International Journal of Animal Science and Technology
2017; 1(1): 19-34
http://www.sciencepublishinggroup.com/j/ijast
doi: 10.11648/j.ijast.20170101.14

Effects of GnRH on Conception Rate at the Time of Artificial Insemination in Crossbred Dairy Cows

Sm. Ahasanul Hamid[1, 2], S. M. Kamruzzaman[2]

[1]School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Japan

[2]Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi, Bangladesh

Email address:

ahasanulhamid@gmail.com (S. A. Hamid)

To cite this article:

Sm. Ahasanul Hamid, S. M. Kamruzzaman. Effects of GnRH on Conception Rate at the Time of Artificial Insemination in Crossbred Dairy Cows. *International Journal of Animal Science and Technology*. Vol. 1, No. 1, 2017, pp. 19-34. doi: 10.11648/j.ijast.20170101.14

Received: November 20, 2017; **Accepted**: December 7, 2017; **Published**: January 3, 2018

Abstract: The present study was conducted to investigate the influence of GnRH (Gonadotropin Releasing Hormone) administration on conception rate at the time of artificial insemination (AI) in healthy crossbred dairy cows. A total 90 cows were recruited and a preset questionnaire was answered by the owners. The cows were divided randomly in to two equal groups. GnRH treated group was injected intramuscularly (i/m) in the neck muscle with GnRH (Gonadorelin Acetate) at the time of AI. Again the treated group was divided randomly into three equal groups (15 cows each) for three different doses of GnRH (200, 250, 300 μg). The necessary information was taken from the owner with pretested questionnaire. The overall 71.11% conception rate was found in GnRH treated group on the other hands 55.56% conception rate was in control groups and having significant effect ($P<0.05$). There were significant effect ($P<0.05$) of age and milk yield and there were no significant effect of doses, timing, body condition score and parity within GnRH treated group. The study revealed that use of GnRH improved the conception rate in crossbred dairy cows at the time of AI.

Keywords: Conception Rate, Crossbred Dairy Cows, GnRH and Artificial Insemination

1. Introduction

Production of livestock is the segments of agriculture constituting means of developing living standards of exponentially increasing poor farmers in many regions of developing world (Sere and Steinfeld, 1995; FAO 2009). Those production sectors can dramatically reduce poverty and huge demands of food. To get desired productions, development in management, environment, genetics, health and reproductive management is essential (Azage *et al*., 1981).

For ensuring of upgrading reproductive performance, understanding of reproductive traits is essential in bovine species (Hogland, 2013). These parameters are; age of first service, number of services per conception, days-open and calving interval and these are common for profitable dairy cattle production (Azage *et al*., 1981 and Stevenson *et al.,* 2000). Instead of, low reproductive performances hinders genetic development as local breed which were not crossed provides to less milk production, lengthy calving interval, low sexual maturity, small lactation length and thus such factors directly suffers the farmers and gross domestic production of a nation (Mukosa-Mugerwa *et al*., 1993).

Bangladesh is most densely populated and the fourth largest agricultural country in the world (Habib, 2001). The economy of Bangladesh depends huge on agriculture. Livestock production is one of the four major components viz., crops, livestock, fisheries and forestry of agriculture plays animportant role in national economy. At present livestock sector accumulates 6.5% to GDP on the basis of added value through production of milk, meat, hide, skins and eggs. The country earns about 13% of foreign currency through hide and skins export (Alam, 1991). In addition, the sub-sector supplies an average of 42.54% of the animal protein in the form of milk, meat and eggs (BBS, 1998).

Livestock population in Bangladesh was mainly of cattle, buffalo, goat, sheep, chicken and ducks with respective populations are 24.5 million, 0.82 million, 34.5 million, 2.00 million, 139.55 million and 70.12 million (BBS, 2001). So, it was clear that the total number of livestock in Bangladesh was not small in proportion to 160 million of its human population, but the major problem is the animal output individually. This low productivity of the native livestock was mainly due to less improved genetic materials. Bangladesh now produces 2.28 MMT of milk and 1.04 MMT meat of against demand of 12.58 MMT milk and 1.04 MMT meats (DLS, 2008). With the growth of human population and more urbanization, the demand of meat and milk will also increase in future dramatically. In a nutshell, there was wide scope for milk and meat production if it was produce profitably under good supervision.

It was calculated that daily per capita demand of milk was 250 ml and annual requirement stands at about 12.58 million MT in the country (DLS, 1991). But it was alarming that the present daily per capita availability of milk was only 40 ml and total annual production was calculated at 2.28 million MT. Thus, annual deficit of milk in the country was about 10.30 million MT (DLS, 1998). To fulfill the deficit, the country has to import milk and milk products from aboard every year costing large amount of foreign money TK. 1310 million (DLS, 1998). So, decision was made to reduce this deficit by developing dairy farm at privately as well as upgrading high quality breeds or genotype of the dairy cattle under subsistence farming. In addition, cattle holds the first position in livestock sector in Bangladesh, so effort should be given on cattle development programmed.

Economy of dairy farming largely depends on a good conception rate after insemination or natural service. The twelve-months calving interval was advantages for maximum milk yield per cows per year with good economic return (Opsomer *et al.,* 1996). One of the major constraints of profitable dairy farming was low conception rate (Alam and Ghosh, 1994; Mehedi, 2002, Sarder, 2008).

Reproduction in dairy cows has lowered during the last few decades due to many factors including inaccuracy of estrus detection, improper timing of insemination and delayed ovulation problems. Another factor contributing to lowpregnancy rates is embryonic loss. Alongwith many possible etiologies, low plasmaprogesterone concentration has been relatedto early embryonic death (Mann andLamming, 1999). Some studies have been shown the effect of GnRH on reproductive performances in dairy cows when treated on the day of AI (Shahneh *et al*., 2008), early luteal phase (Beltran and Vasconcelos, 2008) and/or mid luteal phases (Szenci *et al*., 2006).

The estrus cycle of cows takes about 21 days (18-24 days). The top moment of the cycle is the estrus and the ovulation (Broers P, 1996, Driancourt MA, 2001, Sali G, 1996, Youngquist RS, 1997). These procedures are controlled through the neuronal-endocrinal system. The absence or the delay of the ovulation is one of the facts that lower the conception of the cows (Morrow AD, 1986, Emma CL Bleach 2004). Many factors as; the nutrition, breeding conditions, different pathologies, stress caused by high temperature, the milk yield, the age, and the breed etc, have a negative influence on the ovulation process. In present days the relations between system hypatalmo -hypofisar - ovaries are well known (Milvea RA, 1996, Ginther OJ, 2000, Perry TC, 1993). Onthe estrus, the mature follicle grows up and about 12 hours after it ovulates (Younqguist SR, 1997, Roche FJ, 2001, Sali G, 1996, Broers P, 1996).

Only knowledge of semen deposition in the reproductive tracts will not result in high conception rate and high milk yield since factors such as female fertility and heat detection accuracy must be considered in conjunction with the AI. Using AI, many exotic breeds were crossed with local one with improved milk yield by many folds having good situations (Foote, 2002). However, this technology is not without problems. So the above condition, major problem observed is low conception rate using AI. This was the main problem that needs a solution to increase pregnancy rate in crossbred cattle (Morrel, 2011). According to the various literatures, major reproductive problems identified were, delayed ovulation, anovulation, anestrus, cystic ovaries and early embryonic death which cause cows to show repeated estrus cycle and low conception rate (Maureral, 1985). Such conditions were observed due to hormonal deficiency; GnRH produced by hypothalamus which controls synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). These two hormones have combined effects on follicular development, ovulation and corpus luteum functions (Douglas, 1998).

Many techniques were taken to treat reproductive problems associated with such hormonal imbalances (Mwaagna, 2004). One of these techniques was the use of synthetic GnRH analogue that has been used to modify reproductive efficiency of normal dairy cows which has been investigated widely. Synthetic buserelin acetate has an added advantage of improved half life in the blood circulation, greater stability to enzymatic degradation, increased receptor affinity, prolonged biological potency and less antigenic than the other molecules for repeated use(Cline, 2002 and Ehler *et al*., 2013). Exogenous administration of these hormones at different period of estrus of cow may lead to treat disease condition or cysts in the ovary or may rectify the hormonal insufficiency to these cows (Morgan, *et al*., 1993; Bearden and Fuquay, 1997). The GnRH had been used as treatment for conception rates, embryonic mortality and treatment for cystic ovaries (Gustafsson *et al.,* 1986).

GnRH is a protein produced by hypothalamus. This hormone controls the synthesis and release of LH and FSH both are produced from the pituitary gland. The combined action of these two hormones regulates follicular development, ovulation and corpus luteum (CL) function. For many years, these GnRH agonists have been used to manipulate reproduction functions in dairy cattle (Douglas, 1998). Various GnRH agonists have been used with variable success for enhancing conception rates and shortening the interval to first postpartum ovulation in dairy cattle. The different economical and more reliable methods are used to evaluate financial returns from the use of GnRH at time of insemination to enhance fertility of dairy cows.

There are several factors which are affecting fertility in crossbred dairy cows in addition withhigh milk production, postpartum disorders and low body condition score (Vacek *et al.,* 2007; Jilek *et al.,* 2008). GnRH is a protein hormone secreted by hypothalamus. It controls the production and release of LH and FSH those are originating from the pituitary gland. These two hormones control follicular growth, ovulation and corpus luteum (CL). GnRH agonists have been used to manipulate reproductive function in dairy cattle (Douglas, 1998). GnRH and its various analogues administered at the time of AI are the familiar treatments in various management programs for cross bred dairy herds, prescribed by veterinarians (Stevenson *et al*., 1984; Chenault, 1990; Stevenson *et al*., 1990; Morgan and Lean, 1993).

Effective improvement of the conception rate after administration of GnRH has been attributed to the prevention of an ovulation failure or a small variation in the time between the onset of estrus and ovulation by increased preovulatory LH surge (Mee *et al.,* 1993; Kaim *et al.,* 2003). Some scientist found that conception rate was improved after administration of GnRH (Kaim *et al.,* 2003; Lee *et al.,* 1983; Nakao *et al.,* 1983; Lopez-Gatius *et al.,* 2006). On the other hand, others reported there is no effect on conception rate (Stevenson *et al.,* 1984; Lee *et al.,* 1985; Chenault, 1990; Mee *et al.,* 1990; Perry and Perry, 2009).

1.1. Advantages of GnRH Administration at the Time of AI

Administration of GnRH analogue during, before and after the time of AI following detection in standing estrus in crossbred dairy cows may increase conception rate.

The main purpose of rearing crossbred dairy cows to get one calf per year from a dam will be fulfilled.

Increase the number of crossbred dairy cows and milk production also.

Increased demand for milk and meat will be fulfilled.

Grossly marginal farmers will get handsome profit from their crossbred dairy cow's production.

1.2. Importance

Milk and meat are the products of great demand all the year round. As a result of this demand, farmers have been given an incentive to produce milk and meat around the year. In order to achieve this demand for milk and meat, crossbred dairy farmers needed to change their breeding policy. Enhancement of conception rate through administration of GnRH analogue on standing estrus of crossbred dairy cows enabled the changed of breeding policy. Production of more calves per year on farms will change the demand of milk and meat production. In addition improvement of conception rate using GnRH analogue will help in reduce cost involve in breeding policy.

No work was done to investigate the effects of GnRH analogue on enhancement of conception rate of crossbred of dairy cows and therefore the present study was conducted with the aim of enhancing the conception rates in crossbred dairy cows with specific objectives of: observing the effects of GnRH on improvement of conception rate on treated groups as compared to control and to evaluate the effects of dose, parity, age, milk yield, timing and body condition score variation between treated groups.

Therefore; my present study is designed to investigate the potential effect of GnRH on conception rate in crossbred dairy cows when administered during, one hour before and one hour after the time of AI. Finally the study shows benefits obtained from treatment of hormones.

1.3. Objectives

To know the Conception rate of crossbred (LxHF) dairy cows in Bangladesh may be increase with the practice of GnRH administration at the time of AI.

Investigate the potential effect of GnRH on conception rate in crossbred dairy cows.

2. Review of Literature

Low conception rate is a tremendous problem in dairy cows worldwide including Bangladesh. This problem was solved through various techniques. Many researcher have been taken worked on pure and cross breed dairy cows using hormonal drugs by different strategies. Historically, use of GnRH or its analogue has been promoted as a tool for those farmers who want to get superior percentage of conception rate after AI in their cows. However, this aspect was peanuts compared to the economic returns available when GnRH or its analogue was used as a reproductive management tool. Many GnRH therapy irrespective of days of administration resulted in an overall enhancement in conception rate of 83.33 as against 33.33 percent in control group of cows (Mahesh *et al*., 2010).

The use of the hormonal substances is still one of the best treatment to decrease the delay of the ovulation together with AI, thus increases the conception rate in dairy cows (Luigjturmalaj *et al*., 2014). Some studies have been shown the effect of GnRH on reproductive performances in dairy cows when treated on the day of AI (Shahneh *et al*., 2008), early luteal phase (Beltran and Vasconcelos, 2008) and/or mid luteal phases (Szenci *et al*., 2006).

Economy of dairy farming largely depends on a high conception rate after insemination. The one year calving interval was advantages for highest milk yield per cows per year with better economic return (Opsomer *et al.,* 1996). One of the major constraints of profitable dairy farming was low conception rate (Alam and Ghosh, 1994; Mehedi, 2002, Sarder, 2008). It was observed that the loss in gross margin (milk sell over feed cost) tended to be increased when calving interval exceeded twelve months.

Effective improvement of the conception rate after administration of GnRH has been attributed to the prevention of an ovulation failure or a time variation between the onset of estrus and ovulation by increased LH surge (Mee *et al.,* 1993; Kaim *et al.,* 2003). Some researchers found that conception rate was improved after administration of GnRH (Kaim *et al.,* 2003; Lee *et al.,* 1983; Nakao *et al.,* 1983; Lopez-Gatius *et al.,* 2006). On the other hand, others reported there is no effect on conception rate (Stevenson *et al.,* 1984; Lee *et al.,* 1985; Chenault, 1990; Mee*et al.,* 1990; Perry and Perry, 2009).

Subfertility problems were of great concern to dairy producers worldwide. About 37% cows were affected with infertility and infertility causes every year approximately 47 crore taka economic losses in Bangladesh (Samad, 2001). The estimation of the effects of these conditions on milk production, fertility and survivability were of great importance to assess cost-benefits of diagnosis, treatments and prevention efforts (Bar and Ezra, 2005). Gynecological problems were related to infectious diseases, hormonal imbalance, nutritional deficiencies and pathological conditions of the genital tracts. Subfertility cows were often suffering from anoestrus, repeat breeding and lack of fertility (Alam and Rahman, 1979, Dewan and Rahman, 1987; Shamsuddin *et. al.,* 1988; Ahmed *et. al.,* 1989 Mollah *et. al.,* 1989, Rahman, 1993).

Reproduction process is regulated through communication between the endocrine system and the nervous system. Hormones are chemical messengers produced in various locations in the body, which control or regulate the function of cells, tissues, and organs. Hormones are released into the blood and exert their effect by binding to a specific receptor on or in target tissues. Receptors, or receptor domains, for protein hormones (LH and FSH) are located on the plasma membrane of the target cell. When the domain becomes occupied, configurationally changes occur (Ginther *et al*., 1996). Both pituitary and steroid hormones regulate the synthesis, storage, and release of hypothalamic hormones through three feedback mechanisms: a long, short, and ultra-short loop (Shupnik, 1996). Sexual promoters (estradiol (E2), progesterone (P4), and testosterone) are produced by the gonads of the male and female to help regulate the function of the hypothalamus and the anterior pituitary (Shupnik, 1996). The ovary is dependents on secretion of gonadotropins from the anterior pituitary. Hypothalamic neurons synthesize neuropeptides that stimulate or inhibit release of hormones from the anterior pituitary, which eventually influences ovarian function (Nett, 1990). The E2 exert their effects on the GnRH receptors to stimulate release of LH (Thiery and Martin, 1991).

There is an important interrelationship between nutrition and production performance of dairy cows. This interrelationship has far reaching effects on the physiological functioning of the reproductive system, which is constantly under the influence of the neuro-endocrine system. Ruminants have the ability to convert low quality forages to useful products and the excess nutrients can be stored to be retrieved at a later time to maintain production (Short et *a1*., 1990). The energy requirement just before parturition for a dairy cow weighing 600 kg is 85 MJ/day (Sporndly, 1995), it increases shot upafter parturition and at the peak of lactation the demands are at least three fold compared to that of late gestation (Osterman, 2003).

Negative energy balance results in mobilization of body fat and loss of body condition score (BCS), which is strongly associated with the length of VWP to conception, reduced serum progesterone concentrations and pregnancy rate (Butler, 2005). The reproductive performance of cows may be negatively associated with the magnitude and severity of negative energy balance during early lactation (Nebel and McGilliard, 1993) first ovulation (Canfield and Butler, 1991) and formation of luteal tissue. The latter influences progesterone concentrations in milk and early embryonic development (Shamsuddin *et al*., 2001). Therefore, poor BCS is generally 35 resulted from negative energy balance and there was negative relationship between negative energy balance and development of luteal tissue and embryonic development (Tenhagen *et al*., 2000). A significant negative correlation was observed between weight gain and days postpartum period (Ghosh, 1990). Change in BCS after calving, rather than BCS at calving has a significant effect on the percentage of dairy cows exhibiting a prolonged VWP (Shamsuddin *et al*., 2005). BCS loss was greater in animals supplemented with concentrate prior to calving (Kokkonen, 2005).

Luigj Turmalaj *et al*., (2014) reported that high temperature over 35°C (heat stress) is one of the factors which influence in general reproduction processes and specially the ovulations process. The researchers have thought to experiment the use of hormonal substances (analogue synthetic of GnRH). Two cows of Simmental and their crossbred groups were made up. All the cows have the same breeding conditions. Their age is about 3-6 years old and they are 60 days after their parturition, they are also in normal healthy condition and with estrus normal cycle. The experimental group (IA + GnRH), the cows of this group were inseminated and they have been treated with Fertagyl (Intervet, 0.1 ng/ml) 2.5 ml/animal, (IM). The B group of control (IA), the cows of this group has been inseminated without hormonal above. The artificial insemination of the cows was performed about 12 hours after the beginning of the estrus. It resulted that (for two cows groups) the rate of fertilization for the experiment group is 80% against 60% of the control group ($P>0.05$). Thus, the treatment of the cows by using synthetics analogue of GnRH and at the same time the artificial insemination influences positively on their conception rate.

GnRH is a naturally occurring hormone. During the normal estrous cycle of a non-pregnant animal, GnRH is synthesized and release from the anterior pituitary gland. This release of GnRH acts on ovary and induces ovulation and luteinization of dominant follicles and causes the smaller follicles. A new follicular wave was subsequently recruited and a dominant follicle gradually emerges. GnRH will destroy the follicular cyst which a structure in the ovary that interferes with the normal hormonal cycle of cows and prevents the cows from returning to estrus.

Fricke (2005) stated that hormonal intervention is a good method for improving reproductive management. Induction of estrus cycles, ovulation and synchronization of estrus and ovulation and elimination of the necessity of estrus detection were possible by hormone treatments using specified Techniques. However, hormonal interventions with breeding program increase conception rate by increasing the number of cows at service not by increasing conception rate.

Youngquist *et al*., (1988) stated that when artificial insemination is used, the most common cause (about 90%) of anestrous is failure to observe estrus, while 10% due to an abnormality that suspended the estrus cycle. It becomes difficult to identify the accurate time of insemination. In practice, some of the animals would have been inseminated at wrong time (Alam and Ghosh 1994 and Shamsuddin *et al.,* 2001).

Faisal Omar Ahmed *et al*., (2015) reported that the number of services per conception was significantly (P <0040) improved for the GnRH treated crossbred dairy cows as compared to the control group crossbred dairy cows. Moreover the days open and calving interval were significantly (P <001) minimized compared to the control. A total number of 45 crossbred dairy cows (4-8 years) were taken to determine the influence of administration of GnRH on the above traits. The cows were divided randomly into three equal groups (15 cows each). Group A and B were injected intra muscularly with GnRH or $PGF_{2\infty}$ 200 microgram and 500 microgram respectively on the same day. Other group kept as the control group.

Mahesh S. Dodamani *et al.,* (2010) stated that twenty four cows were randomly allocated into 4 groups of six each. The animals of groups I, II and III were injected with 250 µg of buserelin acetate (Receptal®) on two occasions i.e. once on day of estrus and second dose on days 10 or 12 or14 respectively in I, II and III groups following breeding, while the animals of group IV served as control. Among the physical characters of estrualcervico-vaginal mucous, typical arborization pattern (80.95% in pregnant vs. 55.56% in non-pregnant cows) and marginally high spinnbarkeit readings (24.67+2.7cms in pregnant and 22.21+1.32 cms in non-pregnant cows) favored better fertility, although the differences between the groups were statistically insignificant. However, the pH of estrualcervicovaginal mucous did not indicate any effect on fertility and it ranged between 8.00 to 9.00. The cows of treatment groups I, II and III registered a considerably higher conception rate of 83.33 percent each, while in control group cows had only 33.33 percent.

Birhanu *et al*., (2014) reported that the use of GnRH injection 12 days post AI and can improve pregnancy rate in repeat breeding cross bred dairy cows. A total of forty four repeat-breeding cows from twenty herds were selected and assigned randomly in to two equal groups, A and B: one treated and one control group. Group A (n=22) cattle were treated intramuscularly with 10 µgbuserelin acetate 12 days post AI. Group B (n=22) cattle were inseminated once during estrus exhibition with single service and considered as control. Dairy cows were examined for pregnancy after three months through rectal palpation. Pregnancy rates recorded were 55%, and 32% in A and B respectively. The conception rates of treated group exceed control group by 23% respectively. Treated group showed a significant statistical variation (P<0.05) as compared to control groups.

BirhanuHailu *et al*., (2015) revealed that the use of GnRH analogue at the time of AI can improve conception rate. Sixty six cross breed dairy cows which were apparently healthy, exhibits estrus regularly were selected and taken randomly in to three equal groups (A, and C); two treated groups and one control group. Group A (n=22) cattle were administered intramuscularly a 20 µgbuserelinacetateat along with AI and Group B (n=22) cattle have injected with dose of 10µg Buserelin acetate (GnRH analogue). Group C (n=22) cattle were receive no treatment and considered as a control group, given a single service. These cows were tested for pregnancy after three months by rectal palpation. Additional history was taken from herd owners. The pregnancy rates recorded were 68%, 59% and 32% in the cattle grouped with A, B and C respectively. The conception rates of the treated groups exceeds control group by 36% and 27% respectively. Treated groups showed a significant statistical variation (P<0.05) as compared to control group. Dose variation between two treated groups has an effect on conception rate with significant variation. Other observed finding was that treated first and second parity cows became more responsive to therapy than those with more than two parity with a significant variation (P<0.05).

Anjum *et al.,* (2010) revealed that the administration of GnRH analogue increase the conception rate in repeat breeding crossbred cows when it was injected at the time of AI. A total of 64 cows maintained at the Government Farm Okara were included in the study. Age of these cows ranged from 44 to 232 months and their lactation number ranged from 1–13. The experimental cows were divided into control and treatment groups. In this experiment 32 cows received no treatment at the time of AI while in other group 32 cows received Dalmarelin (GnRH analogue) therapy at the time of AI. The cows were examined after 60 days post insemination per rectum. A total 12 animals out of 32 confirmed pregnant after rectal palpation having conception rate 37.5% in control group and in treatment group 22 animals out of 32 confirmed pregnant after rectal palpation having conception rate 68.75% (P < 0.0242/x2: 5.082).

M.P. Beltran *et al*., (2008) reported that Treatments with GnRH or hCG increased conception rates in cows with rectal temperature below 39.7°C (CG: 10.1%, n=26, GG: 36.8%, n=27; and HG: 32.8%, n=21), but not in cows with rectal temperature above 39.7°C (CG: 15.2%, n=26; GG: 17.8%, n=28; and HG: 24.4%, n=30). Holstein cows (n=158), at 213±112 days in milking and averaging 26±9kg of milk per day, were randomly assigned to one of three treatment groups: control (CG, n=52, saline), GnRH (GG, n=55, 100g gonadorelin), and hCG (HG, n=51, 2500IU) given five days after artificial insemination (AI). Rectal temperature was taken at the time of AI and blood samples were taken five, seven, and 12 days after AI. Pregnancy was tested between 42 and 49 days after AI. Concentration of progesterone (P4) in serum (ng/ml, mean±SE) for CG, GG, and HG were, respectively, 2.7±0.4, 2.5±0.4, and 3.2±0.5 on day 5; 4.8±0.4, 4.2±0.4, and 5.7±0.5 on day 7; and 5.2±0.4, 6.9±0.4, and 8.5±0.5 on day 12 after AI. P4 concentration had proportional increase in serum between days 5 and 7 after AI (CG: 178%, GG: 168%, and HG: 178%), suggesting that the treatments did not induce a luteotropic effect on the existing corpus luteum (CL). Concentrations of P4 increased between days 7 and 12 in cows treated with GnRH and hCG (GG: 164%, and HG: 149%, P<0.01); but not in control cows (GC: 18%, P=0.31).

M. Kaim *et al*., (2003) stated that GnRH at onset of estrus increased LH surges; prevented delayed ovulation, and may increase subsequent progesterone concentrations. Treatments with GnRH increased conception rate in primiparous cows, during summer, and in cows with lower body condition. 146 cows received either saline, 250 μg of GnRH, or 10 μg of the GnRH analogue, Buserelin. Cows were observed for estrus detection, blood samples were taken, and ovulations were recorded by ultrasound. In controls, 76% of cows had intervals from estrus to ovulation of ≤ 30 h and 24% had intervals > 30 h. Treatment with either GnRH or GnRH analogue increased magnitude of LH surges and decreased time intervals from estrus to LH surge or to ovulation. Higher plasma progesterone was observed in the subsequent estrous cycle in GnRH administered cows compared to control cows with delayed ovulations. Experiment 2 included 152 primiparous and 211 multiparous cows in summer and winter. Injection of GnRH analogue at OE increased conception rates (CR) from 41.3 to 55.5% across seasons. In summer, GnRH treatment increased CR from 35.1 to 51.6%. Across seasons, GnRH increased CR from 36.0 to 61.5% in cows with lower body condition at insemination and GnRH increased CR (63.2 vs. 42.2%) in primiparous cows compared to controls. Use of GnRH eliminated differences in CR for cows inseminated early or late relative to OE and increased CR in cows having postpartum reproductive disorders.

P. K. Sarker *et al*., (2015) reported that GnRH may be the choice of treatment for anestrous and following conception rate. The GnRH treated heifers showed the highest (60.00%) proportion of cyclicity and conception rate (46.67%) whereas vitamin mineral solution treated heifers showed the lowest cyclicity (33.33%) and conception rate (26.67%), (P<0.05). Heifers with BCS 3.5 had the highest (57.14%) rate of oestrus induction and (57.14%) rate of conception (P<0.05) whereas heifers with BCS 2.5 had the lowest (41.67%) rate of oestrus induction and (20.83%) rate of conception (P>0.05). Heifer showed highest rate (50.00%) of cyclicity and (38.89%) rate of conception when their age ranges from 36 to 42 months where as heifer showed lowest (37.50%) rate cyclicity and (31.58%) rate conception when their age ranges from 24 to 29 months (P>0.05).

Iftikhar A. *et al*., (2009) stated that use of GnRH analogue improved the conception rate in repeat breeding crossbred cows when administered at the time of AI. The experimental cows were divided into two groups A and B, with 32 cows in each group. Cows of group A received no treatment at the time of AI and served as control, while 32 cows of group B received Dalmarelin therapy at the time of AI. The cows were examined for pregnancy 60 days post insemination per rectum. A total of 12 animals out of 32 were pregnant with a conception rate of 37.5% in control group and in treatment group 22 animals out of 32 were pregnant, having conception rate of 68.75% (P<0.05).

Jaswal *et al*., (2013) reported that gonadotropin releasing hormone analogue increase conception rate in cows when administered either at the time of AI or day 5 or 12 post AI. Overall, 531 dairy cows were inseminated with the aim to know their reproductive performance following administration of GnRH analogue on different days of estrous cycle with different doses. These cows were divided into six treatment and one control group. Depending upon different treatment groups, Buserelin acetate (GnRH analogue) was injected at a dose of 10.5 µg or 21.0 µg on different days (0, 5 or 12) of estrous cycle in these cows. Control cows were inseminated without any treatment. The highest CR was recorded when 10.5 µg GnRH analogue was administered on day 12 post AI. The doses of 10.5 µg or 21.0 µg were equally effective when instituted on day 12 post AI.

3. Materials and Methods

3.1. Description of Study Areas

The study was conducted at four metro thanas of Rajshahi district in Bangladesh. Rajshahi district are located at the North side and 261 kilometers away from Dhaka city, having low, flat, and fertile land with 26°C mean annual temperature and average annual rainfall varies from 1429 to 4338 mm (BBS, 2002). The cows were selected from four metro thanas were Motihar, Boalia, Rajpara, Shahmukdhmat Rajshahi district. Ninety cows were selected from those metro thanas. Livelihood of the people in and around the city depends on trade, civil servants and agricultural practices. Dairy farming is one of the major farming systems practiced in the city. Small holder farmers around the city supply moderate amount of milk to urban dwellers and some of the farmers in the nearby area practice a mixed crop livestock production system. The area is moderately covered with bushy and low weed vegetations. Both small holder farmers and commercial dairy farms owners were included the study. In addition communication system of the study areas were well enough to reach any selected cows herd within 45 to 60 minutes with auto vehicle.

Crossbred cattle are mainly Holstein Friesian and Sahiwal with local indigenous. Approximately 20% animals are crossed in Bangladesh. Indigenous cattle are found everywhere of the country has no definite characteristics and constitute about 80% of indigenous cattle population of the country. Their coat color varies from red, grey, white, black or a mixture of them in different proportion. They are of various sized animals possessing high level of phenotypic variation for most of the economic traits. They are of sole source of draught power of the subsistence farming system of the country. For the identification of the Holestein Friesian crossbred we used the phenotypic characters that were color, no hump and chute, size etc. The cows were selected from four metro thanas were Motihar, Boalia, Rajpara, Shahmukdhmat Rajshahi district. A total ninety healthy Holstein Friesian crossbred dairy cows were selected from those metro thanas.

3.2. Data Collection Procedure

A questionnaire was developed for proper recording of estrous dairy cows and thorough gynecological examination was done (Apendix-1). To achieve the results of conception rate in cattle the influencing factors were classified according to the dose, time of GnRH administration, age, parity, body condition score, milk yield.

The method or techniques were classified into following main two groups-

I. GnRH treated group; n= 45
II. Control group; n=45

Doses of GnRH:

Again various doses of GnRH analogue administration classified into three groups follows –

I. Group I; 200 µg (n=15)
II. Group II; 250 µg (n=15)
III. Group III; 300 µg (n=15)

GnRH analogue was injected intramuscularly in every treated cow.

Time of GnRH administration:

Timing of GnRH administration on occurrence of estrus within AI in cows classified into three categories as follows-

I. Group I: 1 hour before AI (n=15)
II. Group II: During AI (n=15)
III. Group III: 1 hour after AI (n=15)

Age:

Again, the age of these cows were determined from birth register and examined by teeth and reading the corneal ring. After confirmation of age of these cows and then classified as follows –

I. Group I: 2.5-3.5 years (n=11)
II. Group II: 4.0-5.0 years (n=25)
III. Group III: 5.5-6.5 years (n=09).

Parity:

Parity of these cows was determined by the information from farmer and observation of corneal ring of cows. After determined of parity of these cows were classified as follows-

I. Group-I; 1st parity (P_1): Cows give one calf her life (n= 10)
II. Group-II; 2nd parity (P_2): Cows give two calf her life (n= 17)
III. Group-III; 3rd parity (P_3): Cows give three calf her life (n=18)

Body Condition Score:

The scoring of body condition involved a manual assessment of the thickness of fat covered and prominence of bone at the tail head and loin area. The tail head score by feeling for the amount of fat around the tail head and the prominence of the pelvic bones. The loin scored by feeling the horizontal and vertical projection of the vertebrae and the amount of fat in-between. The body condition score (BCS) varied from 1.00-5.00, according to five scale point system outline by Wildman *et al.,* (1982). In this scale emaciated cow were scored 1.00; thin cows 2.00; medium or average cows 3.00; fat or good cows 4.00 and very fat or obese cows were scored 5.00. The body condition score (BCS) divided into grossly two groups in this study-

I. Group-I (BCS 3): Medium (n=26) and
II. Group-II (BCS 4): Good (n=19)

Milk Yield:

Then the cows were classified again according to their milk yield such as:

I. Group-I: 07-<10 Liters per day
II. Group-II: 10-more Liters per day

3.3. Management of Animals

These all 90 animals were maintained under almost same routine feeding and management conditions. The routine feeding consisted of available green fodder plus concentrate ration according to the daily milk yield. Vaccination was carried out as per vaccination schedule.

3.4. Heat Detection

Estrus detection of cows was carried out intensively twice daily by experienced herd persons. Only those animals were selected and recorded in heat which stood still while being mounted by other female cows. The animals were also observed for behavioural symptoms like frequent urination, bellowing, raised tail, restlessness and licking of external genitalia. Different visible external changes like vulvular edema and absence of wrinkles on vulvular lips, vaginal hyperaemia, wetness and mucus discharge were also observed.

3.5. Study Procedures

Farmers were made aware about mucosal discharges from vulva, restlessness, frequent urination; stand to be mounted and raised tails during estrus exhibition. As soon as such symptoms were observed, they informed to make phones to local inseminator immediately and included in the study. Based on the above criterion; selection process of animals for the study was carried out and assigned randomly to control and treatment groups. The first group I (n=15) was inseminated along with intramuscular injection of 200 µg (2ml) GNRH analogue (Ovurelin® Renata Ltd.). Second group II (n=15) was also inseminated along with injection of 250 µg (2.5 ml) of GNRH analogue (Ovurelin® Renata Ltd.). Third group III (n=15) was also inseminated along with injection of 300 µg (3 ml) of GNRH analogue (Ovurelin® Renata Ltd.). Control group, (n=45) receive no treatments except single AI.

Other basic groups were maintained along with the above groups where GnRH analogue administration was done in three different times these are 1 hour before AI, during AI and 1 hour after AI. In that time related groups, each group containing three type doses equally maintained. In a sentence, 1 hour before AI group containing 200, 250, 300 µg doses are five in numbers each. Others groups are same as above one. Other groups are randomly maintained when they were found. After cows were followed for 60 days post AI, pregnancy diagnosis was carried out using a rectal palpation in cows' herds with history of failure to return to estrus. The cows were considered as pregnant if were able to palpate the fetal membrane, amniotic vesicles, cotyledons and fetus otherwise the cows were classified as negative.

3.6. Artificial Insemination by Frozen Thawed Semen

Frozen semen from Friesian bulls was used and evenly allotted to the cows in those the groups. The semen was stored in liquid nitrogen at temperature of –196°C. The semen was thawed at temperature 37–40°C in water bath for 10–15 seconds and after washing the external genitalia with water the cows were inseminated. Sterilized Insemination Gun with disposable plastic sheath was used for insemination of the entire animal. All the hygienic measures were adopted to check the possibility of infection at the time of artificial insemination.

3.7. Experimental Period

The duration of study was six months from the start of experiment (from October 2016 to March 2017).

3.8. Mechanism of Action of GnRH

GnRH is a naturally occurring hormone. During the normal estrous cycle of a non-pregnant animal, GnRH is synthesized and release from the anterior pituitary gland. This release of GnRH acts on ovary and induces ovulation and luteinization of dominant follicles and causes the smaller follicles to undergo artesia. Effective improvement of the conception rate after administration of GnRH has been attributed to the prevention of an ovulation failure or a small variation in the time between the onset of estrus and ovulation by increased preovulatory luteinizing hormone (LH) surge. GnRH will destroy the follicular cyst which a structure in the ovary that interferes with the normal hormonal cycle of cows and prevents the cows from returning to estrus. GnRH increases progesterone level indirectly and helps in pregnancy maintained and improves the conception rate.

3.9. Pregnancy Diagnosis

Animals which did not return to estrus after 30 days post insemination were examined per rectum 60 days post insemination for the diagnosis of pregnancy. The cows were considered as pregnant if were able to palpate the fetal membrane, amniotic vesicles, and fetus otherwise the cows were classified as negative.

3.10. Statistical Analysis

Study variables were collected from dairy cows owners by asking breed type, insemination and service history, parity, management condition, milk yield, time and day of estrus exhibition and previous condition of dairy cows. During this study period, pregnancy diagnosis was made through rectal palpation and positive and negative results were recorded. Some data were collected from farm records about animals' previous milk yield, clinical cases, origin of the animal and breeding type. The experimental study was of a randomized type in which data was collected from treated and controlled study animals. The raw data was sorted and computed and statistically analyzed to calculate the effect of using GnRH hormonal drugs on the estrous cycle in relation doses, time of GnRH administration, age, parity, body condition score, milk yield. Collected data were compiled by Microsoft Excel 2007 and then Statistical Package for Social Science (SPSS) software 20.0 version.

4. Results

Out of 90 crossbred (LxHF) dairy cows included in this study, 57 cows were found to be pregnant. A total of 32 cows from the GnRH treated group were found as pregnant with the conception rate of 71.11%. In control group, from the 45; 25 cows were conceived with the 55.56% as indicated in Table 1. GnRH analogue (gonadorelin acetate) was injected in three different doses within AI after confirming estrus and healthy status of those crossbred (LxHF) dairy cows.

4.1. Effects of GnRH on Conception Rate in Crossbred (LxHF) Dairy Cows

The improvement of conception rate by using GnRH analogue (gonadorelin acetate) is shown table 1 and figure 1. It was shown that the proportion of conception rate higher and lower number and percentage share were tabulated 32 & 71.11% and 25 & 55.56% in GnRH treated and control groups respectively. Researcher has found from table 1 that the calculated value of F ratio was 10.50, on the other hand the tabulated value of ANOVA at ($P<0.05$) level of significance was 5.99. Since the calculated value of the test was higher than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect on conception rate in relation to GnRH administration at the time of artificial insemination in crossbred (LxHF) dairy cows.

Table 1. *Effects of GnRH administration on conception rate in crossbred dairy cows.*

Group	Number of cows	Number of conceived cows	Percentage of conception rate
GnRH treated	45	32[a]	71.11%
Control	45	25[b]	55.56%

[a,b]F ratio within the same column differed significantly by chance with a $P<0.05$.

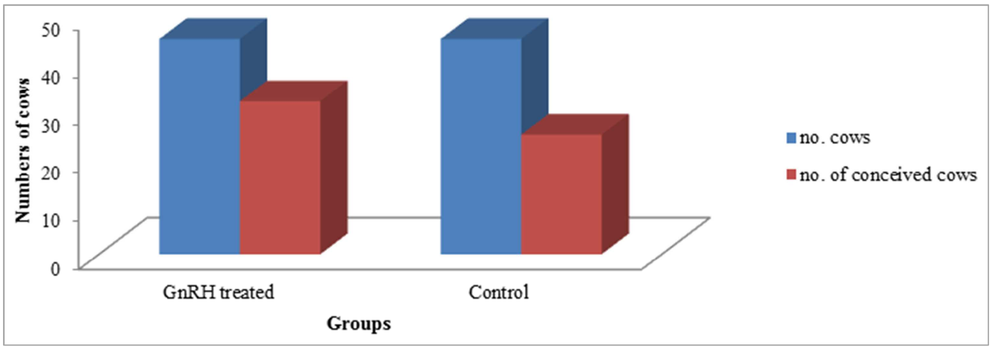

Figure 1. *Conception rate in relation to GnRH administration.*

4.2. Effects of Doses of GnRH Analogue (Gonadorelin Acetate) on Conception Rate

Conception rate in relation to doses ofGnRH analogue (gonadorelin acetate) in table 2 & figure 2, it was observed that the conceived cattle number and percentage share were recorded 12 & 80%, 11 & 73.33% and 09 & 60% in 200 µg, 250 µg and 300 µg respectively. Researcher has found from table 2 that the calculated value of F ratio was 1.024, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 4.26. Since the calculated value of the test was lower than the tabulated value, so researcher may accept the null hypothesis that means there was no significant effect on doses ofGnRH analogue relation to conception ratein crossbred (LxHF) dairy cows at the time of artificial insemination.

Table 2. *Effects of various doses of GnRH administration on conception rate in crossbred dairy cows.*

Doses (µg)	Number of cows	Number of conceived cows	Percentage of conception rate
200	15	12[a]	80%
250	15	11[b]	73.33%
300	15	09[c]	60%

[a,b,c] F ratio within the same column was not differed significantly by chance with a P<0.05.

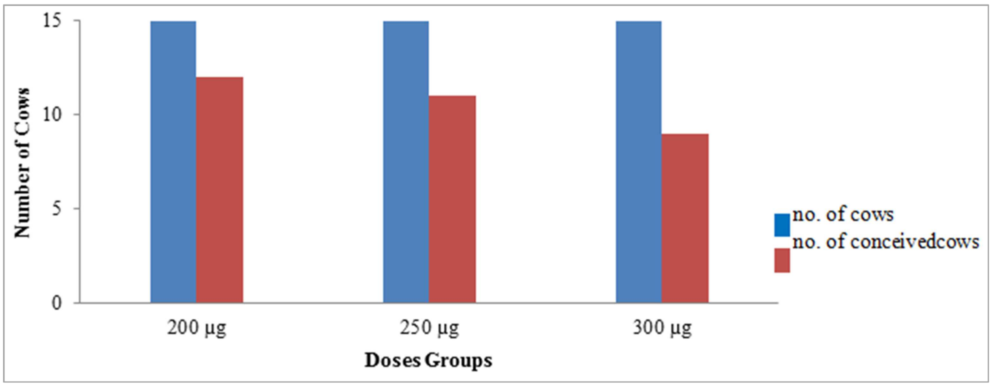

Figure 2. *Conception rate in relation to doses ofGnRH analogue.*

4.3. Effects of Timing of GnRH Administration on Conception Rate

The effects of timing of GnRH analogue administration on treated group cows on occurrence of estrus and conception rate are presented in table 3 & figure 3. It was observed that the conceived cattle number and percentage share were recorded 11 & 73.33%, in 1 hour before AI and 1 hour after AI groups; on the other hand, 10 & 66.67%in during AI group. Researcher has found from table 3 that the calculated value of F ratio was 0.120, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 4.26. Since the calculated value of the test was lower than the tabulated value, so researcher may accept the null hypothesis that means there was no significant effect on timingofGnRH analogue administration relation to conception ratein crossbred (LxHF) dairy cows at the time of artificial insemination.

Table 3. *Effects of timing of GnRH administration on occurrence of estrus and conception rate in crossbred dairy cows.*

Time period	Number of cows	Number of conceived cows	Percentage of conception rate
1 hour before AI	15	11[a]	73.33%
During AI	15	10[b]	66.67%
1 hour after AI	15	11[c]	73.33%

[a,b,c] F ratio within the same column was not differed significantly by chance with a P<0.05.

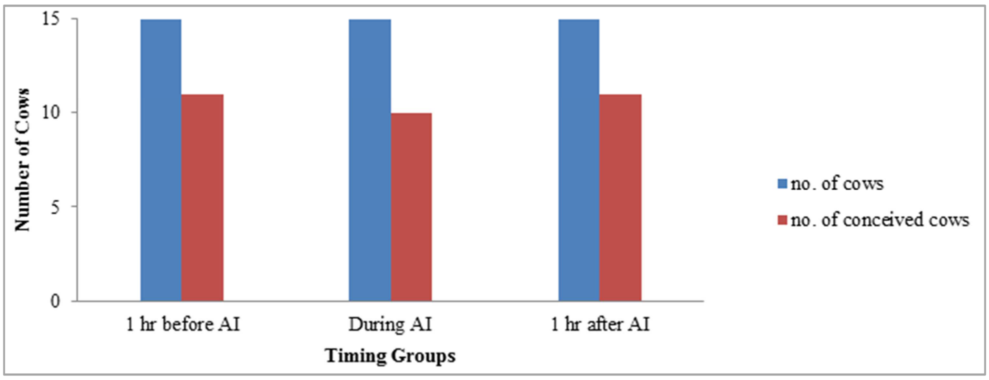

Figure 3. *Conception rate in relation to timing ofGnRH analogue administration.*

4.4. Effects of Body Condition Score (BCS) on Conception Rate After Using GnRH in Crossbred (LxHF) Dairy Cows

The effects of body condition score on GnRH treated group cows on occurrence of estrus and conception rate are presented in table 4 & figure 4. It was shown that the proportion of conception rate higher and lower number and percentage share were tabulated 19 & 73.08% and 13 & 68.42% in 3 (Medium) and 4 (Good) body condition score groups respectively. Researcher has found from table 4 that the calculated value of F ratio was 3.927, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 5.99. Since the calculated value of the test was lower than the tabulated value, so researcher may accept the null hypothesis that means there was no significant effect on conception rate in relation to body condition score on GnRH treated crossbred (LxHF) dairy cows group at the time of artificial insemination.

Table 4. *Effects of body condition score of GnRH treated crossbred dairy cows on conception rate.*

Body Condition Score	Number of cows	Number of conceived cows	Percentage of conception rate
3 (Medium)	26	19[a]	73.08%
4 (Good)	19	13[b]	68.42%

[a,b]F ratio within the same column was not differed significantly by chance with a P<0.05

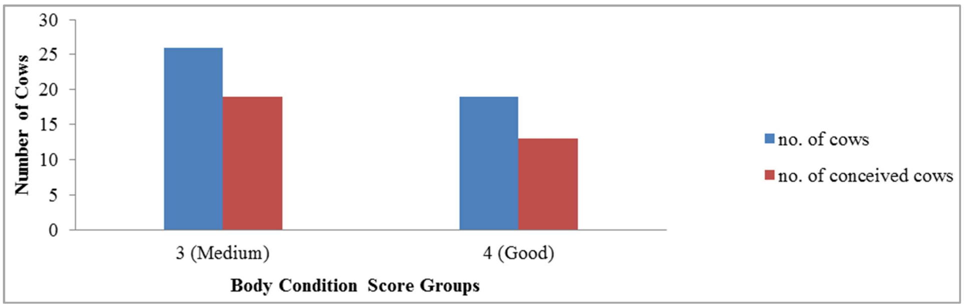

Figure 4. *Onceptionrate in relation to BCS of GnRH treated cows.*

4.5. Effects of Parity on Conception Rate After Using GnRH Analogue

The influences of parity of GnRH treated group cows on conception rate are presented in table 5 & figure 5. It was shown that the proportion of conception rate and percentage share were tabulated 07 & 70.00%; 12 & 70.79% and 13 &72.22% in 01, 02 and 03 parity groups respectively. Researcher has found from table 5 that the calculated value of F ratio was 3.207, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 4.26. Since the calculated value of the test was lower than the tabulated value, so researcher may accept the null hypothesis that means there was no significant effect on conception rate in relation to parity on GnRH treated crossbred (LxHF) dairy cows group at the time of artificial insemination.

Table 5. *Effects of parity of GnRH treated crossbred dairy cows on conception rate.*

Parity	Number of cows	Number of conceived cows	Percentage of conception rate
01	10	07[a]	70.00%
02	17	12[b]	70.79%
03	18	13[c]	72.22%

[a,b,c] F ratio within the same column was not differed significantly by chance with a P<0.05.

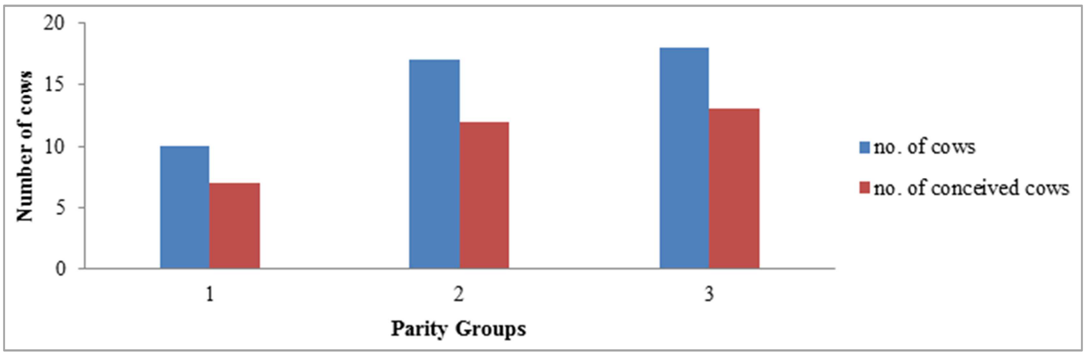

Figure 5. *Conception rate in relation to parityofGnRH treated cows.*

4.6. Effects of Age of GnRH Treated Crossbred Cows on Conception Rate

Conception rate in relation to age of GnRH treated cows in table 6 & figure 6 it was observed that the conceived cattle number and percentage share were recorded 07 & 63.67%, 17 & 68.00% and 08 & 88.89% in 2.5-3.5 years, 4.0-5.0 years and 5.5-6.5 years respectively. Researcher has found from table 6 that the calculated value of F ratio was 13.00, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 4.26. Since the calculated value of the test was higher than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect on conception rate in relation to age after using GnRH at the time of artificial insemination in crossbred (LxHF) dairy cows.

Table 6. *Effects of age of GnRH treated crossbred dairy cows on conception rate.*

Age (Years)	Number of cows	Number of conceived cows	Percentage of conception rate
2.5-3.5	11	07^{a}	63.67%
4.0-5.0	25	17^{b}	68.00%
5.5-6.5	09	08^{c}	88.89%

[a,b,c] F ratio within the same column differed significantly by chance with a P<0.05.

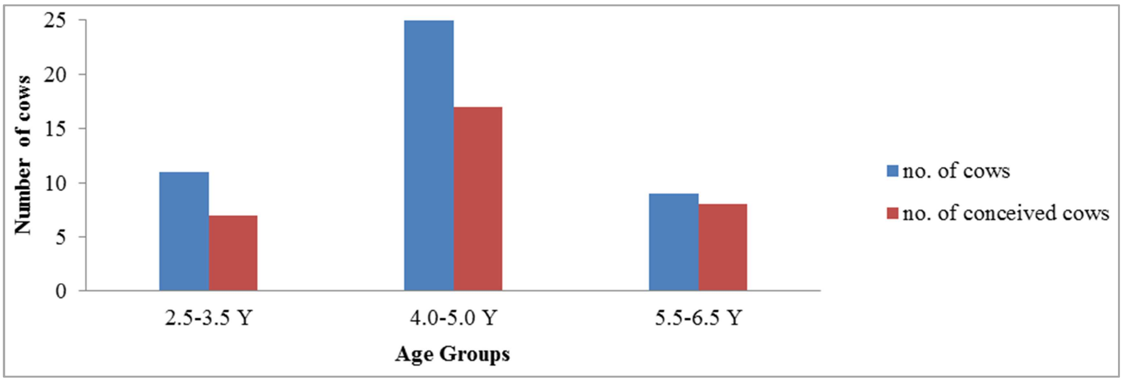

Figure 6. *Onceptionrate in relation to age of GnRH treated cows.*

4.7. Effects of Milk Yield of GnRH Treated Crossbred Cows on Conception Rate

The effects of milk yields on GnRH treated group cows on occurrence of estrus and conception rate are presented in table 7 & figure 7. It was shown that the proportion of conception rate higher and lower number and percentage share were tabulated 21 & 72.41% and 11 & 68.75% in 07-<10 liters and 10-more liters milk yield groups. Researcher has found from table 7 that the calculated value of F ratio was 7.229, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 5.99. Since the calculated value of the test was higher than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect on conception rate in relation to milk yield after using GnRH at the time of artificial insemination in crossbred (LxHF) dairy cows.

Table 7. *Effects of milk yield of GnRH treated crossbred dairy cows on conception rate.*

Milk yield (Litre)	Number of cows	Number of conceived cows	Percentage of conception rate
07-<10	29	21^{a}	72.41%
10-more	16	11^{b}	68.75%

[a,b] F ratio within the same column differed significantly by chance with a P<0.05.

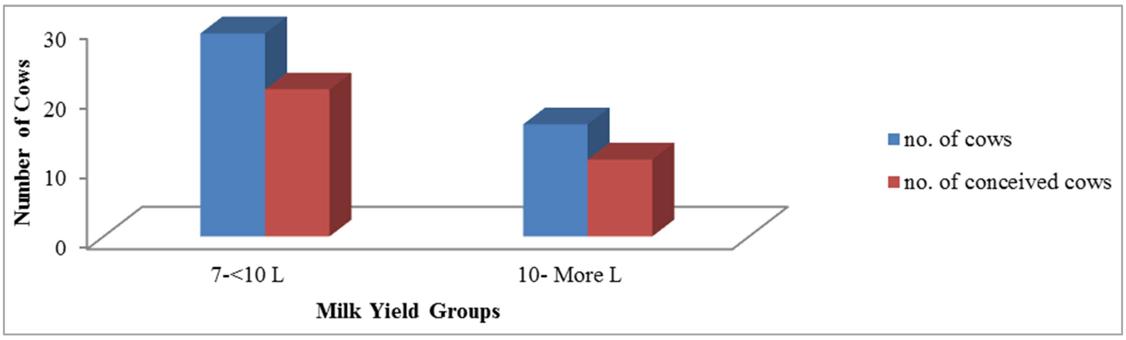

Figure 7. *Onceptionrate in relation to milk yieldofGnRHtreated cows.*

5. Discussion

It was hypothesized that GnRH analogue (Ovurelin® as gonadorelin acetate) treatment at the time of artificial insemination improves conception rate. The aim of the study was to estimate the effects of GnRH treatment at the time of AI in healthy crossbred (LxHF) dairy cows. The effective factors of improvement of conception rate were doses of GnRH, time of administration with in AI, body condition score, parity, age and milk yield. Among those age and milk yield were showed variance significantly in GnRH treated group and F ratio is likely to occur by chance with a P<0.05. The overall conception rate was 71.11% in GnRH treated group where as 55.56% in control group and showed variance significantly and F ratio is likely to occur by chance with a P<0.05 in the results. This work is almost similar to the Raoyos (1995) reported that conception rate for hormone treatment after AI in cows to be 70% compared to 50% in control cows.

Mann and Lamming (1985) demonstrated on the treatment of cows with 10µg GnRH analogues Buserelin (Receptal; Hoechst) 12 days after insemination. Phatak *et al*., (1986) and Stevenson *etal*., (1984) reported that cows and heifer treated with GnRH at times of AI pregnancy rate 25% more in treated than control cow and heifer. Our results are in agreement with the findings that the use of GnRH at the time of AI increases conception rate in dairy cows (Shahneh *et al*., 2008).

It was previously thought and suggested that using GnRH at occurrence of heat might prevent ovulation failure or reduce the time interval to ovulation (Coulson *et al*., 1980; Nakao *et al*., 1984). Besides, our study differed from the other researcher's observation (Perry and Perry, 2009) who reported that GnRH treatment had no effect on conception rate if administered at the time of artificial insemination. An extreme delay in ovulation could affect fertilization and also delay the establishment of luteal function (Larson *et al*., 1997) and consequently reduce fertility. Thus, if GnRH treatment were given at estrus, failure or delay of ovulation might be prevented and conception rate might increase. On the other hand, if GnRH is administered at the time of AI, especially when detection of estrus is not carried out frequently, the administration is likely, in most cases, to be rather late in estrus, with unknown effects on the timing of ovulation, and a prospective decline in plasma progesterone levels in the subsequent luteal phase (Ryan *et al*., 1994).

The improvement in fertility due to GnRH treatment in the present study has two possible explanations. Firstly, the positive effect of GnRH at the time of AI is mediated by the improved ovulation rate (Yaniz *et al*., 2004). Cows treated with GnRH have a LH surge, which is maximum two hours after treatment (McDougall *et al*., 1995) and causes a fourfold increase in plasma LH concentration after 2-2.5 h (Osawa *et al*., 1995). Since the preovulatory surge of LH normally occurs about 6 h after onset of estrus (Schams *et al*., 1977), treatment with GnRH at insemination may have induced a secondary surge of LH before or after the spontaneous preovulatory surge of LH. That added increment of LH may be helpful to the events associated with conception rate.

Secondly, progesterone is a vital hormone during early pregnancy that promotes embryo development and controls the luteolytic mechanism (Mann and Lamming, 1999). A single GnRH dose at AI can increase subsequent plasma progesterone concentration (Kaim *et al*., 2003) which is related to hypertrophy and hyperplasia of the luteal cells. GnRH injection at the time of estrus causes LH surge and following ovulation LH increases blood flow to ovaries, causing ovarian hyperemia. Therefore, CL formation occurs rapidly and progesterone production increases significantly (Rosenberg *et al*., 2003). In various studies, progesterone concentration has either increased, decreased or remained unchanged during the luteal phase, following GnRH administration (Lee *et al*., 1985; Lucy and Stevenson, 1986; Mee*et al*., 1993; Ryan *et al*., 1994; Ullah*et al*., 1996). In our study we have shown that the small dose variation like as 200, 250 and 300 µg were not significant that means among those doses each are almost similar to others. In our study we also diverse very small time variation with AI that are 1 hour before AI, during AI and after AI; in those cases we found no significance. However, our thoughts were similar to the above two explanation and it was improves conception rate.

It has been shown previously, in first postpartum AI cows, that when GnRH is administered at estrus, the GnRH-induced LH peak coincides with the spontaneous LH peak, and the resulting merged peak is higher than either the spontaneous LH peak in the control group or the peak induced late in estrus by GnRH (Rosenberg *et al*., 1991). In contrast, in most other studies GnRH administered at AI induced a second LH peak and did not increase the spontaneous one (Lee *etal*., 1985; Lucy and Stevenson, 1986; Ryan *et al*., 1994). These findings could be related to the fact that heifers have a smaller preovulatory LH surge than virgin heifers (Gustafsson*et al*., 1986) and, therefore, an increase in the spontaneous surge that results from the administration of GnRH at estrus affects the conception favorably.

The improvement in the conception rate with the use of GnRH during luteal phase has been attributed to the fact that GnRH on day 5 induces ovulation of the first wave dominant follicle, thus forming an accessory CL (corpus luteum) and enhancing progesterone production early in the cycle. This increase in progesterone secretion caused by GnRH may facilitate embryonic development (Mann and Lamming, 1999). Exogenous administration of GnRH could initiate the endogenous increase in progesterone (Mehni *et al*., 2012) via increasing numbers and sizes of CLs following luteal phase administration of GnRH (Willard *et al*., 2003). This increase in CL number and thus total CL tissue area is most likely responsible for the increased serum concentration of progesterone observed with GnRH treatment.

The results of the present study in which the use of GnRH during the time of AI enhanced the conception rate in the crossbred (LxHF) dairy cows are also in agreement with the earlier findings (Beltran and Vasconcelos, 2008). However, other researchers observed that GnRH did not improve conception in dairy cows (Szenci *et al*., 2006; Khoramian*et al*., 2011). Administering GnRH or it's analogues to cows at thetime of AI increase conception rate in some experiments, but notin others (Mee*et al*., 1990; Stevenson *et al*., 1990). Withlate AI, cows treated early in estrus had a higher conception ratethan those treated late in estrus (Rosenberg *et al*., 1991). The latter study, conducted in the winter, andthat by Ullah *et al*. (1996) under heat-stress conditions, oth indicated that GnRH treatment at estrus significantlyimproved conception rate, particularly when the conception rate of untreatedcows was low.

Anjum I.. (2010) revealed that there was no significant effect of age and parity number (p>05). In group A (control) the average age of 32 animals was 83 months ±6.77 and age range was from 48–232 months. The average parity number was 2.44 ± 0.38 and the range number was from 1–13. Whereas, in group B (Treatment) the average age was 87 months ±5.42 and range was 44 to 186 months. The average parity number was 2.47 ± 0.44. The parity number ranged from 1–11. In our present study we also found no significant among parity where we used only 1, 2 and3 number of parity in GnRH treated healthy crossbred (LxHF) dairy cows. But we have shown the significant improvement of conception rate among age variation in GnRH treated group, in where the conceived cattle number and percentage share were recorded 07 & 63.67%, 17 & 68.00% and 08 & 88.89% in 2.5-3.5 years, 4.0-5.0 years and 5.5-6.5 years respectively.

Conception rate was higher in cows with high BCS than in cows with low BCS (Moreira *et al*., 2000). The higher response to GnRH in cows with high BCS, found in the latter study, was attributed to an earlier postpartum resumption of cyclicity. Butler (2000) suggested that the low fertility of the dairy cow under negative energy balance is associated with low estrogen and LH secretion, which could be due to low glucose, insulin and IGFI secretion, and that part of the low-fertility syndrome could be related to low secretion of progesterone as well. In our study we used only medium and fat cows having body condition score 3 and 4 respectively and observed no significant between two groups in the case of improving conception rate using GnRH analogue. But we have shown the significant improvement of conception rate among milk yield variation in GnRH treated group, in where the proportion of conception rate higher and lower number and percentage share were tabulated 21 & 72.41% and 11 & 68.75% in 07-<10 liters and 10-more liters milk yield groups. Researcher has found the calculated value of F ratio was 7.229, on the other hand the tabulated value of ANOVA at (P<0.05) level of significance was 5.99.

The findings of our study revealed that the use of GnRH analogue (gonadorelin acetate) therapy with the time of AI improved in pregnancy rate in crossbred (LxHF) dairy cows. The scored conception rates were 71.11% and 55.56% in GnRH treated and control groups respectively. Use of GnRH at the time of AI increases the spontaneous LH peak, prevents delays in ovulation, and induces postovulatory progesterone. Thus GnRH has the potential to increase conception rate. In conclusion our study revealed that administration of GnRH at the time of artificial insemination improved the conception rate in crossbred dairy cows.

6. Conclusion

The study was carried out to evaluate the effects of GnRH (Ovurelin® as Gonadorelin Acetate) administration at the time of AI on conception rate in crossbred (LxHF) dairy cows. The findings of our study revealed that the use of GnRH (Gonadorelin Acetate) along with AI improved the conception rate in crossbred (LxHF) dairy cows. The scored conception rates were 71.11% and 55.56% in the GnRH treated and control groups respectively. There were significant effects of GnRH on age and milk yield on improvement of conception rate in GnRH treated crossbred (LxHF) dairy cows group. There were no significant effects of GnRH on GnRH doses (200, 250 and 300 µg), timing with AI (1 hr before, during and 1 hr after), BCS (3/medium and 4/good), and parity (01, 02 and 03) on improvement of conception rate in GnRH treated crossbred (LxHF) dairy cows group.

From the present study it might be concluded the following-

I. GnRH administration at the time of AI improved conception rate in crossbred (LxHF) dairy cows.
II. Age between 5.5-6.6 years within GnRH treated group showed better result, scored 88.89% of conception rate and 07-<10 liters milk yield within GnRH treated group also showed better result, scored 72.41% of

conception rate.

Recommendations

Farmers can easily adapt the technique of GnRH analogue administration along with AI for the improvement of conception rate in crossbred dairy cows.

The GnRH is effective in high milk yielding and middle aged crossbred dairy cows for improving conception rate so it can be easily applied for better conception rate.

Before administration the farmers must be treated the cows with anthelmintics.

The vaccination, good housing system, balanced diet must be maintained before GnRH treatment protocol.

The diseased, cachectic, very young and aged female cattle should avoid from this treatment.

Any infectious diseases and nutritional deficiency cattle should not use GnRH techniques.

GnRH analogue can be effectively used to overcome low conception rate in crossbred dairy cows.

References

[1] Alam J 1991. Livestock development strategies in Bangladesh. The Socioeconomic Perspective. Proceeding of workshop on Livestock Development in Bangladesh. BLRI, Savar, Dhaka.

[2] Alam MGS and Gosh A 1994. Reproductive performance in cows: it's relationship to parity and season. Bangladesh Veterinary Journal 12:51-61.

[3] Alam MGS and Rahman A 1979. Diseases of the genital tract of indigenous cows in Bangladesh. Tropical Animal Health Production 11: 179-180.

[4] Anjum IA 2010. Improvement of Conception R in Crossbred Cattle by Using GnRH analogue Therapy. Agriculturatropicaetsubtropica vol. 43.

[5] BBS 2001. Bangladesh Bureau of Statistics.

[6] BBS 2002. Bangladesh Bureau of Statistics.

[7] BBS 2006. Bangladesh Bureau of Statistics.

[8] BBS-Bangladesh Bureau of Statistics 1998. The Bangladesh census of agriculture and livestock-1983/84. Vol. 1. Structure of Agricultural holding and livestock population Statistical Division, Ministry of Planning, GOB, Dhaka.

[9] Birhanu 2014. Effects of Buserelin Acetate on Conception rate in Repeat Breeding Dairy Cattle.ndian Journal of Animal Reproduction 35.

[10] Birhanu2015. Effects of Gonadotropin Releasing Hormone analogue in Enhancements of Pregnancy in repeat Breeding Dairy cows in and around Mekelle, Tigray, Ethiopia. Animal and Veterinary Sciences. 3: 12-17.

[11] Butler W R2005. Nutrition, negative energy balance and fertility in the postpartum dairy cows. Cattle Practice 13: 13-18.

[12] Butler W R 2000. Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduion Science. 60–61:449–457.

[13] Butler W R and R D Smith 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. Journal of Dairy Science 72:767–783.

[14] Cline A M2002. Efficiency of Synthetic Gonadotropin Releasing Hormone analogue for the control of ovulation during estrus synchronization protocols. Animal Science. Physiology of Reproduction.

[15] Conception rates in Holstein cows treated with GnRH or hCG on the fifth day post AI during summer. Veterinary Zoology. 60: 580-586.

[16] Coulson A D E Noakes, J Hamer, and T Cockril. 1980. Effect of gonadotrophin releasing hormone on levels of luteinising hormone in cattle synchronised with dinoprost. Vet. Rec. 107:108–109.

[17] Dewan ML and Rahman MM 1987. Studies on relation of placenta in Savar dairy farm. Bangladesh Veterinary Journal. 21: 49-53.

[18] DLS- Directorate of Livestock Services. 1991. A report of livestock. Directorate of Livestock Services, Dhaka.

[19] DLS-Directorate of Livestock Services. 1998. A report of livestock. Directorate of Livestock Services, Dhaka.

[20] Dougall S, Williamson N and Macmillan K1995. GNRH induces ovulation of dominant follicle in primiparous dairy cows undergoing anovulatory follicle turn over. Animal ReproductionScience. 39: 205-214.

[21] Douglas WS 1998. Use of GnRH to enhance pregnancy rates and shorten the postpartum interest us interval in dairy cattle. Ohio Veterinary Newsletter. 25: 4–6.

[22] Driancourt MA 2001.egulation of ovarian follicular dynamics in farm animals. Theriogenology. 55: 1211-1239.

[23] Ehlers K, Halvorson L and Glob liber 2013.onadotropin Releasing Hormone and Gonadotropin Releasing Hormone Receptors. ISSN: 1756-2228.

[24] F Lopez-Gatius, p santolaria, A Martino, F Deletang, F De Rensis 2006. The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. Theriogenology 65: 820-830.

[25] Faisal Omer Ahmed 2015.anagement of Postpartum Reproductive Performance with Administration of GNRH or PGF2α in Cross Bred Dairy Cows. Journal of Agriculture and Veterinary Science. 8: 45-49.

[26] Foote R2002. The history of Artificial Insemination, Selected notes and notables, American society of Dairy Sciences.

[27] Frick MP 2005. Systematic synchronization and resynchronization systems for reproductive management of lactating dairy cows. Proceeding of the 4th Annual Arizona Dairy Production Conference, October 11, Tempe, AZ, USA. PP: 33.

[28] Ginther OJ2000. Selection of the dominant follicle in cattle‖. Biology of Reproduction. 62: 920-927.

[29] Gustafsson H, Larsso, H Kindah and A Madei1986. Sequential Endocrine Changes and Behavior during estrus and met estrus in repeat breeder and virgin heifer's. Animal ReproductionScience.10:261.

[30] Gustafsson, H., K. Larsson, H. Kindahl, and A. Madej. 1986. Sequential endocrine changes and behaviour during oestrus and metoestrus in repeat breeders and virgin heifers. Animal Reproduction Science. 10:261–273.

[31] Habib MA 2001. Study on the Red Chittagong Cattle –an animal Genetic Resource of Bangladesh. M. Thesis. Department of Animal Breeding and Genetics, Faculty of Animal Husbandary, BAU, Mymensingh, Bangladesh.

[32] Iftikhar A2009. Improvement of Conception rate in Crossbred Cattle Using GnRH Analogue Therapy. Pakistan Veterinary Journal. 29: 93-94.

[33] Jaswal2013. The effect of administration of gonadotropin releasing hormone analogue at estrus or during luteal phase on reproductive performance of dairy cows maintained under sub-temperate climate. Iranian Journal of Veterinary Research. 14: 57-60.

[34] Jilek F, Pytloun P, Kubesova M, Stipkova M, Bouska J, Volek J, Frelich J, Rajmon R2008. Relationships among body condition score, milk yield and reproduction in Czech Fleckvieh cows. Czech Journal of Animal Science. 53: 357–367.

[35] Kaim M, Bloch A, Wolfenson D, Brawtal R, Rosenberg M, Voet H, Folman Y 2003. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. Journal of Dairy Science. 86: 2012–2021.

[36] Kokkonen T 2005.nergy and protein nutrition of dairy cows during the dry period and early lactation: Production performance and adaptation from pregnancy to lactation. Academic dissertation for PhD, Faculty of Agriculture and Forestry, University of Helsinki, Finland.

[37] Larson S F, W R Butler, and W B Currie 1997. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. Journal of Dairy Science. 80:1288–1295.

[38] Lee C N, J K Kritser, and R L Ax 1985. Changes of luteinizing hormone and progesterone for dairy cows after gonadotropinreleasing hormone at first postpartum breeding. Journal of Dairy Science. 68:1463–1470.

[39] Lopez-Gatius F, Santolaria P, Martino A, Deletang F, Rensis FD 2006. The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. Theriogenology. 65: 820–830.

[40] Lucy M C, and J S Stevenson 1986. Gonadotrophin-releasing hormone at estrus: Luteinizing hormone, estradiol and progesterone during the periestrual and postinsemination periods in dairycattle. Biology of Reproduction. 35:300–311.

[41] LuigjTurmalaj 2014. Role of GnRH on Ovulation during summer's Months in Cows. Anglisticum Journal. Volume: 3.

[42] MP Beltran2008. Conception rate in Holstein cows treated with GnRH or hCG on the fifth day post artificial insemination during summer. Veterinary Zootecnology. 60: 3.

[43] Mahesh S Dodamani 2010. Evaluation of Pre and Post Artificial Insemination effect of GnRH Hormone on conception of repeat breeder Deoni Cows. Veterinary World. 3: 209-211.

[44] Mann GE and Lamming GE 2001. Relationship between the maternal endocrine environment, early embryo development and the inhibition of the luteolytic mechanism in the cow. Reproduction. 121: 175-180.

[45] McDougall S, Willamson NB and MacMillan KL 1995. GnRH induces ovulation of dominant follicle in primiparous dairy cows. Animal Reproduction Science. 39: 205-214.

[46] Mee MO, Stevenson JS, Scoby RK1990: Influence of gonadotropin-releasing hormone and timing of insemination relative to estrus on pregnancy rates of dairy cattle at first service. Journal of Dairy Science. 73: 1500–1507.

[47] Mee M O, J S Stevenson, B M Alexander, and R G Sasser 1993. Administration of GnRH at estrus influences pregnancy rates, serum concentration of LH, FSH, estradiol-17β, pregnancy specific protein B, and progesterone, proportion of luteal cell types, and in vitro production of progesterone in dairy cows. Journal Animal Science. 71:185–198.

[48] Mee M O, J S Stevenson, R K Scoby and Y Folman 1990. Influence of gonadotrophin-releasing hormone and timing of insemination relative to estrus on pregnancy rates of dairy cattleat first service. Journal Dairy Science. 73:1500–1507.

[49] Mehedi MH 2002. Improvement of conception in repeat breeding cows using synthetic GNRH. MS Thesis. Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

[50] Mehni SB, Shabankareh HK, Kazemi- Bonchenari M and Eghbali M 2012. The comparison of treating Holstein dairy cows with progesterone, CIDR and GnRH after insemination on serum progesterone and pregnancy rates. Reproduction of Domestic Animal. 47: 131-134.

[51] Mollah WA, Rahman A, Alam MGS, Sen MM, &Islam A 1989. Studies on bovine fertility, infertility and health status in an area of Bangladesh. Livestock Advisor. 14: 27-33.

[52] Moreira F, C Risco, M F A Pires, J D Ambrose, M Drost, M DeLorenzo, and W W Thatcher 2000. Effect of body condition on reproductive efficiency of lactating dairy cows receiving a timed insemination. Theriogenology. 53:1305–1319.

[53] Morrel M 2011. Artificial Insemination; Current and the Future Trends: Swedish University of Agricultural Science, Uppsala, Sweden.

[54] Morrow A D1986. Current Therapy in Theriogenology. 243-246, 247-249.

[55] Nebel R L and McGilliard M L 1993.nteractions of high milk yield and reproductive performance of dairy cows. Journal of Dairy Science. 76: 3257-3268.

[56] Opsomer G, Mijten P, Coryn M, Druif ADE 1996. Post partumanestrous in dairy cows. Veterinary Quarterly. 18:68-75.

[57] Osawa T, Nakao T, Kimura M, Kaneko K, Takagi H, Moriyoshi M and Kawata K 1995. Fertirelin and Buserelin compared by LH release, milk progesterone and subsequent reproductive performance in dairy cows treated for follicular cysts. Theriogenology. 44: 835-847.

[58] Osterman S2003. Extended calving interval and increased milking frequency in dairy cows. Doctoral thesis, SwedishUniversity of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, swedeen.

[59] P K Sarker 2015. Effects of GnRH Analogue and Vitamin AD3E on Induction of Cyclicity in Anoestrus Heifers. Bangladesh Journal of Vererinary Medicine.13: 33-40.

[60] Perry GA, Perry BL 2009. GnRH treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. Theriogenology. 71: 775–779.

[61] Perry GA and Perry BL 2009. Gonadotrophin releasing hormone treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. Theriogenology. 71: 775-779.

[62] Rahman MF, Hossain MS and Prodhan MAM 1993. Reproductive problems of cattle in Bangladesh. Bangladesh VeterinaryJournal. 10: 71-73.

[63] Roche F J2001. Follicular growth and hormonal control. Animal Reproduction and Fertility. 17-25.

[64] Rosenberg M, S Y Chun, M Kaim, Z Herz, and Y Folman 1991. The effect of GnRH administered to dairy cows during oestrus on plasma LH and conception in relation to the time of treatment and insemination. Animal Reproduction Science. 24:13–24.

[65] Rosenberg RM, Wolfenson D and Bloch2003. Effects of GnRH administered to cause at the onset of estrus on timing of ovulation and conception. Journal Dairy Science. 86: 2012-2021.

[66] Ryan D P, S Snijders, T Condon, M Grealy, J Sreenan, and K J O Farrell 1994.ndocrine and ovarian responses and pregnancy rates in dairy cows following the administration of gonadotrophin releasing hormone analog at the time of artificial insemination or at mid-cycle post insemination. Animal Reproduction Science. 34:179–191.

[67] Saacke R G, J C Dalton, S Nadir, R L Nebel, and J H Bame 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Animal Reproduction Science. 60– 61:663–677.

[68] Sali G 1996. Teriogenologia bovine.164-173.

[69] Samad MA 2001. PoshuPalon O Chiskistsavidya. LEP Publications. 2nd edition, BAU, Mymensingh. pp:747-750.

[70] Sarder MJU 2008. Occurrence of reproductive disorders in crossbred cows of north Bangladesh. Published in proceeding paper of 15th International Congress on Biotechnology and animal Reproduction, Bangladesh Agricultural University, Mymensingh, Bangladesh. P:74-75.

[71] Schams D, Shellenberger E, Hoffman B and Karg H 1977. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. ActaEndocrinol. 86: 180-192.

[72] Shahneh AZ, Mohammadi Z, Fazeli H, Babak MMS and Dirandeh E 2008. The effect of gonadotrophin releasing hormone injection on plasma progesterone concentration, conception rate and ovulation rate in Iranian Holstein cows. Journal of Animal Veterinary Advance. 7: 1137-1140.

[73] Shamsuddin M, Alam MGS and Ahmed JU 1988. Reproductive disorder of cross bred cows. Bangladesh Veterinary Journal. 22:21-28.

[74] Shamsuddin M, Bhuyan MMU, Sikder TK, Sugulle AH, Chandra PK and Alam MGS 2001. Constraints limiting the efficiency of artificial insemination of cattle in Bangladesh. International Atomic Energy Agency. 1220:9-27.

[75] Short R., Bellows R A, Staigmiller R B, Berardinelli J G and Custer E E 1990. Physiological mechanism controlling anestrus and fertility in postpartum beef cattle. Journal of Animal Science. 68: 799-816.

[76] Sporndly R1995. Feed Tables for Ruminants. Rapport 235.epartment of Animal Nutrition and Management.wedish University of Agricultural Sciences, (SLU), Uppsala, Sweden.

[77] Szenci O, Takacs E, Sulon J, Melode SN and Beckers JF 2006. Evaluation of GnRH treatment 12 days after AI in the reproductive performance of dairy cows Theriogenology. 66: 1811-1815.

[78] Tenhagen B A, Birkelbach E and Heuwieser W 2000.erum progesterone-levels in postpartum dairy cows after repeated application of the prostaglandin F2, analogue D (+) cloprostenol.merican Journal of Veterinary Medicine.47: 213-220.

[79] Ullah G, J W Fuquay, T Keawkhong, B L Clark, D E Pogue, and E J Murphey 1996. Effect of gonadotrophin-releasing hormone at estrus on subsequent luteal function and fertility in lactating Holsteins during heat stress. Journal of Dairy Science. 79:1950–1953

[80] Vacek M, Stadnik L, Stipkova M 2007. Relationships between the incidence of health disorders and the re-production traits of Holstein cows in the Czech Republic. Czech Journal of Animal Science. 52: 227–235.

[81] Wildman E E, Jons P, WagnerR L, Boman H F and Leseli T N 1992. Dairy cow body condition scoring system and its relationship to selected produced characteristics. Journalof dairy Science. 65: 455–459.

[82] Willard S, Gandy S, Bowers S, Graves K, Elias A and Whisnant C 2003. The effects of GnRH administration post insemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. Theriogenology. 59: 1799-1810.

[83] Yaniz, JL, Murugavel K and Lopez-Gatius F 2004. Recent developments in oestrous synchronization of postpartum dairy cows with and without ovarian disorders. Reproduction of Domestic Animal. 39: 86-93.

[84] Youngquist RS 1998. Anoestrus and infertility in cow. In: Fertility and infertility in Veterinary Practice. 4th edition, Edited by Laing JA Morgan WJB, Wagner WC, Bailliere and Tindall, London pp: 100-105.

[85] Youngquist S R 1887. Current Therapy in Large Animal Theriogenology.