



Exploring Prevalence and Contributing Risk Factors of Repeat Breeding Syndrome of Crossbred Dairy Cows in Bangladesh

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ABSTRACT

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The dairy sector suffers significant financial losses due to repeat breeding syndrome (RBS), a significant reproductive disease in dairy cattle. For a very long time, repeat breeding has been regarded as one of the most critical reproductive issues in cattle. The objective of this study is to investigate the frequency and identify the risk factors related to repeat breeding in dairy cows in the Bogura district of Bangladesh. Additionally, the study aims to assess the influence of repeat breeding on reproductive performance in this population. This study collected indigenous and crossbred 179 cow's data using a pre-structured questionnaire. The cows that needed more than three consecutive inseminations to conceive were considered repeat breeders, and those that needed ≤ 3 inseminations were regular cyclic cows. The incidence rate of RBS in this study was 33.50%. In BCS 2.5 to 3.0, the incidence rate was significantly higher (58.30%) in repeat breeders than in others. Cows with < 5-litre average daily milk production and reared in intensive farming systems with moderate to poor levels of hygienic management have a significant ($p < 0.005$) effect of repeat breeding syndrome. Different reproductive diseases have a significant ($p < 0.001$) effect on the conception rate of cows. Stillbirth (77.87%) followed by fetal death (76.47%) were recorded as major reproductive obstacles in repeat breeding, whereas pyometra (71.43%), followed by abortion (66.67%), dystocia (70.00%) and endometritis (57.14%) were the major recorded complications in repeat breeders. The rate of conception would go up if cows had optimal BCS, good hygiene and management practices were used, and farmers were trained on how to manage cattle reproduction. This would lower the repeat breeding syndrome.

1. INTRODUCTION

To get the best profit from dairy cows, decent pregnancy rate after insemination/mating need to maintain and the herd must deliver one calf a year. In Bangladesh, numerous reproductive disorders, poor conception rate, delayed conception, and prolonged inter-calving intervals are documented as key constraints of commercial dairy farming (Jeong *et al.*, 2022; Shamsuddin *et al.*, 2001b). Repeat breeding syndrome is an important complication of the reproductive system of cows, which significantly reduces reproductive performance due to the increased number of services per conception, and prolonged inter-calving interval, as a result, the percentage of culling increases followed by financial

damage to the owners (Bartlett *et al.*, 1986; García-Ispuerto *et al.*, 2007; Lafi *et al.*, 1992). A cow is known as a repeat breeder when that has a regular oestrus cycle, does not exhibit or possess any palpable clinical abnormalities, does not have any atypical vaginal discharge, is less than 10 years of age, and most importantly the cow has a minimum single parity and has failed to conceive despite (at least three or more) successive insemination either with proven bull or good quality semen (Zemjanis, 1980). The prevalence of repeat breeding fluctuates depending on the geographical location, environment and management system (Bartlett *et al.*, 1986; Kimura *et al.*, 1987). According to (Asaduzzaman *et*

al., 2016; Nishi et al., 2018) the prevalence of repeat breeding syndrome in cows was 11.5% and 15.2% respectively, whereas (Båge et al., 2002; Bartlett et al., 1986; Bulman and Lamming, 1978; Moss et al., 2002; Yusuf et al., 2010) found ranged from 5% to 30% in tropical conditions. The incidence in dairy cows was 8.9% (Bulman and Lamming, 1978) and 24% (Bartlett et al., 1986) in the USA, while in Sweden it was reported at 10% (Båge et al., 2002; Gustafsson and Emanuelson, 2002). More recently, in the USA it's approximately 20% (Levine, 1999).

Though several studies (Gustafsson and Emanuelson, 2002; Heuwieser et al., 1997; Kimura et al., 1987, Moss et al., 2002; Pursley et al., 1998; Silvia, 1994) were conducted to reveal the precise reasons for repeat breeding syndrome it remains unclear, it assumes that multifactorial causes are responsible for this reproductive disorder. The risk factors involve managerial errors like inadequate estrus detection (Heuwieser et al., 1997; Pursley et al., 1998) poor quality semen, improper timing and technique of insemination (Hallap et al., 2006; Morrell, 2006); intrinsic errors like infection of uterus/cervix/vagina (Moss et al., 2002), hormonal maladies (Båge et al., 2002; Gustafsson and Emanuelson, 2002; Lopez-Gatius et al., 2004), delayed ovulation/ovulatory failure/defective ova, any type of anatomical defects in the reproductive tract such as oviduct (Kimura et al., 1987; Silvia, 1994); extrinsic factors like breed, age, parity, BCS and milk yield, etc. any alteration in hormonal concentration like high estrogen concentration during estrus, abridged luteinizing hormone (LH) concentration prior to LH surge (Sood et al., 2015; Pérez-Marín et al., 2007). Furthermore, different peri- and postpartum complications such as abortion, stillbirth, endometritis, pyometra, metritis, dystocia, early embryonic death, etc. also the associated factors of repeat breeding (Bonneville-Hébert et al., 2011; Kasimanickam et al., 2021; Salasel et al., 2010). However, so far very few works have been done to reveal the risk factors and the hidden causes of this vital reproductive condition. The objective of the present study is to determine the incidence of repeat breeding and associated risk factors and their effect on reproductive performance in dairy cows in Bangladesh.

2. MATERIALS AND METHODS

2.1 Study design

The study was conducted during the period from June to August 2022 in several dairy farms from different Upazila of Bogura district, Bangladesh. The farms were chosen as sampling locations based on the random sampling method. The data were collected directly through face-to-face interviews with the

farmers and visual observation of the cow with the help of a pre-structured questionnaire. The productive and reproductive data were collected through personal interaction with the owners. The questions were asked straightforwardly with explanations of questions where necessary.

2.2 Identification of repeat breeding cows

The cows identified as repeat breeders in this study based on their history taken from the owners, especially the cows that fail to conceive after at least three or more successive services with bulls of known fertility or artificial insemination with excellent quality semen. Some other clinical examination was done during data collection the repeat breeders had a normal estrus cycle, their ovaries free from any palpable abnormalities, possess normal vaginal discharge, must have at least single parity, and had less than ten years old.

2.3 Study approaches

The collected data were divided into two groups, normal cyclic cows and repeat breeder cows for the convenience of calculation of the study. To determine the effect of parity on postpartum cyclicity the animals were divided into 5 groups (0, 1, 2, 3 and >4). Body condition score (BCS) is a widely used method for assessing the amount of fat that remains subcutaneously which implies the nutritional and health status of the animal. The BCS scoring system is done by visual observation of the lumbar vertebral process (LVP) and back muscle over the pin bone. The scale ranges from 1.5 to 4.0 where 1.5-2.0 indicates very thin back muscles with LVP visible from a long distance, 2.5-3.0 indicates moderate back muscle with LVP slightly visible, and 3.5-4.0 indicates the presence of sufficient back muscle with LVP not visible. For hygienic condition-related variation, cows were categorized into good, moderate, and poor groups, where good groups regularly clean animal sheds with regularly empty and clean feed and water troughs, use separate units for different groups, and proper health care. Moderate groups clean their cowshed and feed and water troughs regularly but didn't use separate units for different groups. Poor groups didn't clean the environment, feed and water troughs regularly, and keep all aged animals in the same shed or unit.

2.4 Statistical analysis

The obtained data were cleaned, loaded, and stored on the Excel spreadsheet 2016. Then the data of quantitative variables were arranged and transferred to the software SPSS (Statistical Package for the Social Sciences, version: 1.0.0.1406) to calculate the Mean and Standard Error (SE) of each crossbreed and Independent sample T-test, and ANOVA was performed to know significance level

(at 5% significance level) among breeds for different risk factors.

3. RESULTS

3.1 Comparison of normal cyclic cows and repeat breeder cows

The results of the overall prevalence of normal cyclic cows and repeat breeder cows were 66.5% (n = 119) and 33.5% (n = 60), respectively (Figure). Postpartum interval extended more than 90 days in repeat breeder cows than that of regular cyclic cows, which declines the reproductive performance of repeat breeders. Table 1 reflects the comparison of normal cyclic cows and postpartum anestrus cows according to breeds. Most of the crossbred cows have observed a repeated number of AI than those of Indigenous.

3.2 Effect of different risk factors on cyclicity in cows

Cow having more than 4 parities shows the highest percentage (65.22%) of normal cyclicity, at which parity the percentage of repeat breeding cows also topmost (Table 2). In case of BCS, the highest percentage (52.90%) of normal cyclic cows was found within the groups of 2.5-3.0 BCS, while the lowest percentage (14.30%) was for the groups of 1.5-2.0 BCS. In repeat breeder cows, the highest percentages (58.30%) belong to the 2.5 - 3.0 BCS group, while the lowest percentage (16.70%) is within the 1.5-2.0 BCS group. The study also shows that the cows from the less than 4 years age group express the highest (37.82%) percentage of normal cyclicity which decreased with the advancement of age. In contrast, in case of repeat breeder cows uppermost (31.70%) percent of cows <4 years of age and repeat breeding increased with the progression of age. In terms of the number of artificial insemination, the cows need to inseminate 3 or more times seems repeat breeder whereas 2 or less than 2 inseminations were normal in terms of cyclicity. On the other hand, the cow's gives less than 5 litter milk on an average

daily were the highest (33.60%) percentage in terms of cyclicity whereas cows that produced more than 20 liters of milk were the lowest. Similarly, the highest 30.00% of the repeat breeder cows produced <5 liters of milk on average. The cows were reared in the intensive system where the highest percentage in both normal cyclic and repeat breeders. The farm owners who maintained moderate hygienic conditions in their farms show the highest (52.10%) percentage of normal cyclicity, in contrast, farms with poor hygienic management express the lowest percentage. Similar results were found in case of repeat breeder cows in terms of hygienic management.

3.3 Effect of feeding on normal cyclic cows and repeat breeder cows

The study shows that cows with no grass feeding have the highest (83.33%) percentages of normal cyclicity whereas cows with 16-30kg grass feeding cows show a maximum (35.62%) amount of repeat breeding. Roughage feeding (5-8kg) cows show uppermost (76.67%) cyclicity whereas 50% of the cows with >8kg roughage display the topmost amount of repeat breeding (Table 3).

3.4 Effect of reproductive diseases on cyclicity of cows

Like other risk factors, the prevalence and comparison of prevalence percentages of various reproductive diseases between cyclic and repeat breeder cows were observed. The results of the overall effect of reproductive diseases/conditions on cyclicity are presented in Table 4. There was a positive association between repeat breeding syndrome and reproductive diseases or conditions. In this study, most of the cows showed repeat breeding syndrome in relation to pre and postpartum conditions like abortion (66.67%), dystocia (70.00%), fetal death (76.47%), stillbirth (77.87%), endometritis (57.14%), and pyometra (71.43%) than those of normal cyclic cows.

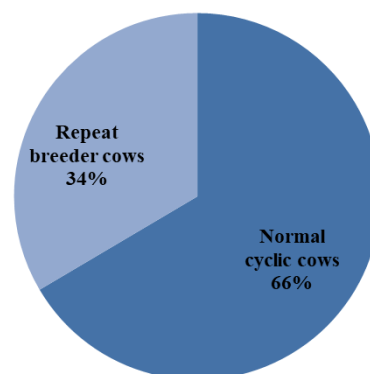


Fig. diagram explained the prevalence of normal cyclic cows and repeat breeder cows

Table 1. Breed wise comparison between normal cyclic cows and repeat breeder cows

Breed	Normal cyclic cows		Repeat breeder cows		Level of significance
	n	%	n	%	
Indigenous	34	28.60	7	11.70	**
Crossbreed	85	71.40	53	88.30	
Total	119	100.00	60	100.00	

n = Frequency, % = Percentage, Row wise different superscript letters denote significant level at ** $P < 0.01$

Table 2. Prevalence of normal cyclic cows and repeat breeder cows with different risk factors

Parameters	Indicators	No. of cows	Normal cyclic cows		Repeat breeder cows		Level of significance
			n	%	n	%	
Parity	0	21	15	12.61	6	10.00	NS
	1	55	38	31.93	17	28.33	
	2	43	25	21.01	18	30.00	
	3	37	26	21.85	11	18.33	
	>4	23	15	65.22	8	34.78	
BCS	1.5-2.0	27	17	14.30	10	16.7	*
	2.5-3.0	98	63	52.90	35	58.3	
	3.5-4.0	54	39	32.80	15	25.0	
Age (years)	<4	64	45	37.82	19	31.70	NS
	4-5	33	20	16.81	13	21.70	
	5-6	32	24	20.20	8	13.33	
	6-7	22	13	10.92	9	15.00	
	7-8	17	9	7.60	8	13.33	
	>8	11	8	6.72	3	5.00	
Number of A.I.	1	73	73	61.34	0	0.00	***
	2	46	46	38.76	0	0.00	
	3	9	0	0.00	9	15.00	
	>3	51	0	0.00	51	85.00	
Average Milk Production/Cow/Day	1-2	21	15	12.60	6	10.00	*
	<5	58	40	33.60	18	30.00	
	5-10	18	11	9.24	7	11.70	
	10-15	27	15	12.60	12	20.00	
	15-20	43	29	24.47	14	23.33	
Farming System	>20	12	9	7.66	3	5.00	*
	Intensive	160	107	89.92	53	88.33	
Hygienic Management	Semi-intensive	19	12	10.10	7	11.70	*
	Good	49	39	32.80	10	16.70	
	Moderate	104	62	52.10	42	70.00	
	Poor	26	18	15.13	8	13.33	

n = Frequency, % = Percentage, Row wise different superscript letters denote significant level at * $P < 0.005$, ** $P < 0.01$, *** $P < 0.001$

Table 3. Effect of feeding on normal cyclic cows and repeat breeder cows

Parameters	Amount (kg)	No. of cows	Normal cyclic cows		Repeat breeder cows		Level of significance
			n	%	n	%	
Grass	0	12	10	83.33	2	16.67	NS
	1 – 15	83	54	65.06	29	34.94	
	16 – 30	73	47	64.38	26	35.62	
	>30	11	8	72.73	3	27.27	
Concentrate	0	26	15	57.69	11	42.31	NS
	0.1 – 2.0	53	40	75.47	13	24.53	
	2.1 – 4.0	17	12	70.59	5	29.41	
Roughage	>4.0	83	52	62.65	31	37.35	NS
	0	25	17	68.00	8	32.00	
	1 – 4	80	49	61.25	31	38.75	
	5 – 8	60	46	76.67	14	23.33	
	>8	14	7	50.00	7	50.00	

n = Frequency, % = Percentage, Row wise different superscript letters denote significant level at * $P < 0.005$

Table 4. Effect of reproductive diseases on cyclicity of cows

Parameters	No. of cows	Normal cyclic cows		Repeat breeder cows		Level of significance
		n	%	n	%	
No	116	77	66.38	39	33.62	
Abortion	6	2	33.33	4	66.67	
Dystocia	10	3	30.00	7	70.00	
Fetal death	17	4	23.53	13	76.47	***
Still birth	9	2	16.67	7	77.78	
Endometritis	14	6	42.86	8	57.14	
Pyometra	7	2	28.57	5	71.43	

n = Frequency, % = Percentage, Row wise different superscript letters denote significant level at *** $P < 0.001$

4. DISCUSSION

The calving interval is increasing as a result of repeat breeding syndrome in Bangladesh because it delays the conception of cows. Postpartum interval extended more than 90 days in repeat breeder cows than that of regular cyclic cows, which declines the reproductive performance of repeat breeders. The prevalence (33.5%) of repeat breeding syndrome in dairy cows in the present study was closely aligned with the recent finding of (30.0%) Jeong *et al.*, (2022). However, it's relatively higher than the previous findings of Nishi *et al.*, (2018) and Sarder *et al.*, (2010), who reported 15.2% and 20.2% repeat breeders respectively. Although the prevalence is ranged from 14.0% to 24.0% in various countries (Bartlett *et al.*, (1986); García-Ispuerto *et al.*, (2007); Yusuf *et al.*, (2010)). In the present study due to differences in the study area, study population, management system, and productive and reproductive characteristics of the animals, the incidence rate is high.

In this study, most of the crossbreed cows have observed a repeated number of AI than those of Indigenous and also genotypes had a highly significant ($p < 0.05$) effect on repeat breeding in dairy cows. The result of this research was similar to the study of Nishi *et al.*, (2018) where they also perceived that in local cow's occurrence of repeat breeding was lowest (4.10%) and in L×F it was highest (19.62%). Mandefro and Negash (2014) also reported the same findings that in crossbreed cows incidence of repeat breeding is higher than in those of local breeds. In local breeds the frequency is lower may be due to the environment, as local cows are more adaptable to the normal atmosphere than that of cross-breed cows. The study shows that more than 4th parity had the highest occurrence of repeat breeding problems (34.78%) and the lowest in 1st calving (10.00%). This outcome is in alignment with previous findings of Matubber *et al.*, (2018), Asaduzzaman *et al.*, (2016), and Bonneville-Hébert *et al.*, (2011). Jeong *et al.*, (2022) also found a

significant amount of repeat breeding problems in multiparous cows. Though Nishi *et al.*, (2018) reported that at 3rd parity the highest percentage of repeat breeding problems have occurred. In 1st and 2nd parity, the prevalence of repeat breeding syndrome is relatively higher than that of 3rd parity because of low-energy supplements at the post-calving period to meet up regular needs. BCS is one of the most influential factors, which play an important role to increase the risk of repeat breeding syndrome. The present study reveals that there is a significant ($P > 0.05$) effect of BCS on repeat breeding; cows with 2.5-3.0 BCS show the highest percentages of repeat breeding syndrome. Some earlier studies by Matubber *et al.*, (2018), Shamsuddin *et al.*, (2001a), and Nishi *et al.*, (2018) also found similar results. The cows of herds with low BCS likely suffer more from negative energy balance consequently the secretion of reproductive hormones decreases which uplifts the conception rate and early embryonic death afterward repeat breeding.

The study shows that at the early stage of life especially at <4 years aged cows show more repeat breeding syndrome. This is partially supported by the earlier study by Sarder *et al.*, (2010) stated that the prevalence of repeat breeding was higher in >8 year aged cows whereas lower in less than 4 and 4-6 years old cows. This dissimilarity may be due to the estrus detection method, management, and hygienic system of the farms. In separate research both Hodel *et al.*, (1995) and Hewett (1968) found a negative impact of age on fertility, and they also stated that in older cows the rate of repeat breeding syndrome is higher. With the advancement of age, the level of a hypothalamic or pituitary hormone varies and also the ability of ovarian response differs depending on age. Though the study shows there is no significant influence of age on repeat breeders, which is supported by Matubber *et al.*, (2018) and Asaduzzaman *et al.*, (2016). The number of insemination was significantly higher ($P < 0.0001$) in repeat breeders compared to normal cyclic cows. The cows need to

inseminate 3 or more times seem repeat breeder whereas 2 or <2 inseminations were normal in terms of cyclicity. Poor heat detection and a lack of skilled AI technicians in artificial insemination might be responsible for this; there might be some missed services as well as consecutive missed services. According to Pérez-Marín & España (2007), poor heat detection and infertility in cattle are key reasons for repeat breeding syndrome. And poor heat detection plays a significant role in overall reproductive performance like calving interval, conception rates, length of the breeding period, etc. In a previous study, Muxlow (2005) reported that errors in heat detection were caused by some individual factors comprising the skill of the technician as well as sound knowledge about signs of heat, proper time of observation, heat checks per day, and timely reporting of observations, etc. The present study found that cows with low (<5 litter) and medium (15-20 litter) milk yield mainly suffer from repeat breeding syndrome. But according to Jeong *et al.*, (2022), milk yield was not a risk factor for repeat breeder cows; they found a negative relation between reproductive performance and higher milk production. Though in earlier studies Buckley *et al.*, (2000) and Carthy *et al.*, (2016) were both unable to reveal any relation between reproductive performance and milk production during the early stage of the lactation period. The current study shows that the cows reared in an intensive system were the highest percentage in both normal cyclic and repeat breeders. This result is differing from the findings of Matubber *et al.*, (2018) and Khan *et al.*, (2016). This variation may be due to some other factors like heat detection, feeding management, etc. In the present study, the farm owners who maintained moderate hygienic conditions in their farms show the highest (52.10%) percentage of normal cyclicity, in contrast, farms with poor hygienic management express the lowest percentage. Similar results were found in case of repeat breeder cows in terms of hygienic management. According to Tesfaye *et al.*, (2013) routine deworming and vaccination can reduce the risk of repeat breeding. So, along with hygienic management deworming and vaccination also must be followed to get better reproductive performance from cows.

The present study shows that repeat breeding syndrome has occurred in the cows given more green grass and roughage than that concentrate. This is in partial alliance with the earlier finding of Padder *et al.*, (2018), who stated that the cows feed with only concentrate like straw and broken rice were more prone to be repeat breeders than those cows receiving both green grass and concentrate. Diet has an intense

influence on the reproductive performance of cows (Butler, 2005; Shamsuddin *et al.*, 2001b). Furthermore, persistent deficits in dietary energy and protein can cause chronic pressure on the hypothalamic-pituitary-ovarian axis, which will have an adverse effect on pregnancy rates (Siddiqui *et al.*, 2002). According to this study, it can be suggested that farmers should provide a better diet through concentrate and green grass supplementation. However, further experiments are required to explain this factor more accurately. There was a positive association between repeat breeding syndrome and reproductive diseases or conditions. The study summarizes that cow having difficulties at last calving was found more susceptible to repeat breeding than those having no difficulties at last calving. This finding is supported by the earlier study by Bonneville-Hébert *et al.*, (2011), Padder *et al.*, (2018), and Jeong *et al.*, (2022).

5. CONCLUSIONS

In conclusion, the overall prevalence of repeat breeding in dairy cows in Bogura district was 33.5%, where crossbreed cows were more prone to repeat breeder. Number of parities, age, breed, body condition score, feeding with more roughage, poor hygienic management, and pre and post-partum diseases were identified as significant risk factors for repeat breeding in the current study. The farmers should be paid attention to proper heat detection technique and AI as well as breeding management to reduce the occurrence of repeat breeding syndrome.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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