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Optimization of Estrus Synchronization Protocol for Target Breeding to Decrease Voluntary Waiting Period in Lactating Cows

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ABSTRACT

Effective estrus detection and artificial insemination (AI) are necessary for profitable management of dairy herd. In current study, 45 crossbred lactating cows have been selected with the complaint of unobserved oestrus for more than sixty days postpartum. All cows had functional corpus luteum as examined by transrectal ultrasonography. Cows were treated with $PGF_2\alpha$ analogue and AI was performed with observed oestrus and then single dose of GnRH was administered. Similar synchronization protocol has been repeated after 14 days in cows that did not repose to first treatment. Remaining cows received additional $PGF_2\alpha$ after 14 days of second treatment and timed AI was performed following GnRH administration. Among 45 cows, 28.89% showed estrus after first treatment and 78.79% responded to second hormonal intervention. A higher conception rate (88.89% vs 26.66 and 72.72%) was observed in cows after triple administration of $PGF_2\alpha$ and timed AI. We noticed a significant differences in body condition score (BCS, 1～5 scale), postpartum period, and daily milk production between cows that either responded of non-responded following first and second hormonal treatment. In addition, there was a significant positive correlation between daily milk production and BCS, age and postpartum days, milk production and estrus/BCS, and milk production/BCS/estrus and conception rate. Depending upon the findings we conclude that hormonal intervention with $PGF_2\alpha$ and GnRH enhances postpartum ovarian cyclicity and help decreasing the days open of dairy herd. Therefore, this finding might provide an excellent guideline for target breeding system for profitable dairy herd management.

(Key words : Estrus synchronization, $PGF_2\alpha$, GnRH, Postpartum anoestrus, Ultrasonography, Voluntary waiting period)

INTRODUCTION

Dairy farming plays a pivotal role in the village micro economy of Bangladesh. The major factor limiting reproductive performance in many dairy herds is the failure to detect estrus in a timely and accurate manner (Rahman *et al*., 2012a; Lee *et al*., 2013). Efficient and accurate estrus detection is essential to optimize the economic management of individual cows for profitable dairy operation (Rahman *et al*., 2012b). Increases herd size, lower milk yield and average days open have been reported as primary contributors of the reproductive efficiency that has been experienced by many dairy herds, as reproductive performance appears to be directly related to herd management (Britt, 1985). Therefore, for a profitable dairy farming, more attention and skill need to be devoted to increasing reproductive efficiency.

To maximize lifetime milk yield, the goal for most dairy operations is to generate the highest possible number of offspring in the shortest interval without compromising the well being of the dam and/or neonate. Therefore, establishment of a voluntary waiting period (VWP) and accurate detection of estrus leads to a decreased calving interval (Lucy *et al*., 1986). Inter calving interval should not exceed 365 days (Opsomer *et al*., 2000; Shamsuddin *et al*., 2006) to achieve satisfactory

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economic benefit from a dairy industry. Increased calving intervals results from reproductive inefficiency, leading to a decrease in profits and calving interval should be between 12 and 13 months for optimal milk production and profit (Allalout, 1979; Dailey *et al*., 1986). Prolong interval (>85 days) between calving and onset of ovarian function is regarded as one of the most important gynecological problems responsible for failure to maintain optimum reproductive efficiency, which in turn, causes economic loss to the owner of the dairy farmers (Shamsuddin *et al*., 2006). In Bangladesh, the main constraints of cattle reproduction are prolonged postpartum anestrus resulting from inefficient nutritional management and poor oestrus detection (Shamsuddin, 1995) extending calving interval. Increased rates of estrus detection and proper timing of insemination increase pregnancy rate within predetermined time limits that leading to shorter calving intervals. Lucy *et al*. (1986) suggested that methods of estrus synchronization which optimize first service conception rate and reduce variability in days to first service may reduce the variability and length of calving intervals for all cows in a dairy herd.

Detection of estrus is often very difficult due to short periods of standing estrus, silent estrus, changing nutritional regimens, environmental temperatures, and estrus onset during the late night to early morning hours. In Bangladesh 40% cows remain undetected when they were in estrus (Shamsuddin *et al*., 2006). Synchronization protocol is a proactive response for optimizing reproductive management in the dairy herd. Therefore, the present study was designed to investigate the effect of selective estrus synchronization protocol for lactating dairy cows with more than 60 days postpartum period. Simultaneously we investigated the correspondence relation between body condition score (BCS), age, daily milk production, and postpartum period with hormonal treatment for oestrous synchronization. In addition, we also demonstrated the importance of using secondary heat symptoms of cows to improve heat detection.

MATERIALS AND METHODS

The present study was conducted in the registered dairy farms of the Community-based Dairy Veterinary Foundation (CDVF) located in the Sathkhira district of Bangladesh during March 2011 to May 2012.

Animal Selection and Management

We selected 45 crossbred (Friesian X nondescript indigenous zebu) lactating cows from a total of 70 cows with the complaint of unobserved oestrus for more than sixty days postpartum period from 30 dairy farm families. These selected cows had corpus luteum (CL) in there ovaries as confirmed by transrectal ultrasonography. All cows calved normally and had no history of peripaturierit diseases or disorders. Routine deworming against round worms and liver flukes were practiced and the cows were vaccinated routinely against foot and mouth disease, anthrax, and hemorrhagic septicemia. The cows were housed almost 24 hours in their rearing sheds with the proper facilities of natural ventilation and were fed concentrate and straw. Total feeds and forages were given in two splits every day. All cows were milked by hand twice daily at an interval of 9 hours and were suckled approximately for 4 hours daily. Date regarding age of cows and daily milk yield per cow were recorded by interviewing the farmers and /or by taking records from the farm register. The nutritional status of the cows were determined by scoring the body condition score (BCS) of the cows using 1～5 scales (0.5 fraction between 2 scores) on the basis of bony prominence and deposition of subcutaneous fat as described by Rahman *et al*. (2012a). The cows with <2.0 body condition score (BCS) were not selected for this study.

Examination of Genital Organs of Cows by Ultrasonography

To determine the ovarian cyclicity, all cows were examined transrectally by ultrasonography. B-mode real-time ultrasonography (Tringa Linear VET®, Esaote Pie Medical, Genova, Italy) was used transrectally to examine ovaries. The detail procedure of using B-mode real-time ultrasonography has been described in Rahman *et al*. (2012a). Briefly, 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. All fecal materials from the rectum were evacuated prior to introduction of the transducer. The probe face was lubricated with a suitable coupling medium (Ultrsonic Gel® for Medical use-250 mg) and was usually covered by a lubricated plastic sleeve before insertion. The probe was then progressed cranially along the rectal floor to overlie the reproductive tract. The ultrasound screen and the human eye were at similar level for accurate interpretation of ultrasound images.

Treatment Protocol and Artificial Insemination (AI)

Cows with the presence of CL in ovaries with normal uterus were regarded as silent oestrus cows that were undetected by the farmers. The schematic presentation of estrus synchronization and AI is resented

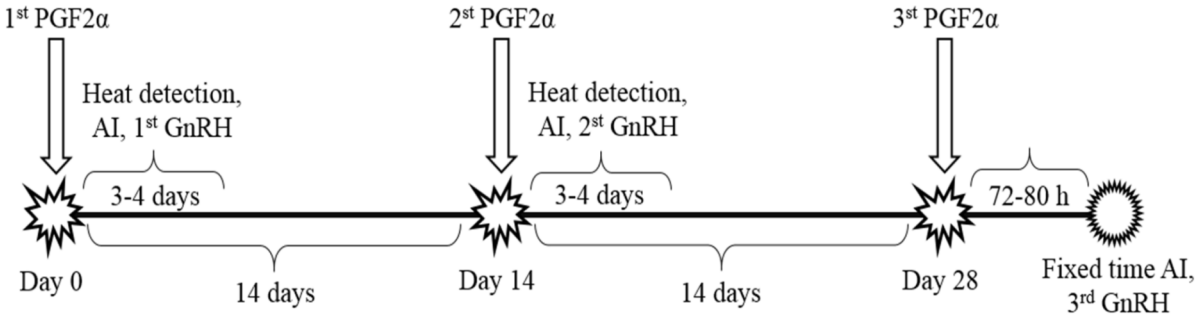

Fig. 1. Experimental design and schedule for estrus synchronization and artificial insemination of cows.

in Fig. 1. The selected cows were treated with $PGF_2\alpha$ analogue (Alfaprostol, Gabbrostim®, Porto Empedocle, Italy). The day of first $PGF_2\alpha$ injection was regarded as Day 0 followed by AI in observed oestrus after the single injection of GnRH (Gonadorelin, Fertagyl®, Intervet International BV, European Union). The cows that did not show estrous signs were treated with same manner that initiated at Day 14. Repeatedly at day 28, cows failed to response to second treatment, were treated with $PGF_2\alpha$ analogue followed by timed artificial insemination (TAI) within 70～80 hours after a single injection of GnRH analogue. All selected cows reared with balanced diet containing maize (50%), wheat bran (30%), oil cake (10%), rice polish (8%) and Vitamin-Mineral premixes (2%).

Pregnancy Diagnosis

The cows were examined transrectally by using B-mode ultrasonography for pregnancy diagnosis between 80～90 days after AI and results were recorded.

Statistical Analysis

All data were normally distributed and variances were homogenous. Numerical data were presented as mean±SEM. The data were analyzed using ANOVA to identify differences among treatments, SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Differences among treatments were examined using the multiple range test. Pearson correlation coefficients were calculated to determine the associations between several parameters in response to the hormonal interventions. $p<0.05$ was considered statistically significant.

RESULTS

Effect of Different Treatments on Postpartum Anoestrus Cows

Several information, such as average age, BCS, postpartum period, daily milk production, percentage of estrus, and conception rate according to three different treatment protocols were presented in Table 1. We did not observed any difference among three treatment groups in term of age and postpartum period. However, significant differences in BCS, and milk production were noted. The percentage of estrus and conception rate were 28.89 and 26.66%, respectively following single injection of $PGF_2\alpha$ and GnRH. Interestingly, following second treatment (double $PGF_2\alpha$ and Gn-

Table 1. Average age, BCS, postpartum period, daily milk production, percentage of estrus, and conception rate according to the three treatment protocols

Groups	Treatments	Age (years)	BCS (1～5 scale)	Postpartum period (days)	Milk production (kg/day)	Estrus cows (%)	Conception rate (%)
1 (n=45)	D0	4.05±0.18	3.02±0.07^a	130.26±6.40	11.02±0.6^a	28.89^a(13/45)	26.66^a(12/45)
2 (n=33)	D0+D14	4.04±0.22	2.82±0.07^b	134.31±6.91	8.90±0.39^b	78.79^b(26/33)	72.72^b(24/33)
3 (n=9)	D0+D14+D28	4.05±0.49	2.16±0.08^c	143.55±14.62	7.22±0.81^c	-	88.89^c(8/9)

n; number of cows, BCS; body condition score, D0; first injection of $PGF_2\alpha$, D0+D14; first plus second injection of $PGF_2\alpha$, D0+D14+D28; first plus second plus third injection of $PGF_2\alpha$. Data were analyzed using ANOVA to identify differences among treatments, using Tukey's test. Values with different superscripts ($^{a\sim c}$) characters indicate statistically significant ($p<0.05$) differences among groups.

RH), number of cows respond was increased in term of estrus behaviors (78.79%) and conception rate (72.72 %). Simultaneously we noticed a significantly higher conception rate (88.89%) in cows following administration of triple doses of $PGF_2\alpha$ and GnRH with TAI (Table 1). It is important to note that the number of cows showed oestrus was not recorded in treatment group 3 as TAI was performed within 70～80 h.

Difference between Different Parameters of Cows Responded to Treatments

We analyzed data regarding age, BCS, postpartum period, and daily milk production in cows responded to either first or second hormonal interventions. A significant difference (p<0.05) was noticed for the BCS, days postpartum period, and milk production while compare between responded and non-responded groups after 1st injection of $PGF_2\alpha$ and GnRH (Table 2). A similar difference (p<0.05) was also noticed when we analyzed the same parameters after 2nd injection of $PGF_2\alpha$ and GnRH (Table 3). Interestingly, we did not notice any difference of cow's age between responded and non-responded groups following both treatment interventions (Table 2 and 3).

Correlation among Different Parameters considered in This Study

During the hormonal treatment we noticed a strong positive correlation among milk production, BCS, estrus and conception rate that was statistically significant (p <0.05) (Table 4). Simultaneously postpartum period showed a negative correlation with BCS and milk production although there relation was insignificant (p< 0.05). In addition, there was significant (p<0.05) positive relation between age and postpartum period.

DISCUSSIONS

It has been found that nutritional status significantly effects postpartum reproductive activity of animal. The reproductive efficiency of cows during VWP is largely dependent on proper energy balance (Tufarelli *et al*., 1996). The VWP allowed involution of the cervix and uterus after calving. The reproductive tract typically returns to normal size by 35 days after calving, while the uterus takes approximately 45 days to return to normal. The VWP may be intentionally extended in an attempt to alleviate the occurrence of lower conception rates in high producing early postpartum cows. The evaluation of ovarian follicular growth by transrectal ultrasonography coupled with measurement of the levels of reproductive hormones has allowed to study three functionally critical follicular diameters during follicular growth; such as emergence (4 mm), deviation (9 mm), and ovulation (10～20 mm). Classification of anovulatory follicle on the basis of these three critical points is logical that aids in rational diagnosis, and assist the treatment of the underlying physiologic condition (Wiltbank *et al*., 2002).

In current study, together with number of cows showed estrus and conception following hormonal interventions, we also studied different parameters such as

Table 2. Differences of age, BCS, postpartum period, and milk production compare with responded and non-responded groups after 1st injection of $PGF_2\alpha$ and GnRH

Groups / no. of animals	Age (years)	BCS (1～5 scale)	Postpartum period (days)	Milk production (kg)
Respond (n=13)	4.07±0.29	3.50±0.00[a]	120.30±14.32[a]	16.23±0.76[a]
Non-respond (n=32)	4.04±0.22	2.82±0.07[b]	134.31±6.91[b]	8.90±0.39[b]

n; number of animal. The data were analyzed using ANOVA to identify differences among treatments. Values with different superscripts ([a,b]) indicate statistically significant (p>0.05) differences among groups.

Table 3. Differences of age, BCS, postpartum period, and milk production compare with responded and non-responded groups after 2nd injection of $PGF_2\alpha$ and GnRH

Groups / no. of animals	Age (years)	BCS (1～5 scale)	Postpartum period (days)	Milk production (kg)
Respond (n=23)	4.04±0.26	3.08±0.02[a]	130.69±7.8[a]	9.56±0.38[a]
No respond (n=9)	4.05±0.49	2.16±0.08[b]	143.55±14.62[b]	7.22±0.81[b]

n; number of animal. The data were analyzed using ANOVA to identify differences among treatments. Values with different superscripts ([a,b]) indicate statistically significant (p>0.05) differences among groups.

Table 4. Correlation among the values of different parameters following different hormonal interventions in postpartum cows

Parameters		BCS	Postpartum period (days)	Age (years)	Estrus (%)	Conception (%)
Milk production	*r* value	0.721	−0.092	0.033	0.347	0.380
	p value	0.000	0.410	0.763	0.001	0.000
BCS	*r* value		−0.150	0.012	0.330	0.302
	p value		0.169	0.910	0.002	0.005
Postpartum period (days)	*r* value			0.366	−0.074	0.124
	p value			0.001	0.500	0.254
Age (years)	*r* value				0.003	0.129
	p value				0.980	0.235
Estrus	*r* value					0.925
	p value					0.000

Pearson correlation coefficients (*r*) were calculated to determine the associations between several parameters in response to the hormonal interventions.
p-value indicate the probability of correlation. $p<0.05$ was considered statistically significant correlation.

age, BCS, postpartum period, and daily milk production. Because all of these factors are important that could be associated with the occurrence of anestrous (Ferguson, 1996). Our study showed that cows having BCS >2.5 triggers better postpartum ovarian activity than those having BCS 1.0～2.5 (Table 2 and 3). We also observed increased postpartum period in cows having low BCS (Table 1). Consistent with current findings a significant negative correlation was also observed between weight gain and days postpartum period by other investigator (Ghosh, 1990). Shamsuddin *et al*. (2006) have reported that change in BCS after calving, rather than BCS at calving has a significant effect on the percentage of dairy cows exhibiting a prolonged VWP.

We also noticed that cows with >7.00 kg milk yield per day triggers their postpartum ovarian cyclicity earlier than those produce <5.00 kg (Table 2 and 3). Supporting to the current findings Taylor *et al*. (2004) also reported a negative correlation between milk yield and return to cyclicity in multiparous cows. In contrast, Harrison *et al*. (1990) reported that high milk production is antagonistic to the expression of oestrus behavior of cows. We did not consider parity in the current study. High milk production is not a major factor in delaying conception, except in the first parity cows (Grohn *et al*., 2000). Gröhn and Rajala-Schultz (2000) have been reported that calving to first ovulation interval is significantly longer in cows producing more milk and is associated with higher circulating concentrations of growth hormone and Q-hydroxy butyrate and lower concentrations of insulin and glucose. Moreover, repeat breeding is more prevalent in crossbred than pure breed cows in Bangladesh (Hossain, 2002). Therefore, the possible explanation is that the burning of fatty acids due to negative energy balance under-fed or high production animals causes a release of progesterone from in fat tissues, which in turn inhibits follicular growth and signs of oestrus (Schopper *et al*., 1993).

Another novel finding of the current study is hormonal treatment of postpartum anestrus cows increase the conception rate gradually (26.66, 72.72, and 88.89%). Fricke (2005) reported that hormonal interventions increase pregnancy rate by increasing the number of cows at service not by increasing conception rate. Hormonal interventions aim to induce ovulation and estrus by stimulating the maturation of ovarian follicles by inducing LH surge (Rhodes *et al*., 2003). All cows used in this study did not response to same hormonal treatment at a time. Therefore, the success of estrus induction with $PGF_2\alpha$ depends upon the presence of a functional CL during the diestrus stage of the estrous cycle. These cows have normal oestrus, ovulation, and formation of a CL, with prolonged luteal function due to a lack of luteal regression. A contributing factor may be the lack of an estrogenic dominant follicle at the expected time of luteal regression. In that regard, estradiol from a dominant follicle is believed to induce the formation of uterine oxytocin receptors, leading to pulsatile release of PGF (Mwaanga *et al*., 2000). Many factors have been suggested to increase the risk of a prolonged luteal phase, including parity, dystocia, and

health problems during the first month of lactation, heat stress, and perhaps ovulation soon after calving. Uterine infection or pyometra can prolong the life of the CL and activity in the early postpartum period (Sangsritavong *et al*., 2002). The reduction in LH pulse frequency may be the result of increases in the negative feedback effect of estradiol on LH pulse frequency. This can occur due to increased availability of estradiol receptors in the hypothalamus or increased sensitivity of hypothalamus to the negative feedback effect of estradiol, as well as other factors (Ambrose *et al*., 2007). In addition, there may be suppression of GnRH pulses, and it can be hypothesized that there is decreased GnRH neuronal activity (similar to prepuberal anoestrus).

In conclusion, good nutrition management in both pre- and postpartum periods, efficient and accurate heat detection, prevention and treatment of reproductive and metabolic diseases should be considered very seriously for better reproduction efficiencies. Hormonal treatments under various protocols are capable to improve the reproductive performances and thus the reproductive goals can be achieved. On the other hand, the success of estrus induction with $PGF_2\alpha$ depends upon the presence of a functional CL in ovaries during estrous cycle. When $PGF_2\alpha$ is used for synchronization of estrous in lactating cows most of cows respond to after second hormonal treatment. However, there are significant differences in parameters in terms of BCS, postpartum period and daily milk production among cows, responded and non-responded to hormonal treatment. Since a strong positive correlation exists among milk production, BCS, estrus as well as conception rate in lactating cows. Therefore, age, BCS, daily milk production and postpartum period must be considered seriously for better selection of hormonal treatment in target breeding program.

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