Milk Progesterone Levels in Cows with Normal or Prolonged Estrous Cycles, Referenced to an Early Pregnancy Diagnosis

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ABSTRACT. Progesterone levels in skim milk sampled from 17 pregnant and 39 non-pregnant cows three times weekly for 4 to 9 weeks after insemination were measured by an enzyme immunoassay. The lengths of estrous cycle in non-pregnant cows were 19 days in 8, 21 days in 14, 22 days in 1, 23 days in 3, 26 days in 3, and 30 to 60 days in 10 cows, respectively. A significantly lower (p<0.01) progesterone level of skim milk in non-pregnant cows, as compared with the level in the pregnant, was observed only on the day of estrus. The milk progesterone contents 2 days before and after the estrus were high enough to indicate the active corpus luteum function. In the 10 cows which returned to estrus 30 to 60 days after insemination, an occurrence of the embryonic death after 20–22 days after the service was suspected. The variation of the length of the estrous cycle as well as the occurrence of embryonic mortality could be most probable cause of the false positive diagnosis of pregnancy when the milk progesterone test was applied on the 20th or 21st day after insemination.

Progesterone level in milk as well as the level in blood of the cows is generally considered as a clear indicator of corpus luteum function [10, 12, 14]. Milk progesterone assay has now been applied to diagnosis of pregnancy, detection of estrus and diagnosis of subfertility [2–5, 7, 8, 10–12, 14–16].

Milk progesterone assay as a tool of an early pregnancy may be used for the purpose of maintaining and/or improving reproductive efficiency in cows [4]. The accuracy of the pregnancy diagnosis based on milk progesterone assay is reported as 60.0–84.5% [4, 7–9, 18] for positive diagnosis and 87.0–100% [4, 7–9, 18] for negative cases. The accuracy rate of milk progesterone test for non-pregnancy is indicating its practical application. However, the reduced accuracy rate for positive diagnosis may indicate it involves a limit to some extent for the practical use. Some workers presumed that the false positive diagnosis for pregnancy seems largely due to embryonic death which results in a prolonged estrous cycle [5, 7, 13, 18].

By a recent report, however, the variation of the length of the normal estrous cycle could also be a cause of the incorrect positive diagnosis by milk progesterone assay [20]. The present study was aimed to investigate the changes in milk progesterone levels after insemination in cows with normal or prolonged estrous cycles, comparing with the levels in pregnant cows to speculate the possible causes of false positive pregnancy diagnosis in milk progesterone test.

MATERIALS AND METHODS

Animals: Seventeen pregnant cases and 39 non-pregnant cases of total 56 Holstein-Friesian cows in the dairy herd of Rakunogakuen University inseminated between June of 1980 and February of
1981 were used.

**Milk samples:** Milk samples were obtained three times weekly for 4 to 9 weeks after insemination. About 5 ml of milk was collected randomly from a quarter before the morning milking and let stand at room temperature. The sample was centrifuged (1,580 xg, 10 minutes) within 6 hours after collection to separate cream and skim milk. The upper cream layer was discarded and remained fraction of skim milk was transferred into another tube and stored at −20°C until analysis.

**Milk progesterone assay:** Progesterone levels in skim milk were measured by an enzyme immunoassay as reported elsewhere [17–19].

**RESULTS**

Of 56 cases, 17 cows became pregnant, 29 cows returned to estrus 19 to 26 days after insemination and 10 cows also returned to estrus in 30 to 60 days.

**Milk progesterone levels in 17 pregnant cows:**

![Progesterone levels in skim milk of 17 pregnant cows after insemination.](image)

**Notes:** The upper, middle and lower lines indicate the highest, mean and lowest milk progesterone levels respectively.

Progesterone levels in skim milk elevated 2–3 days after insemination and thereafter remained rather high ranging between 1.0 ng/ml and 7.4 ng/ml (Fig. 1).

**Milk progesterone levels in 26 cows with a normal estrous cycle:** Changes in milk progesterone levels in 8, 14, 1 and 3 cows with 19, 21, 22 and 23 days estrous cycle respectively, in comparison with the lower limit of the range of progesterone levels in 17 pregnant cows, are shown in Fig. 2.

In the cows returning 19 days after insemination, milk progesterone levels decreased significantly (p<0.01) on the day of estrus and a rise of progesterone level was observed as early as 2 days after the estrus, 21 days after the insemination. The cows having 21 days estrous cycle also showed a significant decline of milk progesterone level (p<0.01) only on the day of estrus. The progesterone contents in milk were still high 2 days before estrus and the contents were already ele-
MILK PROGESTERONE LEVELS IN COWS

Fig. 2. Progesterone levels in skim milk of 26 cows with a normal estrous cycle.

Notes: —■— Mean level with standard deviation
■——■ Lowest level in 17 pregnant cows

vated 2 days after the estrus. The similar trends were observed also in the animals returning to estrus 22 or 23 days after service.

Milk progesterone levels in 13 cows with a prolonged estrous cycle: Progesterone levels in milk of 3 cows with 26 days cycle and 10 cows showing estrus during 30 to 60 days after insemination are shown in Fig. 3. In all cases, milk progesterone levels declined on the day of estrus, while the levels still remained high on 21 days after insemination.

Milk progesterone levels in pregnant and non-pregnant: Progesterone levels in skim milk sampled on 21 days after insemination were 2.0±0.5 (S.D.) ng/ml in pregnant cows, 1.7±0.7 ng/ml in cows returning to estrus 19 days after insemination, 0.7±0.2 ng/ml in cows with 21 days estrous cycle, 2.0±0.7 ng/ml in cows coming into estrus 22 to 26 days after insemination and 1.9±0.5 ng/ml in cows with prolonged estrous cycles beyond 30 days.

Discussion

The mean length of the estrous cycle in the cows is known to be 21 days on the average with a range from 18 to 24 days [21].

Milk progesterone levels remaining low during estrus increased drastically 4 to 5 days after estrus in several reports [1, 6, 12, 14]. Pennington et al. [20], however, reported that unexpectedly high levels of progesterone in milk occurred 2 days before the next estrus. Foote et al. [8] also reported similar observations on milk progesterone levels. Results of the present study are comparable with the findings of the above authors [8, 20]. Progesterone levels in milk increased significantly 2 days after estrus and remained rather high until 2 days before the next estrus. Progesterone levels in milk sampled only once on 20 or 21 days after insemination in cows with 18 or 19 days estrous cycle or in cows with 22 days or longer cycle may be high enough to be diagnosed falsely as pregnant.

Thus, deviations of the length of the estrous cycle are considered to be one of the causes of false-positive diagnosis in pregnancy test by milk progesterone assay.

These findings indicate that milk sample for pregnancy diagnosis should be collected as near as possible to the expected day of next estrus as stated by Pennington et al. [20].
Milk progesterone levels in cows suspected of embryonic death in this study are similar to the results reported by Bulman & Lamming [5], Kummerfeld et al. [12] and Ball [2]. The incidence of the embryonic death estimated by milk progesterone assay in the herd used in this study was 17.9%, higher than each figure reported by Kummerfeld et al. [13], Ball [2] and Bulman & Lamming [5], but lower than that obtained by Cox et al. [7]. The accuracy rate of positive diagnosis in milk progesterone test for pregnancy varies herd to herd, possibly depending largely on the incidence of embryonic mortality.

To improve the accuracy of pregnancy diagnosis by milk progesterone test, two or three times sampling around the expected day of next estrus may be necessary, while considering the other causes of incorrect diagnosis such as embryonic death.

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要約
乳牛の正常性周期および異常性周期における乳汁中progesterone濃度の変動: 中尾敬彦・杉橋章義・
河田啓一郎・横山伸彦・角田修男（鹿島学園大学獣医学科）——授精後20～22日目の乳汁中progester-
one（P4）測定により乳牛の早期妊娠診断を実施しようとする際に起こる易い誤診の原因を明らかにする目的
で、授精後4～9週間の妊娠牛17頭と非妊娠牛39頭から、2、3日間隔で採取した乳汁についてP4
を測定し、これらの値の変動を比較した。妊娠牛17頭では、P4値は授精後2～3日目から1.0ng/ml
以上に増加し、その後も1.0～7.4ng/mlの高値を維持した。非妊娠牛39頭の内8頭では授精後19日目
に、14頭では21日目に、1頭では22日に、3頭では33日目に、3頭では26日目に、そして10頭では
30～60日目にそれぞれ発情が再帰した。非妊娠牛において、P4値の低下（1.0ng/ml未満）は発情の当
日のみに認められ、発情の2日前および2日後のP4値は1.0ng/ml以上で、妊娠牛との区別は困難であっ
た。また、授精後30～60日目で発情が再帰した牛では、この間P4値は1.0ng/ml以上を維持して
いたことから、胚の死滅が推測される。このように、授精後20～22日目の乳汁中P4測定による妊娠診
断においては、性周期の変動、および胚の死滅にと推測される理由のために誤診が起こり易いこと
が確認された。