreases are well-reproduced by “MemCalc”, because it has been demonstrated that the decreases were specific phenomena based on periodic structures. Meanwhile, the specific periods observed also showed the interesting phenomenon that many oocytes, which possess the ability to develop individually, are induced by exogenous FSH. In mice, it was reported that ovulated oocytes are selected by a mechanism involving ovarian angiogenesis and non-selective oocytes caused apoptosis [9–11]. In addition the oocytes in small follicles (≤ 4 mm) in the bovine ovary are used for in vitro fertilization and have the ability to develop to term [12]. In this study, it is reasonable to postulate that many oocytes (≤ 4 mm) before the selective stage, developed rapidly at these specific decrease periods without the interference of large follicles (> 4 mm).

From the frequency distribution of MEM power spectra, five peaks were detected in this study. This frequency distribution indicated that variation of uiFOL is generalized to accumulate each periodic value (day) for each cow. Therefore, the peaks demonstrated by the frequency distribution corresponded to the ovarian follicular dynamics such as the three waves in the estrous cycle. The first peak corresponded to about 21 days and the third, fourth and fifth peaks corresponded to multiples of the 21-day cycle. The first estrus day after parturition of four cows for 105 to 504 days and of seven cows for 14 to 98 days were 44.5 ± 3.4 days respectively (Table 1). The number of recovered embryos were over 10, and these results well resemble the data on embryos recovered following superovulation treatment at 92 to 98 days after parturition [2].

### Table 1. Mean number of embryos in cows superovulated using FSH

<table>
<thead>
<tr>
<th>group of cows</th>
<th>number of cows</th>
<th>number of embryos recovered</th>
<th>number of embryos transferrable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>7</td>
<td>15.9 ± 5.8</td>
<td>11.0 ± 2.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>7</td>
<td>15.3 ± 5.3</td>
<td>11.0 ± 6.3</td>
</tr>
</tbody>
</table>

Group 1 and Group 2 cows were administrated with FSH at 304 to 309 days and 469 to 505 days postpartum respectively. Recovered embryos were classified as either transferrable (normal development) or non-transferrable from the morphological aspect.

**Discussion**

It has been reported that there are three wave of ovarian follicular development occurring at about 7-day intervals in the bovine estrous cycle, and during the early postpartum period of a cow periodical follicular wave can also occur [1, 7, 8]. Since a long-term observation at a short interval, such as 3 days, would negatively influence the reproductive function of a cow, we introduced 7-day intervals in this study. In fact, when cows were observed at 3-day intervals for about 100 days postpartum in a preliminary experiment, the development of the three waves in estrous cycle regularly occurred several times, but subsequent estrous cycles became irregular, and after a time, the three waves did not appear (data not shown). Accordingly, although 7-day intervals could not detect the short periodic development of follicular population, the obtained results appear to be physiological.

Although there were several other decrease points in each cow, such as at 168, 252, 350 and 476 days, the periods where the number of uiFOL of two pairs in each observation period decreased synchronously were only 303 to 308 and 495 to 500 days. In addition, when superovulatory treatments were done at these periods, many embryos were recovered. On the other hand, a previous report showed that no correlation between the number of uiFOL at the start of superovulatory treatment and that of embryos was observed at any period except for a specific decrease such as 120 to 207 days postpartum [2]. Therefore, it is important for both synchronizing decreases and high embryos production to identify specific decrease period in addition to the 92 to 98 day period. In this study, although superovulation treatment was not tested during the 208 to 500 day period (except for these decrease periods), it was suggested that two periods, 303 to 308 and 495 to 500 days postpartum, were the specific decrease periods having both synchronizing decreases and high embryos production.

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and 37.6 ± 4.9 days respectively. In addition, we have been reported that the first ovulation after parturition occurs at 43.5 days [13]. Therefore, the postpartum cow requires much time to recover estrous cycle. In present study, the frequency distribution demonstrates that the ovarian follicular dynamics is based on a 21-day cycle, and that it starts from parturition day. Consequently, it is reasonable to propose that the periodicity of ovarian follicular dynamics starting from parturition, in relation to the resumption of estrous cycle play important role in inducing periodicity of estrous.

In conclusion, it was confirmed that the cows have a variety of periodic structures corresponding to biological rhythms. These findings are significant for reproductive physiology and can be beneficial for animal reproduction in the clinical field.

References