MANAGEMENT OF ANOESTROUS IN CROSSBRED CATTLE

A THESIS
BY

MD. KHURSHID ALAM
REGISTRATION NO.: 1705479
SEMESTER: JULY-DECEMBER, 2019
SESSION: 2017-2018

MASTER OF SCIENCE (MS)
IN
THERIOGENOLOGY

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER 2019

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Submitted to the
Department of Medicine, Surgery and Obstetrics
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In partial fulfillment of the requirements for the degree of

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER 2019

Dedicated
To My
Beloved Parents, Brother
And
Sister-In-Law

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In the Name of ALLAH (SWT), the most Beneficent, the most Merciful. All the praises and thanks be to Allah, the lord of the "Alamin (mankind, jinns and all that exists). –Surah Al-Ftaihah (1-2). At first I would like to express my all praise and deepest sense of gratitude to almighty Allah, most gracious, most merciful and supreme creator of the universe, who enabled me to undertake and complete this thesis work for the degree of Master of Science (MS) in Theriogenology.

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December, 2019 ***The Author***

ABSTRACT

This study was conducted at two upazillas (Faridpur, Santhia) of Pabna District to investigate the prevalence of anoestrous and determine the comparative effectiveness between hormonal and non-hormonal treatment for anoestrous cows and heifers. Out of 230 animals, 48 (20.9%) some where as well animals were confirmed to be anoestrous through questionnaire and rectal palpation. All selected animals were divided into two classes (heifers and cows). All animals were treated by Triclabendazole (900mg) & levamisole (600mg) (Renadex, Renata limited, Bangladesh) @ 1 bolus per 75kg. According to the design of treatment, each class randomly allocated into 3 groups, in which Group I were treated with Nutritional supplements that include balance diet, Vit-ADE (Vit-AD_3E vet. Nutech limited, Bangladesh), Selenium (E-Sel, Square limited, Bangladesh and mineral, (DCP Plus, Opsonin Limited, Bangladesh). The animals of Group II were treated with a single dose (5 ml/animal) GnRH analogue (Gonadorelin 100 µg, Fertilon®, Techno Limited, Bangladesh) and the animals of Group III were treated with a single dose of 5 ml GnRH analogue (Gonadorelin 100 µg, Fertilon®, Techno Limited, Bangladesh) 2.5 ml $PGF_{2\alpha}$ (Cloprostenol Na 250 µg, Ovuprost®, Renata Limited, Bangladesh) followed by after 7 days. The overall prevalence of anoestrous was 20.9 %. Prevalence of anoestrous was higher in cows (21.1 %) than heifers (20.5 %). The highest prevalence of oestrus response (85.7 %) in cows were shown in Group III (with 5 ml GnRH followed by 2.5 ml $PGF_{2\alpha)}$. The response of estrous in heifers and cows after the treatment were 755.3 ± 31.9 and 675.7 ± 29.2 in Group I respectively. After the treatment, estrous was observed in heifers and cows that were 529.0 ± 1.6 and 478.4 ± 8.1 in Group II respectively. The onset of estrous (hrs) in heifers and cows after the treatment were 57.2 ± 0.7 and 50.9 ± 0.9 in Group III respectively. There were highly significant ($P < 0.05$) differences in the onset of estrous among different groups. The conception rate was less in hormonal treatment (1.4 ± 0.2 and 1.29 ± 0.2, respectively than the nutritional treatment (1.50 ± 0.2 and 1.29 ± 0.2, respectively) in both heifers and cows. Pregnancy rate was higher in hormonal treatment (75 % and 83.3 %, respectively) than the nutritional treatment (71.4 % and 81.8 %, respectively) in both heifers and cows. There was no significance ($P < 0.05$) differences in between conception and pregnancy rates were observed among different groups. It might be suggested that both nutritional and hormonal treatments were effective -for the treatment of anoestrous, however, cows were showed estrous earlier by the hormonal treatment than nutritional treatment.

CONTENTS

CONTENTS (Contd.)

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In order to increase the milk production in the tropical regions of the world, cattle crossbreeding programme have been used as one of the main strategies and temperate breeds have been introduced in many developing countries (Ehui *et al.*, 1995). High reproductive efficiency of cows is very important for achieving the maximum return from dairy farming. Any abnormality in reproductive system can interrupt animal production performance (Maruf *et al.*, 2012). Reproductive disorders such as anoestrous of crossbred dairy cattle specially Friesian cows significantly reduce about 27% of their fertility which is of great concern of dairy producers worldwide because anoestrous adversely affect the future fertility.

Fertility and breeding efficiency of dairy animals play a vital role in dairy economics (Peter *et al.*, 2009). Any deviation in the breeding rhythm results in a progressive economic loss due to widening of dry period during the life span of animal (Dudhatra *et al.*, 2012). Profitability in a dairy herd is dependent on onset of puberty and calving interval (Paul *et al.*, 2012). Delayed puberty and prolonged calving interval are considered to be the important factors to reduce profitability. A calving interval of 365 days has been suggested (Esslemont *et al.*, 1985) for optimum production. The cows therefore must become pregnant by 80-85 days postpartum, if the above target is to be achieved. Unfortunately, this goal is seldom realized because the interval from calving to conception is prolonged by a number of factors including poor detection of oestrus (Barr, 1974) poor breeding techniques (Graham *et al.*, 1966) reproductive tract infections (Holt *et al.*, 1989) and ovarian dysfunction (Britt *et al.*, 1980). Prevalent form of ovarian dysfunction is anoestrous. A long interval from calving to first oestrus is the major factor contributing to the reduced reproductive efficiency (Casida *et al.*, 1971; Edgerton *et al.*, 1980). This concept has been established for at least 20 years and has attracted extensive research attention. Postpartum infertility and anoestrous in cattle were first recognized as problems over 75 years ago (Hammond *et al.*, 1927). Anoestrous is a period of sexual quietude in which the animal fails to exhibit normal estrous cycles and no manifestation of heat (Boyd *et al.*, 1977) and it is one of the major causes of economic losses in both the dairy and beef industries. Cows are regarded as physiologically anoestrous for a few days (up to 60) following parturition, whereas, lack of estrous after 60 days postpartum is termed pathological anoestrous.

Postpartum anoestrous has been reported to occur in approximately 30% of dairy cows (Archbald *et al.*, 1990; Humblot *et al.,* Thibier *et al.*, 1980). The normal postpartum dairy cow ovulates by 27 days postpartum (Savio *et al.*, 1990); therefore, dairy cows that do not have functional luteal tissue by 32 days postpartum are considered to be in postpartum anoestrous. Early onset of cyclicity is important because the more the estrous cycles a cow has before 30 days postpartum the fewer services per conception are required (Thatcher *et al.,* Wilcox *et al.*, 1973). Puberty in heifers is the age at first ovulation and at which regular oestrus cycle begins. In dairy cattle the possibility of improving the economic efficiency of milk production by the early mating of heifers is clearly a valid objective but factors affecting puberty in cattle have not received a great deal of attention in the past relative to other aspects of bovine reproduction.

Several methods of oestrus and ovulation induction are being used hormonal manipulation of anoestrous and improving reproductive efficiency. Considering the above facts and circumstances, the present study was undertaken with the following objectives -

Research Objectives:

- ❖ To investigate the prevalence of anoestrous in crossbred cattle at the selected area.
- ❖ To determine the comparative effectiveness between nutritional supplements and hormonal treatment for anoestrous in crossbred cattle.

CHAPTER II

REVIEW OF LITERATURE

The modern dairy cow is an important livestock species with a remarkable ability to produce tens of thousands of liters of milk during her lifetime. Understandably, the profitability of dairy farming is closely integrated with the reproductive success of the dairy cow. However, suboptimal fertility of the modern dairy cow is a problem that afflicts the dairy industry worldwide, with reproductive failure often being the primary reason for culling dairy cows. In order to achieve this calving interval an optimum voluntary waiting period of 65 days is recommended followed by conception within 85 to 90 days of the postpartum (Opsomer *et al.,* 1998; Noakes *et al.,* 2000). Failure to having an optimum fertility leads to lower production efficiency through uneconomical milk production and reduced number of offspring.

Only a small proportion of cows resumed their ovarian cyclicity by Day 60 postpartum (Shamsuddin *et al.,* 2006). Furthermore, of the ones that resumed ovarian cyclicity 40% were not detected in estrous even when they had completed one or more ovarian cycles (Shamsuddin *et al.,* 1995; Shamsuddin *et al.,* 2001). The calving interval extends by 21 days in cows when one estrous is missed with an estimated economic loss of $43 (Shamsuddin *et al.,* 2006b) its effects are greater than most dairy farmers realize. Although, the significant effects of anoestrous heifers and cows are well known, effective remedies in Bangladeshi household cows is not well studied. Different methods of hormonal and non-hormonal treatment for anoestrous have been completed by the practitioners but there are no comparative studies on their effectiveness in cows.

2.1 Anoestrous

Anoestrous is a period of sexual quietude in which the animal fails to exhibit normal estrous cycles and no manifestation of heat (Boyd *et al.,* 1977). The cow is not observed in estrous either because she has not come into estrous (not cycling) or because estrous was not detected (cycling).

2.2 Classification of anoestrous

Based on ovarian activity, anoestrous cow has broadly been classified into ovulatory, an ovulatory and inactive. (Robert *et al.,* 1971) divided the anoestrous cow or heifer into two class viz., Class I–cow with a normal functional corpus luteum and Class II–cow with no functional corpus luteum. (Witbank *et al.,* 2002) classified an ovulatory anoestrous into three classes i.e. anovulation with follicular growth up to emergence; anovulation with follicular growth up to deviation but not ovulatory one; anovulation with follicular growth up to ovulatory size. Further, (Peter *et al.,* 2009) divided anoestrous into four types viz., Type–I, characterized by emergence of follicular wave and growth of follicle up to pre deviation stage; Type–II, characterized by deviation of follicle and follicle grow up to dominance stage followed by atresia; Type–III, characterized by persistent dominant follicle (persistent of large follicle) i.e. follicle grow up to pre ovulatory or ovulatory size or more but fails to ovulate or regress (follicular and luteal cyst) and Type–IV, characterized by normal ovulation and formation of corpus luteum but corpus luteum persist beyond the expected time of regression (persistent corpus luteum, PCL) resulting into anoestrous.

Based on the above information's and for the ease, anoestrous has been classified accordingly for better understanding.

2.2.1 Physiological anoestrous

Animals remain anoestrous during certain physiological stages which does not related to infertility viz., before puberty, during pregnancy, lactation and early postpartum period. Accordingly, physiological anoestrous has been classified into pre–pubertal, gestational, locational and anoestrous in cows.

2.2.2 Prepubertal anoestrous

The follicular waves in pre–pubertal animals are similar to that of adult but follicles grow in response to FSH secretion only up to the stage where they have a theca interna and then regress. Such heifers remain in anoestrous before the onset of puberty. The reasons of pre–pubertal anoestrous includes low LH pulse frequency that results in insufficient growth of follicles; inhibitory effect of opioids on LH secretion and high threshold for positive feedback effect of estradiol on LH surge (Noakes *et al.,* 2009).

2.2.3 Gestational anoestrous

The elevated level of progesterone during pregnancy exerts negative feedback effect on GnRH secretion from hypothalamus and reduces LH pulse frequency resulting into anoestrous. However, some cattle and buffaloes exhibit oestrus during early pregnancy (known as gestational oestrus) which is seen most often during first four months of pregnancy. The prevalence of gestational oestrus has been recorded as 3.3 to 20.3% and 6.1 to 14.4% in Indian cattle (Luktuke *et al.,* 1964; Chauhan *et al.,* 1976; Kaikini *et al.,* Fasihuddin *et al.,* 1984) and buffaloes (Luktuke *et al.,* 1964), respectively. Usually cow or buffalo exhibits gestational heat only once during pregnancy, however, few animals show twice or thrice in same gestation.

2.2.4 Postpartum anoestrous

Following parturition, all the females undergo through anoestrous for a variable but short period of time, known as postpartum anoestrous. The period of postpartum anoestrous is usually longer in buffalo than the cattle under similar management conditions (Dobson *et al.,* Kamonpatana *et al.,* 1986; Jainudeen *et al.,* Hafez *et al.,* 1993), probably due to low LH secretion during early postpartum period (Perera *et al.,* 2011). Under normal conditions, buffaloes resume cyclicity by 30–90 days (Perera *et al.,* 2011), however; only about 45% of Indian buffaloes resume cyclicity within 90 days postpartum and rest 55% remain in anoestrous for about 150 days (El–Wishy *et al.,* 2007). Most of the dairy cows resume ovulatory oestrus cycle within 15–45 days postpartum (Butler *et al.,* Smith *et al.,* 1989; Forde *et al.,* 2011). The physiological postpartum anoestrous cannot be avoided and is useful to allow uterine involution prior to first postpartum anoestrous.

High lactation suppresses the fertility in almost all the mammals (Warnick *et al.,* 1950; Baker *et al.,* 1953). Higher level of prolactin in high yielding animals suppresses GnRH secretion and ultimately reduces production of gonadotrophins from pituitary, resulting into anoestrous.

2.2.5 Pathological causes of anoestrous

Certain pathological conditions i.e. ovarian agenesis, dysgenesis or derangement of follicular–luteal dynamics leads to anoestrous causing infertility and pose a herd problem. Such conditions may be congenital or acquired (Kumar *et al.,* 2014).

Postpartum anoestrous can be classified into different types based on either ovarian follicle dynamics or progesterone profiles.

2.2.6 Congenital and hereditary causes of anoestrous

Congenital and hereditary form of anoestrous is upshot of ovarian agenesis or dysgenesis. Ovarian agenesis or aplasia (absence of ovary) is extremely rare condition and probably crop up due to inherited autosomal dominant gene. Bilateral aplastic or gonad less heifers appear normal until breeding age but fail to show oestrus and normal development of udder at puberty and are sterile. Such reports from India are meager. Ovarian dysgenesis has been identified as ovarian hypoplasia and freemartin. Ovarian hypoplasia (incomplete development of ovary) is caused by single autosomal recessive gene with incomplete penetration. It may be unilateral or bilateral. The affected ovary is characterized by lack of primordial follicles reserve either partial (partial hypoplasia) or complete (complete hypoplasia) (Settergren *et al.,* 1964, 1997).

Bilateral complete hypo plastic females remain in anoestrous whereas partial hypo plastic animals exhibit oestrus, conceive and produce viable calves but transmit this undesirable character to the next generation therefore must be avoided. The prevalence of ovarian hypoplasia in Indian cattle has been reported between 0.08–4.3% (Kodagali *et al.,* 1969; Narasimha Rao *et al.,* Murthy *et al.,* 1972; Nair *et al.,* Raja *et al.,* 1974; Kumar *et al.,* Agarwal *et al.,* 1986) whereas it is less than 1 per cent in Indian buffaloes (Damodaran *et al.,* 1956) with a slightly high prevalence (1.46%) in Jaffarabadi buffalo (Kodagali *et al.,* Kerur *et al.,* 1968). Higher prevalence of ovarian hypoplasia (10–23%) has been reported in exotic cattle (Lagerlof *et al.,* Boyd *et al.,* 1953). In Free martin (sterile heifer born co–twin with bull calf) the ovaries usually fails to develop and remain hypo plastic resulting into anoestrous.

2.2.7 Anoestrous due to persistent corpus luteum (PCL)

In this type of anoestrous, the follicular growth proceeds through all the developmental stages and undergo ovulation and CL formation which subsequently turn into anoestrous due to failure of luteal regression. This is probably due to absence of estrogenic dominant follicle at the time of luteal regression (Wiltbank *et al.,* 2002) secreting adequate estradiol to induce the formation of uterine oxytocin receptors and consequently resulting in to pulsatile release of $PGF_{2\alpha}$ for luteolysis (Knickerbocker *et al.,* 1986; Thatcher *et al.,* 1989; McCracken *et al.,* 1999). Persistent corpus luteum (PCL) is mostly associated with uterine pathology such as endometritis, pyometra, resorption, maceration, mummification and uterine unicorns (Lynn *et al.,* 1966; Noakes *et al.,* 1990). Retained corpus luteum may also be associated with embryonic death when death of embryo occurs after maternal recognition of pregnancy where corpus luteum persists until desorption of embryo. In uterine unicorns, CL formed on the ovary apse lateral to the missing uterine horn persists due to a lack of a luteolytic signal from the missing uterine horn. PCL results in anoestrous due to inhibitory effect of progesterone secreted by the corpus luteum on hypothalamo–hypophyseal axis for the secretion of gonadotrophins.

2.2.8 Sub–oestrus/silent oestrus/quite ovulation

Sub oestrus or silent oestrus or quite ovulation is clinically characterized by failure of overt symptoms of oestrus, though the animal is surprisingly normal. Under these conditions, follicular development and ovulation occurs normally in animals without the manifestation of overt signs of oestrus. Sub oestrus is common during the post pubertal period in heifers and early post–partum (30 to 120 days) in high yielding dairy cows. Progesterone secreted from regressing CL of previous cycle potentiates the action of estrogen and seems to favors the manifestation of oestrus in next cycle (King *et al.,* 1976; Allrich *et al.,* 1994). Thus, lack of progesterone priming results in sub–oestrus. Such conditions have been frequently reported in dairy buffaloes especially in summer months (Shah *et al.,* 1990; Badr *et al.,* 1993; Singh *et al.,* 2013) and may be the one of the reasons of prolonged calving interval in buffaloes (Singhal *et al.,* 1984; Barkawi *et al.,* 1986). The concentration of estrogen determines intensity of behavioral signs of oestrus which is low in high yielding dairy cow (Lopez *et al.,* 2004). Lower concentration of estrogen may be either due to higher metabolism and clearance with a high metabolic load (Sangsritavong *et al.,* 2002) or sub–optimal follicular growth (Awasthi *et al.,* 2007). The probable cause of silent oestrus is sub–optimal secretion of estradiol by mature follicles or higher threshold of estrogen in central nervous system to display the symptoms of oestrus in that particular individual animal. Silent oestrus also appears to be hereditary in predisposition in certain breeds (Lagerlof *et al.,* 1951). Other causes of sub oestrus are heat stress, nutritional deficiencies, overweight, foot lesions, aging and ergotism (fescue toxicity) but most common cause considered for sub oestrus is the failure of oestrus detection.

2.2.9 Anoestrous due to failure to observe oestrus/unobserved oestrus

Oestrus detection is critical aspect of dairy herd management where artificial insemination is being practiced. The length of estrous cycle and oestrus period varies among breeds and within a breed (Rottensten *et al.,* Touchberry *et al.,* 1957; Roxström, *et al.,* 2001). It also varies with season, nutrition, lameness, presence of bull, housing, herd size and production status (King *et al.,* 1976; Lopez *et al.,* 2004; Diskin *et al.,* 2008; Walker *et al.,* 2008). Earlier, it was reported that intensity and duration of standing oestrus is shorter in *Bos indicus* cattle as compared to *Bos taurus* cattle (DeAlba *et al.,* 1961; Plasse *et al.,* 1970), probably due to small follicular diameter (Lyimo *et al.,* 2000; Bo *et al.,* 2003). However, recent studies indicate that there is no difference in intensity and duration of oestrus between *Bos Taurus* and *Bos indicus* cows (Bastos *et al.,* 2010). In high yielding cows, many times the oestrus cycles become irregular (Bartha *et al.,* 1971) in terms of its intensity and duration of standing oestrus (Lopez *et al.,* 2004), resulting in low oestrus detection rates. The condition may be due to low estrogen concentration (Lymio *et al.,* 2000; Lopez *et al.,* 2004), insulin and IGF–I mediated deficiency of follicular growth (Butler and Smith, 1989; Lucy, 2001) or increase metabolism and clearance of estrogen with high metabolic load (Sangsritavong *et al.,* 2002). The short period of oestrus often fails to notice by the farmers.

2.3 Factors contributing to anoestrous in heifers and cows

2.3.1 Nutrition

Inadequate consumption of dry matter (i.e., energy) resulting in negative energy balance is an important contributing factor to anoestrous in dairy cattle.11,38,48 Soon after calving, the energy needs of the newly lactating cow increase dramatically due to the higher energy requirements associated with milk production, pushing the cow into a catabolic metabolism. Energy balance is the net result of energy intake minus energy expended for maintenance, growth, and milk yield.

Nutritional status of animals affects the follicular growth, maturation and ovulation (Diskin *et al.,* 2003). Under nutrition is the one of the most prevalent cause of anoestrous in heifers. Extended postpartum period of anoestrous (>150 days) are usually observed in cattle of tropical area under free range rearing system, probably due to shortage of feed and good quality fodder. Reduced feed intake during late gestation or/and early postpartum period or negative energy balance (NEB) due to very high metabolic load following parturition especially in high yielders delays postpartum restoration of LH pulsatility, resulting into prolonged postpartum anoestrous (Wiltbank *et al.,* 1962; Easdon *et al.,* 1985; Connor *et al.,* 1990; Hegazi *et al.,* 1994). Under high metabolic load, nutrients are utilized for production rather than reproduction (Ferguson *et al.,* 2001). In addition to NEB, the deficiency of minerals like calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn) and manganese (Mn) are also associated with anoestrous (Hidiroglou, 1979; Campbell *et al.,* 1999). It is well established that minerals play an intermediate role in the action of hormones and enzymes at cellular level and its deficiency ultimately affect the reproductive performance of females (Bearden *et al.,* 2004).

Occurrence of anoestrous is not only due to underfeeding or malnutrition but also occurs owing to high feed intake which promotes high metabolism and clearance of ovarian steroids (estrogen and progesterone) from body by enhancing the hepatic perfusion (Sangsritavong *et al.*, 2002), especially in high yielders. Low estrogen in high yielding animals result in sub oestrus as intensity of oestrus behaviors is directly related to its concentration (Lymio *et al.,* 2000: Lopez *et al.,* 2004). Excessive clearance of ovarian steroids also leads to anovulation (Walsh *et al.,* 2007) and delayed luteal regression (Opsomer *et al.*, 2000; Petersson *et al.,* 2006).

2.3.2 Parasitic infestations

Heavy parasitism is one of the stressful conditions and is more common in growing than in adult cattle. It affects the future productive and reproductive efficiency in infested animals (Heath *et al.,* 1997). Parasitic infection like fascioliasis, theileriosis schistosomiasis and trypanosomiasis infection in animals cause anaemia and weight loss and ultimately results into anoestrous. Recently, it has been found that *Neospora caninum* infection (Neosporosis) is widely prevalent among dairy herds and has significant association with anoestrous (Bruhn *et al.*, 2013).

2.3.3 Body condition score (BCS)

Body condition score is the measures of nutritional status of animals and is an important factor influencing the reproductive performance (Baruselli *et al.,* 2001). Extremes of BCS (very low and very high) at pre–calving, calving and early postpartum period delay onset of cyclicity (Butler *et al.,* Smith *et al.,* 1989; Markusfeld *et al.,* 1997; Pryce *et al.,* 2000). However, BCS at calving is a better indicator of resumption of postpartum cyclicity than prepartum BCS (Whitman *et al.*, 1975; Lalman *et al.,* 1997). For optimum reproductive performance, BCS of 3.5 (on five point scale) is required at calving (Bhalaru *et al.*, 1987). Restricted feed intake during late gestation and early postpartum period result in low BCS, consequently, leads to prolonged postpartum anoestrous (Dziuk *et al.,* Bellows *et al.,* 1983; Robinson *et al.,* 1990).

2.3.4 Environmental stress

Environmental stress (extreme cold and heat) affect the development of follicles and manifestation of oestrus both in heat stress affects folliculogenesis, follicular fluid micro environment and oocyte quality. In buffaloes, decline in feed intake during summer results in reduced secretion of gonadotrophins (El–Sawaf *et al.,* 1979). Besides this, high environmental temperature causes hyper–prolactinaemia and suppressing the secretion of gonadotrophin which leads to alteration in ovarian folliculogenesis and steroidogenesis. Among cattle, reproductive functions are suppressed more in *Bos indicus* than *Bos Taurus* during winter (Randel *et al.,* 2005); however, such effect of extreme cold has not been reported in buffaloes.

2.3.5 Lactation

High yielding cattle and buffalo shows significantly longer postpartum anoestrous period (Harrison *et al.,* 1989; El–Azab *et al.,* 1984) or weaker signs of oestrus (Harrison *et al.,* 1990). (El–Fadaly *et al.,* 1980) reported that buffalo producing >8 liters milk per day had longer postpartum anoestrous (107 ± 36 days) than those producing <8 liters per day (77 ± 30 days). However, others have reported no significant correlation between milk yield and postpartum anoestrous period (El–Keraby *et al.,* 1981; Kawthar *et al.,* 1985).

2.3.6 Genotype

The resumption of postpartum cyclicity depends upon species as well as breeds. The postpartum anoestrous period is shorter in milked dairy cows as compared to suckled beef cows but suckled dairy cows have longer postpartum anoestrous period than beef cows (Short *et al.,* 1990). The period of postpartum anoestrous is usually longer in buffalo than the cattle (Dobson *et al.,* Kamonpatana *et al.,* 1986; Jainudeen *et al.,* Hafez *et al.,* 1993). How genotype affect the resumption of postpartum cyclicity is not fully understood, however, it may be due to physiological differences among breeds species, difference in milk production and feed intake (Short *et al.,* 1990).

2.3.7 Parity

A longer postpartum anoestrous period have been reported in primiparous than pluriparous buffaloes (Ali and El–Sheikh *et al.,* 1983; Barkawi *et al.,* 1984). Moreover, as the parity increases, the postpartum anoestrous period decreases (El–Sheikh *et al.,* Mohamed *et al.,* 1965; El–Wishy *et al.,* El–Sawaf *et al.,* 1971; Shah *et al.,* 1989; Mahdy *et al.,* 2001). However, others have reported there is no correlation between parity and postpartum anoestrous period (El–Fouly *et al.,* 1976; Borghese *et al.,* 1993).

2.3.8 Periparturient diseases

Periparturient Diseases such as abnormal calvings, metritis, mastitis and ketosis also influence onset of postpartum cyclicity (Fonseca *et al.,* 1983; Opsomer *et al.,* 2000). Delayed uterine involution also holdup resumption of ovarian activity. Postpartum uterine infection (clinical or sub clinical) suppress GnRH release and possibly LH secretion (Peter *et al.,* Bosu *et al.,* 1988; Peter *et al.,* 1990; Mateus *et al.,* 2002), probably due to inflammatory response (Sheldon *et al.,* Dobson *et al.,* 2004; Herath *et al.,* 2006; Williams *et al.,* 2007) and thus, ovarian activity remains suppressed in uterine infections.

2.4 Diagnosis of anoestrous

2.4.1 History

Based on the information viz., failure of displaying the overt signs of oestrus by the animals after attaining puberty or 60–90 days post-partum; symptoms of estrous shown with cyclicity which subsequently ceased and revert in to anoestrous. Such cases are diagnosed when presented for pregnancy diagnosis. Many times, owner's complaint that they are not able to detect estrous or have not seen any signs of estrous in that particular animal since long (Kumar *et al.,* 2014).

2.4.2 Progesterone estimation

True anoestrous is usually characterized by a lack of ovarian progesterone production (Peter *et al.,* 2009). Presence of basal level (0.5–1 mg/ml) of progesterone in the blood samples at an interval of 8–10 days further confirms the diagnosis. If the concentration of progesterone is more than 1ng/ml, it is suggestive of presence of corpus luteum and anoestrous in such situation might be due to unobserved estrous/silent estrous/persistent corpus luteum.

2.4.3 Per rectal examination

Pregnancy can be a prominent cause of anoestrous and therefore must be ruled out by careful examination of ovary and uterus when any animals present for gynaecological examinations. On per rectal examination**,** ovaries are smooth, small and inactive with the absence of corpus luteum in true anoestrous cattle and buffaloes (Agarwal *et al.,* 2004), however, follicles may develop up to maturation stage (Roche *et al.,* 1998; Ghuman *et al.,* 2010). Functional corpus luteum can be palpated in case of silent estrous/unobserved as well as in anoestrous due to persistent corpus luteum.

2.5 Management of anoestrous

Before planning a course of action to manage anoestrous, it is important to assess the prevalence of anoestrous at herd level. Anoestrous postpartum beef cows, if treated, can recover from anoestrous with normal fertility, (Lucy *et al.,* Billings *et al.,* Butler *et al.,* 2001). Whereas dairy cows treated for anoestrous have much lower fertility, (Gumen *et al.,* 2003) possibly due to an associated negative energy balance. Therefore, every effort should be taken to return the cows to positive energy balance before first insemination. Anoestrous can be treated according to their cause, however; there is no single panacea to correct it. Various therapeutic agents including hormonal and non–hormonal compounds have been used extensively for the restoration of cyclicity in anoestrous cattle by several workers with varying degree of success (Glotra *et al.,* 1970; Deshpande *et al.,* 2000). In order to ensure effective treatment, the health and nutritional status of the animals must be in good conditions. Besides deworming, the supplementation of vitamins, minerals and antioxidants in feed are useful to improve health status of the animals.

2.5.1 Non hormonal treatments

2.5.1.1 Nutritional interventions

Interactions between nutrition and reproductive processes are complex (Chagas*et al.,* 2007a; Lucy *et al.,* 2007) and it is often difficult to precisely study effects of specific nutrients on ovarian function. Excessive energy intake during the close-up dry period can result in changes in metabolism that may predispose cows to decreased dry matter intake and higher circulating NEFAs during the immediate peri partal period (Overton *et al.,* 2012) therefore monitoring feed intake in close-up dry cows is also quite important. Specific nutritional or nutraceutical interventions to reduce the prevalence of postpartum anoestrous may include feeding diets high in starch (Gong *et al.,* 2002; Dyck *et al.,* 2002) or daily oral administration of mono propylene glyco (Chagas *et al.,* 2007b) during the postpartum period. Adding starch (Gardner *et al.,* 2001) or oilseeds enriched in long-chain fatty acids such as linoleic or linolenic acid (Colazo, 2009) during the prepartum period have also reduced the interval from calving to first ovulation. Thus, several options exist for dietary manipulations that could mitigate negative energy balance and reduce anoestrous. However, results are variable and research findings may not always translate into practical or economic solutions under commercial herd settings.

2.5.1.2 Plant based heat inducers

Plants have been used for the treatment of animals since long back. Plants synthesize varieties of phytochemicals such as alkaloids, glycosides, terpenes and tannins (secondary metabolites) as a part of their normal metabolic activity and many of these have therapeutic actions when consumed by animals. Many plants are rich source of vitamins and minerals whereas some have estrogenic property which is useful in restoration of cyclicity in anoestrous animals. Many plants such as *Murraya koenigii* (curry leaves), *Nigella sativa* (kalonji), *Abroma augusta* (Ulatkambal), *Saraca asoca* (Ashoka), *Trigonella foenum–graecum* (Methi), *Bambusa aruninacea, Carica papaya, Asparagus recemosus, Leptadenia reticulate, Courupita guianesis, Pergulacia daemia, Semecarpus anacardium cucumber, and jute plants* either alone or in combinations have been fed to treat the anoestrous animals with variable response on induction of oestrus (Kabir *et al.,* 2001; Das *et al.,* 2002; Rajkumar *et al.,* 2008; Kumar *et al.,* Punniamurthy *et al.,* 2009). (Kabir *et al.,* 2001) reported 50% oestrus induction in anoestrous buffaloes using mixture of *Abroma augusta* (root) and *Nigella sativa* (seed) in 2:1 ratio. (Rajkumar *et al.,* 2008) reported higher success rate in anoestrous cattle i.e. 83.3 and 66.7% using Methi seed (@ 200g/day/cow for 20 days) and bark of Ashoka tree (@ 50g/day/cow for 20 days) respectively.

2.5.1.3 Utero–ovarian massage

Utero–ovarian massage is the oldest, simplest, cheapest and effective method to induce oestrus in anoestrous cattle (Romaniuk *et al.,* 1973; Rahawy *et al.,* 2009; Mwaanga *et al.,* 2010). Oestrus induction in cattle andbuffalo varies between 40 to 80% following utero–ovarian massage daily/on alternate day/weekly for 3–4 weeks (Mwaanga *et al.,* 2004; Naidu *et al.,* 2009; Gupta *et al.,* 2011). The mechanism by which ovarian massage induces cyclicity is not clearly understood, however, probable mechanism includes: activation of intrinsic intra–ovarian factors; enhancement of blood circulation to the ovaries and uterus that increases the availability of hormones and growth factors; stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation and luteolysis, if CL is present (Romaniuk *et al.,* 1973; Lobb *et* *al.*, Dorrington *et al.*, 1992; Monget *et al.*, Monniaux *et al.*, 1995; Mwaanga *et al.*, 2010).

2.5.2 Hormonal treatments

2.5.2.1 GnRH treatment in pubertal and postpartum anoestrous

GnRH plays a crucial role in regulating ovarian activity during the cow's normal oestrus cycle as well as in initiating gonadal activity prior to the onset of puberty and after periods of anoestrous (Gordon *et al.*, 1996).

2.5.2.2 GnRH treatment in pubertal anoestrous heifers

Deen *et al.* (1996) reported follicular activity in response to GnRH infusion (Buserelin @ 80 ng / hour) administered for 7 days in pubertal crossbred heifers. Delayed pubertal crossbred heifers were injected with 5 ml of Receptalm (Buserelin acetate) intramuscularly (Thakur and Bhatt *et al.*, 1999). The interval between injection and induction of oestrus was 23.50 ± 1.98 days whereas conception rate was 6.7 percent. Zebu crossed heifers treated with GnRH agonists from 14 to 23 months of age failed to conceive but showed normal conception patterns when introduced into mating herds at approximately 26 months of age. It was concluded that GnRH agonist bio implants have considerable potential for both pro-fertility and antifertility application in catties (D-Occhio *et al.*, 2000). The effect of Receptal Vet (GnRH) for induction of oestrus and subsequent effect on conception rate was studied (Patra *et al.*, 2001) in delayed matured and post-pubertal anoestrous heifers with smooth and quiescent ovaries. Oestrus was induced in 80 and 70 of the cases by I/v and I/m administration with the resultant conception rate being 80.

2.5.2.3 GnRH treatment in postpartum animals

Garverick *et al.*, (1980) reported that cows having follicles larger than15mm in size at the time of GnRH administration did only ovulate while those having follicles below 10mm size remained unovulated.

Edwards *et al.* (1983) observed that the presence of responsive follicle on the ovaries during GnRH treatment is essential for inducing ovulation and ovarian activity. (Short *et al.*, 1988) also expressed similar views. Uterine involution at the time of treatment was estimated by palpation per rectum. Twenty percent of the cows examined were classified as having delayed uterine involution (abnormal). By analyzing milk progesterone patterns it was determined that 38% of the animals were in the luteal phase of an estrous cycle when treated. Cows without luteal tissues (< 1 mg of progesterone / ml of milk) given 8 or 32 µg of HOE 766 increased in progesterone to > 1 mg / ml within 7 days in 77 and 72 % of the cows compared with 40 and 57 % for cows receiving 0 and 2 µg (P < 0.05). This increase in progesterone was followed by a normal estrous cycle within 4 weeks in a higher proportion of cows treated with the two higher doses of the GnRH analogue (87 and 86 %) compared with 67 and 70 % of those receiving 0 or 2 µg of the analog (P < 0.005).

Pattabiraman *et al.* (1986) treated postpartum anoestrous cows with single injection of 5ml of Receptal (GnRH) with 60% results. The interval between injection and induction of oestrus was 10-22 days. They also reported that intensity of oestrus in such animals were mild to moderate. 65% of cows treated showed either moderate to mild signs of oestrus.

Archbald *et al.* (1990) Studies on the abnormal postpartum ovarian function in dairy cows and effectiveness of the exogenously administered GnRH in treating this condition were done. The presence of a palpable CL and retrospective determination of plasma progesterone (P.O concentration > Ing / ml were the criteria used to assess the ovarian activity. Cows possessing a palpable CL and [^concentration >l mg / ml on day 0 were determined to be cycling normally. The animals were treated 100 µg I/m. GnRH. Reproductive parameters evaluated were the percentage of cows pregnant within 180 days after calving and at the end of the study, number of days open and number of services per conception. The results of this study indicated that the prevalence of abnormal postpartum ovarian function in the herd was 30.2 % and the non-treated cows experienced more days open and required more services per conception than the treated cows those that were cycling normally on the initial examination and those that responded spontaneously by day 14.

96 postpartum crossbred cows were subjected to an intramuscular injection of GnRH analogue, buserelin (20 µg) or saline at two different postpartum stages of 15-20 or 30-35 days (Deen *et al.*, 1996). This induced varying degrees of synchronous FSH and LH surges. The gonadotropic response was higher in 30-35 days’ postpartum group as compared to 15-20 days.

Rise was observed in 1 out of 2cows of 15-20 clays group mid none out of 6 cows of 30-35 days’ group. The reproductive efficiency did not improve in the treated cows over the control. Despite its stimulatory action on gonadotropin release Gnarl treatment did not induce ovarian activity in majority of the treated animals. It was concluded that GnRH could induce ovarian activity only when a responsive follicle is present in the ovary. Similarly, Garverick *et al.* (1980) reported GnRH induced ovulation in postpartum dairy cows when large follicles were present at the time of treatment.

Mehta and Mehta (1999) studied effect of single injection of Receptal in total 10 early lactating noncyclic cows (30-60 days' pp) having ovarian follicles (approx. 9- 10mm). The cows were examined daily per rectum for 10 days after treatment to monitor any follicular development, oestrus and ovulation. Visual examinations were made up to 90 days postpartum for oestrus. They reported similar result of oestrus induction and ovulation (2/3 out of 10) in treatment and control groups.

The single intramuscular injection of GnRH analogue (10 to 20μg Buserelin) has been used effective in induction of oestrus and concurrent ovulation with variable response (45.5 to 87.5%) within 4–22 days (Dhoble *et al.,* Gupta *et al.,* 1986; Pattabiraman *et al.,* 1986; Sonwane *et al.,* 1995; Nautiyal *et al.,* 1997; Markandeya *et al.,* Patil *et al.,* 2003; Prahalad *et al.,* 2010).

To achieve better response, GnRH has been combined with other drugs such as phosphorus injection (Tonophosphan), prostaglandin, estradiol and progesterone (Shams *et al.,* 1991; Rhodes *et al.,* 2003; Sirmour *et al.,* 2006). The Ovsynch protocol or GPG regimen (GnRH–PG–GnRH), developed (Pursley *et al.,* 1995) to synchronize ovulations in dairy cows has been widely used to treat anoestrous cattle and buffaloes and results are also promising.

2.5.2.4 Prostaglandin based treatment

Prostaglandin ($PGF_{2\alpha}$) is the treatment of choice for persistent corpus luteum and sub oestrus. Natural or synthetic analogue of $PGF_{2\alpha}$ as a single dose has been used with a reasonable degree of success for management of silent estrous in cattle (Nautical *et al.,* 1998). An intramuscular injection of 25mg (total dose) of natural $PGF_{2\alpha}$ or 250 to 500 micrograms of synthetic ones is required to regress the CL in cattle. However, a lower dose of $PGF_{2\alpha}$ (5mg) are also effective to regress the CL through intra–Volvo–submucosal (IVSM) (Dhaliwal *et al.,* 1988; Narasimha Rao *et al.,* Venkatramaiah *et al.,* 1990). Alternatively, Ovsynch protocol as describe (Pursley *et al.,* 1995) may be used to treat sub oestrus or unobserved oestrus.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

This study was conducted at two upazillas (Faridpur, Santhia) of Pabna district of Bangladesh. The research units (Faridpur, Santhia) are located on 24°47' N latitude and 89°50' E longitude, respectively. The duration of the experiment was one year and was conducted during the period from July 2018 – June 2019.

3.1.1 Study population

Out of 230 animals, 48 animals were confirmed to be anoestrous through per rectal palpation of the reproductive tract twice at 10 days interval.

3.1.2 Selection of household and experimental animal

Two hundred thirty heifers and cows from 70 households were randomly used at two upazillas (Faridpur, Santhia) of Pabna district in Bangladesh.

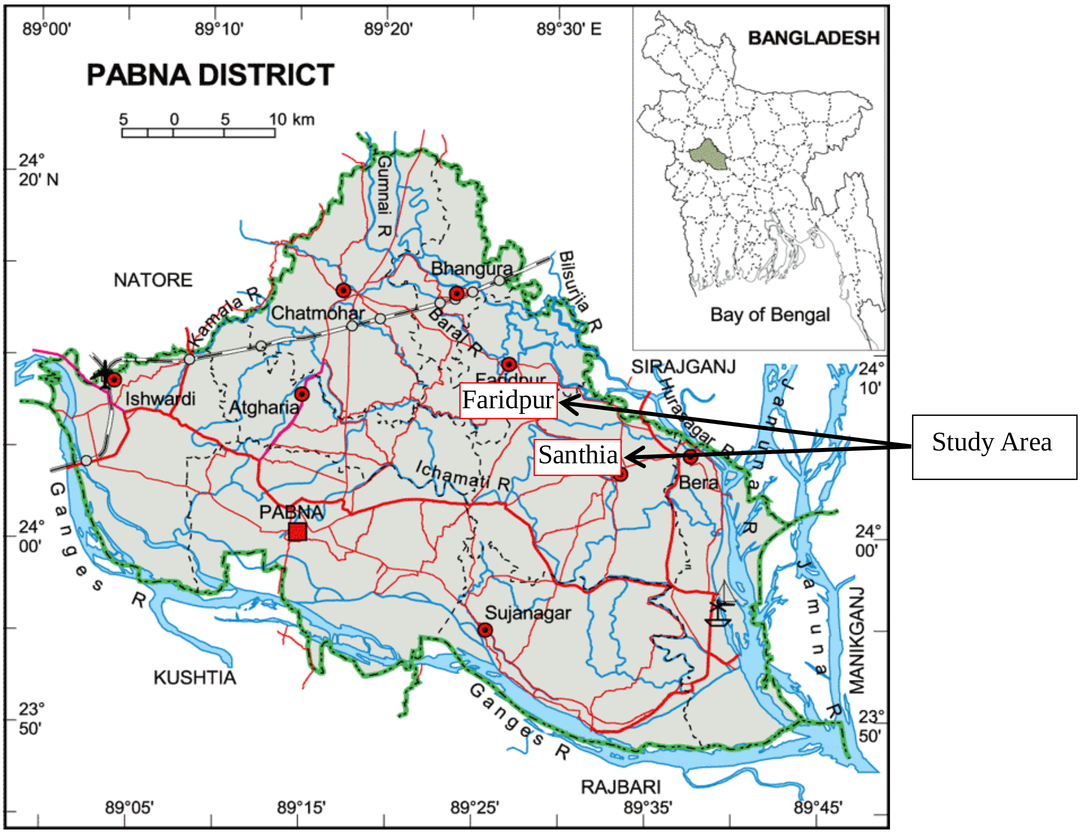

Fig. 1: Map of Faridpur and Santhia Upazillas of Pabna district showing the experimental site

3.1.3 Preparation of questionnaire and collection of data

The information on calving history, interval from calving to the examination, parity, feeding and management was obtained from the owners using a structured questionnaire to collect data for the selection of experimental animals for anoestrous in smallholder farms. Body condition score (BCS) of the animals (range: 1–5) was recorded. The number of parity and ages of cows were determined by interviewing the farmers and by dental examination of cows, respectively. For all the animals under study, body condition was scored in order to assess the nutritional status of the cows. The nutritional status of the cows was determined by scoring the body conditions of the cows using 1 to 5 scales with 0.5 increments (Ferguson *et al.*, 1994). Physical examination and rectal palpation were performed for the diagnosis of anoestrous.

Fig. 2: Collection of data

Fig. 3: Data recording

Fig. 4: Physical examination of animal

Fig. 5: Rectal palpation of animal

3.2 Experimental design

3.2.1 Anoestrous heifers

In case of pubertal heifers two upazillas (Faridpur, Santhia) of Pabna district of Bangladesh at the age group of between 2.5 to 3.0 years and body weight of 250 to 300 Kg which still had not shown the sign of oestrus and inactive ovaries (i.e., minimal follicular development, anovulation, and absence of a CL); ovarian hypo function (i.e., persistent dominant follicle); cystic ovarian degeneration (i.e., follicular or luteinized follicular cyst); and persistent CL (i.e., lack of luteal regression) in one of the ovary at two examinations made by rectal palpation 11 days apart were selected (Ghuman *et al.*, 2010).

3.2.2 Anoestrous cows

In case of postpartum cow, which had not shown the signs of oestrus even after 3 months of parturition and inactive ovaries (i.e., minimal follicular development, anovulation, and absence of a CL); ovarian hypo function (i.e., persistent dominant follicle); cystic ovarian degeneration (i.e., follicular or luteinized follicular cyst); and persistent CL (i.e., lack of luteal regression) in one of the ovary at two examinations made by rectal palpation 11 days apart were selected (Ghuman *et al.*, 2010).

3.2.3 Grouping of animals

All the selected animals were classified into two groups (heifers and cows) and further grouped into three sub-groups (group 1, 2 and 3)

Group I: Nutritional supplements (Traditional feed - Maize grain-20%, Wheat bran-20%, mastered oil cake-20%, Rice polish-10%, Khesari-29%, Iodine salt-1%, 250gm/day Boots and Ad-libitum water with vitamin-mineral premix e.g. Vit-ADE, Selenium)

Group II: 5 ml GnRH treatment

Group III: 5 ml GnRH +after 7 days 2.5 ml PGF2α treatment

3.2.4 Treatment of anoestrous

Twenty four cows those were at poor body condition were treated with nutritional supplement without monitoring per rectal palpation. Optimum reproductive performance, Body Condition Score (BCS) of 3.5 (on five point scale) is required at calving (Bhalaru *et al.*, 1987).

Body Condition Score 3: Area between tail and pins is deeply sunken, insides of pins are hollow.

Body Condition Score 3.5: Area between tail and pins is deeply sunken, Insides of pins are not hollow.

Body Condition Score 4: Area between tail and pins is sunken, Backbone is a bumpy, sharp ridge.

Twenty four cows were treated with hormone using per rectal palpation on the basis minimal follicular development, anovulation, and presence or absence of a corpus luteum (CL). 12 cows which had minimal follicular development with absence of corpus luteum (CL) were treated with a single dose of GnRH analogue. Another 12 cows were treated with GnRH analogue + $PGF_{2\alpha}$ which had persistent corpus luteum (CL).

Group I: Animals of this group were fed with concentrate feed supplement at (Maize powder, Wheat bran, mastered oil cake, Rice polish, Molasses Iodine sal). Routine deworming against round worms and liver flukes was done by using Triclabendazole (900mg) & levamisole (600mg) (Renadex, Renata Limited) @ 1 bolus per 75kg. The cows and heifers were also injected with Vit-ADE (Vit-AD_3E vet. Nutech limited) @ 10 ml per 100 kg at every alternative day for 15 days with additional supplement of Selenium (E-Sel, Square Limited) @ 10 ml per 100 kg twice daily for 10 days and mineral (DCP Plus, Opsonin Limited) @ 20 gm per 100 kg for 10 days.

Also fed 250 gm boots per day. Green grasses, straw and ad-libitum water was provided.

Group II: The animals of group II were injected intra muscularly with 5 ml GnRH analogue (Fertilon®, Techno Limited).

Group III: The animals of this group were injected intra muscularly with 5 ml GnRH analogue (Fertilon®, Techno Limited) followed by 2.5 ml $PGF_{2\alpha}$ (Ovuprost®, Renata Limited) at 7 days interval.

Renadex

Vitamin AD$_3$E

E-Sel

Fertilon

Ovuprost

Fig. 6: Drug used for treatment of anoestrous animals

3.3 Oestrus detection

The follow-up visit was conducted regularly after initiation of the treatment for heat detection by observing oestrus signs (Mucus discharge from the vulva, swelling of vulva, reddening of vaginal mucosa, standing for mounting by bull/cow, vocalization and finally rectal palpation).

3.4 Artificial insemination

Artificial insemination was performed for selected animal that showed estrous sign. All the animals were followed for at least 3 months post-treatment or two natural cycles after induced oestrus.

3.5 Diagnosis of pregnancy

The pregnancy was diagnosed when the animals non-return to oestrus and per rectal palpation after 80-90 days after service. Further rectal palpation was performed after 120 days after service. The data collected on response to treatment interventions with respect to oestrus and conception was recorded.

Fig. 7: Treatment of Animal

Fig. 8: Oestrus Animal

Fig. 9: Artificial insemination

Fig. 10: Diagnosis of pregnancy

3.6 Statistical analysis

Data were stoned in the Excel sheet and statistically analyzed by using ANOVA under the statistical Package Social Science (SPSS® version 15.0). One way ANOVA test was performed to determine significant associations among different variable. Chi-Square test was used to determine significant associations among variables where P value was considered at 0.05.

CHAPTER IV

RESULTS

The prevalence of anoestrous in animals from two upazillas (Faridpur, Santhia) of Pabna district was shown in Table 1. The overall prevalence of anoestrous was 20.9%. Prevalence of anoestrous in heifers was 20.5 % and in cows was 21.1%. Anoestrous cases were recorded higher is low body conditioned (BCS 3) animals compared to high body conditioned cows (BCS 5).

Table 1: Prevalence of anoestrous in heifers and cows

Types of animal	Total No. of animal	No. of animal with anoestrous (%)
Heifers	88	18 (20.5 %)
Cows	142	30 (21.1 %)
Total	230	48 (20.9 %)

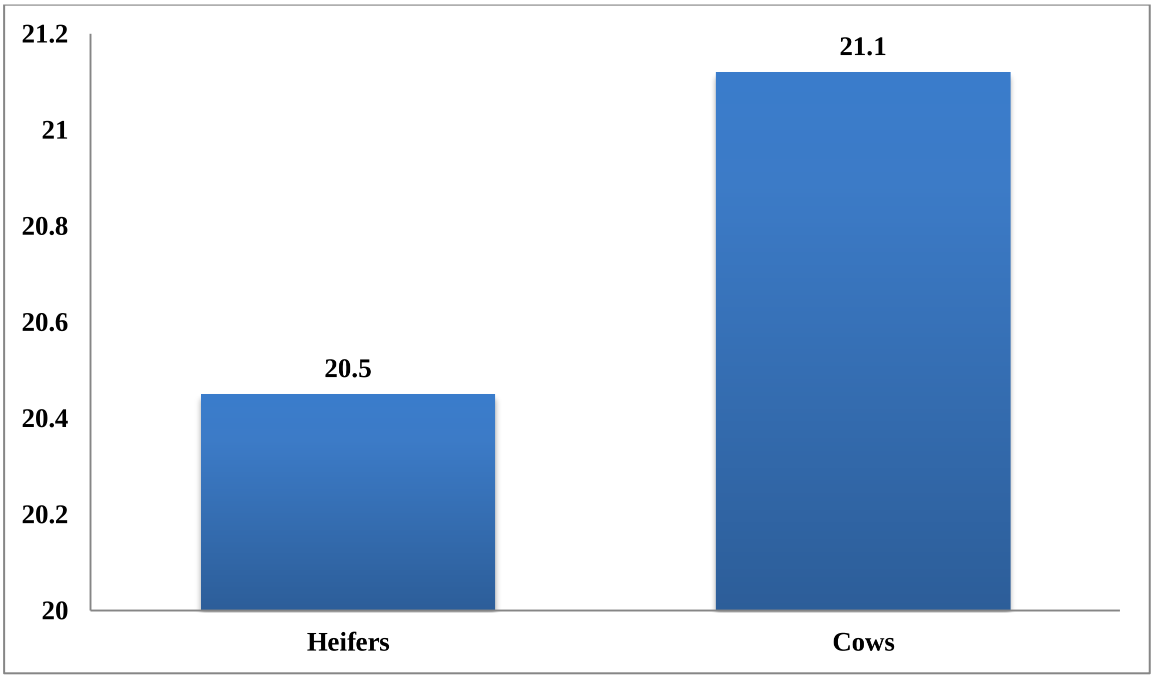

Fig. 11: Prevalence of anoestrous in heifers and cows

The effects of treatment protocols in heifers were illustrated in Table 2 and Table 3. The oestrus animals treated with nutrition was not significant ($P < 0.05$) compared to animals treated with hormone (70.0% vs. 80.0% and 80.0% respectively). The response of estrous in heifers after the treatment was 755.3 ± 31.9 in Group I, 529.0 ± 1.6 in Group II, and 57.2 ± 0.7 in Group III. There were highly significant ($P < 0.05$) differences among the different groups. No significance differences ($P < 0.05$) were observed in service per conception between different groups. The lowest value was 1.4 ± 0.2 in group II and highest value was 1.6 ± 0.2 in group III. The pregnancy rate was also no significant ($P < 0.05$) among different groups.

Table 2: Effects of treatment protocols on onset of oestrus in anoestrous heifers

Traits	Non-hormonal treatment	Hormonal treatment	
	Group I (Nutrition)	Group II (GnRH)	Group III (GnRH + PGF2α)
No. of anoestrous heifers	10	5	5
No. of heifers showed oestrus	7 (70.0 %)	4 (80.0%)	4 (80.0%)
Time of onset of oestrus (hrs)	755.3 ± 31.9[a]	529.00 ± 1.6[b]	57.2 ± 0.7[c]

Values in the same row with different superscripts (a, b, c) are highly significant ($p<0.05$)

Table 3: Effects of treatment protocols on number of service per conception (mean ± SEM) and pregnancy rates (%) in anoestrous heifers

Traits	Non-hormonal treatment	Hormonal treatment	
	Group I (Nutrition)	Group II (GnRH)	Group III (GnRH +PGF2α)
No. of heifers	10	5	5
No. of heifers showed oestrus	7 (70.0 %)	4 (80.0%)	4 (80.0%)
No. of service per conception	1.50 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
Pregnancy rate	71.4 %	75.0 %	75.0 %

Effects of treatment protocols in cows were illustrated in Table 4 and Table 5. The Prevalence of oestrus animals treated with hormone was not significant ($P < 0.05$) compared to those treated with nutritional supplements (71.4 %, 85.7 % vs. 78.6 % respectively). The onset of estrous (hrs) in cows after the treatment was 675.7 ± 29.2 in Group I, 478.4 ± 8.1 in Group II, and 50.9 ± 0.9 in Group III. There were highly significant ($P < 0.05$) differences among the different groups. No Significance differences ($P < 0.05$) in service per conception were observed between different groups.

The lowest value was 1.29 ± 0.2 in group II and highest value was1.4 ± 0.2 in group III. The pregnancy rate was also no significant (P < 0.05) among different groups.

Table 4: Effects of treatment protocols on prevalence of oestrus (%) and onset of oestrus in anoestrous cows

Traits	Non-hormonal treatment	Hormonal treatment	
	Group I (Nutrition)	Group II (GnRH)	Group III (GnRH + PGF2α)
No of cows	14	7	7
No. of cows showed estrous	11 (78.6%)	6 (71.4%)	6 (85.7%)
Time of onset of oestrus (hrs)	675.7 ± 29.2[a]	478.4 ± 8.1[b]	50.9 ± 0.9[c]

Values in the same row with different superscripts (a, b, c) were significantly highly Significant at least (p<0.05)

Table 5: Effects of treatment protocols on no. of service per conception (mean ± SEM) and pregnancy rates (%) in anoestrous cows

Traits	Non-hormonal treatment	Hormonal treatment	
	Group I (Nutrition)	Group II (GnRH)	Group III (GnRH+ PGF2α)
No of cows	14	7	7
No. of cows showed oestrus	11 (78.6 %)	6 (71.4 %)	6 (85.7 %)
No. of service per conception	1.4 ± 0.1	1.29 ± 0.2	1.4 ± 0.2
Pregnancy rate	81.8%	83.3%	83.3%

CHAPTER V

DISCUSSION

Livestock is an important source of income for smallholders and the landless people in Bangladesh. Livestock products such as milk and eggs are steady source of cash income how one live animals are important natural assets for the poor, which can be easily liquidated for cash during emergency. It can be represented by a measure of the ability of a cow to become pregnant within a desired time. The problem of postpartum infertility is one of the well-known drawbacks in cattle production, resulting in substantial financial losses due to prolongation of the service period and culling (Esslemont *et al.,* 1991; Bailey *et al.,* 1999) and also substantially reduce the farmer's financial returns from milk or beef sales. The fertility rates are strongly reliant on endocrine function (Sartori *et al.,* 2004), which can be influenced by the management system and nutrition provided (Boland *et al.,* Lonergan *et al.,* 2003).

In the present study prevalence of anoestrous was 20.9 % overall, (21.1 %) were true anoestrous by 60 days postpartum and (20.5 %) prepartum anoestrous found which is dissimilar with (Shamsuddin *et al.,* 1995; Shamsuddin *et al.,* 2001) 40% were not detected in estrous; higher rates of up to 59% have been reported in individual herds. (Lucy *et al.,* 2001; Stevenson *et al.,* 2006; Walsh *et al.,* 2007). Generally, in the early postpartum period, cows will have a problem with energy balance caused by the increased energy output associated with high milk yield (Robinson *et al.,* 2006).

Nutritionally induced postpartum anoestrous is characterized by turnover of dominant follicles incapable of producing sufficient estradiol to induce ovulation due to reduced LH pulse frequency (Roche *et al.,* 2006). The proportions of the silent oestrus and true anoestrous cases were comparable among different parities; but the true anoestrous cases were higher among the low body conditioned than high body conditioned dews. A negative relationship between parity and number of days from calving to first ovulation in dairy cows under similar body nutritional conditions has demonstrated (Tanak *et al.,* 2008). A poor BCS at calving adversely affects fertility characterized by prolonged postpartum intervals (Shamsuddin *et al.,* 2006).

The results of the study showed that when nutritional treatment was given for induction of estrous, a higher **(**78.6 %) number of cows cows and lower (70.0 %) number of heifer showed estrous within a mean interval of 657.7 ± 29.2 and 755.3 ± 31.9 hours. In addition, 81.8 % cows conceived after receiving nutritional treatment, whereas previous study showed that induction of oestrus in response to nutritional treatment 56.3% (Islam *et al.,* 2013). Lower body condition will affect the circulating levels of hormones which are the major stimulators of estrous behavior and consequently affect fertility levels (Wright *et al.,* 1992). Cows in negative energy balance may have extended periods of anovulation or anoestrous, and show poor oestrus expression which will cause difficulty in detecting oestrus (Scaramuzzi *et al.,* Martin *et al.,* 2008). Consequently, the cow loses body condition, which may affect the first postpartum estrous cycle and the subsequent oestrus. A prolonged anoestrous period can be a sign of temporary depression of ovarian activity. The nutritional status directly affects the development and production of an oocyte, ovulation, estrous cyclicity, fertilization rate, and the development of the fertilized gamete and the whole period of gestation (Robinson *et al.,* 2006).

The results of present study showed that when GnRH hormone treatment was given for induction of oestrus, higher (80%) in heifer and lower (71.4 %) in cows showed oestrus within a mean interval of (529.0 ± 1.6 and 478.4 ± 8.1) hours. In addition, 83.83 % cows conceived after receiving GnRH hormone treatment. Previous studies showed that the ovulation in response to GnRH treatment in postpartum in cattle it was 85% (Wiltbank *et al.,* 1997) which was higher than the present results. In the present study interval after GnRH to ovulation was 478 hours (20 Days) and 529 hours (22 Days) while, 10-22 Days has been reported in cattle (Pattabiraman *et al.,* 1986). Lack of ovarian activity or true anoestrous is considered as one of the major problems to efficient cattle reproduction and cows with true anoestrous may have complete ovarian inactivity (McLeod *et al.,* Williams *et al.,* 1991) demonstrates that cows with true anoestrous by 60 days postpartum need to have some sort of management or hormonal interventions for induction of cyclicity and conception. Accordingly, the present study has demonstrated that a single injection of GnRH analogue has positive effect on induction of estrous and conception in anoestrous cows. GnRH and its agonist act on ovarian follicular development and CL formation indirectly via the induced release of pituitary LH and FSH. Administration of GnRH causes the large follicles to ovulate and induces emergence of anew follicular wave within 3 to 4 days after treatment at any stage of the estrous cycle in cattle.

In the present study it also observed that (80.0 %) of heifers and (85.7 %) of cows showed oestrus when GnRH and PGF2α was used within a mean interval of (57.2 ± 0.7

and 50.9 ± 0.55) hours. It demonstrated that administration of PGF2α or their analogues in cows hasten early resumption of cyclic ovarian activity and thereby, increased the reproduction (Dudhatra *et al.,* 2012). The present relative findings that pregnancy rate 83.3 % with PGF2α and GnRH therapy are far better than the results of (Patel *et al.,* 2007), who recorded 33.3 and 50.0 % oestrus induction response with 50.0 and 66.6 % conception rate in anoestrous buffaloes treated with PGF2α and GnRH.

The present study it showed that the higher proportion of cows showed estrous and no significant difference among different methods. Only between hormonal and nutritional treatment was statistically highly significant ($P<0.05$) observe in from after the treatment to onset of time of oestrus.

Various workers have reported varied response of hormones and nutrition on estrous induction and conception as well as onset of time of oestrus in cows (Patel *et al.,* 2007; Islam *et al.,* 2013; Deshpande *et al.,* 2007). However, the variations observed in oestrus induction response and fertility in different studies could be due to the stage of cycle, product potency, oestrus detection efficiency, nutritional status, general and genital health, breeding time and quality of semen used season/climate and luteal activity or sustainability leading to embryo survival and such other factors.

The relationship between nutritional supplements and reproductive performance of dairy heifers cannot be ignored and this have a far reaching effects on the physiological functioning of the reproductive system, which is constantly under the influence of the endocrine system. However, the mechanism by which nutrition influences reproduction in bovine has not been fully established.

CHAPTER VI

CONCLUSIONS

Livestock is an important part for supporting the livelihoods of poor farmers, consumers, traders and laborers in developing countries like Bangladesh. Fertility improvement is a common goal for many dairy industry or livestock owners.

This study concluded that:

The results could be concluded that overall prevalence of anoestrous in the study area was 20.9 %. Prevalence of anoestrous was higher in cows (21.1%) than the heifers (20.5%). Prevalence of oestrus in hormonal treatment was higher 80 % and 85.7 %, respectively than the nutritional (70 % and 78.6 % respectively) treatment in case of both anoestrous heifers and cows, though there are no significant differences. The response of estrous was highly earlier in hormonal groups (50.9.94 ± 0.55 and 57.2 ± 0.7 respectively) than the nutritional treatment (675.7 ± 29.2 and 755.3 ± 31.9 respectively) in case of both anoestrous in heifers and cows, though there were highly significant differences. Number of service per conception rate was less in hormonal treatment (1.4 ± 0.2 and 1.29 ± 0.2, respectively than the nutritional treatment (1.50 ± 0.2 and 1.29 ± 0.2, respectively) in both heifers and cows. There was no significant variation in service per conception rate among the all treated group therefore; more than one insemination was needed. Pregnancy rate was higher in hormonal treatment (75 % and 83.3 %, respectively) than the nutritional treatment (71.4 % and 81.8 %, respectively) in both heifers and cows. It might be suggested that both nutritional and hormonal treatment was effective for the treatment of anoestrous but cows were showed estrous earlier by the hormonal treatment than nutritional treatment.

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