

# Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows

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## Abstract

The objectives of this study were to derive a useful case definition of delayed resumption of ovarian activity, based on factors associated with reduced fertility, and to assess its impact on subsequent reproductive performance in Holstein cows (*Bos taurus*). Milk samples were collected twice weekly from 219 cows from four commercial herds, and whole-milk progesterone concentrations were determined with an enzyme-linked immunosorbent assay. Ovulation was considered to have occurred 5 d before the first rise of milk progesterone concentration above the basal level. Survival analysis was used to derive a case definition of delayed resumption of ovarian activity postpartum based on factors that were predictive of reduced pregnancy rate. First postpartum ovulation occurring beyond 35 d postpartum was associated with a reduced pregnancy rate (hazard ratio [HR] = 0.50;  $P < 0.001$ ) and was defined as delayed resumption of ovarian activity; overall, 75 (34.9%) cows were in this category. These cows were more likely not to conceive on first artificial insemination (odds ratio [OR] = 2.85;  $P = 0.01$ ) and more likely not to become pregnant within 100 d (OR = 3.30;  $P = 0.001$ ) and 210 d (OR = 3.20;  $P < 0.001$ ) postpartum compared with cows with normal resumption of ovarian activity. Furthermore, 13 (6%) cows that ovulated within 35 d postpartum had a prolonged ( $\geq 14$  d) interval between either first and second or second and third luteal phases postpartum. A prolonged interluteal interval was also associated with a reduced pregnancy rate (HR = 0.35;  $P = 0.02$ ). Days open (mean  $\pm$  SEM) were greater ( $P = 0.0002$ ) in cows with delayed resumption of ovarian activity ( $213 \pm 13$  d) and in cows with prolonged interluteal interval ( $220 \pm 37$  d) than in cows with normal resumption of ovarian activity ( $152 \pm 9$  d). In conclusion, first ovulation occurring beyond 35 d postpartum was defined as delayed resumption of ovarian activity, and the first ovulation occurring within 35 d postpartum but the absence of luteal activity  $\geq 14$  d between two consecutive luteal phases was defined as a prolonged interluteal interval; both abnormalities adversely affected the subsequent reproductive performance of Holstein cows.

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**Keywords:** First ovulation postpartum; Holstein cows; Milk progesterone; Ovarian activity; Reproductive performance

## 1. Introduction

During the past few decades, continued genetic progress for milk production, coupled with nutritional management of high-producing dairy cows, has led to antagonism between high milk production and fertility [1,2]. To have a calving interval of 12 to 13 mo, cows should become pregnant within  $\sim 3$  mo after calving. This requires normal resumption of ovarian activity

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within a few weeks after calving. Numerous recent studies have reported that the reproductive performance of dairy cows was compromised primarily through delayed resumption of ovarian activity postpartum [3–6].

Examination of milk progesterone profiles offers an objective method to characterize postpartum ovarian activity, which can lead to more accurate identification of factors conducive to high fertility and study of syndromes causing subfertility [3]. Although the adverse effect of delayed resumption of ovarian activity based on progesterone profiles is generally accepted, there is no agreement in the literature on what constitutes a delayed resumption of ovarian activity. It still remains unclear until when the first postpartum ovulation should occur to have an optimum reproductive performance under field conditions. Reist et al. [7] considered the normal resumption of estrous cycle within 30 d postpartum as an optimum under practical conditions. Some studies used 3 wk [5] or 4 wk postpartum [8] as a threshold to classify cows into delayed or nondelayed ovulating groups, whereas others have considered the normal resumption of ovarian activity even if the commencement of first luteal activity occurred as late as 56 d [6], 60 d [9], or 65 d postpartum [10]. However, most other studies considered first postpartum ovulation beyond 45 d postpartum (or first rise in progesterone level above basal level beyond 50 d postpartum) as delayed resumption of ovarian activity postpartum [3,4,11–15]. Thus, diagnosis of delayed resumption of ovarian activity postpartum has been hampered by the lack of a universal definition. Therefore, a practical case definition of delayed resumption of ovarian activity, in terms of measured impact on actual reproductive performance is required. Likewise, a substantial proportion of cows even ovulating within the optimum period postpartum may have cessation of cyclicity or formation of an incompetent corpus luteum, as indicated by a prolonged interval between subsequent luteal phases. The objectives of this study were to derive a clinically useful definition of delayed resumption of ovarian activity based on factors that are predictive of reduced pregnancy rate and to assess the impact of delayed resumption of ovarian activity and prolonged interluteal interval on subsequent reproductive performance in high-producing Holstein cows.

## 2. Materials and methods

The Institutional Animal Care and Use Committee of the Yamaguchi University approved all the procedures involving animals.

### 2.1. Animals and management

This study was conducted on 219 Holstein cows (*Bos taurus*) from four commercial dairy herds. Herd A was located in Hokkaido, the northeastern region of Japan. Herd B (in Tottori), and Herds C and D (in Yamaguchi) were located in the middle to southwestern region of Japan. The herd inclusion was dependent on the regular progesterone monitoring program conducted by the Laboratory of Theriogenology, Yamaguchi University, in these herds for reproductive management of postpartum cows. The cows were nonseasonal, year-round calvers, milked twice daily with herd average 305-d milk yield between 9400 and 12,000 kg/cow. The parity of cows ranged from 1 to 7. Cows in Herd A (herd size, 100 cows) and Herd B (herd size, 150 cows) were kept in a loose-housing system under roofed structures (zero grazing system). Cows in Herds C and D (herd size, 20 cows per herd) were kept in 24-h tie-stall barns with rubber mattresses on the floor. A voluntary waiting period of 45 d was generally maintained, and cows detected in estrus after this period were artificially inseminated. Cows were inspected for the signs of estrus by visual observation in the morning and afternoon before milking period and during other farm activities. In Herds A and B, standing to be mounted was interpreted as a sign of estrus, and those cows were inseminated 8 to 14 h later. In Herds C and D, estrus was detected based on secondary signs of estrus (swelling, relaxation, and congestion of vulva, mucus discharge, bellowing, restlessness, or decreased milk yield), and cows were inseminated 8 to 14 h later. All cows were inseminated with frozen-thawed semen of proven sires from the Livestock Improvement Association of Japan. Pregnancy diagnosis was performed 35 to 70 d after insemination (during monthly herd visits) by transrectal palpation of the uterus or transrectal ultrasonography (Tringa Linear Vet, equipped with dual frequency 5/7.5 MHz rectal transducer; Pie Medical Equipment BV, Maastricht, The Netherlands), and pregnant cows were rechecked by transrectal palpation 1 mo after the first diagnosis.

### 2.2. Milk sampling and milk progesterone assay

Milk samples were collected from postpartum cows twice weekly (every Monday and Thursday), starting from 2 wk postpartum and continuing until 80 d (Herd B) and 90 d (Herd A) postpartum or until the confirmation of pregnancy or decision of culling (Herds C and D). A milk sample (~10 mL) was collected as stripping after milking into a plastic tube containing

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