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Research Article

Uprising the effective estrus synchronization protocols with hormones in anestrus dairy and beef cows guided by rectal palpation

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Abstract

The purpose of the study was to evaluate effective estrus synchronization protocols with hormones in anestrus cows guided by rectal palpation. About 200 dairy and beef cattle was selected at private and Govt. dairy farm of Rajshahi region from 1st July 2020 30th June, 2021. For this experiment animals were divided into 7 groups of different protocol and one control. Protocol-I, II and III was singly double and triple PGF2α injection Technique to inject $PGF_{2\alpha}$ was @ 5ml Intramuscular at day 0, 11-14 and 9 & 11-14 days respectively 3 protocol and inseminated heated. Protocol -IV: GnRH–$PGF_{2\alpha}$–GnRH Technique to inject GnRH @ 5ml Intramuscular at day 0 and Check estrous and bred them. The second injection of $PGF_{2\alpha}$ was administered 7 days after first injection similarly estrus and AI. The third injection of GnRH was administered 11-14 days after second injection similarly estrus and AI. Protocol -V: $PGF_{2\alpha}$–GnRH– $PGF_{2\alpha}$ Technique to inject $PGF_{2\alpha}$ @ 5ml Intramuscular at day 0 and the second injection of GnRH was administered 12 days after first injection. The third injection of $PGF_{2\alpha}$ was administered 7 days after second injection similarly estrus and AI after 12-18 hours of fix times with GnRH injection. Protocol VI: GnRH– $PGF_{2\alpha}$ Technique, to inject GnRH @ 5ml Intramuscular at day 0 and Check estrous and bred them. Second injection of $PGF_{2\alpha}$ was administered 7 days after first injection similarly estrus and AI. Protocol -VII: hCG technique to inject hCG @ 1500 i.u. Intramuscularly at 0 day and observed up to 1 month. Protocol VIII: Control group (No treatment) (n=20), animal was observed for one month's how many cows have shown estrus and check it after 120 day by rectal palpation. In results It showed that when hormonal treatment was given for induction of oestrus, higher (77.14%) proportion of cows showed oestrus compared to control counterpart (20.00%). The difference in the induction of oestrus in cows with different treatment protocol groups were 60%, 85%, 80%, 90% and 60% in PG, PG-PG, PG-PG-PG with fixed AI, PG-GnRH-PG with Fixed AI, GnRh-PG and hcG, respectively. It shows that the higher proportion of cows showed oestrus with hormonal treatment. Pregnancy rate of induction of estrous cycle by using different synchronization protocols were observed highest in 88.89% at PG-GnRH-PG fixed time AI technique and lowest 81.25% at GnRH-PG-GnRH fixed time AI protocol. Based on the experimental results. different treatment protocols compare in terms of their effectiveness in inducing estrus

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Introduction

Reproduction is one of the main orders of livestock development. In cattle breeding, one of the most common methods of reproduction management is estrus synchronization. It allows obtaining seasonal calving at the desired period of the year (Haile-Mariam et al., 2023). Nevertheless, the effectiveness of the synchronization largely depends on the properly selected and applied protocols. Therefore, when applying innovative scientific ideas, additional adjustments to cow reproductive system stimulation methods are relevant (Fricke & Wiltbank, 2022). The efficiency of estrus synchronization is influenced by many factors, including the physiological state of cows, lactation, body condition scoring, cyclicity, and the stage of the estrous cycle (Islam et al., 2004; Kadir et al., 2003; Khan et al., 2004; Mazed et al., 2004; Musal et al., 2016). Estrus synchronization (ES) and artificial insemination (AI) continue to be a major influential technology for cattle producers in terms of genetic improvement, reproductive management, and animal reproductive and productive performance (Jinks et al., 2013). ES entails manipulating or controlling females' estrus cycles in order to breed them at roughly the same time (Cushman et al., 2007). ES and its various protocols are useful tools in a cow herd's reproductive management, but they require optimal diet, good body condition, and health, as well as experienced manpower. Inadequacy in any of these areas, on the other hand, can spell disaster for these technologies (Lamb, 2012; Gizaw et al., 2016). Consequently, all synchronization protocol techniques necessitate proper management, regular estrous cycles, and cows in good physical condition, as well as attention to detail and adequate feed (Baset at al., 2003; Mustafa et al., 2022; Rick, 2013). The main causes of failure of the technology are poor nutrition, lack of effective estrus diagnosis, artificial inseminators and breeding control problems, illness, calf suckling, then quiet heat (Stevenson, 2008 & Chaudhari et al., 2012). In the tropics, majority cows, plus hybrids, unsuccessful to calve every 12–13 months afterward the initial calving, much as they do on

smallholder farms (Mekonnin, 2015). The efficiency of the treatment depends on the status of the ovary at the time of administration and the stage of anestrus. Thus, it is apparent that GnRH influences the secretion of pituitary hormones that initiate postpartum development, maturation and eventual ovulation of matured follicles (McLeod and Phillips, 1998 and Ginther et al., 1999). The GnRH and PGF2α based strategies have been demonstrated to be effective in synchronizing estrus in cattle and buffaloes (Lamb et al., 2001 and 2004; Odde, 1990; Amaya et al., 2007). In conclusion, the effectiveness of synchronization protocols involving combinations of PG and GnRH with fixed-time AI shows better results in achieving high estrus and pregnancy rates in cows.

Materials and Methods

The present study was conducted at Department of Veterinary and Animal Sciences, University of Rajshahi from 1st July 2020 30th June, 2021. The animal was selected at private and Govt. dairy farm of Rajshahi region.

Data collection

A format was developed for proper recording the anestrus dairy cows. The prevalence of anestrus was recorded at study area by observing and interviewing. After confirmation of anestrus then used the hormonal drugs against the anestrus dairy and beef cows and recorded total no. of cows was heat shows and also recorded the pregnancy rate after using hormonal drugs. The selected cattle also divided into various factors like factors were identified. The factors was classified according to their breed, age, body condition score, stage of post-partum, overall management, housing, feeding status, health care etc. Before using hormones, all the selected cows have been given broad spectrum anthelmintic and vitamin as per recommended dose. The feeding and housing system kept seminally. Some cows may be in heat at the starting of protocol that cows were excluded from the synchronization techniques.

Estrus synchronization Procedures

Eight protocols were applied on the anestrus cows. The following protocols or treatment given below:

Protocol-I: PGF2α Technique (n=20), $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health or Inj. Repromate, ACI Animal health) was administered @ 5ml Intramuscular at day 0 then wait for 7 days and inseminated only the animal showed heat. Protocol -II: PGF2α– $PGF_{2\alpha}$ Technique (n=20), $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health) was administered at day 0 and Check estrous after first dose of treatment and breed them. Second same injection administered 11-14 days after first injection. Check estrous after second dose of treatment and bred them after 12 hours of standing estrous. Protocol -III: $PGF_{2\alpha}$– $PGF_{2\alpha}$– $PGF_{2\alpha}$ Technique (n=20), $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health or Inj. Repromate, ACI Animal health) was administered @ 5ml Intramuscular at day 0 and Check estrous after first dose of treatment and breed them. The second injection of $PGF_{2\alpha}$ was administered 9 days after first injection. The third injection of $PGF_{2\alpha}$ was administered 11-14 days after second injection. Check estrous after third dose of treatment and bred them after 72-96 hours of 2 times AI. Protocol -IV: GnRH–$PGF_{2\alpha}$–GnRH Technique (n=20), GnRH (Inj. Ovurelin 5 ml Renata Animal Health or Inj. Fetazyl 5 ml im Intervet limited) was administered @ 5ml Intramuscular at day 0 and Check estrous after first dose of treatment and bred them. The second injection of $PGF_{2\alpha}$ was administered 7 days after first injection. The third injection of GnRH was administered 11-14 days after second injection. Check estrous after third dose of treatment and bred them after 16-18 hours of fix times AI. Protocol -V: $PGF_{2\alpha}$–GnRH– $PGF_{2\alpha}$ Technique (n=20), $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health) was administered @ 5ml Intramuscular at day 0 and the second injection of GnRH (Inj. Ovurelin 5 ml Renata Animal Health or Inj. Fetazyl 5 ml im Intervet limited) was administered 12 days after first injection. The third injection of $PGF_{2\alpha}$ was administered 7 days after second injection. Check estrous after third dose of treatment and bred them after 12-18 hours of fix times AI with GnRH injection. Protocol VI: GnRH– $PGF_{2\alpha}$ Technique, (n=20), GnRH (Inj. Ovurelin 5ml Renata Animal Health or Inj. Fetazyl 5 ml im Intervet) was administered@ 5ml Intramuscular at day 0 and Check estrous after first dose of treatment and bred them. Second injection of $PGF_{2\alpha}$ was administered 7 days after first injection. Check estrous after second dose of treatment and bred them after 12 hours of standing estrous. Protocol -VII: hCG technique, (n=20), hCG (Inj. Chorulon 1500-3000 iu im, Intervet limited) was administered @ 1500 i.u. Intramuscularly at 0 day and observed up to 1 month. Protocol VIII: Control group (No treatment) (n=20), animal was observed for one month's how many cows have shown estrus.

Estrus detection procedure

All the animals were monitored for the manifestation of oestrus symptoms three times daily i.e., at 6 am, 2.00 pm and 10.00 pm for six days after each injection. The cows was also monitored both for behavioral symptoms. The important symptoms of heated cows are below:

Symptoms of heat in cows

Symptoms of early stages of heat are not pronounced in cattle but they show activates like smelling other cows, attempting to mount on them and bellowing. They become restless and their vulva gets moist, red and slight swollen. After a lapse of six to eight hours the heat hours the heat becomes more pronounced the cows stands still to be mounted by other cows or bulls. Due to this period is termed as standing heat. This extends to 12-18 hours and shows other symptoms like bellowing nervousness, anorexia, reduction on milk yield, moist and red vulva and clear mucus discharge. The time from the injection of prostaglandin to onset of oestrus was recorded in hours. Duration of estrus was taken in hours from the time of first receptivity (standing firm) of a cow for other animals (time of onset of estrus) to the time of refusal to other animals (end of oestrus).

Artificial insemination

The animals under the group were observed by the farmers closely. When the cows have been shown the signs of oestrus and the farmer informed the AI technicians and AI inseminator inseminate accordingly. Animal find oestrus in the morning was inseminated in the evening and vice versa. Prior to insemination, reproductive health of the cows was examined by rectal palpation of genital tract. Animals showing any abnormalities were recorded. All inseminations were performed by three technicians. Application of first injection of prostaglandin with good condition and presence of CL expect a successful fertile estrus. In unsuccessful estrus induction after first injection of PG then another PG after 10 days was done and cows were

inseminated at 72 hours and 96 hours irrespective of heats signs. Conformation of pregnancy by rectal palpation at 90 and 120 day after last AI.

Results and Discussion

The present study were taken about 160 dairy cow were selected according to their full history like age, milk production, breed, body condition, parity etc from private dairy farms at Rajshahi, Bangladesh. The prevalence of anestrus in cows with respect to different areas is presented in 1 and 2. The Table presents the results of different treatment protocols aimed at inducing estrus (heat) in a group of animals. The protocols include various combinations of prostaglandin (PG), gonadotropin-releasing hormone (GnRH), human chorionic gonadotropin (hcG), and fixed-time artificial insemination (AI). The key metrics compared are the number and percentage of animals in estrus and the number and percentage of negative outcomes (animals not in estrus).
Highest Estrus Induction: The PG-GnRH-PG and Fixed Time AI group had the highest percentage of animals in estrus at 90.0%, followed by the PG-PG and PG-PG-PG and Fixed Time AI groups, both at 85.0%. Lowest Estrus Induction: The control group had the lowest percentage of animals in estrus at 20.0%, showing the effectiveness of the treatment protocols compared to no treatment. Negative Outcomes: The control group also had the highest percentage of negative outcomes at 80.0%, while the PG-GnRH-PG and Fixed Time AI group had the lowest at 10.0%.

Intermediate Results: Other groups, such as GnRH-PG-GnRH and Fixed Time AI, and GnRH-PG, showed intermediate levels of success with 80.0% of animals in estrus. Effectiveness of Protocols: The protocols involving a combination of PG and GnRH, especially with fixed-time AI, tend to be more effective in inducing estrus compared to single hormone treatments or no treatment at all. Comparing these results with those reported by Lamb et al. (2004) & Stevenson et al. (2000). Our study shows similar trends in treatment effectiveness for estrus induction with PG but contrasts in outcomes with hcG treatment. Kojima et al. (2000) drawback of the Select Synch protocol is that between 5 and 20% of the female's exhibit estrus before or immediately after the injection of PGF 2α. This suggests potential variability in treatment efficacy across different studies, possibly influenced by variations in experimental protocols or subject populations.

Table 1. Cross table of the Analysis treatment protocol with its results

Result	Treatment protocol group								Total
	PG	PG-PG	PG-PG-PG and fixed time AI	GnRH-PG-GnRH fixed time AI	PG-GnRH-PG fixed time AI	GnRH-PG	hcG	Control	
Number of Estrus	12	17	17	16	18	16	12	4	112
Percentage Number of Estrus	60.0%	85%	85.0%	80.0%	90.0%	80.0%	60.0%	20.0%	70.0%
Number of Negative	8	3	3	4	2	4	8	16	48
Percentage Number of Negative	40.0%	15.0%	15.0%	20.0%	10.0%	20.0%	40.0%	80.0%	30.0%
Total	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

The Table provides data on the effectiveness of various synchronization protocols in inducing estrus and achieving pregnancy in cows. The metrics include the number of cows showing estrus after hormone treatment, the number of cows that became pregnant, the percentage of cows that became pregnant, and for some protocols, the percentage of cows that conceived via fixed-time artificial insemination (AI).

Highest Pregnancy Rates: The control group showed a 100% pregnancy rate, but this is based on a very small number of cows showing estrus (4 out of 20). Among the treatment groups, PG-GnRH-PG and fixed time AI had the highest pregnancy rate at 88.89%, followed closely by PG-PG (88.23%) and GnRH-PG (87.50%). Estrus Induction: The PG-GnRH-PG and fixed time AI protocol had the highest number of cows showing estrus (18 out of 20), indicating its effectiveness in estrus induction. Fixed-time AI Conception: The GnRH-PG-GnRH and fixed time AI protocol had the highest conception rate via fixed-time AI at 50.00% (2 out of 4), whereas PG-GnRH-PG and fixed time AI had no cows conceived through fixed-time AI.

Overall Effectiveness: While the control group had a high pregnancy rate, the small number of cows showing estrus makes it less comparable. Among the hormone treatments, PG-GnRH-PG and fixed time AI, and PG-PG showed high effectiveness in inducing estrus and achieving pregnancy. Average Pregnancy Rate: The grand total across all treatment groups shows an average pregnancy rate of 85.19%, with a 33.33% conception rate through fixed-time AI. Purohit et al. (2019) obtained results of estrus rates ranging from 41.6 to 91.9% and conception rates from 11.11 to 68.8%, the GnRH-based protocols have been applied to buffaloes in several different countries. Successful economy of a dairy farm production lies in ensuing proper and optimal reproductive rhythm of individual cow within the normal physiological range. Anoestrus not only does lengthen the postpartum interval, but also substantially reduce the farmer's financial returns from milk or beef sales. Seeing this perspective the study was aimed to evaluate the effectiveness of synchronizing protocol for the achieving to come in heat of anoestrus animals. Effects of hormones on anoestrus cows are shown in Table 1 & 2 and Figure 1 and 2. It showed that when hormonal treatment was given for induction of oestrus, higher (88.89%) proportion of cows showed oestrus compared to control counterpart (20.00%). The difference in the induction of oestrus in cows with different treatment protocol groups were 83.34%, 88.23%, 83.34%, 81.25%, 88.89%, 87.50% and 83.33% in PG, PG-PG, PG-PG-PG with fixed AI, GnRh-PG GnRh, PG-GnRH-PG with Fixed AI, GnRh-PG and hcG, respectively. It shows that the higher proportion of cows showed oestrus with hormonal treatment. Similar result was observed by Islam et al. (2013). Previous studies showed that the ovulation in response to GnRH treatment in postpartumin cattle it was 85% (Wiltbank et al., 1997). In the present study interval after GnRH to ovulation was 48 h while, 30-32 h has been reported in cattle (Pursley et al., 1995).

Table 2. Pregnancy rate of induction of estrous cycle by using different synchronization protocols (each group consisting 20 anestrus cows)

Synchronization protocols	No. of cows showed estrus after used of hormones	No. of cows pregnant of estrus cow	% of cow Pregnant	Fixed time AI and % of conceived
PG n=20	12	10	83.34%	-
PG-PG n=20	17	15	88.23%	-
PG-PG-PG and fixed time AI n=20	17	14	83.34%	3* (1)
GnRH-PG-GnRH fixed time AI n=20	16	13	81.25%	4* (2)
PG-GnRH-PG fixed time AI n=20	18	16	88.89%	2*(0)
GnRH-PG n=20	16	14	87.50%	-
hcG n=20	12	10	83.33%	
Grand Total	108	92	85.19%	33.33%
Control n=20	4	4	100%	20.00%

N.B. *mark indicated fixed time AI and parenthesis indicated no. pregnant

Conclusions

These results suggest that combination protocols, particularly those involving multiple doses of PG and GnRH with fixed-time AI, are most effective in inducing estrus, while single hormone treatments and no treatment are less effective. These results highlight the effectiveness of synchronization protocols involving combinations of PG and GnRH, particularly when combined with fixed-time AI, in achieving high estrus and pregnancy rates in cows.

Conflict of interest

There are no conflicts of interest among the authors.

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