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Effect of Age and Different Doses of GnRH and PGF2α Analogue on Oesturs Synchronization and Conception Rate of Crossbreed Heifers

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Abstract: Forty-eight crossbreed heifers were artificially inseminated within 10-20 hours after standing heat to evaluate the effects of different doses of hormones and age on reproductive performance synchronized with GnRH and PGF2α. On day 90 after artificial insemination, the conception rate was confirmed by rectal palpation. There were significant differences (P<0.05) in oesturs response in maximum doses (1.5 ml GnRH used in group III) of the hormone-treated group. The conception rate was significantly higher (P<0.05) in the highest treatment group (1.5 ml, 66.67%) than in low treatment groups (1 ml and 1.3 ml; 42.85% and 44.44%; respectively) in morning synchronization protocol (83.33%). The effect of different doses of the hormone on conception rates was statistical significance on the age of crossbreed heifers (P<0.05), time of initiation of ovsynch (P<0.05), time of insemination (P<0.05), semen placement (P<0.05), body condition score (P<0.05), retrieve to natural oesturs and subsequent pregnancy rate (P<0.05). On the other hand, there was no significant (P>0.05) effect on conception rate by the onset of oesturs and duration of oesturs. The highest oesturs rate and conception rate was observed at 75.00% and 66.67%; respectively by administration of maximum doses of the hormone of body condition score (3) 69.23% of crossbreed heifer. When 1.5 ml GnRH was used in group III, the conception rate was observed higher at 25-30 months of age (77.78%) which were inseminated in the site of the deep cervix (77.77%) with frozen-thaw semen by experience inseminator at 10-14 hours (75%) after the onset of oesturs and naturally retrieve of oesturs on conception rate (75%). It was concluded that the age of the heifers, doses of the hormone, Body Condition Score (BCS), time of initiation of synchronization protocol, time of AI, semen placement and retrieve to oesturs naturally were the most important factors to get maximum oesturs response and conception rate of crossbreed heifers. So, it may be suggested that to achieve the desired rate of oesturs and conception, the farmers should inseminate their crossbreed heifers (BCS, 3 out of 5) with maximum doses of hormone in the deep cervix by technically sound and skilled inseminator at 10-14 hours after onset of oesturs in morning synchronization protocol. GnRH and PGF2α analogs

could successfully induce oesturs response and improve the fertility of a dairy herd. Therefore, ovsynch protocol for crossbreed heifers is needed for further work involving a large number of animals for a definite conclusion.

Keywords: Crossbred, Hormones, Oesturs Synchronization, AI & Conception Rate

1. Introduction

Bangladesh is a low-lying densely populated country of more than 150 million people, 80% of who live in rural areas; the rural poverty rate is 36%, of which 29% are extremely poor [1]. Most people depend on the agricultural sector because agricultural development is the key to alleviating poverty in the country [2]. Dairy cattle rearing is an inseparable and integral part of the agricultural farming and agribusiness system in Bangladesh. The present cattle population of Bangladesh is 25.7 million [3] and the contribution of this sector to the GDP is 3.49% [4]. More than 80 percent of this cattle population is indigenous and the rest of them are purebreds and crossbreds of different exotic breeds (Friesian, Jersey, and Sahiwal) cattle, in which productivity of indigenous cattle is very low in comparison with the pure breed. Generally, the indigenous cow (Red Chittagong, Pabna, Faridpur, etc) produces only 1.86 ± 0.57-liter milk per day [5]. Pure breed cattle rearing requires high management practices and excess feeding costs in the aspect of Bangladesh. To overcome the problem of pure breed in Bangladesh, the number of crossbreed heifers is increasing day by day with the spread of artificial insemination practices throughout the country which can be a vital weapon to contribute a major share of livestock sub-sector and playing multifunctional roles (supply of calf, milk, meat, skin, manure, carting, etc.) in the subsistence production system of Bangladesh and produce 5.94 ± 3.49-liter milk per day per cow [5].

However, there are some problems with rearing crossbreed heifer to gain profitable business in Bangladesh. The main constraints of crossbreed heifer's reproduction are prolonged resulting from insufficient nutrients, age, and difficulties of oesturs detection due to short periods of standing oesturs, silent oesturs, and the onset of oesturs during the late night to early morning hours [6, 7]. Moreover, the demand for livestock products is increasing with population growth and socio-economic changes. But, the productivity of livestock is not increasing for meeting the requirements of the country due to these problems [2]. In this circumstance, rearing crossbreed heifers for profitable reproduction is an emergent issue in Bangladesh. The efficient and accurate oesturs detection is essential to optimize the economic management of individual heifers to yield a profitable dairy operation [8]. For this reason, it requires programmed breeding to bring all the animal's reproductive cycle (oesturs) at the same time. The available option for inducing heat at the same time is oesturs synchronization [9]. Oesturs synchronization is the initiation of the reproductive process at a time so that females can be bred with normal fertility during a short, pre-defined interval [10]. It reduces or some cases eliminate labor for detecting heat and allow the breeder/producers for scheduled breeding that use AI by concentrating the breeding and resulting calving periods. It is enabling more crossbreed heifers to be artificially inseminated using frozen-thawed semen and the length of the breeding season can be reduced successfully [11, 12].

For synchronization of oesturs, there are some protocols involving the use of gonadotropin-releasing hormone (GnRH) and Prostaglandin PGF2 used in dairy cattle. After using these hormones [13], observed the conception and conception rates range from 19.8-37.7% utilizing GnRH at day 0 + prostaglandin F2α after 7 days + GnRH after 36-48 hours in Ovsynch protocol [14, 15]. Synchronization protocol with the combination of hormones (GnRH & PGF2α) is more effective than a single one for the initiation of follicular development and oesturs [16].

Although many researchers use different hormones for breeding programs to increase the reproductive performance of dairy cattle by using different methods for oesturs synchronization. Some researchers introduced only PGF2α hormone and some introduced both GnRH and PGF2α hormones of different breeds in different aged animals but no one researched different doses of hormone in ovsynch protocol to increase heat detection as well as artificial insemination for conception. Therefore, the present study was conducted to evaluate the effects of different doses of GnRH on the reproductive performance of crossbred heifers synchronized with PGF2α. So, the present study was undertaken with the following objectives:

1) To study the effect of GnRH and PGF2α hormone analog on the onset of oesturs in a crossbred heifer.
2) To investigate the effect of oesturs synchronization on the conception rate of crossbred heifers.

2. Materials and Methods

2.1. Description of the Study Area

The area in which an experiment is to be carried out depends on the particular purpose and the possible co-operation from the farmers". Ulipur Upazila in Kurigram district was selected purposively as the experimental area during the period of March 2014 to August 2014. One union namely Doldolia was covered for collecting the necessary information for the study (Figure 1). The reasons for selecting this area for the present study are given below:

1) Availability of household cattle.
2) The area was well communicated which helped involvement and data collection easier for the

researcher.

3) It was expected that cooperation from the farmers in this area would be high so that reliable data could be obtained.
4) Farmers got training on heifer rearing.

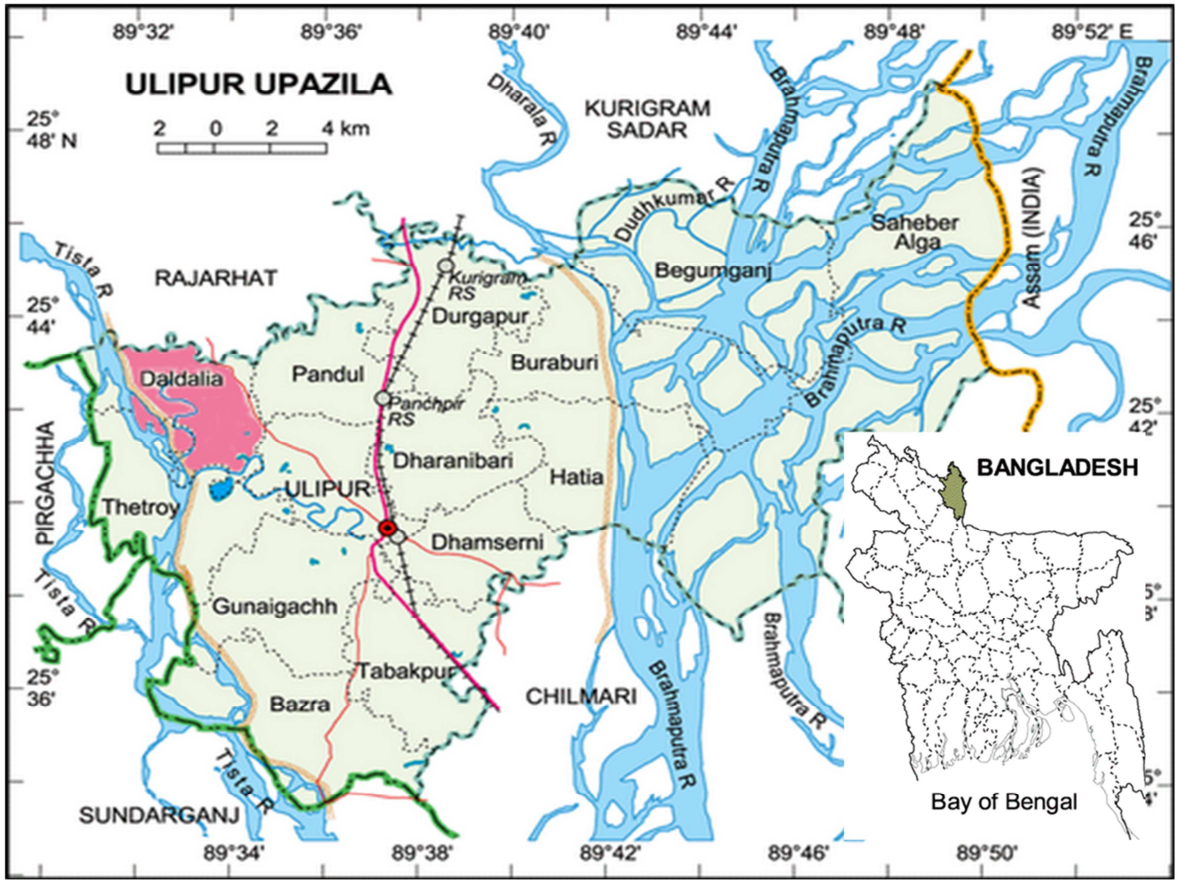


Figure 1. *Location of the Study area (Doldoliaa, Ulipur Upazilla, Bangladesh).*

2.2. Selection of Animal

For this study, 65 crossbred (Local × Shahiwal) heifers were examined during the study period. Animals suffering from clinical reproductive problems like metritis, endometritis, and cystic ovary were not included. Of 65 crossbred heifers a total of 48 heifers were selected and a list of farmers was selected randomly who reared at least one crossbreed (Local × Shahiwal) heifer by observing reddish color, marked dewlap, moderately big hump, well-developed udder and history of AI. A sample of representative farmers should be chosen in such a way that the information from it can meet the purpose of the experiment. Fourty-eight sample farmers were selected for the study, of which 16 sample from Purbo Nowdanga Unnoyan Somitty, 16 samples were from Kanipara Unnoyan Somitty, and 16 were from Uttar Nowdanga Unnoyan Somitty at Doldolia union in Ulipur upazila of Kurigram district, Bangladesh.

Table 1. *Average age and weight of experimental heifers.*

Group Name	Average age during hormonal treatment (Mean)		Average weight at day hormonal treatment (Mean)	
	18-25 months	26-30 month	18-25 months	26-30 months
Group-I	24.40	28.00	132.00	140.72
Group-II	24.60	28.45	130.00	142.00
Group-III	24.75	28.00	132.00	141.33

2.3. Housing, Feeding and Management of the Heifers

All heifers were reared under a semi-intensive system. Heifers were between 18 to 30 months of age presented in Table 1. The approximate age of the heifers was recorded according to the owner's statement during purchase and recorded in a personal file. It was further cheeked through the dentition of the heifers. The animals were fed daily with paddy straw-2kg; concentrate mixture-1/2 kg, cut-and-carry grass-15kg, and milling by-products according to their body weight. Vitamin premixes (Powder Renavet-DB®, Renata Animal Health, Mirpur, and Dhaka in Bangladesh) were also supplied to the heifers with concentrates of about 100-150gm daily on the basis of body weight provided from the project. Water

supply was adlibitum. Routine deworming against roundworms and liver flukes was in practice and the heifers were vaccinated routinely against foot and mouth disease (FMD), anthrax, and hemorrhagic septicemia. The heifers were housed with good facilities for natural ventilation. Feeds and forages were given in two splits per day. The nutritional status of the heifers was determined by scoring the body conditions of the heifers using 1-5 scales on the basis of bony prominence and deposition of subcutaneous fat as described by Nicholson and Butterworth (1986) [17]. The cows with < 2.0 body condition score (BCS) were not selected for this study.

2.4. Determination of Body Condition Scores (BCS) of Heifers

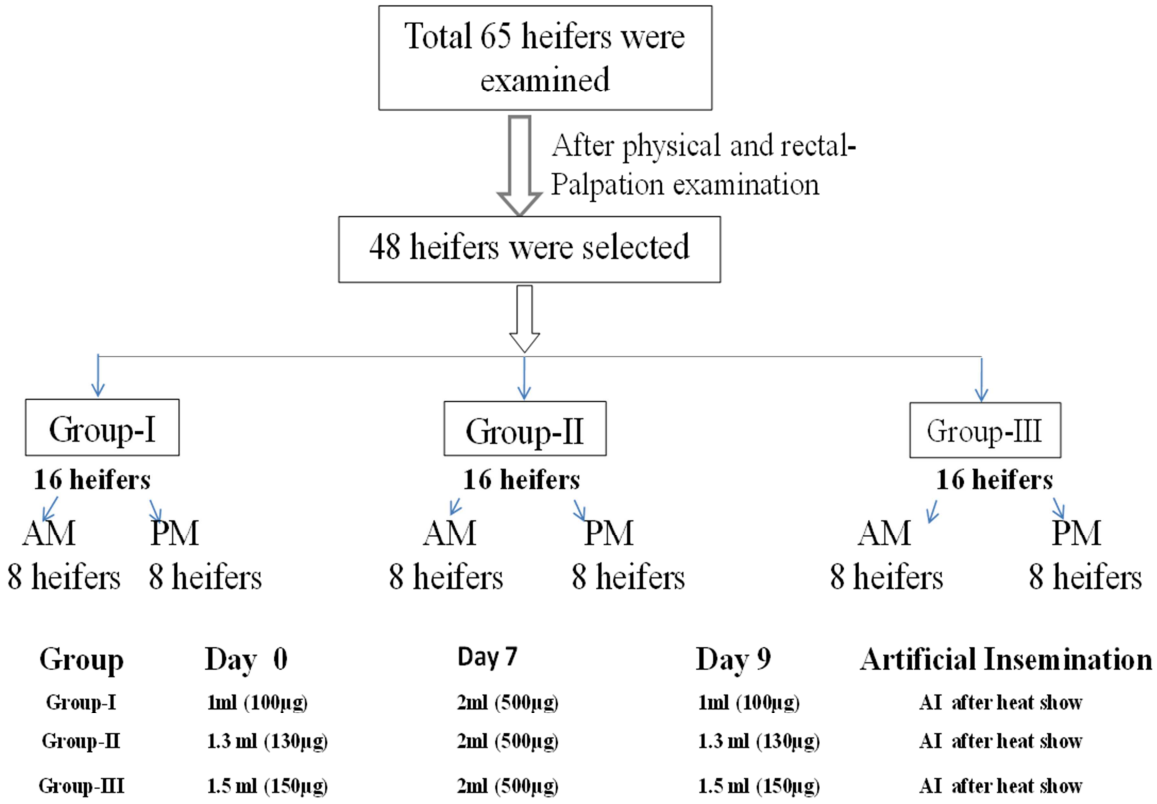
The heifers with 3 or more BCS were selected in this experiment. The BCS of heifers was evaluated and scored according to Anitha *et* al. [18] at the beginning of hormonal treatment. The body condition score (BCS) was evaluated by visual examination of fat deposition in the heifers and scored from 1 to 5 scales. Briefly BCS 1 stood for emaciated animals, 2 indicated individual dorsal spines were pointed to touch; hips, pins, tail-head, and ribs were prominent, 3 represented those heifers, whose ribs were usually visible and with little fat cover, dorsal spines barely visible, 4 was for animals with smooth and well fat layer, but fat deposits were not marked; whereas 5 was for heavy deposit of fat clearly visible on tail-head and brisket, dorsal spines, ribs, hooks, and pins were fully covered and could not be felt even with firm pressure. It was recorded for all heifers during starting of the hormonal treatment, half hours before AI, and conception confirmation time.

2.5. Grouping and Synchronization of Ooesturs

The heifers were randomly divided into 3 groups. Each group consisted of 16 heifers. AM and PM method was used for the timing of estrous induction and insemination. Ovurelin (GnRH) and Ovuprost (PGF2α); (BAYER Laboratories Ltd., Germany) were selected for inducing ooesturs of heifers in this study. For estrous induction, recommended doses of hormones used in group-I were treated as a control group. 100 µg Gonadorelin (1ml Ovurelin®, BAYER Laboratories Ltd., Germany) was administered to the heifers of group-I, at the intramuscular route (i/m) followed by i/m administration of 500 µg Cloprostenol (2ml Ovuprost®, BAYER Laboratories Ltd., Germany) at 7 days interval. Forty-eight hours after Prostaglandin F2α analog administration, 100 µg of GnRH hormone was also administered at the i/m route. AI was carried out by a trained AI technician using frozen-thawed semen collected from BRAC 12-20 hours after the onset of the ooesturs sign. In Group II and Group III, the same protocol was conducted with 1.3ml; 2ml; 1.3ml, and 1.5ml; 2ml; 1.5ml respectively by Ovurelin®; Ovuprost®; Ovurelin® respectively. The first injection was given ignoring the stage of the ooesturs cycle. On day 9 the respective doses were injected into all heifers and artificial insemination was done after observing the ooesturs show. The experimental layout is given below.

2.6. Experimental Layout

Schematic diagram of Experimental is shown below.



Group	Day 0	Day 7	Day 9	Artificial Insemination
Group-I	1ml (100µg)	2ml (500µg)	1ml (100µg)	AI after heat show
Group-II	1.3 ml (130µg)	2ml (500µg)	1.3 ml (130µg)	AI after heat show
Group-III	1.5 ml (150µg)	2ml (500µg)	1.5 ml (150µg)	AI after heat show

Figure 2. *Schematic Diagram of Experimental Layout.*

2.7. Factors Consider on Heifer Reproduction

2.7.1. Onset and Intensity of Oesturs Signs

The heifers were observed closely for detecting the signs of ooesturs at 4 hours intervals after Day 0 of injection for all groups with the help of Veterinary Field Organizers (VFOs), AI technician, s and Farmers which is recorded in the register file. The estrous heifers were detected by using castrated bull for inducing male effect or symptom of ooesturs of the heifer. The onset and signs of ooesturs were recorded on the basis of restlessness, reluctance to feed, shifting the tail, vaginal discharge, mounting to others & blowing. Duration of ooesturs was calculated from the time of onset of ooesturs to the end of ooesturs as a rejection of female to the male. The day of onset of ooesturs was calculated from hormonal treatment to estrous show. The ooesturs detected all groups in the same process as did for induced groups.

2.7.2. Detection of Oesturs

Cows were observed for signs of behavioral oesturs at 4-hr intervals for 9 days after injection of ovurelin®. Animal-induced mounting behavior, vaginal discharge, and reluctance to feed were used to assist in the detection of oesturs. Artificial insemination was performed on all cows in the afternoon and morning following the first observation of oesturs. Conception rates were determined by sustained rectal palpation.

2.7.3. Semen Placement

In the present study, the site of insemination was recorded in two categorical ways to investigate its effect on the conception rate. The categories were the deep cervix and others of the cervix. It was confirmed by asking inseminators while performing inseminations.

2.7.4. Time of Service

This was calculated from the time of the first observed oesturs signs to the time of the first service recorded from the record file of the project.

2.8. Conception Diagnosis

All animals were diagnosed for conception via rectal palpation 90 days post-AI by a trained area AI technician and results were recorded for calculation of the conception rate.

$$\text{Conception rate (\%)} = \frac{\text{Number of pregnant heifers}}{\text{Total number of heifers inseminated}} \times 100$$

2.9. Statistical Analysis

The data was entered in the Excel sheet. Results were calculated as least square mean (LSM) ± standard error mean (SEM) by using the SPSS package program. Comparison among three different doses of hormones (GnRH& PGF2α) on the percentage of heifer showed ooesturs, time of onset of ooesturs, duration of ooesturs, and conception rate trait were analyzed in the population using the general linear model procedure for least square means (LSM) with Statistical Package for the Social Sciences (SPSS) statistics analytical software package (version 20.0; IBM Corp., Armonk, NY). A probability of $P< 0.05$ was considered statistically significant.

3. Result

3.1. Effect of Ovuprost® and Ovurelin® Hormones on the Time of Onset of Oesturs and Duration of Oesturs in Heifers of Ovsynch Protocol

The effect of different doses of Ovuprost® and Ovurelin® hormones on the time of onset and duration of ooesturs of crossbreed heifers were shown in Figure 3. The results of the present study revealed that the mean interval to the onset of ooesturs (LSM ± SEM) after Ovurelin® and Ovuprost® administration was 54.50 ± 3.86, 47.00 ± 3.36 and 40.67 ± 1.85 hours of group-I, group-II, and group-III; respectively. This indicates that there was no significant difference among the three groups after giving different doses of hormonal treatment. Like the onset of ooesturs, the duration of ooesturs (mean ± SEM) of crossbreed heifers after ovurelin® administration was 16.40 ± 0.02 hours, 18.00 ± 0.03 hours, and 19.50 ± 0.04 hours of group-I, group-II, and group-III, respectively. The variation of oesturs duration indicated non-significant effects among the three groups.

3.2. Effect of Different Doses of Hormone on Oesturs and Conception Rate

In the present study, there were used different doses of hormones to calculate the oesturs and conception rate of crossbreed heifers presented in Figure 4. Increasing the doses of Ovurelin® hormone resulted in a higher oesturs response (75%) and conception rate (66.67%). The effect of hormones on oesturs and conception rate of crossbreed heifers was observed that there was highly significance (P<0.05) in group III than in the other two groups.

3.3. Effect of Time of Initiation of Synchronization Protocol & Time of Service

The effect of the time of initiation of synchronization protocol and time of service on the conception rate of a crossbred heifer of different groups was shown in Figures 5 & 6. In the present study, the average conception rate of crossbred heifers was 64.44% at the morning ovsynch protocol whereas 38.33% at the evening ovsynch protocol (Table 2). This result indicated that the conception rate was significantly higher (P<0.05) at morning ovsynch than at evening ovsynch. The effect of time of service on the conception rate of crossbreed heifers observed that time of service is one of the most important factors influencing the conception rate. The average conception rate was significantly (p<0.05) higher at 10-14 hours (61.66%) followed by at 15-20 hours (36.11%) after the onset of oesturs (Table 2).

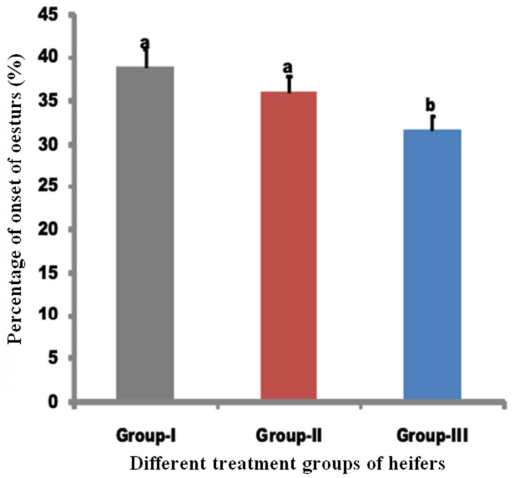


Figure 3. *Effect of Ovuprost® and Ovurelin® hormones on the time of onset of oesturs in heifers. Each bar of percentage value with error bar represents the mean ± SEM of oesturs & conception rate. Different letters above the error bars denote statistically non-significant differences (P>0.05).*

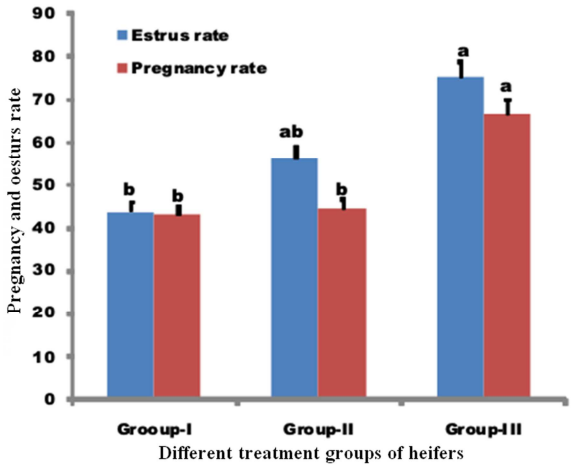


Figure 4. *Effect of different doses of Ovurelin® and Ovuprost® hormone on oesturs and Conception rate in ovsynch protocol of heifers. Each bar of percentage value with error bar represents the mean ± SEM of oesturs & conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).*

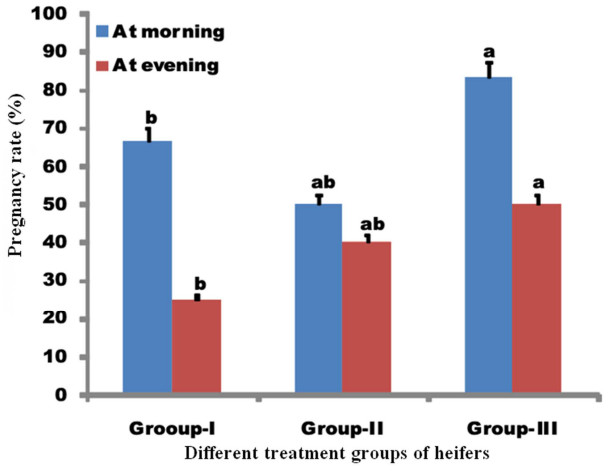


Figure 5. *Effect of time of initiation of synchronization protocol on conception in crossbreed heifers. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).*

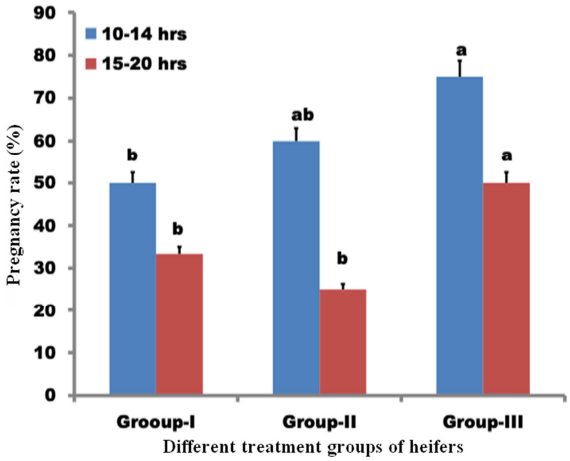


Figure 6. *Effect of time of artificial insemination of ovsynch protocol on conception rate of crossbreed heifers. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).*

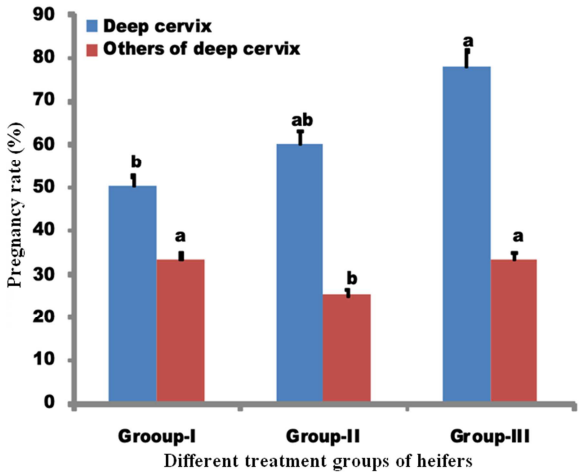


Figure 7. *Effect of site of semen placement on conception rate of dairy heifer after hormonal treatment. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).*

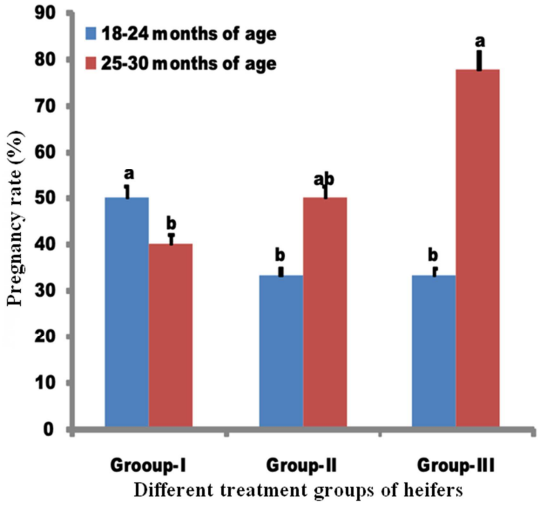


Figure 8. *Effect of age on oesturs synchronization and conception rate after hormonal treatment. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).*

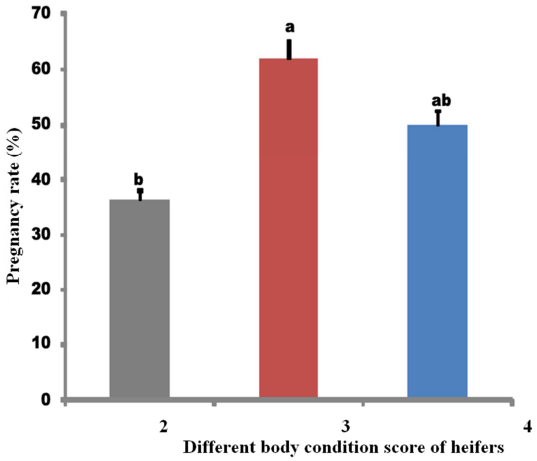


Figure 9. Effect of Body Condition Score (BCS) on Conception rate in heifers after hormonal treatment. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).

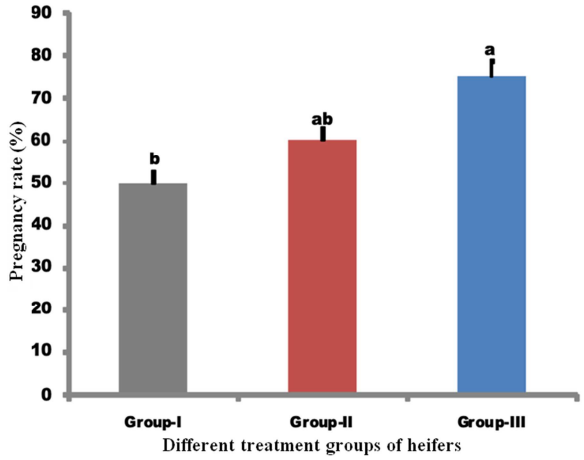


Figure 10. Effects of different doses of hormones on conception rate after first natural oesturs. Each bar of percentage value with error bar represents the mean ± SEM of conception rate. Different letters above the error bars denote statistically significant differences (P<0.05).

Table 2. Effect of time of initiation of synchronization protocol & time of AI on conception in dairy heifers.

Topics	Factors	Pregnant rate (%)	Level of significance
Time of AI (After onset of oesturs)	10-14 hours	61.66[a]	Significant
	15-20 hours	36.11[b]	
Time of initiation of ovsynch protocol	At morning	64.44[a]	Significant
	at evening	38.33[b]	

Values within the same column with different superscripts differ (P<0.05).

3.4. Effect of Site of Semen Placement

Faulty insemination technique is a significant factor causing low conception rates in many herds. Correct semen placement is critical. But the conception rates of crossbreed heifers were higher for those who deposited semen in the deep cervix than in other positions. The effect of the site of insemination on the conception rate of heifers observed there was highly significant (P<0.05) among the heifers presented in Figure 7. The conception rate of group-III heifers was 77.77% vs 33.33% for deep cervix and others than cervix; respectively (Figure 5) whereas 50% vs 33.33% conception rate in group-I. This result indicated a marked difference in conception rate by placing semen in the deep cervix and others.

3.5. Effect of Age on Conception Rate of Heifers

The effect of age on the conception rate of crossbreed heifers was observed and there was statistically significant (P<0.05) conception rate among the heifers of different ages (Figure 8). The highest conception rate was observed at 25-30 months of age (77.78%) with the administration highest doses of hormone and gradually decreasing the conception rate by decreasing hormonal doses.

3.6. Effect of Body Score Condition on Conception Rate Successful of Heifers

The effect of body condition score on the conception rate of crossbreed heifer was observed that the average conception rate was higher in BCS-3 (61.90%) than in other BCS (BCS= ≤2, 36.11% and BCS=≥4, 50%). The present result indicates that there was statistical significance (P<0.05) among the heifers of different groups after giving different doses of hormone (Figure 9).

3.7. Return to Oesturs Naturally and Subsequent Conception Rate

All the non-pregnant animals in these groups returned to oesturs usually and subsequently artificially inseminated. There was a significant (P<0.05) difference in conception rate among the three groups after the hormonal administration of heifers (Figure 10). The interval was found to be highest (26 days) in the lowest treatment group, whereas it was the lowest (19 days) in the highest treatment group but the overall average interval to second oesturs was the same.

In the current study, it was also stated that injected GnRH and PGF2α doses cause different effects on the interval from hormonal administration to first natural oesturs. These results clearly showed a positive effect of large quantities of GnRH on subsequent next oesturs (Figure 9).

4. Discussion

4.1. Effect of Ovuprost® and Ovurelin® Hormones on the Time of Onset of Oesturs & Duration of Oesturs in Heifers of Ovsynch Protocol

Several researchers [19-24] have reported that the average

onset of ooesturs was 96 ± 15.08, 94, 93.3 ± 6.1, 96, 97, 120 and 79.1 hours, respectively by using prostaglandin for ooesturs synchronization in Sahiwal heifers, which was higher than the findings of the present study. However, some other researchers [25, 26] have observed an average interval of onset of ooesturs 56.0 ± 12.74 and 50.4 ± 4.9 hours, respectively in crossbred heifers, which is similar to the present study (Figure 6). Moreover, some other researchers [27, 28] also reported that the average interval of onset of ooesturs was 38.0± 4.27 and 42.0± 5.8 hours, respectively in dairy cattle. These variations can be attributed to differences in body condition score, climate, method, frequency of ooesturs detection, and presence of large follicles at the time of treatment of the animal. Similar results were found in the present study that the average ooesturs duration was 17.3 ± 4.6 hours (range from 9 to 24 hours) in dairy heifers [29, 30]. Chaudhry (1985) reported an ooesturs duration of 24.7 ± 1.6 hours in Holstein Frisian Cattle [31]. M. Amjad et al., (2006) reported that the duration of ooesturs was 23.25 ± 1.99 vs. 20.33 ± 1.45 hours between two groups of Sahiwal heifers [19]. Some other researchers have observed that the average duration of ooesturs was 10 and 10.6 hours in indigenous heifers, which indicated lower than present study [25, 32].

4.2. Effect of Different Doses of Hormone on Ooesturs and Conception Rate

Many investigators have reported that there was no significant difference (P>0.05) in ooesturs response among all treatment groups injection of PMSG at 2, 2.5, 3, 3.5, or 4 IU/kg BW, on ooesturs response in crossbreed heifers [33]. Zonturlu et al., (2011) and Sá Filho et al., (2010) followed by an injection of PGF2α in crossbreed cows but the present study indicated a 43-75% variation also reported non-significant effect on ooesturs response among all treatment groups (2.5 µm/kg, 3 µm/kg and 4 µm/kg of PGF2α) which varied from 70-90% [34, 35]. Lower ooesturs response was recorded by some other scientists than the present study, as these workers reported ooesturs response as 55, 62.2, 66.7, and 64.32%, respectively in crossbreed cows [22, 36, 37]. The conception rate of the crossbred heifer was significantly lower (P>0.05) in the highest treatment group (4 IU/kg, 25%) than in lower treatment groups (2 and 2.5 IU/kg, 71.4 and 66.7%) has been stated by Shu-Bin Fu (2013) [33]. Some other investigators [38, 39] have reported that lower conception rates in lower dose groups may be associated with not-mature follicular development, a decrease in the percentage of fertilized ova, and embryo quality in different species. The success of ooesturs results and conception can be affected by the quality and dose of the synthetic product of prostaglandin and the time of injection [40].

4.3. Effect of Time of Initiation of Synchronization Protocol & Time of Service

The highest conception rate (60.5%) of crossbred heifers has been noted at 4 to 12 hours after artificial insemination [41] which was similar to the present study. It also reported a similar result to the present study that the conception rate of dairy heifers was observed at 60.26%, and 39.42% when inseminated at 11-14 hours and 15-18 hours; respectively [42]. The present result was higher than Barel et al., (2005) [43] who reported 50% and 58.82% conception rates of crossbred heifers when inseminated at 6 to >10 hours, and 14 to above hours respectively. Significant variations in conception rates 58.82%, 69.69%, and 33.70% were observed when the cow was inseminated at early, middle, and later ooesturs, respectively [44].

4.4. Effect of Site of Semen Placement

The finding of the present study was dissimilar from the finding of M. M. R. Mufti (2010) [45] who reported a 67.44% vs 56.82% conception rate for semen deposited in the body of the uterus deposited at the middle of the cervix of crossbreed cattle. In another study, a marked difference was found in terms of conception rate while placing semen into the cervix (35.20%) and vagina (10.00%); respectively [46]. They also found a 22% reduction in conception rate if the inseminator could not penetrate through the cervix and deposited the semen outside the cervical os which was in agreement with the present work.

4.5. Effect of Age on Conception Rate of Heifers

It has been stated that the conception rate of crossbred animals varies from 37-71% but is higher at a younger age [47]. Similar results were reported by some scientists [48] where they found the highest conception rate (74%) of crossbreed heifers of 2-4 years of age. Lower results were reported than the present study that the conception rate of 2 years, 3 years, 4 years, and more than 13 years old were 55.90, 60.50, 63.00, and 42.00%, respectively for dairy cows [49]. Miah et al., (2004) [42] studied that the conception rate of younger heifers varied significantly (P<0.05) with the rest of all the heifers. However, the present result indicated that this protocol is useful to maximize the production of heifers by using ooesturs synchronization at 25-30 months of age in a large dairy herd.

4.6. Effect of Body Score Condition on Conception Rate Successful of Heifers

Significant negative correlation between conception rate and ooesturs rate in crossbred animals (BCS -3.02 ±.07 out of 1-5 score), whereas cows showed 28.89% ooesturs and 33.33% conception rate [50]. It is reported that the conception rate was significantly higher (68%) in heifers (BCS at 3-4 scale from 1-9 scale) producing more milk and is associated with higher circulating concentrations of growth hormone and lower concentrations of insulin and glucose [51]. It is also reported that a 62% change in BCS after conception, rather than during AI has a significant effect on the percentage of dairy heifers exhibiting ooesturs [52].

4.7. Return to Ooesturs Naturally and Subsequent Conception Rate

All the non-pregnant animals in these groups returned to

ooesturs usually and subsequently artificially inseminated. There was a significant (P<0.05) difference in conception rate among the three groups after the hormonal administration of heifers (Figure 10). The interval was found to be highest (26 days) in the lowest treatment group, whereas it was the lowest (19 days) in the highest treatment group but the overall average interval to second ooesturs was the same. However, the conception rates after the first natural ooesturses ranged from 50 to 75% which was similar to the result of Quintero-Elisea et al., 2011 [53].

In the current study, it was also stated that injected GnRH and PGF2α doses cause different effects on the interval from hormonal administration to first natural ooesturs. These results clearly showed a positive effect of large quantities of GnRH on subsequent next ooesturs (Figure 9). There are few studies regarding hormone administration on returning to first natural ooesturs. For this reason, further studies can be designed to explain this phenomenon.

5. Conclusion

It was concluded that the age of the heifer, time of initiation of ovsynch protocol, time of insemination, and site of insemination were the most important factors to get the maximum conception rate of crossbreed heifers. So, it may be suggested that to achieve the desired ooesturs and conception rate, the farmers should be inseminated their heifers at the site of the deep cervix by a technically sound and skilled inseminator at 10-14 hours after the onset of ooesturs of morning ovsynch protocol. The age of the heifers and body condition score will be difficult to control from the standpoint of the small farmer's economies and the biology of heifers. Whereas, the time of initiation of synchronization protocol, doses of the hormone, time of insemination, and semen placement remained to give top emphasis in order to achieve desired ooesturs response and conception rate of crossbreed heifers.

The information related to ooesturs synchronization by rural farmers in Bangladesh is very poor. A detailed study is needed in different districts of Bangladesh to recommend ooesturs synchronization programs for rural poor farmers as an income-generating activity. Though as per findings and results, the following recommendations may be mentioned for ooesturs synchronization in a rural area of our country:

a) Young-aged farmers are more proactive, devoted, and adaptive to new technology rather than old-aged farmers.
b) A farmer should be given priority to maintaining doses of the hormone as well as the time of initiation of the ooesturs synchronization protocol (in the case of the ovsynch protocol).
c) A farmer having more experience in cow rearing was found to have more efficiency in managing the farms (especially heat detection) which results in higher profitability compared to the farmers having less experience.
d) A farmer should be inseminated their heifers in the site of the deep cervix by a technically sound and skilled inseminator 10-14 hours after the onset of ooesturs.
e) Farmers should be waited at least 21 days for normal ooesturs after using the scheduled hormone because most of the heifers show heat normally and subsequently conception for the effect of 1st scheduled doses of the hormone.
f) Vaccination is an important factor for small-scale heifer rearing (especially ooesturs synchronization). Regular vaccination is essential to keep the heifer healthy thus increasing profitability.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

This work was carried out in collaboration with all authors. All authors contributed equally, read and approved the final manuscript.

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