—Original Article—

Repeatability of antral follicle count according parity in dairy cows

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Abstract. Ovarian reserve in cattle can be predicted by an indicator, the antral follicle count (AFC), which is easily determined via ovarian ultrasonography. However, the repeatability of AFC measurements in the same individual taken approximately 1 year apart after first parity remains unclear. This study, thus, aimed to clarify the between-lactation repeatability of AFC after first parity in dairy cows. We measured the AFC of the same individual cows consecutively across both first and second parity, both second and third parity, and both third and fourth parity in 31, 37, and 26 heads, respectively. The values of the intraclass correlation coefficients (ICC) for the AFCs in first–second and second–third parity cows were more than 0.8, and the value of the ICC for the AFCs in third–fourth parity cows was significantly lower than that in first–second parity cows ($P = 0.01$). Subsequently, based on the average number of AFCs measured at some points from first to third parity, we classified the cows into three tertiles: <11 (low), 11–15 (intermediate), and ≥ 15 (high). We then compared the reproductive performance of the first through third parity cows among the groups. The hazards of pregnancy by 200 days postpartum were higher in the high group than in the other groups ($P < 0.05$). Our findings demonstrate that between-lactation repeatability of AFC from first through third parity in dairy cows is very high, and that cows with an AFC of ≥ 15 have a better reproductive performance than cows with a low AFC.
Key words: Antral follicle count, Dairy cows, Ovarian reserve, Repeatability

In cattle, the pool of primordial follicles located in the ovaries, known as the ovarian reserve, is formed during fetal development, and remains relatively unchanged until cattle are around 4–6 years old, after which it declines [1, 2]. In cattle, the reserve is associated with both fertility [3–7] and responsiveness to superovulation [8–13]. The inherent reproductive capacity of cattle, of which the reserve is an indicator, could be used in the selection of superior cattle for fertility and superovulation to increase the efficiency of reproductive management on farms.

Two indicators are used to predict the ovarian reserve in cattle: circulating concentrations of anti-Müllerian hormone (AMH), and the antral follicle count (AFC), which is determined by ovarian ultrasonography [14]. AMH, which is produced exclusively by the granulosa cells of the growing follicles in females, is a 140 kDa glycoprotein belonging to the transforming growth factor β superfamily [15]. It is, therefore, a reliable endocrine marker of the population size of growing follicles in cattle [8]. Several reports indicate that there is a relationship between circulating concentrations of AMH and reproductive characteristics including reproductive performance [7] and the ovarian response to superovulation [8, 9, 12]. However, it is difficult to measure circulating concentrations of AMH in real time on farms. The AFC, determined by ultrasonography, and being positively associated with the total number of all follicles, is defined as the number of follicles ≥ 3 mm in diameter [14, 16]. The AFC, similar to the AMH concentration, is related to reproductive characteristics including reproductive performance [3–6], ovarian response to superovulation [10, 11, 13], oocyte yield after ovum pickup [17, 18], and function of the corpus luteum [19]. Moreover, the AFC can be suitably assessed at any stage of the estrous cycle [3] and can be measured in real time on farms.

The AFC is highly repeatable within individuals but is highly variable among individuals [10, 16, 20]. For example, one study measured the AFC in the same individuals every 15 months and reported high repeatability, despite lengthy intervals between measurements [16]. However, this finding is based on a comparison of the AFC of the same individuals as heifers and primiparous cows; the between-lactation repeatability of the AFC spaced approximately 1 year apart for the same individuals in their first or later parities remains unclear. To use the AFC as a constant indicator of the inherent reproductive capacity of cattle, it is crucial to understand the between-lactation repeatability of the AFC after first parity.

In this study, we aimed to clarify the repeatability of AFC in dairy cows after first parity. We examined both the AFC values of individual cows with different parities and the general relationship between AFC and reproductive performance in dairy cows.

Materials and Methods

This study was conducted at the Konsen Agricultural Experiment Station located in Nakashibetsu, Hokkaido, Japan (43.5N, 145.0E), where a milking herd of approximately 80 Holstein cows produce on average 30 kg of milk per cow per day. The Animal Care Committee for Laboratory Animals of the Konsen Agricultural Experiment Station approved all procedures employed in this study.

Animals

We enrolled 108 postpartum lactating Holstein cows in the study between June 2010 and February 2016. The cows were housed primarily in a free-stall barn and were fed a total mixed ration diet consisting of grass silage, wheat straw, and concentrated feed, with free access to water and a salt-based mineral supplement, in accordance with the Japanese Feeding Standard for dairy cattle [21]. When a cow acquired a disease (for instance, mastitis, hoof disease, or anorexia), that cow was temporarily moved to the tie-stall barn for treatment.

Measurement of AFC

Measurement of AFC was performed 85 ± 26 days (mean $\pm$ SD) postpartum, as previously described [4]. We identified the ovulation date by performing transrectal ultrasonography with a 5.0 MHz linear transducer (HS-101V, Honda Electronics, Toyohashi, Japan) three times a week after 2 weeks postpartum. At 4.5 ± 1.0 days after ovulation, i.e., during the first wave after ovulation, when fluctuation of AFC is considered to be relatively small [16], an operator performed transrectal ovarian ultrasonography with a 7.5 MHz linear transducer (Prosound 6, Aloka, Tokyo, Japan), recording the ultrasonography video image of each ovary on a portable video recorder (E-Balance, Tokyo, Japan). The recorded videos were played on a personal computer, and all follicles ≥ 3 mm in each ovary were counted, and the mean total number of follicles per cow was defined as the AFC.

Reproductive management

After a voluntary waiting period of 50 days postpartum, we performed artificial insemination (AI) with unsorted frozen–thawed semen without embryo transfer. We treated the cows diagnosed with ovarian and uterine disease with hormones. Specifically, 4 cows diagnosed with metritis were treated with prostaglandin F2α (PGF2α) at 21 ± 2 days postpartum; 11 cows diagnosed with endometritis were treated with PGF2α or estradiol benzoate (EB) at 43 ± 5 days postpartum; 18 cows diagnosed with ovarian quiescence were treated with gonadotropin-releasing hormone, EB, or pregnant mare serum gonadotropin at 67 ± 20 days postpartum; 3 cows diagnosed with silent heat were treated with PGF2α at 92 ± 14 days postpartum; and 12 cows diagnosed with retained corpus luteum were treated with PGF2α at 124 ± 44 days postpartum. Fifty three cows were initiated into the timed artificial insemination program at 121 ± 31 days postpartum. We diagnosed pregnancy using transrectal ultrasonography 60 days after AI, based on the detection of an embryo with a beating heart.

Experimental design

In analysis 1, we investigated the between-lactation repeatability of AFC after first parity for the same individuals. We measured AFC of the same individual consecutively during both first and second parity (2.2 ± 0.2 and 3.3 ± 0.3 years old), both second and third parity (3.3 ± 0.3 and 4.4 ± 0.3 years old), and both third and fourth parity (4.3 ± 0.2 and 5.4 ± 0.3 years old) in 31, 37, and 26 individuals, respectively.

In analysis 2, we investigated the association between AFC and reproductive performance. Since we had previously confirmed the high within-individual repeatability of AFC between the first and second parity and second and third parity in analysis 1, in analysis 2, we used the average AFC of each individual cow measured at various points from the first to third parity to represent the constant AFC of that individual cow. We classified the cows into three groups based on their AFC's tertile: < 11 (low), 11–15 (intermediate), and ≥ 15 (high). We then compared the reproductive performance from first through third parity among groups.

Statistical analysis

We assessed the relationship between the AFCs in different parities using correlation analyses. To assess between-lactation repeatability of the AFC, we calculated intraclass correlation coefficients (ICCs) and their 95% confidence intervals. ICC values range from 0 to 1, and adequate repeatability is generally indicated by values exceeding 0.80 [22]. Using the Benjamini-Hochberg procedure, we compared values of the ICC using a z-score test with false discoveries [23].

We quantified the association between the AFC classification and calving to first service interval using a fixed effects linear model, in which calving to first service interval was the dependent variable, while parity ($1, \geq 2$), treatment history for disease before first service (no, yes), and AFC classification were included as predictor variables. We determined the association between AFC classification and the remaining reproductive performance indicators (other than calving to conception intervals) using nonlinear models. Logistic regression using a logit link function was used to quantify the association between AFC classification (predictor variable) and the percentage of cows treated with hormones, pregnancy rate at first service, and pregnancy rate at 100 and 200 days postpartum. Using these results, we predicted the probability whether cows in each AFC classification would require hormone treatment or would have a successful pregnancy. We included parity and disease treatment history before first service and conception as predictor variables in the model. We also used survival analysis with Cox's proportional hazards model to determine the association of AFC classification and calving to conception intervals, thereby estimating the possible hazards associated with a cow being pregnant at a given time. We used the interval in days between calving and pregnancy as the time variable in the model. Cows were right-censored if not diagnosed as being pregnant before 200 days postpartum. AFC classification, parity, and disease treatment history before conception were all included as predictor variables in the model. We performed all analyses using the software R, version 3.4.0 for Windows [24]. In all analyses, we considered a P-value of < 0.05 to be statistically significant.

Results

Analysis 1

Figure 1 shows the relationships between the AFCs in different parities. We observed a significant positive correlation between the AFCs across different parities ($P < 0.01$). Data on the ICC values are summarized in Table 1. ICC values for the AFCs in first–second and second–third parities were more than 0.8. The ICC values of the AFCs in the third–fourth parity were significantly lower than those in the first–second parity ($P < 0.05$).

Analysis 2

Figure 2 shows the frequency distribution of AFC values. The mean AFC for all cows was 14.1 ± 5.7, and AFC values ranged
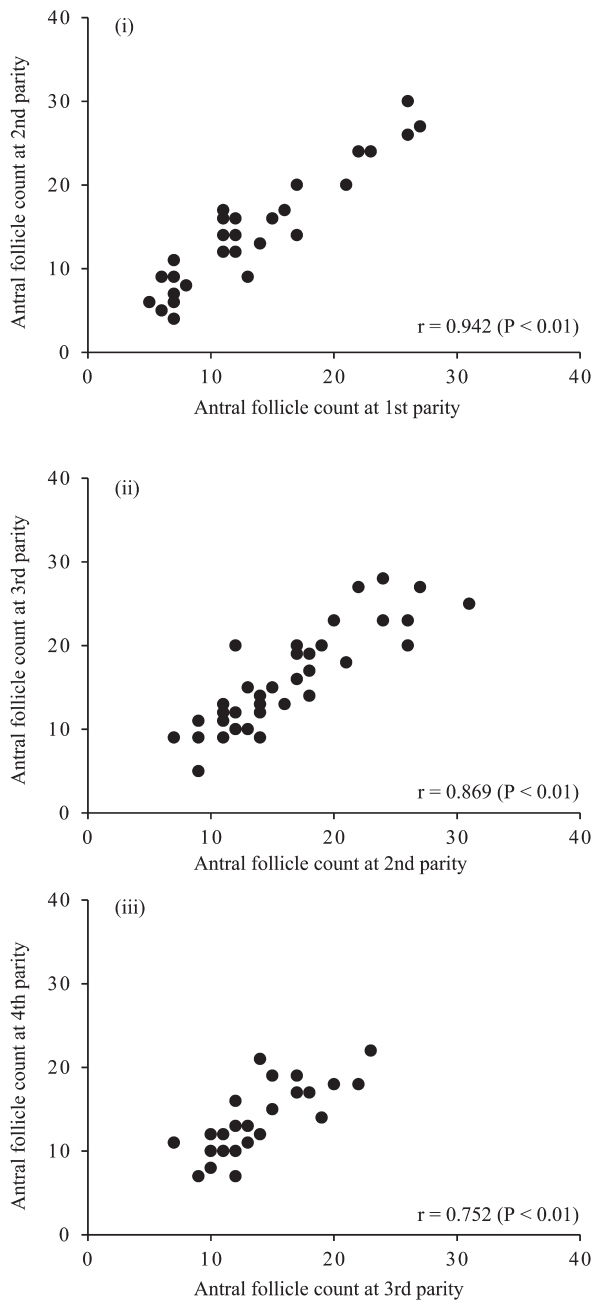
Fig. 1. Relationship between the antral follicle counts (AFCs) of the same individuals across different parities. (i) The AFCs of the same individual in both first and second parity (n = 31), (ii) in both second and third parity (n = 37), (iii) in both third and fourth parity (n = 26).

Table 1. Intraclass correlation coefficients (ICCs) for AFCs in different parities in dairy cows

Parameter	ICC
AFC in first and second parity	0.93 (0.85–0.97) *a
AFC in second and third parity	0.87 (0.76–0.93) ab
AFC in third and fourth parity	0.76 (0.53–0.88) b

* Values in parentheses are 95% confidence intervals.
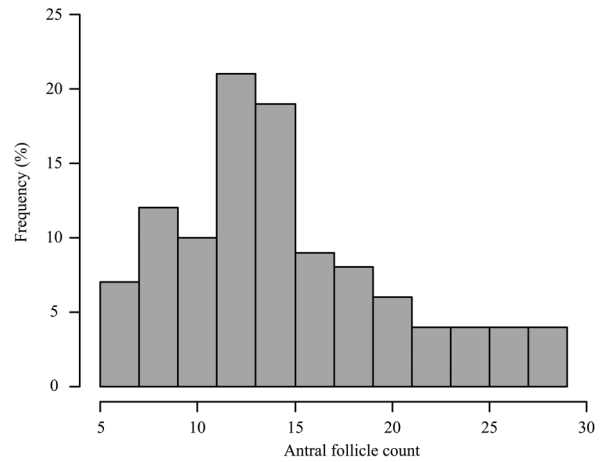a, b Different letters indicate significant difference (P < 0.05).

Fig. 2. Frequency distribution of antral follicle count detected using ovarian ultrasonography in 108 lactating dairy cows.

from 5 to 28. Table 2 summarizes the association between AFC and reproductive performance. No differences in the percentage of cows treated with hormones were evident among the AFC groups. In the high group, calving to first service interval was shorter than that in the low group (P < 0.05). Moreover, the pregnancy rate by 100 days postpartum was also higher in cows of the high group than in those of the low group (P < 0.05). Figure 3 shows survival curves for the interval to pregnancy in cows from the high, intermediate, and low groups. The hazard of pregnancy by 200 days postpartum was higher in cows from the high group than in those from the other groups (P < 0.05).

Discussion

In the present study, we examined the between-lactation repeatability of the AFC after first parity in dairy cows. This is, to the best of our knowledge, the first study to clarify the high between-lactation repeatability of AFC from first to third parity in dairy cows. Burns *et al*. (2005) [16] demonstrated high repeatability of AFC for the same individuals as heifers and primiparous cows. Therefore, it is reasonable to assume that, during the transition from heifer to third-parity cow, the AFC for the same individual remains relatively constant. We also examined the relationship between AFC ICC values for first–second parity cows and body weight at first calving and peak milk yields during first parity (Supplementary Tables 1 and 2: online only). Differences in body weight at first calving and peak milk yield during first parity did not affect AFC ICC values during transition from first to second parity. Although primiparous cows require not only lactation but also nutrition for growth [21], our data indicate high AFC ICC values in cows transitioning from first to second parity, regardless of body weight at first calving and peak milk yield during first parity.

Table 2. Characteristics determined by ultrasound scanning and reproductive performance in dairy cows with high (≥ 15), intermediate (11–15), and low (< 11) antral follicle counts

Variable	Antral follicle count		
	Low	Intermediate	High
Number	49	71	69
Lactation number	1.6 ± 0.7	1.7 ± 0.8	1.6 ± 0.7
Percentage of cows treated with hormones (%)	44.9	50.7	39.1
Calving to first service interval (days)	76.6 ± 22.6 [b]	76.5 ± 20.7 [ab]	69.9 ± 19.2 [a]
Pregnancy rate at first service (%)	42.9	49.3	55.1
Pregnancy rate by 100 d postpartum (%)	51.0 [b]	50.7 [ab]	71.0 [a]
Pregnancy rate by 200 d postpartum (%)	90.6	89.9	94.7
Median calving to conception interval (days)	98.0 [b]	100.0 [b]	75.0 [a]

Values are means ± SD. [a, b] Values with different superscripts within the same row are significantly different (P < 0.05).
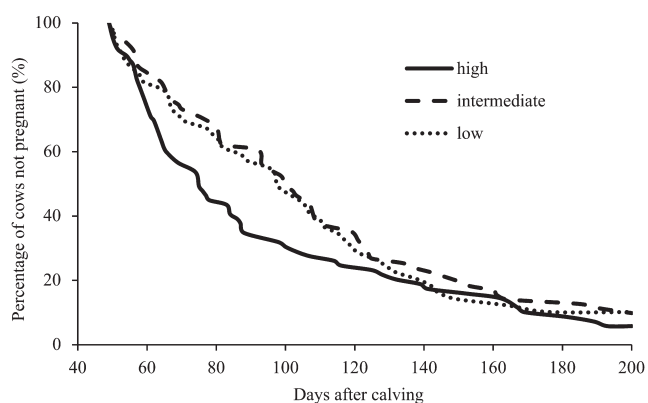

Fig. 3. Survival probability of pregnancy by 200 days postpartum in first, second, and third parity cows according to antral follicle count categorized as low (< 11), intermediate (11–15), or high (≥ 15) in 108 lactating dairy cows. The probability of pregnancy by 200 days postpartum was higher in the high group than in the other groups (P < 0.05).

Although we observed a positive correlation between AFCs in cows transitioning from third to fourth parity, AFC ICC in this group was less than 0.8 and significantly lower than the ICC value of cows transitioning from first to second parity. Ovarian reserves in cattle remain relatively constant until they reach the age of about 4 to 6 years and declines thereafter [1, 2]. Moreover, Cushman *et al.* (2009) [3] demonstrated a decline in AFC from the age of about 5 years in beef cows. As the fourth-parity cows, in which AFC was measured in the present study, were aged about 5 years, the AFC might already have begun to decline in some of them.

The mean AFC in the present study was approximately 14, which is similar to that of a previous report [5]. However, Mossa *et al.* (2012) [4] demonstrated the mean AFC in dairy cows to be approximately 19, ranging from 4 to 61 follicles per cow. The differences between these results and ours may be because of disparities in the timing of our measurements of AFC, which was about 1 day later than in the study by Mossa *et al.* (2012) [4]. AFC during the first follicular wave after ovulation reaches the highest value immediately after ovulation, and decreases until the start of the second follicular wave [16]. Conversely, AFC in dairy cows is a moderately heritable genetic trait [25]. This may further account for the differences in AFC distribution among reports, as genetic differences in cattle strains between farms could be responsible for variation in AFCs. Moreover, a restriction on maternal nutrient availability during the first trimester of gestation results in female offspring with smaller ovarian reserves [26]. Differences between the AFC distribution reported by numerous studies may also be related to the nutritional status of the dams during pregnancy. Future studies should, therefore, examine in detail the relationship between the nutritional status of dams during pregnancy and AFCs in female offspring.

In the present study, cows with an AFC of ≥ 15 conceived promptly after 50 days postpartum relative to cows with lower AFCs. This result is consistent with a previous report [4]. For cows with high AFC, progesterone concentrations are typically higher during estrous cycles and endometrium is thicker during the early luteal phases of the estrous cycles relative to that in the cows with low AFC [19]. A significant positive association exists between progesterone concentrations 4–6 days after ovulation and the probability of embryo survival [27], and the percentage chance of successful pregnancy following AI is higher for cows with thick endometrium than for cows with thin endometrium [28]. These findings provide indirect evidence for differences in reproductive performance among the different AFC groups reported in the present study. Conversely, Jimenez-Krassel *et al.* (2017) demonstrated that dairy heifers, with an AFC of ≥ 25, have suboptimal fertility and a shorter productive herd life than herdmates with lower AFCs [29]. Because the proportion of cows with an AFC of ≥ 25 was only 7% of the cows enrolled in the present study, we could not assess the adverse effects of cows having higher a AFC in the present study. Future studies should, therefore, verify the adverse effects of very high AFC on dairy cows.

In conclusion, we verified that between-lactation repeatability of AFC from first to third parity in dairy cows is very high. However, for some fourth-parity cows, AFC may begin to decline. We also confirmed that the reproductive performance of cows with an AFC of ≥ 15 was better than that of cows with lower AFCs. Unfortunately, the adverse effects of very high AFCs remain unclear.

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