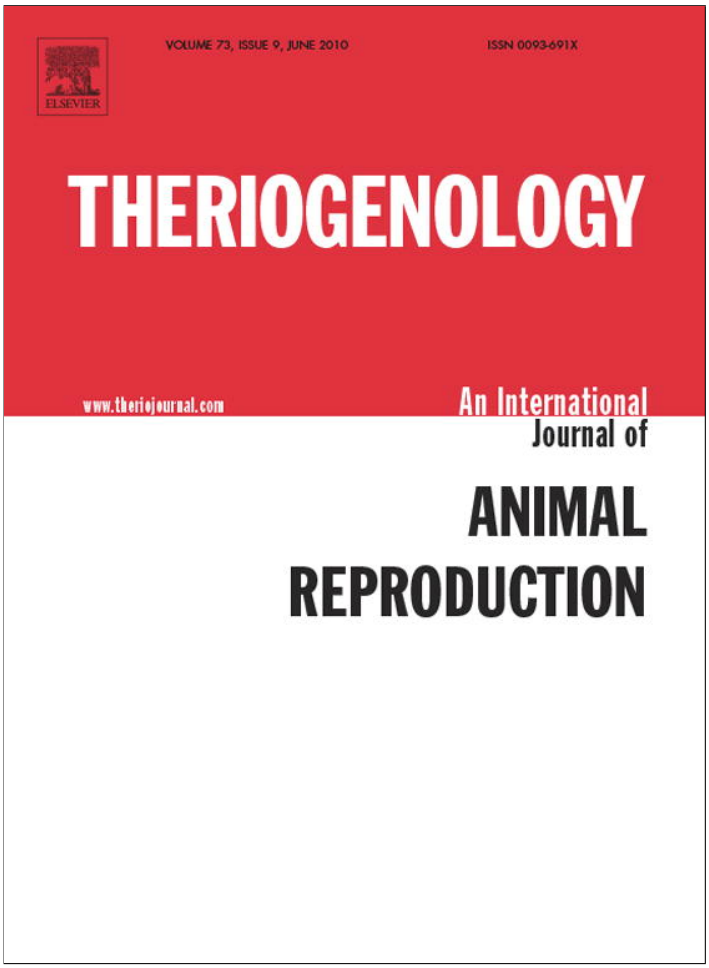

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Reproductive performance of repeat breeders in dairy herds

Muhammad Yusuf, Toshihiko Nakao *, RMS Bimalka Kumari Ranasinghe, Gokarna Gautam, Su Thanh Long, Chikako Yoshida [1], Kana Koike, Aki Hayashi

Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8515, Japan

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Abstract

The objectives were to characterize repeat breeding in dairy cows, including reproductive performance and risk factors. Data from 613 Holstein Friesian cows in nine dairy herds across Japan were enrolled. A repeat breeder was defined as a cow that did not become pregnant after three inseminations, despite no clinically detectable reproductive disorders. In contrast, cows that became pregnant within three inseminations were considered to have normal fertility. Of the 613 cows, 87.3% eventually became pregnant after repeated AI (maximum calving to conception interval was 435 d). Mean (±SEM) first AI conception rate, days in milk at first AI, calving to conception interval and service per conception were 38.3%, 82 ± 2 d, 125 ± 3 d, and 2.0 ± 0.1 times, respectively. Normal fertility cows (n = 479) required only 114 ± 3 d to conceive and 1.7 ± 0.1 inseminations per pregnancy, whereas repeat breeders (n = 86) required significantly more days to conceive (211 ± 10) and more inseminations per pregnancy (4.7 ± 0.2). Based on survival analysis, it took 94 d after calving for 50% of normal fertility cows to become pregnant, compared to 155 d for repeat breeders. For repeat breeders, 31.4, 50.0, and 58.1% became pregnant within 210, 300, and 435 d after calving, respectively. The risk factors for repeat breeding were parity (relative risk [RR] = 0.809; P = 0.058), resumption of postpartum ovarian cycles (RR = 1.928; P = 0.009), and days in milk at first AI (RR = 0.991; P = 0.039). In conclusion, repeat breeder dairy cows had very poor reproductive performance. Lower parity, abnormal resumption of postpartum ovarian cycles, and shorter days in milk at first AI were risk factors for repeat breeding.

Keywords: Repeat breeding; Infertility; Pregnancy; Reproductive performance; Holstein Friesian cows

1. Introduction

Repeat breeding has long been considered one of the important reproductive disorders in cattle. Incidences of repeat breeding in lactating dairy cows varied among regions, environments, and management. In 1978, Bulman and Lamming [1] reported that the incidence of repeat breeding in dairy cows was 8.9%, whereas it was 24% according to the report of Bartlett et al. in 1986 [2]. More recently, the incidence of repeat breeders was reported as 10% in the Swedish dairy cow population [3,4].

The causes of the repeat breeding are multifactorial [4–10]. For example, it will be increased by inadequate estrous detection [8,9] resulting in errors in timing of insemination in relation to the onset of standing estrus, or insemination of cows not in estrus. Other potential factors include quality of semen and insemination technique [11,12], uterine and/or cervical/vaginal infections [10], endocrine disorders [3,13,14], ovulation failures [6,7], obstructed oviducts, defective ova,

* Corresponding author. Tel.: +81 83 9335935; fax: +81 83 9335935.
E-mail address: tnakao@yamaguchi-u.ac.jp (T. Nakao).
[1] Present address: Field Center for Sustainable Agriculture and Forestry, Faculty of Agriculture, Niigata University, Niigata, 959-1701, Japan.

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anatomical defects of the reproductive tract [7], and early embryonic death [3,15]. However, the specific causes of the repeat breeding are not clear [4,16]. A multifactorial problem involving a number of extrinsic factors as well as intrinsic factors coupled to the individual animal [4] could also be a cause. Since several factors affect the incidence of repeat breeding in dairy cows, it is difficult to make generalizations regarding predominant causes [7].

Increased capability for milk production has been associated with a reduced fertility in lactating dairy cows [17] through changes in reproductive physiology [18], resulting in an increased number of services per conception [19–22]. Consequently, the incidence of repeat breeding should have increased [23].

To our knowledge, most reports of repeat breeding in cows described incidence, causes, and treatment, but lacked detailed characterization of reproductive performance of repeat breeding. Therefore, it is necessary to describe the reproductive performance of repeat breeding in modern dairy cows. The objectives of the present study were to characterize repeat breeding in dairy cows, including reproductive performance and risk factors.

2. Materials and methods

2.1. Animals and herd management

A total of 613 Holstein Friesian cows (from nine dairy herds across Japan) that calved between January 2004 and December 2007 were enrolled. Cows in seven herds were housed in free-stall barns, whereas the other two herds were housed in tie-stall barns with a paddock. Herd size ranged from 20–60 lactating cows. Average 305-d milk production per cow ranged from approximately 8,700–10,200 kg. Cows were milked twice daily and fed total mixed rations. Feedstuffs consisted of grass or corn silage, hay, concentrate, and mineral supplements. Rations were consistent with NRC recommendations. The parity of the cows ranged from one to eight. Cows detected in estrus were inseminated (by owners) approximately 12 h later, using frozen/thawed semen from proven Holstein Friesian or Japanese black sires.

2.2. Reproductive management

Once monthly, a reproductive health program was implemented by the authors (Herds A, C, D, and G) or by local veterinarians (Herds B, E, F, H, and I). Postpartum cows were designated for examination at each visit. Cows within 30 d after AI were examined with a vaginoscope, and not subjected to transrectal palpation. Cows not detected in estrus within 35 d after AI were examined for pregnancy by transrectal palpation or ultrasonograpy. Vaginoscopy was conducted using a glass speculum (4 cm in diameter and 35 cm in length) and a light source; any cervical and/or vaginal discharges were collected into a plastic Petri dish using a plastic pipette. The discharge was considered normal, if it was clear or slightly cloudy without any pus flakes and no foul smell. However, mucopurulent or purulent discharge was considered abnormal, consistent with endometritis. Trans-rectal palpation of the genitalia was conducted to assess ovarian structures and uterine conditions. Ovarian cysts was defined as one or more follicle-like structures $>$ 25 mm in diameter without a concurrent CL. Ovaries without palpable structures (i.e., ovarian follicles $>$ 10 mm and/or a functional CL) were considered inactive. Milk progesterone profiles were retrospectively used to confirm clinical findings. Reproductive disorders diagnosed during a clinical examination, e.g., pyometra, ovarian cysts, endometritis, and urovagina, were immediately treated.

2.3. Definition of repeat breeder and reproductive end points

A repeat breeder was defined as a cow which did not become pregnant after three inseminations, despite no clinically detectable reproductive disorders. Normal fertility and the other types of fertility were derived according to the different number of inseminations, conception, and presence or absence of clinical reproductive disorders (Table 1).

The following reproductive end points were used to characterize reproductive performance:

- Days in milk at first AI: number of days from calving to first AI
- First AI conception rate: number of cows that conceived at first AI, divided by number of cows which received first AI
- Pregnancy rate within 100, 150, 210, 300, and 400 d: number of cows that conceived within 100, 150, 210, 300, and 400 d, divided by the total number of cows inseminated postpartum
- Proportion of cows conceived eventually (by 435 d): number of cows that conceived by 435 d, divided by the total number of cows inseminated postpartum
- Calving to conception interval: number of days from calving to conception
- Services per conception

Table 1
Types of fertility in lactating dairy cows, based on number of AI, conception, and clinically detectable reproductive disorders.

Type of fertility	Definition	No. cows (%)
Normal	Cows which conceived within three inseminations	479 (78.1)
Cull	Cows culled after one or two infertile inseminations	42 (6.9)
Reproductive disorder	Cows having reproductive disorders* and not conceiving within three inseminations	6 (1.0)
Repeat breeding	Cows which did not conceive after three inseminations and had no clinical reproductive disorders	86 (14.0)

* Pyometra, ovarian cyst, endometritis, and urovagina.

2.4. Data collection

The following data were recorded for each cow: herd, parity, season of calving, ease of calving, resumption of postpartum ovarian cycles within 80 d, days in milk at first AI, postpartum reproductive disorders (retention of fetal membranes, pyometra, ovarian cysts, endometritis, and urovagina). In two of the nine herds which had been registered in a Dairy Herd Improvement program (DHI), milk yield and body condition scores (BCS) within 30 d, between 31 and 60 d, and between 61 and 90 d postpartum were also recorded. In order to determine the resumption of ovarian cycles, milk samples from each cow were collected twice weekly (Monday and Thursday), from the second week after calving to diagnosis of pregnancy or culling. A total of approximately 10 mL of fore-milk was collected from all four quarters at the morning milking. The milk was put into a plastic tube (1.5 x 10.5 cm) containing 15 mg potassium dichromate (Wako Pure Chemical Industries, Ltd, Osaka, Japan), and stored at 4 °C. Every 2 wk, milk samples were sent to our laboratory for determination of whole-milk progesterone concentrations, using a direct ELISA [24]. The intra- and inter-assay coefficients of variations were 12.1 and 16.3%, respectively. For each cow, progesterone profiles were used to derive the types of resumption of postpartum ovarian cycles, as follows:

- Normal: first ovulation occurred within 35 d after calving, followed by two or more normal estrous cycles
- Delayed first ovulation: ovulation occurred $>$ 35 d after calving
- Prolonged luteal phase: first or second ovarian cycle with luteal activity $>$ 20 d without a preceding insemination
- Short luteal phase: cyclicity characterized by one or more ovarian cycles with luteal phase activity $<$ 10 d (except the first cycle)
- Cessation of cyclicity: ovarian cycles ceased for $>$ 14 d, consistently low progesterone concentrations, and erratic

2.5. Statistical analyses

Days in milk at first AI, calving to conception interval, service per conception, service intervals, and milk yield and body condition scores (BCS) within 30 d, between 31 and 60 d, and between 61 and 90 d postpartum were analyzed with one-way ANOVA. Chi-square analysis was used to compare, between normal fertility and repeat breeding, the pregnancy rate within 100, 150, 210, 300, and 400 d postpartum. Chi-square was also used to compare characteristics of resumption of postpartum ovarian cycles within 80 d, between normal fertility cows and repeat breeders, and between repeat breeders that finally conceived and failed to conceive, and parity between repeat breeders that finally conceived and failed to conceive.

Variables potentially affecting the incidence of repeat breeding are shown (Table 2). Data for each type of fertility were compared by multinomial logistic regression. The type of normal fertility at each variable was used as a reference. Furthermore, differences in median days nonpregnant between repeat breeding and normal fertility cows at each class at different variables were measured by Kaplan-Meier survival analysis. Furthermore, this analysis was also used to compare, between the two types of fertility, cows that became pregnant.

Survival analysis was also used to examine the number of service per conception, with each service regarded as a ''time period'' and pregnancy regarded as principal outcome [25]. The criteria of censored animals were similar; those cows that did not conceive and were culled. The proportions of cows censored were compared using Chi-square analysis. All calculations were performed using the statistical package SPSS 12.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results

A total of 649 cows were examined in the present study; 36 (5.5%) were culled without being inseminated and they were excluded from further analysis.

Table 2
Risk factors assessed for possible effects on the incidence of repeat breeding in lactating dairy cows.

Risk factor	*N* classes	Class description (*N* per class)	Mean ± SD (range)
Herd	9	A (101), B (94), C (63), D (59), E (43), F (71), G (80), H (37), I (65)	
Parity	4	Parity 1 (201) Parity 2 (156) Parity 3 (112) Parity 4 or more (134)	
Season of calving	4	Winter (161) Spring (130) Summer (157) Autumn (165)	
Resumption of postpartum ovarian cycles	2	Normal (297) Abnormal (316)	
Days in milk at first AI	Continuous	(613)	82.1 ± 36.5 (23-286)

3.1. Reproductive performance in nine dairy herds

Of a total of 613 cows in nine commercial dairy herds, the proportion of cows that eventually conceived within 435 d postpartum was 87.3%. First AI conception rate, days in milk at first AI, calving to conception interval and service per conception (mean ± SEM) were 38.3%, 82 ± 2 d, 125 ± 3 d and 2.0 ± 0.1 times, respectively (Table 3).

3.2. The incidence and reproductive performance of repeat breeding

The incidence of repeat breeding was 14% (Table 1), ranging from 5–24% among herds ($P < 0.01$).

Normal fertility cows which conceived within three inseminations required only 114 ± 3 d to conceive after calving and 1.7 ± 0.1 inseminations per pregnancy (Table 3). Conversely, repeat breeders required longer to conceive (211 ± 10 d, $P < 0.01$) as well as more inseminations per pregnancy (4.7 ± 0.2, $P < 0.01$). There were no significant differences between the two groups for service intervals from first to second services, and from second to third services (Table 4). The group with reproductive disorders required 250 ± 28 d to conceive and 5.5 ± 0.5 inseminations per pregnancy. Based on survival analysis, the rate at which repeat breeders became pregnant after calving was lower than in normal cows (log rank test, $P < 0.001$, Fig. 1). By 94 d postpartum, 50% of normal

Table 3
Reproductive performance of lactating dairy cows (in nine herds) with varying types of fertility (numbers with variability are mean ± SEM).

	Types of fertility				Total
	Normal	Cull*	Reproductive disorder**	Repeat breeding	
No. cows inseminated	479	42	6	86	613
Days in milk at first AI	81 ± 2[a]	107 ± 7[b]	84 ± 10[ab]	74 ± 4[a]	82 ± 2
First AI conception rate (%)[1]	49.1	–	–	–	38.3
Pregnancy rate ≤100 d (%)[2]	50.1	–	–	1.2	39.3
Pregnancy rate ≤150 d (%)[2]	75.2	–	16.7	11.6	60.5
Pregnancy rate ≤210 d (%)[2]	94.8	–	16.7	31.4	78.6
Pregnancy rate ≤300 d (%)[2]	99.8	–	66.7	50.0	85.6
Pregnancy rate ≤400 d (%)[2]	99.8	–	100.0	58.1	87.1
Cows conceived by 435 d (%)	100.0	–	100.0	58.1	87.3
Calving to conception interval (d)	114 ± 3[a]	–	250 ± 28[b]	211 ± 10[b]	125 ± 3
Service per conception	1.7 ± 0.1[a]	–	5.5 ± 0.5[b]	4.7 ± 0.2[b]	2.0 ± 0.1

[a,b]Within a rows, means without a common superscript differed ($P < 0.01$).
*Reasons for culling included mastitis, high somatic cell count, infertility, severe urovagina, endometritis, etc. Interval from calving to culling (±SD) was 183 ± 72 d; ranged from 64-414.
**Pyometra, ovarian cyst, endometritis, and urovagina
[1]Number of cows conceived at first AI divided by number of cows which received first AI.
[2]Number of cows conceived within 100, 150, 210, 300, and 400 d, divided by total number of cows inseminated postpartum.

Table 4
Service intervals in lactating dairy cows with normal fertility and repeat breeding.

	Type of fertility		*P*-value
	Normal	Repeat breeding	
No. cows	479	86	–
Mean ± SD service intervals (d) (Range)			
1st to 2nd	45.2 ± 31.0 (6–156)	45.4 ± 21.4 (15–92)	0.974
2nd to 3rd	39.3 ± 26.2 (9–132)	37.2 ± 17.5 (15–69)	0.720
3rd to 4th	–	36.3 ± 20.5 (10–91)	–
4th to 5th	–	49.2 ± 18.2 (23–87)	–
5th to 6th	–	23.6 ± 11.8 (6–47)	–

cows were pregnant with services per conception (mean ± SEM) of 1.6 ± 0.1, whereas median days nonpregnant and service per conception for repeat breeders were 155 d and 4.1 ± 0.1 times, respectively (Fig. 2). Detailed information regarding the incidences of repeat breeding and median days nonpregnant according to various factors and classes between cows with repeat breeding and normal fertility are shown (Table 5).

Of the 86 repeat breeders, pregnancy rate within 210 and 300 d postpartum were 31.4 and 50.0%, respectively (Table 3); 50 cows (58.1%) conceived eventually, with a calving to conception interval of 211 ± 10 d. The other 36 cows were culled after 4.6 ± 0.3 infertile breedings; mean (± SEM) interval from calving to cull was 266 ± 15 d (range, 141–402). Service intervals were not significantly different between cows that finally conceived and cows that failed to conceive from first to sixth services (Table 6).

3.3. Risk factors for the incidence of repeat breeding

Based on multinomial logistic regression analysis, parity, resumption of postpartum ovarian cycles within 80 d, and days in milk at first AI affected the incidence of repeat breeding (Table 7). For increased parity or days in milk at first AI, the relative risk for repeat breeding decreased by a factor of 0.809 and 0.991, respectively. Likewise, for cows with abnormal resumption of postpartum ovarian cycles, the relative

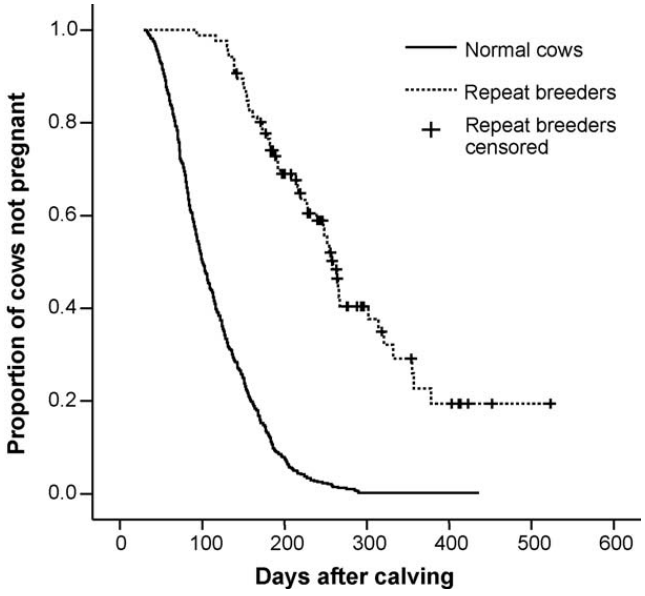

Fig. 1. Kaplan-Meier survival analysis for proportion of lactating dairy cows not pregnant, according to fertility status. The pregnancy rate was higher for normal versus repeat breeding cows ($P < 0.01$). Median days to conception were 100 for normal cows (n = 479) and 194 for repeat breeders (n = 86) (Log rank statistic, 167.49, 1 df, $P < 0.001$). The proportions censored were 0, and 41.9%, respectively ($P < 0.001$).

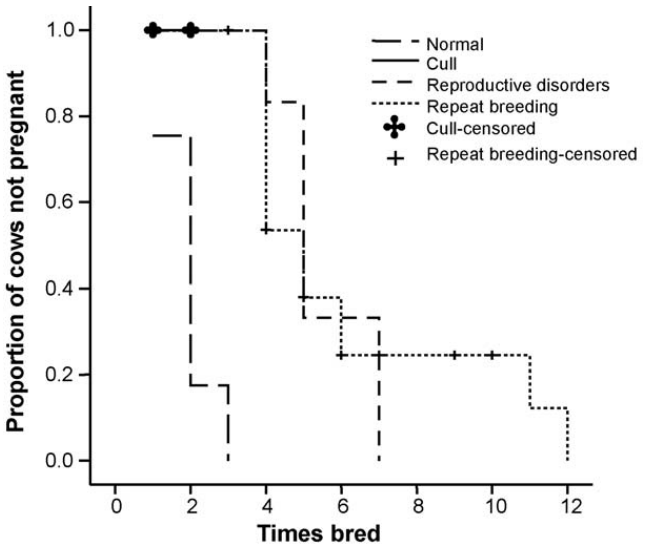

Fig. 2. Kaplan-Meier survival analysis of number of inseminations for lactating dairy cows. Times bred (±SEM) and days to conception (±SEM) for 50% of cows to become pregnant were 1.6 ± 0.1 and 93.5 ± 1.7 for cows in the normal group, and were 4.1 ± 0.1 and 154.8 ± 4.9 for repeat breeders. The symbol representing censored data means the point where some animals were culled. The proportions censored in cull and repeat breeding cows were 100.0 and 41.9%, respectively.

Table 5
Kaplan-Meier survival analysis for effects of various factors on median days open in repeat breeding or normal fertility lactating dairy cows.

Factor	Class	No.	Repeat breeding (%)	Median days open (± SEM)			
				Repeat breeding (95% CI)	Normal (95% CI)	Statistic	Log rank *P*
Herd	A	101	17.8	248 ± 23 (204–292)	95 ± 12 (72–118)	20.55	<0.001
	B	94	7.4	254 ± 28 (200–308)	144 ± 12 (121–167)	5.25	0.0219
	C	63	11.1	187 ± 42 (104–270)	95 ± 9 (78–112)	7.41	0.0065
	D	59	5.1	321 ± 47 (230–412)	147 ± 15 (118–176)	5.99	0.0114
	E	43	20.9	132 ± 25 (83–181)	64 ± 5 (54–74)	7.57	0.0059
	F	71	7.0	154 ± 31 (93–215)	83 ± 3 (78–88)	1.50	0.2202
	G	80	23.8	161 ± 13 (135–187)	97 ± 6 (86–108)	4.37	0.0366
	H	37	10.8	173 ± 16 (141–205)	128 ± 5 (117–139)	1.74	0.1874
	I	65	21.5	228 ± 33 (163–293)	92 ± 8 (76–108)	26.31	<0.001
Parity	1	201	19.4	248 ± 10 (229–267)	96 ± 4 (88–104)	82.31	<0.001
	2	156	12.2	321 ± 68 (187–455)	99 ± 5 (88–110)	38.94	<0.001
	3	112	9.8	266 ± 3 (261–271)	128 ± 10 (108–148)	18.18	<0.001
	4 or more	134	11.2	357 ± 103 (155–559)	92 ± 6 (79–105)	26.72	<0.001
Season of calving	Winter	161	16.1	321 ± 48 (226–416)	99 ± 9 (82–116)	58.95	<0.001
	Spring	130	13.8	257 ± 6 (245–269)	122 ± 7 (108–136)	35.42	<0.001
	Summer	157	17.8	239 ± 24 (192–286)	96 ± 5 (85–107)	41.26	<0.001
	Autumn	165	8.4	216 ± 21 (175–257)	94 ± 6 (83–105)	27.22	<0.001
Resumption of postpartum ovarian cycles	Normal	297	10.8	228 ± 41 (148–308)	85 ± 5 (76–94)	64.42	<0.001
	Abnormal	316	17.1	267 ± 22 (225–309)	114 ± 5 (104–124)	101.33	<0.001
Days in milk at first AI	Continuous	613	14.0	194 ± 10 (93–378)	100 ± 3 (29–435)	5.64	0.0176

risk to become repeat breeding increased by a factor of 1.928.

Characteristics of the resumption of postpartum ovarian cycles within 80 d are shown (Table 8). In repeat breeders, the incidence of abnormal resumption was higher than in normal cows (P = 0.019). Repeat breeders had a higher incidence of delayed first ovulation than in normal cows (P = 0.003).

Among repeat breeders, cows that failed to conceive had a higher incidence of abnormal resumption than

Table 6
Characteristics of resumption of postpartum ovarian cycles within 80 d, parity, and insemination intervals in repeat breeder dairy cows that eventually conceived, or failed to conceive and were culled.

	Repeat breeders		*P*-value
	Conceived	Failed to conceive and were culled	
No. cows	50	36	-
Normal resumption (%)	46.0	25.0	0.069
Abnormal resumption (%)	54.0	75.0	0.069
Delayed first ovulation (%)	36.0	44.4	0.504
Prolonged luteal phase (%)	12.0	22.2	0.244
Others (%)	6.0	8.3	0.691
Parity			
1	56.0	30.6	0.027
2	18.0	27.8	0.303
3	12.0	13.9	1.000
≥4	14.0	22.0	0.392
No. services (± SEM)	4.7 ± 0.2	4.6 ± 0.3	0.723
Mean ± SD) service intervals (d) (Range)			
1^{st} to 2^{nd}	40.6 ± 18.8 (17–84)	54.0 ± 24.0 (15–92)	0.114
2^{nd} to 3^{rd}	35.6 ± 14.9 (15–62)	40.0 ± 22.3 (17–69)	0.557
3^{rd} to 4^{th}	34.2 ± 17.8 (12–81)	41.3 ± 26.9 (10–91)	0.453
4^{th} to 5^{th}	46.2 ± 15.1 (26–65)	51.7 ± 21.5 (23–87)	0.645
5^{th} to 6^{th}	23.3 ± 17.2 (6–47)	24.0 ± 5.5 (20–32)	0.936

Table 7
Relative risk and 95% confidence interval of variables included in a multinomial logistic regression model for the incidence of repeat breeding in lactating dairy cows.

Variable	Type of fertility	Relative risk	95% confidence interval	*P*-value
Herd	Normal	Reference		
	Cull	0.809	0.695–0.941	0.006
	Reproductive disorder	0.728	0.496–1.065	0.104
	Repeat breeding	1.013	0.930–1.105	0.753
Parity	Normal	Reference		
	Cull	1.039	0.774–1.395	0.799
	Reproductive disorder	0.927	0.446–1.928	0.840
	Repeat breeding	0.809	0.651–1.007	0.058
Season of calving	Normal	Reference		
	Cull	0.724	0.537–0.978	0.035
	Reproductive disorder	1.283	0.599–2.748	0.521
	Repeat breeding	0.854	0.693–1.052	0.138
Resumption of postpartum ovarian cycles	Normal	Reference		
	Cull	0.985	0.497–1.953	0.967
	Reproductive disorder	2.552	0.428–15.216	0.304
	Repeat breeding	1.928	1.173–3.169	0.009
Days in milk at first AI	Normal	Reference		
	Cull	1.013	1.006–1.021	<0.001
	Reproductive disorder	0.998	0.975–1.022	0.856
	Repeat breeding	0.991	0.983–1.000	0.039

cows that finally conceived (75 vs. 46.0%, P = 0.069, Table 6). Percentage of Parity 1 cows was significantly higher in repeat breeders that finally conceived than in those that failed to conceive (P = 0.027).

In the two herds (n = 199 cows) that were involved in DHI program, milk yield and BCS within 30 d, between 31 and 60 d, and between 61 and 90 d postpartum, did not differ between normal and repeat breeders. Among repeat breeders, BCS between 31 and 60 d postpartum in the cows that failed to conceive was lower (P = 0.002) than in cows that eventually conceived. However, there was no difference in milk yield between cows that finally conceived and cows that failed to conceive for the following postpartum intervals: < 30 d, 31–60 d, and 61–90 d postpartum.

Table 8
Characteristics of resumption of postpartum ovarian cycles within 80 d in lactating dairy cows with normal fertility and repeat breeding.

	Type of fertility		P-value
	Normal	Repeat breeding	
No. cows	479	86	-
Normal resumption (%)	51.1	37.2	0.019
Abnormal resumption (%)	48.9	62.8	0.019
Delayed first ovulation (%)	23.8	39.5	0.003
Prolonged luteal phase (%)	13.2	16.3	0.494
Others (%)	11.9	7.0	0.262

4. Discussion

Reproductive performance of the 613 cows from nine herds which were involved in the current study, including first AI conception rate (38.3%), calving to conception interval (125 ± 3 d), and the proportion that eventually conceived (87.3%), seemed comparable with previous reports [26–28]. The incidence of repeat breeding in this study was 14%, ranging from 5 to 24% among nine herds (substantial variation among herds). This incidence was consistent with values previously reported [1–4,29–31].

Although the incidence of repeat breeding [1–4,29–31], its causes [3,4,6,10,13], and treatment [15,23,32–34] have already been reported, the present study is apparently the first to provide a detailed assessment of reproductive performance in repeat breeders. That their reproductive performance was poorer than in normal fertility cows was predictable *a priori*, based on the definition of repeat breeders. It was noteworthy that of 86 repeat breeders, only 27 (31.4%) conceived within 210 d postpartum and 50% became pregnant within 300 d postpartum. Furthermore, only 58.1% of repeat breeders eventually became pregnant, even though they were still inseminated after 300 d postpartum. Their calving to conception interval and expected calving interval were 211 ± 10 d and 491 ± 10 d, respectively, and there was no significant increase in pregnancy rate of repeat breeders when they were inseminated after 300 d postpartum. Caraviello et al. [35] reported a

survey on reproductive management of dairy cattle on large commercial farms in the USA. Cows were exposed to bulls (for natural service) after 8.8 ± 0.9 failed inseminations or 232 ± 9 d postpartum; cows not pregnant at 300 d postpartum were culled (intervals from calving to culling were 326 ± 36 d). In the present study, 31.4 and 50.0% of repeated breeders were pregnant by 210 and 300 d after calving, respectively.

Some factors associated with repeat breeding have been reported [4–10]. In the present study, due to a lack of information in some herds, we considered herd, parity, season of calving, resumption of postpartum ovarian cycles within 80 d, and days in milk at first AI as potential factors affecting the incidence of repeat breeding. We confirmed that lower parity, abnormal resumption of postpartum ovarian cycles, and shorter days in milk at first AI were the risk factors increasing the incidence of repeat breeding.

In the present study, the incidence of repeat breeding was 19.4% in Parity 1 cows, but it decreased to 12.2 and 9.8% for Parities 2 and 3, respectively. A higher incidence of repeat breeding in the first lactation may have been due to a high incidence of abnormal resumption of ovarian cycles in first-lactation cows [36].

Abnormal resumption of postpartum ovarian cycles increased the risk of repeat breeding in the present study, consistent with previous reports [1]. Lamming and Darwash [27] reported that at least one abnormal ovarian pattern before insemination contributed to a delayed conception, higher number of services per conception, lower first service conception rate, and a reduced total conception rate in comparison to cows with a normal progesterone pattern. Taylor et al. [36] also reported that delayed first postpartum ovulation increased the number of services per conception and days open in comparison to the cows with normal resumption of ovarian cycles. Bulman and Lamming [1] reported that the incidence of abnormal resumption of ovarian cycles in repeat breeders was 70%, which was comparable to the incidence of abnormal resumption in repeat breeders in the present study.

It is not well understood how abnormal resumption of postpartum ovarian cycles causes subfertility. Britt [37] proposed that preantral follicles may be biologically imprinted by physiological changes associated with a negative energy balance in the early postpartum period and, therefore, at maturity, produce lower-quality oocytes. Metabolic changes in follicular fluid of the dominant follicle in dairy cows with negative energy balance during early postpartum period may affect the quality of both oocyte and granulosa cells [38]. Corpora lutea may secrete less progesterone, resulting in lower fertility. As a consequence, it is likely that the repeat breeders were suffering from a temporary endocrine imbalance resulting in ovulation failure, fertilization failure, or early embryonic loss [1,3,15].

The causes of repeat breeding also include poor fertility semen, incorrect timing of AI [39], or asynchrony of estrus and ovulation [7]. The use of semen from a bull with high fertility [39,40], or straws containing semen from a number of bulls [39], or insemination more closely to the time of ovulation [7], improved fertility of repeat breeders. In two studies [41,42], conception rates of 33 and 50% were achieved when repeat breeders were inseminated with semen from high-fertility bulls.

To increase fertility in dairy cows, strategies to attenuate the incidence of abnormal resumption of postpartum ovarian cycles and repeat breeding are necessary. Management of the dry period [43] and nutritional strategies during transition and early postpartum periods [44] were proposed to improve fertility in dairy cows. Furthermore, shortening and even eliminating the dry period may improve energy status of cows and increase reproductive efficiency [43,45].

In conclusion, repeat breeders had very poor reproductive performance, despite repeated insemination. Lower parity, abnormal resumption of postpartum ovarian cycles, and shorter days in milk at first AI were risk factors for repeat breeding.

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References

[1] Bulman DC, Lamming GE. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. J Reprod Fert 1978;54:447–58.

[2] Bartlett PC, Kirk JH, Mather EC. Repeated insemination in Michigan Holstein Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Theriogenology 1986;26:309–22.

[3] Båge R, Gustafsson H, Larsson B, Forsberg M, Rodríguez-Martínez H. Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. Theriogenology 2002;57:2257–69.

[4] Gustafsson H, Emanuelson U. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. Acta Veterinaria Scandinavica 2002;43:115–25.

[5] ÓFarrel KJ, Langley OH, Hartigan PJ, Sreenan JM. Fertilization and embryonic survival rates in dairy cows culled as repeat breeders. Vet Rec 1983;112:95–7.
[6] Kimura M, Nakao T, Moriyoshi M, Kawata K. Luteal phase deficiency as a possible causes of repeat breeding in dairy cows. Br Vet J 1987;143:560–6.
[7] Silvia WJ. Embryonic mortality and repeat breeder cows. In: Proc National Reprod Symposium, 27th Annual Conf of American Association of Bovine Practitioners; 1994.p. 151–60.
[8] Heuwieser W, Oltenacu PA, Lednor AJ, Foote RH. Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. J Dairy Sci 1997;80:2766–74.
[9] Pursley JR, Silcox RW, Wiltbank MC. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J Dairy Sci 1998;81:2139–44.
[10] Moss N, Lean IJ, Reid SWJ, Hodgson DR. Risk factors for repeat-breeder syndrome in New South Wales dairy cows. Prev Vet Med 2002;54:91–103.
[11] Hallap T, Nagy S, Jaakma Ü, Johannisson A, Rodriguez-Martinez H. Usefulness of a triple fluorochrome combination Merocyanine 540/Yo-Pro 1/Hoechst 33342 in assessing membrane stability of viable frozen-thawed spermatozoa from Estonian Holstein AI bulls. Theriogenology 2006;65: 1122–36.
[12] Morrell JM. Update on semen technologies for animal breeding. Reprod Dom Anim 2006;41:63–7.
[13] Gustafsson H. Studies on follicular dynamics and hormonal asynchrony around ovulation as a potential cause of repeat breeding. Reprod Dom Anim 1998;33:139–40.
[14] Lopez-Gatius F, Yaniz JL, Santolaria P, Murugavel K, Guijarro R, Calvo E, Lopez-Bejar M. Reproductive performance of lactating dairy cows treated with cloprostenol at the time of insemination. Theriogenology 2004;62:677–89.
[15] Villarroel A, Martino A, BonDurant RH, Dèletang F, Sischo WM. Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. Theriogenology 2004;61:1513–20.
[16] Katagiri S, Takahashi Y. Changes in EGF concentrations during estrous cycle in bovine endometrium and their alterations in repeat breeder cows. Theriogenology 2004;62:103–12.
[17] Butler WR. Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci 2000;60–61: 449–57.
[18] Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gumen A. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 2006; 65:17–9.
[19] Macmillan KL, Lean IJ, Westwood CT. The effects of lactation on the fertility of dairy cows. Aust Vet J 1996;73: 141–7.
[20] Roche JF, Mackey D, Diskin MD. Reproductive management of postpartum cows. Anim Reprod Sci 2000;60–61:703–12.
[21] Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA, Lamming GE. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000;70:487–501.
[22] Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 2001;84:1277–93.
[23] Dochi O, Takahashi K, Hirai T, Hayakawa H, Tanisawa M, Yamamoto Y, Koyama H. The use of embryo transfer to produce pregnancies in repeat-breeding dairy cattle. Theriogenology 2008;69:124–8.
[24] Isobe N, Yoshimura T, Yoshida C, Nakao T. Incidence of silent ovulation in dairy cows during post partum period. Deutsche tierärztliche Wochenschrift 2004;111:35–8.
[25] Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 2005;64:1879–88.
[26] Santos JEP, Cerri RLA, Ballou MA, Higginbotham GE, Kirk JH. Effect of timing of first clinical mastitis occurence on lactational and reproductive performance of Holstein dairy cows. Anim Reprod Sci 2004;80:31–45.
[27] Lamming GE, Darwash AO. The use of milk progesterone profiles to characterize components of subfertility in milked dairy cows. Anim Reprod Sci 1998;52:175–90.
[28] Lopez-Gatius F. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology 2003; 60:89–99.
[29] Stevenson JS, Call EP, Scoby RK. Double insemination and gonadotropin-releasing hormone treatment of repeat-breeding dairy cattle. J Dairy Sci 1990;73:1766–72.
[30] Pérez-Marín CC, España F. Oestrus expression and ovarian function in repeat breeder cows, monitored by ultrasonography and progesterone assay. Reprod Dom Anim 2007;42: 449–56.
[31] Kendall NR, Flint APF, Mann GE. Incidence and treatment of inadequate postovulatory progesterone concentrations in repeat breeder cows. The Vet J 2009;181:158–62.
[32] Morales-Roura JS, Zarco L, Hernández-Cerón J, Rodríguez G. Effect of short-term treatment with bovine somatotropin at estrus on conception rate and luteal function of repeat-breeding dairy cows. Theriogenology 2001;55:1831–41.
[33] Selvaraju S, Agarwal SK, Karche SD, Srivastava SK, Majumdar AC, Shanker U. Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. Anim Reprod Sci 2002;73:141–9.
[34] Kharche SD, Srivastava SK. Dose dependent effect of GnRH analogue on pregnancy rate of repeat breeder crossbred cows. Anim Reprod Sci 2007;99:196–201.
[35] Caraviello DZ, Weigel KA, Fricke PM, Wiltbank MC, Florent MJ, Cook NB, Nordlund KV, Zwald NR, Rawson CL. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J Dairy Sci 2006; 89:4723–35.
[36] Taylor VJ, Beever DE, Bryant MJ, Wathes DC. Metabolic profiles and progesterone cycles in first lactation dairy cows. Theriogenology 2003;59:1661–77.
[37] Britt JH. Follicular development and fertility: potential impacts of negative energy balance. In: Proc Nat Reprod Symposim; 1994.p. 103–12.
[38] Leroy JLMR, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PEJ, Dewulf J, de Kruif A. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. Theriogenology 2004;62: 1131–43.
[39] Noakes DE, Parkinson TJ, England GCW. The 'repeat breeder' syndrome. In: Noakes DE, Parkinson TJ, England GCW, editors. Veterinary Reproduction and Obstetrics. Elsevier; 2009. p. 463–75.
[40] Roberts SJ. The repeat breeder cow. In: Roberts SJ, editor. Veterinary Obstetrics and Genital Diseases (Theriogenology). Woodstock, VT: Published by Roberts SJ; 1986. p. 559–72.

[41] Tanabe TY. Effect of crossbreeding on embryonic survival in normal and subfertile dairy cattle. Fertil. Steril. Proc. VII World Conf. Fertil. Steril., Tokyo and Kyoto, Japan. Excerpta Medica, Amsterdam, Internat. Congr. Ser. No. m 1973:722-4.
[42] Tanabe TY, Hawk HW, Hasler FJ. Comparative fertility of normal and repeat-breeding cows as embryo recipients. Theriogenology 1985;23:687–96.
[43] Grummer RR. Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology 2007;68:5281–8.
[44] Roche JF. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim Reprod Sci 2006;96:282–96.
[45] Peter AT, Vos PLAM, Ambrose DJ. Postpartum anestrus in dairy cattle. Theriogenology 2009;71:1333–42.