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Competitive Research Grant

Sub-Project Completion Report
on
Development of estrus synchronization protocols using hormones in anestrus cows for improved reproductive performance

Project Duration
May 2016 to September 2018

Department of Veterinary and Animal Sciences, University of Rajshahi

Submitted to

Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
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Project Implementation Unit:
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

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National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
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Acronyms

%	= Percentage
@	= at rate of
<	= Less than
>	= Greater than
×	= Cross
µl	= Micro liter
ACI	= Advance chemical industries
AI	= Artificial Insemination
AM	= Ante meridiem
BARC	= Bangladesh Agricultural Research Council
BAU	= Bangladesh Agricultural University
BCS	= Body condition score
CL	= Corpus luteum
cm	= Centimeter
Co-PI	= Co-Principal Investigator
DLS	= Department of Livestock Services
ELISA	= Enzyme linked Immuno-sorbent Assay
et al.	= Etalia
FN	= Friesian
FAO	= Food and Agricultural Organization
GDP	= Gross Domestic Product
GnRH	= Gonadotropin releasing hormone
HF	= Holstein Friesian
IAEA	= International Atomic Energy Agency
Im	= Intramuscular
Inj	= Injection
Kg	= Kilogram
L×J	= Local × Jersey
L/LO	= Local
L×F	= Local × Friesian
Lab	= Laboratory
ml	= Milliliter

n/No. = Number

NATP = National Agricultural Technology Programme

ng = Nano gram

P = Probability

PG = Prostaglandin

$PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$

Phy = Physical

PI = Principal Investigator

PIU = Project Implementation Unit

PM = Post meridiem

RU = University of Rajshahi

SD = Standard deviation

Sig = Significant

SL = Sahiwal

SPSS = Statistical Package for the Social Sciences

t = T-test

PCR = Project Completion Report

CRG = Competitive Research Grants

USAID = United State Agency for International Development

MHz = Mega HertZ

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Executive Summary

Anestrus is a major problem of the dairy industries in Bangladesh and the farmers are failing to crop one calf per cow per year that in turn is resulting in a great economic loss to the farmers. The aim of the present study was therefore to determine the prevalence of anestrus in cows in relation to different risk factors and to develop effective estrus synchronization protocols using Prostaglandins F2α analogue, GnRh and hCG. The study was carried out in the private and government dairy farms under Rajshahi and Natore districts during the period from 01-07-2017 to 30-6-2018. A total of 294 cows were selected for the study. The data was collected directly from the dairy farm owners by using structured questionnaires. To study the prevalence of anestrus in relation to different risk factors the breed, age, parity, body weight, body condition score (BCS), stage of anestrus, housing, feeding, health care, farming system, location, etc. of the cows were determined. Gynecological examinations of cows were made on the basis of history, clinical examination and visual observation. Out of 294 cows 160 were diagnosed as anestrus cows which were selected for synchronization study through clinical management. The anestrus cows were divided into eight protocol groups containing 20 cows in each group. The protocol groups were numbered as Protocol-I, II, III, IV, V, VI, VII and VIII for hormonal treatment with PGF2α, PGF2α-PGF2α, PGF2α-PGF2α-PGF2α (Fixed time AI), GnRH-PGF2α-GnRH (Fixed time AI), PGF2α-GnRH-PGF2α (Fixed time AI), GnRH-PGF2α, hCG and no treatment at all (control group) respectively. After the treatment the cows from each protocol groups were observed for the heat to come and then inseminated using artificial insemination (AI) technique. Finally the pregnancy was diagnosed using ELISA, ultrasound and rectal palpation techniques. The overall prevalence of anestrus in cows was around 54%. The prevalence of anestrus was least in indigenous cattle (50%). The highest occurrence of estrus was observed in the cattle group of cross-bred (55%), <4 years of age (60%), <200 kg body weight (60%), medium body condition (55%) and in the heifer (62%). The highest occurrence of anestrus in relation to various management factors was found to be with the group with poor housing system (69%), farm size of <10 cows (72%), poor quality feed supply (71%), least fodder supply (63%), poor health care (71%), herbal medicine used for treatment (60%), anthelmintics not used (83%), least (<3 years) farming experience (70%) and untrained farmer (60%) in the study areas. The lowest rate of anestrus in dairy cows was seen where fodder availability was abundant (47%), good health care was provided (48%), allopathic medicine was practiced (52%), DLS health service was availed (28%), regular preventive control measures were taken (51%), regular deworming was practiced (52%), with the farmers with long farming experience (47%) and with the trained farmers (52%). On the whole, 77% anestrus cows came into estrus after hormone treatment under 7 protocol groups whereas; only 20% anestrus cows came into estrus from the control group. The rhythmic percentages of estrus induction with the treatment protocols by injecting PGF2α, PGF2α -PGF2α, PGF2α -PGF2α -PGF2α, GnRH–$PGF_{2\alpha}$-GnRH, $PGF_{2\alpha}$-GnRH-$PGF_{2\alpha}$, GnRH-$PGF_{2\alpha}$, hCG and without treatment (control group) were 60%, 85%, 85%, 80%, 90%, 80%, 60%, and 20% respectively. The serum ELISA and the Ultrasound techniques were found to be the techniques of choice for early pregnancy diagnosis in dairy cows. For midterm pregnancy diagnosis, Ultrasound, ELISA and Rectal palpation methods were found to be very useful while, for late pregnancy diagnosis, Visual observation and Rectal palpation methods were found to be the methods of choice for pregnancy diagnosis in dairy cows.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project:
Development of estrus synchronization protocols using hormones in anestrus cows for improved reproductive performance

2. Implementing organization:

Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

Principal Investigator:
Dr. Md. Jalal Uddin Sarder
Professor, Dept. of Veterinary & Animal Sciences
University of Rajshahi, Rajshahi-6205, Bangladesh
Phone: +880721711578 (office), +880721812516 (Residence)
Cell: +8801929231933, Email: jalalnusa@yahoo.com

Co-principal investigator:
Dr. Rashida Khaton
Associate Professor, Dept. of Veterinary & Animal Sciences
University of Rajshahi, Rajshahi-6205, Bangladesh
Phone: +880721711141 (office)
Cell: 01717609564, Email: rashidavet@yahoo.com

4. Sub-project budget (Tk):

4.1	**Total:**	Tk. 18,00,000.00
4.2	**Revised (if any):**	Tk. 17,25,020.00

5. Duration of the sub-project:

5.1	**Start date (based on LoA signed):**	12 July, 2017
5.2	**End date:**	30 September 2018

6. Justification of undertaking the sub-project:

One of the major goals of dairy farmers is one calf per cow per year. This type of high reproductive efficiency is very much important for achieving maximum return from the dairy animals. The reproductive performance of high yielding cows with high genetic merit declines in many dairy industries in Bangladesh due to low pregnancy rate in cows (Alam *et al.,* 1994, Shamsuddin *et al*., 2001). The productivity of cattle could be low because of poor nutrition (Alam *et al*., 2006), and incorrect detection of estrus (Roelofs *et al*., 2010; Macmillan, 2010; Paul *et al*., 2011). However, the specific reasons for the decline might be directly related to or exacerbated by environmental and management conditions. The same management advantages are less relevant to reproductive management where each animal must be considered as an individual within its own estrus cycle so that estrus, ovulation, insemination, fertilization and conception occur within a sequence that is within restricted behavioral and biological limits. Estrus detection and animal identification have become increasingly difficult in large dairying operations to the extent that decisions related to the breeding management of individual cows are usually made by "barn" staff working with cows that

may only have limited periods to interact in an unrestricted manner (Macmillan, 2010). This situation can limit the likely expression of the classic symptoms of behavioral estrus; namely, standing when mounted by a herd mate. Increasing incidence of early lactation anovulatory anestrus combined with a greater likelihood of early embryonic death without a return to estrus has been reported by Macmillan, 2007.

Various combinations of synthetic hormones are being used in inducing ovarian activity postpartum and increasing conception rates but with variable responses. This variation of hormonal response may be due to the influence of age, nutritional status of the cow, body condition and seasonal variations. Elevated temperature has also a depressing effect on ovarian functions and fertility rate. However, hormonal studies have established that the secretion and release of pituitary hormones are under the influence of hypothalamic hormones. The effectiveness 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 gonadotropin-releasing hormone (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 synchronization systems currently used in the reproductive management of dairy cattle involve hormone treatments, which may influence the inter-estrous interval in normally cycling cows. Progesterone or potent progesterone analogues are used in combination with a luteolytic agent involving an estrogen or synthetic prostaglandin F2α (PGF2α). The estrogen is administered at the start of the progesterone treatment, whereas the prostaglandin is injected close to or at the end of the treatment.

Anestrus not only does lengthen the postpartum interval, but also substantially reduce the farmer's financial returns from milk or beef sales. Anestrus is a major problem of the dairy industries in Bangladesh and the farmers are failing to achieve one calf per cow per year that in turn is resulting in a great economic loss to the farmers. This project therefore was undertaken for clinical management of anestrus in cows and improving the reproductive performances of dairy cows through proper and timely diagnosis and judicious use of hormones such as progesterone, gonadotropins, GnRH, $PGF_2\alpha$ and non-hormonal agents for the management of various reproductive disorders in cattle for sustainable dairy farming in Bangladesh.

7. Sub-project goal:

The goal of the sub-project was to develop a suitable synchronization protocol for improving the reproductive performances of anestrus cows.

8. Sub-project objective (s):

- To study the prevalence of anestrus in relation to different risk factors in heifer and postpartum cows.
- To develop the effective estrus synchronization protocols using hormones.
- To evaluate the useful pregnancy diagnosis techniques in cows by using different tools.

9. Implementing location (s): Rajshahi and Natore districts

10. Methodology in brief:

The present study was conducted at the Department of Veterinary and Animal Sciences, University of Rajshahi from 1st July 2017 30th June, 2018. A total of 294 dairy cows were selected from different private and government dairy farms of Rajshahi and Natore districts region.

10.1 Study of the prevalence of anestrus in relation to different risk factors in heifers and postpartum cows:

To study the prevalence of anestrus in relation to different risk factors the breed, age, parity, body weight, body condition score (BCS), stage of anestrus, housing, feeding, health care, farming system, location, etc. of heifer and postpartum cows were accurately determined. The cows (294) under study were classified as follows:

According to the breeds of cows-

Group-I: Indigenous breed (n=14)
Group-II: Crossbred (n=280)

According to the genetic composition-

Group-I: Indigenous (n=14)
Group-II: Indigenous × Friesian (n=265)
Group-III: Indigenous × Sahiwal (n=15)

According to the age of the cows-

Group-I: <4 years (n=116)
Group-II: 4-<6 years (n=101)
Group-III: 6 years and above (n=33).

The body weights of dairy cows were calculated according to the Shaeffer's formula adopted by McNitt (1983).

$$\text{Body weight (Kg)} = \frac{\{\text{Length (inch)}\} \times \{(\text{Heart Girth (inch)}\}^2}{300 \times 2.2}$$

The cows were again classified according to their body weight such as-

Group-I: <200kg body weight (n=114) and
Group-II: >200 (n=180)

The scoring of body condition involved a manual assessment of the thickness of fat covered and prominence of bone at the tail-head and loin area. The tail-head scoring was done by feeling for the amount of fat around the tail-head and the prominence of the pelvic bones. The loin scoring was done by feeling the horizontal and vertical projection of the vertebrae and the amount of fat in-between. Assessment relied mainly on the tail-head but was refined by the loin score if both were very different.

According to the body condition the cows were grossly divided into three groups-

Group-I: Poor (n=44)
Group-II: Medium (n=168) and
Group-III: Very Good (n=82)

Parity of cows were classified as follows-

Parity $_0$ (P_0): Heifer not given birth yet (n=77)
1st parity (P_1): Cows gave birth to one calf in her life (n=82)
2nd parity (P_2): Cows gave birth to two calves in her life (n=78)
3rd parity (P_3): Cows gave birth to three calves in her life (n=38)
4th parity (P_4): Cows gave birth to >4 calves in her life (n=19)

Post partum heat /anestrous of these cows were determined from birth register and history of cows and information from owners of cows.

According to the status of given birth or not given birth the cows were divided into two groups as follows-

Group-I: Heifer (n=77) and
Group-II: Cow (n=217)

The housing system of dairy cows were divided into three classes-

Class-I: Poor (muddy house) (n=75)
Class-II: Medium (semi- concrete house) (n= 51) and
Class-III: Good (concrete house) (n=168)

The cattle shed of dairy cows were divided into three classes-

Class-I: Kancha (n=74)
Class-II: Pakka (n= 54) and
Class-III: Both (n=66)

According to the number of cows in a farm the farms were divided into three groups-

Group-I: < 10 cows (n=67)
Group-II: 10-< 20 cows (n=131) and
Group III: > 20 cows (n=96)

According to the quality of feed supplied the cows were divided in to three groups-

Group-I: Poor (n=7)
Group-II: Medium (n=103) and
Group-III: Good (n=184)

According to fodder availability the cows were divided in to three groups-

Group-I: Least (n=80)
Group-II: Medium (n=60) and
Group-III: Abundant (n=154)

According to the health care services provided the cows were divided in to three groups-

Group-I: Poor (n=7)
Group-II: Medium (n=103) and
Group-III: Good (n=184)

According to the type of medicine used the cows were divided into two groups-

Group-I: Allopath (n=190) and
Group-II: Herbal (n=104)

Service providers of dairy cows were divided into three groups-

Group-I: DLS (n=50)
Group-II: Private (n=54) and
Group-III: Own (n=190)

According to the control measures taken the cows were divided into three groups-

Group-I: Regular (n=139)
Group-II: Irregular (n=137) and
Group-III: None (n=18)

According to the experience of farmers on dairy farming the farmers were divided into-

Poor: <2 years of cattle farming (n=44)
Moderate: 3-5 years of cattle farming (n=54) and
Good: > 5 years of cattle farming (n=196)

According to the status of de-worming the cows were divided into three groups-

Group-I: Regular (n=139)
Group-II: Irregular (n= 137) and
Group-III: None (n=18)

According to the training status the dairy farmers were divided into two groups-

Group-I: Trained (n=201) and
Group-II: Untrained (n= 93)

10.2 Development of effective estrus synchronization protocols using hormones:

Selection of anestrus cows

Out of 294 cows under study a total of 160 cows were detected to be anestrus and those were selected for synchronization study. The cows were selected on the basis of age, milk production, breed, body condition, parity, etc from the different private and government dairy farms at Rajshahi and Natore districts. The dairy cows in the different farms were kept more or less with the same feeding and housing system under routine vaccination and de-worming schedule. Heat/ estrus detection and artificial insemination were done by the skilled inseminator. Confirmation of pregnancy was done using ultrasound machine, the ELISA kits or rectal palpation techniques. The detail procedure is given below.

Determination of anestrous in cows

Anestrus is the cessation of estrous cycle. It is mainly of two types; 1) physiological and 2) pathological. For this study the pathological anestrus cows were targeted. The cows were categorized as anestrous cows when they failed to mate even after attaining the age of 2.5 years in case of crossbred heifer and 3 years in case of indigenous heifer. But in case of cows, those are in production, anestrous was considered when they failed to mate after 60 days have passed from the date of parturition.

Procedure of confirmation of anestrus

For the confirmation of anestrous dairy cows history of the cows was taken from owners, register books and other documents of the farms were checked, the farm attendants were interviewed, the physical appearance, and the gynecological conditions were examined thoroughly.

Data collection

A format was developed for proper recording of the history of anestrus cows. The prevalence of anestrus cows was recorded at the study area by observing the cows and interviewing the related persons. After confirmation of anestrus, the hormonal drugs were used to treat the cows. Total number of cows came to heat along with the pregnancy rate were recorded for the hormone treated cows.

Estrus synchronization procedure

Before using hormones all the selected cows were treated with broad spectrum anthelmintics and vitamin as per recommended dose. The feeding and housing system kept similar. The cows those came to heat at the starting of hormonal treatment were excluded from estrus synchronization process. Three types of hormones viz., prostaglandins $F_2\alpha$ analogue (PG $F_2\alpha$), gonadotropin releasing hormone (GnRH) and human chorionic gonadotropin (hCG) were used to synchronize estrus of the selected cows. A short description of the hormones used for the treatment is given below:

Commercial hormone (injectable)	Recommended dose	Route of administration
Prostaglandins:		
Ovuprost® (Renata)	5±1 ml	Intramuscular (im)
Repromate® (ACI)	5±1 ml	Intramuscular (im)
GnRH:		
Ovurelin® (Renata)	5 ml	Intramuscular (im)
Fertagyl ® (Intervet)	5 ml	Intra muscular (im)
hCG:		
Chorulon ® (Intervet)	1500 -3000 iu	Intramuscular (im)

Protocolwise treatment cost is provided below:

Protocol group	Hormonal drugs used	Treatment cost	AI cost	Total Cost (Tk)
Protocol-I: $PGF_{2\alpha}$	Inj Ovuprost 5 ml @ 85 taka/ml	425.00	500.00	925.00
Protocol-II: $PGF_{2\alpha}$– $PGF_{2\alpha}$	Inj Ovuprost 10 ml@ 85 taka/ml	850.00	500.00	1,550.00
Protocol-III: $PGF_{2\alpha}$– $PGF_{2\alpha}$– $PGF_{2\alpha}$	Inj Ovuprost 15 ml @ 85 taka/ml	1,275.00	1,000.00	2,275.00
Protocol-IV: GnRH–$PGF_{2\alpha}$– GnRH	Inj. Ovurelin 10 ml (@ 70 taka/ml) & Inj. Ovuprost 5 ml @ 85 taka/ml	1,125.00	1,000.00	2,125.00
Protocol-V: $PGF_{2\alpha}$–GnRH– $PGF_{2\alpha}$	Inj. Fertazyl 5±1 ml (@ 750 taka/5ml & Ovuprost 10 ml	1,600.00	1,000.00	2,600.00
Protocol-VI: GnRH– $PGF_{2\alpha}$ Technique	Inj. Ovurelin 5±1 ml (@70tk/ml) Inj. Fertazyl 5±1 ml(@750/5ml)	1,100.00	500.00	1,600.00
Protocol-VII: hCG	Inj. Chorulon 5ml @ 650 taka/5ml	650.00	500.00	1,150.00
Protocol-VIII: Control	None	0	500.00	500.00

Note: In control group, only 20% animal came into heat normally. So, the cost was accounted only for 20% cows.

The anestrus cows were divided into eight different protocol groups for hormone treatment as described below with a flow chart shown in Figure 1:

Protocol-I:

$PGF_{2\alpha}$ Technique (n=20)

The $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health or Inj. Repromate, ACI, Animal health) was administered at day 0. After waiting for 7 days the cows showing the symptoms of estrus or heat were inseminated using AI.

Protocol -II:

$PGF_{2\alpha}$– $PGF_{2\alpha}$ Technique (n=20)

The $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health) was administered at day 0. Estrus was checked regularly and the positive cows were inseminated. The second injection with $PGF_{2\alpha}$ (Ovuprost) was administered at 11-14 days after the first injection. Then the estrus cows were inseminated using AI after 12 hours of standing estrus.

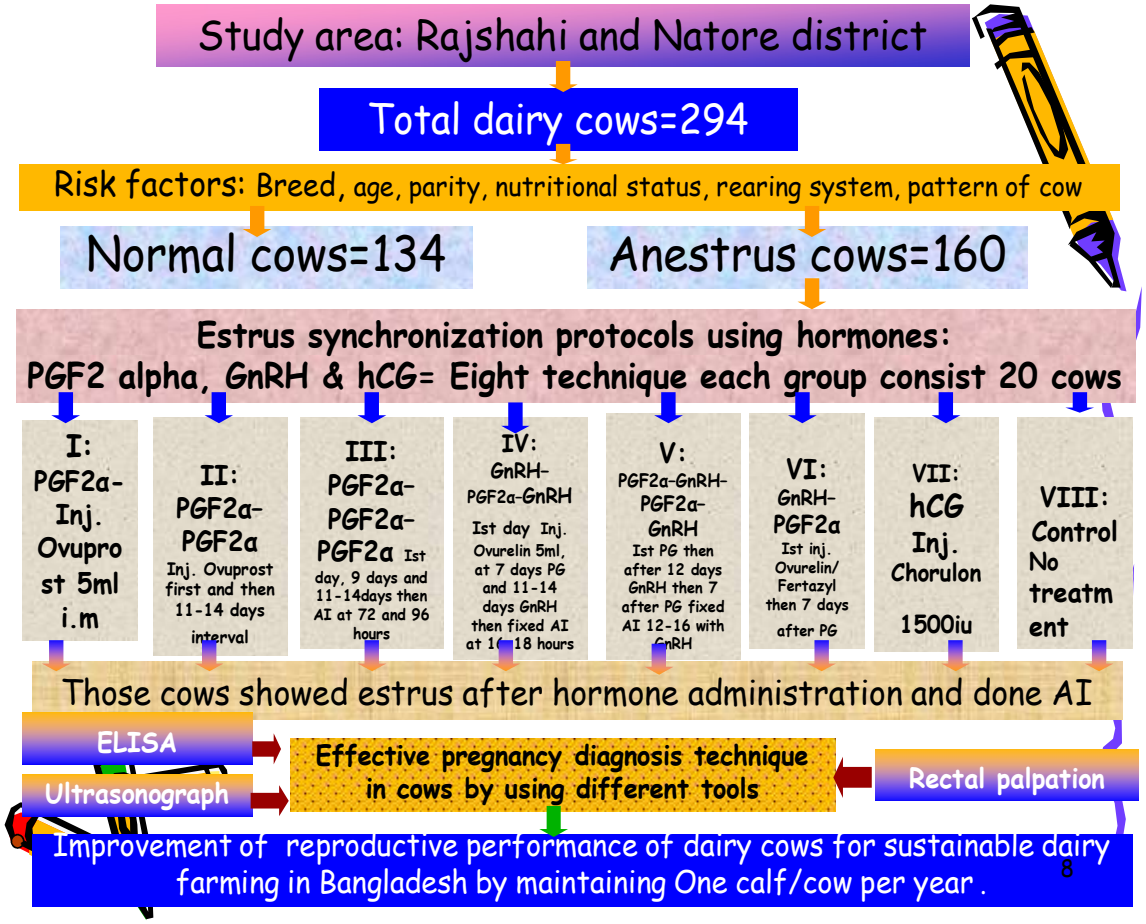

Figure 1. Flow chart of estrus synchronization protocols using hormones

Protocol -III:

$PGF_{2\alpha}$– $PGF_{2\alpha}$– $PGF_{2\alpha}$ Technique (n=20)

The $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health or Inj. Repromate, ACI Animal health) was administered at day 0. Estrus was checked regularly and the positive cows were inseminated. The second injection with $PGF_{2\alpha}$ was administered at 9 days after first injection and the third injection with $PGF_{2\alpha}$ was administered at 11-14 days after second injection. Estrus was checked after the third injection and the positive cows were inseminated two times.

Protocol -IV:

GnRH–$PGF_{2\alpha}$–GnRH Technique (n=20)

The GnRH (Inj. Ovurelin 5 ml, Renata, Animal Health or Inj. Fetazyl 5 ml, Intervet limited) was administered at day 0, the estrus was checked and estrus cows were inseminated through AI, second injection of $PGF_{2\alpha}$ was administered at 7 days after first injection. The third injection of GnRH was administered at 11-14 days after second injection. Estrus was checked and after 16-18 hours positive cows were inseminated (fixed time AI).

Protocol -V:

$PGF_{2\alpha}$–GnRH– $PGF_{2\alpha}$ Technique (n=20)

The $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health) was administered at day 0 and the second injection of GnRH (Inj. Ovurelin 5 ml, Renata Animal Health or Inj. Fetazyl 5 ml, Intervet limited) was administered 12 days after first injection. The third injection of $PGF_{2\alpha}$ was administered 7 days after second injection. Estrus was checked and positive cows were inseminated (fixed time AI) after 12-18 hours, along with GnRH injection.

Protocol VI:

GnRH– $PGF_{2\alpha}$ Technique, (n=20)

The GnRH (Inj. Ovurelin 5ml, Renata, Animal Health or Inj. Fetazyl 5 ml, Intervet) was administered at day 0, estrus was checked and the positive cows were inseminated. Second injection of $PGF_{2\alpha}$ (Inj. Ovuprost, Renata, Animal health) was administered 7 days after first injection. Estrus was checked and positive cows were inseminated after 12 hours of standing estrous.

Protocol -VII:

hCG Technique, (n=20)

The hCG (Inj. Chorulon 1500-3000 iu, Intervet limited) was administered intramuscularly at 0 day and the cows were observed up to 30 days for estrus. The cows showing estrus were inseminated.

Protocol VIII:

Control group (None) (n=20)

The control animals, where no hormone was used, were observed for one month for the estrus or heat to be expressed and the cows showing estrus were inseminated.

Estrus detection procedure

All the animals were monitored for the manifestation of estrus 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 described below:

Signs of heat in cows

Signs of heat at early stages are not pronounced in cows but they show some behavioral changes like, smelling other cows, attempting to mount on them and bellowing. The cows become restless and their vulvas get moist, red and slightly swollen. After a lapse of six to eight hours the heat becomes more pronounced and the cows stand still to be mounted by other cows or bulls. Due to this behavior the period is termed as standing heat. This extends up to 12-18 hours and shows other signs like bellowing nervousness, anorexia, reduction of milk yield, moist and red vulva and clear mucus discharge.

The time from the injection of prostaglandin to the onset of estrus 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 estrus).

Artificial insemination:

The cows under different protocol groups were closely observed by the farmers. When the cows showed the signs of estrus the farmer informed AI technicians for inseminating the cows using AI technique. Cows showing estrus 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 the genital tract. Any abnormalities found in the genital tract during rectal palpation were recorded. All inseminations were performed by three technicians.

Application of first injection of prostaglandin (PG) in cows with good body condition and presence of corpus luteum (CL) was expected for a successful fertile estrus. In case of unsuccessful estrus induction after first injection of PG, a second injection of PG was done after 10 days and the cows were inseminated between 72 and 96 hours irrespective of heat signs.

10.3 Evaluation of useful pregnancy diagnosis techniques:

The aim of this study was also to compare commercially available blood-based pregnancy tests for early pregnancy diagnosis in dairy cattle using transrectal ultrasonography and rectal palpation followed by confirmation of the pregnancy using ELISA kit through the determination of progesterone in the serum at 35 days after insemination.

Pregnancy diagnosis using ultrasonography

The pregnancy diagnosis was performed by transrectal ultrasound 30 days after AI using 6.0 MHz linear transducer (Made in Poland). The procedure in brief is given below:

As ultrasonograph examination of the reproductive tracts of cows largely depends on the operators skills on per rectal palpation, animals were first examined by rectal palpation and then by ultrasongraphuy to confirm the clinical diagnoses. Ultrasonography was performed by using a protalble ultrasound machine with liner array dual frequency probe (3.5 MHz). Real-time B-Mode ultrasound 3.5 MHz (frequency conversed from 3.5 -7.0 MHz) scanner (Esaoate Piemedical, Tringa Linvear) was used. After adequate restraining, the scanner was placed at a sensible distance from the cow on the side opposite to the operators arm engaged in rectal palpation. All the fecal materials from the rectum were evacuated prior to insertion of the transducer. The transducer face was lubricated with a suitable coupling medium (Ultrasonic Gel* for Medical use) 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. The ultrasound screen and operator's eye were a similar level for accurate interpretation of ultrasound image. Presence of fetus, fetal fluid, condition CL and uterus were examined on the day of diagnosis. The ultrasonography images were saved a multimedia kit attached to the instrument and subsequent transferred to a computer. By observing different types of images taken by ultrasound machine, diagnosis was done on the basis of echogenicity of the image. Optimum images were selected from the memorizer. Size of fetus and fetal fluid and CL images were measured by using electronic calipers (vertical and horizontal measures) and their echo characteristics were described and compared.

Pregnancy diagnosis through rectal palpation

Palpation of the uterine contents rectally is probably the most commonly used method for pregnancy diagnosis. Pregnancy diagnosis can be conducted as early as 30 days after service in heifers and 35 days in cows, although much practice is necessary to be able to determine pregnancy at that stage. Due to accumulation of fluids within the gravid uterine horn, one of the initial signs of pregnancy is a difference in size of the horns (uterine horn asymmetry). Also, it is possible to feel the slipping of the chorioallantoic membrane (fetal membrane) along the greater curvature of the uterus (membrane slip). As pregnancy progresses, it becomes possible to feel the fetus. After about day 150, the fetus stay too far forward in the body cavity to palpate the entire fetus although fetal structures can be palpated. Beginning at about day 90, it becomes possible to feel the placentomes, the structures are formed by the union of maternal caruncles and fetal cotyledons. From day 120, one can palpate the vibration of uterine artery, called fremitus, caused by the increase in blood flow in the artery. Diameter of the uterine artery increases from about 3-4 mm in a non-pregnant cow to about 10-15 mm in late pregnancy. Uterine position, diameter and structures felt by rectal palpation according to the stage of pregnancy are presented in Table-A.

Table-A: Pregnancy diagnosis through palpation of the uterine contents rectally

Stages of pregnancy (days)	Uterine position	Palpable structures
35-40	Pelvic floor	Uterine asymmetry/membrane slip
45-50	Pelvic floor	Uterine asymmetry/membrane slip
60	Pelvis / Abdomen	Membrane slip
90	Abdomen	Small placentomes / fetus (10-15 cm long)
120	Abdomen	Placentomes / fetus (25-30 cm long)/ Fremitus
150	Abdomen	Placentomes / fetus (35-40 cm long)/ Fremitus

Rectal palpation has the advantage of being an accurate, fast, relatively cheap pregnancy diagnosis method.

The following fertility parameter was studied

$$\text{Estrus detection Rate (\%)}: \quad \frac{\text{No. of detected in estrus}}{\text{No. Assigned}} \times 100$$

Estrus percent was calculated for each interval of interest

$$\text{Conception rate (\%)}: \quad \frac{\text{No. of conceived/ pregnant}}{\text{No. of detected estrus and AI}} \times 100$$

Conception rate was calculated for first service only.

The early pregnancy diagnosis of cows was confirmed using ELISA blood serum tests as follows:

ELISA blood serum tests for early pregnancy detection in dairy cows

Materials for ELISA test kits

- ✓ Goat anti- rabbit IgG-coated microtitre wells, 96 wells
- ✓ Progesterone Reference Standards: 0, 0.5, 3.0, 10, 25 and 50 ng/ml, liquids, 0.5 ml each, ready to use.
- ✓ Rabbit Anti-progesterone Reagent, 7ml.
- ✓ Progesterone-HRP Conjugate Concentrate (11x), 1.3ml.
- ✓ Progesterone-HRP Conjugate Diluent, 13ml.
- ✓ TMB substrate, 12ml.
- ✓ Stop solution, 12ml.
- ✓ Wash buffer concentrate (50x), 15ml.
- ✓ Control set (optional).

Components required

- ✓ Precision pipettes: 5µl~ 40 µl, 50 µl~ 200 µl and 1.0ml
- ✓ Disposable pipette tips.
- ✓ Distilled or deionized water.
- ✓ Vortex mixer or equivalent.
- ✓ Absorbent paper or paper towel.
- ✓ Linear- linear graph paper.
- ✓ Microtiter plate reader.

Specimen collection and preparation

- ✓ Serum was used. No special pre-treatment of sample was necessary.
- ✓ Serum was stored at 2-8°C for up to 24 hours, and was frozen at -10°C or lower for longer periods.

Sample, containing sodium azide was not used in the assay.

Storage of test kit and instrumentation

Unopened test kits was stored at 2-8°C upon receipt and the microtiter plate was kept in a sealed bag with desiccants to minimize exposure to damp air. An opened test kit was remaining stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range (0-2.5) O.D. or greater at 450 nm wavelength was used for absorbance measurement.

The procedure of the test in brief is stated below:

1. 2.5 ml blood was taken from jugular or ear vein of the dairy cows for serum preparation which was stored at -20^{o} C for later use.
2. Secured the desired number of coated wells in the holder
3. 25 μl of standard specimens were dispensed, and the controls into appropriate wells.
4. 50 μl of rabbit anti progesterone reagent were dispensed to each well.
5. 100 μl of working progesterone–HRP conjugate reagent was dispensed into each well and thoroughly mixed for 30 seconds followed by incubation at room temperature (18-22°C) for 90 minutes.
6. The micro wells rinsed and flicked 5 times with washing buffer (1x).
7. 100 μl of TMB substrate were dispensed into each well, gently mixed for 10 seconds and then incubated at room temperature (18-22°C) for 20 minutes.
8. The reaction was stopped by adding 100 μl of stop solution to each well.
9. Mixed gently for 30 seconds until all the blue color changed to yellow completely.
10. Absorbance at 450 nm with micro titer well reader was read within 15 minutes.

Statistical Analysis:

The raw data was sorted, computed and statistically analyzed by one way ANOVA using SPSS computer package version 19.0 to calculate the effect of hormones on the estrous cycle in relation to breed, age, body weight, body condition, lactation status, management system and farm size. Data pertaining to the induction of estrus was analyzed by using unpaired t-test (Steel and Torrie, 1982).

11. Results and discussion:

11.1 Prevalence of anestrus in relation to different risk factors in heifers and postpartum cows:

The present study was conducted with 294 dairy cows which were selected according to their history e.g., age, milk production, breed, body condition, parity, etc. from different private and government dairy farms at Rajshahi. The prevalence of anestrus in cows with respect to different risk factors is presented in Table 1 to 18. The Effect of breed on prevalence of anestrus in cows is presented in Table-1. The overall prevalence of anestrus in cows was 54% and numerically the lowest occurrence was seen in indigenous (50%) and the highest occurrence was seen in cross-bred (55%) cows. The genotype of cows was found to have significant effect on anestrus (Table-2).

Table 1. Effect of breed on prevalence of anestrus in cows

Breed	No. of Observation	No. of normal cow	No. anestrus cow	% anestrus cow
Indigenous	14	7	7	50.00%
Crossbred	280	127	154	55.00%
Total	294	134	160	54.42%

Table 2. Effect of genotypes on prevalence of anestrus in cows

Genotypes/genetic makeup	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Indigenous	14	7	7	50.00%$^{bc??}$
Indigenous× Frisian	265	122	143	53.96%^{b}
Indigenous× Sahiwal	15	5	10	66.66%^{a}
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

Effect of age of cows on occurrence of anestrus is presented in Table 3. Among the affected cows, significantly lower proportion of cows (39%) in the age group of > 6 years suffered from anestrus than that of cows in the age group of 4-6 years (51%) and <4 years (60%).

Table 3. Effect of age on the prevalence of anestrus in cows

Age group (Year)	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
< 4	160	64	96	60.00%^{a}
4 to 6	101	50	51	50.49%^{b}
> 6	33	20	13	39.39%^{c}
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

The highest occurrence of anestrus was observed in cows with the body weight of <200 kg (60%) and the lowest in >200 kg (51%) body weight group (Table 4). The variation in the occurrence of anestrus in cows was not significant at P>0.05 level.

Table 4. Effect of body weight on the prevalence of anestrus in cows

Body weight	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
<200 kg	114	46	96	59.64%
>200 kg	180	84	51	51.49%
Total	294	134	160	54.42%

Effect of body condition score (BCS) of cows on the occurrence of anestrus are presented in Table 5. Among the affected cows, significantly lower proportion of cows (51%) suffered from anestrus with good BCS (2.5-3.5) than that of cows (55%) with medium BCS (2 to 2.5) and poor BCS <2.0 (55%). Similar to herd BCS, individual cows with low BCS was found to suffer more from negative energy balance resulting in increased occurrence of anestrus. Accordingly, negative effect of poor BCS on conception rate in cows has been documented by Shamsuddin *et al*. (2001).

Table 5. Effect of Body condition score on the prevalence of anestrus in cows

Body condition (BCS)	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Poor (<2.0)	44	20	24	54.54%
Medium (2 to 2.5)	168	74	92	54.76%
Good (2.5-3.5)	82	40	42	51.21%
Total	294	134	160	54.42%

The effect of parity on the prevalence of anestrus in cows is presented in the Table 6. The highest occurrence of anestrus was recorded in heifer group (62%). The lowest was observed in 3rd parity (42%) followed by 4th parity and above (53%), 2nd parity (59%) and 1st parity (61%). The variation in the occurrence of anestrus among cows with different parity is probably due to the differences in sample size and other factors like breed, genetic makeup, weight, body condition, housing condition, farm size, etc. that could not be studied due to time constraints.

Table 6. The effect of parity on the prevalence of anestrus in cows

Parity	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Heifer	77	29	48	62.33%[a]
1st Parity	82	32	50	60.97%[ab]
2nd Parity	78	36	42	58.94%[b]
3rd Parity	38	22	16	42.10%[d]
4th Parity and above	19	9	10	52.63%[c]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c, d in the same column differs significantly with each other (P<0.05)

Effect of state of the animals on occurrence of anestrus is presented in Table 7. Among the affected animals the lower proportion (52%) suffered from anestrus in cow group than that of heifer group (62%).

Table 7. Effect of state of animal on the prevalence of anestrus

State of Animal	No. of Observation	No. of normal animal	No. of anestrus animal	% anestrus animal
Heifer	77	29	48	62.33%[a]
Cow	217	105	112	51.61%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

The effect of housing condition on the prevalence of anestrus in cows is presented in the Table 8. The prevalence of anestrus in cows was found to differ significantly (P<0.05) between the cows with good housing condition (43%) and poor (69%) & medium (69%) housing condition.

Table 8. Effect of housing condition on the prevalence of anestrus in cows

Housing condition	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Poor	75	23	52	69.33%[a]
Medium	51	16	35	68.62%[a]
Good	168	95	73	43.45%[b]
Total	294	134	160	54.4%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

Effect of floor condition of cows on the rate of anestrus is presented in Table 9. The prevalence of anestrus cows was significantly lower (P<0.05) when the cows were kept on Pacca floor (30%) followed by mixed (57%) and Kacha floor (66%).

Table 9. Effect of floor condition on the prevalence of anestrus in cows

Floor condition	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Kacha	74	25	49	66.21%[a]
Pacca	54	38	16	29.62%[c]
Mixed	66	71	95	57.22%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

The prevalence of anestrus in cows with respect to farm size is presented in Table 10. The occurrence of anestrus was significantly lower (P<0.05) in the farms having 10 to 20 cows (47%) than that of the farms with >20 cows (51%) and <10 cows (72%). This may be explained by the fact that cows in small farms (<10 cows) might have been neglected by the owner and large farms (>20 cows) lacked proper attention than that was received by the medium farms with 10 to 20 cows (Hewett, 1968). A study by Walsh *et al*. (2007) showed that the cow-level prevalence of anovulation is 19.5% with a herd-specific range from 5 to 45%. Moreover, farms having less number of breedable cows had better heat detection than that of the farms having more breedable cows. Further, it is likely that farms with low number of breedable cows had low sub-clinical uterine infection than that of the more breedable cows resulting in anestrus.

Table 10. Effect of farm size on the prevalence of anestrus in cows

Farm size	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
< 10 cows	67	19	48	71.64%[a]
10 to 20 cows	131	70	61	46.56%[c]
> 20 cows	96	45	51	53.12%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

Effect of feed quality on the prevalence of anestrus in cows is presented in Table 11. Significantly higher (P<0.05) proportion of cows were affected with anestrus those fed with poor (71%) and medium (65%) quality feed followed by the cows fed with good (48%) quality feed.

Table 11. Effect of feed quality on the prevalence of anestrus in cows

Feed quality	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Poor	7	2	5	71.42%[a]
Medium	103	36	67	65.04%[b]
Good	184	96	88	47.82%c
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

Effect of fodder availability on the prevalence of anestrus in cows is presented in Table 12. Among the cows under study, significantly (P<0.05) lower percentage (47%) was found to suffer from anestrus with abundant supply of fodder than that of cows supplied with medium (62%) and least (63%) quantity of fodder.

Table 12. Effect of fodder availability on the prevalence of anestrus in cows

Fodder availability	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Least	80	30	50	62.50%[a]
Medium	60	23	37	61.66%[a]
Abundant	154	81	73	47.40%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

Effect of health care on the prevalence of anestrus in cows is presented in Table 13. Significantly (P<0.05) higher prevalence of anestrus was observed in cows under poor health care group (71%), while the lowest was recorded in good health care group (48%).

Table 13. Effect of health care on the prevalence of anestrus in cows

Application of health care	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Poor	7	2	5	71.42%[a]
Medium	103	36	67	65.04%[b]
Good	184	96	88	47.82%[c]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b, c in the same column differs significantly with each other (P<0.05)

Effect of the types of medicine used for cows on incident of anestrus is presented in Table 14. The cows treated with allopathic medicine affected lowest (52%) with anstrus than those treated with herbal medicine (60%). However, these differences were not statistically significant.

Table 14. Effect of the type of medicine used on the prevalence of anestrus in cows

Medicine Type	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Allopathic	190	92	98	51.57%
Herbal	104	42	62	59.61%
Total	294	134	160	54.42%

The prevalence of anestrus in cows with respect to service provider is presented in Table 15. The presence of anestrus was significantly (P<0.05) lower in the farms where service was provided by DLS (28%) than that of the farms received service from private veterinarians (59%) and based on own knowledge (60%).

Table 15. Effect of service provider on the prevalence of anestrus in cows

Service provider	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
DLS	50	36	14	28.00%[a]
Private vets	54	22	32	59.25%[b]
Own knowledge	190	76	114	60.00%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

Effect of control measures against infectious diseases of cows on incidence of anestrus is presented in Table 16. Among the cows under study significantly (P<0.05) lower percentage (51%) was affected with anestrus where regular control measures were taken than that of the irregular (54%) and none (60%) group.

Table 16. Effect of control measures against infectious diseases on the prevalence of anestrus in cows

Control measures	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Regular	142	69	73	51.40%[a]
Irregular	74	34	40	54.05%[b]
None	78	31	47	60.25%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

The prevalence of anestrus in cows with respect to deworming practice is presented in Table 17. The occurrence of anestrus in cows was lower in regular deworming group (52%) than that of the cows where owners practiced irregular deworming (53%) but the difference was not significant. However, significant difference (P<0.05) was found with the group of cows where farmers did not use any anthelmintics at all (83%).

Table 17. Effect of deworming practices on the prevalence of anestrus in cows

Practice of deworming	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Regular	139	67	72	51.79%[b]
Irregular	137	64	73	53.28%[b]
None at all	18	3	15	83.33%[a]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other (P<0.05)

The prevalence of anestrus in cows with respect to farming experience of farmers is presented in Table 18. The prevalence of anestrus was significantly (P<0.05) higher in the cows with low (70%)

and medium (69%) experienced farmers having farming experience of < 3 years to 5 years than that of the farmers with > 5 years (47%) experience.

Table 18. Effect of farming experience of farmers on the prevalence of anestrus in cows

Farming experience (years)	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
<3	44	13	31	70.45%[a]
3-5	54	17	37	68.50%[a]
>5	196	104	92	46.93%[b]
Total	294	134	160	54.42%

Percentage of anestrus cows with different superscript letters a, b in the same column differs significantly with each other's (P<0.05)

Effect of training of farmers on the prevalence of anestrus in cows is presented in Table 19. The cows reared by trained farmers were less affected (52%) with anestrus than that of the cows handled by untrained farmers (60%) but the results were not statically significant.

Table 19. Effect of training of farmer on the prevalence of anestrus in cows

Training of owner	No. of Observation	No. of normal cow	No. of anestrus cow	% anestrus cow
Trained	201	97	104	51.74%
Un trained	93	37	56	60.21%
Total	294	134	160	54.42%

11.2 Development of effective estrus synchronization protocols using hormones:

Successful economy of a dairy farm production lies in ensuring proper and optimal reproductive cycle of individual cow within the normal physiological range. Anoestrus not only lengthen the postpartum interval, but also substantially reduce the farmer's financial returns from milk or beef sales. Considering this perspective the study was aimed to evaluate the effectiveness of US monitoring-based diagnosis and subsequent treatments of postpartum anestrus. Effects of hormones on anoestrus cows are shown in Table 20 and 21. It showed that when hormone treatment was given for induction of oestrus, higher (77%) proportion of cows showed oestrus compared to control counterpart (20%). The difference in the induction of estrus in cows with different treatment protocol groups were 60%, 85%, 85%,80%, 90%, 80% and 60% in PG, PG-PG, PG-PG-PG with fixed AI, GnRH-PG-GnRH 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 hormone treatment. Similar results were observed by Islam *et al.* (1997).

Table 20. Induction of estrous and pregnancy rate by using different synchronization protocols

Synchronization protocols	**No. of cows showed estrus (%)**	**No. of cows pregnant of the estrus cows**	**% cow pregnant**	**Fixed time AI (% conceived)**
PG (n=20)	12 (60)	10	83.33	-
PG-PG (n=20)	17 (85)	15	88.24	-
PG-PG-PG and fixed time AI (n=20)	17 (85)	14	82.34	3* (1)

GnRH-PG-GnRH fixed time AI (n=20)	16 (80)	13	81.25	4* (2)
PG-GnRH-PG fixed time AI (n=20)	18 (90)	16	88.89	2*(0)
GnRH-PG (n=20)	16 (80)	14	87.50	-
hcG (n=20)	12 (60)	10	83.33	
Grand Total	108 (77.14)	92	85.19	33.33%
Control (n=20)	4 (20)	4	100	-

*N.B. *marks indicate fixed time AI and parenthesis indicate no. pregnant*

Table 21. Cross table of the analysis of treatment protocol with its results

Result	Treatment group								Total
	PG	PG-PG	PG-PG-PG fixed time AI	GnRH-PG-GnRH fixed time AI	PG-GnRH-PG fixed time AI	GnRH-PG	hcG	Control	
Estrus	12	17	17	16	18	16	12	4	112
% within result	10.7	15.2	15.2	14.3	16.0	14.3	10.7	3.5	100
% within treatment group	60.0	85	85.0	80.0	90.0	80.0	60.0	20.0	70.0
% of Total	7.5	10.6	10.6	10.0	11.3	10.0	7.5	2.5	70.0
Negative	8	3	3	4	2	4	8	16	48
% within result	16.6	6.3	6.3	8.3	4.2	8.3	16.6	33.3	100
% within treatment group	40.0	15.0	15.0	20.0	10.0	20.0	40.0	80.0	30.0
% of Total	5.0	1.8	1.8	2.5	1.2	2.5	5.0	10.2	30.0
Count	20	20	20	20	20	20	20	20	160
% within result	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	100
% within treatment group	100	100	100	100	100	100	100	100	100
% Total	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	100

11.3 Evaluation of pregnancy diagnosis techniques in cows:

The mean levels of progesterone throughout the pregnancy and non pregnancy are shown in Table 22 to 24 and Figure 2. In all cows the major peak of progesterone occurred on the day of 90-120 days (9.85±0.8) and lowest in early pregnancy at 22-30 days (3.80±1.64), but in non pregnant cows it was about 1.50±0.30. The mean level of progesterone in pregnant cows was 7.30±2.03. When compared with genotype, the mean levels of progesterone were 6.34±2.99 & 6.02±2.54 in crossbred and in indigenous cows.

Table 22. Progesterone level at different stages of pregnancy period in dairy cows (n=90)

Stages of Pregnancy	No. of observation	Progesterone level (Mean ± SD) ng/ml
22-30 days	23	3.80±1.64[d]
31-60 days	23	5.09±1.93[c]
61-90 days	22	6.51±2.51[b]
91 - 120 days	22	9.85±0.8[a]
Total	90	6.27±2.90

Figure indicates (Mean ± SD) Standard deviation and a, b, c, d means superscripts are statistically significant (P<0.05) in between rows

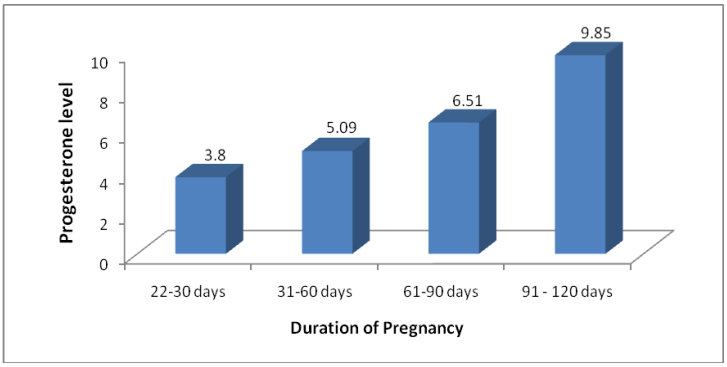

Fig. 2: Progesterone level at different stages of pregnancy period in dairy cow.

Table-23. Effect of breed on Progesterone level of pregnancy period in dairy cows

Breed of Cow	No. of observation	Progesterone level (Mean ± SD) ng/ml
Crossbred	70	6.34±2.99
Indigenous	20	6.02±2.54
Total	90	6.27±2.90

Table-24. Progesterone level in pregnant and non pregnant dairy cows

Pregnancy status	No. of observation	Progesterone level (Mean±SD)
Pregnant	74	7.30±2.03
Non-Pregnant	16	1.50±0.30
Total	90	6.27±2.90

The progesterone level of cows at different stages of their estrus cycle is shown in Table 25 and 26. The average progesterone level (Mean ± SD) of cattle was 7.30±2.03 & 1.50±0.30 in pregnant and no-pregnant cows in the study area (Table 24). When come in estrus the average progesterone level (Mean ± SD) was 0.762±0.39 and 4.833±2.16 in estrus and anestrus cows (Table 25) respectively. Qureshi MS *et al.* (2000) partially agreed with the current result and they observed milk progesterone at estrus was 0.30 ± 0.98.

Table-25. Progesterone level of estrus and anestrus in dairy cows

Status of cow	No. of observation	Progesterone level (Mean ± SD)
Estrus	41	0.762±0.39
Anestrus	36	4.833±2.16
Total	77	5.595±1.78

Table-26. T test for Progesterone level of estrus and anestrus dairy cow **(One-Sample Test)**

	Test Value = 0					
	test value	df (degree of freedom)	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Anestrus	13.420	35	.000	4.83361	4.1024	5.5648
Estrus	12.486	40	.000	.76220	.6388	.8856

The pregnancy diagnosis of cows was confirmed using ELISA, ultrasound and rectal palpation techniques. Efficacy of the techniques is shown in Table 27. In ELISA 98% cows were found pregnant where as 93% cows were found pregnant in ultrasound techniques but 100% cows were pregnant in rectal palpation.

Table-27. Conformation of pregnancy diagnosis by different technique

Technique of Pregnancy diagnosis	**No. of observation**	**No. Conformation by rectal palpation** (%)
ELISA	90	88 (97.77%)
Ultrasound	42	39 (92.85%)
Rectal Palpation (after 90 days)	95	95 (100%)

12. Research highlight/findings:

- The prevalence of anestrus cows was recorded to be 54.42%, which is alarming for sustainable dairy farming in Bangladesh because, the farmers' expectation of one calf/cow/year is hampered.
- The crossbred Sahiwal, <4 years age groups, <200 kg body weight, poor body condition, 1st parity cows and heifers were found at high risk of suffering from anestrus. However, lower prevalence of anestrus was observed in indigenous cattle (50%) compared to crossbred cows (55%).
- Good housing system, medium size farm, good quality feed, sufficient fodder, good health care, allopathic medicine, DLS health service, regular preventive measure, regular deworming, >5 years farming experience and trained farmers met with the less occurrence of anestrus in cows.
- With the PG-GnRH-PG treatment protocol 90% of the anestrus cows came to estrus followed by PG-PG and PG-PG-PG fixed time AI (85.0%) treatment protocol.
- The ELISA (97.77%) was found to be an effective early pregnancy diagnosis method in dairy cows with minimum cost.

- Ultrasound method (92.85%) was also found to be a good technique for early pregnancy diagnosis in dairy cows.
- Rectal palpation method (100%) was the best technique of pregnancy diagnosis when used after 90 days of pregnancy in cows.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	GD1 (Furniture)	68,000.00	100%	67,500.00	
	GD2 (Computer and accessories)	1,07,000.00	100%	106500.00	
(b) Lab & field equipment	GD3 (Lab or Field equipments)	1,34,250.00	100%	1,33,250.00	
	GD4 (Hormone)	2,90,000.00	100%	2,89,100.00	
(c) Other capital items	-	-	-	-	

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
			-	-	
			-	-	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	369637	349276	357137	12500		
B. Field research/lab expenses and supplies	678040	664331	653709	24331		
C. Operating expenses	139231	137656	131013	8218		
D. Vehicle hire and fuel, oil & maintenance	105936	96568	104600	1336		
E. Training/workshop/seminar etc.	0	0	0	0		
F. Publications and printing	74980	2829	0	74980		
G. Miscellaneous	125026	120900	123490	1536		
H. Capital expenses	307150	303709	303709	3441		

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To study the prevalence of anestus in relation to different risk factors in heifer and postpartum cows	• Questionnaire preparation and Survey • History, Clinical examination and observation	- About 54% of the cows were recorded to be anestrus. - Crossbred Sahiwal, <4 years age groups, <200 kg body weight, poor body condition, 1st parity cows and heifers were found to be at high risk of suffering from anestrus.	The knowledge will help dairy farmers to take care of their cows against anestrus problem that will help achieving one calf/cow/year
To develop effective estrus synchronization protocols using different hormones	• Grouping of Animals • Estrus synchronization • Treatment of selected anestrous cattle with the combination of different hormones.	- With the PG-GnRH-PG treatment protocol 90% of the anestrus cows came to estrus followed by PG-PG and PG-PG-PG fixed time AI (85.0%) treatment protocol.	The knowledge of hormone treatment protocol will help the field veterinarians to treat anestrus cows efficiently.
To evaluate the useful pregnancy diagnosis technique in cows by using different tools	• Pregnancy diagnosis by ELISA, Ultrasound methods and Rectal palpation • Comparison of the efficacy of different pregnancy diagnosis methods.	- Ultrasound & ELISA are useful for early pregnancy diagnosis. - Ultrasound, ELISA and Rectal palpation methods are useful for midterm pregnancy diagnosis. - Visual and Rectal palpation methods are useful for late pregnancy diagnosis.	The knowledge will help the field veterinarians to choose the most effective methods of pregnancy diagnosis at the different stages of pregnancy.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.		one	MANUAL OF REPRODUCTIVE DISEASES IN DAIRY COWS (vfxi cÖRbb †ivM-e¨vwa welqK cyw¯Ívv)
Journal publication		one	Effect of individual cows factor on prevalence of anoestrus in heifer and postpartum cows at Rajshahi region of Bangladesh (2019). Bangladesh Livestock Journal. 4:27-35.
Information development			
Other publications, if any MS thesis in Obstetrics under the Dept. of Veterinary and Animal Sciences, RU.		one	Pregnancy diagnosis in dairy cow by ELISA technique in Bangladesh

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Suitable estrus synchronization protocol for treating anestrus cows developed. With the PG-GnRH-PG treatment protocol 90% of the anestrus cows came to estrus and with PG-PG or PG-PG-PG fixed time AI treatment protocols 85.0% anestrus cows came to estrus.

ii. **Generation of new knowledge that help in developing more technology in future**

More than 54% of the cows in Bangladesh are found to be suffering from anestrus.
Crossbred Sahiwal, <4 years age groups, <200 kg body weight, poor body condition, 1st parity cows and heifers are found to be at high risk of suffering from anestrus.

iii. Technology transferred that help increased agricultural productivity and farmers' income

None

iv. Policy Support

The knowledge generated through this sub-project, related to the prevalence of anestrus cows and the risk factors of the cows suffering from the disease will help policy makers in the development of control measures against anestrus in cows to save dairy industries in Bangladesh.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring (description & output of consultation meeting, monitoring workshops/seminars etc.):

- Progress Review workshop on CRG sub-projects, PIU-BARC, NATP-2 under livestock division, BARC was held on 24-25/04/2018.
- Progress Review Workshop on CRG Sub Project, PIU-BARC, NATP-2 Under Livestock Division, BARC, was held on 23 September, 2018.

ii) Field Monitoring (time & No. of visit, Team visit and output):
Monitoring Status

Monitoring team	Date(s) of visit	Total visit till date (No.)	Remarks
Technical Division/ Unit, BARC	02/04/2017	02	1. Dr. Shah Md. Ziqrul Haq Chowdhury, Member Director (Livestock), BARC. 2. Dr. Md. Rafiqul Islam, PSO (Livestock), BARC
PIU-BARC, NATP-2	07/03/2017	02	Two Officers from NATP
Internal Monitoring	15/06/2017	02	Prof. Md. Saiful Islam, Ex dean, and Prof. Saleha Jesmin, Dean, Faculty of Agriculture

H. Lesson Learned

Rainy season was found not to be suitable for estrus synchronization protocols of cows at field level.

I. Challenges (if any)

1. Tagging of the cows for identification was difficult at farmer level.2.
3. Maintaining uniformity in management was very difficult at farm level.
4. Pregnancy diagnosis was found very difficult at farm level by using Ultrasound.
5. Some owners sold their cows after pregnancy that made record keeping for the study difficult.

Signature of the Principal Investigator

Professor Dr. Md. Jalal Uddin Sarder
Department of Veterinary and Animal Sciences
University of Rajshahi
Rajshahi-6205, Bangladesh

Signature of the Co-principal Investigator

Dr. Rashida Khaton
Associate Professor
Department of Veterinary and Animal Sciences
University of Rajshahi, Rajshahi-6205,
Bangladesh

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal

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Appendex-1

Plate 1: Diagnosis of anestrus cows by rectal palpation

Preparation for hormone injection

Injection of hormone in a L x F cross breed cow for esturs synchronization

PI is observing BCS of Cows

Plate-2: Activities showing the process of estrus synchronization

Blood collection for serum preparation

Blood stored in refrigerator for serum collection

Numbering of samples for ELISA

Preparation of ELISA plates

Plates showing ELISA results

Reading of ELISA plates

Plate-3: Activities showing sample preparation and ELISA test for pregnancy diagnosis.

Pregnancy diagnosis by Ultrasound method

BAU Professor helping pregnancy diagnosis by Ultrasound method

Ultrasound monitor reading for pregnancy diagnosis

Plate-4: Activities showing pregnancy diagnosis by Ultrasound method

PI is briefing about project activities to BARC expert members at project Office.

BARC expert members are visiting a dairy farm.

BARC expert member is interviewing a dairy farmer.

Plate-5: Filed visit by expert members from BARC

Plate-6: Calves from estrus synchronized dairy cows at the study areas