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# Reduced Pregnancy Rates in Lactating Cows Inseminated Following Short or Prolonged Luteal Phases

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**Abstract:** The objectives of this study were to describe the luteal phase during the estrous cycle preceding first insemination (1st AI) and to assess its association with the pregnancy rate. From forty-one cows milk samples were collected three times a week starting 2nd week postpartum until cows returned to estrus or were diagnosed pregnant after 1st AI. Progesterone concentrations in defatted milk were measured by enzyme immunoassay. Based on the duration of the luteal phase in the cycle preceding 1st AI, 27 (65.9%) cows had normal luteal phase (12-19 days), 8 (19.5%) had prolonged ($\geq$20 days; PLP), 5 (12.2%) had short (<12 days; SLP) and in one cow there was no luteal phase. Peak progesterone values were lower in cows with SLP than in cows with PLP or normal luteal phase (1.50, 2.27, 2.20 ng mL$^{-1}$, respectively $p<0.01$). About 5 (62.5%) of the eight cows with PLP in the preceding cycle repeated the PLP in the cycle subsequent to 1st AI. Of the 15 cows not conceiving to the 1st AI, 10 (66.7%) had abnormal luteal phases (SLP, PLP and absent) while only 4 (15.4%) of 26 cows which conceived had abnormal luteal phases ($p<0.01$). The incidence of abnormal phases increased in cows in third parity than first or second parity ($p<0.05$). In conclusion, inseminating cows after short or prolonged luteal phase was associated with reduced pregnancy rate.

**Key words:** Dairy cow, luteal phase, pregnancy rate, cycle, insemination, Japan

## INTRODUCTION

Once cows have been correctly detected in estrus, it is vital to obtain high pregnancy rate per service to achieve high reproductive efficiency. Low conception rates after insemination are multifactorial problems that could be attributed to management and nutrition factors (Larson *et al*., 1997; Butler, 1998) or they maybe due to abnormal hormonal patterns (Bulman and Lamming, 1978; Vanroose *et al*., 2000; Hommeida *et al*., 2004). Low peripheral concentrations of progesterone 4-5 days after insemination (Kimura *et al*., 1987; Shelton *et al*., 1990; Larson *et al*., 1997) or during mid-luteal phase (Hansel, 1981; Fonseca *et al*., 1983; Lamming *et al*., 1989) have been reported to result in reduced fertility in cows. Moreover, lower progesterone concentrations during the luteal phase of the preceding estrous cycle in non-pregnant cows in comparison with pregnant cows were reported (Folman *et al*., 1973; Fonseca *et al*., 1983). Supplementation of progesterone during the luteal phase prior to insemination resulted in a higher conception rate in a previous study (Rosenberg *et al*., 1990). In contrast, some studies did not show similar findings (Noseir *et al*., 1992; Sreenan and Diskin, 1983). However, information on the association of the luteal phase of the estrous cycle preceding insemination on pregnancy rate is limited. The objectives of this study were to describe the luteal phase (duration and peak values) in the estrous cycle preceding the 1st AI and to assess its association with pregnancy rate.

## MATERIALS AND METHODS

**Animals:** This study was performed at Setouchi Field Science Centre Farm, Hiroshima University. Forty-one lactating Holstein Frisian cows in their first to third lactations were used. The cows were housed in roofed free stalls and fed a ration consisting of hay, silage and

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concentrate prepared to meet their production requirements. They were machine-milked twice daily, 08:30 and 16:00 h and produced averaged 100 day milk yield of 35.6±5.8 kg day$^{-1}$.

**Milk samples collection and progesterone assay:** Milk samples were collected thrice a week (Monday, Wednesday and Friday), starting in the 2nd week postpartum until the cows either returned to estrus or were diagnosed pregnant after 1st AI. Milk fat was separated by centrifugation at 1700×g for 30 min and the defatted milk was stored in 4 mL plastic tubes at -30°C until assay. The progesterone was assayed in defatted milk samples in duplicates by enzyme immunoassay (Zulu *et al*., 2000). A luteal phase was defined as the time during the preceding cycle with defatted milk progesterone concentrations ≥1.0 ng mL$^{-1}$. Based on duration, luteal phases were grouped into; normal when defatted milk progesterone concentrations of ≥1.0 ng mL$^{-1}$ continued for 12-19 days; Prolonged (PLP), Luteal Phase of ≥20 days; Short (SLP), Luteal Phase of <12 days and absent when progesterone concentrations remained <1.0 ng mL$^{-1}$.

**Reproductive management:** Detection of estrus was started after a 40 days voluntary waiting period and based on visual observation and use of Heat Mount Detector (HMD) (Kamar Heatmount Detector®, Kamar, Inc., Steamboat Spring Co., USA). Cows were observed 3 times a day for at least 30 min each and any secondary estrous signs or mounting activity were recorded. Estrus was defined when cow stood to be mounted or when the color of HMD was turned uniformly red. When detected in estrus cows were inseminated artificially following subsequent milking by recto-vaginal method. Pregnancy was diagnosed by palpation per rectum 35 days after insemination or later.

**Statistical analysis:** Peak progesterone concentrations and days to 1st AI between different types of luteal phase were compared by one-way ANOVA and differences determined using Duncan's Multiple Range test. Chi-square test was used to compare conception rates.

## RESULTS AND DISCUSSION

**Characteristics of the luteal phase in the cycle preceding 1st AI:** Characteristics of luteal phase of the cycle preceding first insemination are shown in Table 1. Based on the duration of the luteal phase, 27 (65.9%) of 41 luteal phases were defined as normal, 8 (19.5%) were Prolonged (PLP), 5 (12.2%) were Short (SLP) and in one (2.4%) cow there was no luteal phase (Fig. 1). Peak progesterone values during luteal phase were lower in cows with SLP than in cows with PLP or normal luteal phase (p<0.01) (Table 1). About 5 (62.5%) of the eight cows with PLP in the preceding cycle repeated the PLP in the cycle subsequent to 1st AI (Fig. 2a-e).

Table 1: Characteristics of the luteal phase during the cycle preceding 1st AI and pregnancy rate in lactating cows

| Luteal phase type | Number (%) | Luteal phase length (d) | Peak $P_4$ (ng mL$^{-1}$) | Days to 1st AI | Pregnancy rate (%) |
|---|---|---|---|---|---|
| Normal | 27 (65.9) | 15.1±1.9$^b$ | 2.20±0.64$^a$ | 73.8±22.6 | 81.5$^a$ |
| Prolonged | 8 (19.5) | 29.1±1.3$^a$ | 2.27±1.04$^a$ | 73.0±24.5 | 37.5$^b$ |
| Short | 5 (12.2) | 6.8±1.1$^c$ | 1.50±0.80$^b$ | 70.0±22.1 | 20.0$^b$ |
| Absent | 1 (2.4) | 0.0 | 0.17 | 69.0 | 0.0 |
| Total | 41 (100.0) | 16.95 | 2.14±0.74 | 72.9±22.0 | 63.4 |

Results are shown as mean±SD; values in the same column with different superscripts (and) different (p<0.05)

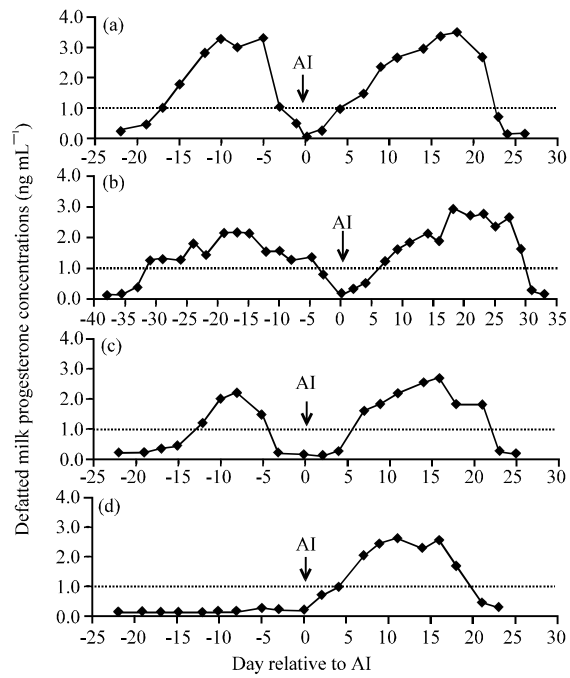


Fig. 1: Representative defatted milk progester one profile of lactating dairy cows with normal (a), prolonged (b), short (c) or absent (d) luteal phase in the cycle preceding insemination (AI = day 0)

**Luteal phase of the preceding cycle and conception rate:** Out of the 15 cows not conceiving, 10 (66.7%) had abnormal (short, prolonged or absent) luteal phase in the cycle preceding 1st AI while only four (15.4%) of 26 cows that conceived showed abnormal luteal phase (p<0.01) (Table 2). Days to 1st AI were not different among the groups. Peak progesterone concentration in the preceding cycle was lower in cows not pregnant than in cows that became pregnant (1.82±0.62 vs. 2.31±0.76 ng mL$^{-1}$, p<0.05). The incidence of abnormal luteal phases increased in cows in third parity than first or second parity (p<0.05) (Fig. 3).

In cows, progesterone concentrations that increase few days after estrus and remain elevated for about 2 weeks are the hallmark of a normal estrous cycle (Lamming and Darwash, 1998). In this study deviant luteal phases of <12 days (short) or >19 days (prolonged) were observed in the cycle preceding first insemination in 13 of 41 cows and were found to be associated with reduced pregnancy rate.

Occurrence of short luteal phase has been reported in anestrous postpartum cows following spontaneous or gonadotropin-induced ovulation (Garverick and Smith, 1986; Hunter, 1991). The incidence of short luteal phase was reported to be 34% after first ovulation (Smolders *et al*., 1996) and 0.5-3.7% (Opsomer *et al*., 1998; Shrestha *et al*., 2004) in subsequent cycles in postpartum dairy cows. It has been associated with infertility when observed after insemination (Inskeep, 1995) may be due to associated premature luteolysis (Hunter, 1991). However, the mechanism by which short luteal phase in one estrous cycle can adversely affect conception rate after the subsequent insemination is not clear. Shaham-Albalancy *et al*. (2001) reported that cows having low but constant and low but increasing progesterone concentrations in one estrous cycle showed an increased PGF2α secretion late in the luteal phase in the subsequent cycle than cows with higher progesterone concentrations. Whether short luteal phase in the preceding cycle can induce the same action needs to be investigated. However, peak progesterone concentrations in cows with short luteal phase remained the lowest among the groups in this study.

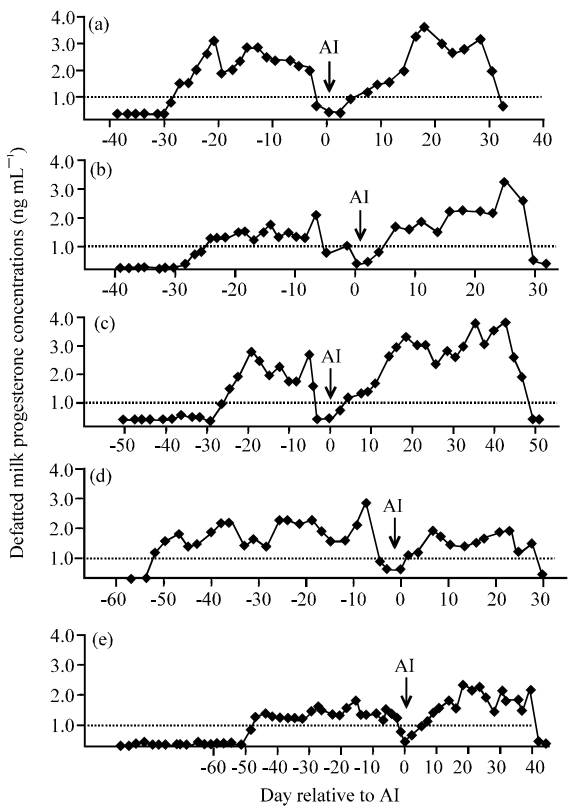


Fig. 2a-e: Defatted milk progesterone profiles in 5 cows with prolonged luteal phase in the cycles preceding and subsequent to insemination (AI = days 0)

In lactating cows, once ovulation resumes in early postpartum period, the most common ovarian abnormality is the PLP (Opsomer *et al*., 1998; Roche *et al*., 2000). Prolonged luteal phase was reported to be associated

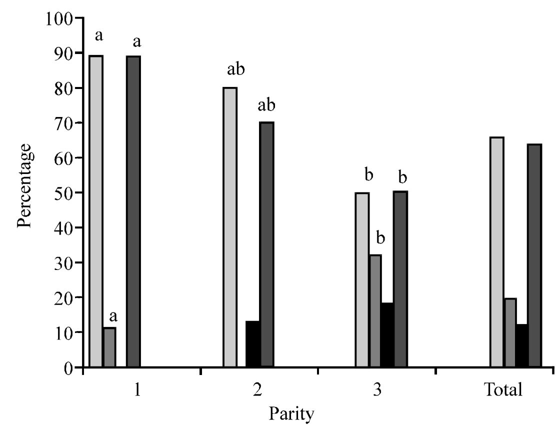


Fig. 3: Incidence of normal, prolonged and short luteal phase during the cycle preceding 1st AI and pregnancy rate in dairy cows classified by parity. Different letters (a-c) indicate difference among parities (p<0.05)

Table 2: Luteal phase during the cycle preceding and the cycle subsequent to 1st AI in lactating dairy cows and pregnancy rate

| Preceding cycle luteal phase | No. of cows | Subsequent cycle luteal phase (%) Normal | Prolonged | Short | Sub-total | Pregnancy rate (%) |
|---|---|---|---|---|---|---|
| Normal | 27 | 23 (85.2) | 3 (11.1) | 1 (3.7) | 4 (14.8) | 18.5 |
| **Abnormal** | | | | | | |
| Prolonged | 8 | 3 (37.5)$^{c}$ | 5 (62.5)$^{c}$ | 0 (0.0) | 5 (62.5)$^{c}$ | 37.5$^{b}$ |
| Short | 5 | 2 (40.0)$^{b}$ | 2 (40.0) | 1 (20.0) | 3 (60.0)$^{b}$ | 20.0$^{c}$ |
| Absent | 1 | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.0 |
| Sub-total | 14 | 6 (42.9)$^{c}$ | 7 (50.0)$^{c}$ | 1 (7.1) | 8 (57.1)$^{c}$ | 28.6$^{c}$ |
| Total | 41 | 29 (70.7) | 10 (24.4) | 2 (4.9) | 12 (29.3) | 63.4 |

Values in the same column with superscripts, $^{bc}$differed from normal; b = p<0.05 and c = p<0.01

with abnormal uterine environment (Opsomer *et al*., 2000; Shrestha *et al*., 2004) and inadequate estradiol concentrations secreted from a mature follicle or resulting from increased estradiol metabolism (Wilson *et al*., 1998; Lucy, 2001). The lower fertility in cows with prolonged luteal phase in the preceding cycle in this study agreed with Lamming and Darwash (1998) who reported that prolonged luteal phase postpartum was associated with lower first service conception rate and more services per conception.

In cows, the length of the luteal phase appears to be the primary determinant of number of follicular wave during an estrous cycle (Ahmad *et al*., 1997; Townson *et al*., 2002) and cows with shorter cycles may be more apt to have two waves (Ginther *et al*., 1989; Townson *et al*., 2002) and cows with longer cycles three waves (Fortune, 1993; Townson *et al*., 2002). Moreover, lactating cows were reported to predominantly have two follicular waves in the estrous cycle preceding insemination rather than three waves (Taylor and Rajamahendran, 1991; Ahmad *et al*., 1997; Townson *et al*., 2002). The lower fertility in cow with two-relative to three-follicular waves (Townson *et al*., 2002) would be theoretically exacerbated by longer luteal phases in high producing dairy cows. It might also be possible that the suboptimal uterine environment that possibly had interfered with luteolysis before 1st AI was still acting to cause pregnancy failure after insemination.

Peak progesterone concentrations in the preceding cycle were lower in non-pregnant cows than in cows that became pregnant was in agreement with previous reports (Folman *et al*., 1973; Fonseca *et al*., 1983). In cows treated with progesterone, low dosages of progesterone maintained the large follicle of the first (Ahmad *et al*., 1995) or second wave (Stock and Fortune, 1993) which prolonged the pre-ovulatory secretion of estradiol-17β (Sirois and Fortune, 1990). Decreased fertility (Mihm *et al*., 1994) or early embryonic death (Ahmad *et al*., 1995) ensued when follicles were persistent and pre-ovulatory estradiol was elevated during the last 6 days before ovulation.

## CONCLUSION

About one-third of the cows in this study had prolonged or short luteal phase during the estrous cycle preceding 1st AI. Inseminating cows following prolonged or short luteal phase was associated with reduced pregnancy rate.

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