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Risk factors for postpartum anestrus in crossbred cows in Bangladesh

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Abstract: Ultrasonography and a structured questionnaire were used in a cross-sectional study to gather data on the prevalence and risk factors for anestrus in crossbred cows at ≥ 60 days postpartum in 273 smallholder farms. The prevalence of anestrus was 18%. The odds ratio (OR) for true anestrus was 17.52 and 2.81 times higher ($P < 0.05$) in cows with poor (≤ 2.0) and excessive (> 3.5) body condition score (BCS), respectively, compared to those with optimal BCS (2.5–3.5), 2.82 times higher in suckled than in nonsuckled cows ($P = 0.03$), and 2.53 times higher in cows that calved during the cold season than in those that calved during the hot season ($P = 0.03$). The OR for anestrus was 1.62 times higher ($P = 0.017$) in cows managed by an employee than in those managed by the farmers themselves ($P = 0.001$), and 2.66 times higher ($P = 0.003$) in small farms (≤ 5 cows) than in large farms (≥ 11 cows). The OR was 0.71 to 0.46 times lower in farms having a guaranteed market to sell milk than those with an uncertain traditional milk market ($P < 0.05$). Maintaining optimal BCS of cows, farmers' training on management of cattle reproduction, and development of a market linkage to sell milk would improve the number of cows for breeding by 60 days postpartum.

Key words: Postpartum, anestrus, risk factors, crossbred cows

1. Introduction

The economic success of a dairy farm commonly depends on the success of establishing an optimal calving interval of approximately 1 year (1). In order to achieve the optimal calving interval in dairy cows, an anestrus period after calving should not exceed 65 days (2). Factors associated with an increased calving interval are related to the dairy cow herself (uterine-ovarian abnormalities) (2) or may reflect serious shortcomings in management (inadequate estrus detection) (3,4). In developing countries, dairying is an important economic activity that provides supplementary income and high employment of family labor (5). In general, farmers follow a dominant marketing channel to sell their milk according to their geographic location and proximity to possible purchasers (6), although in some areas farmers collectively operate their farm through a cooperative system (6). Prolonged postpartum anestrus is the main constraint of cattle reproduction (7), resulting from inefficiencies in nutritional management (8) and poor estrus detection (4,9), both giving rise to a significantly extended calving interval. Environmental factors like heat stress and poor condition of barns also potentially prolong

the postpartum anestrus (3). Only a small proportion of cows resume their ovarian cyclicity by 60 days postpartum (4). Furthermore, 40% of cows that resume cyclicity are not detected in estrus even when they successfully complete one or more ovarian cycles (4) although there is a good prospect of efficient heat detection. Missing one estrus extends the calving interval by 21 days and is accompanied by an estimated loss of \$43 in smallholder farms (5). Hence, it is clear that postpartum anestrus in crossbred cows demands particular attention, but corresponding assessments of the prevalence and risk factors have, to the best of our knowledge, not previously been performed in crossbred cows. Therefore, the objectives of the present study were to determine the cow-level prevalence of anestrus and to examine various risk factors supposed to be significantly associated with them in smallholder farms in Bangladesh.

2. Materials and methods

2.1. Study population

Postpartum anestrus was defined as lack of estrus in a cow that had calved 60 or more days (3). It was evaluated in

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crossbred (Holstein × Zebu, $n = 1286$) cows of smallholder farms ($n = 273$) in the Chittagong, Joypurhat, and Sirajgonj districts of Bangladesh. The farms were registered with the Community-based Dairy Veterinary Foundation (CDVF) of Bangladesh Agricultural University, which provides scheduled preventive and emergency veterinary services to registered farms. Cows were hand milked twice daily; however, during the last part of lactation, many farmers milked their cows only once a day. An intensive dairy production system with tie-stall barns was practiced. Animals were generally fed a mixed ration consisting of agricultural by-products (wheat bran, rice polish, and oil cakes), rice straws, green fodders, and cut-and-carry grass. All cows in the randomly selected farms were taken into consideration to determine anestrus in the study population. Sample size was determined based on an estimated anestrus prevalence of 20% and an allowable error of 5% in the estimated prevalence. Descriptive statistics relative to the geographic area where the study took place are given in Table 1.

2.2. Measurement of cyclicity

Cows not detected in estrus at 60 or more days postpartum were examined by transrectal ultrasonography to identify the underlying disorders. The ultrasonographic examinations were performed using a real-time B-mode ultrasound (PharVision Micro V10, Tequesta, FL, USA). The cows were examined again 10 days later when the result of the first examination was not conclusive. The findings were interpreted as follows: a) cows having 2 small ovaries without a CL at both examinations and without any abnormal finding in the uterus were grouped as true anestrus; b) cows were considered subestrus if a functional CL was found on at least 1 of the ovaries at either of the 2 examinations; c) cows with follicular or luteal structures more than 2.5 cm in diameter on both examinations were diagnosed with cystic ovaries; and d) cows with purulent material in the uterus with a CL on either of the ovaries were diagnosed with pyometra.

2.3. Data notation

Data on the following parameters were carefully recorded on a cow basis (body condition, suckling status, calving season, parity, milk production, and milking frequency) and herd basis (frequency of anestrus cows, herdsman's sex and education, milk market, amount of income from the dairying for livelihood of the farmer, farm size, floor type, bull in farm, supplementation of vitamin/mineral premix in the ration, green grass availability, and time and duration of estrus observation) for every farm. All information was collected by the same person, by interviewing the farmers using a structured questionnaire, retrieving the requested data from the farm record book, or physical measurement throughout the study. Body condition score (BCS) of cows was given using a 1 to 5 scale (1 = emaciated, 5 = obese)

with 0.5 increments (10) by the same person throughout the study. The milk market was recorded as traditional, cooperative, and ensured traditional (Pala group) based on the prevailing marketing channels (6). Pala is an informal group of farmers in remote areas collaborating for collective milk marketing (6). In the Pala marketing channel of the area studied, the farmer groups affiliated with CDVF had a higher bargaining power to negotiate a reasonable milk price.

2.4. Data management and statistical analysis

After a descriptive analysis, the likelihood of the biologically relevant risk factors was analyzed by using multiple logistic regression (Minitab version 15.1, Minitab Inc., State College, PA, USA). The continuous independent variables were transformed into categorical variables before being used in the statistical models. Cows diagnosed with cystic ovaries ($n = 10$) and pyometra ($n = 6$) were not included in the models. Daily milking frequency (once or twice), bull in farm (presence or absence), time of estrus observation (morning or evening; before or after milking), supplementation of vitamin/mineral premix in the ration (used or not), and green grass (available or not) were firstly tested for possible univariate associations with anestrus ($P \geq 0.10$) and were excluded. Only risk factors that had univariate associations ($P < 0.10$) were subsequently evaluated in the multiple explanatory multivariable models of logistic regression analysis to generate odds ratios (ORs) with respective 95% confidence interval (CI). The statistical model (true anestrus vs. subestrus) for evaluating the cow-level risk factors consisted of BCS, sucking, calving season, parity, and milk production. In another statistical model (anestrus vs. non-anestrus) the farm-level risk factors for anestrus such as the herdsman, farm size, milk market, estrus observation, sex and education of the herdsman, and dairying in livelihood were evaluated. Differences with $P \leq 0.05$ were considered significant in the final model. Goodness-of-fit of the model was assessed by using the comparison of the deviance of the model to a χ^2 distribution.

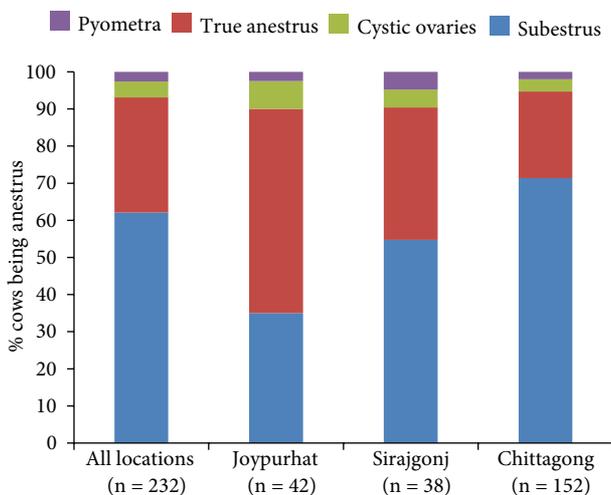
3. Results

3.1. Prevalence of anestrus

Statistical analyses revealed that 18% (232/1286) of cows were anestrus at ≥ 60 days postpartum (Table 1). Of the cows reported to be anestrus ($n = 232$), 62% (144/232) were subestrus as evidenced by the presence of a CL on at least 1 ovary, and 31% (72/232) were true anestrus as defined by not having a CL in 2 examinations 10 days apart. In the 16 remaining anestrus cows, 4% (10/232) were diagnosed as suffering from cystic ovaries and in only 3% (6/232) pyometra was diagnosed. Ultrasonographic diagnoses of utero-ovarian conditions of the cows that were reported to be anestrus by the farmers are shown in the Figure.

Table 1. Prevalence of postpartum anestrus in crossbred cows in relation to geographical localization.

Descriptors	All locations	Chittagong	Joypurhat	Sirajgonj
Total cows in the study population	1286	979	169	138
Not anestrus (pregnant or bred but not diagnosed pregnant or <60 days in milk)	1054 (82%)	829 (84.7%)	129 (76.3%)	96 (69.6%)
Anestrus (cows at ≥ 60 days postpartum but not detected in estrus by the farmer)	232 (18%)	150 (15.3%)	40 (23.7%)	42 (30.4%)
Subestrus (cycling cows not detected in estrus evidenced by a CL on either of the ovaries)	144 (11.2%)	107 (10.9%)	14 (8.3%)	23 (16.7%)
True anestrus (acyclic cows with no CL found at 2 ultrasonography examinations 10 days apart)	72 (5.6%)	35 (3.6%)	22 (12.9%)	15 (10.9%)

**Figure.** Utero-ovarian conditions of cows being anestrus

3.2. Cow-level risk factors

OR estimate for true anestrus was 17.52 and 2.81 times higher ($P < 0.05$) in cows with poor (≤ 2.0) and excessive (> 3.5) BCS, respectively, compared to those with optimal BCS (2.5–3.5). The OR estimate was furthermore 2.82

times higher in suckled than in nonsuckled cows ($P = 0.03$). Cows that calved during the cold season (November to March) had 2.53 times higher OR in comparison to those that calved during the hot season (April to October) of the year ($P = 0.03$). ORs with their confidence intervals of the cow-level risk factors for true anestrus are shown in Table 2.

3.3. Farm-level risk factors

OR estimates for anestrus was 1.62 times higher ($P = 0.017$) in cows managed by an employee than in those managed by the farmers themselves ($P = 0.001$). Cows in small farms (≤ 5 cows) had 2.66 times higher OR ($P = 0.003$) than those in large farms (≥ 11 cows). The OR was lower in farms having a guaranteed market to sell milk (farmers in cooperative association selling to formal processor, OR = 0.71; farmers in group selling to sweetmeat industries, OR = 0.46) than those with an uncertain traditional milk market ($P < 0.05$). The OR for anestrus was 1.95 times higher in cows watched for estrus for ≤ 20 min than in cows watched for > 20 min ($P = 0.039$). OR with their confidence intervals of the farm-level risk factors for anestrus are shown in Table 3.

Table 2. Cow-level risk factors for true anestrus in crossbred cows.

Variable	Category	Definition	Percent cows true anestrus	Adjusted odds ratio (95% CI)	P-value
Body condition	Optimal BCS	Body condition 2.5–3.5	10.7	1 (referent)	-
	Poor BCS	Body condition < 2.5	78.5	17.52 (9.59–43.27)	< 0.001
	Excessive BCS	Body condition > 3.5	23.1	2.81 (1.05–7.52)	0.040
Suckling	Absent	No suckling during last month	22.6	1 (referent)	-
	Present	Calf suckled during last months	37.7	2.82 (1.14–6.95)	0.025
Calving season	Hot season	April to October	28.0	1 (referent)	-
	Cold season	November to March	47.5	2.53 (1.09–5.84)	0.030

Table 3. Farm-level risk factors for postpartum anestrus on smallholder farms.

Variable	Category	Definition	Percent anestrus	Adjusted odds ratio (95% CI)	P-value
Herdsman	Self	Owner himself or family member manages farm	17.6	1 (referent)	-
	Hired	Employee as manager	20.3	1.62 (1.09–2.42)	0.017
Farm size	Large	Farms with ≥ 11 cows	14.8	1 (referent)	-
	Medium	Farms with 6–10 cows	14.8	1.13 (0.73–1.75)	0.593
	Small	Farms with 1–5 cows	25.6	2.66 (1.41–5.04)	0.003
Milk market	Traditional	No specific buyers	30.2	1 (referent)	-
	Cooperative	Farmers in association selling to formal processor	24.6	0.71 (0.42–0.98)	0.037
	Ensured traditional	Farmers in group selling to sweetmeat industries	15.3	0.46 (0.25–0.84)	0.011
Estrus observation	More	Daily ≥ 20 min	15.5	1 (referent)	-
	Less	Daily < 20 min	23.4	1.95 (1.03–3.68)	0.039

4. Discussion

In total, 18% of cows were reported by the farmer as anestrus in the present study. This is comparable with an earlier report in Bangladesh (8), although a higher prevalence (35%) of anestrus was reported in an earlier study (11). The prevalence of the forms of anestrus in different herds is dependent on various factors (12) but major causes of anestrus are subestrus and true anestrus (11). Subestrus is more likely because of poor detection system or high incidence of silent estrus (4,13). Inaccurate estrus detection is also an important problem in Bangladesh (4,9). Visual checks are the most frequently used methods to detect a cow in estrus, often resulting in relatively low on-farm detection rates (14). Moreover, the most prevalent ovarian dysfunctions like delayed onset of postpartum ovarian activity and cessation of cyclical ovarian activity after a period of normal ovarian function (2,15) contribute significantly to the prevalence of anestrus. Prevalence of ovarian cysts and pyometra is minimal (11) and are comparable to the result of the present study.

Cows with poor and excessive BCS were more likely to remain in true anestrus at 60 or more days postpartum than cows with optimal BCS in the present study. Although energy balance was not measured, these data clearly demonstrate that changes in energy status as evidenced by BCS are important factors regulating when cows resume cyclicity after calving (16). Underfeeding and poor BCS led to high incidence of true anestrus (2). Cows with excessive BCS undergo increased mobilization of body fat and accumulate more triglycerol in the liver, and revealed a longer postpartum interval to first estrus (17). Although energy status partially determines when cows first ovulate postpartum, it is likely that other factors might also be involved. Primiparous cows are more likely

to be true anestrus than multiparous in the first 60–70 days postpartum (16,18) for significantly less intense cycles in them (19). They have greater concentrations of blood nonesterified fatty acids in postpartum than multiparous cows for a greater loss of BCS (20), and these differences have been associated with a longer calving to conception interval (21). In a review (14), it was reported that younger cows display silent estrus less often than older cows; however, this was not proven in the present study. Monitoring of BCS on a regular basis at early lactation could also be a useful approach to identify cows' cyclic status. When cows with poor BCS are identified, intervention measures could be taken early enough to correct for energy deficiencies to avoid marked adverse effects on reproductive efficiency. Suckling cows had a higher risk for true anestrus at postpartum than nonsuckling cows in the present study. The adverse effects of frequency and duration of suckling on the initiation of postpartum cyclicity is well documented (4). Suckling interferes with hypothalamic release of GnRH, provoking a marked suppression in pulsatile LH release, resulting in extended postpartum anestrus (22). Although cattle are not seasonal breeders, cows that calved in the winter were at the greatest risk of true anestrus for delayed resumption of postpartum ovulation (2,23). It can be postulated that alterations in photoperiodic stimulation (24) and nutritional changes (18) associated with specific times of the year are potential explanations for the effect of season on delayed onset of estrus in lactating crossbred cows. Increase milk production delayed the resumption of ovarian function in the cows in part because of increased catabolic state (17) and reduced estrus behavior even if BCS loss is moderate (25). However, milk yield is closely associated with dry matter intake and energy intake accounted for most of the variation in energy balance in postpartum cows (26).

Estrous detection is the most important managerial variable involved in anestrus in postpartum cows (19), maintaining a short calving interval and defining the time interval to insemination (27). It was worse in farms when an employee managed them than those managed by farmers themselves in the present study. The high rate of anestrus cases is a result of the stockman's failure to properly observe and record animals in estrus, which substantially contributes to subestrus occurrence (12). The maintenance of a good level of knowledge of both primary and secondary estrus symptoms and the observations of cows at the appropriate time of day is critical for farm staff (19). The intensity of estrus symptoms that are expressed by the cow and hence can be observed by the herdsman is a very subjective matter and therefore difficult to scientifically investigate (3). Any adjustment in procedures to improve the success of the detection of estrus will also potentially improve fertility. Ensured milk marketing facilities influenced farmer to be more attentive to detect cows in estrus and thereby reduced the prevalence of postpartum anestrus in their farms. The organized marketing channel in which farmers can get a fair price would encourage them to farm better (6) and thus marketing guides them towards new production opportunities (5). A democratic organization like a dairy cooperative can play a vital role for poor rural farmers in terms of better access to fair market prices for their products and thereby improvement in income (6). The insignificant effect of farmers' income on the prevalence of postpartum anestrus may be explained by the fact that although large farmers earn more from dairying they have some other income generating parallel business. Conversely, although small farmers earn less money from dairy farming, this constitutes a major portion in terms of their family income.

Prevalence of postpartum anestrus was higher in farms with fewer cows and this is explained by the fact that mounting activity and the duration of estrus are minimal when only one animal is in estrus and it is easily overlooked (28). Experimental examples of this social influence have also been reported, demonstrating the effect of synergy in the expression of estrus due to the presence of companion cows (14). The duration and intensity of the displayed estrus is highly variable among individuals

and is greatly influenced by the number of cows that are in estrus simultaneously (14,25). However, the manpower input per cow decreases with increasing herd sizes and reduced levels of staffing available on farms (29), which increases the need for more practical and focused estrus detection methods. Therefore, large groups of animals and concentrated calving patterns would increase the likelihood of natural synchronization of estrus and improve the probability of accurate estrus detection. Increased duration of estrus observation decreased risks of postpartum anestrus as the accuracy and efficiency of direct observation are affected by the frequency, duration, and timing of the observation periods (28). Additionally, with the duration of estrus being reported to be as low as 4 h (19), a short estrus period could be missed between observational periods. The farm staff, who have a standard operating procedure for estrus detection, realized a marginally higher (73%) detection rate by observing the animals during four 20-min observational periods per 24-h period, with additional observations during the milking periods (30).

5. Conclusion

A high percentage of the cows were identified as being anestrus at 60 or more days postpartum. The higher proportion of subestrus among cows determined as anestrus indicates that poor estrus detection is a much more serious problem than true anestrus. Maintaining optimal BCS of cows, farmers' training on management of cattle reproduction, and development of a market linkage for the farmers to sell their milk would improve number of cows for breeding by 60 days postpartum.

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