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Practical Aspects of the Fertility of Dairy Cattle

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Abstract. To establish sustainability in the dairy industry, it is important that cows become pregnant at a biologically optimal time and at an economically profitable interval after calving. In this review, the results obtained from Holstein cattle in an experimental herd for dairy research are summarized. First, the effect of age at first calving of heifers on productive and reproductive performance was examined. A reduction in calving age from 25.1 to 21.5 months with the same growth rate during the first 12 months after birth had no negative effects on the heifers' performance. Second, the postpartum follicular dynamics of lactating cows were traced in relation to their fertility, and the emergence and fate of cystic ovarian follicles were examined. The premature initiation of ovarian activity does not always improve the fertility of cows as indicated by the number of days open. Third, the occurrences of anestrus ovulation during the early postpartum period were analyzed with reference to the frequency of reversion to anestrus. The premature onset of estrous activity also did not improve fertility, and relapse back into anestrus after the onset of the estrous cycle often occurred during the breeding period. Fourth, some indices for the occurrence of postpartum reproductive events were evaluated as an indicator of the reproductive performance of lactating cows. The milk yield and percentage of body weight loss could be indicators for reproductive events. Finally, the potency of a pedometer system for the detection of typical and atypical estrous behaviors of heifers and lactating cows was evaluated in terms of efficiency and accuracy. The location of the pedometers and housing conditions for the animals affected the estrus detection of the system. These results represent the reproductive potential of modern high-yielding dairy cattle and provide a baseline to evaluate their reproduction.

Key words: Dairy cow, Estrus, Fertility, Milk production, Ovulation

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The challenges facing dairy cattle fertility consist of two heterogeneous factors: the biological changes in the dairy cattle *per se* [1–3] and the continual changes in dairy herd management and the economic environment of the dairy industry [4–6]. The biological factor encompasses some globally common features concerning the genetic improvement of modern high-yielding dairy cattle, such as metabolic profiles and reproductive function [7–10]. However, the second, *i.e.*, human or economic, factor differs among nations, areas and individual herds, and management decisions largely impact the fertility measurement in relation to the changing biological and economic demands [11–15]. In this review, previously obtained results concerning the reproductive physiology and performance of modern dairy cattle are summarized and discussed for practical implications.

The body growth capacity of dairy heifers has been steadily increased by selection for milk yield, and a reduced age at the first calving offers management advantages and decreases the replacement costs; for example, it results in lower overhead costs, decreased overcrowding, and increased production per day of herd-life [16]. A study in the United States indicated that heifers calving at >26 months of age produced similar amounts of 305-day milk as did heifers calving at 24 months of age [17].

After the successful first calving, high reproductive efficiency requires each cow to calve at an interval that maximizes the economic output of the milk production in the face of declining reproductive efficiency over the last two decades of the past century [18, 19]. Rapid progress in genetics and management in the dairy industry has resulted in increased milk production per cow. Metabolic demands for more milk production negatively impact the reproductive function of postpartum cows [20]. The cows with the greatest milk production have the highest incidence of infertility, but epidemiological studies indicate that, in addition to milk production, other factors likely contribute to the decreasing reproductive efficiency of dairy herds, and the expression and detection of estrous behavior is an important factor in determining dairy cows' fertility. Recently, Le Blanc [21] questioned whether the fundamental fertility (*i.e.*, the capacity for reproductive function and successful pregnancy) of dairy cows has in fact declined, as opposed to the success of the management systems and people at meeting the metabolic, nutritional, housing, and social needs of increasingly productive animals but with no less inherent capacity to achieve and maintain pregnancy.

Many factors can influence the function of the ovaries of postpartum dairy cattle [22]. The resumption of ovarian activity plays an important role in their subsequent fertility [23, 24]. Since the ultrasound imaging technique has been applied to the study of bovine follicular dynamics [25–27], it has become clear that most postpartum follicular development occurs in a wave-like manner in

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normal cyclic cattle [28, 29]. Recent studies have revealed that postpartum anovulatory anestrus in dairy cows can be attributed not to the lack of follicular development, but rather to the failure of a dominant follicle to ovulate [18]. The first dominant follicle is selected within 10 days after calving in nearly all dairy cows, independent of their subsequent reproductive performance [26, 30], and the first ovulation can be observed within 2 weeks after calving in dairy cows [28–30].

For postpartum dairy cattle, especially high-yielding cows, the development of anovulatory ovarian cysts during the early lactation period is a significant cause of anestrus [31, 32]. Anovulatory cysts have been further classified as follicular or luteal cysts, and the former are more common than the latter. Follicular cysts are usually defined as fluid-filled cysts that are more than 25 mm in diameter and persist for at least 10 days in the absence of a corpus luteum. The incidence of this condition has been reported to be between 6 and 19% in lactating dairy cows [31]. Ovarian follicular cysts in lactating dairy cattle have been thought to contribute to an extended calving interval [33], and follicular cysts that develop during the early postpartum period often recover spontaneously [34–36]. The ovaries of high-yielding dairy cows frequently contain numerous large anovulatory follicles, which quickly become atretic during the early postpartum period [37]. The persistence of this condition is presumed to be observable as repeated waves of anovulatory large follicles and is believed to delay the resumption of ovulatory activity. Hence, the occurrence of repeated follicular waves has the potential to affect the fertility of postpartum cows. Information on this topic, including follicular dynamics and subsequent fertility, is also limited [30].

Postpartum anovulatory anestrus is regarded as a normal facet of bovine reproduction because the high concentrations of progesterone that prevail throughout pregnancy and the peripartum elevation of the estrogen level cause negative feedback suppression of the hypothalamo-pituitary axis, with the result that follicular activity in the ovaries of full-term pregnant cows is minimal. Furthermore, anestrus ovulations often occur during the early postpartum period [38, 39], but only limited information on the frequency and timing of the events occurring in modern high-yielding dairy cows is available.

A long-term solution for the fertility problem of high-producing dairy cattle should include the improvement of their genetics for dairy reproduction through a balanced genetic selection strategy [40]. However, a short-term solution is needed to maintain the dairy industry in the short term. The body condition score (BCS) is an easy and inexpensive, but also subjective, method to evaluate the body tissue reserves of lactating cows independently of the frame size and body weight (BW) [41]. During the early lactation period, the mobilization of body reserves for milk production induces a negative energy balance that has been reported to affect the reproductive performance of dairy cows [20]. However, the effects are inconsistent; for example, some studies have reported that the BCS had no effect on reproductive indices [42, 43], whereas others have reported significant effects [44, 45]. Domecq *et al.* [46] reported that greater BCS changes during the early lactation period were associated with a reduced probability of conception at the first service in multiparous cows, but not in primi-

parous cows. Thus, many factors, such as the feeding system and level, system of milk production, the cow's genetic background, and parity, might influence these reported results.

Compared to the attention paid to the relationship between the BCS and the reproductive performance of dairy cattle, there have been few reports regarding associations between the more objective measure of the BW and reproductive traits. Senatore *et al.* [47] reported the significant effects of the BW at parturition and BW loss during the early lactation period on the reproductive performance of primiparous cows. Under pasture-based seasonal calving conditions, Buckley *et al.* [45] analyzed the field data from 74 dairy herds and suggested that BW is potentially an important determinant of the likelihood of reproductive success.

The exact detection of estrus, which often occurs during the late post-partum period in high-yielding cows, is an important factor for improving the reproductive performance of modern high-yielding dairy cows [48]. Measurement of the increase in an animal's activity around estrus is one of the most reliable methods of detecting estrus, and radiotelemetric pedometers have recently become available for the automatic, continuous recording of the activity of cattle in terms of the number of steps taken [49–51]. Generally, the main challenge in automatic estrous detection is reducing the number of false positive alerts [9, 50]. A previous study utilizing pedometry as a technique for estrous detection indicated that an animal's type of housing may influence the magnitude of its change in physical activity [52], and recently López-Gatius *et al.* [53] also concluded that factors related to the cow itself and to its management greatly contribute to the level of walking activity at estrus. A pedometry system can be useful for detecting estrus in dairy cows housed in tie stall barns but a number of false positives can be expected [54]. Because the location of the pedometer on an animal's body influences the type of activity being measured [55], the location of the pedometer (attached to the neck or leg) may also influence the efficiency and accuracy of estrous detection.

Age at First Calving

To confirm the effect of genetic improvement on the productive and reproductive performance after first calving, the relationships between the age and the BW at first calving, first lactation yield, and reproductive performance of 267 Holstein heifers were retrospectively analyzed for one experimental herd [56]. According to the history of genetic improvement that had been applied to the herd, the heifers born from 1979 to 1997 were divided into 3 groups of birth phases (early, 1979–86; middle, 1987–91; and late, 1992–97). The age at first calving did not differ among the 3 groups. However, the BW at first calving and milk yield significantly increased from the early to the late phase. In the early group, the milk yield increased as the age at first calving increased, while no correlation was found between milk production and age at first calving in the middle and late groups. In the middle group, the number of services required to conceive increased as the BW at first calving increased. In the late phase, the number of services required to conceive increased as the age at first calving increased. As a result, the number of days open increased as the age, BW at first calving and milk yield increased. These results suggest that

early breeding is not detrimental to the first lactation, is associated with better reproductive performance, and will improve the productivity of recent dairy heifers. This retrospective study using replacement heifers of the Japanese Holstein breed also suggested that an age at the first calving of later than 24 months results in no increase in milk yield and has a detrimental effect on the reproductive performance of heifers during their first lactation period [56].

The increasing growth rate accompanying the increasing yield enables the age at the first calving of heifers to be reduced; however, it was reported based on statistical data that the reduction of the age at first calving from 24 to 22 months has a negative effect on the first lactation milk yield and milk fat production [57]. Van Amburgh *et al.* [58] reported that an experimental reduction of the age at first calving to 21.3 months with a prepubertal growth rate of 1,000 g/day resulted in a reduction in milk yield compared with heifers who had been fed to gain 600 g/day, but the reduction was associated with a lower BW at calving for heifers calving at 21.3 months. The results also suggested that the reproductive performance of the heifers was not negatively affected by the age at first calving or the BW at first calving.

An increased risk of calving difficulty, which results in impaired fertility during the first lactation period, is another drawback sometimes associated with accelerated growth for early breeding in heifers [59]. Thus, another study evaluated the effects of a difference in the age at the first breeding (early at 12.3 ± 0.5 ; late at 15.9 ± 1.2 months) on the fertility after the first calving using heifers with a prepubertal growth rate of 900 g/day under the same feeding and management conditions. This study also compared the milk yields and productivity of cows that first calved at different ages (early at 21.5 ± 0.5 ; late at 25.1 ± 1.2 months) up to their third lactation [60].

With the exception of the average dairy gain at 18 months due to fetal growth (early, 883 ± 18 g vs. late, 829 ± 25 g), no differences in body growth were found between the two breeding groups. The difference in mean age at conception of heifers between the two breeding groups (3.6 months) caused nearly identical differences in their mean ages at the first to third calvings. No significant differences were detected between the two breeding groups in calving difficulty, fertility parameters, gestation lengths or calving intervals up to the third lactation. The milk yields were similar in the two breeding groups, but the production period up to the completion of the third lactation in the early breeding group was smaller. Thus, the reduction in the calving age from 25.1 to 21.5 months with the same growth rate of approximately 900 g/day during the first 12 months after birth had no negative effects on the fertility or milk production of heifers during the first to third lactations. This result is consistent with previous results showing that the growth rate during the prepubertal (BW of 90 to 320 kg) period, which was increased from 680 to 940 g/day, did not significantly affect the milk yield during the first and second lactations [58].

Weekly changes in the BW and BCS after the first calving are shown in Fig. 1. The nadirs of the mean BW and BCS were recorded at 4 and 8 weeks postpartum for each breeding group, respectively. Compared to late-bred heifers, the mean BW of early-bred heifers was lower throughout the pre-calving to postpartum 10 weeks, and the mean BCS in the early-bred heifers was

Fig. 1. Weekly changes in body weight (a) and body condition score (b) in heifers after the first calving. Each point indicates the mean $\pm$ SD (shown by vertical bars). In heifers bred from 12 months of age ($\bullet$; AI-12), the means of the body weights were consistently lower than in the heifers bred from 15 months of age ($\blacksquare$; AI-15) throughout the measuring period ($P < 0.01$). The means of the body condition scores in the AI-12 heifers at 2, 4 and subsequent weeks postpartum were lower than those in the AI-15 heifers ($P < 0.05$) [60].

lower at 2 and 4 weeks and later. The values at the nadir of the BW and BCS in the early-bred group were 514 kg and 2.70, respectively. If inadequate management condition reduces these nadir values, then the productivity and fertility will be endangered. Therefore, an early breeding program for heifers will be successful when adequate management practices can be applied during their first lactation period. Monitoring of the BCS or BW will help to maintain acceptable management conditions.

Studies during the last three decades have shown that rapid growth rates in young heifers can reduce mammary development and subsequent milk production [61, 62]. However, a higher BW gain due to a higher genetic potential for growth is positively related to milk yield [62]. The negative effect of a high feeding level before puberty occurs in all breeds, but the level of feeding that causes reduced milk yield is variable. For modern Holstein

Table 1. Reproductive performance and milk yield of postpartum dairy cows differing in parity

	Primiparous	Multiparous	Total
Days to			
First ovulation	26.7 ± 3.2	35.5 ± 3.5	30.9 ± 2.4
First detected estrus	47.8 ± 3.7 ^a	63.1 ± 4.4 ^b	55.2 ± 3.0
First service	65.7 ± 2.9 ^a	77.8 ± 4.0 ^b	71.5 ± 2.6
Uterine diameter involution	17.3 ± 0.9	18.3 ± 0.8	17.8 ± 0.6
Duration (days)			
First ovarian cycle ¹	15.1 ± 1.1	19.1 ± 1.3	17.0 ± 1.1
Second ovarian cycle ²	20.7 ± 0.9 ^a	23.1 ± 0.7 ^b	21.8 ± 0.6
Number of services per conception			
By 100 days	1.39 ± 0.12	1.40 ± 0.16	1.39 ± 0.10
By 180 days	1.65 ± 0.17	1.59 ± 0.17	1.62 ± 0.12
Total	1.96 ± 0.23	1.67 ± 0.19	1.82 ± 0.15
Days open			
By 100 days	73.1 ± 4.1	77.5 ± 3.7	75.1 ± 2.8
By 180 days	83.1 ± 5.4	96.4 ± 7.3	89.6 ± 4.6
Adjusted days open ³	93.6 ± 7.6	99.0 ± 7.3	96.2 ± 5.2
Number of cows (%)			
Total	26 (52)	24 (48)	50 (100)
Pregnant by 100 days	18 (69)	15 (63)	33 (66)
Pregnant by 180 days	23 (88)	22 (92)	45 (90)
Milk yield (kg)			
Daily (1 to 10 weeks)	30.0 ± 0.8 ^a	42.0 ± 1.5 ^b	35.8 ± 1.2
305-day	7932 ± 214 ^a	10708 ± 348 ^b	9265 ± 281

Mean ± SEM. ¹ From the first to second ovulation. ² From the second to third ovulation. ³ Cows that did not have a positive pregnancy diagnosis within 180 days in milk were allocated a days open value of 21 days greater than the interval from calving to their last unsuccessful service. ^{a,b} Values in the same row with different superscripts are significantly different ($P < 0.05$) [78].

heifers that the recommended average age at first calving is ≤ 24 months with a BW > 560 kg after calving to maximize lactation performance and to reduce rearing costs [63, 64]. Our results support this recommendation and also recommend the monitoring of the growth of Holstein heifers around their first breeding age as well as the estimation of the BW from the hip width as a useful tool for rapid estimation in the field [65].

Postpartum Resumption of Ovarian Activity

The average interval from parturition to postpartum first ovulation was reported to be approximately 2 weeks in the 1960s [34, 66] and was then prolonged to 3 weeks during the 1970s–90s [23, 67]; recently, it has been prolonged to 4 weeks or more [19, 68].

Follicular dynamics during the normal estrous cycle have been extensively studied as an adjunct to develop effective estrus-synchronization strategies [69]; however, less information is available on postpartum follicular dynamics [28, 70]. Moreover, the relationship between early follicular dynamics and subsequent fertility is not clear in modern dairy cows. Most cattle exhibit 2 or 3 follicular waves during the normal estrous cycle [69]. However, a one-wave and a four-wave cycle are sometimes observed [26, 71]. Townson *et al.* [72] reported that fertility tended to be greater in lactating cows that were inseminated after ovulation of the third wave dominant follicle compared with those that were inseminated after ovulation of the second wave dominant follicle.

Since Thatcher and Wilcox [73] reported that the occurrence of multiple estrous periods (up to 4) during the first 60 days postpartum was associated with an increased conception rate, the early resumption of cyclic ovarian activity and the occurrence of luteal concentrations of progesterone have been considered beneficial to fertility. However, whether the early resumption of ovarian activity improves the fertility of dairy cows is still controversial [23, 74–76]. Early ovulation, which was assessed by the milk progesterone-determined luteal activity prior to 21 days postpartum, was associated with reduced pregnancy rates and prolonged calving to conception intervals in multiparous dairy cows [24, 77].

To clarify the process for the resumption of ovarian activity, the early postpartum follicular dynamics were characterized using 50 (26 primiparous and 24 multiparous) lactating Holstein cows, and the effect of the timing of the resumption of ovarian function on the subsequent reproductive performance of the cows, including the expression of estrus, pregnancy rate, and days open, was examined [78]. The ovaries and uterine horns of postpartum lactating cows were examined by ultrasonography thrice weekly until the first services occurred after a 45-d voluntary waiting period. No fertility differences were detected between primiparous and multiparous cows (Table 1). The frequency of the first ovulation after various numbers of follicular waves were: 46% after one wave, 22% after 2 waves, 12% after 3 or 4 waves, and 20% after ≥ 5 waves or in cows developing follicular cysts. Before the second ovulation, 44% of cows had one wave, 38% had 2 waves, 14% had 3 or 4 waves, and

Table 2. Fertility and milk yield in postpartum lactating dairy cows having various numbers of follicular waves before the first ovulation

Item	Number of follicular waves before the first ovulation			
	1	2	3 or 4	>4 ¹
Days to				
First ovulation	18.1 ± 1.0 ^a	29.2 ± 1.5 ^b	38.5 ± 4.1 ^c	57.6 ± 4.4 ^d
First detected estrus	42.8 ± 2.3 ^a	50.4 ± 5.1 ^a	70.5 ± 4.1 ^b	79.6 ± 5.1 ^b
First service	63.1 ± 2.3 ^a	68.6 ± 6.5 ^a	74.7 ± 2.2 ^{ab}	92.2 ± 5.4 ^b
Uterine diameter involution	19.9 ± 0.9 ^a	15.9 ± 0.7 ^b	15.5 ± 1.7 ^b	16.3 ± 1.2 ^b
Number of services per conception				
By 100 days	1.44 ± 0.13	1.57 ± 0.20	1.00 ± 0.00	1.33 ± 0.33
By 180 days	1.68 ± 0.19	1.91 ± 0.25	1.40 ± 0.40	1.30 ± 0.23
Total	1.96 ± 0.23	1.91 ± 0.25	2.00 ± 0.68	1.30 ± 0.21
Days open				
By 100 days	72.6 ± 4.7	74.4 ± 6.7	72.3 ± 2.3	84.2 ± 4.1
By 180 days	79.5 ± 5.6	97.5 ± 11.0	90.6 ± 18.4	99.5 ± 8.7
Adjusted days open ²	92.2 ± 8.2	97.5 ± 11.0	103.5 ± 19.8	99.5 ± 8.7
Number of cows (%)				
Total	23 (46)	11 (22)	6 (12)	10
Pregnant by 100 days	16 (70)	7 (63)	4 (67)	6 (60)
Pregnant by 180 days	19 (83)	11 (100)	5 (83)	10 (100)
Milk yield (kg)				
Daily (1 to 10 weeks)	32.7 ± 1.7 ^a	36.6 ± 2.1 ^{ab}	36.7 ± 3.2 ^{ab}	41.6 ± 2.0 ^b
305-day	8519 ± 402 ^a	9390 ± 516 ^{ab}	9553 ± 908 ^{ab}	10671 ± 515 ^b

Mean ± SEM. ¹ Includes cows that developed follicular cysts. ² Cows without a positive pregnancy diagnosis by 180 days in milk were assigned a days open value equal to 21 days after their last unsuccessful service. ^{a,b,c,d} Values within rows with different superscripts differ ($P < 0.05$) [78].

4% had ≥ 5 waves. Before the third ovulation, the frequency of cows ovulating after one wave was dramatically decreased to 4%, that of cows ovulating after 2 waves was increased to 71%, and that of cows ovulating after 3 or 4 waves was increased to 25%. Although a 4-wave pattern before the third ovulation was observed in one cow, no cow had ≥ 5 waves before the third ovulation. Most of the cows regained normal ovarian follicular dynamics after the second ovulation.

The mean number of days until the first ovulation was increased as the number of follicular waves increased before ovulation. The duration of the first ovarian cycle (from the first to second ovulation) was less when the second ovulation occurred after one wave; however, those occurring after 2, 3, or 4 waves were within the normal range. For the second ovarian cycle (from the second to third ovulation), the mean intervals after 3 or 4 waves were greater than those that occurred after only 1 or 2 waves. Table 2 shows the fertility and milk yield in cows having various numbers of follicular waves before the first ovulation. Cows having the longest intervals from calving to the first ovulation produced the most milk and also had prolonged intervals until the first estrous activity. Differences in the follicular dynamics before the first ovulation altered the intervals until the first estrus, first service, and uterine involution, but these differences did not affect the pregnancy rate, number of services, or days open. Our finding that the first postpartum insemination after 3 follicular waves tended to result in greater pregnancy rates than insemination after 2 follicular waves was consistent with the results that were reported by Townson *et al.* [72].

The early resumption of ovarian activity promoted by reduced milking frequency did not shorten the calving to conception inter-

val [79]. It has been suggested that early luteal activity negatively impacts the involution of the uterus after calving [80] and reduces fertility in dairy cows [24]. From parturition until first ovulation, a period when the concentrations of progesterone are minimal, the uterus is resistant to infections, and purulent uterine infections seldom develop during this period. After ovulation, when concentrations of progesterone increase, the uterine immune system becomes down-regulated, creating a uterus that is susceptible to infection [80]. In our results (Table 2), the days required for uterine diameter involution in cows having one wave were greater than for those ovulating after two or more waves. Delayed uterine involution might be caused by early exposure of the uterus to progesterone and might explain why no difference was detected in the number of days open among the four groups that were classified by the number of follicular waves before the first ovulation [81].

Emergence of Cystic Follicles

Since ultrasound imaging techniques have been applied to the monitoring of bovine ovarian structure, the accuracy of diagnosis for follicular cysts has improved [82, 83], and the development and turnover of cysts can be accurately traced [84]. Although there is extensive information on the dynamics of follicular cysts that have already developed or been induced and on the endocrine status of cows bearing cysts [31], there has been very limited information on the developing and atretic processes of spontaneously occurring follicular cysts [29].

A previous study [78] summarized the postpartum follicular dynamics. Subsequently, the fate of the cystic ovarian follicles

Fig. 2. Follicular dynamics and corpus luteum growth in 5 cows that developed follicular cysts: (a) cow FC-1, (b) cow FC-2, (c) cow FC-3, (d) cow FC-4, and (e) cow FC-5. The diameters of the follicles and corpora lutea are plotted with solid lines for the right ovary and with dotted lines for the left ovary [85].

occurring spontaneously among the 50 cows during the early postpartum periods was traced by characterizing the dynamics of the cystic follicular waves and the fertility of the cows bearing cystic follicles after self-recovery was evaluated [85]. The anovulation cases in other cows with repeated follicular waves before the first postpartum ovulation, which seemed to represent one type of folli-

cular cyst [86], were also characterized for their follicular dynamics and evaluated for fertility.

Among 50 cases where the early postpartum follicular dynamics were examined [78], 15 cases showing cystic follicles, some of which subsequently transitioned to follicular cysts, were observed before and after the first postpartum ovulation. Ten of the 15 cases

Fig. 2. continued

were classified as cystic follicles, while the other 5 cases were diagnosed as follicular cysts. In 8 of the 10 cows, the cystic follicles emerged before the first ovulation and were ovulated or regressed. With the exception of one case, the ovulatory cystic follicles were the first detected dominant follicles. The mean maximum diameter of the 5 ovulated cystic follicles was 37.8 mm, while that of the 3 atretic follicles was 29.0 mm. The mean growth rate of the ovulatory cystic follicles was greater than that of the atretic follicles (1.89 ± 0.23 vs. 1.35 ± 0.20 mm/day).

In 4 of the 5 cows that were diagnosed as having follicular cysts, the cysts emerged before the first postpartum ovulation; however, in the fifth cow, the cysts emerged after the first ovulation was completed (Fig. 2). Ten cysts were observed in 5 cows, and their mean growth rate was 1.55 ± 0.68 mm/day. None of the cows displayed nymphomaniacal behavior while the follicular cysts were present. In the first case (cow FC-1), a single cyst developed on each ovary, followed by two additional cysts growing on the right ovary. With 3 cysts on the right ovary, the first and second postpartum ovulations occurred on the left side without estrous activity. Before the third ovulation on the left side, the cow showed its first estrous activity, was inseminated and conceived. In the next two cases (cows FC-2 and -3), the first postpartum ovulation occurred while there were single cysts on the right and left ovary. In cow

FC-2, the first and second ovulations were twin ovulations. The cow showed estrus activity at the third ovulation and was inseminated but did not conceive. In cow FC-3, a twin ovulation occurred that was accompanied by estrous activity after 42 days of the first ovarian cycle, during which a new cyst developed, again on the left ovary. The cyst observed in cow FC-4 was not atretic but ovulated without estrous activity after growing for 21 days; subsequently, a normal corpus luteum developed and regressed during a normal ovarian cycle. In cow FC-5, a cystic follicle developed after the first ovulation, but it disappeared. After the corpora lutea regressed, a single cyst emerged on each ovary. While the cysts were still present, the second ovulation occurred 49 days after the first ovulation and was accompanied by the first postpartum estrus.

Later first postpartum ovulations were observed in 6 different cows that had 5 to 13 repeated waves of follicles, and second ovulations occurred during ovarian cycles that lasted only 8 to 11 days (Fig. 3). The first postpartum estrus was detected at the first ovulation in cow RF-2; at the second ovulation in cows RF-1, RF-3, and RF-6; and at the third ovulation in cows RF-4 and RF-5. Each cow, except for cow RF-1, was inseminated at the third ovulation with no hormonal treatment; cow RF-1 was inseminated at the fourth ovulation because it was anestrus at the third ovulation. All six cows conceived at the first-service insemination.

Fig. 3. Follicular dynamics and corpus luteum growth in cows with repeated waves of follicles before the first postpartum ovulation. (a) Five waves emerged before the first ovulation (cow RF-1), (b) six waves emerged before the first ovulation (cow RF-2), (c) six waves emerged before the first ovulation (cow RF-3), (d) seven waves emerged before the first ovulation (cow RF-4), (e) 10 waves emerged before the first ovulation (cow RF-5), and (f) 13 waves emerged before the first ovulation (RF-6). The diameters of the follicles and corpora lutea are plotted with solid lines for those emerging from the right ovary and with dotted lines for those emerging from the left ovary [85].

Parkinson [86] interpreted the above results to mean that the development of a cyst is symptomatic of a more generalized disturbance of the ovarian function, which ranges from a series of anovulatory follicle waves of normal duration, through the temporary appearance of anovulatory cystic structures that develop and regress in a cyclical manner, to anovulatory cysts that persist in the ovary for extended periods of time.

The reproductive traits of these cows are summarized in Table 3. The mean milk yield of the cows that developed follicular cysts was higher than that of the cows with cystic follicles. The first

postpartum ovulation was delayed by the development of follicular cysts and by the emergence of repeated waves of follicles, the latter causing a more severe delay in the resumption of the ovarian cycle. The mean duration of the first ovarian cycle was prolonged by the development of follicular cysts but was shortened by having repeated waves of follicles. The timing of the first detected estrus and the first insemination was delayed in the cows with follicular cysts or with repeated waves of follicles, but the number of services required for conception was lower in the cows with repeated waves of follicles than it was in those with cystic follicles. As a result,

Fig. 3. continued

there were no significant differences between the mean numbers of days open. The mean interval to uterine diameter involution was shorter in the cows with repeated waves of follicles than it was in the cows with cystic follicles or follicular cysts. When the cows were inseminated at a later time because of the development of follicular cysts or repeated waves of follicles, the improvement in their conception rates at the first insemination tended to compensate for their prolonged postpartum interval to estrus.

Anestrus Ovulation; Incidence and Reversion

From a comparison of fertility between New Zealand dairy cattle (grazing management system) and North American dairy cattle (confinement management system), it was speculated that the length of the postpartum anovulatory anestrus *per se* may not be a

major factor contributing to infertility in cattle unless the anestrus period extends into the breeding period [40]. Parkinson [86] noted that the problematic noncycling condition can be a consequence of prolonged anovulation, ovulations without estrus, or reversion to an anestrus state after a previous detection of being in estrus. This relapse back into anestrus does have a significantly negative impact on the reproductive performance of dairy cows even though the resumption of the estrous cycle occurs during the early postpartum period.

One of the short-term strategies for increasing fertility in dairy cows is the intensive reproductive management of estrous and ovulation by hormonal treatments [87]. Although these treatments increase the estrus detection rate and the submission rate to insemination, the conception and pregnancy rates are not always able to be increased. Meta-analysis of hormonal synchronization datasets

Table 3. Productive and reproductive variables in cows with cystic follicles (CF), follicular cysts (FC) and repeated waves (RF)

Variable	CF (n=10)	FC (n=5)	RF (n=6)
305-day milk yield (kg)	9,241 ± 1,698 ^a	11,693 ± 1,398 ^b	10,038 ± 1,422 ^{ab}
First ovulation (days postpartum)	27.4 ± 4.4 ^a	45.4 ± 8.1 ^b	64.2 ± 14.0 ^c
First ovarian cycle (days)	17.8 ± 6.0 ^a	28.3 ± 16.5 ^b	9.5 ± 1.0 ^a
Second ovarian cycle (days)	21.3 ± 1.2	23.5 ± 3.4 ¹	19.2 ± 5.4
First estrus (days postpartum)	43.5 ± 5.0 ^a	81.8 ± 7.0 ^b	78.5 ± 6.4 ^b
First insemination (days postpartum)	66.3 ± 6.2 ^a	96.4 ± 26.6 ^b	94.8 ± 14.0 ^b
Number of services required to conceive (pregnancy rate, %)	2.10 ± 0.57 ^a (100)	1.60 ± 0.89 ^{ab} (100)	1.00 ± 0.00 ^b (100)
Days open	98.0 ± 29.9	111.0 ± 38.4	94.8 ± 14.0
Uterine diameter involution (days postpartum)	19.3 ± 3.6 ^a	18.6 ± 3.9 ^a	14.3 ± 1.5 ^b

Mean ± SEM. ¹ n = 4. ^{a,b,c} Values within rows with different superscripts differ (P<0.05) [85].

Fig. 4. Frequency distributions of the different times of each postpartum ovulation stage in fertile dairy cows [89].

has demonstrated that the overall pregnancy rates are not improved by this form of intervention [88]. Depending on the postpartum intervals at the onset of the treatment programs, some cows cannot respond to a hormonal treatment because of the absence of the corpus luteum or matured follicle [68]. Estrus synchronization programs that rely on the detection of estrus for the correct timing of insemination also require an inherent capability of the cow to express behavioral estrus.

To provide a reference for improving and studying the reproductive performance of modern high-yielding dairy cattle, the frequency of anestrus ovulations during the early postpartum period in relation to their subsequent fertility was described using data from 92 lactations with 368 ovulations, and the frequency and timing of the occurrence of relapse back into anestrus after having

resumed normal cycling activity was identified [89]. As summarized in Fig. 4, there were cows representing 4 different stages of ovulations at any one time (either the first to fourth or the second to fifth) during weeks 10 to 13. This finding that at a certain number of weeks postpartum the individual cows could be at very different stages of ovulation could be helpful in analyzing the possible causes of unexpected outcomes from the hormonal treatment programs and in developing successful hormonal programs in dairy herds.

The cows that were observed to be standing to be mounted were considered in standing estrus, and the cows showing mounting activity accompanied by other signs but not observed to be standing were considered in mounting estrus. Ovulations without standing estrus or mounting estrus activity were considered anestrus ovula-

tions. Only 17% (15% standing and 2% mounting estrus) of the ovulations during the initial 4 weeks postpartum were accompanied by a detected estrus. While the frequencies of standing estrus increased to 36% at the second 4-week interval (weeks 5–8), 52% at the third (9–12), 63% at the fourth (13–16), and 84% at the fifth interval or later (more than 16 weeks), the frequencies of mounting estrus increased to 15% at the second 4-week interval and to 27% at the third, but then decreased to 20% at the fourth and 14% at the fifth interval or later. The ratios of mounting estrus to the total number of estrus events (standing estrus + mounting estrus) increased from 11% in the first 4-week interval to 29% in the second and 34% in the third and then decreased to 24% in the fourth and 14% in the fifth or more 4-week intervals. These results suggest that intensive detection not only for standing estrus but also for

mounting estrus can increase the submission rate to insemination.

Only 10% of the first ovulations were accompanied by standing estrus behavior; the percentage of ovulations that were accompanied by standing estrus increased to 41%, 61%, 70%, and 74% at the second, third, fourth, and fifth (or more) ovulations, respectively (Fig. 5). The percentage of first ovulations that were detected with mounting estrus was 1%; this increased to between 19 and 24% for the subsequent ovulation sequences. The percentages of anestrus ovulations consistently decreased from 89% to 7% between the first and fifth or more ovulation sequences. Of the total of 368 ovulations observed, 46% were accompanied by standing estrus, 17% by mounting estrus, and 37% were anestrus ovulations.

Fourteen of 92 cows (15%) relapsed into anestrus ovulations after the onset of postpartum estrus was detected (either as standing estrus or mounting estrus). One cow relapsed thrice, and another relapsed twice, resulting in a total of 17 relapses (13% of the total anestrus ovulations). Sixteen of the 17 relapses were observed during the second to fourth 4-week intervals (weeks 5–16). The percentages of anestrus ovulations that were relapsed anestrus ovulations during each 4-week interval were as follows: 0% in the first, 3% in the second, 29% in the third, 80% in the fourth, and 100% in the fifth or more 4-week interval. There was no effect of parity on the relapse to anestrus. The mean number of days for which the cow's anestrus relapse lasted before it returned to estrus was 48.1 days. The early resumption of the estrous cycle with a relapse back into anestrus may be a possible cause of the contradictory effect of the onset of estrous activity on the reproductive performance.

The reproductive parameters of the cows resuming estrous activity at different ovulation stages are shown in Table 4. As would be expected, the number of days between the parturition and the first detected estrus was lowest for the group that showed estrus at the first ovulation and increased as the ovulation stage at which the first estrus had occurred increased. However, the lowest mean number of days open was recorded in the group that showed signs of estrus at the third ovulation because of the relatively higher con-

Fig. 5. Frequency distributions of estrous behavior at different ovulation stages [89].

Table 4. Reproductive parameters and milk yield in postpartum dairy cows that resumed estrous activity at the first to fifth ovulations and were subsequently pregnant

Number of ovulations	First	Second	Third	Fourth or Fifth	Total
Number of cows (%)					
detected first postpartum estrus ¹	10 (11)	50 (54)	26 (28)	6 (7)	92 (100)
relapse back into anestrus ²	3 (30)	8 (16)	2 (8)	1 (17)	14 (15)
pregnant at first service	4 (40)	28 (56)	17 (65)	1 (17)	50 (54)
Weeks to first ovulation ³	5.4 ± 0.7	4.9 ± 0.3	4.7 ± 0.5	4.3 ± 0.9	4.8 ± 0.2
Days to first detected estrus	35.5 ± 5.4 ^a	49.3 ± 2.4 ^b	64.7 ± 3.3 ^b	96.3 ± 7.0 ^c	55.2 ± 2.3
Days to first service ⁴	78.6 ± 5.6 ^{ab}	74.3 ± 2.5 ^a	71.2 ± 3.5 ^a	96.5 ± 7.2 ^b	75.3 ± 1.9
Number of services per pregnancy	2.30 ± 0.30	1.66 ± 0.14	1.46 ± 0.19	2.17 ± 0.39	1.71 ± 0.16
Days open	118.4 ± 10.4 ^a	92.4 ± 4.7 ^{ab}	85.0 ± 6.5 ^b	126.7 ± 13.5 ^a	95.4 ± 3.6
Milk yield (kg/305-day) ⁵	8536 ± 542	9497 ± 242	9720 ± 336	9763 ± 700	9473 ± 179

Mean ± SEM. ¹ Estrus was defined as observation of a cow standing to be mounted or observation of mounting activity accompanied by other symptoms with no standing activity and was confirmed by the subsequent ovulation within 2 days after the activity. ² An anestrus ovulation was observed after the resumption of the estrous cycle was detected. ³ Ovulations were confirmed by rectal palpation or ultrasound scanning. ⁴ Postpartum first service was initiated after the 45-day voluntary waiting period. ⁵ The mean of the first estrus in the first ovulation group was lower than the pooled mean (9,587 ± 186 kg) of the other three groups ($P = 0.03$). ^{a,b,c} Values within rows with different superscripts differ ($P < 0.05$) [89].

ception rate at the first service and the lower number of services required to become pregnant. The mean milk yield of the group that showed estrus at the first ovulation was lower than the pooled mean of the other three groups. These results indicate that an onset of estrous activity that is too early or too late may increase the number of days open in high-yielding dairy cows.

Indices for the Occurrence of Reproductive Events

Recent genetic improvement of milk yield has resulted in negative effects on cow reproduction [90–92], partly because of homeorhetic adaptation, which is the orchestrated or coordinated control of metabolism of the body tissues that are necessary to support a physiological state [93]. Increases in milk yield have prolonged the intervals to postpartum at the first ovulation, estrus and insemination [1, 20, 78, 94–96]. However, how the increased milk yield of lactating cows impacts the conception rate [94, 97–101] and the number of days open remain controversial [102–106].

In previous results, the premature resumption of ovarian activity as identified by the first ovulation postpartum shortened the intervals to the resumption of estrus activity and the first service but did not improve the reproductive performance as indicated by the number of days open at the experimental herd level [78, 85]. However, at the practical herd level, the delayed resumption of estrus activity combined with poor estrus detection and relapse back into anestrus often results in a first service that is too late and in an increased number of days open [85, 86]. Thus, a rough estimation of the relationships between productivity and the intervals to postpartum reproductive events will be helpful for the management practice of dairy reproduction.

The results from our experimental herd indicate that the intervals to the BCS nadir and the loss of BCS at nadir as relative values were significantly correlated with the intervals to the first ovulation and first estrus, respectively. However, they cannot be practical indicators for the initiation of ovulation and estrus under field conditions because weekly BCS estimation is necessary to determine these relative BCS values [107]. As a productive parameter, the 305-day milk-yield (MY305) is significantly correlated with the day of first ovulation, estrus and insemination (Fig. 6). The mean day of first ovulation and estrus can be easily predicted from the 305-day yield. For practical considerations of initiating the synchronization of the ovarian or estrous cycle, the resumption of ovulation activity (postpartum first ovulation) and estrous activity (postpartum first estrus) occur on average at MY305 (t) times 3.3 (10/3) and MY305 times 6 days, respectively. For example, the average intervals until the postpartum ovulation and estrous activity in cows yielding 9 t (9,000 kg) or 12 t (12,000 kg) are expected to be 30 and 54 days or 40 and 72 days, respectively.

In contrast, the intervals to conception (days open) could not be predicted from MY305 or BCS, but they did have a significant correlation with the milk fat to protein ratio at 4 months postpartum [107]. However, this relationship could not represent a practical predictor for the number of days open because some cows would conceive within 4 months postpartum.

Although MY305 reflects only the production performance (output) level of lactating cows, the BCS and BW reflect both the

Fig. 6. Linear regression of the intervals of days postpartum to first ovulation (y_a), first detected estrus (y_b), and first service (y_c) on the 305-day milk yield (x) [107].

production performance and the dry matter intake (input). Because changes in the gut fill with advancing lactation contribute to changes in the BW, postpartum dairy cows require an even longer period of time to return to precalving values [108]. The results of our study indicated that the changes in the BCS and BW measurements from prepartum to 10 weeks postpartum were parity-dependent, but the changes in the BW loss as percentages of the prepartum value were parity-independent [109]. In the primiparous cows, the BW nadir was recorded at 30 days postpartum with a

Fig. 7. Weekly changes in the body weight change ratio (BW loss, %) of cows with early (○) and late (□) reproductive traits. Each point indicates the mean $\pm$ SD (shown by vertical bars). Early was defined as the first ovulation, first detected estrus, and insemination occurring within 21, 42, and 63 days in milk, respectively; the establishment of pregnancy within 84 days in milk was considered to be early conception. Otherwise the events were considered to occur late [109]. * Significantly different between the early and late occurrence groups ($P < 0.05$). ** Significantly different between the early and late occurrence groups ($P < 0.01$).

15.5% loss, and at 15 days later (at 45 days postpartum), the BCS nadir was recorded with a 0.55-point loss, while in multiparous cows the BW nadir at 33 days was recorded with a 17% loss and the BCS nadir at 58 days was recorded with a 0.71-point loss. Therefore, the percentage of BW loss, rather than the BCS, was considered a parity-independent indicator during the early postpartum period in lactating cows. Early recovery of the BW might be a result of increased dry matter intake that enhanced the gastrointestinal fill of the cows. The recovery of the BW, which includes gastrointestinal contents, was initiated at 30–33 days postpartum, regardless of parity. The recovery of the body fat reserves, which is initiated at the BCS nadir, was delayed by approximately 15 and 25 days from the BW nadir in primiparous and multiparous cows, respectively.

As shown in Fig 7, cows with late occurrence of the first ovulation postpartum, estrus, and insemination underwent severe BW loss compared to their early counterparts; however, this difference was not found between early and late conceiving cows. The occurrences of ovulation, estrus, and insemination mainly depend upon the ovarian activity that is affected by the negative energy balance during early lactation, while other factors, such as uterine involution and timing of insemination, should be involved with the establishment of pregnancy [78]. The application of an automatic BW measuring system to dairy herds is expected to provide an effective tool for monitoring reproductive events in dairy herds, with the exception of the number of days open.

Detection of Estrous Activity by Pedometry

As described above, modern dairy cows with high production performance have longer intervals from calving to the first ovulation, estrous activity and insemination. Moreover, the expression of standing heat, which is the most typical estrous behavior, has become less frequent during the early postpartum period. Additionally, the ratio of mounting estrus events to the total number of estrus events was highest at the third postpartum month, during which most of the first services are initiated, and relapses back into anestrus often occurred during and after this period. Thus, it should be emphasized again that the intensive detection not only of standing estrus but also of mounting estrus could increase the submission rate to insemination. An activity-monitoring device would be an effective tool for the accurate and efficient detection of mounting estrus because mount-detecting devices remain reliant on the standing estrus activity.

In a preliminary study using dairy heifers, the efficiency and accuracy of an estrous detection method using a new pedometry system were estimated because dairy heifers generally show estrous activity more clearly and intensively than do lactating cows. [110]. The efficiency and accuracy of this system were evaluated using the estrous detection ratio and the true positive ratio, respectively, and the efficiency and accuracy of the data from the pedometers that had been attached to different locations on the same heifers were compared under different rearing conditions.

To estimate the reliability of the estrous detection system using pedometry, a detection index was calculated from the efficiency and accuracy results as follows:

Fig. 8. Evaluation of the efficiency and accuracy of estrous detection by observing increases in the number of steps using a pedometer. The efficiency of estrous detection = $(PDE / TOE) \times 100 (\%)$. The accuracy of estrous detection = $(PDE / TPA) \times 100 (\%)$ [110].

$$\text{Detection index (DI)} = (\text{Efficiency}) \times (\text{Accuracy}) / 100,$$

where Efficiency = $(PDE) / (TOE) \times 100 (\%)$, Accuracy = $(PDE) / (TPA) \times 100 (\%)$, PDE = the number of pedometer-detected estruses, TOE = the number of total observed estruses, and TPA = the number of total pedometer alerts (Fig. 8).

As shown in Fig. 9, the estrous activity of the whole-day grazing heifers was detected by the hind leg pedometers with a level of reliability that was similar to that achieved by the hind leg pedometers under paddock conditions. In contrast, the neck pedometers attached to the grazing heifers detected estrous activity with a high efficiency but lower accuracy (DI=32) than the neck pedometers under paddock conditions (DI=59). Under pasture conditions, the neck pedometers count the neck activity of heifers that accompanies not only walking but also grazing behavior, which might result in a lower accuracy of estrous detection. These findings indicate that the use of a neck pedometer for estrous detection is impractical in grazing heifers and likely in lactating cows as well.

Under a tie-stall rearing condition, the front leg pedometers had the highest estrous detection potency (Fig. 9) when compared with hind leg (DI = 61) and neck (DI = 31) pedometers. Placement of the pedometer on a front leg is recommended for heifers that have been reared in tie stall barns because the pedometers can be kept clean and it is relatively easy to attach and detach the devices.

In lactating cows under a free-stall housing condition, which is similar to the paddock rearing condition for heifers, the leg pedometer produced acceptable results for detecting estrous activity (Fig. 9). The neck pedometer might also be a practical method for estrus detection in lactating cows that are housed in free-stall barns (DI = 78) [111]. However, under the whole-day intensive grazing condition utilizing a different pasture daily, the estrous detection performance in lactating cows using a leg pedometer was lower (Fig. 9) than that in the heifers because of the daily changes in the distance from the milking facility to the pasture. It is recommended that changes in the distance between the pasture and a milking facility be limited as much as possible when this estrus detection system is applied to lactating cows under a whole-day grazing condition. In contrast, in lactating cows under rationed grazing (4 h per day with 7- to 10-day intervals of grazing rotation) with a free-stall condition, nearly the same potency for estrous detection was recorded as under the free-stall only condition (Fig. 9).

In the system used in this study, the total number of steps over the most recent 24 h was divided by the mean daily value during

Fig. 9. Estrous detection indices obtained by leg pedometers in heifers and lactating cows under different housing conditions [110, 111].

the reference period to provide the activity rate for each 1-h interval. When the activity rate exceeded the threshold level, the cattle number, date, and time were displayed on a monitor as pedometer-detected estrus. To improve the reliability of this estrus detection system for lactating cows kept in daily rotated grazing, other algorithms for the identification of pedometer-detected estrus are needed. Elimination of the activity data from the calculation of the activity level during the daily movement to pasture and a milking facility [55, 112] or implementation of essentially a new algorithm [113, 114] may be expected to improve the reliability of estrus detection in lactating cows under a daily rotational grazing system.

Concluding Remarks

There have been many reports and reviews on the relationship between improved milk yield and declining fertility in modern dairy cattle, some of which have contradicted others. It may be true that a higher milk yield affects the fertility in dairy cattle under certain situations but not others. Good management practice can eliminate the harmful effect of a higher yield on fertility [21]; however, the higher yield can also increase the risk of declining fertility due to the occurrence of metabolic and related diseases in a herd with poor practical management. Thus, improved yield may be a major factor for transitioning a normal-fertility herd to a subfertility herd.

It is normal for mammals to store body fat during pregnancy and release body fat during early lactation independent of the feeding level. A certain degree of negative energy balance in early lactation cannot be prevented, and it is supposed that this natural level of energy mobilization does not affect health and reproduction [115]. Although the negative energy balance that accompanies high milk

production may be a cause of declining fertility through direct or indirect pathways, it is important to determine the critical level of negative status as a threshold for declining fertility.

The application of hormonal treatment programs, such as estrus or ovulation synchronization, is an effective tool to overcome the fertility problems of modern dairy herds; however, it should be noted that the persistent employment of these techniques may hide deficiencies in the management of husbandry [116]. Furthermore, consumers have a growing interest in animal health and animal welfare issues, and they have ethical concerns regarding the use of hormones in addition to antibiotics, particularly if they are used as performance enhancers [117].

The goal of reproductive management is to have cows become pregnant at a biologically optimal time and at an economically profitable interval after calving [118]. At least at the experimental herd level, the premature initiation of ovarian activity and estrous activity did not improve the reproductive performance of postpartum dairy cows. The results reviewed in this paper were retrieved from a healthy herd with a relatively sound management practice for experimental use, and the management factors under the field conditions affect these reproductive characteristics. Therefore, these results cannot be applied directly for understanding and improving farm-level reproductive performance; however, they represent the reproductive potential of modern high-yielding dairy cows and provide a baseline to discuss their reproduction.

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