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Underlying Disorders of Postpartum Anoestrus and Effectiveness of their Treatments in Crossbred Dairy Cows

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ABSTRACT

The objectives of this cross-sectional study were to determine the underlying disorders and effective treatment of postpartum anoestrus. Rectal examination accompanied by ultrasonography was performed twice in a 10-day interval on crossbred (Holstein×Zebu) anoestrus cows at = 60 days postpartum to diagnose their cyclic status. Cows diagnosed as silent estrus and true anoestrus were allotted to different treatment and Artificial Insemination (AI) protocols. The pregnancy was diagnosed thereafter by ultrasonography between 30-35 days post AI. The prevalence of anoestrus was 18.5% (83 out of 448 cows) as reported by the farmers with silent estrus 53.0% (n = 44), true anoestrus 42.2% (n = 35), cystic ovarian disease 2.4% (n = 2) and uterine infections 2.4% (n = 2) as the underlying disorders. Treatment of silent estrus was revealed estrus and conception; respectively, in 52 and 43% cows carefully monitored of estrus signs for AI (Group I, n = 23) and in 73 and 45% prostaglandin treated cows with AI on observed estrus (Group II, n = 11). Prostaglandin treatment followed by timed AI with GnRH administration at first AI (Group III, n = 10) revealed 60% conception. The difference in rates of conception was not significant among different treatment groups ($p>0.05$). Treatment of true anoestrus was revealed estrus and conception; respectively, in 82 and 64% nutritionally supplemented cows (Group IV, n = 11) and in 50 and 40% suckling restricted cows (Group V, n = 10). GnRH treatment followed by PGF2α administration and timed AI with GnRH at first AI (Group VI, n = 9) revealed 44% conception. Control anoestrus cows (Group VII, n = 5) without any treatment did not show estrus. The difference in rates of conception was significant ($p<0.05$) between the groups. In conclusions, close monitoring of silent estrous cows for estrus and nutritional supplementation for true anoestrus could be effective tools for successful reproduction.

Key words: Dairy, cows, anoestrus, ultrasonography

INTRODUCTION

Success in dairy farming depends to a large extent on the establishment of a year-round calving interval in cows (Hanzen *et al*., 1994; Shrestha *et al*., 2004). In order to achieve this calving interval an optimum voluntary waiting period of 65 days is recommended followed by conception within 85 to 90 days of the postpartum (Opsomer *et al*., 1998; Noakes, 2000). Failure to having an optimum fertility leads to lower production efficiency through uneconomical milk production and reduced number of offspring (Lamming and Darwash, 1998). Hence, cows not seen in heat during

underlying disorder of the prolonged postpartum anoestrus (Zdunczyk *et al*., 2002; Shrestha *et al*., 2004). Prolonged postpartum anestrus is the main constraint of cattle reproduction in Bangladesh (Rahman and Haque, 2001; Haque *et al*., 2002) that results from inefficiency in nutrition management and oestrus detection (Kamal, 2010) leading to longer calving interval (Sultana *et al*., 2001; Al-Amin and Nahar, 2007). Only a small proportion of cows resumed their ovarian cyclicity by Day 60 postpartum as determined by milk progesterone assay (Shamsuddin *et al*., 2006a). Furthermore, of the ones that resumed ovarian cyclicity 40% were not detected in oestrus even when they had completed one or more ovarian cycles (Shamsuddin, 1995; Shamsuddin *et al*., 2001). The calving interval extends by 21 days in cows when one oestrus is missed with an estimated economic loss of $43 (Shamsuddin *et al*., 2006b) its effects are greater than most dairy farmers realize. Although, the significant effects of postpartum anoestrus are well known, the prevalence of underlying disorders and effective remedies in Bangladeshi crossbred cows is not well studied. Different methods of hormonal and non-hormonal treatment for anoestrus have been employed by the practitioners but there is no comparative studies on their effectiveness in cows. Hence, the objectives of this study were to determine the prevalence of underlying disorders and to evaluate the effective treatment of the postpartum anoestrus in crossbred dairy cows.

MATERIALS AND METHODS

Animal selection and management: This study on crossbred (Holstein × Zebu) cows (n = 448) was conducted at randomly selected smallholder dairy farms (n = 30) at Chittagong district in Bangladesh. The cows were examined during a six-month period from July to December 2010 covering summer and winter season. The cows were generally kept in an intensive management system. The cows were housed in tie-stall barns and mainly fed on rice straw, concentrates and cut-and carry grasses. Total feeds and forages were given in two splits per day. Average milk yield was 4000 L per lactation of about 300 days. Routine deworming against round worms and liver flukes was in practice. The cows were vaccinated routinely against foot-and-mouth disease, anthrax and hemorrhagic septicemia. The cows were housed almost 24 h in their rearing sheds having the facilities of natural ventilation. All cows were milked by hand twice daily at an interval of 8-10 h and were suckled approximately for 4 h daily. The nutritional status of the cows was determined by scoring the body conditions of the cows using 1 to 5 scales with 0.5 increments (Ferguson *et al*., 1994).

Clinical examinations: Cows that did not show any visible signs of estrus at = 60 day postpartum were examined by a real-time B-mode transrectal ultrasonography (Pharvision Micro V10®, Esaote Pie Medical, USA) twice in a 10-day interval. After adequate restraining, the scanner was placed at a sensible distance from the cow on the side opposite to the operator's arm engaged in rectal palpation. The transducer face was lubricated with a suitable coupling medium (Ultrasonic Gel® for Medical use-250 mg) and was usually covered by a lubricated plastic sleeve before insertion. The transducer was then progressed cranially along the rectal floor to overlie the reproductive tract. Cows were considered as 'silent estrous' if a CL was found on at least one of the ovaries at either of the two examinations. Cows having two small ovaries without a CL at both examinations and without any abnormal finding in uterus were grouped into the 'true anoestrus' category. Presence of follicular or luteal structures at least 2 cm in diameter on both examinations were interpreted as cystic ovaries. Presence of purulent material in uterus with a CL in either of the ovary was

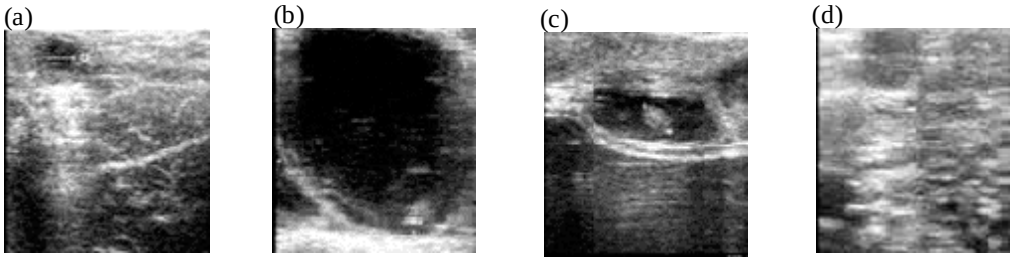

Fig. 1(a-b): Photographs showing utero-ovarian conditions of cross-bred cows, (a) Corpus luteum, (b) Luteal cyst, (c) 30-days embryo and (d) Uterine infection

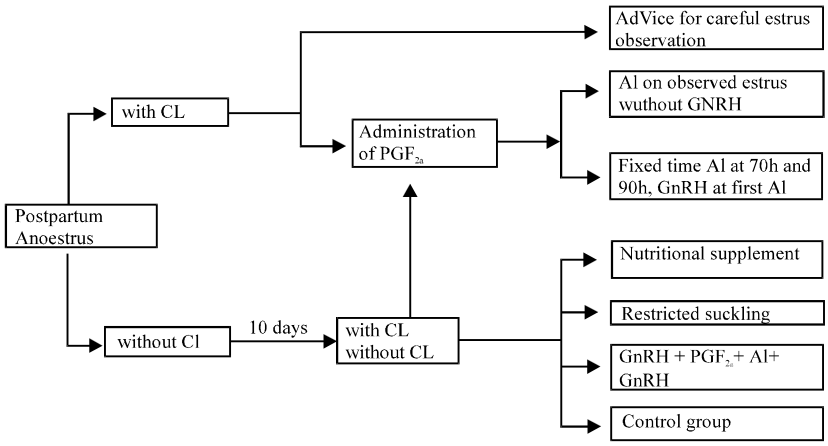

Fig. 2: Schematic presentation of the experimental design

diagnosed as uterine infections. Cystic ovaries and uterine infections were not considered further other than prevalence study (Fig. 1a-d).

Treatment protocol and artificial insemination: The experimental design is presented in Fig. 2. Silent estrus cows were either closely monitored for estrus twice daily for 15 days with AI on observed estrus (Group I); or treated with PGF2α analogue (Alfaprostol 8 mg; Gabrostim®, VETEM SpA, Porto Empedocle, Italy) followed by AI on observed estrus (Group II); or treated with the PGF2α analogue followed by timed AI at 70 and 90 h with a single dose of GnRH analogue (Gonadorelin 500 μg, Fertagyl®, Intervet International BV, European Union) at first AI (Group III). True anoestrus cows were either supplemented with balanced diet [Maize (50%), Wheat bran (30%), Oil cake (10%), Rice polish (8%) and Vitamin-Mineral premixes (2%) for one month with AI on observed estrus (Group IV); or suckling was restricted (Group V); or treated with a single dose of GnRH analogue on Day 0 (day of injection) followed by administration of PGF2α analogue on Day 12 and TAI at 70 and 90 h with GnRH analogue at first AI (Group VI); or control without any treatments (Group VII). All injections were given intramuscularly in the shoulder region. The cows were examined between 30-35 days after AI by using B-mode real-time ultrasonography for pregnancy diagnosis.

Statistical analysis: The data was analyzed to determine the positive fertility result. Data on silent estrus, true anoestrus, cystic ovaries and those with uterine infections was used to determine

the prevalence. The data collected on response to treatment interventions with respect to estrus and conception were entered in Microsoft Excel 2003 and descriptive statistics were performed. The fertility indices were analyzed by paired t-test using MINITAB version 13 statistical program. The difference between values was considered significant when the P value was less than 0.05.

RESULTS

Prevalence of underlying disorders: The prevalence of anoestrus was 18.5% (83 out of 448 cows) with silent estrus 53.0% (n = 44), true anoestrus 42.2% (n = 35) as their underlying disorders. The proportions of the silent estrus and true anoestrus cases were comparable among different parities but the true anoestrus cases were higher among the low body conditioned than high body conditioned cows (Table 1). A few cystic ovarian disease 2.4% (n = 2) and uterine infections 2.4% (n = 2) were also recorded.

Effectiveness of treatments: The effectiveness of different treatments of anoestrus cows on estrus and conception are presented in Table 2. Treatment of silent estrus was revealed estrus and conception; respectively, in 52 and 43% cows carefully monitored for estrus signs with AI on observed estrus (Group I, n = 23) and in 73 and 45% prostaglandin treated cows with AI on observed estrus (Group II, n = 11). Prostaglandin treatment followed by timed AI with GnRH administration at first AI (Group III, n = 10) revealed 60% conception. The difference in rates of conception was not significant among different treatment groups ($p>0.05$). Treatment of true anoestrus was revealed estrus and conception; respectively, in 82 and 64% nutritionally supplemented cows (Group IV, n = 11) and in 50 and 40% suckling restricted cows (Group V, n = 10). GnRH treated cows followed by PGF2α administration and timed AI with GnRH at first AI (Group VI, n = 9) revealed 44% conception. Control anoestrus cows (Group VII, n = 5) without any treatment did not show estrus. The difference in rates of conception was significant ($p<0.05$) between the groups.

Table 1: Number (proportion) of silent estrus and true anoestrus by parity and BCS

Anoestrus by parity			Anoestrus by BCS		
Parity	Silent estrus	True anoestrus	BCS	Silent estrus	True anoestrus
1 (n = 25)	13 (52)	12 (48)	2.5 (n = 41)	18 (44)	23 (56)
2-3 (n = 34)	19 (56)	15 (44)	3.0 (n = 23)	14 (61)	9 (39)
4-5 (n = 20)	12 (60)	8 (40)	3.5 (n = 15)	12 (80)	3 (20)
Total (n = 79)	44 (53)	35 (42.2)	Total (n = 79)	44 (53)	35 (42.2)

Values in brackets are percentage

Table 2: Effects of treatments of anoestrus cows on cyclicity and conception

Type of anoestrus	Type of treatments	Number of cows treated	No. (%) inseminated	No. (%) conceived
Silent estrus	Monitor estrus carefully+AI on observed estrus	23	12 (52)	10 (43)
	$PGF_{2\alpha}$+AI on observed estrus without GnRH	11	8 (73)	5 (45)
	$PGF_{2\alpha}$+ timed AI+GnRH	10	10 (100)	6 (60)
True anoestrus	Nutrition supplement	11	9 (82)^{ab}	7 (64)^{b}
	Restricted suckling	10	5 (50)^{a}	4 (40)^{a}
	GnRH+$PGF_{2\alpha}$+timed AI+GnRH	9	9 (100)^{b}	4 (44)^{a}
	Control (received no advice/treatment)	5	0 (0)	0 (0)^{a,b}

Value in brackets are percentage, proportion values with superscripts within same column differ significantly ($p<0.05$)

DISCUSSION

Underlying disorders of anoestrus: The present investigation demonstrated that estrus in about half of the cows (53%) remain undetected as confirmed by ultrasonography. This finding is in agreement with previous study (Shamsuddin *et al*., 2001) where approximately 40% estrus remains undetected in Bangladesh. Another study (Opsomer *et al*., 2000) also highlighted the high incidence of anoestrus postpartum (62%) in high-yielding dairy cows suggesting a strong influence of heat detection. Other management problems leading to anoestrus in dairy herds are too few observations per day, observations at the wrong time of the day, too little time spent per observation and lack of knowledge of both primary and secondary signs of estrus (Van Eerdenburg *et al*., 1996; Roelofs *et al*., 2010). The silent estrus may occur due to poor expression of estrus which is difficult to identify by the farmers. A number of environmental conditions also restrict the behavioral manifestation; cows show less mounting activity that are crowded or housed on slippery alleys and any surface that makes footing tenuous (Leonard *et al*., 1994; Roelofs *et al*., 2010). A high proportion of cows (42%) were true anoestrus by 60 days postpartum in present study might be regarded as unusual. This is one of the major constraints for profitable smallholders' dairying in Bangladesh (Shamsuddin *et al*., 2001). The reason might be nutritional deficiency (Hossain *et al*., 2004) leading to inactive ovaries (Talukder *et al*., 2005) and abnormal progesterone profiles (Opsomer *et al*., 1998). Nutritionally induced postpartum anoestrus is characterized by turnover of dominant follicles incapable of producing sufficient estradiol to induce ovulation due to reduced LH pulse frequency (Roche, 2006). The proportions of the silent estrus and true anoestrus cases were comparable among different parities; but the true anoestrus cases were higher among the low body conditioned than high body conditioned cows. A negative relationship between parity and number of days from calving to first ovulation in dairy cows under similar body nutritional conditions has demonstrated (Tanaka *et al*., 2008). A poor BCS at calving adversely affects fertility characterized by prolonged postpartum intervals (Shamsuddin *et al*., 2006a).

Effects of treatments: It was observed that about half of the silent estrus cows can be detected in estrus by close observation and then most of them conceived if followed by AI without any hormonal treatments. Rate of estrus detection was somewhat comparable to previous report (42%) in dairy cows (Cartmill *et al*., 2001) but lower (20%) in crossbred cows (Ahuja *et al*., 2005) and higher (76%) in beef cattle (Kojima *et al*., 2000). The rate of estrus detection in the present study is unacceptable when attempting to get a large percentage of anestrous crossbred cows bred by AI after detected estrus. The reason for the different estrous response may be the cyclic status of cows at treatment, response to PGF2α, genotype of the cows and heat stress (Ahuja *et al*., 2005). Determination of estrus also appears to be important in conception success (Rae *et al*., 1999). A primary reason for low pregnancy rate in dairy cows after administration of PGF and TAI is inappropriate ovarian function prior to, or following treatment (Waldmann *et al*., 2006).

It was observed that cyclicity in high proportion (82%) of cows with true anoestrus can be induced using nutritional supplementation. The conception rate in these cows was also significantly higher than that of other means (restricted suckling and hormonal interventions) used in this study. The supplementations at postpartum have been reported to enhance ovarian rebound after calving and reduce the length of postpartum anoestrus in cows (McNamara *et al*., 2003). The restoration of normal ovarian activity takes place when the state of under-nutrition or negative

energy balance is reversed (Sabo *et al.*, 2008). Extremes of body condition (<1.5 or >4) will almost invariably reduce reproductive performance although changes in condition are more important than actual condition at any time point (Pryce *et al.*, 2000). In suckled cows it is reported that temporary calf removal reduces the interval from calving to first ovulation (Rhodes *et al.*, 2003). Isolation of calves from their mothers reduces the postpartum interval but such treatments results in reduced expression of estrus and shorter estrus cycles unless done in conjunction with progesterone treatment (Mackey *et al.*, 2000). GnRH may cause ovulation or no effect on follicle development, depending on the animal's stage of follicle development at treatment (Crowe, 2008). Thus, when GnRH is used as part of an ovsynch protocol (GnRH-PGF2a-GnRH treatment) in postpartum anoestrous cows, the effectiveness of the treatment is wholly dependent on the presence or absence of a dominant follicle at the time the first GnRH injection is administered (Crowe, 2008).

CONCLUSION

Close observation for silent estrus and nutritional supplementation for true anoestrus could be an effective tool for management of postpartum anoestrus in smallholder dairy farms. Instead of only advising the hormonal drugs, the practitioners has to look after the management factors such as estrus detection and the plane of nutrition at postpartum in order to prevent prolonged anoestrus. Good fertility in high yielding dairy herds can only be achieved when management is excellent.

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