PRACTICES AND EFFECTIVENESS OF ARTIFICIAL INSEMINATION IN CATTLE AT KALIAKOIR UPAZILLA, GAZIPUR

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CERTIFICATE

This is to certify that the thesis entitled, ***"PRACTICES AND EFFECTIVENESS OF ARTIFICIAL INSEMINATION IN CATTLE AT KALIAKOIR UPAZILLA, GAZIPUR"*** *submitted to the Faculty of Animal Science & Veterinary Medicine,, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in ANIMAL SCIENCE****, embodies theresult of a piece of bona fide research work carried out by* ***MD. ROKONUZZAMAN****, Registration No.* ***12-04896*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 01-12-2019
Dhaka, Bangladesh

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Dedicated to
My Beloved Parents

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ABSTRACT

Artificial insemination (AI) was the first great biotechnology applied to improve reproduction and genetics of farm animals. It has had an enormous impact worldwide in many species, particularly in dairy cattle. To get more meat, milk from dairy cow, AI play an important role to develop different breeds across the country. This study was conducted from May 2018 to April 2019 at upazilla livestock office, Kaliakoir, Gazipur to determine the effectiveness of Artificial insemination in cattle. A total of 150 cows were selected to identify the potential risk factors such as breed, age, body condition score (BCS) & season on conception rate. The study showed that overall pregnancy rate was 55.33%, The highest pregnancy rate (59.52%) was observed in Sahiwal cross, which was significantly ($p<0.05$) higher than that of Friesian cross (47.92%) cows. Cows of 2< - <4 years of old revealed a significantly ($p<0.05$) higher pregnancy rate (56.47%) than others. The higher pregnancy rate (63.08%) in cows of BCS 3.5 than lower 2.5 BCS (29.17%) cows showed significantly ($p<0.05$) higher pregnancy rate (71.05%) when AI was done in spring (March-April) compared to winter (November-February) calving cows (58.14%). Good BCS significantly enhances the pregnancy rate in cattle. The study suggested that the spring (March-April) may be the best season for good fertility of cattle in Bangladesh.

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LIST OF ABBREVIATIONS AND SYMBOLS

1^{st} =First

2^{nd} = Second

3^{rd} =Third

A =Angus

AF =Africander

AI =Artificial Insemination

AW =Average Weight

B =Brahman

BR =Brangus

CFS =Calving to first service

CI =Confidence interval

et al. =And his associates

DMRT=Duncans multiple range test

F =Frisian

G =Gram

H =Hour

HF =Holstein Friesian

Kg =Kilogram

L =Local

SE = Standard Error

Chapter I

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CHAPTER I

INTRODUCTION

Livestock plays a vital role in the national economy of Bangladesh. The share of the livestock sub-sector, in 2017-18, to the national gross domestic products (GDP) was 1.54%, which was 14.23% of agricultural GDP (Bangladesh Economic Review, 2018). Share of Livestock in Agriculture GDP (current prices) 13.62% . Employment (Directly) 20%, Employment (Partly) 45% .Total Cattle Population 240.86 Lakh (DLS, 2017-18).

To meet the increasing demand and ensure adequate amount of protein from animal source and thus, food security at household level the famers need to increase their productivity. The existing low productive local cattle, low production of milk and meat and low investment in the sector are the major challenges towards improvement of livestock sector (Bormann *et al.* 2006; Ferguson *et al.* 1996). In addition, lack of appropriate breeds, suitable breeding policy and shortage of feeds and fodders throughout the year are also hindering the productivity (Schilling *et al.* 1998; Khan *et al.* 2008). The government intervention to overcome those problems by importing high yielding temperate breeds could not bring a solution to increase productivity. This instigates to search for alternative options; one of which is to infuse the exotic blood into the best local cows through either upgrading or crossbreeding.

The dairy cows play an important role to improve the livelihood of poor and marginal people of Bangladesh. AI is one of the tools for genetic improvement through male line. In Bangladesh the conception rate (CR) using fresh and frozen semen is 45.3% and 57.3% respectively (Rahman *et al.*, 1995). Shamsuddin *et al.*, (2001) reported higher conception rate (54%) in cows inseminated with frozen semen than that (37%) in cows inseminated with chilled semen. The average conception rate of local nondescript and crossbred cows with Holestein-Friesian and Sahiwal breed were 42.5% and 45.2% to 53.1%, respectively (Shamsuddin *et al.*, 1997) The productivity of cattle is low because of poor genetics (Gonzalez *et al.*, 2001), poor nutrition (Brosaster *et al.*, 1998), weak herd health veterinary services and marketing access (Shamsuddin *et al.*, 2001). The season of insemination might be the important factors to get maximum conception rate in cows (Miah *et al.*, 2004). In the summer season, heat stress (29ºC) of dairy cattle markedly affects the pregnancy rate (25.4%, Ricardo *et al.*, 2004). Bormann *et al.*, (2006) studied the productive performance of indigenous and crossbred cows in Bangladesh and demonstrated the overall AI per pregnancy was 1.7. AI per pregnancy of Local, L×HF and L×SL was 1.6, 2.2 and 1.6, respectively. It is supported by Shamsuddin *et al.*, (2001) who reported that the local cows required fewer services per conception than the crossbred animals.

Economies of dairy farming largely rely on a good pregnancy rate after AI. The low conception rate and other fertility indices after AI are affected by health status of the bull, semen collection, processing, preservation, transportation, proper heat detection, AI at correct time, insemination in friendly uterine environment and keeping of AI record. (Paul *et al.* 2010) reported that the efficiency and skillness of AI technician play the most important role for pregnancy rate of cows. Furthermore, the parity, breed and age of the inseminated cows also found to affect the conception rate after AI. Kaliakoir, Gazipur is a densely cattle populated area. Proper management of cattle by introducing AI could be a tool for emancipation of the people. Therefore, the present study was undertaken to determine the conception rate of dairy cows in Kaliakoir, Gazipur with overall improvement of conception rate after AI, reduced number of services required per conception and assessment of age, parity, breed and season of the cows on conception rate.

Considering the above facts and circumstances, this study was designed with

the following two objectives:

1. To evaluate AI practice in cattle based on various breed.
2. To assess the potentiality of AI practice in Kalikoir upazilla, Gazipur.

CHAPTER II

REVIEW OF LITERATURE

2.1 Pregnancy rate

The percentage of cows / heifers diagnosed pregnant out of total number of cows/ heifers inseminated. Shamsuddin *et al*., (2006) reported an average conception rate of 46.2% in Mymensingh district of Bangladesh. The Author expressed that the nutritional condition of the cow at calving and thereafter, weaning age of calves, frequency of suckling, cattle rearing system, accuracy of oestrus detection, interval between oestrus and AI , the oestrus signs and semen quality were the important determinant of conception rate. They also told that the technician's skill and sire were very important factors in judging conception rate. Some other scientist reported that conception rate depends mainly on skillness of inseminator, estrus detection of cows, quality and quantity of spermatozoa in semen, proper semen thawing procedure, placement of semen in the uterus, calving to service interval and herd size (Hodel *et al.*, 1996; Jainudeen and Hafez, 2001; Talukder *et al.* , 2001; Shamsuddin *et al.* , 2001).

Mekonnen *et al.* (2010) suggested that the first service non return rate (NRR) in the retrospective study was found to be 86.6% (n= 2511), which was much higher than the NRR (48.2%; n=81) recorded in the field follow-up study. The first service NRR of 48.2% recorded in the follow-up study which is supposed to be due to pregnancy was much lower than first service pregnancy rate (34.5%). This may be attributed to factors including failure of return to oestrus or farmers choice of natural mating in cases of insemination failure. Analysis of the breeding records of 622 dairy cows indicated that the mean duration of calving to first service (CFS) in local breed cows (mean ± SD: 241 ± 18.5 days; n=205) was significantly higher (P<0.01) than those of crossbreed cows (mean ± SD: 182 ± 9.9 days; n=417) indicating the low reproductive efficiency of local breed cows. Only 4.5% of dairy cows (n=28) had CFS less than 80 days.

Hassan *et al.* (2003) expressed that a commercial dairy farm in the central region of Saudi Arabia was evaluated for overall reproductive performance and inseminators' effect. Average days to first service and average days open were 70 and 105, respectively. Heat detection rate was low (61%) and first service conception rate average was (51%), while second and third service conception rates were precipitously lower, 38 and 14% respectively. Overall conception rate was 74% and an average service/conception ratio was 2.5. There were no significant inseminator effect regarding interestrous interval, pregnancy rate and number of services per conception. However, pregnant cow's average days open was significantly higher for cows inseminated by one inseminator (86.8 days) than cows inseminated by second inseminator (77.4 days). These results indicate that the reproductive performance of the herd as a whole can be improved by increasing heat detection rate.

2.2 Factors affecting pregnancy rate

2.2.1 Breed

Miah *et al.* (2004) mentioned that the experiment was conducted for a period of 1.5 years at the district Artificial Insemination Centre, Sylhet, Bangladesh. They observed the highest pregnancy rate was found in the cows which were indigenous local cows (46.1%) of second parity and inseminated by local x Holstein-Friesian bulls (44.4%) at 11-14 hours after onset of estrus (60.3%) in the spring season (53.1). It was concluded that the time and season of insemination might be the most important factors to get maximum conception rate of the cows. Thus it was suggested that for the farmers to achieve the desired rate of conception, they should inseminate their cows in spring season and during the time 11-14 hours after onset of oestrus.

Haque *et al.* (2002) observed on some indigenous cows, which reared in urban area to evaluate the various milking and reproductive performances. The effects of management level were significant on AW ($P<0.001$), PPHP ($P<0.01$), CI ($P<0.001$) and daily milk yield ($P<0.05$). The overall mean values were 245.8 + 3.8 days for AW, 35.3+0.40 months for AFH, 37.20.7 monthly for conception, 46.2+0.4 monthly for AF calving, 1.3+0.1 for no. of services for conception, 182.9+ 4.8 days for PPHP, 15.5+0.2 months for CI, 2.5+0.3 kg for daily milk yield and 250.6+4.5 days for lactation length.

Sarder *et al.* (1997) found reproductive and productive performance of 313 progenies. Information were collected from 40 artificial insemination subcentres or points under the District AI centre, Rajshahi at greater Rajshahi district over a period from 1993 to 2002. The mean reproductive and productive parameters such as body weight of progeny, age at puberty, age at first service, age at first calving, service per conception in heifer, gestation length, birth weight of progeny's calf, post-partum heat period, days open, wastage day, service per conception, milk production per day, lactation length, lactation yield, dry period, weaning period and caving intervals were 18.8 kg, 27.9 month, 29.2 month, 38.7 months, 1.68, 278.7 days, 20.7 kg, 139 days, 160 days, 20.3 days, 1.6, 5.1 liters/day, 10.4 liters/day, 282 days, 1445 liters, 146 days, 10.6 months and 438 days, respectively. Genetic groups of progeny LxF, LxFxSL, LxSL, LxSxSL and LxFxSxSL had significant ($P<0.05$) effect on all the reproductive and productive performances female progeny except service per conception in heifer, post-partum heat period, service per conception and wastage days. LxF cross-progeny showed excellent reproductive and productive performance and most of these parameters were poor performance observed in LxSxSL cross-bred of progeny under field condition. Breed of sire of progeny F, SL, LxFxF, SLxF, LxF. LxSLxF and SxSL had significant ($P<0.05$) effect on all the reproductive and productive performance except service per conception in heifer, wastage and service per conception.

2.2.2 Age

Ettema & Santos (2001) observed that milk production, health, and economic performance among Holstein heifers during first lactation on 3 commercial dairy farms in California. Heifers (n = 1905) were moved to the breeding group between 360 and 390 d of age and grouped retrospectively according to age at first calving (AFC) as low (≤700 d), medium (701 to 750 d), and high (≥751 d). Within farm, growing heifers were managed similarly, as were lactating primiparous cows, for the first 310 d in lactation. Heifers were fed to gain 0.70 to 0.80 kg/d from 4 mo of age to breeding, and 0.8 to 0.9 kg/d from breeding to 252 to 258 d of pregnancy. First calving at <700 d was associated with reduced yields of milk and milk components. Cows in the high age group produced more milk fat and true protein than medium and low cows. Incidence of stillbirths was highest for cows in the low group (19.8%), but stillbirths were also a concern for those calving at medium (16.1%) or high age groups (13.5%). Both low and high cows had lower conception rates at first postpartum AI, and abortions averaged 9.8% across groups. Days open and number of inseminations were lower for medium than low cows. Incidence of mastitisand lameness was lowest for cows in the medium group. Culling and mortality rates were not affected by AFC, but among those that died, cows in the low group tended to die earlier postpartum than cows in the high group. Heifers in the medium group had an adjusted income value numerically higher by $138.33 and

$98.81 compared with those in the low and high groups, respectively. First calving at <700 d compromised first lactation yields of milk and milk components and impaired reproductive performance. However, extending AFC beyond 750 d did not improve lactation, reproduction, or health of primiparous cows. Although not preassigned to age groups before start of breeding, Holstein heifers managed as in this study had the highest economic return when calving between 23 and 24.5 month of age.

Raheed (2002) found the age in month of different breeds of dairy cattle and it was 67.1f19.9, 70.5±24.6, 62.2f25.0, 67.5±22.8, 68.8±22.8 and 49.Of12.0 months in the LXL, LXSL, LXF, VJR, LxSLXF and LXFXXF breeds, respectively. Apart from the LxFxF crossbred, all the dairy cattle belonged to five years and above age group. Analysis of variance for the trait shows the breeds did not differ significantly (P>0.05).

Bakhinov and Sabostin (1995) in Russia revealed CR to 1st insemination of 39.1, 38.0, 34.8% for heifers, younger and older cows, respectively. Mullah *et al.* (2010) reported an increased conception rate with advancing parity from parity 2 up to 6, and then declined at parities 7 and 8. Post partum first ovulation was earlier in cows that had >2 calving or of over 5 years old than in those of 3-5 years old cows (Eduvie, 1985). Khan *et al.* (2008) reported that 9 years old cows had significantly decreased ($p \leq 0.000$) conception rate than other groups.

2.2.3 Body weight

Kaim *at al.* (2010) observed that In three experiments 250 high-yielding dairy cows were fed for 18 weeks after parturition either a low-protein (LP) diet containing 150 to 160 g crude protein per kg or a high-protein (HP) diet containing 190 to 200 g crude protein per kg. In all three experiments cows were fed an average of 2·6 to 2·9 kg crude protein per day on the LP diet and 3·3 to 3·7 kg crude protein per day on the HP diet. Inclusive of maintenance, crude protein intake was, on average, 71 to 83 g crude protein per kg milk on the LP diet and 84 to 112 g crude protein per kg milk on the HP diet.

Protein intake did not affect the post-partum decrease in body weight; however, cows in their 4th and later lactations lost significantly more weight than cows in their 2nd and 3rd lactations ($P < 0·01$). Number of lactation did not affect the levels of rumen fluid ammonia or plasma urea, but older cows had significantly higher milk yields than younger ones. In all the experiments the first oestrus was observed, on average, between 38 and 43 days after parturition. The first insemination was carried out, on average, between 69 and 75 days after calving. Protein intake or age did not affect the intervals between parturition and the first observed oestrus or insemination. Proportional conception rates of cows fed the LP and HP diets were 0·566 and 0·431, respectively ($P < 0·05$). Conception rates of cows in their 2nd and 3rd lactations were 0·582 and 0·515 for cows on the LP and HP diets, respectively, whereas conception rates of cows in their 4th and later lactations were 0·526 and 0·288, respectively ($P < 0·02$). Within the HP-fed group the difference in conception rate between cows in their 2nd and 3rd lactations and those in their 4th and later lactations was also significant ($P < 0·01$). The proportion of cows pregnant 126 days after parturition was 0·786 and 0·645 for cows fed the LP and HP diets, respectively ($P < 0·05$). The proportion of cows in their 2nd and 3rd lactations which were pregnant, was 0·792 and 0·702 for cows of the LP and HP groups, respectively. Pregnancy rates for cows in their 4th and later lactations were 0·769 and 0·515 in the LP and HP groups, respectively ($P < 0·05$).

Sarder *et al.* (1997) observed that the cross-bred cows weighed more (264-400 kg) than the local nondescript cows (178). Holstein-Friesian (HF) cross-breed cows yielded 2.5 kg more milk daily than that of local cows. Regarding the onset of post partum estrus the local cows required the longer time (149 days) and the HF cross-bred cows required the shortest interval (119). Consequently, the local cows remained open for the longest period (158).

2.2.4 Body condition score (BCS)

Marcos *et al.* (2007) found that One of the causes of poor fertility in high producing dairy cows is inadequate progesterone. Therefore, we determined the efficacy of an intravaginal insert containing 1.55 g of progesterone (PRID) given before and/or after timed AI (TAI) on ovarian response, plasma progesterone concentrations, pregnancy per AI (P/AI) and pregnancy losses.

Lactating dairy cows at three locations were assigned (Day 0) to an Ovsynch protocol with (N = 294) or without (N = 314) a PRID. The Ovsynch protocol consisted of two injections of 100 μg gonadorelin (GnRH) 9 days apart and one injection of 500 μg cloprostenol(PG) 7 days after the first GnRH treatment. Insertion and removal of PRID occurred concurrent with the first GnRH and PG treatments, respectively. Timed AI was carried out 12 to 16 hours after the second GnRH. Ovarian status of a subset of 217 first service cows had been presynchronized with 2 treatments of PG 14 days apart with the last PG given 12 days before the first GnRH of the Ovsynch protocol. Body condition score (scale of one to five) was recorded at TAI. Ultrasonographic examinations were done in all cows at first GnRH, at PG, at TAI, and 24 hours after TAI for response to treatment and at 32 and 60 days after TAI for confirmation of pregnancy. At 4.5 days after TAI (Day 14), cows that responded to PG and ovulated after the second GnRH treatment were reassigned to receive (N = 223) or not receive (N = 229) a PRID for 7 days. Blood samples were taken for progesterone determination at PG treatment, at TAI, and post TAI on Days 14 and 21. The PRID treatment pre-TAI reduced the percentage of cows ovulating before TAI (5.8% vs. 11.1%), and significantly increased P/AI in nonpresynchronized cows (41.3% vs. 25.1%). Cows ovulating in response to the first GnRH treatment, cyclic cows, and cows with body condition score of 2.75 or more had increased P/AI, but the addition of a PRID pre-TAI to these cows did not increase P/AI. The PRID treatment post TAI did not affect P/AI, but reduced pregnancy losses (6.1% vs. 11.4%) between

32 and 60 days of gestation. The reduction in pregnancy losses tended (P = 0.10) to be significant in acyclic cows receiving a PRID than in those not receiving a PRID (5.6% vs. 33.3%). Plasma progesterone concentrations at PG treatment and on Day 21 (11.5 days after TAI) were linearly associated with P/AI. In conclusion, progesterone supplementation pre-TAI increased P/AI in nonpresynchronized cows. Progesterone supplementation post TAI reduced pregnancy losses, particularly in acyclic cows.

Yaudan *et al*. (2007) revealed relationships between body condition score (BCS) and body weight (BW) in dairy cows with reproduction variables in pasture-based, seasonal-calving dairy herds. Over 2,500 lactation records from 897 spring-calving Holstein-Friesian dairy cowswere used in the analyses. Eleven BCS- and 11 BW-related variables were generated, including observations at calving, nadir, planned start of mating (PSM), and first service, as well as days to nadir and the amount and rate of change between periods. The binary reproductive variables were cycling by PSM, mated in the first 21 d from PSM, pregnant to first service, and pregnant in the first 21, 42, and 84 d of the seasonal mating period. Generalized estimating equations were used to identify BCS and BW variables that significantly affected the probability of a successful reproductive outcome. After adjusting for the fixed effect of year of calving, parity (for cycling by PSM only), and the interval from calving to either first service or PSM, reproductive performance was found to be significantly affected by BW or BCS at key points, and by BCS and BW change during lactation. All reproductive response measures were negatively affected when BCS and BW measures indicated an increased severity and duration of the postpartum negative energy balance. In particular, cycling by PSM was positively associated with calving BCS, whereas pregnancy at 21, 42, and 84 d post-PSM were positively associated with nadir BCS and BW gain post-PSM, and negatively associated with BCS loss between calving and nadir. The results highlight the important role that BCS and BW loss has on reproductive performance, especially in seasonal-calving dairy systems because of the short period between calving and PSM.

Body condition score at calving has played an important effect on pregnancy rate during a controlled breeding season (Lalman *et al.*, 1997). The feeding practices of animals are reflected by the BCS of the animals. Providing adequate quantity of balanced diet to animals will help to gain good BCS resulting in satisfactory conception rate. Higher conception rate in cows with good BCS than that in cows with poor BCS has been documented by Shamsuddin *et al.*, (2001) in Bangladesh. The main cause of poor reproductive performance could be due to poor health management, incorrect nutrition during and after calving (Dziuk and Bellows, 1983). Inadequate dietary intake and decreased utilization of some nutrient may result in delayed onset of ovarian activity by preventing release of gonadotropin from the pituitary, (Nolan *et al.*,1988; Collins *et al.*,, 1992; Osawa *et al.*, 1996). Balanced nutrition with better management help to maintain general health condition of the cow that stimulate the endocrine system through the activation of the cow that stimulate the endocrine system through the activation of the hypothalamo-pituitary-ovarian axis to work properly and thereby improved reproductive performance (Morrow, 1980; Fitzpatrick, 1994). Energy availability has been considered to regulate gonadal activity by modulating the release of GnRH, LH at various reproductive phases. It was observed that reduced nutrition decreases energy intake to inhibit gonadal function through the suppression of GnRH/LH secretion in ruminants (Crockett *et al.*, 2000). Experimental models with Sheep have demonstrated that fasting or glucose deprivation suppresses pulsatile LH release. From those experiments, the information on energy deficiency is considered to be detected by specific central sensors and conveyed to the hypothalamus to regulate by specific central sensors and conveyed to the hypothalamus to regulate LH release as well as food intake (Maeda *et al.*, 2003). It is not surprising that post-partum ovarian activity was more closely associated with milk production than with total digestible nutrient intake (Whitemore *et al.*, 1974). Thus, it seems logical that the interval to first estrus is related to energy balance. But supplying sufficient energy to avoid body weight loss and to support optimum reproduction is a huge challenge with high producing taurine breed cows. However, managing feeding to minimize the negative effects of an energy deficit can help improve reproductive performance.

2.2.5 Season

Rahman *et al.* (1995) Heat stress has adverse effects on the reproductive performances of dairy cattle and buffaloes. The dairy sector is a more vulnerable to global warming and climate change. The temperature humidity index (THI) is the widely used index to measure the magnitude of heat stress in animals. The objective of this paper was to assess the decline in performances of reproductive traits such as service period, conception rate and pregnancy rate of dairy cattle and buffaloes with respect to increase in THI. The review stated that service period in cattle is affected by season of calving for which cows calved in summer had the longest service period. The conception rate and pregnancy rate in dairy cattle were found decreased above THI 72 while a significant decline in reproductive performances of buffaloes was observed above threshold THI 75. The non-heat stress zone (HSZ) (October to March) is favorable for optimum reproductive performance, while fertility is depressed in HSZ (April to September) and critical HSZ (CHSZ) (May and June). Heat stress in animals has been associated with reduced fertility through its deleterious impact on oocyte maturation and early embryo development. The management strategies *viz*., nutrition modification, environment modification and timed artificial insemination protocol are to be strictly operated to ameliorate the adverse effects of heat stress in cattle and buffaloes during CHSZ to improve their fertility. The identification of genes associated with heat tolerance, its incorporation into breeding program and the inclusion of THI covariate effects in selection index should be targeted for genetic evaluation of dairy animals in the hot climate.

Berman (2003) observed Environmental heat stress, present during warm seasons and warm episodes, severely impairs dairy cattle performance, particularly in warmer climates. It is widely viewed that warm climate breeds (Zebu and Sanga cattle) are adapted to the climate in which they evolved. Such adaptations might be exploited for increasing cattle productivity in warm climates and decrease the effect of warm periods in cooler climates. The literature was reviewed for presence of such adaptations. Evidence is clear for resistance to ticks and tick-transmitted diseases in Zebu and Sanga breeds as well as for a possible development of resistance to ticks in additional breeds. Development of resistance to ticks demands time; hence, it needs to be balanced with potential use of insecticides or vaccination. The presumption of higher sweating rates in Zebu-derived breeds, based upon morphological differences in sweat glandsbetween breeds, has not been substantiated. Relatively few studies have examined hair coat characteristics and their responses to seasonal heat, particularly in temperate climate breeds. Recently, a gene for slick hair coat has been observed that improved heat tolerance when introduced into temperate climate breeds. No solid evidence exists that hair coat in these lines is lighter than in well-fed warm climate-adapted Holsteins. Warm climate breeds and their F1 crosses share as dominant characteristics lower maintenance requirements and milk yields, and limited response to improved feeding and management. These characteristics are not adaptations to a feed-limited environment but are constitutive and useful in serving survival when feed is scarce and seasonal and high temperatures prevail. The negative relationship between milk yield and fertility present in temperate climates breeds also prevails in Zebu cattle. Fertility impairment by warm conditions might be counteracted in advanced farming systems by extra corporeal early embryo culture. In general, adaptations found in warm climate cattle breedsdid not increase heat dissipation capacity, but rather diminished climate-induced strain by decreasing milk production. The negative relationship between reproductive efficiency and milk yield, although relatively low, also appears in Zebu cattle. This association, coupled with limited feed intake, acting over millennia, probably created the selection pressure for a low milk production in these breeds.

Howlader *et al*. (2003) observed In dairy cows inseminated during the hot months of the year, there is a decrease in fertility. Different factors contribute to this situation; the most important are a consequence of increased temperature and humidity that result in a decreased expression of overt estrus and a reduction in appetite and dry matter intake. Heat stress reduces the degree of dominance of the selected follicle and this can be seen as reduced steroidogenic capacity of its theca and granulosa cells and a fall in blood estradiol concentrations. Plasma progesterone levels can be increased or decreased depending on whether the heat stress is acute or chronic, and on the metabolic state of the animal. These endocrine changes reduce follicular activity and alter the ovulatory mechanism, leading to a decrease in oocyte and embryo quality. The uterine environment is also modified, reducing the likelihood of embryo implantation. Appetite and dry matter intake are both reduced by heat stress thus prolonging the postpartum period of negative energy balance and increasing the calving-conception interval, particularly in high producing dairy cows. The utilization of cooling systems may have a beneficial effect on fertility but dairy cows cooled in this way are still unable to match the fertility achieved in winter. Recent studies suggest that the use of gonadotropins to induce follicular development and ovulation can decrease the severity of seasonal postpartum infertility in dairy cows.

Ingraham *et al*. (1994) emerged that the pregnancy rate of dairy cows declined from 55 to 10% when the temperature-humidity index (THI) increased from 70 to 84. Soumya *et al.,* (2001) observed that heat stress delayed puberty in heifers, caused anestrus in cows, depressed estrous activity, induced abortions, and increased prenatal mortality. This decrease in fertility is caused by elevated body temperatures that influence ovarian function, expression of estrus, oocyte health, and embryonic development (Biggers *et al.,* 1987; Lucy, 2002). Dunlap and Roth *et al.,* (2001) found 0% conception in beef cows that had an average body temperature of 40°C or above, whereas Biggers *et al*. (1987) reported pregnancy rates of 82, 67, and 55% for cows with body temperatures of 38.9, 39.2, and 39.8°C, respectively. Other reasons for impaired reproductive performance in cows during hot weather may include decreased intensity of estrus, failure to ovulate, lack of implantation, embryo disintegration, and fetal abortion (Stott, 1974; Fabio *et al.*, 2002). Previous research involving the effects of heat stress on reproduction has been conducted using dairy cows (Balachandran *et al*., 1983; Ingraham *et al.*, 1994; Holmes *et al.*, 2002).There are very few studies that have assessed the effects of heat stress on pregnancy rate of beef cows managed in a pasture setting (Sprott *et al.,* 2001). Dairy cows' tolerance to high temperatures is diminished during lactation due to increased internal metabolic heat production associated with high feed intake and milk synthesis.

CHAPTER III

MATERIALS AND METHODS

3.1 Selection of study area:

This study was conducted from May, 2018 to April, 2019 at upazilla livestock office Kaliakoir, Gazipur.

3.2 Animal selection

The study was carried out in upazilla livestock office, Kaliakoir, Gazipur during the period of May 2018 to April 2019. One hundred & fifty cows of different breeds, age, BCS were considered for evaluation.

3.3 Grouping of animal

3.3.1 Breed of cows: Three breeds were selected as follows:

(i) Local (Indigenous cow): Well developed hump.

(ii) Friesian cross: Predominant black and white color, ranging from all black to all white. The hump is not developed.

(iii) Sahiwal cross: Characteristics reddish brown colour, pendulous udder.

3.3.2 Age of animal

The age of cows was determined by observation of teeth eruption. The age of Cows were considered into the following three groups:

(i) 2 - <4 years

(ii) 4< - <6 years

(iii) 6< years

3.3.3 Determination of BCS of animals

The BCS of the Cows/ heifers was determined by eye estimation. The BCS was measured by 1-5 scale.

3.3.4 Season of year of AI

Seasons of artificial insemination were divided into following four groups.

i) Summer season (May - July)

ii) Rainy season (August - October)

iii) Winter (November - January) and

iv) Spring (February - April)

3.4 Techniques of artificial insemination with frozen semen:

3.4.1 Equipment required for artificial insemination

a. Tank- Aluminum vacuum-insulated vessel used to hold semen and liquid nitrogen.(Fig-1)

Fig-1: liquid nitrogen tank

Source:https://liquidnitrogentank.com/How_to_Measure_Liquid_Nitrogen_Level.php

b. Canister- Removable cylinder with a mesh or solid bottom to hold semen in the tank. It has a long, hooked handle to permit straw identification and access from the mouth of the tank.

c. Mini-goblet- Plastic cylinder with a sealed base, and which fits into the canister. It held up to twenty-five straws in a bath of liquid nitrogen.

d. Straws- Each straw contains enough semen to inseminate a cow once (Fig-2). The volume of semen in the mini-straw was 0.25 ml, which normally contains 20 million sperm cells with a usual minimum of 40% live at thaw.

Fig-2: Straw with AI gun

Source: http://www.livestocktool.com/product/cattle-farming-equipments1/ veterinary-instruments-for-cattle/artificial-insemination-gun-in-cattle-equipment.html

3.4.2 A hygiene checklist

3.4.2.1 Before and during the insemination:

New plastic glove, new sheath, fresh paper towel were used. The 'cut end' of loaded gun was kept uncontaminated; did not allowed it to contact any surface in the dairy or, breeding race. The vulva was wiped as clean as possible. The lips of the vulva were parted and introduced the point of the gun as clean as possible. Insemination was avoided too deeply and excessive movement of the gun inside the cow was avoided, as these actions are likely to cause minor injuries that allow a better chance for infection to enter and reduce the chance of the cow holding. Free hand was washed if contaminated by dung or mucus. Fresh paper was used. Then it was preceded as for points above.

3.4.2.2 Insemination technique

Always it was ensured that kit box and its contents were cleaned and it contained gloves and paper towel, insemination gun, sheaths, thaw box, thermometer and lubricant.

3.4.3 Positioning the cow

The chance of AI success is greatly increased when the cow is relaxed (Fig-3), it should stand on a level surface with plenty of grip. The cow should also be appropriately restrained use an area which is already familiar to the cow and the facility should be well lit, with provision made for food and water. Minimize any changes in the routine and do not mix cows from separate groups at the point of insemination. Keep them in their group until they return to the herd.

Fig-3: Positioning of cow for AI

3.4.4 Thawing the straw

Before thawing the straw, check the water temperature; it should be at 37^{o}C, or as instructed by the semen company. Particular care should be taken with sexed semen; for optimum results it may require slightly longer and warmer thawing frost (up to line) temperatures. The straw should be removed from the flask with forceps and submerged in the water. Leave it in for 20-30 sec for a 0.25 ml straw and 40 sec for a 0.5 ml one. After withdrawal, wipe it dry and place it in the gun, which should have been pre-warmed by rubbing between the hands. Only thaw one straw at a time. Cut the crimped straw end at a 90^{o} angle, then slide on to the plastic sheath and secure with the collar. Hold the gun vertically and gently press the plunger upwards, until the semen rises to the top.

3.4.5 Preparation

Clean the cow’s vulva with a paper towel and put on a full-arm glove and lubricant. Insert your arm into the cow, by forming a cone with your fingers while keeping the tail aside with your other hand. Gently work out any excess dung and if the rectum becomes distended with gas or the cow strains excessively, withdraw the arm and consider re-serving a few hours later. The cow must be relaxed during the procedure to avoid injury, as the rectum wall is a delicate structure. If the bladder is full, wait and try again once the cow has urinated.

3.4.6 Finding the cervix

The initial landmark is the cervix and this should be located before inserting the gun. The cervix is normally found on the pelvic rim, but in older cows, it may have moved slightly to one side. Consult the vet if the cervix is pulled down inside the abdomen and is difficult to manipulate, as the cow could be pregnant or suffering from a uterine infection.

3.4.7 Inserting the AI gun

After locating the cervix, use the elbow to exert downward pressure on the vagina. This will part the lips of the vulva, in preparation for the AI gun (Fig-4). The lips should be wiped clean, with the gun inserted past the vestibule and into the vagina. To avoid the opening to the urethra, run the gun tip gently along the roof of the vagina, until the cervix is reached. The gun should be inserted almost vertically at 45^0 angle and finish horizontally. Diagram showing how to insert the AI gun into a cow's cervix. The gun should be inserted as soon as possible after priming, to preserve semen quality. Insemination is a two-stage process. Guide the AI gun, so that it is engaged in the cervix Pass the cervix over the AI gun. The blind pocket around the cervix can make it difficult to the gun into the cervical canal entrance. To resolve this, push the cervix as far forward as possible, while closing the pocket with your grip. Once the tip is in the canal, you should feel a gritty sensation.

Fig- 4: Inserting AI gun

3.4.8 Lining up the AI gun

Line up the gun with the cervix and pass it through the canal, manipulating the cervix back over the tip of the gun.

3.4.9 Reaching the cervix

Once the gun is just through the cervix, you should feel a release in resistance to the gun. The semen should be deposited into the short chamber of the uterine horns, which are located on the other side of the cervix. If you put your index finger over the end of the cervix, you will feel where the top pokes through and this is the area where the semen should be deposited. It is body of uterus where the cervix ends and the uterus begins diagram showing how to know when the cow's cervix has been reached. Care should be taken to avoid placing the semen. In addition, the walls of the uterus are extremely delicate and easily damaged. Deposit the semen slowly, by counting 5,4,3,2,1.

3.5 Confirmation of pregnancy

Pregnancy diagnosis by rectal palpation:

All the animals under this study were subjected to pregnancy diagnosis by rectum palpation (Fig-5) after 60-90 days post AI by visiting owner's house. The results of the pregnancy diagnosis were recorded to find out the conception rate. The pregnancy was confirmed by observing the asymmetry of the horn, palpation of the fetus and slipping of fetal membrane.

Fig-5: Pregnancy diagnosis

3.6 Record keeping and data analysis

All the findings of the study were recorded and the data were analyzed statistically. The data generated from this experiment were entered in Microsoft Excel® Worksheet, organized and processed for further analysis. The data were analyzed by using SPSS software, SPSS Statistics V22. Descriptive statistics were performed to calculate the SE mean and percentages of total conception rate using frozen semen.

Reproductive indicates:

- Service per Conception $= \dfrac{\text{Total number of service}}{\text{Total number of cow conceived}}$

Conception rate calculation/estimated success rate of AI:

Conception rates (CR) are estimated from the proportion of pregnancies confirmed by rectal examination of the genital tract at day 60 of post-insemination among the total number of cows/heifer inseminated artificially with frozen semen in a specified period of time.

Reproductive indicates:

- Conception rate $= \dfrac{\text{No. of cows/heifer pregnant}}{\text{No. of cows/heifer inseminated}} \times 100\%$

CHAPTER IV

RESULTS & DISCUSSION

A total of 150 cows were inseminated with frozen semen brought to the Upazilla Livestock Office, Kaliakoir, Gazipur. In this study the number of Local and Friesian cross cows inseminated were large in number than Sahiwal cross breed. Among the age group 2< - <4 years were relatively larger than other groups. Majority of the animals had a BCS of 3 to 3.5.

Table-1: Description of demographic variables (Breed, age and BCS) of cows inseminated artificially.

Variable	Category level	Number of Observation
Breed	Local	60
	Sahiwal cross	42
	Friesian cross	48
Age (years)	2 - <4	85
	4< - <6	40
	6<	25
BCS	2.5	24
	3	49
	3.5	65
	4	12

4.1 Effects of breeds on pregnancy rate:

Effects of breeds on pregnancy rate of inseminated cows are presented (Table-2). There was a significant ($P<0.05$) breed effect on pregnancy rate, the pregnancy rate was higher in Sahiwal cross (59.52%). The lower pregnancy rate was observed in Friesian cross (47.92%).

Table-2: Effect of breeds on pregnancy rate in cows

Breeds	No. of cows inseminated	No. of cows pregnant	Pregnancy rate (%)		SEM
			Overall	Within group	
Local	60	35	23.33	58.33^{a}	0.064
Sahiwal cross	42	25	16.67	59.52^{a}	0.077
Friesian cross	48	23	15.33	47.92^{b}	0.073
Total	150	83	55.33		

Values bearing different letters within a column differ significantly ($P< 0.05$)

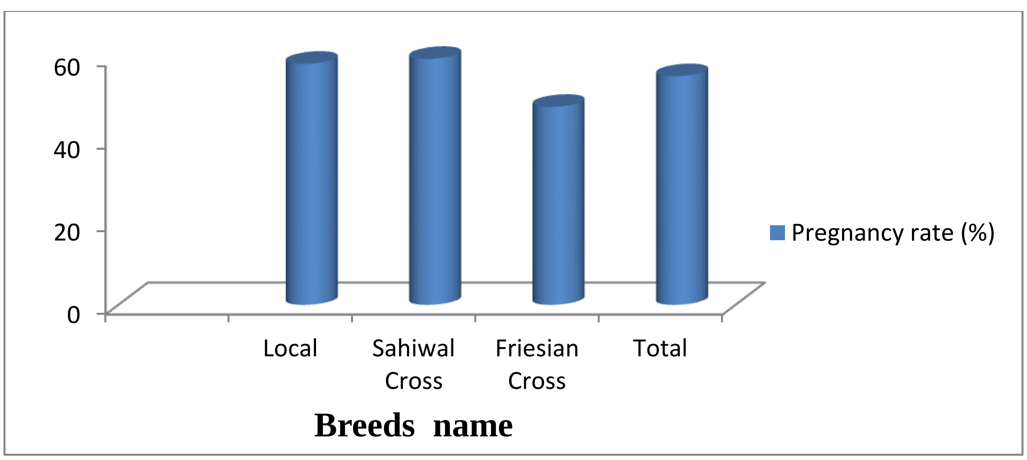

Fig-6: Effects of breeds on pregnancy rate

The pregnancy rates in different breeds of cows were Local (58.33%), Sahiwal Cross (59.52%), Friesian cross (47.92%), The association between breeds of cows and PR/AI was significant (P<0.05). It is supported by Shohiduzzaman *et al.* (2012) observed a pregnancy rate Local, Sahiwal cross, Friesian cross respectly 56.3%, 55.6%, 47.3%. Khatun *et al.* (2014) found AI per pregnancy of Local (52.9%) and Friesian cross (62.3%) cows were relatively higher than that of Sahiwal cross (40%) at 1% level of significant (p<0.01).

Reynolds *et al.* (1999) observed pregnancy rate of different breeds Local 52.9%, Sahiwal cross 40% & Friesian cross 62.3%. Miah *et al.* (2004) stated that the experiment was conducted for a period of 1.5 years at the district Artificial Insemination Centre, Sylhet, Bangladesh. They found the highest pregnancy rate was observed in the cows which were indigenous local cows (46.1%) of second parity and inseminated by local x Holstein-Friesian bulls (44.4%).

The average conception rate of local nondescript and crossbred cows with Holestein-Friesian and Sahiwal breed were 42.5% and 45.2% to 53.1%, respectively, Shamsuddin *et al.* (1997)

Gwazdauskas *et al.* (1975) observed a pregnancy rate of 33.8, 34.6, 37.0, 35.5 and 48.4% for Ayrshire, Brown Swiss, Guernsey, Holstein- Friesian and Jersey respectively. This is supported partially by Freer (1997) and Bratton *et al*. (1994).

Gosh (1995) found different breeds of cows L x HF = 50%, L x SL = 44% & L x J = 44% . The reasons for low pregnancy rate in Friesian cross is environment factor, susceptibility, humidity.

4.2 Effects of age on pregnancy rate

Effects of age of cows on pregnancy rates are presented (Table-3). The pregnancy rate with respect to different ages varied. The pregnancy rate in cows at 2< - <4 years age was higher than that of other age groups.

Table-3: Effects of Age on pregnancy rate in cows

Age group (years)	No. of cows inseminated	No. of cows pregnant	Pregnancy rate (%)		SEM
			Overall	Within group	
2< - <4	85	48	32	56.47^{a}	0.054
4< - <6	40	22	14.67	55^{a}	0.080
6<	25	13	8.67	52^{b}	0.102
Total	150	83	55.33		

Values bearing different letters within a column differ significantly (P< 0.05).

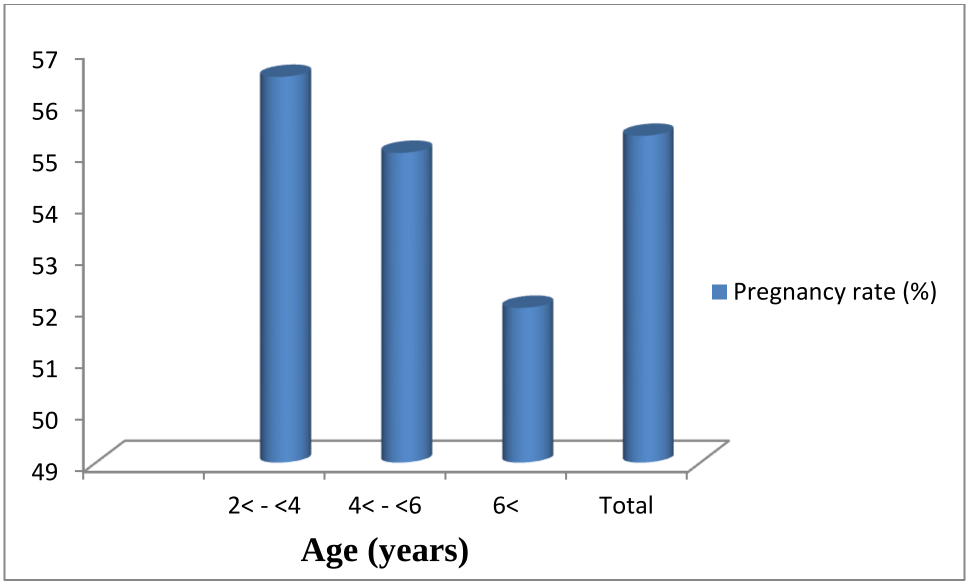

Fig-7: Effects of age on pregnancy rate

The pregnancy rate in cows 2< - <4 years, 4< - <6 years, and 6< years of age were 56.47%, 55%, and 52%, respectively. It shows that the pregnancy rate of 2< - <4 years & 4< - <6 years of old was higher (56.47% and 55%) than that of 6< years (52%). It is supported by Shohiduzzaman *et al.* (2012) found the pregnancy rate in cows 2-3 years, 3-5 years, and 5-7 years of age was 50%, 56.8%, and 51.9%, respectively.

Khatun *e. al.* (2014) found pregnancy rate of different age group <2, 2-3.9, 4-5.9, 6-7.9 years were 42.85%, 56.96%, 54.54%, 55.55% .

Spalding *et al.* (1975) reported that a slightly increase in the fertility of cows up to 3 to 4 years of age and decline after 4 years of age and marked decline in fertility in the cow over 7 years of age. The reason for low pregnancy rate in young cows in the present study may be explained by the fact that these cows may have suffered more from negative energy balance than middle aged grown cows. Moreover, the older cows might have more chance to get subclinical uterine infection resulting in lower conception rate.

4.3 Effects of BCS on pregnancy rate:

Effects of BCS on pregnancy rates of cows are presented (Table- 4). The pregnancy rate with respect to cows having different BCS ranged. The highest pregnancy rate was observed in BCS group 3.5. The significantly lowest pregnancy rate was observed in cows having lower BCS (2.5) compared with other groups.

Table-4: Effects of Body condition score (BCS) on pregnancy rate in cows

BCS	No. of cows inseminated	No. of cows pregnant	Pregnancy rate (%)		SEM
			Overall	Within group	
2.5	24	7	4.67	29.17[d]	0.095
3	49	29	19.33	59.18[b]	0.071
3.5	65	41	27.33	63.08[a]	0.060
4	12	5	3.33	41.67[c]	0.149
Total	150	83	55.33		

Values bearing different letters within a column differ significantly ($P < 0.05$)

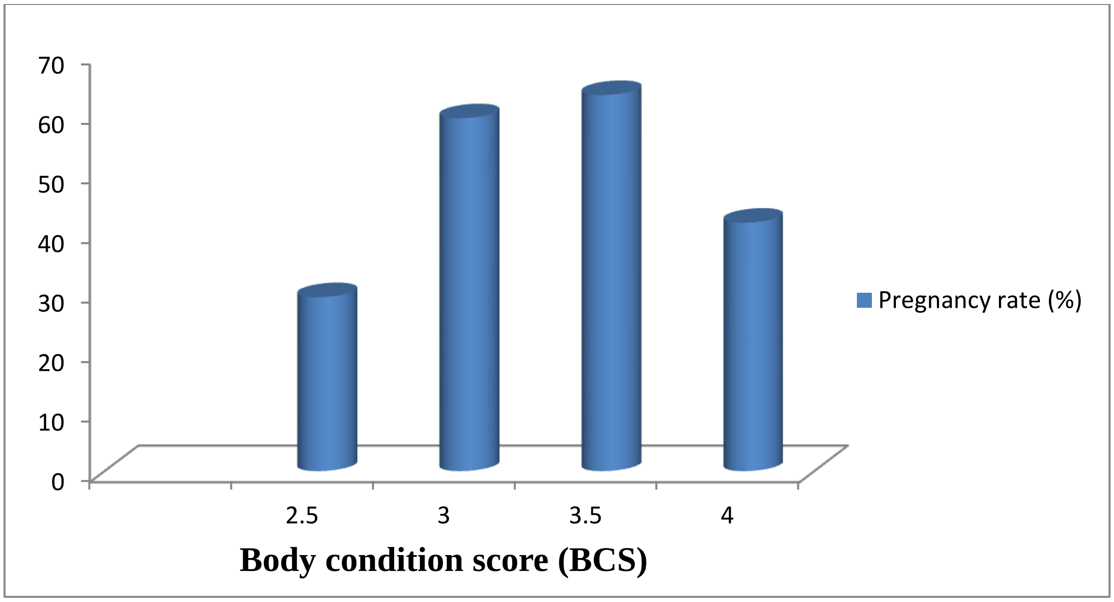

Fig-8: Effects of BCS on pregnancy rate

It is supported by Shohiduzzaman *et al.* (2012) who found the highest pregnancy rate 64.5% in cow with BCS 3.5 & lowest pregnancy rate 31% with BCS 2.5 .Woldu *et al.* (2011) found pregnancy rate at 41.2% of 2.5 BCS, 48.8% of 3- 3.5 BCS & 50% of 4 BCS.

Providing adequate quantity of balanced diet to animals will help to gain good BCS resulting in satisfactory conception rate. Higher pregnancy rate in cows with good BCS than that in cows with poor BCS has been documented by Shamsuddin *et al.* (2001) in Bangladesh. It was found in a number of studies that nutrition manipulation can result in changes in gonadotrophin secretion.

4.4 Effect of season on pregnancy rate in cows:

Effects of season on pregnancy rates of cows are presented (Table- 5). The pregnancy rate with respect to cows in different season ranged from 43.24 to 71.05%. The highest pregnancy rate was observed in Spring season. The significantly lower pregnancy rate (P<0.05, 43.24%) was observed in cows in summer season.

Table-5: Effects of season on pregnancy rate:

Seasons	No. of cows inseminated	No. of cows pregnant	Pregnancy rate (%)		SEM
			Overall	Within group	
Summer	37	16	10.66	43.24^{c}	0.083
Rainy	32	15	10	46.87^{c}	0.090
Winter	43	25	16.67	58.14^{b}	0.076
Spring	38	27	18	71.05^{a}	0.075
Total	150	83	55.33		

Values bearing different letters within a column differ significantly (P< 0.05)

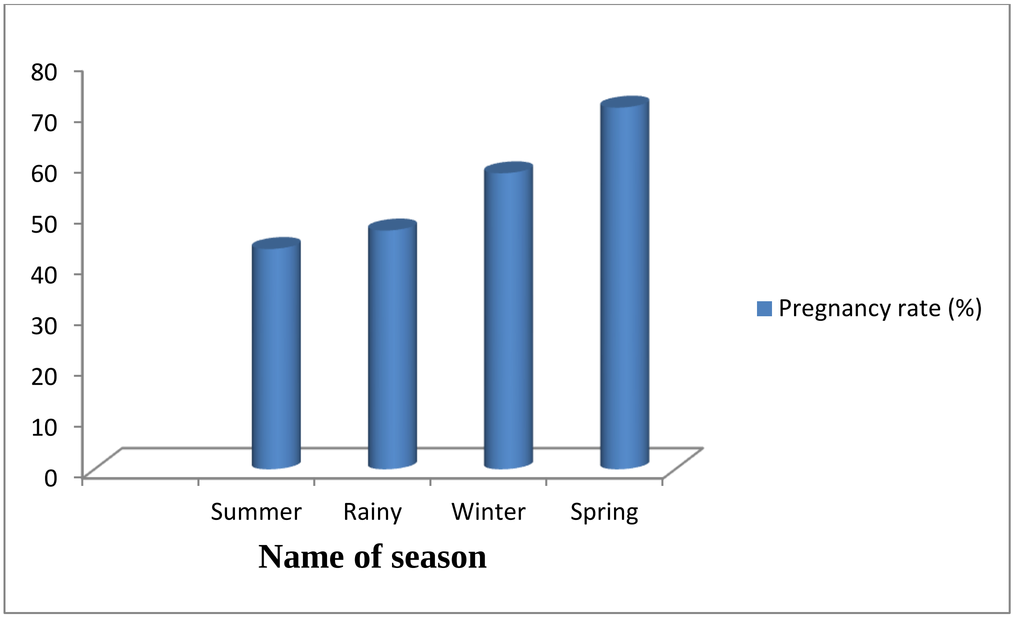

Fig-9: Effects of seasons on pregnancy rate

The study was divided into four seasons of a year such as summer (May - July), rainy (August - October), winter (November - January) and spring (February - April) and the pregnancy rate in different seasons were 43.24%, 46.87%, 58.14% and 71.05% respectively. In the summer season, heat stress markedly affects the pregnancy rate (25.4%) of dairy cattle (Ricardo *et al.*, 2004). Availability of green grass & other fodders are decreased in rainy season due to environmental calamities like flood. So, pregnancy rate decreased due to low nutrition. On the other hand, in winter & spring season more availability of vegetable wastes in the land, greed grasses & other fodders than rainy season which increases the pregnancy rate. The season of insemination might be the important factors to get maximum conception rate in cows (Miah *et al.*, 2004).

This finding is agreed with Quintela *et al.* (2004) who stated calving season was a significant factor for low PR/AI. The results showed that autum calving predispose to lower pregnancy rates than other calving season (Quintela *et al.*, 2004). It is reported that small ovarian follicles are susceptible to heat stress (Badinga *et al.*, 1995; Stevenson *et al*., 1995) and that takes above 40-50 days for small antral follicles to develop into large dominant follicle (Lussier *et al.*, 1987). Ahmed *et al.*, (1987) studied the seasonal effect on conception rate of cows in Bangladesh and recorded the highest conception rate (62.1%) in spring followed by summer (51.6%), winter (47.8%) and rainy (41.5%).

This study showed that overall pregnancy rate was 55.33%. The highest pregnancy rate (59.52%) was observed in Sahiwal cross higher than that of Friesian cross (47.92%). Cows of 2< - <4 years of old revealed a significantly higher pregnancy rate than others. The higher pregnancy rate (63.08%) was in cows of BCS 3.5 than lower BCS 2.5 cows (29.17%). Cows showed significantly higher PR (71.05%) when AI was done in spring (March-April) compared to winter (November-February) calving cows (58.14%). Good BCS significantly enhances the pregnancy rate in cattle. The author suggested that the spring (March-April) may be the best season for good fertility of cattle in Bangladesh.

CHAPTER V

SUMMARY & CONCLUSION

Livestock plays a vital role in the national economy of Bangladesh. The share of the livestock sub-sector, in 2017-18, to the national gross domestic products (GDP) was 1.54%, which was 14.23% of agricultural GDP. Employment (Directly) 20%, Employment (Partly) 45% . The study was conducted from May, 2018 to April, 2019 at upazilla livestock office, Kalikoir, Gazipur. A total of 150 cows were selected to evaluate AI practice in cattle based on various breed, age, BCS & season to assess the potentiality of AI practice in Bangladesh. The results of the pregnancy diagnosis were recorded to find out the conception rate. The pregnancy was confirmed by observing the asymmetry of the horn, palpation of the fetus and slipping of fetal membrane. All the findings of the study were recorded and the data were analyzed statistically. The data generated from this experiment were entered in Microsoft Excel® Worksheet, organized and processed for further analysis. The data were analyzed by using SPSS software, SPSS Statistics V22. Descriptive statistics were performed to calculate the SE mean and percentages of total conception rate using frozen semen. Conception rates (CR) are estimated from the proportion of pregnancies confirmed by rectal examination of the genital tract at day 60 of post-insemination among the total number of cows/heifer inseminated artificially with frozen semen in a specified period of time. Overall pregnancy rate was 55.33% . The highest pregnancy rate

(59.52%) was observed in Sahiwal cross, which was significantly ($p<0.05$) higher than that of Friesian cross (47.92%) cows. Cows of 2< - <4 years of old revealed a significantly ($p<0.05$) higher pregnancy rate than other. The higher pregnancy rate (63.08%) was in cows of BCS 3.5 than lower BCS 2.5 cows (29.17%; $p<0.05$). Cows showed significantly ($p<0.05$) higher PR (71.05%) when AI was done in spring (March-April) compared to winter (November-February) calving cows (58.14%). Good BCS significantly enhances the pregnancy rate in cattle. The author suggested that the spring (March-April) may be the best season for good fertility of cattle in Bangladesh. Other factor which may increase or decrease the conception rate sexual health status of the female reproductive organ, proper maintenance of liquid nitrogen in the container & faulty technique of using of frozen semen in AI practice for increasing conception rate, we have to take proper initiative such as proper heat detection, supply of high quality semen, accurate record keeping, good husbandry practice, supply of proper nutrition, trained & skilled technician at field level.

CHAPTER VI

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APPENDIX-I

Summer Season

SL. NO.	Date of data collection	Breeds of cow	Age (month)	BCS	Pregnancy Diagnosis
1	06.05.2018	Local	32	3	P
2	06.05.2018	Local	41	3	P
3	06.05.2018	Friesian cross	49	3.5	N
4	07.05.2018	Sahiwal cross	70	3	N
5	07.05.2018	Local	67	2.5	N
6	07.05.2018	Friesian cross	48	3.5	N
7	23.05.2018	Sahiwal cross	73	3.5	P
8	23.05.2018	Local	41	2.5	N
9	24.05.2018	Friesian cross	52	3.5	P
10	05.06.2018	Sahiwal cross	44	3	P
11	05.06.2018	Local	50	3.5	N
12	05.06.2018	Friesian cross	49	3.5	N
13	05.06.2018	Local	42	3	P
14	06.06.2018	Local	74	3	N
15	06.06.2018	Sahiwal cross	43	3.5	N
16	19.06.2018	Friesian cross	54	3.5	P
17	19.06.2018	Local	43	2.5	N
18	19.06.2018	Friesian cross	45	3.5	N
19	19.06.2018	Sahiwal cross	55	3.5	P
20	20.06.2018	Local	32	2.5	P
21	20.06.2018	Friesian cross	54	4	N
22	20.06.2018	Local	73	2.5	N
23	10.07.2018	Local	31	2.5	N
24	10.07.2018	Local	74	3	P
25	11.07.2018	Friesian cross	56	4	N
26	11.07.2018	Sahiwal cross	55	3.5	P
27	12.07.2018	Local	77	2.5	N
28	12.07.2018	Sahiwal cross	43	3	N
29	12.07.2018	Sahiwal cross	37	3	N
30	12.07.2018	Sahiwal cross	67	3.5	P
31	25.07.2018	Friesian cross	45	3	N
32	25.07.2018	Sahiwal cross	39	3	N
33	25.07.2018	Friesian cross	50	3.5	P
34	25.07.2018	Local	35	2.5	P
35	26.08.2018	Sahiwal cross	51	3.5	P
36	26.07.2018	Friesian cross	46	3.5	N
37	26.07.2018	Friesian cross	42	3.5	P

P = Positive, N = Negative

APPENDIX-II

Rainy Season

SL. NO.	Date of data collection	Breeds of cow	Age (month)	BCS	Pregnancy Diagnosis
1	08.08.2018	Sahiwal cross	52	3	N
2	08.08.2018	Sahiwal cross	38	3.5	N
3	08.08.2018	Friesian cross	58	3.5	P
4	08.08.2018	Local	34	3.5	N
5	09.08.2018	Friesian cross	69	3	N
6	09.08.2018	Sahiwal cross	44	3	P
7	09.08.2018	Local	34	3	P
8	28.08.2018	Friesian cross	41	3.5	N
9	28.08.2018	Sahiwal cross	52	3	P
10	28.08.2018	Sahiwal cross	41	3	N
11	28.08.2018	Local	68	2.5	P
12	28.08.2018	Friesian cross	61	3.5	P
13	28.08.2018	Sahiwal cross	73	3	N
14	29.08.2018	Local	55	3	P
15	29.08.2018	Local	74	2.5	N
16	29.08.2018	Friesian cross	49	3.5	P
17	10.09.2018	Friesian cross	44	3	N
18	10.09.2018	Local	42	3	P
19	10.09.2018	Sahiwal cross	44	3	N`
20	11.09.2018	Local	43	2.5	N
21	11.09.2018	Sahiwal cross	51	3	P
22	11.09.2018	Sahiwal cross	39	3	N
23	15.09.2018	Local	74	2.5	N
24	15.09.2018	Friesian cross	46	3	P
25	15.09.2018	Sahiwal cross	58	3.5	P
26	16.08.2018	Friesian cross	58	4	N
27	16.09.2018	Local	78	2.5	N
28	25.09.2018	Friesian cross	51	4	P
29	25.09.2018	Sahiwal cross	42	3	P
30	26.09.2018	Friesian cross	50	4	N
31	26.09.2018	Local	34	3	P
32	26.09.2018	Local	35	3.5	N

P = Positive, N = Negative

APPENDIX-III

Winter Season

SL. NO.	Date of data collection	Breeds of cow	Age (month)	BCS	Pregnancy Diagnosis
1	04.10.2018	Local	70	3	N
2	04.10.2018	Local	39	3	P
3	04.10.2018	Friesian cross	44	3.5	P
4	04.10.2018	Local	75	2.5	N
5	05.10.2018	Local	29	2.5	P
6	05.10.2018	Sahiwal cross	36	3	P
7	05.10.2018	Friesian cross	55	4	N
8	28.10.2018	Local	34	3	P
9	28.10.2018	Sahiwal cross	59	4	N
10	28.10.2018	Friesian cross	44	3.5	N
11	29.10.2018	Local	39	3	P
12	29.10.2018	Sahiwal cross	52	3.5	P
13	29.10.2018	Local	37	3	P
14	03.11.2018	Sahiwal cross	47	3.5	N
15	03.11.2018	Local	78	2.5	N
16	03.11.2018	Friesian cross	40	3.5	P
17	04.11.2018	Sahiwal cross	46	3.5	P
18	04.11.2018	Local	72	2.5	N
19	04.11.2018	Local	37	2.5	N
20	04.11.2018	Sahiwal cross	34	3	P
21	04.11.2018	Local	74	3	P
22	10.12.2018	Friesian cross	45	3.5	P
23	10.12.2018	Local	67	3.5	N
24	11.12.2018	Friesian cross	46	3	N
25	11.12.2018	Sahiwal cross	39	3.5	P
26	11.12.2018	Sahiwal cross	41	3.5	P
27	07.01.2019	Friesian cross	45	3.5	P
28	07.01.2019	Sahiwal cross	78	3	N
29	07.01.2019	Local	38	3	P
30	08.01.2019	Friesian cross	42	3.5	N
31	08.01.2019	Friesian cross	45	3.5	P
32	22.01.2019	Local	74	2.5	N
33	22.01.2019	Sahiwal cross	34	3.5	P
34	22.01.2019	Friesian cross	46	3.5	P

35	22.01.2019	Friesian cross	41	3	N
36	23.01.2019	Sahiwal cross	37	3	N
37	23.01.2019	Local	72	2.5	P
38	23.01.2019	Friesian cross	40	3.5	N
39	12.02.2019	Sahiwal cross	36	3.5	P
40	12.02.2019	Friesian cross	67	3	N
41	13.02.2019	Friesian cross	41	3.5	P
42	13.02.2019	Local	47	3.5	P
43	13.02.2019	Friesian cross	58	4	P

P = Positive, N = Negative

APPENDIX-IV

Spring Season

SL. NO.	Date of data collection	Breeds of cow	Age (month)	BCS	Pregnancy Diagnosis
1	03.03.2019	Sahiwal cross	44	3.5	N
2	03.03.2019	Friesian cross	46	3.5	P
3	04.03.2019	Local	74	3	P
4	04.03.2019	Local	40	3.5	N
5	04.03.2019	Sahiwal cross	46	3.5	P
6	04.03.2019	Friesian cross	61	4	N
7	04.03.2019	Local	76	3	P
8	10.03.2019	Local	39	2.5	N
9	10.03.2019	Friesian cross	45	3.5	P
10	10.03.2019	Sahiwal cross	72	3.5	P
11	13.03.2019	Local	47	3.5	N
12	13.03.2019	Local	42	3.5	P
13	13.03.2019	Local	74	3	P
14	13.03.2019	Friesian cross	52	4	P
15	13.03.2019	Sahiwal cross	44	3.5	N
16	14.03.2019	Friesian cross	53	4	P
17	14.03.2019	Local	35	3.5	P
18	14.03.2019	Sahiwal cross	39	3.5	P
19	27.03.2019	Friesian cross	40	3.5	N
20	27.03.2019	Local	72	2.5	P
21	28.03.2019	Local	36	3.5	P
22	28.03.2019	Friesian cross	51	4	P
23	28.03.2019	Local	39	3.5	P
24	07.04.2019	Local	38	3.5	N
25	07.04.2019	Friesian cross	45	3.5	N
26	07.04.2019	Sahiwal cross	40	3.5	P
27	07.04.2019	Sahiwal cross	47	3.5	P
28	08.04.2019	Local	42	3.5	P
29	08.04.2019	Sahiwal cross	42	3.5	P
30	08.04.2019	Local	77	2.5	N
31	16.04.2019	Local	72	3	P
32	16.04.2019	Local	54	3	P
33	16.04.2019	Friesian cross	46	3	N
34	17.04.2019	Local	74	3	P
35	17.04.2019	Friesian cross	40	3.5	P
36	17.04.2019	Sahiwal cross	39	3.5	P
37	23.04.2019	Local	72	2.5	P
38	24.04.2019	Local	39	3	P

P = Positive, N = Negative