

## Evaluation of the Corpus Luteum Size Throughout the Cycle by Ultrasonography and Progesterone Assay in Cows

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**Abstract:** The purpose of this study was to assess the correlation between the sizes of the corpus luteum (CL) with or without a cavity throughout the estrous cycle and progesterone (P<sub>4</sub>) levels in cows.

Images of the CL were obtained by ultrasonography at intervals of 24 h in 10 Holstein cows after their first postpartum estrus. Progesterone levels were measured by radioimmunoassay (RIA). The duration of the estrous cycle was determined to be short (13 days), normal (approximately 22 days) and long (30 days) in 1, 7, and 2 cows, respectively. Measurements were evaluated based on the established cycle durations.

The size of the CL was measured from the beginning of the second day after ovulation. The CL reached its maximum size on days 6, 9, and 10 in short (SC), normal (NC) and long (LC) cycles, respectively. The P<sub>4</sub> levels were below 1 ng/ml on days 0-4 in SC, and 0-2 in NC and LC, and reached a maximum on days 8 (7.31 ng/ml), 10 (9.81 ng/ml) and 18 (6.91 ng/ml) in SC, NC and LC, respectively. The correlation between the size of the CL and the level of P<sub>4</sub> was r: 0.72, 0.92, and 0.54 in cows indicating SC, NC, and LC, respectively.

In conclusion, CL size and P<sub>4</sub> level in cows were correlated. The correlation between the diameter, area, volume and perimeter of the CL was significant.

**Key Words:** Corpus luteum, dairy cattle, estrous cycle, progesterone, ultrasonography

### İneklerde Siklus Boyunca Corpus Luteum Boyutunun Ultrasonografi ve Progesteron Ölçümü ile İzlenmesi

**Özet:** Bu çalışmanın amacı, ineklerde östrus siklusu boyunca corpus luteum (CL) (kaviteli veya kavitesiz) boyutları ile progesteron düzeyleri arasındaki ilişkinin saptanmasıdır.

Postpartum ilk östrusunu göstermiş 10 Holstein inekte ultrasonografi kullanılarak 24 saat aralıklarla CL görüntüleri alındı. Progesteron düzeyi radioimmunoassay (RIA) tekniği ile belirlendi. İneklerin 1 tanesinde kısa (13 gün), 7 tanesinde normal (ortalama 22 gün) ve 2 tanesinde uzun (30 gün) siklus görüldü. Ölçümler bu siklus uzunluklarına göre değerlendirildi.

Corpus luteum boyutları, ovulasyondan sonraki 2. günden itibaren ölçüldü. Corpus luteum maksimum boyutlara kısa siklusta (KS) 6. günde, normal siklusta (NS) ortalama 9. günde, uzun siklusta (US) 10. günde ulaştı. Progesteron düzeyi KS'de 0-4, NS ve US'de 0-2 günler arası 1 ng/ml'nin altındayken, KS'de 8. günde (7,31 ng/ml), NS'de 10. günde (9,81 ng/ml), US'de 18. günde (6,91 ng/ml) maksimum düzeye ulaştı. Corpus luteum boyutlarıyla P<sub>4</sub> değeri arasındaki korelasyon KS'de 0,72 NS'de 0,92 ve US'de 0,54 olarak saptandı.

Sonuç olarak ineklerde CL boyutları ile P<sub>4</sub> arasında korelasyon saptandı. Corpus luteum çapı ile alan, hacim ve çevre arasındaki korelasyon önemli bulundu.

**Anahtar Sözcükler:** Corpus luteum, sütçü inek, östrus siklusu, progesteron, ultrasonografi

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## Introduction

Ultrasonographical studies of the corpus luteum (CL) have revealed it to display a dynamic pattern based upon development and regression. The CL is classified as young (1-4 days), mature (5-16 days), and regressed (17<sup>th</sup> day) until complete luteolysis (1).

Young CLs are hard to differentiate from the stroma of the ovarium due to their small and irregular structure. They display a grayish black image with echogenic spots. The mature CL is separated from the ovarium with definite borders and is easily imaged in granular structure and hypoechogenic appearance. It can protrude over the ovarium. The regressed CL is hard to differentiate due to the decrease in echogenicity difference between tissues in time and the indefiniteness of the border between luteal tissue and the stroma of the ovarium (1-4).

The increase in  $P_4$  level has been reported to occur at similar rates with the enlargement in CL size. However, the decrease in CL size was found to occur at a different rate compared to the decrease in  $P_4$  level (5). Battocchio et al. (6) have reported a classification system applicable to ultrasonographically detected CLs with regard to  $P_4$  levels and have estimated 95% correlation. The correlation between the diameter of the CL and  $P_4$  levels was regarded as significant in the phases of development and regression in heifers and only the development stage in cows (7). A decrease in  $P_4$  level was determined on days 16 (2-wave cycle) and 23 (3-wave cycle), whereas the CL size was observed to regress on days 17 and 24 (8).

Plasma  $P_4$  levels were  $6.13 \pm 0.67$  ng/ml at mid-cycle, and  $0.22 \pm 0.11$  ng/ml during CL regression (4). The mean amounts of progesterone produced by young, mature and regressed CLs were  $1.7 \pm 0.6$ ,  $8.9 \pm 1.0$ , and  $0.9 \pm 0.3$  ng/ml, respectively (1).

The cavities forming inside the CL were non-pathologic. Thus, the presence of a cavity was reported not to affect the milk-plasma  $P_4$  by many researchers (4,5,9).

The aim of this study was to assess the correlation between CL and  $P_4$  by daily ultrasonographic examination of the CL throughout the estrous cycle.

## Materials and Methods

The study involved 10 Holstein cows (4 to 6 years old) with milk production of 25-30 kg/day. An ultrasonographic examination was performed using B-mode real-time ultrasonography (PieMedical 100 Falco, 6-8 MHz linear probe). After parturition, the cows were closely observed. The diagnosis of ovulation in cows with normal cycles was established ultrasonographically starting from day 35 post-parturition, and closely followed. Ultrasonographic examinations were performed at 24 - h intervals, especially after milking in the morning. The day on which ovulation was determined to occur was numbered day 0. Ovulation was determined to have occurred upon the absence of the Graaf follicle at the ultrasonography examination. As described by Pierson and Ginther (10), the average of the widest and longest parts was used in determining the CL and its diameter. The cavity volume and area of the CL were measured by AXES method on ultrasonography. In CLs with cavities, the area and volume of luteal tissue were calculated by subtracting the cavity values from the total values.

Blood samples were collected daily from the V. subcutanea abdominis and centrifuged at 3000 rpm for 10 min to obtain sera. Serum samples were stored at -20 °C in Eppendorf tubes. Progesterone levels were measured by radioimmunoassay (RIA) technique. Progesterone levels were measured using a commercial RIA kit (Immunotech, Marseille, France). Intra - and interassay coefficients of variation were 5.4% and 9.1%, respectively. All the samples were studied in one assay. The values were read on a gamma counter. The values were then converted into ng/ml on a computer.

Evaluation of data included the calculation of arithmetical mean values of daily measurements. The correlation between CL values and  $P_4$  levels was determined using Spearman's non-parametric test.

## Results

Short (13 days), normal (approximately 22 days) and long (approximately 30 days) estrous cycles were determined in 1, 7, and 2 cows, respectively. The data were evaluated based on the cycle duration. Corpora lutea were detected on day 2 after ovulation: 1 from the 2 cows with long cycles, and 4 from the 7 cows with normal cycles. Below are samples of images at different phases of the estrous cycle (Figures 1-3).

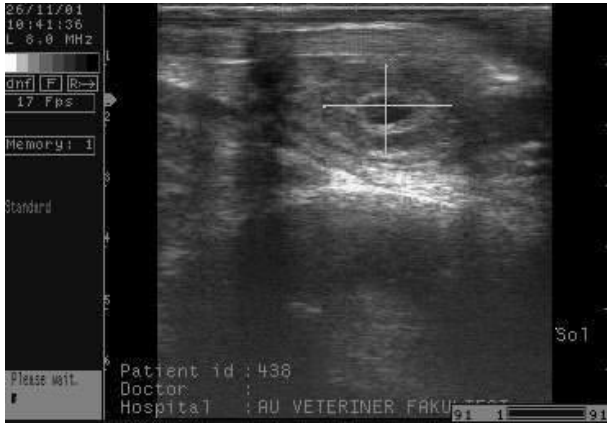


Figure 1. A young CL on day 2 of the estrous cycle.

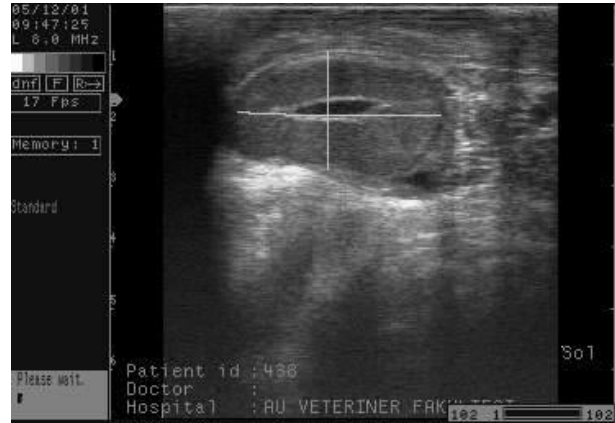


Figure 2. A mature CL on day 11 of the estrous cycle.



Figure 3. A regressed CL on day 19 of the estrous cycle.

The sizes of CLs reached the maximum level on days 6, 9, and 10 in the animals displaying SC, NC, and LC, respectively. The mean diameter of the CL on day 2 of the estrous cycle was 1.15 cm for SC,  $1.06 \pm 0.18$  cm for NC, and  $1.63 \pm 0.15$  cm for LC.

On the other hand, maximum CL diameters were 2.16 cm for SC,  $2.00 \pm 0.11$  cm for NC, and  $2.18 \pm 0.09$  cm for LC. The values obtained before the ovulation day were 1.28, 1.21 and 1.29 cm (Figure 4).

The mean area of the luteal tissue (with and without a cavity) on day 2 of the estrous cycle was  $0.94 \text{ cm}^2$  for SC,  $0.84 \pm 0.25 \text{ cm}^2$  for NC, and  $2.42 \pm 0.28 \text{ cm}^2$  for LC. However, maximum luteal tissue areas were 3.68,  $4.25 \pm 0.52$ , and  $4.87 \pm 0.12 \text{ cm}^2$  in SC, NC, and LC, respectively. Values measured before the day of ovulation were 1.10 (SC), 0.88 (NC) and  $1.26 \text{ cm}^2$  (LC).

The mean volume of the luteal tissue on day 2 of the estrous cycle was  $0.63 \text{ cm}^3$  for SC,  $0.48 \pm 0.19 \text{ cm}^3$  for NC, and  $2.26 \pm 0.31 \text{ cm}^3$  for LC. On the other hand, maximum luteal tissue volumes were  $4.41 \text{ cm}^3$  for SC,  $6.12 \pm 1.39 \text{ cm}^3$  for NC, and  $7.52 \pm 0.14 \text{ cm}^3$  for LC. The values measured before the day of ovulation were 0.73 (SC), 0.47 (NC), and  $0.84 \text{ cm}^3$  (LC).

The mean perimeter of the CL on day 2 of the estrous cycle was 3.46,  $3.29 \pm 0.56$ , and  $3.73 \pm 0.48$  cm for SC, NC, and LC, respectively. Nevertheless, maximum CL perimeters were 6.97 cm for SC,  $6.80 \pm 0.38$  cm for NC, and  $6.60 \pm 0.25$  cm for LC. The values measured before the day of ovulation were 3.80 (SC), 3.66 (NC), and 4.14 cm (LC).

The increase on the last day shown in the above graphic cows with a LC was due to only one cow remaining on day 30.

The  $P_4$  level was below 1 ng/ml from days 0 to 4 in SC, whereas it was below 1 ng/ml on days 0-2 in NC and LC. The  $P_4$  level reached a maximum level of 7.31, 9.8 and 6.91 ng/ml on days 8 (SC), 10 (NC) and 18 (LC), respectively.

The  $P_4$  level was below 1 ng/ml on the last 3 days prior to ovulation in all the groups. As the duration of the cycle increased, fluctuations in  $P_4$  level were observed (Figure 5).

In cows with SC, the correlation coefficient between  $P_4$  level and CL diameter was  $r: 0.72$ ; between  $P_4$  level and luteal area,  $r: 0.69$ ; between  $P_4$  level and volume,  $r: 0.69$ ; and between  $P_4$  level and perimeter measurements,  $r: 0.69$  ( $P < 0.05$ ).

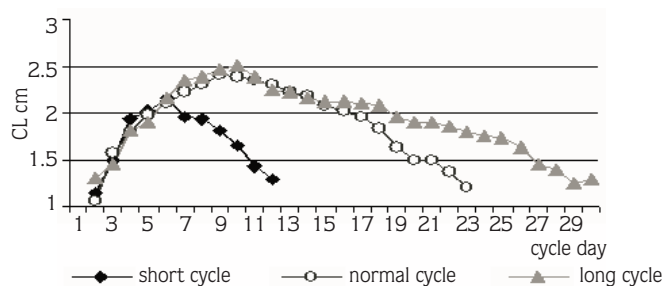


Figure 4. Average CL diameters (cm) measured according to cycle duration.

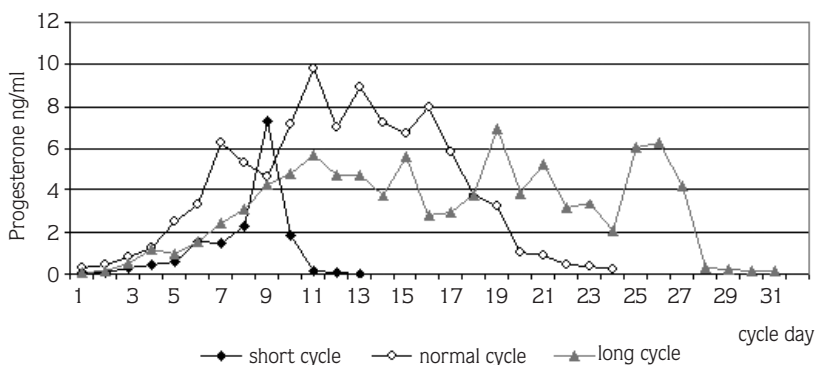


Figure 5. Average P<sub>4</sub> levels (ng/ml) measured according to cycle duration.

For cows in NC, the coefficients were r: 0.92, 0.88, 0.88 and 0.83, respectively (P < 0.01).

For cows in LC, the coefficients were r: 0.54 (P < 0.01), 0.50 (P < 0.05), 0.45 (P < 0.05) and 0.49, respectively (P < 0.01).

The correlation between the diameter and the area, volume and perimeter of the CL was considered significant (P < 0.01).

### Discussion

Young CLs are hard to differentiate from the stroma of the ovary since they present a gray-black ultrasonographic appearance. Mature CLs are separated from the ovary with distinct borders, and therefore are easily detectable and display a hypoechogenic appearance with a granular structure. Similar to young CLs, the echogenicity difference between tissues decreases over time, and the border between the luteal tissue and the stroma of the ovary becomes indefinite; therefore, the differentiation of the regressed (old) CL becomes more

difficult (1- 4). In this study, since the location of the CL was already known, such difficulties were not encountered. However, due to the aforementioned reasons, at any time and during the examination of any animal, problems may be encountered in detecting the CL and the duration of the examination may be prolonged. Thus, detection of the CL requires experience on the part of the operator.

Taylor and Rajamahendran (8) have classified cycles as those with 2 and 3 waves and have monitored CL regression and the decrease in P<sub>4</sub> level. Cycles may be classified according to the duration of follicular waves or CL formation and regression. The animals included in this study were divided into 3 groups: cows with short, normal and long estrous cycles, depending on the presence of the CL.

Battocchio et al. (6) have claimed that the difference between CL diameter and P<sub>4</sub> level may be significant in estrous cycle phases other than diestrus. In our study, the CL was detected on days 3 - 4; however, an exact measurement was not obtained. The P<sub>4</sub> level was within

a range of 1.1-1.7 ng/ml at this phase. The CL was definite and the  $P_4$  level was 4 - 7.3 ng/ml in the diestrous phase (days 7 - 17). During the last 3-4 days prior to ovulation, the  $P_4$  level decreased to 1 ng/ml from 3.6 ng/ml. However, in the aforementioned study, it was stated that errors occurred in the detection of developing and regressing CLs, due to the unavailability of daily monitoring and subsequent non-differentiation of CLs in these 2 phases. In this study, the CL was detected beginning from the day of post-ovulation but it could not be measured because of its indefinite borders. Thus, the measurements were obtained from day 2 onwards. A similar disadvantage was valid for the regressing CL as well. Since echogenicity differences decreased towards the end of the cycle, difficulties were encountered during measurement. However, since monitoring was performed daily, no difficulty was encountered in detection.

Battocchio et al. (6) reported correlations between  $P_4$  level and CL to be 70%, 67% and 89%, during the phase in which CL could not be detected the developmental phase and mid-cycle, respectively ( $P < 0.001$ ). In this study, the correlation between CL diameter and  $P_4$  level was 65.1% in long cycles ( $P < 0.01$ ). In long cycles, the correlation between  $P_4$  levels and CL was more significant (90%,  $P < 0.01$ ). Values of CL diameter measured with regard to correlation may provide information on the functionality of the CL. However, Kastelic et al. (5) have stated that luteal function cannot be exactly defined based on CL diameters measured by ultrasonography and that,

in that respect, the  $P_4$  level may serve as a more accurate parameter. Although individual differences were determined in this study, except for the animal with a short cycle, a statistically significant correlation was found between CL diameter and  $P_4$  level in cycles with normal and long duration. Therefore, we think that the diameter of the CL may provide information on the functionality of the aforementioned structure. Assey et al. (7) have reported a significant correlation between CL diameter and  $P_4$  level at phases of development and regression in heifers and only in the developmental phase of cows. In this study, the classification was based on cycle duration, not on the cycle phases.

The cavity and luteal tissue were reported to be maximum on the day 9 post-ovulation. In our study, we also found out that cows with NC reach maximum values on day 9.

In conclusion, depending on the experience of the operator, it can be stated that the CL can be detected by taking it into consideration in the early cycle; the dominant follicle and ovulation location may assist in the detection of CL. Despite differences in echogenicity, the CL can be detected in early and late cyclic phases and due to the significant correlation between CL size and  $P_4$  levels in normal (22 days) and long (30 days) cycles, the functionality of CL could be assessed. Therefore, ultrasonography proved efficient in the monitoring of reproduction.

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