



Incidence of double ovulation during the early postpartum period in lactating dairy cows



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ABSTRACT

In lactating cattle, the incidence of twin calving has many negative impacts on production and reproduction in dairy farming. In almost all cases, natural twinning in dairy cattle is the result of double ovulation. It has been suggested that the milk production level of cows influences the number of ovulatory follicles. The objective of the present study was to investigate the incidence of double ovulations during the early postpartum period in relation to the productive and reproductive performance of dairy cows. The ovaries of 43 Holstein cows (26 primiparous and 17 multiparous) were ultrasonographically scanned throughout the three postpartum ovulation sequences. The incidence of double ovulation in the unilateral ovaries was 66.7%, with a higher incidence in the right ovary than in the left, whereas that in bilateral ovaries was 33.3%. When double ovulations were counted dividing into each side ovary in which ovulations occurred, the total frequency of ovulations deviated from a 1:1 ratio (60.3% in the right side and 39.7% in the left side, $P < 0.05$). In multiparous cows, double ovulation occurred more frequently than in primiparous cows (58.8% vs. 11.5% per cow and 30.0% vs. 3.8% per ovulation, respectively $P < 0.01$). The double ovulators experienced more anovulatory repeated waves of follicles before their first ovulations than the single ovulators, which resulted in an extension of the period from parturition to third ovulation (81.5 days vs. 64.2 days, $P < 0.05$). In the multiparous cows, the double ovulators exhibited higher peak milk yield ($P < 0.01$) with lower milk lactose concentration ($P < 0.05$), indicating the prevalence of a more severe negative energy balance during the postpartum 3-month compared to the multiparous single ovulators. Our results showed that, regardless of their parity, double ovulation had no impact on the reproductive performance of the cows. Two multiparous cows that experienced double ovulation during the early postpartum period subsequently conceived twin fetuses. It can be speculated that the incidence of double ovulations during the early postpartum period partly contributes to the increased incidence of undesirable twin births in multiparous dairy cows.

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1. Introduction

There have been numerous reports on differences in the functions of the right and left ovaries in mammalian species. The right ovary in humans, for example, produces more ovulations [1], whereas there is no difference in ovulation responses between the right and left ovaries in rabbits [2]. In dairy cattle, pregnancy is established more frequently in the right uterine horn than in the left, indicating that the right ovary is functionally more active than the left [3,4]. Since Casida, et al. reported their results in 1935 [5], it has been known that the right ovary has a greater total follicular

volume than the left ovary. It was concluded from an extensive study on the genitalia of heifer calves that the activity of the right ovary was higher even before the heifers had reached sexual maturity. Although several factors may be responsible for the imbalance between unilateral ovaries [6], the precise mechanism remains unknown.

Most mammals precisely regulate their number of ovulations; *Bos taurus* cattle have two or three follicular waves during normal interovulatory intervals [7]. In each case, the follicles increase in diameter during a common growth phase until a deviation occurs. Follicular deviation is characterized by continuous growth of the largest follicle until it becomes the dominant follicle, and also by a decrease in growth rate and regression of the remaining subordinate follicles [8]. Occasionally, twin follicular selections within a wave become dominant resulting in a phenomenon that has been

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termed codominance [9]. Because both codominant follicles have deviated from the subordinate follicles, and have acquired ovulatory capacity, they are responsive to ovulatory stimuli, leading to the occurrence of double ovulations. If both oocytes derived from such ovulations are successfully fertilized and pregnancy is maintained, dizygotic twin births will occur. It has been reported that most twins in dairy cattle are dizygous [10,11]. According to one recent study, the frequency of monozygotic twin births in dairy cows was only 5.5% of the total twin births [12]. Thus, it is speculated that almost all twins in dairy cows are of the dizygotic type resulting from double ovulation.

Twin calving is generally considered to be undesirable for dairy farming because of the increased risk of pre-term birth, dystocia, abortion, retained placenta, and metritis [13]. Although twinning can result in more calves born per dam, cows produce slightly less milk after twin calving and are culled more often than single-calving cows. It is known that the mean productive lifespan of twin-calving cows tends to be shorter than that of non-twinners [14]. Because there is greater pregnancy loss during the first trimester of gestation in twin-bearing cows compared to single-bearing cows, a recent study attempted to establish a method to eliminate one of the twin embryos by means of intraluteal instillation of PGF_{2α} [14].

Earlier studies have shown that double ovulation rates in dairy cows is established within 2.9%–14.3% of cases based on the results obtained by manual counting of the number of corpus luteum [15,16]; however, these studies presented no information about the milk production level and reproductive performance of the cows in relation to the ovulation number. More recent studies using high-producing dairy cows have indicated that double ovulation rates have been increasing in the range of 13.3%–22.4% [17–20]. The increased level of milk yield might be one of the major factors contributing to the increased incidence of double ovulation observed in lactating dairy cows [19,21]; however, another study has demonstrated an antagonistic association between milk production level and ovarian activity [20], which indicates that the presence of other factors might be involved in this phenomenon. Although there have been many studies investigating the occurrence of double ovulation, to the best of our knowledge, none of these has specifically focused on the number of ovulations during the early postpartum period in lactating dairy cows.

Modern dairy cows produce vast amounts of milk as a consequence of significant genetic improvements, combined with nutritional management optimized toward lactation. However, the increase in feed intake corresponding to high milk production occurs over a long interval, so that the producers may not achieve a positive energy balance (EB) until 8–12 weeks postpartum [22]. It is generally accepted that high-producing dairy cows typically experience negative EB during the early lactation period. Negative EB often results in atypical ovarian activities, such as double ovulation and anovulatory repeated waves of follicles (RWF), which prolong the postpartum anovulatory period [23]. Close interrelationships between multiple metabolic activities in dairy cows, including negative EB and ovarian activity during the early postpartum periods, have been reported [24]. Additionally, a recent study has indicated the utility of milk content data as indices of EB and ovarian activity [25]. The synthesis of milk lactose depends on glucose supplied to the mammary glands, and its concentration may reflect the energy status of lactating cows because it is synthesized almost entirely from plasma glucose. Recently, it has also been reported that milk protein concentration reflects the energy status of cows during the early lactation period [26].

In a previous study, we demonstrated that most lactating dairy cows regained normal ovarian follicular dynamics after the second ovulation, regardless of the number of ovulations (single or double)

and the ovulation side [27]. In the present study, we reanalyzed the dataset from our previous study to investigate the side on which ovulation occurred and the incidence of double ovulation during the early postpartum period. We also compared the productive and reproductive traits of double ovulators with those of single ovulators.

2. Materials and methods

2.1. Animals

Data presented in this study were collected from 48 lactating (26 primiparous and 22 multiparous) Holstein cows that calved between October 1999 and June 2001 at the National Agricultural Research Center for Hokkaido Region (Sapporo, Japan). The data of two cows were eliminated from the previous dataset, which consisted of 50 cows [27], because of missing values in the milk composition data. During the first 10 weeks after calving, primiparous cows and multiparous cows were housed in a free-stall barn and a tie-stall barn, respectively. All the cows were subsequently housed in the same free-stall barn. Throughout the experimental period, the cows were fed a diet that met all maintenance, growth, and lactation requirements, in accordance with Japanese feeding standards (Agriculture, Forestry and Fisheries Research Council Secretariat, 1999). During summer (May to September), cows were pastured for 3–4 h/day, with the amount of food being reduced to meet the nutritional requirements necessary for this period. Cows were milked twice daily (0900 and 1900 h), and milk yield was recorded daily. Average daily milk yield was calculated for the period covering 7–70 days postpartum, and total milk yield records were corrected for a 305-day lactation period. Milk composition (fat, protein, and lactose) was also measured every month using a mid-infrared spectrometry method (MilkoScan, Foss Japan).

2.2. Ultrasound examinations for monitoring ovarian dynamics

Monitoring of ovaries was performed as previously described [27]. To determine the postpartum interval to each ovulation, the ovaries were examined daily by transrectal palpation in addition to when regular ultrasound examinations were not scheduled. When the largest follicle exceeded 25 mm in diameter, and persisted for >10 days in the absence of a corpus luteum [28], it was classified as a follicular cyst and was not included in data analyses for other normal follicular waves. Five cows developing follicular cysts were excluded, and thus 43 cows were examined in this study. During the postpartum anovulatory period, the occurrence of continuous follicular waves (>4 waves) was defined as anovulatory repeated waves of follicles (RWF) [29].

2.3. Estrus detection and artificial insemination (AI)

All cows were observed twice daily for at least 30 min before milking. Those exhibiting standing estrus, determined with the aid of a heatmount detector (Kamar Inc., Steamboat Springs, CO), or mounting activity accompanied by other symptoms, such as vaginal mucous discharge and swelling of the vulva, were considered to be in estrus. After a voluntary waiting period of 45 days, the cows in estrus were inseminated artificially using frozen-thawed semen from bulls, in which normal fertility had been confirmed. Conception was confirmed by detection of a fetal heartbeat using ultrasonography at 35–40 days after each AI. Because no severe reproductive dysfunction was diagnosed before the first AI, no hormonal treatments were applied to cows during this period. After AI, ovaries were examined daily by transrectal palpation until ovulation was confirmed. When ovulation had not occurred by 24 h

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