Project ID -603

Competitive Research Grant

Sub-Project Completion Report

on

Substantial development of genetic potential for improved productivity in cattle through manipulative reproduction technology

Project Duration

May 2017 to September 2018

Implementing Division/Department with organization
Department of Surgery and Obstetrics
Faculty of Veterinary Science
Bangladesh Agricultural University, Mymensingh

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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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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Acknowledgement
The execution of CRG sub-project has successfully been completed by Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grant fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who have made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

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Acronyms

AI	-	Artificial Insemination
AV	-	Artificial vagina
CASA	-	Computer Assisted Semen Analyzer
ELISA	-	Enzyme-linked Immunosorbent Assay
E2	-	Estradiol
GnRH	-	Gonadotropin Releasing Hormone
IVOS II	-	Integrated Video Optical System
P4	-	Progesterone
PCR	-	Polymerase Chain Reaction
PGF2α	-	Prostaglndin F2 α
RFLP	-	Restricted fragment length polymorphism
SSM	-	Semen separation medium
T	-	Testosterone
VER	-	Vaginal Electrical Resistance
VWP	-	Voluntary waiting period

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Executive Summary

Aims of the project were to examine the socio-economic impact of genetic improvement of cattle via conventional artificial insemination (AI) program in selective areas of Bangladesh, and to implement the manipulative reproduction techniques such as semen processing, estrus synchronization and timed artificial insemination (TAI) at the farm level for the production of sex selective offspring to speed up the genetic improvement of economic traits of cattle.

In total four experiments were conducted under this period. Initially, a survey was conducted in Experiment-1 to study the factors affecting the adoption of AI, the reproductive and productive performances of cattle produced after AI. Farmer's education and the breeds of bull were found to play an important role for the adoption of AI. This study also reports that income per cow was slightly increased after breed up gradation by AI in cattle of some selected areas of Bangladesh.

Experiment-2 was performed to study the factors such as parity, time of insemination, daily milk production, VER of vaginal mucus, serum level of progesterone, testosterone and estrogen at the time of AI, affecting pregnancy rate and production of sex selective offspring in cows inseminated with frozen-thawed semen after observing natural estrus. The results revealed that sex hormones, oestrogen and testosterone, present at the time of AI might play a major role for sex characterization in cattle. It was observed that 45% male and 55% female claves were born after AI with frozen semen.

Experiment-3 was performed to investigate the effect of swim up technique on the quality of spermatozoa in terms of viability%, plasma membrane functional integrity% and motility especially kinetic velocity parameters of bull spermatozoa and fertility rate. Morphometry of spermatozoa collected from fresh and different swim-up fractions of different breeding bulls were also studied with CASA HT IVOS II. Significant difference ($p<0.05$) was seen in sperm morphometry between different bulls and different fractions of semen. A total of 229 AI was performed with fractioned semen in cows/heifers after observing natural estrus. It was found that more male calves were born after AI with Fraction-1 (72.9%) and Fraction 2 (67.4%). In contrast, 65.8% and 85.4% female calves were born after AI with Fraction-3 and Fraction-4 respectively.

Experiment-4 was conducted to study the efficacy of three synchronization protocols in dairy cows of commercial farms. The highest pregnancy (92.3%) and calving rates (89.7%) were obtained in cows of Co-synch protocol group. The overall 42% male and 57% female claves were born after TAI with frozen–thawed semen.

It is very worthy to mention that vaginal speculum incorporated with video camera and AI gun equipped with mobile video monitoring system (BAU AIVision) was developed for the Veterinarians and AI technicians under the financial support of this project.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project:

Substantial development of genetic potential for improved productivity in cattle through manipulative reproduction technology

2. Implementing organization:

Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh

3. Name and full address with phone, cell and e-mail of PI:

Nasrin Sultana Juyena, PhD

Professor, Department of Surgery and Obstetrics

Faculty of Veterinary Science

Bangladesh Agricultural University, Mymensingh

4. Sub-project budget (Tk):

4.1. Total: Tk. 22,35,000.00

4.2. Revised (if any): Not applicable

5. Duration of the sub-project:

5.1. Start date (based on LoA signed): 14 May, 2017 5.2.

5.2. End date: 30 September 2018

6. Justification of undertaking the sub-project:

Manipulative reproduction technology (MRT) has the potential to improve production, reproductive efficiency and rates of genetic improvement in livestock. Artificial insemination (AI) is the only economic technique used for improvement of genetic potential of cattle. Although AI has been in practice in Bangladesh for last 50 years, the efficiency and socio-economic impact of this technique has not been well-documented. Under small farm conditions, a number of socio-economic, organizational, biological and technical factors make AI service more difficult to provide. If the major constraints of this technique can be identified and overcome, the technology would become more widely adopted and contribute to an increased production of milk and meat, leading to better food security and poverty alleviation. Moreover, sex ratio manipulation can sensibly enhance the effectiveness of selection and genetic improvement programs, through production of male or female offspring born after AI. Therefore, the aim of the project was to assess the socio-economicai impact of conventional AI for genetic improvement program and the development of proper strategies for the production of sex selective offspring using MRT such as production of high quality sperm, oestrus synchronization and timed artificial insemination (TAI).

7. **Sub-project goal:** To increase livestock production through sex selective offspring production with genetic improvement.

8. **Sub-project objective (s):**

   i) To examine socio-economical impact of genetic improvement of cattle via conventional artificial insemination (AI) program in some selective areas of Bangladesh.

   Ii) To implement the manipulative reproduction techniques at the farm level for the production of sex selective offspring to speed up the genetic improvement of economic traits of cattle

9. **Implementing location (s):**
   Keranigangj and Savar upazila of Dhaka; Srinagar upazila of Munshigonj; Muktagachha upazila of Mymensingh; Shahjadpur upazila of Sirajgonj; Madarganj upazila of Jamalpur; Bogura Sadar upazila of Bogura; Singair upazila of Manikgonj; Kushtia sadar upazila of Kushtia and Karnaphuli upazila of Chattagram.

10. **Methodology in brief:**

10.1. Socio-economical impact of genetic improvement of cattle via conventional artificial insemination (AI)

<u>Experiment-1</u>

A survey was conducted involving a total of 970 farmers using pre-structured questionnaire to study the demography and socio-economic characteristics of farmers viz. farmer's education, occupation, breed selection, different parameters of heifers and cows born after AI such as age, body weight, parity, feeding system, management, oestrus length, anoestrus, daily milk production, lactation period, voluntary waiting period (VWP), seasonality, sex of offspring, calving interval, pregnancy rate, feed cost and milk prices, etc.

<u>Experiment-2</u>

Experiment-2 was performed to study the factors affecting pregnancy rate and sex selective offspring production in cows inseminated with frozen semen after observing natural oestrus (Figure 1). One hundred forty cows and heifers were allowed for AI in natural estrus at Research Animal Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. Breed, age, parity, milk production, post-partum period, onset of estrus, time of AI, vaginal electrical resistance, etc. were recorded at the time of AI.

Recording of Vaginal Electrical Resistance (VER): VER values were recorded in all animals at the time of AI using Electronic Heat Detector (DRAMINSKI®, Owocowa 17, Poland).

Hormonal analysis**:** Blood was collected to measure Estradiol (E2), Progesteron (P4) and Testosterone (T) level at the time of AI using ELISA kit (NovaTec Immundiagnostica GmBH, Germany).

Ultrasonography of reproductive tract: Ultrasonography of reproductive tract was performed for the observation of uterus, follicular size at the time of AI and pregnancy using Ultrasound Machine (YSVET0206 Veterinary Ultrasound Machine®, Hongkong) in all cows and heifers included in the experiments.

10.2. Use of manipulative reproduction techniques for the production of sex selective offspring

Experiment-3

Experiment-3 was performed to investigate the effect of swim up technique on the production of sex selective offspring in cows. Semen was collected from three adult crossbreds (Jersey X, Sahiwal X, Brahman X) and one purebred bull (Friesian 100%) using artificial vagina (AV) method to evaluate the effect of swim-up technique on quality of spermatozoa in terms of viability%, plasma membrane functional integrity% and CASA motility parameters of bull spermatozoa and fertility. CASA motility parameters included Amplitude of lateral head displacement (ALH), Beat cross frequency (BCF), Linearity (LIN), Average path velocity (VAP), Curvilinear velocity (VCL) and Straight-line velocity (VSL). Fresh ejaculates (n=15) were allowed for modified swim-up separation using semen separation medium (SSM). Four supernatants were collected with 15 minutes interval each and named as first, second, third and fourth fractions (Figure 1). Fertility of swim-up separated fractions was studied following artificial insemination (AI). In the meantime, morphometry of bull spermatozoa was studied.

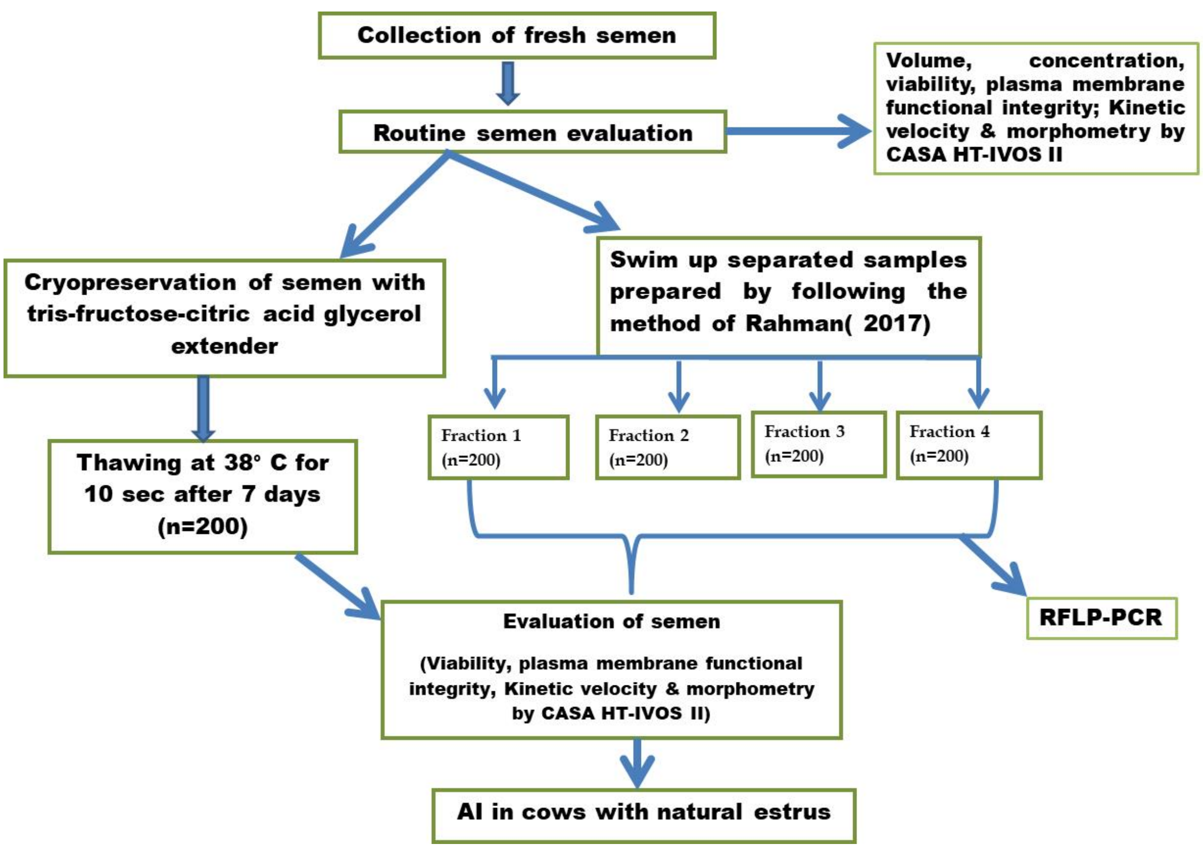


Figure1: Experimental design for semen processing and preservation

Determination of sperm morphometric parameters

Morphometry was analyzed by zoomed on any sperm on the analyzed image on CASA HT IVOS-II screen by moving the cursor over it and clicking when the orange box appears on zoomed cell playback screen. The zoomed cell playback screen was opened showing the enlarged sperm with a red dot identifying its centroid (Figure-2). Individual normal sperm (n=200) morphometric parameters such as Head length (µm)- Distance along the main axis; Head Width (µm)- Distance along the smaller axis; Head perimeter (µm)-Outline of the head, Head elongation- (Head length- Head Width)/(Head length + Head Width); Head area (µm^2) Surface inside the perimeter; Tail length (µm)- Distance of tail, were considered for each fractions.

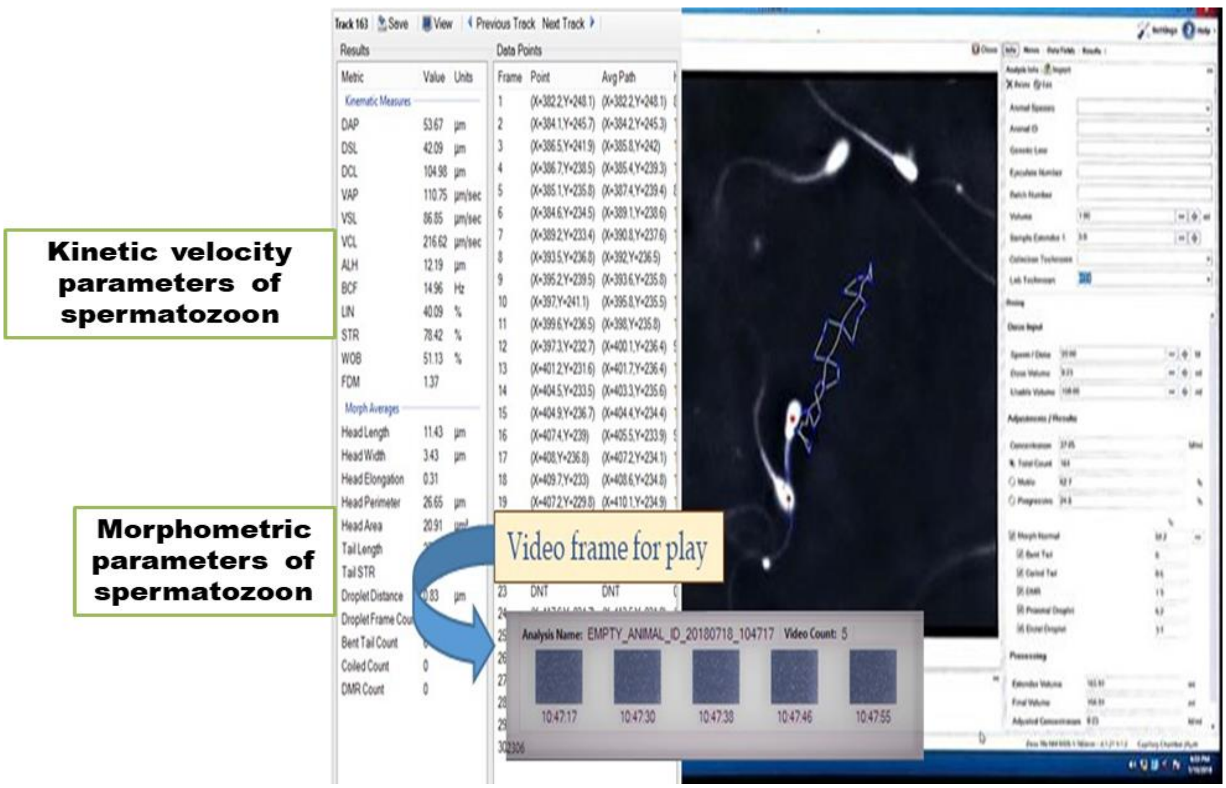


Figure 2: Zoomed cell playback screen for morphometry and kinetic velocity measurements in IVOS II

Restricted fragment length polymorphism (RFLP) PCR

Amplification was performed in a 272º thermal cycler (Applied biosystems, Thermo Fisher Scientific, Germany) using commercial DNA extraction kit (Thermo Fisher Scientific, Baltics UAB, Lithuania). The primers were designed on the basis of a highly conserved region of the *ZFX*/*ZFY* loci of bovine (Thermo Fisher Scientific, Baltics UAB, Lithuania).

Primers used

Forward primer sequence F: 5_-ATA ATC ACA TGG AGA GCC ACA AGC T-3’.
Reverse primer sequence R: 5’-GCA CTT CTT TGG TAT CTG AGA AAG T-3’.

PCR assays were performed following an initial denaturation at 95°C for 5 minutes followed by 35 amplification cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 2 minutes, and a final extension for 7 minutes at 72°C. An aliquot of each PCR reaction sample was digested by *SacI* following the manufacturer's recommendations.

Experiment-4

Experiment-4 was performed to study the efficacy of three synchronization protocols (OVSYNCH, Co-SYNCH, Pre-SYNCH) in milking cows. Total one hundred and twenty cows were synchronized and inseminated in as described in Figure 3. GnRH (Gonadorelin Acetate- Ovurelin®, Renata Limited, Bangladesh), PGF2α (Cloprostenol-Ovuprost®, Renata Limited, Bangladesh) progesterone (Eazi-Breed CIDR®, Cattle Insert, Pfizer Animal Health) were used for this study.

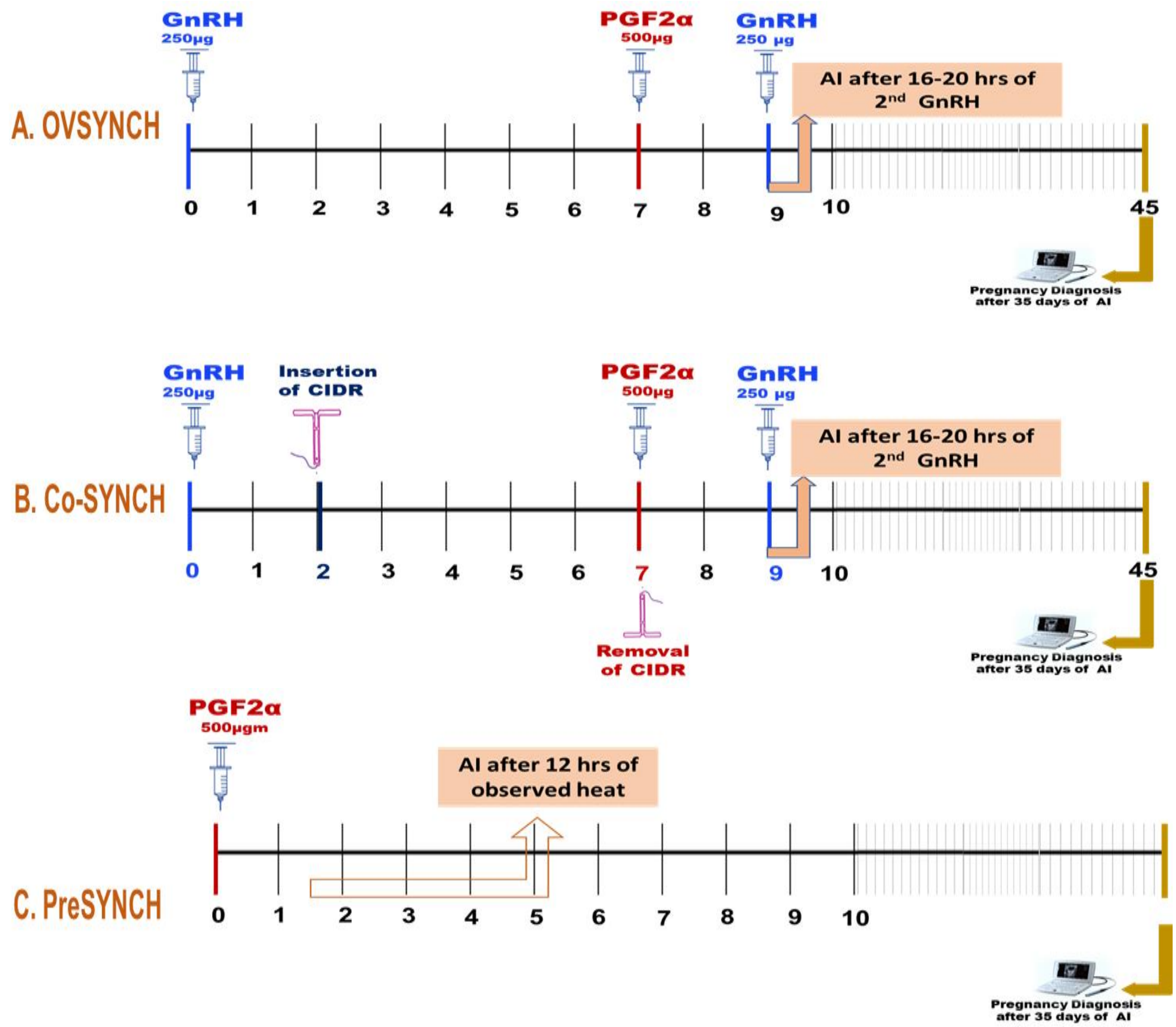


Figure 3: Schematic presentation of OVSYNCH (A. GnRH+PGF2α+GnRH+TAI), Co-SYNCH (B. GnRH+CIDR+PGF2α+GnRH+TAI) and (C. PGF2α+AI) Pre-Synchronization protocols

Statistical analysis

Logistic approach was used to report the socio-economic characteristics of the respondents. The study assumed that, households adoption of artificial insemination is a binary variable, Y, take the value (= 1 when the household adopts artificial insemination; 0 otherwise). We could define y = $\begin{cases}1\\0\end{cases}$ where 1 indicates the positive outcome with probability P and 0 indicates the negative outcome with probability (1 − P). Then the logit transformation is defined as: g(x) = logit (P) = log $[\frac{P}{1-P}]$; which is a function of explanatory variables. Finally, the binary logit model can be expressed as follows:

g (x) = logit (P) = $\boldsymbol{\beta_0 + \sum_{i=1}^{k} \beta_i X_i}$

Where, X is a vector of k independent variables that may affect the households adaption of artificial insemination, β is a vector of regression coefficients to be estimated. However, as we know that econometric analysis, with cross-sectional data often suffers from the problem of multicollinearity which results in inaccurate estimates of parameter. Consequently, the present study uses the variance inflation factor (VIF) in order to detect multicollinearity.

The difference in the reproductive parameters in the corresponding generation (grandmother, mother and daughter) and comparison of VER, follicular diameter, E2 and P4 of pregnant cows was tested using one way analysis of variance (ANOVA) in the study. Two way analysis of variance (ANOVA) followed by the Tukey test was done to find out significant differences in semen parameters of different breeding bulls and different fractions. All the statistical analyses were done using IBM SPSS Statistics 20.0. Differences were considered significant at the level of $P<0.01$ and $P<0.05$.

11. Results and discussions:

11.1. Socio-economical impact of genetic improvement of cattle via conventional artificial insemination (AI)

Factors affecting the adoption of AI using different bull semen by farmers

Total number of farmers from ten different areas of Bogura, Shirajgonaj, Manikgonj, Mymensingh, Dhaka, Kushtia, Jamalpur, Chattagram and Munshigonj districts included for the study are shown in Figure 4.

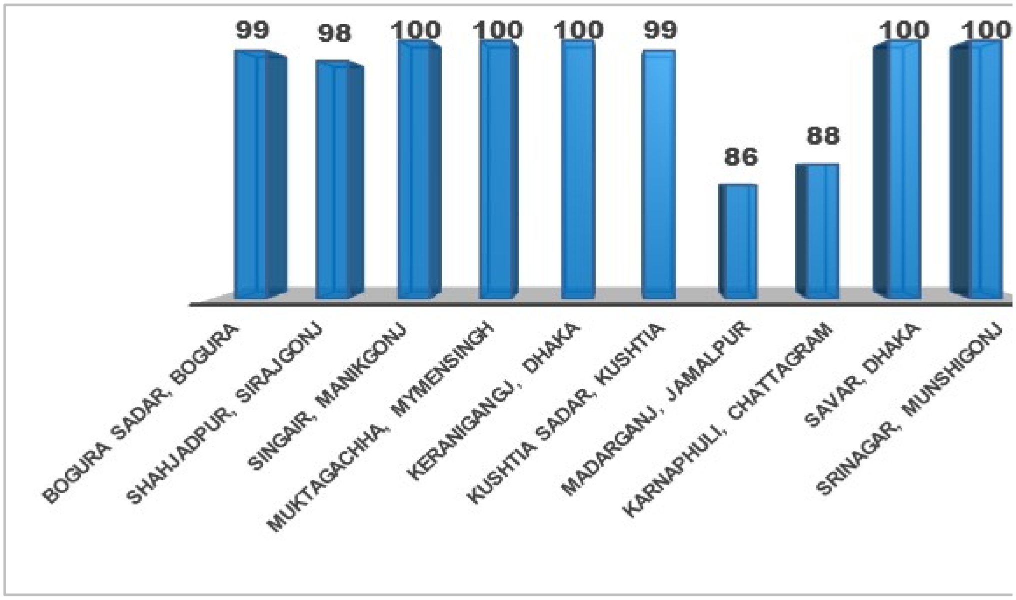


Figure 4: Sample size (n=970) used in this study

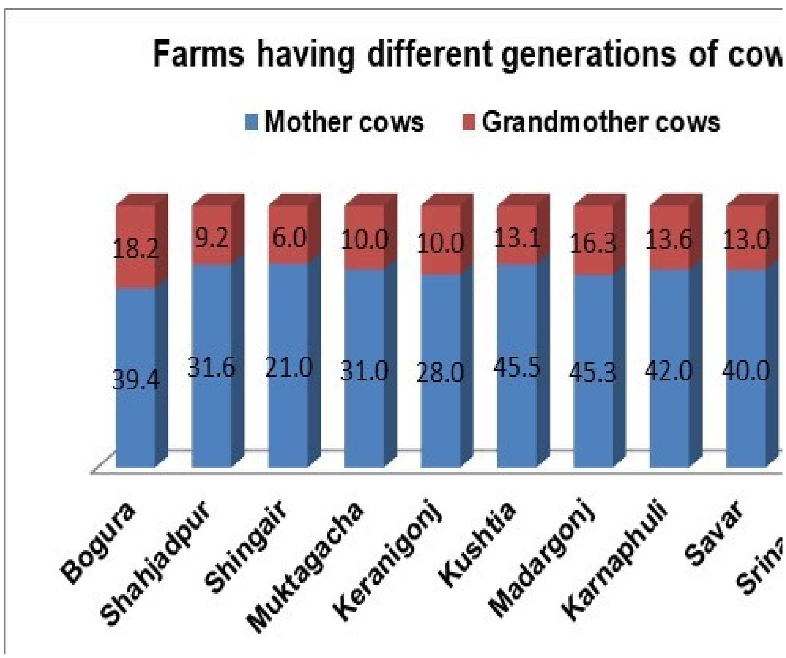


Figure 5: Generations of cows observed in farms of selected areas of Bangladesh

Variables used in the study: The definitions of the variables used in the study with their summary statistics are presented in Table 1. The study found that there was slight variation in the adoption of artificial insemination in our sampled households in rural Bangladesh.

Table 1. Definition and summary statistics of the variables used in the study

Variable	Definition	Mean	SD
Dependent variable			
Artificial Insemination	=1 if the household head adopts artificial insemination; 0 otherwise	0.95	0.21
Explanatory Variables			
Family size	Household size (Persons per household)	5.51	1.34
Education 1	=1 if the household head has no schooling; 0 otherwise	0.20	0.40
Education 2	=1 if the household head has primary education; 0 otherwise	0.30	0.56
Education 3	=1 if the household head has secondary education; 0 otherwise	0.43	0.49
Education 4	=1 if the household head has tertiary education; 0 otherwise	0.07	0.26
Occupation 1	=1 if the main occupation of household head is only dairy farming; 0 otherwise	0.78	0.42
Occupation 2	=1 if the main occupation of household head is Govt./Non Govt. job along with dairy farming; 0 otherwise	0.09	0.28
Occupation 3	=1 if the main occupation of household head is business along with dairy farming; 0 otherwise	0.14	0.34
Working member	Number of working person in the household (Persons per household)	0.99	1.46
Bull selection	=1 if the household select bull by himself; 0 otherwise	0.34	0.47
Cost	Cost incurred by the households for rearing the Cow (BDT)	334.80	103.35
Friesian cross	=1 if the household select Friesian bull for AI ; 0 otherwise	0.88	0.33
Sahiwal cross	=1 if the household select Sahiwal bull for AI ; 0 otherwise	0.05	0.22
Others	=1 if the household select other bull for AI ; 0 otherwise	0.07	0.26

Multicollinearity diagnosis for the explanatory variables

Econometric analysis with cross-sectional data is usually allied with the problem of multicollinearity which can lead to imprecise parameter estimates. The present study used the variance inflation factor (VIF) to detect multicollinearity and concluded that the explanatory variables were free from multicollinearity as the value of VIF for all the variables were less than 10 as seen in Table 2.

Table 2. Multicollinearity diagnosis for the explanatory variables

Variables	Variance Inflation Factor (VIF)
Occupation	1.08
Working Men	1.06
Family Size	1.05
AI Cost	1.05
Education	1.03
Bull Selection	1.02
Breed of Bull	1.02

Logistic model to identify associated factors with adoption of AI

Table 3 represents the results of the logistic regression and the significant likelihood ratio statistic implies that the explanatory variables have contributed well to the adoption of AI in the rural households in Bangladesh. The positive and statistically significant coefficient of education of the household head indicates that households head having secondary and tertiary educations are more likely to adopt AI than the households those had no education. The households whose main occupation was dairy farming were more likely to adopt AI than the households those had business along with dairy farming as their main occupation. On the contrary, the households whose main occupation was government/non-government job along with dairy farming were less likely to adopt AI than the households those had business along with dairy farming as their main occupation. The positive and highly significant coefficient of bull selection indicates that households selecting Friesian bull were more likely to adopt AI than the households those selected other types of bull.

It was evident that education of farmers (specially farmer's with tertiary education) and breed of bull (specially related to more milk production) affected the adoption of AI significantly ($P<0.001$) in this study. Massy *et al*. (2004) observed similar results and stated that adoption happens quickly for the individual with better education, receptive to new ideas, self-confident and younger, and when the farm system is large, profitable, endowed with absorptive capacity, able to transplant information, and linked with other farms and networking. Adoption of breeding technology is considered as a key element in structural changes in the livestock industry (Gillespie *et al.,* 2004), as it directly affects performance (Olynk and Wolf, 2008). Farmer's technology adoption decisions are generally affected by a number of demographic and socioeconomic factors. In an economic sense, farmers adopt technology if the utility associated with adopting is greater than the utility associated with non-adopting. Genetic improvement in dairy cattle is driven primarily by the array of bull genetics provided by the AI industry and secondarily by the choices producers make among the available bulls (Shook, 2006).

Table 3. Logistic model to identify the associated factors with artificial insemination (AI)

Variable	Dependent variable = Artificial Insemination (AI)		
	Parameter estimate	**SE**	**P-value**
Constant	0.6858	3.6707	0.852
Family size	0.0051	0.1364	0.970
Education 1	Reference category		
Education 2	0.1254	0.4461	0.779
Education 3	1.4156***	0.4809	0.003
Education 4	1.8354*	1.0973	0.094
Occupation1	1.6392***	0.4474	0.000
Occupation2	-1.4269***	0.5207	0.006
Occupation3	Reference category		
Working member	0.0066	0.1072	0.951
Bull selection	0.3918	0.3950	0.321
lncost	-0.1008	0.6107	0.869
Friesian cross	2.2409***	0.4408	0.000
Sahiwal cross	-0.8372	0.5167	0.105
Others	Reference category		
Diagnostics			
LR: $\chi 2(11)$	122.38		
Prob> $\chi 2(11)$	0.0000		
Log likelihood	-123.9858		
Pseudo R2	0.3305		
Number of observations	**971**		

*Note: ***, **, * indicate the significance level at 1%, 5% and 10%, respectively.*

Factors affecting production of male and female offspring after conventional AI

The study was conducted to observe the factors affecting production of male and female offspring after conventional AI. The pregnancy rate obtained in this study was 67.14% (n=94/140). Table 4 shows different parameters among the pregnant and non-pregnant cows and heifers observed at the time of AI.

Table 4. Comparison of different parameters among the pregnant and non-pregnant cows and heifers observed at the time of AI

Parameters	Pregnant (n=91)	Non-pregnant(n=49)	P value
Estradiol (pg/ml)	14.77±0.24 (10.76-19.47)	9.26±0.18 (7.66-11.36)	0.000
Progesterone (ng/ml)	0.85±0.01 (0.56-1.21)	2.01±0.09 (1.43-3.10)	0.000
Testosterone (ng/ml)	0.88±0.02 (0.54-1.36)	2.01±0.09 (0.98-2.89)	0.000
Follicular Diameter (mm)	13.78±0.21 (1.20-17.89)	10.15±0.15 (8.56-10.75)	0.000
Vaginal Electrical Resistances (VER) (Ω)	197.26±1.76 (163.33-226.67)	256.41±1.91 (236.67-263.33)	0.000

The value of pregnancy is higher than that observed by Paul *et al*. (2011) and Khan *et al*. (2015) who found 57.3% and 59.3% pregnancy rate, respectively in the island areas of Bangladesh. There were significant (P>0.01) difference in pregnant and non-pregnant cows and heifers in term of VER, follicular size, serum E2 and P4 levels.VER value was 201.1±2.32 in pregnant animals. This finding is slightly higher than that observed by Ahmed (2016). The pregnancy rate was significantly (p>0.01) higher in cows when VER during oestrus was lower. The present finding is in agreement with earlier study of Patil and Pawshe (2011) and Tasal *et al. (*2005), who reported that vaginal electric resistance (VER) was low (230 Ω) during oestrus. During the various stage of oestrus cycle, certain changes occur in composition and relationship of some electrolytes, which leads to ionization of oestrus mucus affecting its electrical conductivity. Based on these characteristics of oestrus mucus, method for determining the electrical resistance of oestrus mucus has been developed, and it helps to detect the optimum time for insemination or mating (Rorrie*et al.,* 2002). We found that diameter of dominant follicle was 13.78±0.21 mm at the time of AI in pregnant cows. This is inconsistent with the findings of Figueiredo *et al*. (1997) and Sartorelli *et al.* (2005). During oestrous cycles in taurine cows, the dominant follicles has been shown to reach a maximum diameter of approximately 10–20 mm (Ginther *et al.,* 1989c) and the largest subordinate follicles to reach maximum diameters of approximately 8 mm (Ginther *et al.,* 1989a). *Bos indicus* cows have smaller dominant follicle diameters (12.3 and 11.3 mm, respectively) in cows with two-wave cycles (Figueiredo *et al.,* 1997; Sartorelli *et al.,* 2005). In a recent study, Keskin*et al.* (2016) have stated that the follicle size can be affected by breed, milk production, parity and season. In addition, pregnancy and embryonic loss in lactating dairy cows were significantly related to follicular size at the time of AI. We found that overall estrogen level in pregnant cows during oestrus was 14.745±0.24 pg/ml which is not similar with the finding of Naik *et al.* (2013). Naik *et al.* (2013) have reported that the overall mean concentration of estrogen in the Punganur cows during estrous cycle on the day of estrus was 20.24±1.17 pg/ml. Ovarian estradiol plays an important role in establishing the timing of uterine receptivity by regulating oviductal secreted glycoproteins (Buhi, 2002; Ozturk *et al.,*2010). Increased preovulatory concentrations of estradiol have been reported to influence sperm transport (Hawk, 1983), embryo quality and survivability, and a >60% improvement in pregnancy success (Atkins *et al.,* 2013). Moreover, follicular diameter shares a positive relationship with peak concentrations of estradiol in cows (Perry *et al.,* 2014). In this study, those animals were pregnant whose progesterone and testosteron levels were below 1 ng/ml in pregnant cows. However, our findings were in agreement with Díaz *et al.* (1986) who reported that P4 levels were lower than 1 ng/ml one day before the next estrus, and levels of 0.4-0.5 ng/ml were obtained on the day of estrus.

Table 5 shows various parameters observed in male and female calves producing cows/heifers (dams) recorded at the time of AI. There were no significant (P>0.05) effects of mothers parity, age and time of insemination on the sex ratio of offspring. However, values of estradiol, progesterone and testosterone varied very significantly (P<0.001), between mothers of male and female calves.

Table 5. Comparison in various parameters observed in male and female calves producing cows/heifers (dams) recorded at the time of AI

Parameters of dam recorded at the time of AI	**Calf sex**		
	Female	**Male**	**P-value**
Dam age	6.07±0.41	6.60±0.36	0.332
Parity	2.18±0.31	2.36±0.32	0.695
Daily milk production (L)	12.68±2.63	10.32±1.28	0.406
Time of AI after observed oestrus (Hrs)	19.48±0.90	18.22±0.77	0.292
Estradiol (pg/ml)	17.01±0.24	13.58±0.15	0.000
Progesterone (ng/ml)	0.89±0.01	0.74±0.02	0.000
Testosterone (ng/ml)	0.79±0.02	1.03±0.02	0.000
Follicular diameter (mm)	15.82±0.20	12.57±0.14	0.000
Vaginal electrical resistances (VER) (Ω)	183.50±2.37	211.97±1.65	0.000

Many farmers assume that early insemination favors female offspring, and late insemination favors male offspring. The terms early and late AI are determined on the basis of the timing of AI relative to the timing of ovulation, which is detected by ultrasonography or predicted by the timing of either the onset of oestrus or hormonal treatment. Martinez *et al*. (2004) conducted 716 AI experiments at 8–18, 8–30, or ≥30 hrs after visual detection of the onset of oestrus, and reported that delayed AI (performed at ≥30 hrs) significantly skewed the sex ratio in favor of male calves (72.1%). Moreover, the oviductal and uterine environments are inevitably affected by maternal physiological conditions, which affect both the primary and secondary sex ratio and the maternal testosterone level probably affects the sex ratio of offspring (Grant *et al*. 2005). Grant *et al*. (2008) reported that when the testosterone concentration in the follicular fluid was high, the enclosed oocyte was more likely to produce male embryos. Furthermore, Grant and Irwin (2005) showed that the concentration of testosterone in the follicular fluid of subordinate follicles was significantly related to the production of male embryos. Several factors have been reported to influence sex ratio in cattle, including the level of testosterone in bovine follicular fluid (Grant *et al.,* 2005; Grant *et al*., 2008), timing of insemination (Martinez *et al*., 2004), and the maturational state of the oocyte at the time of insemination (Agung *et al.,* 2006). Additionally, it has been suggested that in cattle, the oocyte, when ovulated, is already adapted to receive an X- or Y-bearing spermatozoa (Grant and Chamley, 2007). However, in cattle, only limited information is available regarding the sex ratio of the offspring in relation to AI. Novel technologies for obtaining sex-sorted semen are becoming popular. Under these conditions, it would be relatively easy to tackle the enigmatic factors associated with the biased sex ratio of calves, and information obtained in the field as well as in the laboratory may help our understanding of sex biasing factors in cattle.

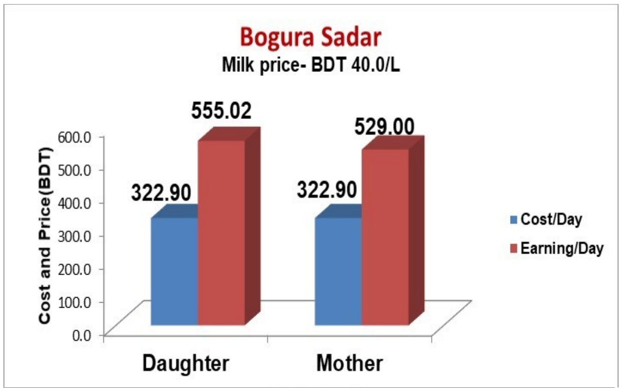
The reproductive and productive efficiency of heifers and cows born after AI as well as productivity and profitability of cattle farms

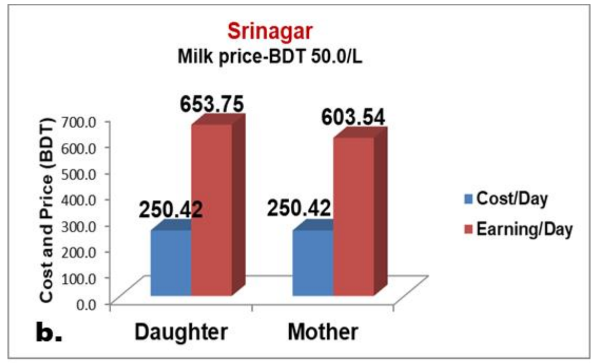
A total of 971 farms were visited where 9,146 cows were present. Among 971 farms, information on three generations of cattle population (grandmother, mother and daughter) was obtained from 117 farms. Reproductive efficiency in terms of sexual maturity, estrous cycle length, estrus, service per conception, parity and calving interval were compared. Results showed that there was significant ($P<0.05$) difference in the time needed for sexual maturity and service per conception (Table 6).

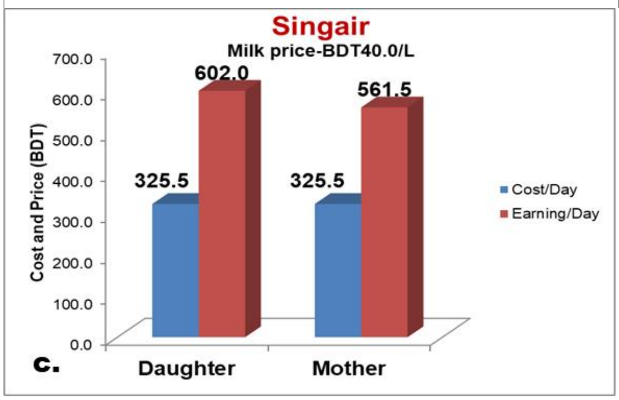
Table 6. Mean ± SE of different reproductive parameters in grandmother, mother and daughter cattle population

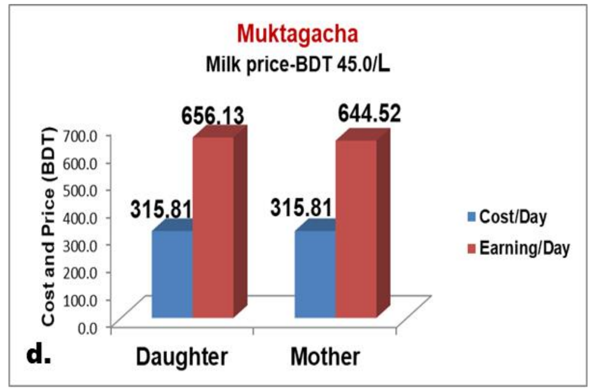
Generation	Sexual maturity	Estrous cycle length	Estrous duration	Service/ conception	Parity	Calving interval
Grand mother	639.33±4.32	21.04±0.05	34.04±0.60	1.44±0.07	7.48±0.08	13.03±0.06
Mother	707.28±5.05	20.82±0.10	33.67±0.68	1.51±0.04	5.43±0.09	13.06±0.07
Daughter	722.76±4.97	20.83±0.10	33.30±0.69	1.61±0.03	2.84±0.07	13.92±0.11
P-Value	0 .00	0.10	0.73	0.03	0.00	0.08

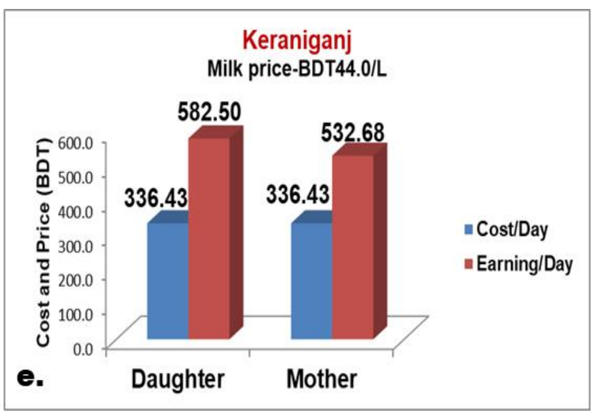
Productivity and profitability from mother and daughter generations in cattle farms were recorded and results are shown in Figures 6 and 7. Results showed that cost/day for mother and daughter were same due to farm management system. It was observed that earning from the daughter's milk was slightly higher in comparison to the that from mothers'milk in Bogura Sadar upazila of Bogura (555.00 BDT vs 529.00 BDT); in Srinagar upazila of Munshiganj (653.75 BDT vs 603.54 BDT); in Singair upazila of Manikgonj district (602.00 BDT vs 561.50 BDT); in Keraniganj upazila of Dhaka (582.50 BDT vs532.68 BDT), in Kustia upazila of Kustia district (443.13 BDT vs 379.38 BDT), in Madarganj upazila of Jamalpur (499.25 BDT vs 422.75 BDT); in Muktagacha upazila of Mymensingh (656.13 BDT vs 644.52 BDT); in Savar upazila of Dhaka (745.77 BDT vs 683.59 BDT).

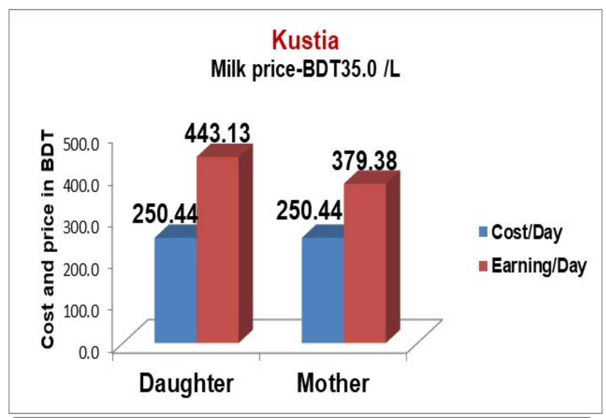

Bogura Sadar
Milk price- BDT 40.0/L
Cost and Price(BDT)
600.0
500.0
400.0
300.0
200.0
100.0
0.0
555.02
529.00
322.90
322.90
Cost/Day
Earning/Day
Daughter
Mother

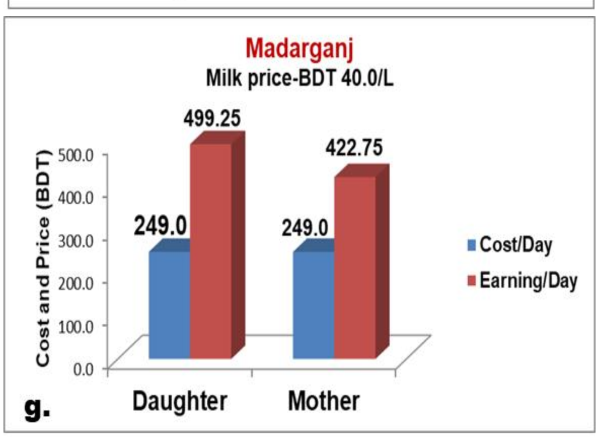

Srinagar
Milk price-BDT 50.0/L
Cost and Price (BDT)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
653.75
603.54
250.42
250.42
Cost/Day
Earning/Day
b.
Daughter
Mother

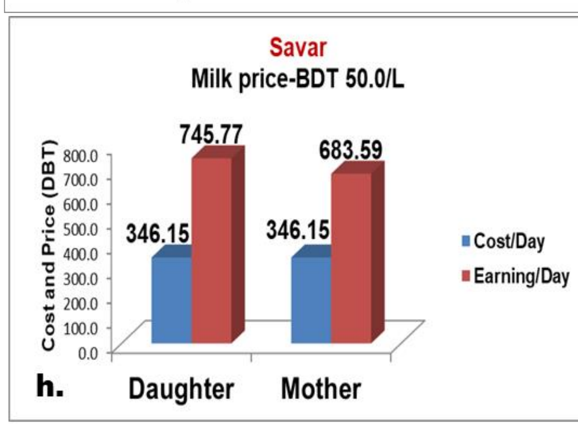

Singair
Milk price-BDT40.0/L
Cost and Price (BDT)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
602.0
561.5
325.5
325.5
Cost/Day
Earning/Day
c.
Daughter
Mother


Muktagacha
Milk price-BDT 45.0/L
Cost and Price (BDT)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
656.13
644.52
315.81
315.81
Cost/Day
Earning/Day
d.
Daughter
Mother


Keraniganj
Milk price-BDT44.0/L
Cost and Price (BDT)
600.0
500.0
400.0
300.0
200.0
100.0
0.0
582.50
532.68
336.43
336.43
Cost/Day
Earning/Day
e.
Daughter
Mother


Kustia
Milk price-BDT35.0 /L
Cost and price in BDT
500.0
400.0
300.0
200.0
100.0
0.0
443.13
379.38
250.44
250.44
Cost/Day
Earning/Day
Daughter
Mother


Madarganj
Milk price-BDT 40.0/L
Cost and Price (BDT)
500.0
400.0
300.0
200.0
100.0
0.0
499.25
422.75
249.0
249.0
Cost/Day
Earning/Day
g.
Daughter
Mother


Savar
Milk price-BDT 50.0/L
Cost and Price (DBT)
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
745.77
683.59
346.15
346.15
Cost/Day
Earning/Day
h.
Daughter
Mother

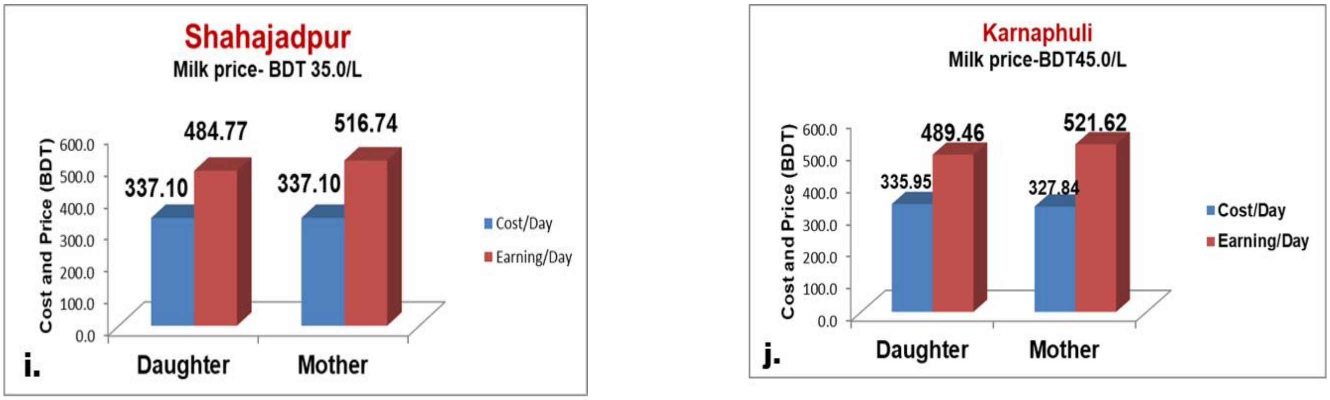


Figure 6: Study areas (a-h) where milk production was slightly increased and areas (i, j) where milk production was decreased in daugter cows

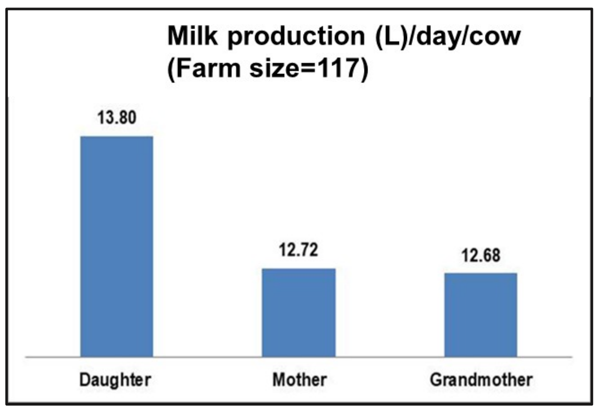


Figure 7: Average milk production (L)/day/cow of three generation observed in several farms

In contrast, earning per day from daughter's milk sell was slightly lower than that of mother in Shahajadpur upazila of Sirajgonj district (484.77 BDT vs 516.74 BDT Figure 6i); in Karnaphuli upazila of Chattagram (489.46 BDT vs 521.62 BDT, Figure 6j). The average cost was 334.80±103.35 BDT in the present study. This result was comparatively less than the result found by Islam *et al*. (2008). This is most probably due to the presence of subsistence level of dairy farming in rural Bangladesh. Khan *et al.* (2001) found the average milk production of daughter cows to be ranging from 0.52 to 3.62 liter per day per cow (mean 1.88±0.25) whereas the milk yield for mother cows ranged from 1.78 liter/cow/day to 10.15 litre/cow/day with an average of (average 6.02±1.16) which is less in comparison to our study. Foltz and Chang (2002) have found production per cow as a strong factor associated with dairy farm profitability.

11.2. Use of manipulative reproduction techniques for the production of sex selective offspring

<u>Optimization of cost effective production of high quality sperm to improve fertility with sex selective offspring</u>

Sperm morphometry of fresh semen collected from different bulls are presented in Table 7. Results of this study showed that head length, head width, head elongation, head perimeter, head area and tail length were 7.94±0.06µm, 3.15±0.04µm, 0.45±0.01µm, 19.21±0.14µm, 13.04±0.17µm^2 and 22.11±0.15µm. Sardar (2005) found that mean value of head length and head width of pure Sahiwal was 9.17±0.55 (µm) and 4.69± 0.4 (µm). Head length is higher and head area is lower than that found in this study. We found head length, head width, head elongation, head perimeter, head area and tail length were 8.28±0.06 (µm), 3.14±0.0 (µm), 0.42±0.00, 20.60±0.23 (µm), 16.86±0.15 (µm^2) and 24.23±0.20 (µm) in Brahman. These values are comparatively lower (except head wide) than that of Rubio-guiuen *et al*. (2007). We found head length, head width, head elongation, head perimeter, head area and tail length were 8.05±0.06 (µm), 3.20±0.03 (µm), 0.42±0.01, 19.85±0.23 (µm), 16.80±0.22 (µm^2) and 22.46±0.14 (µm) in Jersey. These values are lower in comparison to the findings in pure Jersey reported by Beletti *et al.* (2005) and Sundararaman *et al.* (2007). Head length, head width, head elongation, head perimeter, head area and tail length were 8.33±0.07 (µm), 2.67±0.05 (µm), 0.39±0.01, 8.92±0.12 (µm), 12.98±0.16 (µm^2) and 22.39±0.19 (µm) respectively, in pure Friesian of our study. All values are comparatively lower than the reports of Beletti *et al.* (2005). But head length is consistent with that in Frisian. Significant differences (p<0.05) were found between two Jebu bulls namely, Sahiwal and Brahman and between two European bulls namely Jersey and Frisian in terms of head length, head elongation, head perimeter and head area and tail length. In addition, insignificant (p>0.05) differences were found between Jebu, Sahiwal and Brahman, and significant (p<0.05) differences between *Taurus*, Jersey and Friesian in respect to all kinetic parameters (Table 5). The deviation among values of Sahiwal, Brahman and Jersey might be due to genotype difference (pure vs. cross). However, this study supports the hypothesis that sperm biometry also varies among indigenous, crossbred and European bull, stated by Beletti *et al*. (2005) and Roy (2014).

Table 7. Mean sperm morphometry (±SEM) of crossbred (Sahiwal, Jersey and Brahman) and pure-bred (Friesian) bull`s spermatozoa in fresh semen

Sample type	Sahiwal X	Brahman X	Jersey X	Friesian 100%
Head length (µm)	7.94±0.06^{y}	8.28±0.06^{wx}	8.05±0.06^{xy}	8.33±0.07^{w}
Head width (µm)	3.15±0.04^{w}	3.14±0.04^{w}	3.20±0.03^{w}	2.67±0.05^{w}
Head elongation	0.45±0.01^{w}	0.42±0.00^{x}	0.42±0.01^{x}	0.39±0.01^{y}
Head perimeter (µm)	19.21±0.14^{xy}	20.60±0.23^{w}	19.85±0.23^{x}	18.92±0.12^{y}
Head area (µm^2)	13.04±0.17^{x}	16.86±0.15^{w}	16.80±0.22^{w}	12.98±0.16^{x}
Tail length (µm)	22.11±0.15^{w}	24.23±0.20^{x}	22.46±0.14^{w}	22.39±0.19^{w}

Within a single row, values with different superscripts (w, x, y, z) differ significantly among breed (P<0.05).

When kinetic parameters of spermatozoa was considered in fresh semen among bulls, specially between two Jebu bulls Sahiwal and Brahman, insignificant ($p>0.05$) differences were found in respect to all kinetic parameters ALH, VSL, BCF, LIN VAP, VCL, VSL and progressive motility (Table 8). In contrast significant ($p<0.05$) variations were observed between two European Jersey and Friesian bulls in term of kinetic velocity. Moreover, significant ($p<0.05$) differences were observed in values of ALH, VSL and progressive motility of Sahiwal and Friesian. Sahiwal and Jersey bulls varied significantly ($p<0.05$) in term of ALH, LIN VAP, VCL and VSL whereas Jersey differed with Brahman in respect to ALH, BCF, LIN and VCL ($p<0.05$).

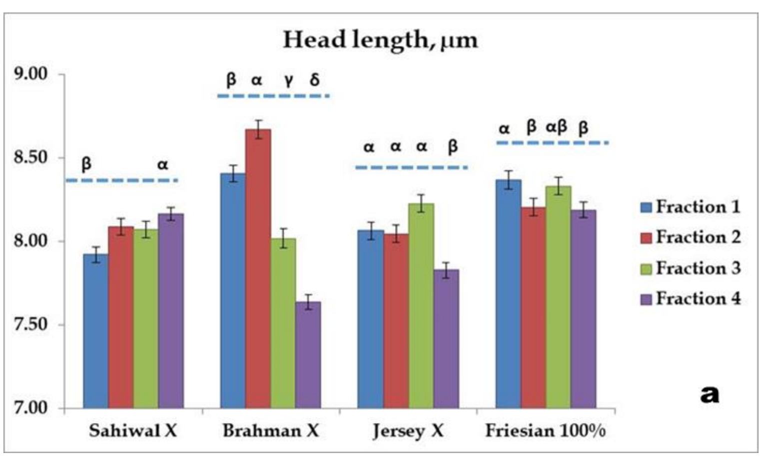
Table 8. Mean sperm Kinetic velocity (±SEM) of crossbred (Sahiwal, Jersey and Brahman) and pure-bred (Friesian) bull`s spermatozoa in fresh semen

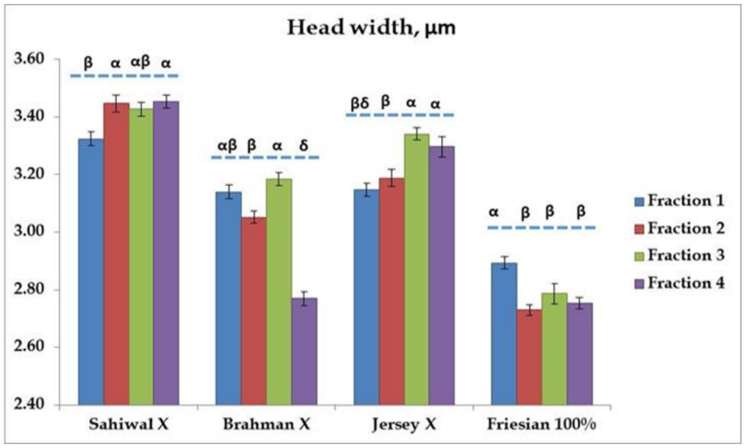
Parameter	Sahiwal X	Brahman X	Jersey X	Friesian 100%
ALH (µm)	9.93 ± 0.31^{y}	10.07 ± 0.32^{y}	8.41 ± 0.46^{z}	11.85 ± 0.34^{x}
BCF (Hz)	33.71 ± 1.17^{xy}	29.49 ± 1.14^{y}	36.79 ± 1.32^{x}	29.67 ± 0.73^{y}
LIN (%)	43.71 ± 1.01^{yz}	39.50 ± 1.26^{z}	54.13 ± 1.67^{x}	45.67 ± 1.45^{y}
VAP (µm/s)	129.87 ± 1.18^{x}	112.58 ± 4.44^{y}	115.05 ± 4.79^{y}	131.24 ± 2.46^{x}
VCL (µm/s)	238.38 ± 7.56^{x}	231.17 ± 3.03^{x}	175.73 ± 1.86^{y}	242.77 ± 3.04^{x}
VSL (µm/s)	99.48 ± 1.04^{y}	92.06 ± 2.04^{yz}	91.27 ± 2.24^{z}	112.63 ± 2.15^{x}

Within a single row, values with different superscripts (x, y, z) differ significantly among breed (P>0.05).

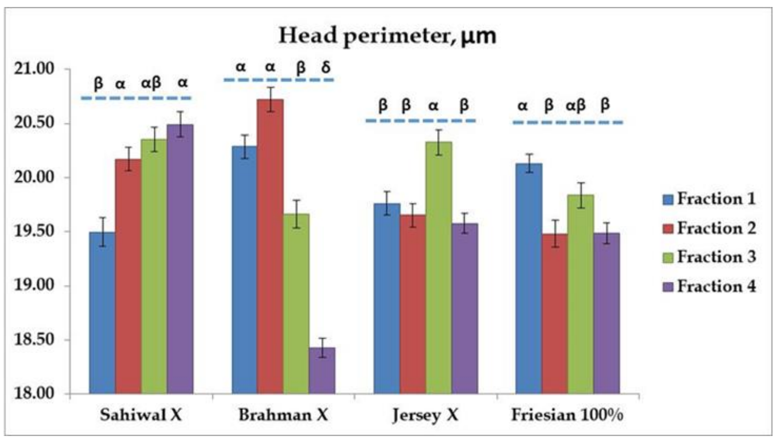
When kinetic velocity of spermatozoa of different fractions was considered no significant ($p>0.05$) variations were found in parameters among fractions within breed. Results are shown in Figure 9.

Figure 8 shows morphometric parameters of individual sperm collected by swim-up separation technique during the study period.

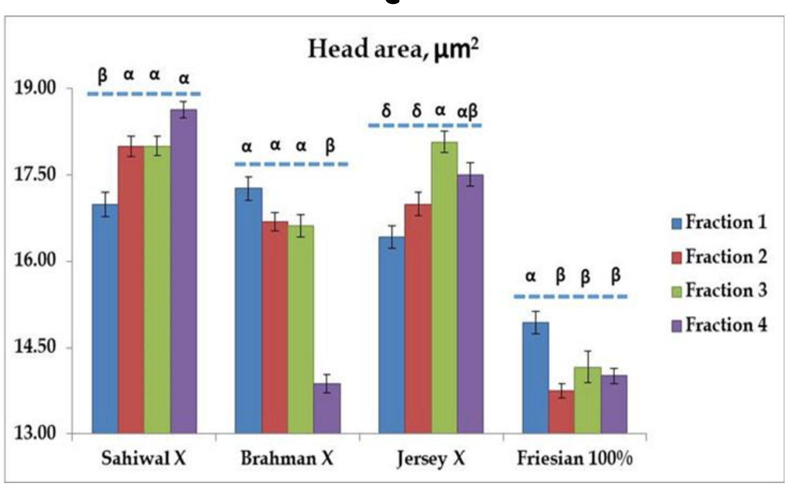



Head width, µm
3.60
3.40
3.20
3.00
2.80
2.60
2.40
β α αβ α
αβ β α δ
βδ β α α
α β β β
Fraction 1
Fraction 2
Fraction 3
Fraction 4
Sahiwal X
Brahman X
Jersey X
Friesian 100%

b


Head perimeter, µm
21.00
20.50
20.00
19.50
19.00
18.50
18.00
β α αβ α
α α β δ
β β α β
α β αβ β
Fraction 1
Fraction 2
Fraction 3
Fraction 4
Sahiwal X
Brahman X
Jersey X
Friesian 100%

c


Head area, µm²
19.00
17.50
16.00
14.50
13.00
β α α α
α α α β
δ δ α αβ
α β β β
Fraction 1
Fraction 2
Fraction 3
Fraction 4
Sahiwal X
Brahman X
Jersey X
Friesian 100%

d

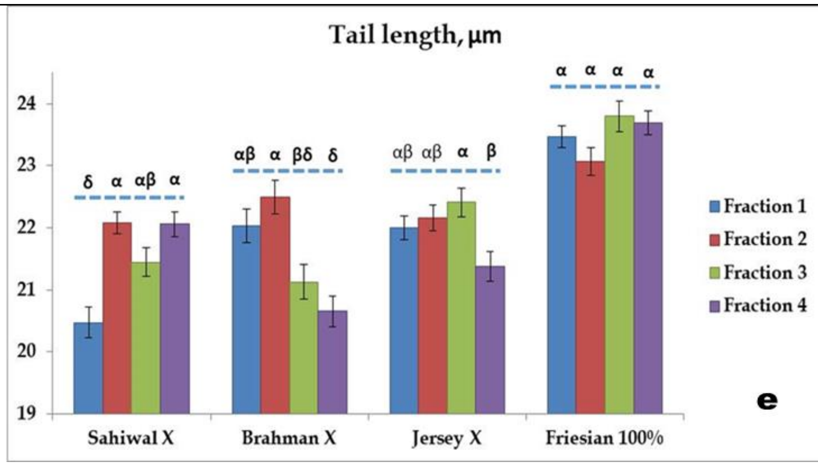
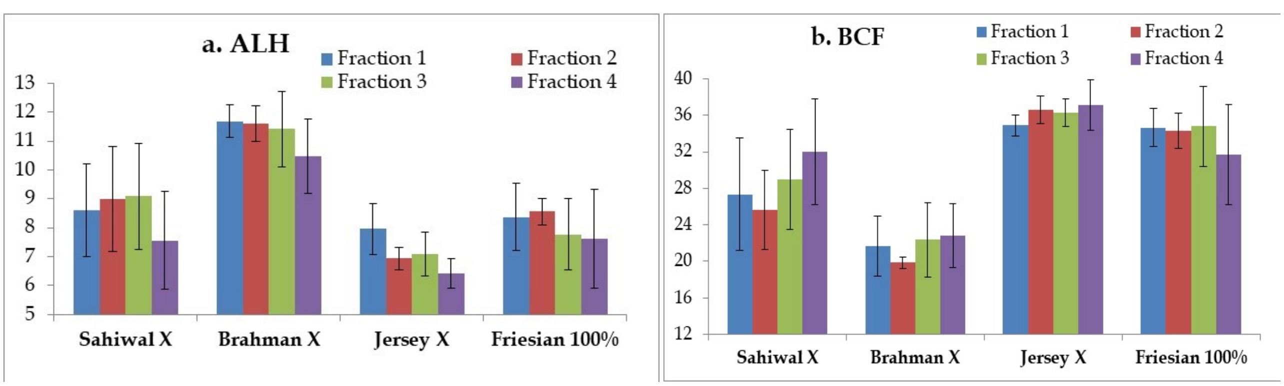


Figure 8: Head length (a), head width (b), head perimeter (c), head area (d) and tail length (e) of four fractions of bull`s spermatozoa (Different superscripts α, β, δ indicates significant differences within breeds).

It was observed that significant variations existed in different morphometric parameters among fractions within and among breeds. Significant ($P<0.05$) bull effects were found for all sperm morphometric measurements between fraction 1 and fraction 2 in Sahiwal, Jersey and Frisian. It is important to mention that modified swim up method was used in this study to separate spermatozoa. Spermatozoa of four fractions were collected and both morphometric and kinetic velocity were studied and analyzed. Swim-up separation techniques involved sperm separation depending on sperm motility characteristics. We separated four fractions in a hypothesis that population of X and Y spermatozoa might be variably present in different fractions according to their motility characteristics.

Considering the morphometry, separated sperm within breeds, the highest values of length, width, perimeter, area and tail length were observed in Fraction 4 of Sahiwal, in Fraction 3 of Jersey and in Fraction 1 of Friesian in this study. However, inconsistent findings were observed in case of Brahman bull in terms of head morphometric parameters. However, to the authors' knowledge, no studies analyzing sperm morphometric characteristics and its relationship with sperm kinetic velocity measured by HT-IVOS II have been reported before. When kinetic velocity of spermatozoa of different fractions was considered insignificant ($p>0.05$) variations were found in parameters among fractions within breed which is shown in Figure 9.



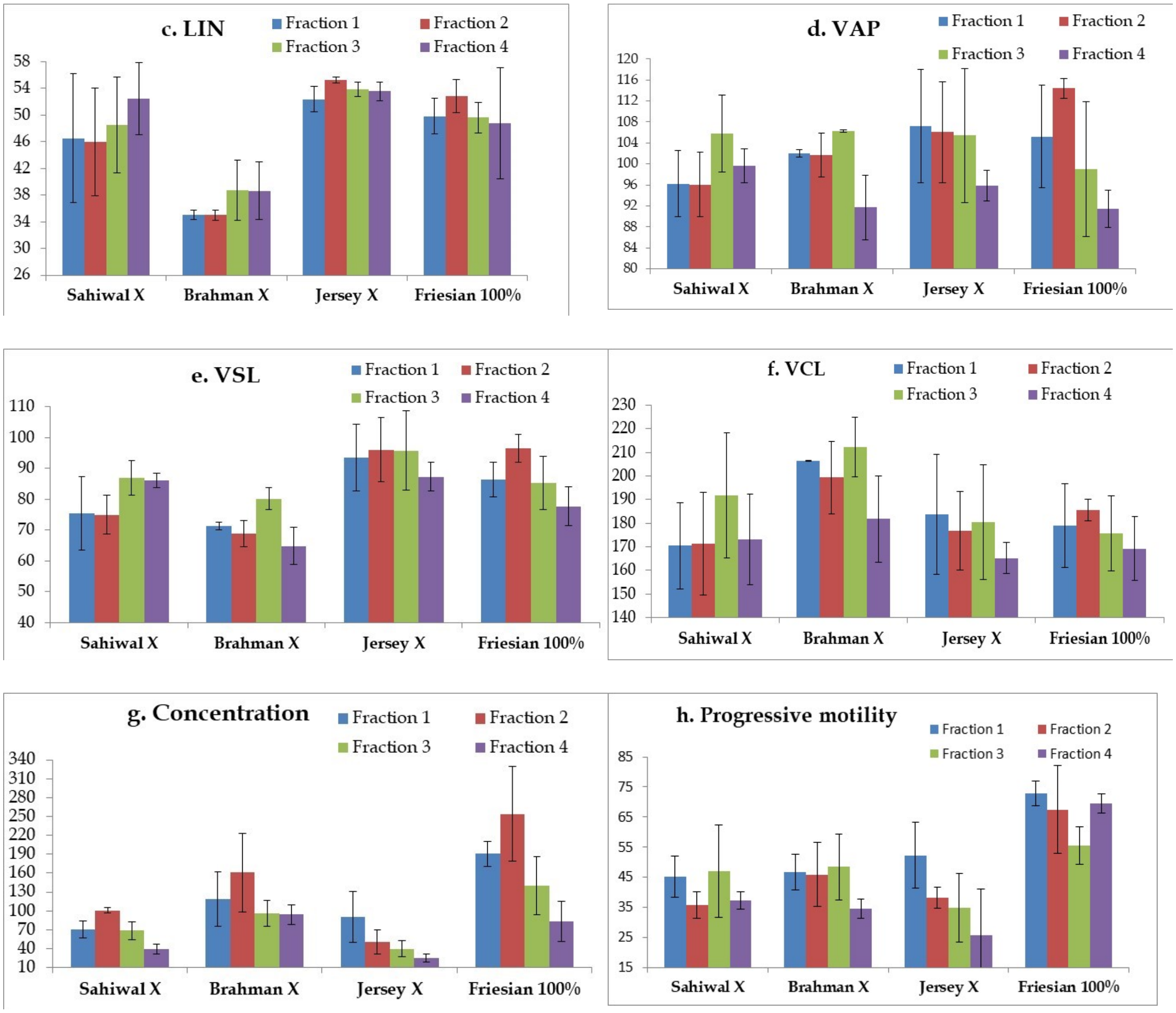


Figure 9: ALH (a), BCF (b), LIN (c), VAP (d), VSL (e) VCL (f), Concentration (g) and Progressive motility (h) of swim up separated semen of bull's spermatozoa (Superscripts α indicates insignificant differences within breed at the level of p>0.05).

Figure 10 presents numbers of fractioned semen samples collected from four bulls. Table 9 shows the sex of calves produced after insemination with different fraction's semen. Table 10 presents head length and head width of spermatozoa observed in fractioned semen. It was observed that 72.9% male calves were born when Fraction-1 sample was used for AI. In contrast, 85.4% female calves were born when Fraction-4 sample was used for AI. Moreover, head width was significantly higher (P<0.01) in semen samples those produced female calves.

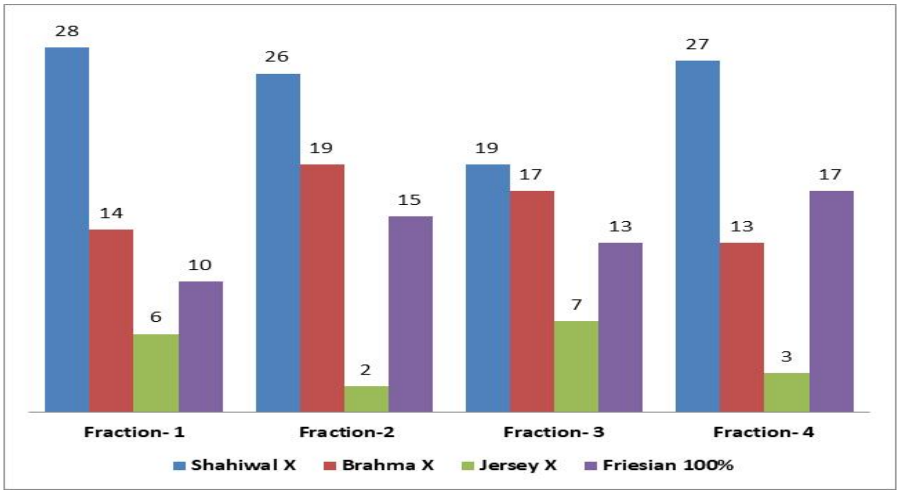


Figure 10: Swim-up separated fractions collected from different bulls used for AI

Table 9: Effects of fractions of semen on pregnancy rate and calf's sex

Fractions	Total AI (n)	Pregnancy rate (%)	Calving rate (%)	Male claves (%)	Female claves (%)
Fraction 1	58	82.75 (n=48)	81.0 (n=47)	72.9 (n=35)	25.0 (n=12)
Fraction 2	62	74.0 (n=46)	74.0 (n=46	67.4 (n=31)	32.60 (n=15)
Fraction 3	49	83.6 (n=41)	83.6 (n=41)	34.2 (14	65.8 (n=27)
Fraction 4	60	80 (n=48)	80 (n=48)	14.6 (n=7)	85.4 (n=41)
Conventional AI	140	65% (n=91)	50% (n=70)	45% (n=31)	55% (n=39)
Synchronized AI	120	85.8% (n=103)	85% (n=102)	42.12% (n=43)	57.8% (n=59)

Table 10. Head length (µm) and Head width (µm) of sperm of different fractions produced male and female calves.

Fractions	Sperm head	Calf sex		
		Female	Male	P-value
Fraction 1	Head length	8.04±0.11	8.05±0.05	0.897
	Head width	3.79±0.18	3.52±0.05	0.056
Fraction 2	Head length	7.83±0.16	8.02±0.05	0.160
	Head width	3.28±0.09	3.20±0.04	0.383
Fraction 3	Head length	8.12±0.01	8.04±0.17	0.518
	Head width	3.36±0.09	3.34±0.19	0.993
Fraction 4	Head length	6.65±0.14	7.48±0.28	0.033
	Head width	3.51±0.05	3.15±0.10	0.008

Ke-hui Cui (1997) found that the length, perimeter and area of the sperm head and the length of the sperm necks and tails of X-bearing spermatozoa were significantly larger and longer than those of Y-bearing spermatozoa. Sperm length determines propulsive efficiency that determines success in fertilization (Katz and Drobbins, 1990). Longer spermatozoa may thus have a greater probability of fertilizing an ovum compared to the shorter ones from the same ejaculate (Gomendio and Roldan, 1991).

Separation of X and Y chromosome-bearing spermatozoa is a permanent point of interest for livestock breeders and scientists in general. Many reports have described hypothetical methodologies for the isolation of sperm populations enriched in each kind of spermatozoa. The aim of this work was to establish an easy and fast method that could be applicable to any procedure of sperm separation. The basis of our methodology was the description of the *ZFX/ZFY* gene. A differential pattern of restricted fragment length polymorphism (RFLP) between the male (447, 344 and 103 bp, respectively) and female (447bp) calves is shown in Figure 11.

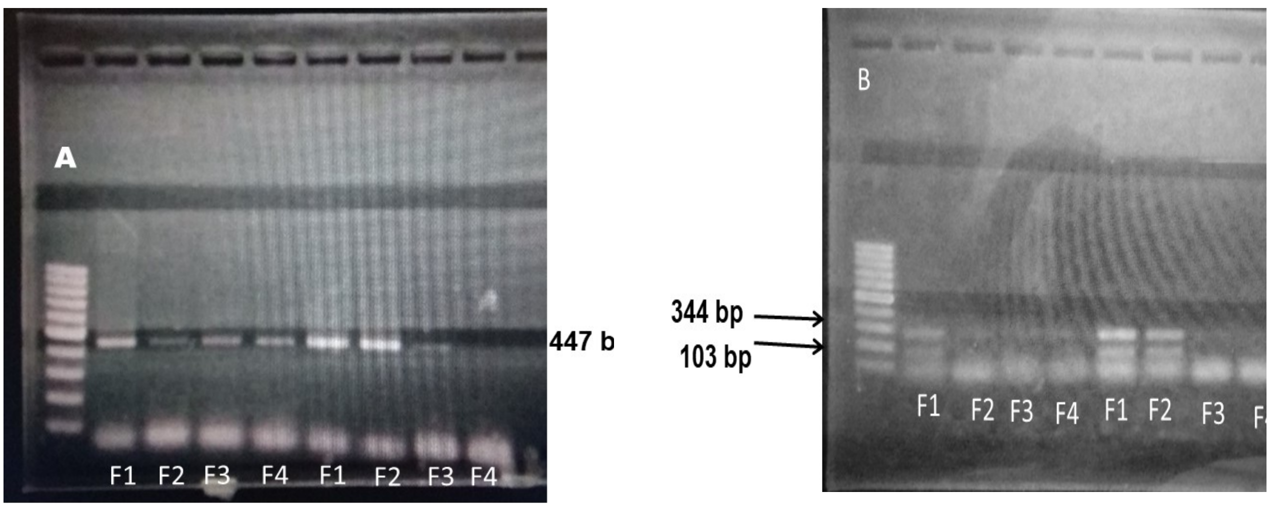


Figure 11: A) Agarose gel separation of *ZFX/ZFY* fragments from PCR amplified genomic DNA standards. B) Sac*I* digestion of amplified fragments (F1- Fraction 1; F2-Fraction 2; F3-Fraction 3, F4-Fraction 4).

Polymerase chain reaction (PCR) was performed based on a quantitative *ZFX/ZFY* gene polyorphism analysis of sex polymorphic patterns described by Aasen and Medrano (1990) in cattle. A 447 base pair (bp) PCR fragment of the *ZFY (indication of Y sperm)* gene digested by Sac*I* produces 2 fragments of 344 and 103 bp, respectively, while the same 447 bp fragment of *ZFX (indication of X-sperm)* remains uncut (Figure a, b). Restricted fragments allowed electrophoretical identification of male and female DNA. We have obtained the densitometric quantification of the Sac*I* restricted fragments obtained following *ZFX/ZFY* gene amplification from bovine genomic DNA and the known percentages of X and Y chromosome standards. This calibration has allowed us to extrapolate and determine the proportion of X and Y chromosomes from a given sperm DNA sample as described by Ollero *et al. (*2000). However, we could not include the densiometric quantification results of all collected fractioned semen samples in this report due to time limitation.

Establishment of proper synchronization protocol for timed artificial insemination

Estrus synchronization allows every animal within a selected group of cows in a herd to receive a programmed series of preparatory injections or treatments and insemination at a pre-determined time regardless of oestrus. Potential benefits from oestrus synchronization in dairy cattle include reduced time devoted to oestrus detection and reduced variability in days from parturition to first service, leading to reduced variability and length of calving intervals within a herd (Waldmann*et al.,* 2006). Synchronization programs have become standard components in the modern breeding management of cows in the dairy herds of most dairy industries in developed countries. It has been recently reported that almost 60% of the AI performed in Brazil was made at fixed time (Baruselli*et al.,* 2012). However, this manipulative breeding program is somehow absent in Bangladesh. Numbers of oestrus synchronization programs are available in cattle based on the use of various hormones like progesterone, prostaglandin F2α and their various combinations with other hormones like estrogen and GnRH. The aim of this experiment was to study the efficacy of OVSYNCH (GnRH+PGF2α+GnRH+TAI), Co-SYNCH (GnRH+CIDR+PGF2α+GnRH+TAI) and Pre-synch (PGF2α+AI) synchronization protocols on fertility of lactating cows. Calving rates were 82.6% in Ovsynch group, 82.8% in Pre-synch and 89.7% in Co-synch group (Table 11).

Table 11. Pregnancy and calving rate obtained after synchronization protocols and TAI in dairy farms

Protocol	OVSYNCH	Pre-SYNCH	Co-SYNCH
Cows inseminated(n)	52	29	39
Pregnant cows (n)	43	24	36
Pregnancy rate	82.6%	82.8%	92.3%
Calves born (n)	43	24	35
Calving rate	82.6%	82.8%	89.7%

We are unaware of data regarding this kind of work in Bangladesh. Kabir *et al.* (2017) have reported a significantly higher conception rate (88.89%) in cows following administration of triple doses of PGF2α and GnRH with TAI in comparison to that of single dose injection of PGF2α. Pursley *et al.* (1997) indicated that highest pregnancy rate (45%) was achieved when insemination was done 16 hrs after the second GnRH injection of OVSYNCH protocols. Macmillan (2010) has reported that conception rates could be increased from 66.1% to 74.6% when CIDR is used from 4 to 9 days after first insemination. Frike *et al*. (2003) conducted an examination of Ovsynch protocol and obtained an AI pregnancy rate of 31%. In this study, the highest pregnancy (92.3%) and calving rates (89.7%) were obtained in cows of Co-synch protocol group. Colazo and Reuben (2014) reported 63% pregnancy rate in heifers inseminated after Co-synch protocol. However, pregnancy rate in these three protocols were higher than that reported in the previous studies (Colazo and Reuben, 2014; Kabir *et al.*, 2017). This variation might be resulted from breed difference, management practices and environmental factors present in the workplace during the study. This demands a further precise study to confirm the present findings. Moreover, selection of appropriate estrus synchronization protocol should be made on the basis of management capabilities and expectations of the farmer.

12. **Research highlight/findings:**

- Farmer's education and breed of bull play an important role for the adoption of AI.
- Only 22% dairy farms maintained three dairy generations (grandmother, mother, and daughter) observed in different study areas.
- Sex hormones, oestrogen and testosterone, present at the time of AI might play a major role for sex characterization.
- This study revealed marked variation in terms of sperm morphometry in the different swim-up separated fractions collected from different breeding bulls.
- 72.9% male and 85.4% female calves obtained from Fraction-1 and Fraction-4 respectively, after dextran swim-up separation of whole semen.
- Sperms with larger head width from different fractions produced more female calves.
- Restriction fragment length polymorphism (RFLP) PCR amplification was optimized to quantify X- and Y- spermatozoa in semen sample.
- The highest pregnancy (92.3%) and calving (89.7%) rates were obtained when cows were inseminated with frozen-thawed semen after oestrus synchronization with Co-synch protocol at commercial farms and it would be a promising manipulative technology for the proper management of dairy farms.
- Vaginal speculum incorporated with video camera and AI gun equipped with mobile video monitoring system was developed for the Veterinarians and AI technicians.

B. Implementation Position

1. **Procurement:**

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment					
(b) Lab &field equipment	Cold cabinet (1)	444100.00	Cold cabinet (1)	444100.00	
(c) Other capital items	Breeding Bulls (2)	249800.00	Breeding Bulls (2)	249800.00	

2. **Establishment/renovation facilities:** Not applicable.

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

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

Description	Number of participant			Duration (Days/weeks/months)	Remarks
	Male	Female	Total		
(a) Training	-	-	-	-	
(b) Workshop “Role of cow’s AI on socio-economic development” (Av_©-mvgvwRK Dbœq‡b Mvfxi K„wÎg cÖRb‡bi f‚wgKv)	100	0	100	1 Day	Participants were AI technicians (32) and Farmers (68)

<u>C. Financial and physical progress (Fig in Tk)</u>

Items of expenditure/ activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	626,643.00	583,293.00	583,293.00	0.00	100.00	
B. Field research/lab expenses and supplies	939,650.00	939,650.00	939,568.00	82.00	99.99	Due to PPR
C. Operating expenses	69,607.00	69,607.00	67,812.00	1,795.00	97.40	Could not be processed in financial year
D. Vehicle hire and fuel, oil & maintenance	0.00	0.00	0.00	0.00	0.0.00	
E. Training/ workshop/ seminar, etc.	30,000.00	30,000.00	30,000.00	0.00	100.00	
F. Publications and printing	90,000.00	15,000.00	15,000.00	0.00	100.00	
G. Miscellaneous	35,000.00	35,000.00	25,000.00	10,000.00	71.43	Could not be processed in financial year
H. Capital expenses	444,100.00	444,100.00	444,100.00	0.00	100.00	
Total	2,235,000.00	2,116,650.00	2,104,773.00	11,877.00	99.40	

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)**
i) Socio-economical impact of genetic improvement of cattle via AI	i) Survey ii) AI after observing natural estrus. iii) Serum level of P4, E2 and T at the time of AI recorded.	i) Farmer's education and breed of bull play an important role for the adoption of AI. ii) 45% male and 55% female calves born after conventional AI. Sex hormones, oestrogen and testosterone, present at the time of AI might play a major role for sex characterization iii) The reproductive and productive performances of three generations of milking cows were recorded. Variation in daily cost /cow and milk price existed in different study areas were known	The knowledge generated through this project could help the policy makers to set up breeding policy and marketing chain of milk in the different study areas of Bangladesh
ii) Implementing the manipulative reproduction techniques	i) Use of Dextran swim-up separation technique to make sperm fraction ii) Use of CASA HT IVOS II to study the sperm morphometry iii) Use of RFLP PCR amplification to identify DNA base of X-and Y spermatozoa in fractioned semen samples iv) Insemination of cows after estrus synchronization with OVSYNCH, Co-synch and Pre-synch protocols	i) Dextran swim-up separated fraction 4 produced 85.4% female calves ii) Sperm produced female calves had significantly ($P<0.01$) higher head width iii) RFLP PCR amplification was optimized to identify DNA base of X-and Y-spermatozoa in fractioned semen samples iv) Highest calving rate was observed in cows synchronized with Co-synch protocols	Large dairy farms could use the estrus synchronization protocols to control and design the breeding plans for dairy cows

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.			
Journal publications	1 submitted 3 under preparation		Submitted i. Morphometry and Kinetic Velocity Analysis of BullSpermatozoa with CASA IVOS II. Under preparation ii. Relationship of pregnancy and sex of offspring with dominant follicular size, level of oestrogen and progesterone at the time of AI in cows iii. Factors affecting adoption of artificial insemination in some selective areas of Bangladesh and its influence on cattle productivity iv. Effect of sperm morphometry and swim-up processing method on sperm and offspring sex ratio
Other publications, if any: MS thesis		3	Master of Science in Theriogenology i. Anup Kumar Sarkar (16 V Therio JD 05) Relationship of pregnancy with dominant follicular size, vaginal electrical resistance and level of oestrogen and progesterone at the time of AI in cows, December 2017. ii. Aynul Hoque (17 V Therio JJ 05) Morphometry and kinetic velocity, analysis of bull spermatozoa with computer assisted semen analyser, June 2018. iii. Md. Mehedi Hasan (16 V Therio JD 01). Factors affecting adoption of artificial insemination in some selective areas of Bangladesh and its influence on cattle productivity, December 2018

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

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

a) Dextran swim-up sperm separation technique for production of sex selective offspring in cows.

b) Estrus synchronization using Co-SYNCH protocol.

c) Vaginal speculum immersed with video camera and AI gun incorporated with mobile video monitoring system (Annexure-II).

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

a) Use of CASA IVOS II frame capture for the study of morphometry of spermatozoa

b) Relation of the production of sex-selective offspring with follicular size, estradiol and testosterone levels present in blood serum of cows/heifers at the time of AI.

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

a) Introduction of synchronization in commercial dairy farms

b) Introduction of heat detector for proper detection of estrus as well as detection of proper time of AI

c) Use of swim-up separated sperm fractions for AI in field condition.

iv. Policy Support

Study on the factors affecting adoption of AI would help policy makers to design breeding policy based on the local progeny performance of cattle in differenct areas for proper utilization of the benefit of AI in Bangladesh.

G. Information regarding Desk and Field Monitoring

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

- Monitoring seminar- 22 April, 2018 in the BAURES conference room, Bangladesh Agricultural University, Mymensingh
- Monitoring workshops-16 May, 2018 in the BARC Auditorium, Farmgate, Dhaka.

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

Time: From 12.30 pm, 4.3.2018 to 2.30 pm 4.3.2018

Team visit: Dr. Shah Md. ZiqrulHaq Chowdhury and Dr. Mohammad Rafiqul Islam

H. Lesson Learned (if any)

For successful completion of this type of breeding study needs more time and budget.

I. Challenges (if any)

Cooperation of the farmers in using their cows for the study was a big challenge.

Signature of the Principal Investigator

Date ………………………….

Seal

Counter signature of the Head of the organization/authorized representative

Date …………………………..

Seal

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ANNEXURE-1

QUESTIONNAIRE

.... Serial No.:	Upazilla:	Date: ....

1. Owner's information:

Name	:	....	Sex	:	....
Number of family member	:	....	Religion	:	....
Educational atainment	:	....	Profession	:	....
Address & Telephone or cell	:	....			

2. Farm information:

Status	:	Single/Multiple	Occupied person	:	…………………………….....
Number	:	Heifer …..... cow …..... bull …..... female calve …..... male calve …..... others .... Total			
Housing system	:	....	Type of rearing	:	....
Type of feed	:	....	Cost/cow/day	:	....
Labor cost	:	....	Health cost /animal	:	………………….
Price of milk (Liter)	:	....	Other	:	....

No of cows/heifers inseminated: Natural:..............Artificial: ....

Selection of bull: Self/ AI technician

Cost/straw

Cost /insemination:.............................

3. Information on reproductive performance of cattle

A. Grandmother

Breed of grandmother	:	....	Sexual maturity (day)	:	....
Oestrus cycle length (day)	:	....	Oestrus duration (hour)	:	....
AI time of cow	:	....	No. of service per conception	:	....
Source of semen	:	Natural/ AI			
1st calving age (month)	:	....	Parity	:	....
Calving interval (month)	:	....	Milk production (liter)	:	....
Lactation length (day)	:	....	Feed cost	:	....

B. Mother

Breed of mother	:	....	Sexual maturity (day)	:	....
Oestrus cycle length (day)	:	....	Oestrus duration (hour)	:	....
AI time of cow	:	....	No. of service per conception	:	....
Source of semen	:	Natural/ AI			
1st calving age (month)	:	....	Parity	:	....
Calving interval (month)	:	....	Milk production (liter)	:	....
Lactation length (day)	:	....	Feed cost	:	....

C. Daughter

Breed of daughter	:	....	Sexual maturity (day)	:	....
Oestrus cycle length (day)	:	....	Oestrus duration (hour)	:	....
AI time of cow	:	....	No. of service per conception	:	....

Source of semen	:	Natural/ AI			
1st calving age (month)	:	....	Parity	:	....
Calving interval (month)	:	....	Milk production (liter)	:	....
Lactation length (day)	:	....	Feed cost	:	....

....

Name & signature of investigator

ANNEXURE-II

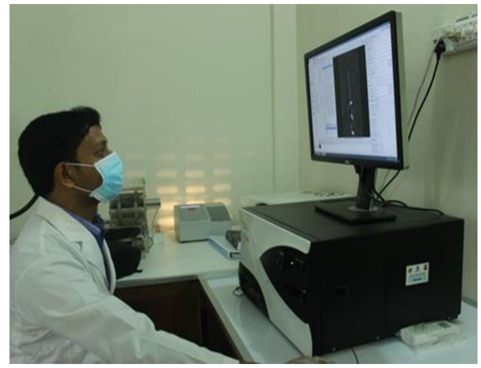
Figure: Vaginal speculumincorporated with video camera and AI gun (BAU AI Vision)

Figure: Use of BAU AI vision

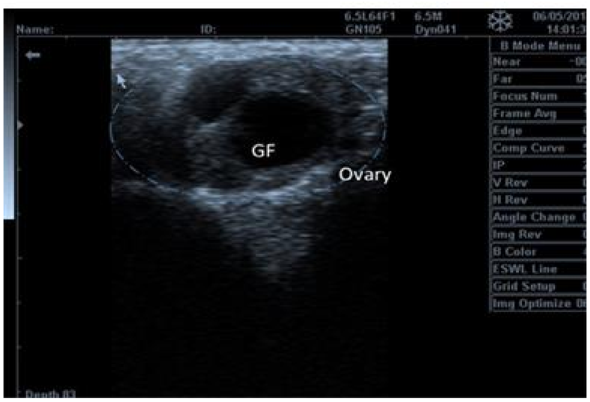
ANNEXURE-III

Pictorial view of research activities:

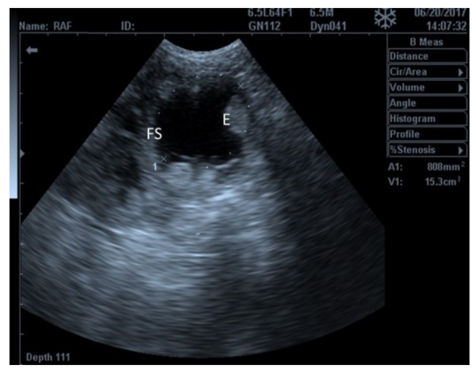
Breeding bulls used for this study.



CASA monitoring system for sperm morphometry



Presence of graffian follicle (GF) at the time of AI



Early pregnancy diagnosis in cows FS-Fetal sac; E-Embryo

Farmer’s workshop at Karnaphuli (Patiya), Chittagong

Interviewing a farmer in a farm at Kustia sadar, Kustia

Pictures of calves born after AI with fractioned semen in the study