DETECTION OF ESTRUS BY MEASURING PROGESTERONE LEVELS IN BUFFALOES DURING POST-PARTUM PERIOD

A THESIS

BY

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DECLARATION

This is to certify that the thesis work entitled as "Detection of Estrus by Measuring Progesterone Levels in Buffaloes during Post-Partum Period" has been carried out in partial fulfillment of the requirement for Master of Science in Biotechnology and Genetic Engineering degree from Biotechnology and Genetic Engineering discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh. The above research work or any part of this work has not been submitted anywhere for the award of any degree or diploma. No other person's work has been used without due acknowledgement.

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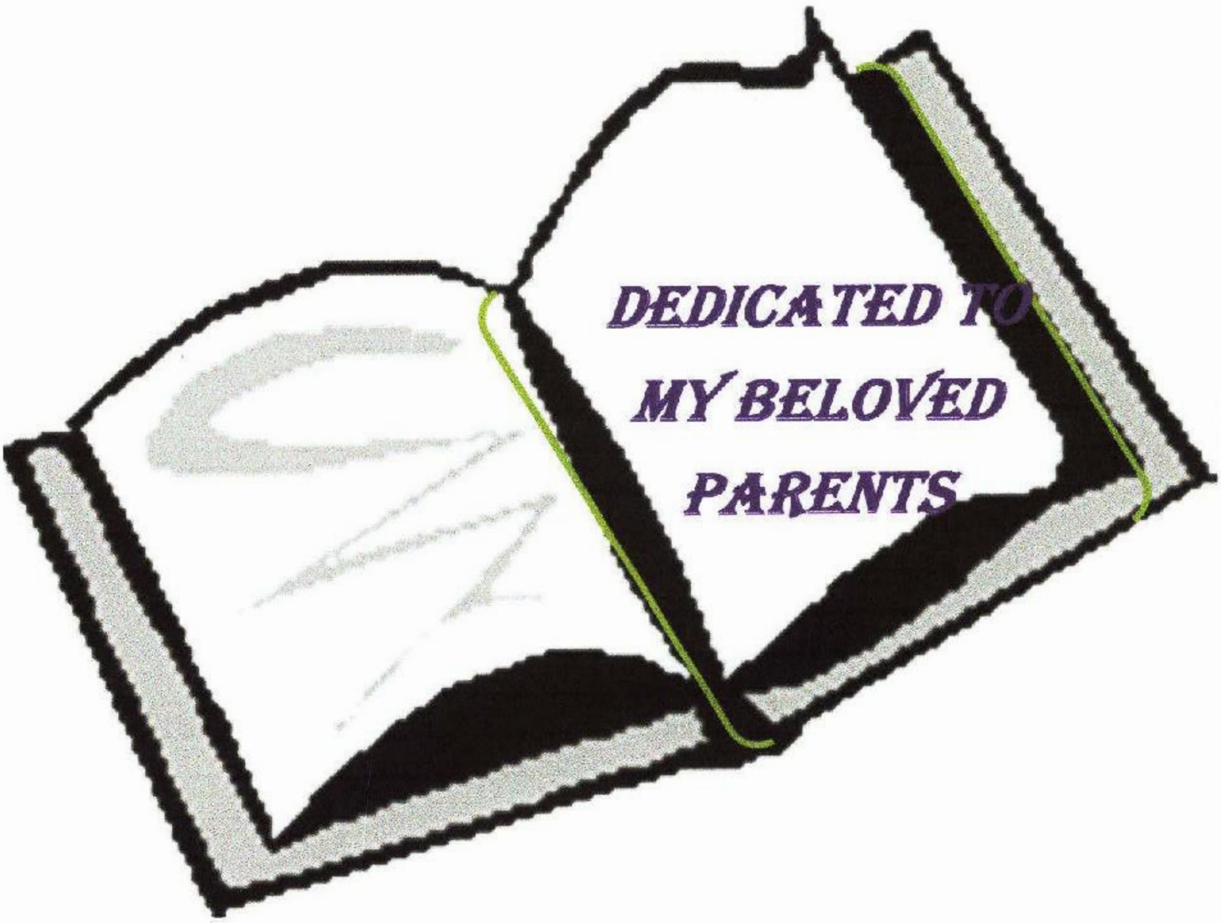
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MY BELOVED
PARENTS

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ABSTRCT

The commencement of early estrous cycle, estrus and pregnancy after calving is fundamental for sustainable buffalo farms, for which proper detection of estrus and correct time of Artificial Insemination (AI) is necessary. This study was conducted to detect estrus of buffalo cows during post-partum period by measuring progesterone levels in serum samples of buffalo cows by means of a commercial hormone analyzer named The ARCHITECT Progesterone assay. Twenty post-partum (45 days post-partum) buffalo cows were selected and serum samples were collected 4 times at 15 days apart. Progesterone level $\leq$ 0.1 ng/ml was observed for 4 buffaloes (45 days postpartum), for 6 buffaloes (60 days postpartum) and for another 4 buffaloes (75 days postpartum). Similarly, 12 out of 20 buffalo cows at 90 days postpartum were also found to have progesterone levels of $\leq$0.1 ng/ml. The progesterone concentration of $\leq$0.1 ng/ml gives an indication to the estrus. The progesterone levels were found significant ($P<0.05$) at 45, 60, 75and 90 post-partum days. The first post-partum heat varies greatly with suckling, nutrition, body condition score at calving, milk yield, parity, season of calving, breed and individual. Repetition of estrus was observed in some buffalo cows at 45 and 90 post-partum days due to not perform servicing and return regular estrus cycle. Acceptance of the male is considered as the most reliable estrus indicator in buffalo. Frequent urination, bellowing, valval swelling, mucus discharge are salient estrus signs in buffalo, but their expression is weak and vary from season to season. This study suggests that the measurement of progesterone could serve as an indicator of the estrus in buffalo cows and to make the findings more accurate and effective further study is essential in buffalo cows with short intervals.

Key words: Buffaloes, postpartum period, progesterone, estrus

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ABBREVIATIONS & SYMBOLS

μL:	Microliter
AI:	Artificial Insemination
BCS:	Body Condition Score
BIRDEM:	Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders
CL:	Corpus Luteum
CMIA:	Chemiluminescent Microparticle Immunoassay
CR:	Conception Rate
FSH:	Follicle Stimulating Hormone
GnRH:	Gonadotropin Releasing Hormone
LH:	Luteinizing Hormone
ME:	Metabolizable Energy
ml:	Milliliter
ng:	Nanogram
P4:	Progesterone
PEI:	Postpartum Estrus Interval
PGF2α:	Prostaglandin F2α
PGFM:	13, 14 Ketodihydro-exo-PGF2α
POI:	Postpartum Ovulation Interval
PUI:	Postpartum Uterine Involution
RLUs:	Relative Light Units
RV:	Reaction Vessel
SPSS:	Statistical Package for Social Sciences
VER:	Vaginal Electrical Resistance
α :	Alpha
β :	Beta
≤ :	Less than and/equal to

Chapter I

INTRODUCTION

INTRODUCTION

Buffaloes are known as "black gold" contributing milk, draft-work and meat, therefore, plays a prominent role in rural economy particularly in Asia. The buffalo is a very important dairy animal in many developing countries like Bangladesh. The buffaloes are suitable for this, because of their high disease resistance and the opportunities of milk production despite of feeding with low quality roughage (Thomas, 2004). The buffaloes have been used for milk production, meat production and draft power for more than 5000 years (Nanda and Nakao, 2003). The production systems are mostly extensive or semi-intensive, with free or tethered grazing in home gardens, fallow fields and communal lands. World's buffalo population is increasing day by day which is estimated to be 202 million represents approximately 11% of all bovine species in world (FAO, 2007), all of these, Asia alone has 174.2 Million (FAO, 2008). This number highlights the importance of using reproductive biotechnologies to improve production in this animal species. Buffaloes are well adapted to sub-tropical and tropical climates and have great potential for meat and milk production both in swamp and in poor soil fertility areas (Valle, 1994; Singh *et al.*, 2000). Buffaloes are seasonally polyestrous animal. The breeding frequency in buffaloes is highest during the winter, decreased in autumn and spring, and is lowest in the summer due to increasing daylight and heat (Shah, 1988). About 20% people of the country are directly engaged in livestock business. The annual production of milk in Bangladesh was 2.7 million ton in the year of 2007- 2008, which was not sufficient to meet up our domestic demand. The per capita milk consumption is 43 ml which is below the requirement of 250 ml. Buffalo alone contributes approximately 96.8% of the total milk to the dairy industry in Asia (Cockrill, 1993), and approximately 12.8% of the total world milk production in spite of being only 11.6% of the total cattle population in the world (FAO-2007). Buffaloes are known to be better at converting poor-quality roughage into milk and meat. They are reported to have a 5% higher digestibility of crude fiber than high yielding cows; and a 4-5% higher efficiency of utilization of metabolic energy for milk production (Mudgal, 1988). It often prefers to cow because of farm net income is higher in buffaloes than from cows (Talukder *et al.*, 2008). If the reproductive management of buffaloes can be improved, it will play an important role in our national economy.

The management of reproduction is a major concern for the dairy industry, because inefficient heat detection causes significant economic losses (Karir *et al.*, 2006). The dairy buffaloes have poor reproduction efficiency attributed mainly to poor expression of estrus symptoms especially during hot summer months and long calving interval. Other problems of reproduction in this animal are late maturity, delay in post-partum estrus, repeat breeding and early diagnosis of pregnancy (Arora & Pandey, 1982a). These problems are confronting the buffalo from becoming a major farm animal in this country. In order to increase the reproductive efficiency of buffaloes, it is necessary to have a comprehensive and accurate knowledge of the regulatory mechanisms involved in the estrous cycle of the animal (Suthar and Dhami, 2010). Ample scope exists for increasing the reproductive efficiency of this animal if the reproductive processes can be controlled and manipulated effectively (Arora & Pandey, 1982a)

The duration of the oestrous cycle in buffalo ranges from 17 to 25 days with a mean of around 21 days (Jainudeen and Hafez, 1993). However, there is greater variability of oestrous cycle length in buffalo, with a greater incidence of both abnormally short and long oestrous cycles, attributed to various factors including adverse environmental conditions, nutrition and irregularities in secretion of ovarian steroid hormones (Kaur and Arora, 1984; Nanda *et al.,* 2003). The postpartum period is characterized by involution of the uterus and re-establishment of ovarian function, intended to prepare the animal for a new pregnancy and uterine involution results from three overlapping processes: contraction, loss of tissue and tissue repair (Kindahl *et al.,* 1999; Yavas and Walton, 2000). The postpartum period in the buffalo like the cow starts with parturition and ends with complete uterine involution and resumption of cyclic ovarian activity and normal estrous expression. As in cattle, uterine involution in buffalo is usually completed in 25–35 days after calving (Jainudeen and Hafez, 1993; Perera *et al.,* 1987). To maintain a calving interval of 13–14 months in buffaloes, successful breeding must take place within 85–115 days after calving. Disturbances during this period due to delay of uterine involution or resumption of estrous activity are likely to prolong the calving interval and reduce the lifetime reproductive and productive efficiency (El-Wishy, 2007). Uterine involution is also a key factor that impacts the time of postpartum anestrus in the water buffaloes. The long postpartum anestrus in the buffalo depends on several factors including: season of calving, uterine involution, suckling, milk yield, nutrition and body condition score at calving (Nam, 2010).

Postpartum anestrus is one of the major causes of reduced fertility in buffalo (Rao and Sreemannarayana, 1982; Samad *et. al.,* 1984). The period of postpartum anestrous is usually longer in buffalo than in cattle under comparative management conditions (Jainudeen and Hafez, 1993). Post-partum anestrous in buffalo is responsible for a long calving interval (Borghese *et al.,* 1993; Campanile et al., 1993) and is a major cause of infertility resulting in economic loss to buffalo breeders in many countries (El-Wishy, 2007). Under optimal conditions buffalo resume ovarian cycle by 30–90 days, but factors such as poor nutrition and body condition (Baruselli *et al.,* 2001), suckling management (Usmani *et al.,* 1990) and climate (Nanda *et al.,* 2003), can delay this considerably. The achievement of early cyclicity after calving is of vital importance in maintaining a dairy industry (Darwash *et al.,* 1997). Fertility improvements of buffalo cows require an adequate knowledge of reproductive behavior (Ireland *et al.,* 2000).

Artificial insemination (AI) in buffaloes has limited use worldwide due to the difficulties in the estrus detection and in finding an adequate moment for this procedure. However, herd management and estrus detection are important issues in AI programs for buffalo (Valle *et al.*, 1994, Singh *et al.*, 2000). As the signs of estrus in buffaloes are less noticeable than in cattle, the estrous detection accuracy is one of the major problems limiting the use of AI in the buffaloes. Covert signs of estrous, the wide variation in duration of estrous (4-64 h) and difficulty in predicting the time of ovulation in female buffaloes (Baruselli, 2001) are major constraints to the broader adoption of AI for genetic improvement in buffaloes. As a result, buffalo number is increasing naturally without any significant growth rate, even without any increase in its milk production. As management-related defect, errors in heat detection (Thakur *et al.* 2013) and suckling may prolong the interval between parturition and onset of estrous which may ultimately affect the pregnancy rate in estrus induced buffaloes (Mondal *et al.* 2007).

Recent efforts to overcome the problem of estrous detection in the buffalo include use of a vasectomized teaser, androgenized females (Drost *et al.,* 1985) plasma and milk progesterone, laparoscopy, use of vaginal electrical resistance (VER) (Gupta and Purohit, 2001) and ultrasonography (Manik *et al.,* 1992).

The present experiment was performed-

- To study about postpartum estrus in buffalo.
- To study progesterone concentration for the detection of estrus.

Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Endocrine and reproductive physiology of buffaloes

Reproductive performance of buffaloes is featured by ineffective. The fertile life of the buffaloes may be delayed due to late puberty. Moreover, silent heat and variable time of ovulation cause the difficulty to the estrous detection which results in either missing estrus, unnoticed or obtaining low conception rate. Due to the decreased level of blood oestradiol-17 beta, the expression of estrus in buffaloes is very poor (Nam, 2010). Buffalo heifers usually attain puberty when they reach about 55-60% of their adult body weight, but the age at which they attain puberty can be highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 1993). Genotype, nutrition, management, social environment, climate, year or season of birth and diseases may be responsible for this.

The study of follicular dynamics and endocrine control of folliculogenesis has provided the basis for enhancing reproductive efficiency in many domestic species (Ginter *et al.,* 2001). Although folliculogenesis studies have been done in the buffalo female (Baruselli *et al.,*1997, Taneja *et al.,* 1996), there is still the need for better understanding of some of these processes in order to improve conception rates following estrus synchronization, superovulation, and artificial insemination, and therefore to reduce the variability in ovulation rate and embryo loss.

The duration of estrus in different breeds of buffaloes vary between 5 and 27 h, and ovulation occurs within 24-48 h (mean 34 h) after onset of estrus, or 6-21 h (mean 14 h) after the end of estrus (Kanai *et al.,* 1990; Perera, 1999). The symptoms observed around the time of estrus include swelling of the vulva and reddening of the vestibular mucosa (Danell, 1987; Kanai *et al.,* 1990). The characteristics of mucus secreted during estrus are less copious than in cattle, does not usually hang as strands from the vulva, and be discharged either when the animal is lying down (Perera *et al.,* 1977) or with the urine (Kanai *et al.,* 1990). Srivastava and Sahni (2003) stated that buffaloes showed more estrus activity in the morning (06:00-07:30 hour) than in the afternoon (14:00- 15:30 hour) or during the night (22:00-23:30 hour). The ovary of buffalo differs from cattle is elongated and considerably smaller. The average dimensions of the buffalo ovary vary between 22-26 X 11-18 X 11-14 mm^3 (Fadle *et al.,* 1974) with a maximum and minimum weight of 6.1 and 2.9 g, respectively (El-Wishy *et al.,* 1971). The ovaries of post-pubertal buffalo heifers have a reservoir of only 10,000-20,000 primordial follicles (Danell, 1987). Per rectum palpation designates that mature follicles tend to protrude from the surface of the ovary and can be mistaken as an early developing corpus luteum (Jainudeen *et al.,* 1983). The CL of the buffalo is often deeply embedded in ovarian stroma and is generally smaller than that of cattle. The CL does not protrude markedly from the surface of the ovary and sometimes lacks a clear crown. Therefore, it is more difficult in buffalo to identify the ovarian structures accurately by rectal palpation than in cattle (Perera *et al.,* 1987; El- Wishy, 2007).

GnRH secretion induces an identical pattern of LH secretion and increases the FSH synthesis at the pituitary level. Pulsatile GnRH secretion controls gonadal development and function, including steroidogenesis in both females and males, while surge GnRH secretion drives ovulation in the females. The GnRH pulse generator drives endocrine signals that allow the onset of reproductive activity, either by the transition from a pre-pubertal state to puberty, or the transition from seasonal anestrous to a full reproductive activity, as well as the maintenance of proper reproductive activity in adult. Later, gonadal steroid or peptide hormones regulate the hypothalamic/pituitary function by feedback mechanisms, completing the loop of endogenous control of reproductive activity.

The pattern of changes in hormone profiles of buffaloes during the estrus cycle closely resembles than that of cattle. The average length of the estrous cycle is 20-22 days for river buffaloes (Singh et al., 2000). The average duration of estrus seems to be slightly longer in river buffalo 23.8 ± 6.2 h (Danell, 1987) than in swamp buffalo 19.9 ± 4.4 h (Shimizu, 1987).

The duration of estrus was estimated to be 14, 18, and 8-10 h in the monsoon, winter, and summer seasons, respectively, in river buffaloes (Janakiraman, 1978). The temporal patterns of both LH and FSH are more or less similar to those in cattle (Kanai et al., 1990), with a pre ovulatory LH surge occurring on the day of estrus and lasting 7-12 h. The concentration of estradiol-17 in blood during the follicular phase of the estrous cycle also appears to be relatively less than that in cattle (Kanai et al., 1990; Roy and Prakash, 2009) and this has been justified as a possible reason for the lesser intensity of estrus exhibited by buffaloes. The peak value of progesterone recorded about 15 days after estrus is 4.0-5.1 ng/ml (Arora and Pandey, 1982b; Takkar *et al.,* 1983). Ovarian follicular growth occurs in wave's pattern. Follicular dynamics is noted as the process of continual growth and regression of antral follicles that leads to the development of the preovulatory follicle (Lucy *et al.,* 1992).

Buffaloes have estrous cycles with 1, 2 or 3 follicular waves, 2-wave cycles are the most common and the number of waves in a cycle is associated with the luteal phase and with estrus cycle length (Baruselli *et al.,* 1997). Awasthi *et al.,* (2006) have stated that one follicular wave patterns are quite usual in the estrous cycles of the river buffalo. The wave pattern of follicular growth is termed as the simultaneous initiation of developing a group (5-7) of follicles in ovaries (Mapletoft *et al.,* 1994). During each wave, small follicles (4 mm in diameter) enter a common growth phase, one follicle of the cohort grows promptly to attain ovulatory diameter (dominant follicle) (Ginther *et al.,* 2003), suppressing the growth of other follicles (subordinate follicles) and preventing developments of a new follicular wave (Armstrong and Webb, 1997). Each antral follicular wave is stimulated by an increase in FSH secretion (Adams *et al.,* 1992) caused by the regression of the dominant follicle of the previous wave (Ginther *et al.,* 2003). The morphologically and functionally dominant follicle (the largest follicle of the final wave, i.e. ovulatory follicle) appears to ovulate under appropriate hormonal conditions (Fortune, 1993). The dominant follicle subsequently acquires LH dependency for its own continued growth and suppresses FSH secretion, starving subordinate follicles of sufficient FSH support (Austin *et al.,* 2003). On the other hand, the small size and slow growth rate of ovulatory follicles in the one follicular wave estrous result in the low concentration of estrogen which is one of the causes of the silent heat in the river buffalo (Awasthi *et al.,* 2007). Beside the follicular model, the partial luteolysis is also pointed out as a probable reason of the silent heat observed in the studies of using PGF2α to induce the luteolysis in buffaloes, (Dhaliwal *et al.,* 1988, El-Belely *et al.,* 1995).

2.2 Importance of hormones in reproduction

Reproduction in mammals is largely controlled by the endocrine glands and the hormones they secrete. They influence all phases of reproduction. The hormonal regulation of reproduction is a complicated and highly balanced system of checks and controls. The various hormones stimulate or inhibit each other and accordingly exert effects on the animal's reproductive organs. In the females estrogens are secreted from maturing graafian follicles in the ovary and are required for coordinating events leading up to mating, conception and parturition. Progesterone, which is secreted by the corpus luteum (CL), is required for maintenance of pregnancy. The consecutive presence of appropriate concentrations of progesterone, estrogens and gonadotropins are necessary for the expression of estrus and ovulation. In the non-pregnant animals, the uterus plays an important role in the regression of CL thereby allowing the repetition of the estrous cycle. The pregnant female has a further endocrine organ, the placenta that may secrete different gonadotropins and steroids depending upon the species. Thus from the numerous studies in other species, it has been established that the reproductive hormones play a crucial role in controlling reproductive events. A clear picture of the endocrine background during various reproductive conditions in buffalo was not established. However the recent application of radio immunoassay for quantitation of hormones in buffalo (Arora and Pandey, 1980) has considerably helped in unfolding the mystery of reproduction control in buffaloes. This increased knowledge of the basic reproductive endocrinology is of fundamental importance for understanding the hormonal interplay accompanying normal reproductive processes and is likely to help in alleviating reproductive problems of endocrine origin in this species.

2.3 Hormones in blood during luteolysis, oestrous cycle and estrus

During estrous cycle progesterone is minimal (0.16 ± 0.02 ng/ml, Mean ± SEM) on the day of estrus (day 0), rises steeply, attains a peak on day 15 (5 .21 ± 0.05 ng/ml) (Bachlaus *et al.,* 1979). A gradual decrease in progesterone occurs and abrupt fall occurs at 43.33 ± 6.33 hours prior to estrus which further declines to lowest level at oestrus (Batra *et al.,* 1981).The oestradiol-17β concentration is 19.32 ± 3.73 pg/ml on the day of estrus and decline to reach a nadir (5.73 ± 3.68 pg/ml) on day 2 of the cycle. A minor rise occurs on day 4 and a more sustained on day 10 (Bachlaus *et al.,* 1979). It shows a gradual increase to a peak concentration of 31.34 ± 1.70 pg/ml at 13.83 ± 1.58 hour before estrus. In non-pregnant buffalo, patterns of progesterone and estradiol 17-β are exactly similar to cyclic buffalo (Arora and Pandey, 1982b). Prostaglandin $F_{2\alpha}$ secreted by the uterus plays an important role in the regression of CL in mammals failing to conceive. The peripheral plasma levels of PGF2α do not present a clear picture of its secretion in relation to the regression of CL since it is rapidly metabolized in the lungs. However, its metabolite i.e., 13, 14 ketodihydro-oxo-PGF2α (PGFM) has been found to be an alternate method for assessing the release of PGF2α (Batra and Pandey, 1981).

The PGFM concentration increases significantly to 990.84 ± 53.9 pg/ml at estrus (day of insemination) and shows minor elevations on 3-4 and 11 days after insemination. The PGFM concentration is significantly affected by different days of the estrous cycle. The androgen during estrous cycle of buffalo does not present a consistent picture. However, in a few buffaloes, high androgenic activity has been reported to occur on the day of estrus, which possibly indicates the role of androgen in eliciting estrous response through estrogens (Arora and Pandey, 1982b).

The concentration of LH is recorded highest on the day of estrus (22.9 1±4.88 ng/m l), which is followed by a sudden drop on the following day (Arora and Pandey. 1982b). It fluctuates at the basal levels throughout the cycle with a significant increase to 24.37 ± 2.05 at ng/ml at l±l hour (0-6 hour) after estrus (Batra *et al.*, 1981). FSH hormone reaches maximum value (52.91 ng/ml) on the day of estrus, declines slowly up to day 6 and then fluctuates within a basal range between 20.74 and 29.58 ng/ml (Heranjal *et al.,* 1979). The prolactin concentration shows wide fluctuations and a peak of 374 ± 67 ng/ml is observed 24.83 ± 8.96 hours before estrus (Batra *et al.*, 1981). It fluctuates between 112 and 366 ng/ml during the rest of the cycle (Heranjal *et al.*,1979). These findings reveal that the day of estrus is the most important stage of the estrous cycle in buffalo since the concentration of gonadotropins or steroids reach their extreme values (highest or lowest) on this day. Also, increasing values of estrogens or androgen coinciding with major prostaglandin activity and low activity of progesterone probably exerts a positive feedback on the hypothalamo-hypophyseal axis resulting in the release of peak levels of gonadotropins during oestrus.

2.4 Problems with reproduction in buffaloes

Buffaloes are said to be seasonal breeders, which is a simplification of the truth. They are polyestral animals and are able to breed the whole year (Ståhl-Högberg and Lind, 2003). Buffaloes have poor reproduction efficiency attributed mainly to poor expression of estrus symptoms especially during hot summer months and due to long calving interval. Buffaloes that calve during the cool season have better reproductive performance than them calving during the hot season. The marked seasonality of buffalo breeding handicaps the commercial milk production which alternate between surplus and shortage (Brakawi *et al.,* 1998; Gupta and Prakash, 1990; Sastry, 1983). Buffaloes may have silent estrus even in non-stressful periods of the year (Kamboj and Prakash, 1993). Successful breeding must take place within 85 - 115 days after parturition to maintain a calving interval of 13 - 14 months in buffaloes (El-Wishy, 2006).

The incidence of anestrus in buffaloes varies between 20-80 %, depending on season. Most female buffaloes which exposed to extreme hot conditions during the summer season cease ovarian activity. During this time the milk production and reproductive efficiency are strongly negative affected, probably due to the combined effects of nutrition, environment and management (Nanda *et al.,* 2003; Sastry, 1983).

Silent estrus is a common problem among buffaloes even under good management (Abdalla, 2003). A study performed by Brakawi *et al.,* (1998) shows that approximately 90 % of the buffaloes resume their ovulatory and estrus activities within 60 days post-partum. The silent estrus period is a big problem and because of this it is difficult to determine the exact time for artificial insemination or servicing. Average calving intervals for Indian buffaloes ranged from 15 to 18 months. The dry period has been reported to be 60 to 200 days, average gestation period was 308-318 days and the average lactation length ranged from 252 to 270 days. As a result of these factors the productive life of a buffalo is only 39 % compared to 52 % in developed dairy breeds. Due to these facts, an important factor to consider in improving milk production in dairy buffaloes is heat detection (Ganguli, 1981; Sastry, 1983).

2.5 Factors affecting efficiency of oestrous cycle in Buffaloes

Beg and Totey, (1999) and Singh *et al.,* (2000) recent studies on estrous cyclicity and estrus behaviour in the buffalo have been reviewed and considerable variations in reproductive traits of buffaloes have been observed in compared with. Several factors, such as dairy business depends on milk production as climate, temperature, photoperiod and nutrition, were indicated as affecting the length of estrous cycle and the degree of heat expression.

2.5.1 Environmental factors

a. Ambient Temperature

Extreme weather conditions such as cold or heat depress the sexual activity and usually less number of conceptions occurs when the temperature is severe. In hot or cold environment the length of estrous cycle is prolonged and duration of heat become shorter as feed intake is drastically reduced compared to the comfortable environment. Decrease thyroid activity during severe summer may indirectly reduce the reproductive efficiency.

b. Season

There is a complex dependency of bovine reproduction on soil, plant and climatic factors particularly in tropical and subtropical parts of the world (Singh et al., 2000). Although buffaloes are polyestrous, they exhibit a distinct seasonal variation in display of estrus, conception rate, and calving rate (Singh and Nanda, 1993). Buffaloes are generally seasonally polyestrous, expression of heat being limited to during winter (October to February) (Sane, 1994). Estrous cycle is controlled by the estrogenic substances. The concentration of the substance present in plants or fodders varies according to different seasons of the year.

Gonadal steroids such as estradiol-17β and progesterone plays pivotal role in the reproductive problems associated with extreme climatic condition in buffaloes. Photoperiod is the principal environmental cue of these changes: decreasing day length in autumn stimulates gonadal activity whereas increasing day length in summer has the reverse effect. There is highly significant difference in the progesterone level at estrus and luteal phase of the cycle between hotter and cooler months (Rao & Pandey, 1982).However estradiol 17β is not altered by different seasons. Similarly, at the onset of estrus, the circulatory LH levels in the hotter months (June-August) are not different than cooler months (October to December).

There is no difference in conception rate for buffaloes coming to estrus in the hotter months as compared to cooler months (Kakar *et al.*, 1980). Sheth *et al.*, (1978) observed that summer sterility in buffaloes is attributed to the 2 to 6 fold higher prolactin levels as compared to medium and peak breeding seasons. Also during peak and medium breeding seasons, there is a sharp fall in prolactin levels after ovulation and lowest levels are observed in luteal phase.

c. Light

The duration and intensity of light to which the animals are exposed also influence the onset of estrous cycle. This effect of light on animal is known as 'sexual photoperiodicity'.

2.5.2 Age and Body Weight

The younger animals show shorter length of estrum and estrous cycle than adult animals. First estrus in young animals is generally not prominent than other stages of life. Body weight of animal also influences the estrous cycle. Different experiments show that buffalo heifers should be bred for the first time either at their age of 30 months or at about 360 kg body weight whichever is earlier.

2.5.3 Nutrition

Very poor nutritional status or starvation of animals for the prolonged period, deficiency of vitamin A and other minerals contents in feed prevent maturation of graffian follicles (Singh *et al.,* 2000).This situation is responsible for abnormal cyclicity of animals or no prominent estrous.

2.5.4 Level of Production

High producing animals suffer from chronic systemic diseases; definitely the estrous cycle is hampered until or unless special preventive measures are taken.

2.5.5 Hormonal Imbalance

Cystic ovarian disease, nymphomania etc., disturb the hormonal (mainly estrogen, progesterone, and LH) balance which, causes either irregularities in the cycle or frequent/ continuous estrum.

2.6 Silent oestrus as the main constraint of buffalo reproduction

Silent estrus is a common problem in buffaloes even under good management (Abdallah, 2003). A study performed by Barkawi *et al.,* (2009) showed that approximately 90 % of the buffaloes resume their ovulatory estrus activities within 60 days post-partum. The silent estrus results in difficulties to determine the exact time for artificial insemination or servicing. Moreover, failure in heat detection leads to a reduced productive life of buffaloes. For instance, average calving intervals of Indian buffaloes ranged from 15 to 18 months, the dry period varied from 60 to 200 days, gestation period was 308-318 days and the lactation length ranged from 252 to 270 days. This means, the productive life of a buffalo is only 39% compared to 52% in developed dairy cattle breeds (Singh, 1993).

2.7 Postpartum period in buffaloes

The postpartum period is characterized by involution of the uterus and reestablishment of ovarian function, intended to prepare the animal for a new pregnancy and uterine involution results from three overlapping processes: contraction, loss of tissue and tissue repair (Kindahl *et al.,* 1999; Yavas and Walton, 2000). The postpartum period in the buffalo like the cow starts with parturition and ends with complete uterine involution and resumption of cyclic ovarian activity and normal estrous expression. Hormonal changes during the peri-parturient period besides regulating lactogenesis and parturition have their impact on postpartum reproductive activity. El-Wishy (2007) have reviewed the hormonal changes during late gestation and postpartum period, initiation of follicular activity after calving and factors which influence uterine involution period. Pituitary release of LH and FSH in response to exogenous GnRH declined progressively with the advancement of pregnancy and low response is observed after parturition regardless of the stable LH and FSH contents in the pituitary, and this could be one of the reasons of long postpartum anestrus in the buffalo (Palta and Madan, 1996). No significant variations observed in basal level of FSH between Days 2, 20 and 35 postpartum (Palta and Madan, 1996). Similarly, no significant variations were observed in the basal LH levels between Days 3 through 90 postpartum or between milked (0.9 ± 0.2 to 1.3 ± 0.2 ng/ml) and suckled (0.9 ± 0.1 to 1.5 ± 0.2 ng/ml) anestrous Murrah buffaloes (Arya and Madan, 2001). Batra and Pandey (1983) reported that basal plasma LH concentration was significantly higher in buffaloes showing estrous than in anestrous animals. Basal values of estradiol-17 were reported between Days 2 and 7 after calving (Eissa et al., 1995; Arya and Madan, 2001) with minor fluctuations thereafter (11 ± 3 to 18 ± 3 pg/ml) until day 45 postpartum (Madan *et al.,* 1984). Fluctuations of total estrogens between 38 ± 10 and 61 ± 5 pg/ml were observed during the first 75 days postpartum in acyclic buffaloes (Soliman *et al.,* 1981) probably reflects waves of follicular growth and atresia. As in cattle, uterine involution in buffalo is usually completed in 25-35 days after calving (Jainudeen and Hafez, 1993; Perera *et al.,* 1987). The stimulus of suckling shortens involution time (Usmani *et al.,* 1990). To maintain a calving interval of 13-14 months in buffaloes, successful breeding must take place within 85-115 days after calving. Disturbances during this period due to delay of uterine involution or resumption of estrous activity are likely to prolong the calving interval and reduce the lifetime reproductive and productive efficiency (El-Wishy, 2007).Uterine involution is also a key factor that impacts the time of postpartum anestrus in the water buffaloes. The long postpartum anestrus in the buffalo depends on several factors including: season of calving, uterine involution, suckling, milk yield, nutrition and body condition score at calving (Nam, 2010).

Perera (2011) have stated that the first postpartum ovulation is frequently followed by one or more short estrous cycles (<18 days) and cessation of oestrous cyclicity occurred after the first or second ovulation in about 25% of animals due to ovulatory failure or prolonged luteal activity.

The response of LH secretion to exogenous GnRH at 25-35 days postpartum, is greater in non-suckled than suckled buffalo (Singh *et al.*, 2006). These considerations indicated a need for estrous synchronization using fixed-time insemination for the implementation of breeding programs in buffalo (Presicce *et al.*, 2004; Ali and Fahmy, 2007). Ovulation and estrous activity after calving are delayed when the positive feedback effects of estradiol on release of LH from the pituitary are reduced due to various factors. Therefore, treatment includes hormonal and management strategies (Rhodes *et al.*, 2003).

Qureshi et al., (2002) reported that BCS and postpartum ovulation interval were correlated with ME intake BCS was higher in estrous buffaloes than anestrous ones. ($P<0.01$). Higher and lower metabolizable energy (ME) intakes are associated with anoestrus, while a moderate energy intake is associated with a postpartum estrus interval (PEI) of less than 75 days (Qureshi *et al.*, 2002). Very recently, Qureshi (2012) has stated that excess intake of crude protein, associated with higher serum urea levels and low energy intake, associated with poor body condition, are the key factors for low reproductive efficiency. Some previous studies showed that high milk yield (8kg of milk per day) (El- Fadaly, 1980, El-Azab *et al.,* 1984) and suckling prolonged time of postpartum anestrus. Arya and Madan, (2001) observed that milked buffaloes had longer acyclic interval of 72 ± 11 days compared with 44 ± 9 days in those who were weaned. Free suckled buffaloes had longest postpartum anestrus interval (82 ± 11 days) in comparison to restricted suckled (69 ± 10 days) and early-weaned buffaloes (50 ± 7 days) (Nordin and Jainudeen, 1991).

Under-nutrition and environmental stress have been recognized as causes of long anestrous periods in buffaloes (Kaur and Arora, 1984). It is suggested that delay in post-partum ovarian activity in ruminants is related to lower level of minerals in blood (Krop, 1993; Manspeaker and Robl, 1993). Deficiency of mineral elements like phosphorus (P), copper (Cu) and zinc (Zn) are associated with subnormal fertility and anoestrous conditions in cows (Campbell *et al.,* 1999). Moreover, Ca, P, Zn and manganese (Mn) have been found affecting post-partum reproduction in cattle (Hidiroglou, 1979).

Data from Egypt, India and Pakistan indicated that only 34-49% of buffaloes showed estrous during the first 90 days after calving and 31-42% remained anestrous for more than 150 days (reviewed by El Wishy, 2007). Anovulatory follicles are reported in postpartum anestrous dairy (El-Wishy, 1979; Abul-Ela *et al.*, 1988; Suthar and Kavani, 1992) and swamp buffaloes (Jainudeen et al., 1983). Restoration of pulsatile LH secretion could be a limiting factor for development and maturation of dominant follicles (Manik *et al.,* 2002) in postpartum buffalo cows. Early studies demonstrated that suckling (Honnapagol *et al.*, 1993; Tiwari and Pathak, 1995), level of milk production (Singh *et al.*, 2006) in postpartum buffalo cows. Early studies demonstrated that suckling (Honnapagol *et al.*, 1993; Tiwari and Pathak, 1995), level of milk production (Singh *et al.*, 1988) and prepartum nutrition (Usmani *et al.*, 1990) did not influence the interval to initiation of follicular activity. The interval to first postpartum estrous is also significantly affected by the season of calving (Madan, 1988; Singh, 1993; Singh and Nanda, 1993). Buffaloes calving in late winter and early summer have lower reproductive efficiency compared to those calving during other periods. The resumption of ovarian activity after calving was significantly delayed in buffaloes that calved from February to May (116-148 days compared to the rest of the year 38-64 days) (Singh and Nanda, 1993).

The achievement of early cyclicity after calving is of vital importance in maintaining a dairy industry (Darwash *et al.*, 1997). During pregnancy, high concentrations of circulating progesterone are associated with a marked reduction of LH content at the anterior pituitary and this is one of the initial limitations on the resumption of normal ovarian activity in post -partum cows (Peters *et al.*, 1981). It is hypothesized that the capability of the pituitary gland to respond to exogenous GnRH is restored by Day 20 postpartum in dairy buffaloes (Palta and Madan, 1996). Therefore, studies on ultrasonographic assessment of ovarian follicular dynamics together with hormonal profiles (progesterone and LH) are needed to define the circumstances under which early return of cyclic activity can be achieved in the postpartum buffalo (El-Wishy, 2007).

2.8 Anestrous in buffaloes

Silent ovulation (lack of overt signs of estrous) and unobserved estrous (poor oestrous detection efficiency) can greatly increase the incidence of anestrous. It is important to distinguish between these conditions (physiology vs. management). Anestrous is broadly classified into physiologic and pathologic (clinical) types,

In type I anestrous, there is growth of follicles to emergence without further deviation or establishment of a dominant follicle. The pathophysiology of this condition is not well understood, but it is presumed to be due to extreme under nutrition. In that regard, under nutrition and severe energy deficit may cause this condition through a lack of essential LH support to sustain follicular growth and dominance (Sangsritavong *et al.*, 2002).

In type II anestrous, there is deviation and growth, followed by either atresia or regression. In certain cases, the regression or atresia occurs only after a follicle has reached a dominant status. Regression of this follicle results in the emergence of a new follicular wave 2 to 3 days later. In these cases, there are sequential follicular waves prior to first ovulation, which may be delayed for a prolonged interval. Some follicles grow further and regress prior to ovulation (Wiltbank *et al.,* 2002).

In type III anestrous, there is deviation, growth and establishment of dominant follicle, but it fails to ovulate and becomes a persistent follicular structure. A single follicular structure >8 mm in diameter was observed in the absence of a CL or cyst in two ultrasonographic examinations 7 days apart (Sangsritavong *et al.,* 2002). This may be due to insensitivity of the hypothalamus to the positive feedback effect of estradiol or to altered follicular responsiveness to gonadotrophic support, mediated via metabolic hormones (e.g., insulin-like growth factor and insulin). Persistent follicular structures may become follicular cysts or they may luteinize (luteal cysts). The latter occurs in 10% to 13% of the cases (Ambrose and Colazo, 2007).

Type IV anestrous, is due to a prolonged luteal phase. These cows have normal oestrous, ovulation and formation of a CL, with prolonged luteal function due to a lack of luteal regression. A contributing factor may be the lack of an estrogenic dominant follicle at the expected time of luteal regression. Regarding this matter estradiol from a dominant follicle is believed to induce the formation of uterine oxytocin receptors, leading to pulsatile release of PGF2α (Mwaanga ES, 2000).

Dairy animals in the tropics face numerous challenges under tropical environments. Rasby et al., (1992) reported that nutrition restriction has a negative influence on LH release. Suboptimal nutrition coupled with stress due to high environmental temperatures may be responsible for long anoestrous in buffaloes (Terzano *et al.,* 2012).

In addition to energy deficits, increased feed intake also suppresses reproduction by promoting steroid hormone metabolism. Increased feed intake enhances hepatic perfusion, thereby promoting clearance of estradiol and progesterone, which can contribute to anovulation (Sangsritavong *et al.,* 2002), ovulation of larger dominant follicles, multiple ovulations (Sartori *et al.,* 2004), poor luteal function and delayed luteal regression, perhaps due to development of estrogen-inactive dominant follicles, resulting in inadequate endometrial PGF2α production. Furthermore, diets high in crude protein, typically in excess of 16% to17%, support high milk yield but may be detrimental to reproductive performance (Tamminga, 2006) showing elevated blood urea concentrations (Butler, 2000).

2.9 Postpartum acyclicity and anestrus

Post-partum anestrous has been recognized as a major reproductive limitation in buffalo. The calving interval in 48-66% of buffaloes has been >14 months as dictated by exposure to the environment and unpredictable management (Perera, 1999). 34–49% of buffaloes showed estrus during the first 90 days after calving and 31–42% remained anestrus for more than 150 days. Anestrous periods due to cessation of cyclic activity (true anestrus) for 3 or more weeks and prolonged luteal activity for 28 days or more were described to occur in about 25 and 8–11% of the buffaloes, respectively, after the first or second ovulation (El-Wishy, 2007). Usmani et al. (Usmani *et al.*, 1990) reported that short cycles were followed by long anestrous and anovulatory periods due to prolongation of the inter-luteal interval to 21–22 days instead of the normal period of 6–7 days, before establishment of regular cyclicity. Underfeeding coupled with high environmental temperature stress was also incriminated for long anestrous and anovulatory periods (Mudgal, 1979; Kaur and Arora, 1984). Inadequate nutrient intake has been found to deplete body energy reserves resulting in extended interval from calving to first estrus.

The period of postpartum anestrous or anestrus is usually longer in buffalo than in cattle under comparative management conditions (Jainudeen and Hafez, 1993). Under optimal conditions buffalo resume anestrous by 30-90 days, but factors such as poor nutrition and body condition (Baruselli *et al.,* 2001), suckling management (Usmani *et al.,* 1990) and climate (Nanda *et al.,* 2003), which also influence nutrition through feed quality and availability, can delay this considerably.

El-Wishy (2006) reported that prolonged postpartum acyclicity (absence of ovarian cyclic activity) and anestrum (absence of overt estrous signs) are major sources of economic loss to buffalo breeders. Studies on the epidemiology of these two problems are highly recommended to achieve successful control. He studied in dairy buffaloes and revealed that first ovulation as detected by rectal palpation and progesterone analysis occurred between 28–71 and 24–55 days, respectively, after calving. Postpartum estrus in the same studies occurred between 44 and 87 days. Reports concerned with data compiled from breeding records of research stations, breeding farms and small holders where estrus is a subjective measure, gave much longer periods. Also data from Egypt, India and Pakistan indicate that only 34–49% of buffaloes showed estrus during the first 90 days after calving and 31–42% remained anestrus for more than 150 days. In swamp buffaloes both postpartum ovulation and estrus are more delayed than in dairy buffaloes. The role of suckling, nutrition, body condition score at calving, milk yield, parity, season of calving and other minor factors were discussed. First postpartum ovulation is frequently followed by one or more short estrous cycles (<18 days). Long anovulatory and anestrous periods due to prolonged inter-luteal phase were reported to occur after short cycles. Also long anestrous periods due to cessation of cyclic activity (true anestrus) for 3 or more weeks and prolonged luteal activity for 28 days or more were described to occur in about 25 and 8–11% of the buffaloes, respectively, after the first or second ovulation. These cycle irregularities certainly impose difficulties on estrus detection programs in postpartum buffaloes.

Postpartum uterine health is most critical component of reproductive and productive efficiency of dairy herds. During early postpartum period uterus is highly vulnerable to even low grade injuries and infections, which may cause delayed uterine involution and further inflammation of the endometrium, depending on the nature of causative agent and its persistence. Thus, such conditions results in delay of the first ovulation postpartum reduce conception rate (CR) to first insemination and increase the risk of culling due to infertility (Sheldon, 2004) even with best managemental practices.

In Nili-Ravi buffalo's 24% incidence of sub-clinical uterine infection was reported by Usmani et al. (Usmani *et al.*, 2001). Such condition are responsible for slower involution of the cervix and uterus, longer mean interval to first postpartum estrus and longer service period in buffaloes of the infected group than in the normal group. Increased milk yield, decreased BCS and decreased body weight were also associated with delayed onset of cyclicity (Mann *et al.*, 2005).

Nutrition plays a considerably important role in reproduction of water buffaloes. Shorter postpartum anestrus period achieved in those buffaloes who were fed high energy prepartum compared with those were not was proved by Salama *et al.*, (1982) and Hegazy *et al.*, (1994a). Nutrition has a close relation to body condition of the water buffaloes. In the buffaloes whose body condition score are low, the self-tissues are used for the production of milk and other daily activities then they do not have enough energy stored for the next estrous cycle. This would elongate the interval of postpartum anestrus. The thin buffaloes were documented to be prolonged the time of acyclic postpartum than those had moderate body condition score; 63 days compared with 47 days (Hegazy *et al.*, 1994b). In both river and swamp buffaloes, those had higher body condition were found to have shorter postpartum anestrus interval than those had lower body condition score (Baruselli *et al.*, 2001).

Season of calving had influence on the reproductive performance. Various workers reported that buffaloes calving in the rainy and monsoon seasons had shorter anestrous periods than other season calvers (Kaur and Arora, 1984; Basu, 1962; Rao *et al.*, 1973). Nevertheless, longer anestrous periods were reported for out of season (156±34 and 91±12 days) than normal breeding season calvers (57±6 and 56±5 days) (Perera et al. 1987; Qureshi *et al.*, 1998). Tomar et al. (Tomar *et al.*, 2002) reported reproductive disorders mostly in summer and rainy seasons. On the other hand Kaushik and Mathur (2005) found problems of difficult calving highest during autumn, followed by rainy and summer months. They reported 8.71% cases of retained placenta followed by abortions (1.18%) and stillbirth (0.44%).

The mean postpartum uterine involution length (PUI), POI and mean postpartum estrus interval (PEI) were 34.30 ± 1.33 days, 59.37 ± 4.76 days and 69.03 ± 6.03 days, respectively. POI was shortest in buffaloes suckled for one month ($P<0.05$) and suckling period had significant positive ($P<0.01$) correlation with POI and PEI. BCS in buffaloes resuming estrus was constantly higher than those failing to resume ovarian cyclicity. Higher BCS was significantly ($P<0.01$) shortened POI and PEI (Qureshi and Ahmad, 2008).

2.10 Estrus Behavior in Buffaloes

Buffaloes are generally seasonally polyestrous, expression of heat being limited to eight months of the year and sexually inactive condition is observed from March to June, when hardly 3% of the heats are recorded. The peak season for estrus in buffaloes is reported to be from October to February. Indian buffaloes are showing more conceptions when both diurnal temperature and relative humidity are low i.e. winter. High temperature affects the conception. Behavioral sign in buffaloes are less obvious than those in cattle; therefore less than one third of buffaloes in heat might be detected by homosexual behavior (Suthar and Dhami, 2010). Unlike cattle obvious and raged sign in buffaloes are not pronounced. Hetero-sexual behavior, particularly standing to be mounted by a bull, is most reliable sign of estrus in buffaloes, whereas homosexual behavior observed only occasionally. Sign such as swelling of vulva, clear transparent mucus discharge, spontaneous milk letdown, bellowing, restlessness (Gunasekaran *et al.*, 2007), frequent urination and raised tail vary in intensity from animal to animal, and in relation to standing estrus.Estrus can be detected on the basis of reactions to a vasectomized male/ teaser or an androgenized female buffalo. Jainudeen, (1988) reported that silent estrus is the common problem in the buffaloes. He also reported that estrus in the buffaloes commences towards late evening, with peak sexual activity occurring during the hours of darkness. The incidence of silent estrus may be higher in herds using AI rather than natural service and this may indicate that the problem may often lie with estrus detection rather than animal itself.

2.11 Methods for estrus detection in buffaloes

Reproduction management is a major concern for the dairy industry, because inefficient estrus detection causes significant economic losses (Karir *et al.*, 2006). There are some different visual and non-visual methods to detect oestrus in both buffaloes and cows. Examples of visual signs are as mentioned before standing to be mounted by herd mates and the secondary signs of estrus. To be able to see these signs of estrus it is required that the cows express the signs for a sufficient time period and the signs need to be carefully observed by the farmer in order to achieve an acceptable oestrus detection rate in the herd (Yoshida and Nakao, 2005).

The tail-paint-mark system where a strip of paint is applied on the rump of the cow can be used for estrus detection. The purpose with this system is to see if the cow has been mounted, because when the cow is mounted the paint becomes scuffed or cracked. The animals should be examined at least daily to determine whether they have been mounted or not. Although several special paints or pastes are commercially available, ordinary high gloss enamel, paste or water-based paint can be used successfully. Where ordinary household paint is used it should be applied every 3 or 4 days. The tail painting method was found to be 88% accurate in a cattle study in which estrus was also determined by regular progesterone assay (Kerr and McCaughey, 1984). Another method also with the purpose of using paint to see whether the cows are in heat or not, are the use of chin ball markers or harnesses worn by a bull or a vasectomised bull. The marker leaves a strip of paint on the back of the cow after mounting (Elmore *et al.,* 1986).

The non-visual methods are for example rectal palpation of the ovaries and uterus, changes in electrical resistance of the reproductive tract tissue, monitoring the follicular development by using ultra sound technique, vaginal pH measurements and measurements of the physical activity and hormonal changes during oestrus. The two latest methods are described in more detail further down in this text. This kind of methods relies on technical equipment or has to be done by a veterinarian. They are very often effective but might be difficult or impossible to use in small scale systems in developing countries.

2.12 Progesterone hormones during estrous cycle and estrus

Progestagens are a group of hormones with similar physiological activities, the most important of which is progesterone (P4), which plays a key role in the regulation of the estrous cycle. Several studies have been reported on peripheral P4 concentrations during estrous cycle in buffalo (Shah and Mehta, 1992; Kamboj and Prakash, 1993; Mondal and Prakash, 2001 and 2002b; Mondal *et al.,* 2003) showing that peripheral plasma P4 profile in buffalo is very similar to that in cattle.

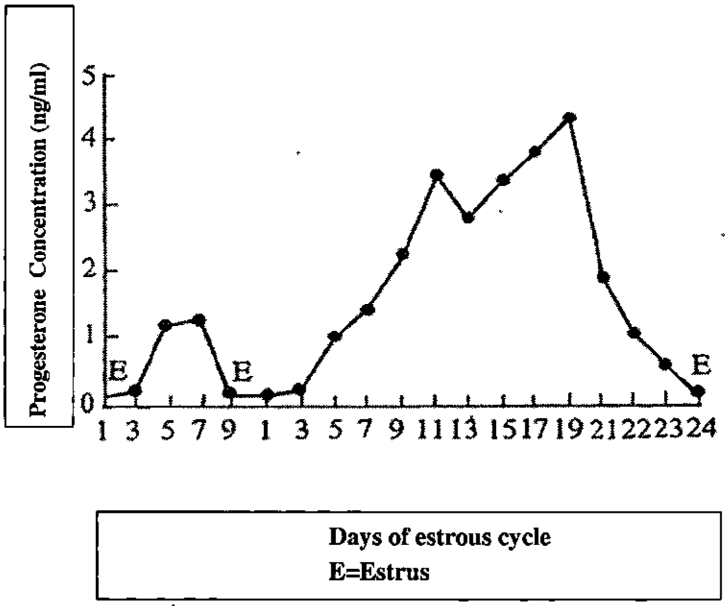


Figure 1: Serum Progesterone levels during 9 days short and subsequent normal oestrus cycle in a buffalo (Chohan *et al.* 1992)

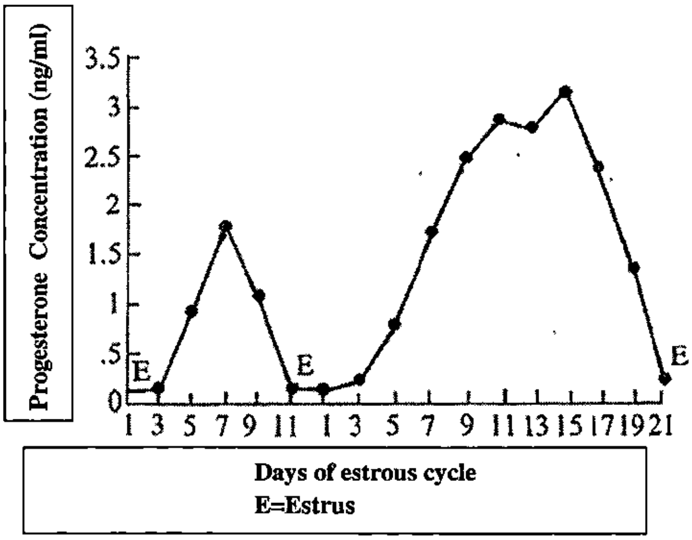


Figure 2: Serum Progesterone levels during 11 days short and subsequent normal oestrus cycle in a buffalo (Chohan *et al.* 1992)

P4 levels rise and fall in coincidence with the growth and regression of corpus luteum (CL) since CL is the source of P4 in cycling buffalo (Ahmed et al. 1977).Peripheral P4 concentrations are lower on the day of oestrus (0.1 ng/ml), rise to peak concentrations of 1.6-3.6 ng/ml on days 13 to 15 of the cycle (Seren *et al.,* 1994; Bachalaus *et al.,* 1979; Ahmed *et al.,* 1977) or even on day 17 (Pahwa and Pandey, 1983) before declining to basal levels at the onset of next estrus; it go down 2 or 3 days before the peak of luteinizing hormone (LH), begin to rise 2 to 4 days after the LH peak while the highest values are characteristic of the luteal phase (diestrus) (5-12 ng/ml and 4-6 ng/ml in Mediterranean and Murrah buffaloes respectively).

P4 levels continue to increase in animals that conceive but drop 3 days before the next estrus in those that fail to conceive (Batra *et al.,* 1979). The onset of the decline in P4 concentrations is variable, depending upon the time of regression of CL.

Peripheral P4 concentrations may change during the seasons (Srivastava et al. 1999): lower P4 levels at estrus as well at midluteal phase in hotter (0.14±0.05 and 2.05±1.16 ng/ml, respectively) than in cooler months (0.49±0.06 and 3.11± 0.20 ng/ml, respectively) are believed to be responsible for the poor expression of estrus and low conception rate during summer season (Rao and Pandey,1982).Others have, however, observed P4 concentrations to be significantly higher during summer compared to those in winter season (Mondal et al. 2001 and 2004). A significant increase in peripheral plasma P4 concentrations during prolonged heat exposure could be from a stress-induced rise in P4 from adrenal cortex (Gwazdauskas *et al.,* 1972; Wagner *et al.,* 1972; Abilay *et al.,* 1975); suboptimal nutrition coupled with stress due to high environmental temperatures may be responsible for long anestrus in buffaloes.

The pattern of P4 concentrations in milk has been found to be similar to that in plasma, although the concentrations in milk are much higher than those in plasma (Kamboj and Prakash, 1993). The average concentration of P4 in milk, which was 0.5ng/ml at estrus increased to18 ng/ml on day 15 and declined thereafter to 4.4 ng/ml 3 days preceding the next estrus in non-pregnant buffaloes (Batra *et al.,* 1979). P4 concentrations in ovarian follicular fluid are much higher than those in peripheral circulation (Palta *et al.,* 1998).

Palta *et al.,* (1998) reported that P4 concentrations (pmol/ml) were not related to follicular diameter and were not different among small (330.99±27.32), medium (384.84±26.20) and large follicles (253.25±32.23). P4 concentrations of ovarian follicular fluid have also been reported to be lowest in monsoon (July to Oct) and highest in summer (March to June) (Mondal *et al.,* 2007).

Hattab *et al.,* (2000) showed that the levels of P4 metabolites in faeces are a valid means to monitor the functionality of the corpus luteum as they reflect the blood levels of P4 with a high correlation coefficient (R = 0.77). In addition, it was reported that the P4 hastens the transportation of oocyte in the oviduct; it has a dominant role in the maintenance of pregnancy, especially during the first phase, and acts in synergy with estrogens by stimulating the growth and development of alveolar tissue of mammary gland.

The blood level of P4 is considered a good indicator of the beginning of puberty and heifers are considered to achieve puberty when plasma P4 levels exceeded 1 ng/ml for two consecutive samples with a low value interval. Many environmental effects influence the age of puberty. In general, all the factors slowing the growth thus prevent the expression of full genetic potential, delay puberty. A buffalo heifer with a good level of nutrition reaches puberty at 19 months (Terzano *et al.,* 1997).

2.13 The production and effects of progesterone

The corpus luteum (CL) develops on the ovarian site where the follicle ruptured and the egg was released around the time of estrus. The CL produces high levels of the hormone progesterone (P4) which is needed to maintain the pregnancy. The CL lacks the enzymes to metabolize progesterone to other steroids and it will therefore diffuse to the plasma and subsequently into the milk (Sjaastad *et al.,* 2003; Karir *et al.,* 2006).

Increased concentration of LH in plasma induces ovulation and the synthesis of progesterone is stimulated by this ovulatory LH peak. When the luteolysis is induced the secretion and concentration of progesterone in plasma falls rapidly. Therefore the progesterone levels can be used for diagnosis of pregnancy. An animal that is not pregnant will have very low levels of progesterone in the plasma and vice versa. Since the range of progesterone concentration in the luteal phase overlaps with the pregnancy concentration repeated measurements are required to determine if the animal is pregnant or not (Sjaastad *et al.,* 2003).

The function of progesterone is to create favorable conditions for the fetus and neonate. Increased levels of progesterone influence the epithelium in the uterus. The glands in the endometrium grow and secrete nutrients, the effects of that are to prepare the uterus to have the right conditions for the development of the embryo. Progesterone prevents uterus contractions that can lead to abortion or too early parturition. Progesterone also contributes to growth and differentiation of the mammary tissue and prepare for milk synthesis (Sjaastad *et al.,* 2003).

2.14 Post-partum hormone levels, monitoring of ovarian activity by progesterone

PGF2α and PGFM decline within a day post-partum and thereafter, no fixed pattern of its secretion is discernible throughout the post-partum period. Also, there is a parallel decrease in the progesterone concentration till completion of regression of CL of pregnancy. This probably suggests that PGFM after parturition may be involved in the regression of CL of pregnancy. The longer the time taken for the CL to regress completely may be due to the significant decline of PGFM after parturition which probably results in delay of post-partum heat in buffaloes. It is, therefore, apparent that buffaloes should be checked for the presence of CL of pregnancy, and if found with CL, should be treated with GnRH for the 'reawakening' of ovarian activity during post-partum

In buffaloes, widespread use of AI service is limited by the difficulties of heat detection. Progesterone levels in blood or milk offer a convenient method for monitoring ovarian activity and oestrus confirmation (*Kamonpatana et. al.,* 1979). A comparison between the progesterone profiles and visible observations of estrus reveals that 50% of the buffaloes show at one silent estrus during the post-partum period. This underlines the subjective character of heat detection and confirms anew that clinical anestrus does not justify a diagnosis for acyclia. The widely exercised practice to inseminate buffalo only at the second heat is backed by the observation that the following cycle is normal. In the majority of the buffaloes investigated, the first cycle was shortened by about 5-6 days and simultaneously progesterone value" remained low. The shortened cycle is possibly caused by inadequate formation of the CL and may be related to an inadequate elevation of the preovulatory LH. However, further research is necessary to establish the true cause of the short cycle (Arora and Pandey, 1982b).

Table 1: Mean values of progesterone in post-partum buffaloes (Yotov, 2013)

Postpartum days	Progesterone Concentration (ng/ml)
	Mean ±SD
1	0.49±0.21
4	0.09±0,00
7	0.10±0.01
10	0.16±0.00
13	0.11±0.08
16	0.15±0.01
19	0.06±0.01
22	0.25±0.16
25	0.17±0.24
28	0.10±0.04
31	0.06±0.08
34	0.15±0.01
37	0.12±0.07
40	0.19±0.16
43	0.13±0.03
46	0.23±0.20
50	0.15±0.07

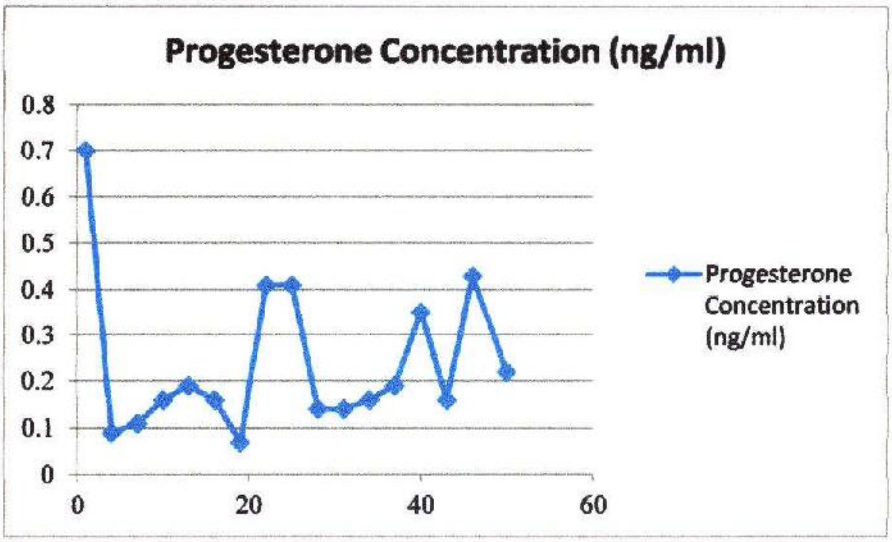


Figure 3: Postpartum progesterone value in buffaloes (Yotov, 2013)

Immediately after the parturition, mean blood progesterone concentrations drop sharply. Blood progesterone level remains < 0.25 ng/ml until the 50th postpartum day and inadequate functional activity of the corpus luteum could remain non-pregnant for a long time after parturition (Yotov, 2013). Plasma progesterone levels confirms the clinical diagnosis of the presence or absence of a palpable CL except that progesterone levels were low (<0.2ng/ml) (Jainudeen *et al.*, 1981).

2.15 Estrous Detection through Progesterone assay

Barnabe *et al.*, (1997) investigated with 15 Murrah buffalo heifers (21-23 months initial age) to find serum progesterone levels during the first estrous cycle in relation to reproductive performance, age and live weight. Weekly blood sampling was done for 21 weeks. 66.6% of the heifers were observed to start cycling when mean progesterone levels reached 3.34 ng/ml (week 12). Fluctuations in mean serum progesterone levels ranged from 0.01-5.2 ng/ml.

A perusal of the studies reported on peripheral progesterone concentrations during estrous cycle in buffalo (Kamonpatana *et al.*, 1976; Ahmed *et al.*, 1977; Bachalaus *et al.*, 1979; Batra *et al.*, 1979; Rao and Pandey,1982; Jainuddin *et al.*, 1983; Pahwa and Pandey, 1983; Kanai and Shimizu, 1984; Shah and Mehta, 1992; Kamboj and Prakash, 1993; Mondal *et al.*, 2001; Mondal and Prakash, 2002a; Mondal and Prakash, 2002b, c; Mondal *et al.*, 2003) indicates that peripheral plasma progesterone profile in buffalo is very similar to that in cattle (Wetteman *et al.*, 1972). Progesterone levels rise and fall in coincidence with the growth and regression of corpus luteum (CL) since CL is the source of progesterone in cycling buffalo (Ahmed *et al.*, 1977). Peripheral progesterone concentrations are minimal on the day of estrus (0.1 ng/ml), rise to peak concentrations of 1.6-3.6

ng/ml on days 13 to 15 of the cycle (Ahmed *et al.,* 1977; Bachalaus *et al.,* 1979) or even on day 17 (Pahwa and Pandey, 1983) before declining to basal levels at the onset of next estrus. Progesterone levels continue to increase in animals that conceive but drop 3 days before the next estrus in those that fail to conceive (Batra et al.,1979). The onset of the decline in progesterone concentrations is variable, depending upon the time of regression of CL. When progesterone concentrations were compared in buffaloes which exhibit silent and those which showed overt estrus, (Mondal and Prakash, 2002b) found that progesterone concentrations increased from 0.42±0.02 and 0.38±0.02 ng/ml during periestrus phase (day 1 to day) 1, day 0 = day of estrus) to 0.66±0.12 and 0.51±0.07 ng/ml during early luteal phase (day 2 to day 5) and then further to 1.55±0.33 and 1.30±0.13 ng/ml during mid-luteal phase (day 6 to day 14) following which these declined to 1.12± 0.27 and 0.66±0.13 ng/ml during late luteal phase (day -4 to day -2) in buffaloes that exhibited overt estrus and silent estrus, respectively.

Chapter III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Selection of Buffalo farm

This study was conducted in a Goverment buffalo farm "Buffalo Breeding and Development Centre, Fakirhat, Bagerhat (Plate 3.1), during a period extending from September to October 2013.

Plate 3.1 Buffalo Breeding and Development Centre, Fakirhat, Bagerhat

3.2 Selection of buffalo cows

The Buffalo Breeding and Development Centre, Bagerhat, have a total of 260 buffaloes. Among them, 20 postpartum (45 days postpartum) buffalo cows were selected to collect serum sample to detect postpartum estrus by measuring of progesterone levels (Plate 3.2)

3.3 Collection of Blood samples

Blood samples were collected from the selected 20 buffalo cows at 15 days apart. 10 ml of blood samples were collected from the jugular vein of the buffalo cows with the help of 10ml needle and syringe (Plate 3.3). A total of 4 times blood samples (total 80 samples) were collected.

Plate 3.2 Buffaloes at the Buffalo Breeding and Development Centre shed

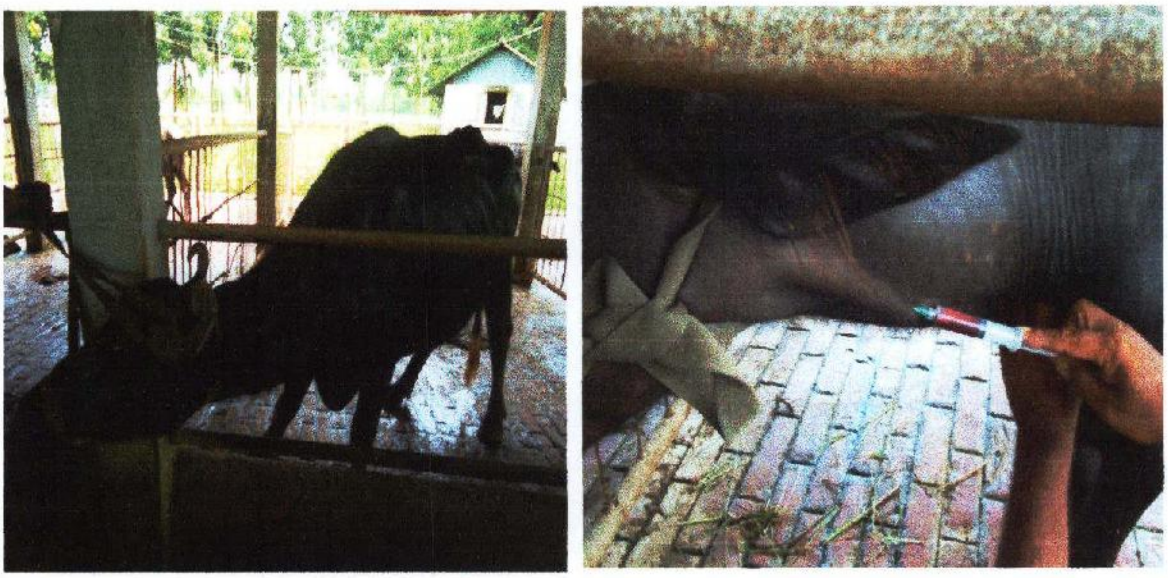


Plate 3.3 Controlling and Collection of blood samples from buffalo

3.4 Collection of serum

The blood samples were kept in the marked test tubes for each buffaloes and put down in a rack vertically to settle down of the cellular components of blood at the bottom of the test tubes and fluid portion at the upper part of the test tubes called serum. These serum samples were separated with the help of a dropper and kept in another marked test tubes for the respected buffaloes (Plate 3.4)

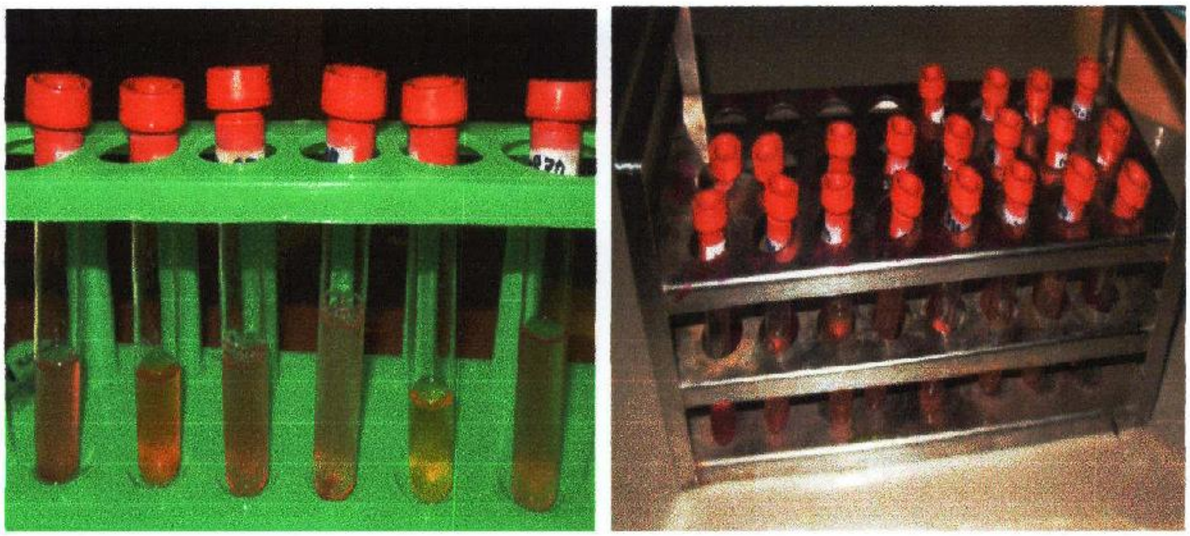


Plate 3.4 Collected serum samples from the blood of buffaloes

3.5 Preservation of serum

Each time twenty serum samples were prepared and kept in marked test tubes and finally preserved in the refrigerator at normal (4°c) until a total of 80 serum samples were obtained (Plate 3.5)

Plate 3.5 Preservation of serum samples in the refrigerator

3.6 Sending of serum samples to the Laboratory (BIRDEM, Dhaka) for analysis of progesterone levels

A total of 80 samples were sent to the laboratory to determine the progesterone level. The serum samples were kept in a cool box until it reaches to the laboratory so that the sample qualities remain normal (Plate 3.6 and 3.7)

Plate 3.6 Serum samples kept in a box with ice

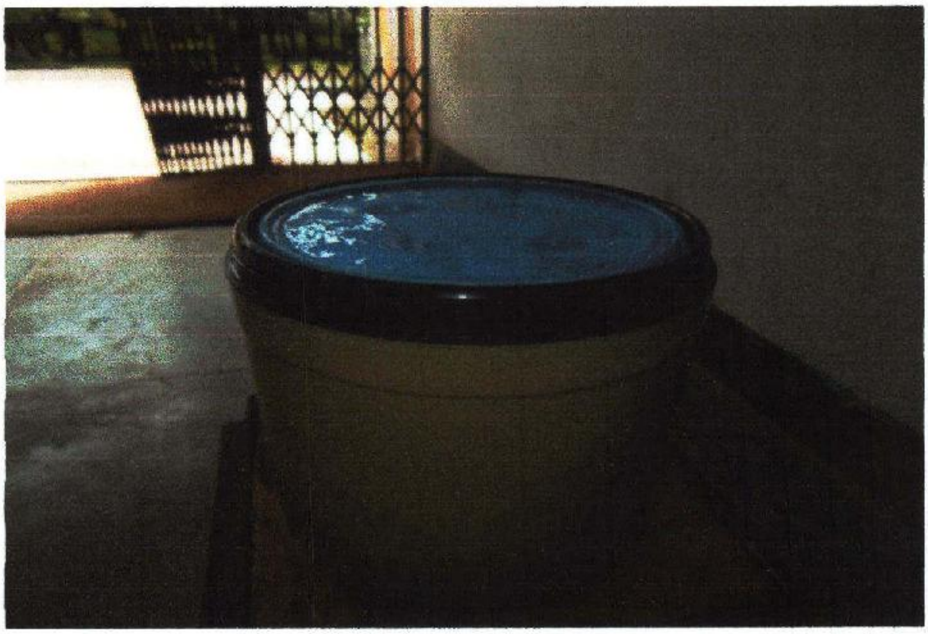


Plate 3.7 Sending of the samples towards laboratory (BIRDEM, Dhaka)

3.7 Analytic procedure of serum samples to find out the progesterone level

The name of the instrument to analyze the serum sample in order to get progesterone value is ARCHITECT Progesterone. The ARCHITECT Progesterone assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of progesterone in serum and plasma.

Biological principles of the assay procedure

The ARCHITECT Progesterone assay is a one-step immunoassay to determine the presence of progesterone in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Sample, anti-fluorescein (mouse, monoclonal) fluorescein-progesterone complex coated paramagnetic microparticles, and anti-progesterone (sheep, monoclonal) acridinium labeled conjugate are combined to create the reaction mixture. Progesterone present in the sample competes with the anti-fluorescein (mouse, monoclonal) fluorescein-progesterone complex coated microparticles for binding with anti-progesterone (sheep, monoclonal) acridinium-labeled conjugate to form antibody-antigen-antibody complexes. After washing, Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of progesterone in the sample and the RLUs detected by the ARCHITECT i optical system.

PROCEDURE

Materials Provided 7K77 ARCHITECT Progesterone Reagent Kit

Assay Procedure

- Loading of the ARCHITECT Progesterone Reagent Kit on the ARCHITECT i System. Verifying that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample cup volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
    - Priority: 100 µL for the first Progesterone test plus 50 µL for each additional Progesterone test from the same sample cup.
    - ≤ 3 hours onboard: 150 µL for the first Progesterone test plus 50 µL for each additional Progesterone test from the same sample cup.
    - > 3 hours onboard: additional sample volume is required.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- ARCHITECT Progesterone Calibrators and Controls must be mixed thoroughly by low speed vortex or inversion prior to use.

- To obtain the recommended volume requirements for the ARCHITECT Progesterone Calibrators and Controls, dispense a minimum of 200 µL of each calibrator or a minimum of 150 µL of each control into each respective sample cup.
- Loading of samples.
- Pressing RUN. The ARCHITECT i System performs the following function:
  - Moving the sample to the aspiration point.
  - Loading a reaction vessel (RV) into the process path.
  - Aspirating and transfers sample into the RV.
  - Advances the Reaction Vessels (RV) one position and transfers microparticles and conjugate into the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adding Pre-Trigger and Trigger Solutions.
  - Measuring of chemiluminescent emission to determine the quantity of progesterone in the sample.
  - Aspirating contents of RV to liquid waste and unloads RV to solid waste.
  - Calculating of the result.

3.8 Statistical analysis

The gathered data were analyzed by a statistical software SPSS 10 version (One sample T test). Differences were considered significant at $P < 0.05$. Values were presented as means, standard deviation and standard error mean.

Chapter IV

RESULTS

RESULTS

The achievement of early cyclicity and pregnancy after calving is of vital importance for buffalo farms. For sustainable breeding program it is necessary to identify estrus and proper time of AI. Apart of monitoring physical signs various methods have been used for estrus detection. Among which serum progesterone assay was used in buffaloes. Serum progesterone concentrations during at 45, 60, 75 and 90 days post-partum are presented in Table 2-5.

Table 2: Serum progesterone concentration (ng/ml) in buffalo cows at 45 post-partum days and estrus (in percentage) expected from the progesterone level

Buffaloes ID No.	Buffaloes Tag No.	Days postpartum	Serum progesterone (ng/ml)	Buffalo cows in estrus		Estrus Percentage (%)
				Yes	No	
S_1	001	45	0.34	-	N	20
S_2	014		0.57	-	N	
S_3	091		0.62	-	N	
S_4	622		0.51	-	N	
S_5	690		0.25	-	N	
S_6	704		0.05	Y	-	
S_7	754		0.61	-	N	
S_8	766		0.50	-	N	
S_9	773		0.38	-	N	
S_{10}	789		0.26	-	N	
S_{11}	791		0.37	-	N	
S_{12}	803		0.55	-	N	
S_{13}	832		0.35	-	N	
S_{14}	837		0.08	Y	-	
S_{15}	864		0.42	-	N	
S_{16}	866		0.1	Y	-	
S_{17}	870		0.28	-	N	
S_{18}	901		0.44	-	N	
S_{19}	904		0.53	-	N	
S_{20}	911		0.06	Y		

From the table 2, it was observed that S_6, S_{14}, S_{16}, S_{20} out of 20 buffaloes (ID No. 704; 837; 866 and 911) have a progesterone level of $\leq$0.1 ng/ml indicating they are in estrus (20%).

Table 3: Serum progesterone concentration (ng/ml) in buffalo cows at 60 post-partum days and estrus (in percentage) expected from the progesterone level

Buffaloes ID No.	Buffaloes Tag No.	Days postpartum	Serum progesterone (ng/ml)	Buffalo cows in estrus		Estrus Percentage (%)
				Yes	No	
S_1	001	60	0.06	Y	-	30
S_2	014		0.34	-	N	
S_3	091		0.03	Y	-	
S_4	622		0.26	-	N	
S_5	690		0.56	-	N	
S_6	704		3.1	-	N	
S_7	754		0.24	-	N	
S_8	766		0.10	Y	-	
S_9	773		0.58	-	N	
S_{10}	789		0.45	-	N	
S_{11}	791		0.02	Y	-	
S_{12}	803		0.20	-	N	
S_{13}	832		0.04	Y	-	
S_{14}	837		3.3	-	N	
S_{15}	864		0.36	-	N	
S_{16}	866		3.2	-	N	
S_{17}	870		0.49	-	N	
S_{18}	901		0.35	-	N	
S_{19}	904		0.08	Y	-	
S_{20}	911		3.1	-	N	

From the table 3, it was showed that S_1, S_3, S_8, S_{11}, S_{13}, S_{19} out of 20 buffaloes (ID No. 001; 091; 766; 791; 832 and 904) have a progesterone level of ≤ 0.1 ng/ml indicating they are in estrus (30%).

Table 4: Serum progesterone concentration (ng/ml) in buffalo cows at 75 post-partum days and estrus (in percentage) expected from the progesterone level

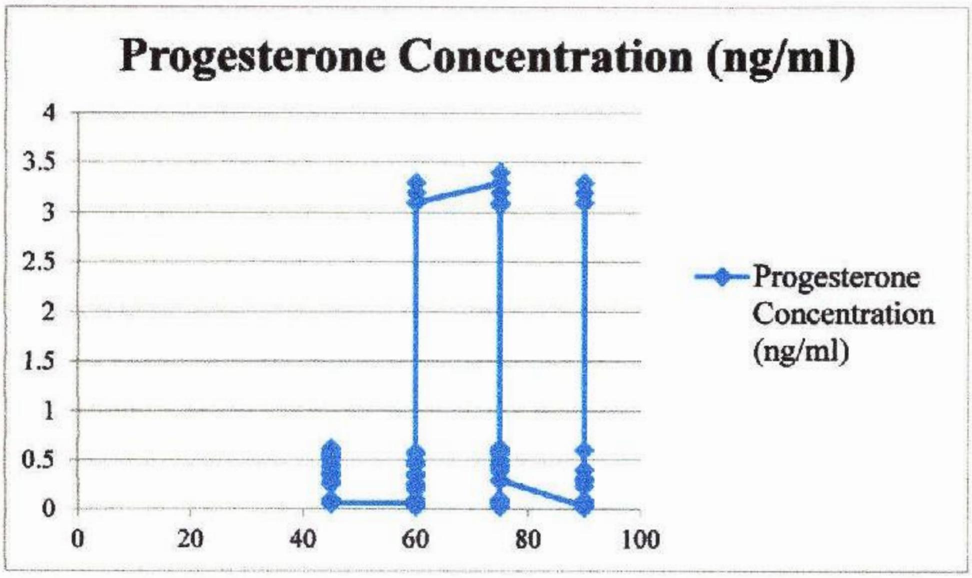
Buffaloes ID No.	Buffaloes Tag No.	Days postpartum	Serum progesterone (ng/ml)	Buffalo cows in estrus		Estrus Percentage (%)
				Yes	No	
S_1	001	75	3.3	-	N	20
S_2	014		0.02	Y	-	
S_3	091		3.08	-	N	
S_4	622		0.60	-	N	
S_5	690		0.08	Y	-	
S_6	704		0.50	-	N	
S_7	754		0.42	-	N	
S_8	766		3.1	-	N	
S_9	773		0.29	-	N	
S_{10}	789		0.10	Y	-	
S_{11}	791		3.4	-	N	
S_{12}	803		0.59	-	N	
S_{13}	832		3.2	-	N	
S_{14}	837		0.45	-	N	
S_{15}	864		0.62	-	N	
S_{16}	866		0.39	-	N	
S_{17}	870		0.06	Y	-	
S_{18}	901		0.56	-	N	
S_{19}	904		3.3		N	
S_{20}	911		0.30	-	N	

From the table 4, it was illustrated that S_2, S_5, S_{10}, S_{17} out of 20 buffaloes (ID No. 014; 690; 789 and 870) have a progesterone level of ≤ 0.1 ng/ml indicating they are in estrus (20%).

Table 5: Serum progesterone concentration (ng/ml) in buffalo cows at 90 post-partum days and estrus (in percentage) expected from the progesterone level

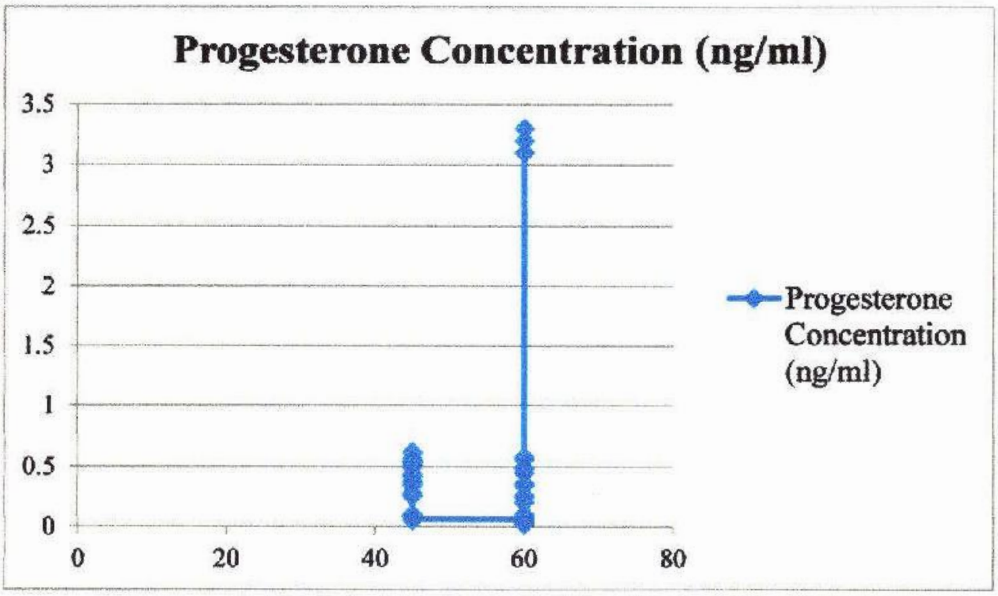
Buffaloes ID No.	Buffaloes Tag No.	Days postpartum	Serum progesterone (ng/ml)	Buffalo cows in estrus		Estrus Percentage (%)
				Yes	No	
S_1	001	90	0.03	Y	-	60
S_2	014		3.2	-	N	
S_3	091		0.60	-	N	
S_4	622		0.09	Y	-	
S_5	690		0.04	Y	-	
S_6	704		0.05	Y	-	
S_7	754		0.03	Y	-	
S_8	766		0.23	-	N	
S_9	773		0.07	Y	-	
S_{10}	789		3.1	-	N	
S_{11}	791		0.31	-	N	
S_{12}	803		0.08	Y	-	
S_{13}	832		0.40	-	N	
S_{14}	837		0.04	Y	-	
S_{15}	864		0.29	-	N	
S_{16}	866		0.03	Y	-	
S_{17}	870		3.3	-	N	
S_{18}	901		0.01	Y	-	
S_{19}	904		0.06	Y	-	
S_{20}	911		0.07	Y	-	

From the table 5, it was explained that S_1, S_4, S_6, S_7, S_9, S_{14}, S_{16}, S_{18}, S_{19}, S_{20} 12 out of 20 buffaloes (ID No. 001; 622; 690; 704; 754; 773; 803; 837; 866; 901; 904 and 911) have a progesterone level of $\leq$0.1 ng/ml indicating they are in estrus (60%).



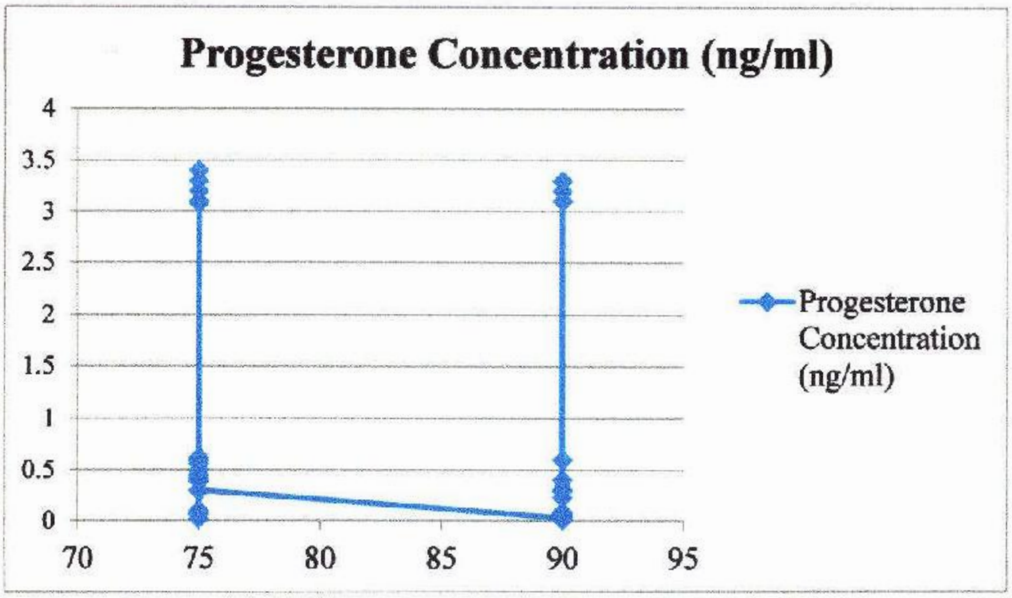
(X axis= Postpartum days and Y axis= Progesterone concentration)

Figure 4: Graphical representation of progesterone levels (ng/ml) of buffaloes during study period



(X axis= Postpartum days and Y axis= Progesterone concentration)

Figure 5: Graphical representation of progesterone levels at 45 and 60 post-partum days



(X axis= Postpartum days and Y axis= Progesterone concentration)

Figure 6: Graphical representation of progesterone levels at 75 and 90 post-partum days

From the above tables (table 2-5) and graphs (figure 4-6) it has found that Progesterone concentration varies between ≤ 0.1 ng/ml and 3.4 ng/ml recorded from 45 to 90 days post-partum. Twenty percent of buffalo cows showed the first postpartum estrus at 45 days. Similarly, 30%, 20% and 60% of buffalo cows showed estrus at 60, 75 and 90 post-partum days respectively. During estrus in buffalo cows the progesterone level showed ≤0.1 ng/ml. Similar values of progesterone has been reported in cattle (Chenault *et al.*, 1975) and in buffaloes (Kamonpatana *et al.*, 1979; Bachlaus *et al.*, 1980; Aboul-Ela 1982). The buffaloes which shown estruses have progesterone level of 3.1 to 3.4 ng/ml on next day 15 of the cycles indicating that they are in normal estrous cycle (Ahmed *et al.*, 1977; Bachalaus *et al.*, 1979).

The source of the transient increase in progesterone is possibly due to the presence of short-lived corpus luteum or luteinized ovarian follicle. Similar postulates were reported by Donaldson et.al. (1970), Corach *et.al.*, (1974) and Werth *et.al.*, (1996). Yelich *et.al.*, (1997) suggested presence of a persistent dominant luteinized follicle during early postpartum period after calving. The transient increase in progesterone that occurred before 1st estrus may possibly have a role in preparation of the reproductive tract of buffalo cows for the next pregnancy (Werth *et.al.*, 1996). Kinder *et.al.*, (1995) found similar increase in progesterone preceding pubertal estrus of heifers and hypothesized that this increase serve for providing a more desirable uterine environment that made conception positive as a result of mating at the pubertal estrus.

The data of progesterone concentration found by testing of serum samples at days 45, 60, 75 and 90 post-partum were analyzed by Statistical software SPSS 10 version (Table 6)

Table 6: Mean, Standard deviation, standard error mean and T-test of progesterone levels of postpartum buffalo cows

T test					
Post-partum period(days)	N	Mean	Std. Deviation	Std. Error Mean	P- value
45	20	0.364	0.185	0.041	0.000*
60	20	0.843	1.209	0.270	0.009*
75	20	1.218	1.364	0.305	0.001*
90	20	0.602	1.131	0.252	0.042*

*Indicates that the value is significant ($P<0.05$).

T test (Table 6) showed significant differences ($P<0.05$) in progesterone levels of serum samples of buffalo cows at 45, 60, 75 and 90 post-partum days where 20%, 30% , 20% and 60% of buffalo cows were assumed to come in estrus respectively (Table 2-5).

Chapter V
DISCUSSION

DISCUSSION

Postpartum anestrus is one of the major problems for successful breeding program in dairy farms. This condition exaggerates in buffalo cows due to covert sign of estrus. Farm personnel can only detect heat in about 20-35% of animals. Therefore, it should take into consideration with care to the factors which create certain difficulties negatively influencing estrus detection. It is reported that high percentage of buffalo cows show silent estrus (Barile, 2005) and the longer period between calving and postpartum cyclicity. Silent estrus is a common problem in buffaloes even under good management and non-stressful periods of the year (Abdallah, 2003). Furthermore, the different duration of estrous (4 to 64 h) hampers the exact detection of ovulation resulting limited application of AI in buffaloes (Neglia *et al.*, 2003). In fact, one of the most important elements for the development of the reproductive efficacy is the proper detection of estrus. Hormonal variations during estrus cycle produce variations in animal behavior. This behavioral pattern is also a consequence of many other factors. Hence, heat detection technique based on animal behavior can produce both false positives and false negatives (Miranda *et al.*, 2009). Most detection methods aren't reliable and not suitable in herd practice. For this reason, the experiments were performed to study progesterone levels as heat detector to monitor estrus and estrus cycle in buffalo cows. During both estrus and met-estrus periods (days 0 and +1, respectively), the levels remained ≤0.1 ng/ml in all animals. This is in accord with other reports in cattle (Chenault *et al.*, 1975) and in buffaloes (Kamonpatana *et al.*, 1979; Bachlaus *et al.*, 1980; Aboul-Ela 1982).

Promdireg et al., 2008 reported that progesterone values followed a similar trend for both 1 and 2 wave cycles during the hot and cool season and detailed progesterone values recorded by him are given in the following table (Table 6)

Table 7: Progesterone values (ng/ml) during estrous cycle with 1 wave and 2 wave follicle development in buffaloes during hot and cool seasons (mean ± SD).

Parameters	1 wave		2 wave		Mean
	Hot	Cool	Hot	Cool	
Estrus	0.11 ± 0.08	0.13 ± 0.05	0.04 ± 0.07	0.09 ± 0.04	0.07 ± 0.03
Mid luteal phase	2.25 ± 0.7	2.06 ± 0.3	2.31 ± 0.4	2.54 ± 0.5	2.37 ± 0.5
Late luteal phase	1.03 ± 0.4	1.14 ± 0.6	0.84 ± 0.5	1.10 ± 0.3	0.98 ± 0.2

Following parturition, all the females undergo through anestrus for a variable but short period of time, known as postpartum anestrus. The period of postpartum anoestrus is usually longer in buffalo than the cattle under similar management conditions (Dobson and Kamonpatana, 1986; Jainudeen and Hafez, 1993), probably due to low LH secretion during early postpartum period (Perera, 2011). Under normal conditions, buffaloes resume cyclicity by 30–90 days (Perera, 2011).

The first post-partum heat varies greatly with suckling, nutrition, body condition score at calving, milk yield, parity, season of calving, breed and individual (El-Wishy, 2007). It has been reported to appear within less than 60 days in some cases and over 230 in others. Average post-partum oestrus in the Murrah breed of India has been reported to be 100 days. The first post-partum oestrus is not always fertile, especially if it comes very near partus (Thomas, 2008). Successful breeding must take place within 85 -115 days after parturition to maintain a calving interval of 13 -14 months in buffaloes (El-Wishy, 2006). Calving interval in buffalo is highly dependent on management, climate and nutrition. It is therefore shorter in some regions and longer in others. In order to shorten the calving interval the female should be serviced again as soon as possible after calving, after providing a sufficient period of rest.

Body condition score (BCS) reflects the overall energy status of the body, depending upon the intake of nutrients and their utilization for milk yield, growth and maintenance. Osoro and Wright (1992) and De Rouen et al. (1994), concluded that BCS at calving significantly affected postpartum reproductive performance in cows. O'Rourke et al. (1991) reported that cows with BCS of ≥8 had a conception rate 33% higher than those with score ≤5 (scale 3-9). Buffaloes in poor BCS had inactive ovaries and long postpartum anoestrus periods (Jainudeen and Wahab, 1987). Bhalaru et al. (1987) reported that conception rates were significantly higher (88.3%) for buffaloes with moderate BCS (2.5 to 3.5) than for females scoring 1 to 2 (65.8%) or 4 to 5 (70.8%). The reason for low reproductive performance in the animals with low BCS was perhaps the non-availability of nutrients for reproduction, being the third candidate in partitioning of nutrients, after health and milk production. In the fat animals the low reproductive performance was perhaps due to abnormal physiological functions during the estrous cycle.

Nutrition plays a major role in the reproductive performance of buffalo, as with other farm animals. However there is a strong possibility that the consequences of poor nutrition are often interpreted as seasonality of breeding in buffalo. Under feeding, over feeding or unbalanced feeding, as well as deficiencies in minerals, vitamins or trace elements will cause reduced fertility in buffalo just as in other farm animals. A poor body condition score at calving affects fertility, characterized by prolonged post-partum intervals, reduced conception rates, and more services per conception (Thomas, 2008). A very low protein diet can cause cessation of oestrus (Agrawal, 2003).

One of the reasons buffalo suffer from long post-partum anoestrus is because their natural behavior of rolling in dirty water pools, and unhygienic shed conditions, cause buffalo to suffer from a high incidence of endometritis. The loose broad uterine ligaments and rolling in water cause torsion of uterus in buffalo. Buffalo also suffer from uterine prolapse and retention of the placenta afterbirth. All these lead to uterine infections, delayed involution of the uterus and endometritis in buffalo resulting in the need for repeat breeding (Thomas, 2008).

The incidence of anestrus in buffaloes varies between 20-80%, depending on season. Most female buffaloes which exposed to extreme hot conditions during the summer season cease ovarian activity (Nanda et al., 2003; Sastry, 1983). Higher incidence of anestrous due to inactive ovaries in buffaloes than in cows has been reported by Tanwar *et al.*, (2003). Sometimes the anestrous is prolonged due to sudden climatic variation such as a fall in temperature, exposure to cold wind, heavy rain associated with low temperature or hot weather without any possibility of bathing or sheltering from the sun (Zicarelli, 1997).

Effect of season of calving on service period is also reported by Dutt and Yadav (1988). There is no seasonal effect on service period in females whose calves are weaned normally, but in females whose calves are weaned at birth, service period is shorter in winter calving than calving in summer and in rainy seasons (Rao et al., 1990). Service period is significantly lower in the weaned calves' buffaloes then in suckled group (Noorclin and Jainuddeen, 1991).

The service period shows a negative trend as parity increased. The lowest mean service period was seen in parity number eight. There was an overall trend of reduction in the length of service period with the increase in parity number in all the dairy farms ((Sethi and Nagarcenkar, 1992). It has been observed in buffaloes that post-partum uterine involution occur sooners with increasing parity which probably leads to better fertility (Naqvi, 2000)

It is important to point that buffaloes are milked twice per day. Lactation may increase circulating progesterone concentrations (Wiltbank *et al.*, 2006) that, in turn, may decrease GnRH-induced LH secretion (Dias, 2008) resulting decreased ovulation efficiency. Ovulation and estrus activity after calving are delayed when the positive feedback effects of estradiol on release of LH from the pituitary are reduced due to various factors. Perera (2011) stated that the first postpartum ovulation is frequently followed by one or more short estrous cycles (<18 days) and cessation of estrous cyclicity occurred after the first or second ovulation in about 25% of animals due to ovulatory failure or prolonged luteal activity. Data from Egypt, India and Pakistan indicated that only 34–49% of buffaloes showed estrus during the first 90 days after calving and 31–42% remained anestrous for more than 150 days (reviewed by El Wishy, 2007).

Moreover, it is likely that suckling significantly increases the interval from parturition to first postpartum estrous in buffaloes. The extension of anoestrous period in buffaloes due to calf suckling is also reported elsewhere (Usmani *et al.,* 1990). Increasing suckling duration and use of oxytocin extended the postpartum ovulation interval (POI) (Qureshi and Ahmad, 2008). Silent estrous is a common problem in buffaloes even under good management and non-stressful periods of the year (Kamboj and Prakash, 1993; Abdalla, 2003).

The buffalo ovary is elongated and considerably smaller than that of cattle. When palpated per rectum, mature follicles protrude from the surface of the ovary and can be mistaken as an early developing corpus luteum (Jainudeen *et al.*, 1983). The corpus luteum of the buffalo is often deeply embedded in ovarian stroma and is generally smaller than that of cattle. The corpus luteum does not protrude markedly from the surface of the ovary and sometimes lacks a clear crown. These characteristics make accurate identification of ovarian structures by rectal palpation in buffalo more difficult than in cattle (El- Wishy *et al.*,1971). Externally detectable physical changes around the time of estrus include swelling of the vulva and reddening of the vestibular mucosa (Danell, 1987; Kanai *et al.*, 1990). Mucus secreted from the cervix during estrus is less copious than in cattle, sometime hang as strands from the vulva. The concentration of estradiol-17β in blood during the follicular phase of the estrous cycle also appears to be relatively less than that in cattle (Kanai *et al.*, 1990; Roy and Prakash, 2009) and this has been suggested as a possible reason for the lesser intensity of estrus exhibited by buffaloes. Considering these perspective, the determination of progesterone could serve as an indicator of the follicular and luteal status in cyclic buffaloes. Progesterone level can be obtained simply by collecting serum and analyzing by means of a hormone analyzer. Regular progesterone assay can be done to detect estrus in buffaloes (Kerr and McCaughey, 1984).

From the experiment, it was obvious that buffalo cows may return to estrous cycle and estrus from 45 to 90 post-partum days. Significant differences of progesterone levels were observed among post-partum serum samples of buffaloes.

In fact, only few trials have been performed to evaluate the progesterone concentration as an estrus detector in buffalo cows. To make the findings more accurate and effective further study is essential in buffalo cows with short intervals.

Chapter VI

CONCLUSIONS

CONCLUSIONS

The correct management of estrus detection requires continuous observation of the herd and trained, responsible and skillful labor. There are different factors which affect the post-partum period of estrus cycle and estrus in buffalo cows. Moreover, due to the absence of the prominent signs of estrus and silent estrus in buffalo cows the reproductive efficiency is not compatible with profits. In herds in which estrus detection is inefficient, there is a decrease in the reproductive performance and a consequent increase in the breeding period and in the calving interval, what causes serious economic losses for the breeder. Therefore, progesterone assay could help the dairy farmers to detect the estrus and subsequent successful breeding. This study fully agrees with the previous findings about progesterone level of ≤ 0.1 ng/ml in serum for the heat detection in buffalo. Progesterone EIA kit (progesterone ELISA kit) has been developed but it is still used mainly in human of developed countries and research which can detect progesterone levels of 0.05 to 50 ng/ml but it is not cost-effective to use in heat detection of animals.

To make the findings of heat detection by measuring progesterone levels more accurate and effective further study is essential in buffalo cows with short intervals considering different types of breeds of buffaloes with short (15-17 days), medium (18-24 days) and long (25-28 days) estrus cycles, age, parity, body condition, nutrition and season.

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