

**INDUCTION OF ESTRUS FOLLOWED BY FIXED TIME  
AI AS A TOOL TO REDUCE NON-PRODUCTIVE  
PERIOD OF ANESTRUS BUFFALO**

**Thesis**

**Submitted to the Guru Angad Dev Veterinary and Animal Sciences University  
in partial fulfillment of the requirements for the degree of**

**MASTER OF VETERINARY SCIENCE  
in  
VETERINARY GYNAECOLOGY AND OBSTETRICS  
(Minor Subject: Veterinary and Animal Husbandry Extension Education)**

**By**

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## **CERTIFICATE – I**

This is to certify that the thesis entitled “**Induction of estrus followed by fixed-time AI as a tool to reduce non-productive period of anestrus buffalo**” submitted for the degree of **M.V.Sc.** in the subject of **Veterinary Gynaecology and Obstetrics** (Minor Subject: **Veterinary and Animal Husbandry Extension Education**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Mahantswamy K. Mallikerimath (L-2016-V-39-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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### **ABSTRACT**

Sixty anestrus Murrah buffaloes were evaluated for the success rate of Progesterone ( $P_4$ ) + Estrogen ( $E_2$ ) + equine chorionic gonadotropin (eCG) protocol (Breeding season, BS=15; Non Breeding season, NBS=15) and estradoublesynch protocol (BS=15; NBS=15). The buffaloes subjected to  $P_4+E_2+eCG$  or estradoublesynch during BS had higher ( $p>0.05$ ) estrus induction response (86.0 and 80.0%, respectively) compared to their counterparts during NBS (73.3 and 66.6%, respectively). Estrus period score at induced estrus in buffalo subjected to either of protocols in BS or NBS was not different ( $p>0.05$ ). Pregnancy rate (induced + spontaneous estrus) was better ( $p>0.05$ ) using  $P_4+E_2+eCG$  compared to estradoublesynch (73.3 vs 63.3%). Pregnancy rate was lower ( $p<0.05$ ) in buffalo exhibiting  $<50$  estrus period score in comparison to those exhibiting 50-100 or  $>100$  score. Interval between start of a protocol and conception in  $P_4+E_2+eCG$  was  $13.9\pm 1.3$  days compared to  $17.5\pm 3.1$  days for estradoublesynch ( $p>0.05$ ). Thus, first objective concluded that  $P_4+E_2+eCG$  protocol has an edge over estradoublesynch protocol for anestrus buffaloes during breeding and non-breeding season. Under second objective, 500 dairy households in rural areas of Punjab were interviewed. About 81.8% dairy households were unaware about any estrus induction or synchronization hormonal protocol and majority (57.9%) belonged to households with  $<5$  animals. Out of households having awareness about hormonal protocol use in dairy animal, the majority (74.7%) belonged to households with  $>15$  animal. Out of dairy farmers using hormonal protocols, the proportion of illiterate was 14.8%. Out of dairy farmers not aware about hormonal protocols, the proportion of illiterate was 52.3%. Data analysis of 91 dairy households who had used hormonal protocols revealed summer (65.9%) and anestrus (82%) as the major underlying reasons for their use in dairy animals. Less success rate (20.2%), high cost (50%) and non-availability of doctor (29.7%) were the reasons for the irregular use of hormonal protocols. The success rate of use of hormonal protocols under field condition in terms of conception was 47.1%. Thus, second objective concluded that major bottlenecks in the acceptability of hormone-based therapeutics strategies are lack of awareness in dairy households especially households with  $<5$  animal, illiteracy and high cost.

**Keywords:** Buffalo, Dairy household, Estrus, Hormones, Season

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**Signature of Major Advisor**

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**Signature of the Student**

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## LIST OF ABBREVIATIONS

%	:	Percentage
<	:	Less than
>	:	Greater than
±	:	Plus minus
≤	:	Less than and equal to
°C	:	Degree Celsius
A.I.	:	Artificial insemination
BCS	:	body condition score
CIDR	:	controlled internal drug release
CL	:	Corpus luteum
CR	:	Conception rate
E <sub>2</sub>	:	Estradiol
eCG	:	Equine Chorionic Gonadotropin
et al	:	et alia (Latin-and others)
FAO	:	Food and Agriculture Organisation
Fig.	:	Figure
FSH	:	Follicle stimulating hormone
FTAI	:	Fixed time artificial insemination
GnRH	:	Gonadotropin releasing hormone
H	:	hour
IGF	:	insulin like growth factor
IM	:	Intra muscular
Inj.	:	Injection
IU	:	International unit
Kg	:	kilogram
LH	:	Luteinizing hormone
Mg	:	Milligram
ml	:	Millilitre
Mm	:	Milimeter
Ng	:	Nanogram

P4	:	Progesterone
PGF2 $\alpha$	:	prostaglandin F2 $\alpha$
PMSG	:	pregnant mare serum gonadotropin
PRID	:	progesterone releasing intravaginal devices
RFM	:	Retention of foetal membranes
Viz.	:	Namely (latin videlicet)
Vs	:	Versus
Mg	:	micro gram

## CHAPTER – I

### INTRODUCTION

Buffalo is a major component of livestock in Asian continent that hosts over 95% of world's buffalo. Rural people in India are mainly engaged in rearing of buffalo, e.g. in Punjab state, out of 80 lakh breedable female bovine population about 94.5% is reared in the rural areas. This population comprises of 74.9% buffalo, 18.9% crossbred cattle and 6.2% indigenous cattle. Despite the position of buffalo farming, growth rate in female buffalo is only 1.4% compared to 5.8% in crossbred cattle (Livestock Census-BAHS 2007).

Most importantly, marginal and small dairy farmers raising 1-3 bovines account to 3/4<sup>th</sup> of livestock wealth and 68% of milk production in India. Moreover, only 19.2% buffalo ownership in India is with large farmers (Vision 2050-CIRB 2015). In addition, if a buffalo fails to conceive at an appropriate time, a farmer is expected to bear a loss of ₹300/day in terms of loss of milk and other managerial expenses. On a conservative estimate, Indian buffalo farmers are losing 20 million tonnes of milk annually on account of seasonal suppression of reproduction. Thus, to have a major impact on total milk production in India, the strategies need to focus on improving the reproductive potential of buffalo reared by marginal and small farmers.

Regardless of having high productive potential, the reproductive performance of buffalo becomes narrow as a consequence of various inherent problems like delayed sexual maturity, silent estrus, reproductive seasonality, poor estrus detection, low conception rate, high thermal and lactation stress, postpartum anestrus and long intercalving interval (Jainudeen and Hafez 2000, Das and Khan 2010) resulting in economic loss to buffalo breeders (El-Wishy 2007). In India, incidence of anestrus is more in buffaloes than cattle, especially during summer and was reported as 36.6% and 59.4%, respectively (Das and Khan 2010). However, even higher incidence was reported in nomadic (83%) than rural buffalo (63%; Brar and Nanda 2004).

Breeding season of buffalo starts in rainy period and winter is the most favorable period, while summer remains the most unfavorable period. A set of data collected from a rural veterinary hospital in Punjab on seasonal variation in artificial insemination (AI) in buffalo suggested that on an average  $90.6 \pm 11.1$  (Monthly range: 41-151) buffaloes were inseminated per month. The maximum inseminations per

month were in October and November, whereas, the minimum inseminations per month were in June and July (Ghuman and Dhama 2017). Seasonal decline in reproductive activity was also manifested by reduced expression of estrus as the cases of abbreviated duration of estrus and unobserved/silent estrus were highest in April (70%) and lowest in December (10%; Prakash *et al* 2005). Moreover, calving interval was longer for buffalo calving in February-June due to delayed resumption of post-calving ovarian activity compared to those calving in July-December (from 38-64 to 116-148 days; Singh *et al* 2000). The minimum and maximum pregnancy rate in buffalo in a calendar year was observed in June and January and was respectively recorded as 27% and 54% (Ghuman and Dhama 2017). During winter, early embryonic mortality in buffaloes was 20% that increased to 45% in summer (Abdoon *et al* 2001). To maximize productive life of a buffalo, she must be bred within 90 days after calving and thus start a new lactation every 13 months. However, their reproductive efficiency is hampered by seasonal reproductive activity. Thus, it is important to develop technologies that can alleviate seasonal suppression of reproductive activity in buffaloes.

An alternative approach suggested that acyclicity in buffalo during summer season can be alleviated by fixed time artificial insemination (FTAI) using synchronization protocols based on progesterone ( $P_4$ ), gonadotropin releasing hormone (GnRH) and prostaglandins ( $PGF2\alpha$ ), although these protocols had variable outcome with respect to conception (Warriach *et al* 2015). Progesterone based protocols either alone or in combination with gonadotropins were effective in inducing ovarian activity in summer anestrus buffalo (Das and Khan 2010). Others suggested that GnRH and  $PGF2\alpha$  based estrus synchronization was successful in cattle and buffaloes (Odde 1990, Lamb *et al* 2004, Amaya-Montoya *et al* 2007). Many studies were also carried out with  $PGF2\alpha$  alone (Stevenson *et al* 1983, Battista *et al* 1984, Brito *et al* 2002).

In buffaloes, there is difficulty in identification of estrus manifestations for the application of AI at an accurate time. The use of FTAI protocols that do not require identification of estrus, are more efficient in buffaloes for increasing their productivity (Pursley *et al* 1995). Administration of GnRH after  $PGF2\alpha$  increased the rate of synchronized ovulation in bovines. It was observed that when  $PGF2\alpha$  is administered on palpation of functional CL, about 60-70% of treated animals, were detected in

estrus within 4 days post PGF2 $\alpha$  (Twagiramungu *et al* 1995). The pregnancy rate achieved in relation to usage of different FTAI protocols in buffaloes ranged between 30-50% (Baruselli *et al* 1997, Pursley *et al* 1997, Baruselli *et al* 2003). A recently completed survey work, at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana has revealed that 77.9% buffaloes failed to conceive within 4 month after calving, thus, leading to an increase in calving interval (AICRP 2015). Using transrectal ultrasound-aided monitoring of ovaries, the buffaloes during summer anovulatory period (non-breeding season) displayed dominant follicles that even after attaining ovulatory size (10.1-15.7 mm) undergo atresia without ovulation (Ghuman *et al* 2010). This suggested that buffaloes could be subjected to FTAI during summer anovulatory period. However, anestrus as well as subestrus buffalo respond poorly to ovsynch or PGF2 $\alpha$  based synchronization protocols especially during non-breeding season (Dadarwal *et al* 2009, Ghuman *et al* 2009). A preliminary comparison of fertility outcome of ovsynch and a progesterone plus estradiol benzoate (P<sub>4</sub>+EB)-based FTAI protocol during non-breeding season in buffaloes reared by small farmers revealed an overall (1<sup>st</sup> AI and re-insemination) conception rate of 25% and 69.8%, respectively (Ghuman and Dhama 2017). Although P<sub>4</sub>+EB-based FTAI protocol was costlier compared to Ovsynch, the former was economical due to much better conception rate.

Induction of synchronized estrus in seasonal anestrus buffaloes may provide a potential alternative for increasing the lifetime productive period of buffalo. Given the eminent role of marginal and small farmers in food security, this practice will ensure sustainability and economic viability of dairy farmers. A significant opportunity will be created to boost rural income in coming decades and hence poverty reduction. In fact, a buffalo farmer can prevent a loss (due to an increase in calving-to-conception interval from 3 to 9 months) of ₹3,470 - 26,870/buffalo by spending ₹1,570 on a hormone-based FTAI protocol.

However, the major bottlenecks in wide application of hormone-based therapeutic strategies at small farmer's doorstep are, a) poor conception rate during non-breeding season, and b) failure of non-conceived buffalo to return to estrus following application of hormone protocols. Moreover, it has been observed usually under field conditions that the veterinary officers are not interested in using a progesterone implant-based estrus synchronization/induction protocol. The protocols

like ovsynch are not effective during summer season in buffalo to establish conception. Hence, this study was planned with the following objectives:

- To select a hormone-based therapeutic strategy to reduce non-productive period in anestrus buffalo under field conditions.
- To assess the acceptability and economic impact of hormone-based therapeutic strategies to overcome anestrus in buffalo.

## CHAPTER – II

### REVIEW OF LITERATURE

#### 2.1 Salient feature of reproductive physiology in the buffaloes

Buffalo heifers usually attain puberty when they achieve 55–60% of their adult body weight (200–300 kg for the swamp type and 250–400 kg for the river type) and is highly variable in age ranging from 18 to 46 months (Perera 2011). The influencing factors for the puberty are genotype, nutrition, management, social environment, climate, year or season of birth and diseases. It was reported that under favourable conditions the riverine and swamp buffaloes exhibit first estrus at 15–18 and 21–24 months of age, respectively (Borghese 2005). Although buffaloes attain puberty later than the dairy cattle, they have a longer reproductive life, which tends to compensate for the economic disadvantage occurred during earlier life.

The ovaries of post-pubertal buffalo heifers have a reservoir of only 10,000–20,000 primordial follicles compared with over 1,00,000 in cattle. The mature ovaries are smaller than in cattle, weighing around 2.5 g when inactive and 4.0 g when active, with fewer tertiary follicles (Danell 1987, Zicarelli *et al* 1997). When palpated per rectum, the mature follicles in swamp buffaloes rarely exceed 8 mm in diameter, and can be mistaken as corpus luteum (CL) which is in early developing stage (Jainudeen *et al* 1983). In buffalo, the CL is smaller than that of cattle and often fail to protrude from the surface of ovary and sometimes lacks a clear crown. These characteristics make accurate identification of ovarian structures by rectal palpation more difficult in buffalo than in cattle (Perera 1987, El Wishy 2007). Ultrasonic imaging indicates that mature follicles range from 1.3 to 1.6 cm in diameter and mature CL from 1.2 to 1.7 cm in diameter (Baruselli *et al* 1997, Brito *et al* 2002, Yindee *et al* 2010).

Folliculogenesis in buffalo is characterized by a wave-like pattern (one 5.89%, two 82.35% or three waves 11.76%) and each wave is characterized by wave emergence, growth, dominance followed by atresia or ovulation (Presicce *et al* 2004, Alejandro *et al* 2014). The diameter of preovulatory dominant follicle is  $1.57 \pm 0.12$ ,  $1.55 \pm 0.16$  and  $1.34 \pm 0.13$  cm for animals with one, two or three waves of follicular growth, respectively (Baruselli *et al* 1997a). However, it may reach upto  $17.00 \pm 4.60$  mm in multiparous buffalo and  $14.00 \pm 2.90$  mm in heifers (Alejandro *et al* 2014). In buffaloes exhibiting regular cyclicality, the estrus cycle length can vary from 16 to 28

days (Baruselli 2001) and the classical duration of estrus is 10–20 h during the breeding season (Campanile *et al* 2010, Perera 2011). The females cycling during the non-breeding season can show irregular pattern in duration of estrus, varying from 2 h to 72 h (Baruselli 2001, Zicarelli 2007). However, regardless of breeding or non-breeding season, the buffaloes show poor expression of estrus and also have less estrus-associated mounting behaviour in comparison with cattle (Roy and Prakash 2009). The time of luteinizing hormone (LH) surge may vary from 1 to 12 h after the onset of estrus in buffaloes and ovulation occurs between 26 and 33 h after the LH surge (Campanile *et al* 2010, Perera 2011). In buffaloes, ovulatory dominant follicles of regular estrus cycles attain a diameter of 13-15 mm before ovulation (Perera 2011). The diameter of the CL was recorded as  $19.58 \pm 4.16$  mm in pluriparous buffalo and  $17.74 \pm 3.32$  mm in buffalo heifers and regression occurs around  $15.22 \pm 5.26$  and  $17.62 \pm 1.68$  days of the estrus cycle, respectively (Alejandro *et al* 2014). Following the establishment of pregnancy, the CL grows at faster rate and exhibits earlier rise in circulating progesterone ( $P_4$ ) concentration compared to its non-pregnant counterparts (Vecchio *et al* 2012).

## **2.2 Impact of seasonal diversities and nutrition on reproductive pattern and endocrine profile of buffaloes**

Summer stress is one of the most important limiting factors for dairying, even in farm condition maintained under controlled environmental techniques. Extreme heat waves of early summer are much more devastating when the animals are not acclimatized (Hansen *et al* 2001, Hansen 2007, Hansen 2009). Heat stress has a severe negative impact on the reproductive capacity of cattle and buffaloes (Tailor and Nagda 2005), through its impact on folliculogenesis and endocrine function (De Rensis and Scaramuzzi 2003, Das and Khan 2010).

Buffalo is a polyestrus animal and is capable of breeding throughout the year, but in many countries a seasonal pattern of ovarian and breeding activity followed by calving was observed (Perera 2011). In tropical regions (like India, Bahamas, Colombia and Mexico), this is attributed to changes in rainfall resulting in feed availability or to temperature stress, where as in temperate regions (like Australia, North Korea, Japan and South Korea), the photoperiod and melatonin secretion play an important role (Kumar *et al* 2015) .

The majority of reproductive activity in buffaloes is minimized during summer season which is known as summer anestrus and varies between 36-60% (Singh *et al* 1988, Campanile *et al* 2010). Others have reported that the prevalence of true anestrus is more in buffalo during summer (41-46%) than in other seasons (7-33%; Singh *et al* 2006). This condition is characterized by cessation of ovarian activity, reduced incidence of estrus behaviour, a decrease in the proportion of buffaloes that undergo regular estrus cycle, shorter duration of estrus (8–10 vs 18 h; Brar and Nanda 2004), apparent prolongation of the interval from estrus to ovulation ( $15.8\pm 0.4$  vs  $14.9\pm 0.4$  h; Janakiraman 1978), fewer ovulatory cycles (71% vs 92%) and finally lower conception rates in summer as compared to winter (Qureshi 2012). Others have also observed that a higher incidence of reduced estrus duration in buffalo during summer (70% in April vs 10% in December; Campanile *et al* 2010).

The major consequence of summer or increased day length in buffalo is smooth or inactive ovaries without any follicle or CL (Nanda *et al* 2003) or follicular growth followed by atresia (Pandey and Raizada 1979). This retarded follicular growth, atresia and anovulation is attributed to lower gonadotropin support during summer (Aboul-Ela *et al* 1988). During off-breeding season, true anestrus buffalo exhibit clear follicle wave pattern with dominant follicle attaining >12mm diameter but failing to ovulate (Ghuman 2014). Furthermore, buffalo oocytes are more sensitive to heat stress than cattle oocytes (Neglia *et al* 2003), which results in poor reproductive efficiency in buffalo during summer (Das *et al* 1996, Nandi *et al* 2001). In Mediterranean buffaloes, majority of the calvings were recorded between the months of July and December and those which calve during the non-breeding season experienced an extended postpartum anestrus period with the delay in resumption of ovarian activity till onset of next breeding season (Barile 2005).

Progesterone is a steroid hormone secreted by the CL with key function in regulation of female reproduction. This hormone directly reflects the functionality of CL (Mondal *et al* 2007). The occurrence of poor luteal activity is the major limiting factor behind reduced reproductive efficiency during summer season (Madan 1990). In fact, a seasonal variation was observed in peripheral P<sub>4</sub> concentrations, with lower levels at estrus as well at mid luteal phase in hot ( $0.14\pm 0.05$  and  $2.05\pm 1.16$  ng/ml, respectively) than in cold months ( $0.49\pm 0.06$  and  $3.11\pm 0.20$  ng/ml, respectively; Srivastava *et al* 1999). The proposed reason is suboptimal nutrition coupled with

stress due to high environmental temperature which impairs CL functionality and thus lowers plasma P4 concentrations, ultimately contributing to a long anestrus period in buffaloes (Ronchi *et al* 2001).

The circulating concentrations of estradiol in a normal estrus cycle are at basal level during the luteal phase (10-20 pg/ml) with minor fluctuations around day 4 and day 10 of the cycle and peak level (30-35 pg/ml) either a day before or on the day of estrus in buffaloes. This peak is responsible for overt estrus signs, by its action on central nervous system (Batra and Pandey 1982). This peak in circulating estradiol seldom occurs during summer compared to winter months, which could be responsible for higher incidence of silent estrus during summer season (Rao and Pandey 1983).

Follicle stimulating hormone (FSH) and LH are directly under the control of hypothalamic neuro-hormones and are also regulated by ovarian steroids. These hormones play a significant role in reproduction, however, their secretory patterns are adversely affected by seasonality (Phogat *et al* 1997). There is lack of LH surge during summer with fluctuating basal levels between 1 and 3 ng/ml (Razdan *et al* 1981). Moreover, the amplitude and frequency of LH secretion during the follicular phase was lower during summer (2.8 pulses/8 h with an amplitude of 2.5 ng/ml) than during the winter (3.6 pulses/8 h with an amplitude of 3.7 ng/ml) season. Also, during the luteal phase, a lower LH pulse frequency was observed in the summer (2.1 pulses/8 h) than in winter (3.2 pulses/8 h; Aboul Ela and Barkawi 1988). This lower pulsatile and surge LH secretion during summer may account for poor follicular growth, lower estradiol concentrations and subsequently, a reduced expression of estrus symptoms, silent estrus or anovulation. During summer, photoperiod and stressful environmental conditions pose a negative impact on hypothalamo-pituitary-ovarian axis, thus directly reducing the pulsatile and estradiol-induced LH secretion (Madan 1990).

Nutritional status of animals affects the follicular growth, maturation and ovulation (Diskin *et al* 2003). Under nutrition is the one of the most prevalent causes of reduced reproductive activity in buffaloes for optimal reproductive performance, the buffaloes must have body condition score (BCS) of 3.5 (1=thin and 5=obese) at calving (Bhalaru *et al* 1987, Hegazy *et al* 1994, Ribeiro *et al* 1997). In swamp

buffaloes, with BCS of 3.3, the resumption of ovarian activity was observed within 90 days postpartum while, the buffalo with a BCS of 2.8 continued to be acyclic (Wongsrikeao *et al* 1990). In dairy buffaloes, the mean interval to first ovulation was shorter in animals with the moderate BCS as compared to thin animals at calving (47 and 63 days, respectively), but no difference was observed in interval to exhibition of overt estrus (75 and 74 days, respectively; Hegazy *et al* 1994). An earlier postpartum estrus was reported in buffaloes with high BCS ( $\geq 4$ ;  $49 \pm 1$  days) than in those with a lower BCS score ( $\leq 3.5$ ;  $77 \pm 2$  days; Baruselli *et al* 2001).

## **2.3 Measures to improve the reproductive efficiency of buffaloes**

Various strategies like optimal management, optimal nutrition, and hormonal therapies can be used to improve the reproductive efficiency in buffaloes (Jainudeen 1988, Zicarelli 1993, Gupta and Das 1999).

### **2.3.1 Optimal management practices**

The alleviation of stress and improvement in nutritional status is a key factor for the optimization of reproductive efficiency in buffaloes during summer (Sastry and Tripathi 1988). In fact, an improvement in management practices is an option which should be considered better than the hormonal treatment to improve the efficiency of buffaloes reared under rural conditions (Pant *et al* 2002).

During hotter part of the day in summer, the provision of shade, fans, sprinklers or wallowing facilities (ponds) in farm premises can reduce the heat stress considerably (Acharya 1988, Di Palo *et al* 2009), and hence the adverse effects of heat stress on buffalo fertility (Jainudeen 1988, Di Palo *et al* 2001, Neglia *et al* 2009). In some studies the, provision of showers in addition to wallowing facilities improved the conception rate by preventing early embryonic mortality as a result of body cooling (Pandey and Raizada 1979, Di Palo *et al* 2009).

The detection of estrus plays an important role in profitability of dairy herds. It is difficult to detect estrus during summer season due to shorter duration and less intensity of estrus. The efficiency of estrus detection can be improved using a combination of estrus detection methods (Crowe *et al* 2018). Increasing the time and number of visual observations for estrus can improve the estrus detection rate (Homer *et al* 2013). Furthermore, during summer month, estrus detection in cooler parts of the

day using a male may improve the efficiency of estrus detection (Acharya 1988, Sastry and Tripathi 1988, Das and Khan 2010).

### **2.3.2 Optimal Nutrition**

The reproductive activity of buffaloes can be kept in normal cyclic state by providing high plane of nutrition with proper mineral supplementation (Lall *et al* 2004). The balanced ration provides adequate energy to reduce herd health and reproductive problems associated with reduced dry matter intake and total digestible nutrients intake during heat stress (Gorniak *et al* 2014). Particularly, the grazing animals had to be supplemented with minerals which are deficient in forages/fodders (Kumar *et al* 2003). The reproductive efficiency during summer can be improved by feeding green fodders/silage/hay, providing free access to night time feeding, grazing only in the morning and late in the afternoon and by mineral mixture supplementation (Sastry and Tripathi 1988).

### **2.3.3 Use of hormonal therapies**

Considering the role of endocrine disturbances in delaying breeding and hampering conception, several hormonal strategies can be employed during summer months to augment the reproductive performance of dairy animal. During the hot season of year, the major constraint in optimal buffalo reproduction is lack of overt estrus which interferes with achievement of optimum calving to conception interval (Khodaei-Motlagh *et al* 2011). In buffaloes, the traditionally used estrus detection measures are inadequate to detect estrus during summer season (Jainudeen and Hafez 2000). Thus, the use of estrus synchronization protocols based on GnRH, P<sub>4</sub> and PGF<sub>2</sub> $\alpha$  are an effective and alternate approach (Warriach *et al* 2015). As per the available literatures, the efficacy of protocols in favour of conception rate varies between breeding (Baruselli *et al* 1999) and non-breeding season (Baruselli *et al* 2003, De Rensis *et al* 2005, Ali and Fahmy 2007, Baruselli *et al* 2010), therefore, various hormone based protocols were developed to increase the pregnancy rate in buffaloes.

#### **2.3.3.1 Progesterone based protocols**

Progesterone exerts negative feedback effect on hypothalamus-pituitary axis, thus restricting the release of gonadotropins (FSH and LH). The sudden withdrawal of P<sub>4</sub> release device results in spontaneous release of gonadotropins, and hence, an

induction of estrus within 2-5 days after P<sub>4</sub> device removal (Adams *et al* 1992, Barile 2012). Progesterone releasing intravaginal devices (PRID) maintained luteal phase plasma P<sub>4</sub> for 15 days in anestrus buffaloes (Singh *et al* 2009). Furthermore, plasma P<sub>4</sub> increases substantially from the day of CIDR insertion (1.5±0.3 ng/ml) to day 3 post-CIDR insertion (3.0±0.3 ng/ml), remained above 2.0 ng/ml till the day of CIDR removal, and decreased substantially (0.9±0.3 ng/ml) about 48 h subsequent to CIDR removal, thus resulting in ovulation of largest follicle which was synchronized in 80% subestrus buffaloes at 48-72 h after CIDR-removal (Ghuman *et al* 2014). Plasma P<sub>4</sub> concentration can be used as a key determinant to differentiate anestrus and subestrus buffalo based on which appropriate exogenous hormonal treatment can be implemented, thus resulting in 50% first service conception rate, 62% overall conception rate, 1.6 services per conception and 62.5% pregnancy rate in true anestrus buffalo inseminated at first estrus subsequent to induced estrus, whereas, 66.6% first service conception rate and 57.1% pregnancy rate was recorded in subestrus buffalo, which were inseminated at induced estrus (Honparkhe *et al* 2008).

The inclusion of exogenous P<sub>4</sub> source such as controlled internal drug release (CIDR) in ovsynch protocol (during the interval between first GnRH and PGF2 $\alpha$  injection i.e day 0 to day 7) was suggested for increasing conception rates in summer anestrus buffaloes (Zaabel *et al* 2009, Ghuman *et al* 2012). In ovsynch plus CIDR protocol, during the period from day 7 to day 10, there was higher growth rate of largest follicle (1.77±0.37 mm/d) with largest preovulatory follicle on day 10, increased first service conception rate (70.6%) compared to ovsynch (1.27±0.28 mm/d and 44.4% respectively) and GnRH-PGF2 $\alpha$ -hCG protocols (1.50±0.24 mm/d and 50% respectively; Ghuman *et al* 2012). The efficacy of estradiol-17 $\beta$  plus CIDR based protocol was higher with respect to emergence of follicular wave (84%), ovulation rate (92%) and first service conception rate (48%) compared to ovsynch plus CIDR protocol (76%, 84% and 37% respectively; Bhat *et al* 2015). Furthermore, the studies in postpartum buffaloes, using ovsynch plus CIDR protocol revealed 80% efficiency in estrus induction as well as in pregnancy rate (Rezk *et al* 2016).

### **2.3.3.2 GnRH- PGF2 $\alpha$ based protocols**

Ovsynch is an estrus synchronization protocol consisting of two injections of GnRH combined with single administration of PGF2 $\alpha$ , first GnRH on day 0 followed by PGF2 $\alpha$  on day 7<sup>th</sup> and second GnRH 48 h after PGF2 $\alpha$ . This protocol is used in

cyclic buffaloes for the synchronization of ovulation followed by FTAI (Paul and Prakash 2005, Singh 2014). The efficacy of ovsynch protocol depends on the stage of follicular development at the time of initial GnRH injection (De Rensis *et al* 2005). First GnRH causes synchronize emergence of a new group of follicles by inducing ovulation. Subsequent to ovulation, the CL formed will result in elevated concentration of P<sub>4</sub> (Baruselli *et al* 2003). The administration of PGF2 $\alpha$  7 days later induces luteolysis. In a study, around 61% buffaloes during the postpartum period (60 days post calving) ovulated in response to the first GnRH administration (Baruselli *et al* 1999). Because of the high ovulation rate following GnRH administration, only few buffaloes exhibited estrus behaviour between day 0 and 7, whereas 70-80% of buffaloes were in estrus 48-72 h after PGF2 $\alpha$  administration (Pursley *et al* 1995 and Brito *et al* 2002). The administration of GnRH 36 to 48 h after PGF2 $\alpha$  increases ovulatory synchronization. The efficacy of ovsynch protocol is significant with respect to pregnancy rates after FTAI in cyclic buffaloes during breeding season, but this protocol is least efficient in summer anestrus buffaloes (Jabeen *et al* 2013). The advantages of ovsynch protocol include no need for estrus detection, and shortening of intercalving periods. The disadvantages include possibility of AI in reproductive disorders, works better for large herds and highest efficiency of protocol was limited to first GnRH administration between day 5 and 9 of estrus cycle (Nowicki *et al* 2017).

Cosynch is a another synchronization protocol, which is similar to ovsynch but AI in cosynch protocol is done at the time of second GnRH (Geary *et al* 2001). The diameter and stage of follicle development at first GnRH injection are also crucial for determining the efficiency of cosynch protocol (Baruselli 2001), and better conception rate was observed when diameter of follicle was >9 mm at the time of first GnRH (Rohilla *et al* 2005). Furthermore, the administration of pregnant mare serum gonadotropin (PMSG), about three days before cosynch protocol (cosynch plus) resulted in the presence of follicle of 10-12 mm diametre at the time of first GnRH (Malik 2012). In addition, cosynch plus had a better outcome with respect to estrus induction and conception rate (93.7% and 56.3%, respectively) compared to cosynch alone (81.3% and 37.5%, respectively; Kumar *et al* 2016). Also, insulin was used for the management of anestrus in buffaloes (Kumar *et al* 2013, Singh 2013), as insulin is

known to stimulate the release of GnRH from the hypothalamus and release of LH from the pituitary (Arias *et al* 1992, Tanaka *et al* 2000).

Presynch protocol was developed by administering one or two injections of PGF<sub>2α</sub> 14 days apart prior to the implementation of ovsynch protocol. The second PGF<sub>2α</sub> injection was administered 12 days prior to the first GnRH of ovsynch protocol. Using presynch protocol, the pregnancy rate was increased by 18% in lactating cyclic cows and the estrus induction rate was 85% (Small *et al* 2009). While in case of buffaloes, the ovulation rate was 85-90% and the conception rate was 35-45% (Phogat *et al* 2016).

Modified ovsynch protocol was also used in buffaloes to overcome the lack of display of estrus. Insulin modified ovsynch protocol had promising results with respect to estrus synchronization (80%) and conception rate (40%) in postpartum buffaloes (Gupta *et al* 2015). Following doublesynch (administration of PGF<sub>2α</sub> two days before ovsynch) protocol, a higher estrus induction response was observed at the time of AI in buffalo (frequent urination 100%, swollen vulva 100% and mucus discharge 82%; Dhindsa *et al* 2014, Mirmahmoudi and Prakash 2014). The attempts were made to increase the profitability in dairy farming through application of doublesynch protocol in buffaloes during summer season resulting in increased pregnancy rate in doublesynch treated (58%) compared to control (39%) buffaloes, thereby, improving the economics of dairy farm by Rs. 3,225 per pregnancy (Dhindsa *et al* 2016).

Another estrus synchronization protocol known as heatsynch was developed for buffaloes which makes the use of a combination of GnRH-PGF<sub>2α</sub>-estradiol benzoate injection (Prakash *et al* 2009). This combination was also successfully tested by using estradiol cypionate in place of estradiol benzoate (Barros *et al* 2000, Fernandes *et al* 2001). In this protocol, FTAI was done at 40 and 60 h interval in buffaloes post estradiol cypionate injection. And the recorded estrus induction in heatsynch protocol was 70-80% (Prakash *et al* 2009). In estradoublesynch protocol, the hormonal administration steps were similar to doublesynch protocol with the replacement of last GnRH injection with estradiol benzoate (day 0: PGF<sub>2α</sub>, day 2: GnRH, day 9: PGF<sub>2α</sub>, day 10: estradiol benzoate) and FTAI was done at 48 and 72 h after the estradiol administration (Biradar *et al* 2014). In anestrous buffaloes, the pregnancy rate after estradoublesynch protocol was higher (60%) than that achieved

after the heatsynch protocol (32.5%; Mirmahmoudi *et al* 2014a). In a recent study, pregnancy rate using estradoublesynch protocol followed by FTAI was 55% in anestrus Gir heifers (Chaudhary *et al* 2018). Furthermore, in buffaloes, the pregnancy rates using estradoublesynch protocol followed by FTAI were 62% for cycling, 64% for anestrus, and 34.5% for control group (Mirmahmoudi *et al* 2014b).

### **2.3.3.3 P<sub>4</sub>+E<sub>2</sub>+eCG based protocol**

The protocols for synchronization of follicular waves and ovulation to FTAI based on the combination of P<sub>4</sub>, estradiol and eCG were able to bypass the reproductive seasonality of female buffaloes (Monteiro *et al* 2018). The combination of P<sub>4</sub> and estradiol at the beginning of FTAI protocol was effective in inducing the emergence of a new follicular wave due to the suppression of both FSH and LH which promotes the atresia of all follicles present in the buffalo ovary (Baruselli *et al* 2010). The necessity of eCG in FTAI protocols during the non-breeding season was confirmed as eCG stimulates the growth and maturation of the dominant follicle, resulting in an increased ovulation rate and P<sub>4</sub> production by the subsequent CL and greater pregnancy rate after FTAI in anestrus buffaloes (Carvalho *et al* 2013). It was also suggested that the addition of eCG to a progesterone-based estrus synchronization protocol in buffaloes under tropical conditions helps to improve conception rate (Murugavel *et al* 2009). It was reported that the efficacy of P<sub>4</sub>+EB, PGF<sub>2</sub> $\alpha$  and eCG protocol during the non-breeding season resulted in a pregnancy rate of 50% in a single FTAI (Carvalho *et al* 2016).

## **2.4 Field application of FTAI**

Buffalo is considered as an animal of poor reproductive efficiency due to several features including delayed puberty and sexual maturity, subestrus, anestrus and seasonality pattern in breeding. The term subestrus is used for the unobserved and silent estrus responsible for prolonged calving interval and culling at breeding age in buffaloes. It is erroneously considered as anestrus in field conditions and responsible for tremendous economic losses to the farmers by decreasing calf crop and milk production (Bisen *et al* 2018). Incidence of anestrus in dairy buffaloes was reported 21.05 to 35.13% under organized farms and 37.64% in unorganized rearing systems (Sachan 2013, Singh 2013). A survey was conducted to investigate the incidence of

anestrus in 6105 Murrah and upgraded Murrah buffaloes in and around the district of Jabalpur, Madhya Pradesh, India. Among these, 4708 and 1397 buffaloes were surveyed in organized and unorganized rearing system, respectively. The incidence of anestrus was recorded 25.84% and 40.15% in organized and unorganized rearing system, respectively (Kumar *et al* 2013).

The estrus synchronization and FTAI was introduced as an alternative to improve reproductive efficiency in buffaloes of unorganized rearing systems. Various estrus synchronization protocols using P<sub>4</sub>, PGF<sub>2</sub> $\alpha$  and GnRH in different combinations were tried to overcome estrus detection problems and facilitate AI program (Singh *et al* 2000). Murrah buffaloes were treated with ovsynch and ovsynch plus norprolac protocol and FTAI was carried out 12 and 24 h post second GnRH. Along with ovsynch protocol, norprolac (10 mg/animal i.m.) was administered daily for 10 days in ovsynch plus norprolac protocol. Out of 11, 5 buffalo (45.45%) were pregnant in ovsynch protocol whereas 6 out of 11 (54.54%) buffalo were pregnant in ovsynch plus norprolac protocol (Roy and Prakash 2009). Similar study was, carried out in 400 Murrha buffalo of Guntur district of Andhra Pradesh where buffaloes were treated with ovsynch protocol and low pregnancy rate was observed to first AI, whereas, 77.70% conception rate was observed after overall three subsequent inseminations (Bhosrekar 2006)

The impact of the GnRH preparations used by the field veterinarians to treat a 499 buffaloes with the history of anestrus and infertility belonging to different agroclimatic zones of Haryana was analyzed. The overall estrus induction rate was 100% in animals treated with Ovsynch, followed by CIDR plus GnRH (98.20 $\pm$ 1.80%), then CIDR plus GnRH-PGF<sub>2</sub> $\alpha$  (96.87 $\pm$ 3.12%) and CIDR plus GnRH-PGF<sub>2</sub> $\alpha$ -GnRH protocols. The overall conception rate was higher in animals of CIDR plus GnRH group, thus, justifying that the use of GnRH in combination with P<sub>4</sub> based protocol can be used to improve estrus induction and pregnancy rate in buffaloes under field conditions (Vikash *et al* 2016).

Under field conditions, true anestrus Surti buffaloes treated with four different hormonal protocols (n=11 each), viz., doublesynch, estradoublesynch, Triu-B/PRID, PRID+PMSG and untreated control revealed 81.8-100% estrus induction response. The respective conception rates obtained following AI at induced estrus was

27.3, 27.3, 18.2 and 45.4%, with an overall conception rate of three cycles as 63.6, 54.5, 63.6 and 81.8%, which was much higher than control buffalo (18.2%; Patel *et al* 2018).

## CHAPTER – III

### MATERIALS AND METHODS

#### 3.1 To select a hormone-based therapeutic strategy to reduce non-productive period in anestrus buffalo under field conditions

##### 3.1.1 Experimental location

The present study was carried out at rural buffalo dairy farms of Punjab, and analysis work was carried out in the Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

##### 3.1.2 Experimental design

The study was conducted on 60 lactating Murrah buffaloes with the history of anestrus during the three-month period before the start of study. The selected buffaloes were in the age group ranging from 3-8 year, and in 2nd to 4th parity with body weight ranging from 350 to 500 kg. Buffaloes were daily fed with 10-15 kg of chopped green fodder, 2-3 kg of concentrates, 50-60 g of mineral mixture and had free access to drinking water. The study was conducted during breeding (Winter: November 2017 to February 2018) and non-breeding (Summer: May 2017 to August 2017) seasons. The selected buffaloes were grouped randomly as below:

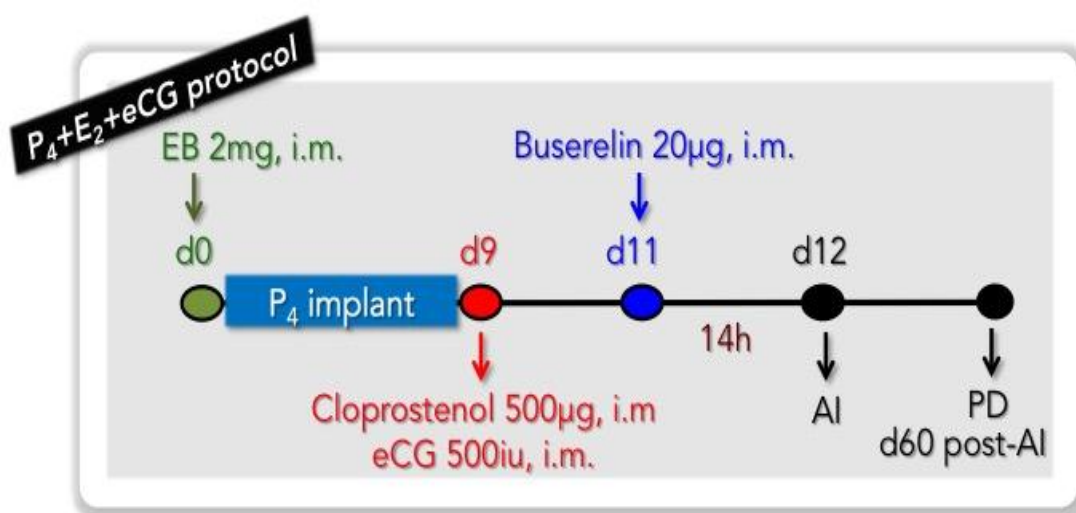
Groups	Treatment	Subgroups	Numbers of buffaloes
<b>I</b>	<b>Progesterone plus estrogen plus equine chorionic gonadotropin (P<sub>4</sub>+E<sub>2</sub>+eCG) protocol</b> (n=30)	Breeding I <sub>B</sub>	15
		Non-breeding I <sub>NB</sub>	15
<b>II</b>	<b>Estradoublesynch protocol</b> (n=30)	Breeding II <sub>B</sub>	15
		Non-breeding II <sub>NB</sub>	15

##### 3.1.3 Research methodology

Based upon history of anestrus, the buffaloes were confirmed for anestrus by rectal palpation twice at 12 day interval. Thereafter, all the buffaloes were dewormed using a per-oral bolus (panacur/fenbendazole) at 10 day interval, and were fed 50g mineral mixture (agrimin forte) per day for 30 days. These buffaloes were randomly

subjected to hormone-based therapeutic strategies (as tabulated) for the induction of estrus followed by fixed-time AI (FTAI) as detailed below:

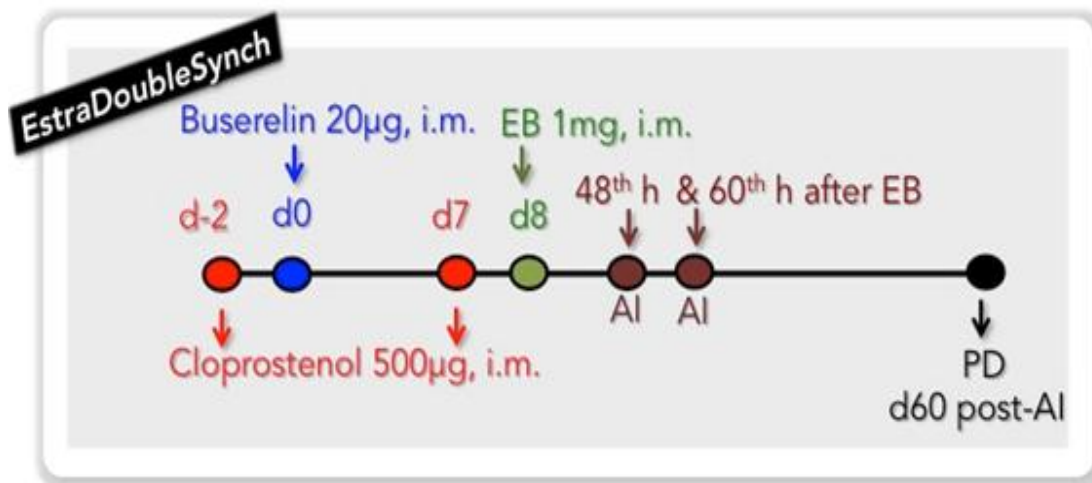
*P<sub>4</sub>+E<sub>2</sub>+eCG protocol (Figure 1):* On day 0 of protocol, each buffalo was administered (i.m.) 2 mg estradiol benzoate and 500 µg PGF<sub>2α</sub> analogue (cloprostenol sodium), and 500 IU eCG was administered (i.m.) on day 9. Sustained progesterone release device (0.96 g hydroxy progesterone implant) was placed intravaginally from day 0 till day 9. On day 11, 20 µg GnRH analogue (buserelin acetate) was administered (i.m.) and AI was done 14 h after GnRH analogue administration.



**Figure 1: P<sub>4</sub>+E<sub>2</sub>+eCG protocol. Treatment on day 0, 9 and 11 was done at 7 pm and AI was done at 9 am (cost of protocol = ₹1850.00)**

*Estradoublesynch protocol (Figure 2):* Two days before the start of protocol, 500 µg PGF<sub>2α</sub> analogue (cloprostenol sodium) was administered (i.m.). On day 0, 7 and 8 of protocol, each buffalo was administered (i.m.) 20 µg GnRH analogue, 500 µg PGF<sub>2α</sub> analogue and 1 mg estradiol benzoate, respectively, and AI was done 48 and 60 h after estradiol benzoate administration.

Under both the protocols, the buffaloes failing to conceive and returning to estrus was re-inseminated at observed spontaneous estrus without any additional hormonal treatment. In non-return cases, the pregnancy was confirmed per rectally at day 60 of last AI.



**Figure 2: Estradoublesynch protocol (cost of protocol = ₹1550.00)**

### 3.1.4 Observations

The success rate of estrus induction hormonal strategy during breeding and non-breeding season was based upon the estrus induction response, pregnancy rate at induced as well as subsequent two spontaneous estruses, buffalo turning to be anestrus at pregnancy diagnosis and the interval between start of protocol to fertile estrus.

#### 3.1.4.1 Estrus induction response

The characteristics of induced and spontaneous estrus were recorded using a estrus scoring chart. The farmers were well educated about the estrus signs and their importance through on-site training. Thereafter, the farmer is observed the buffaloes for various visual signs of estrus, twice per day (early morning and late evening) for 30 min. The scoring parameters of visual estrus signs were: seeking other buffalo (3 points), restlessness (5), mounted but not standing (10), sniffing the vagina of another buffalo (10), chin resting (15), mounting other buffalo (35), standing estrus (100) and mounting head-side of other buffalo (45). The scoring scale was adopted for recording the observations of genital tract such as vulva swelling (+2: 30 points, +1: 20 points), vulva moist and congested (+2: 20, +1: 10), uterine tone (+3: 30, +2: 20, +1: 10) and cervico mucous discharge (present: 100, absent: 0). The observed score for behavioral and genital tract alterations were recorded for each buffalo. If the sum of score was >50 during two consecutive observation periods then the buffalo was considered to be in estrus.

#### **3.1.4.2 Pregnancy rate**

Pregnancy rate is a new and recent method of measuring fertility in buffaloes as compared to traditional estimation of days open and conception rate. Pregnancy rate measures the percentage of buffalo eligible to become pregnant and actually do become pregnant in a given time period (Van Raden *et al* 2004).

#### **3.1.4.3 Interval between start of protocol to fertile estrus**

This period was calculated for each hormonal strategy used in the present study. The interval in terms of days was calculated from the start of protocol (Gp I: day 0; Gp II: day -2) till the day of AI (at induced or spontaneous estrus) which lead to pregnancy in buffalo.

#### **3.1.5 Statistical analysis**

The numerical data is represented as  $AV \pm SE$ , and the differences were considered significant at  $P < 0.05$ . Statistical analysis was performed using GIMMIX procedure of SAS version 9.3 (SAS/STAT) statistical software. The data of various responses like estrus induction response, estrus period score, fate of buffalo failing conceive at induced estrus and the interval between start of protocol and pregnancy was analyzed between therapeutic strategies as well between seasons, using ANOVA. The pregnancy rate and the relation between estrus period score and pregnancy rate was subjected to chi squared test.

### **3.2 To assess the acceptability and economic impact of hormone-based therapeutic strategies to overcome anestrus in buffalo**

#### **3.2.1 Experimental location**

The study was carried out at rural dairy farms of Punjab state.

#### **3.2.2 Experimental design**

The data was collected from the dairy farms and there surrounding areas where estrus induction/ FTAI work was carried between year 2010-2016.

#### **3.2.3 Research methodology**

The comprehensive questionnaires were designed (questionnaire 1 and 2) and a personal interview was conducted at each of the farms (about 500 dairy households). The emphasis was laid on the seasonal pattern of reproductive activity in buffaloes especially number of days spent in anestrus by buffalo, knowledge regarding

hormone-based strategies to overcome anestrus and the farmers' experience in getting the animal pregnant through hormone-based strategies. Also, the follow-up investigations were carried out regarding the adoption of these practices by the farmers whose animal were subjected to estrus induction/synchronization protocols.

**Questionnaire 1: Dairy household response with regard to field application and acceptability of hormonal protocols (estrus induction/synchronization) to overcome anestrus in dairy animal.**

Name of the owner: ..... Date:.....

Address: .....

<b>Herd Size</b>	<5 animals	6-15 animals	>15 animals	
<b>Total dairy animals</b>				
<b>Cross-bred cattle</b>				
<b>Indigenous cattle</b>				
<b>Buffalo</b>				
<b>Farmer education (Tick)</b>	Illiterate	10 <sup>th</sup> pass	Graduate	
<b>Are you aware about estrus synchronization protocols (Tick)</b>	Yes		No	
<b>If yes, then used are not (Tick)</b>	Yes		No	
<b>The number of time protocol used (Tick)</b>	Once	Twice	Thrice	When required
<b>Cost of protocol beared at first time (Tick)</b>	Farmer	GADVASU		Others
<b>Animal conceived by protocols</b>	Total		Conceived	
<b>Cost of protocol beared subsequently time (Tick)</b>	Farmer	GADVASU		Others
<b>If using regularly, reasons (Tick)</b>	Anestrus	Less labour cost		Profit
<b>If not using regularly, reason (Tick)</b>	Less success rate	Costly		Doctor non-availability
<b>Suggestions by farmer for increasing acceptability of protocol (Tick)</b>	Increase success rate	Decrease cost		Cost bearing by govt

**Questionnaire 2: Dairy household survey regarding the outcome of dairy buffalo subjected to estrus induction/synchronization protocol**

<b>Reproductive status of animal</b> (Tick)	Anestrus	Repeat breeding		Cycling
<b>In anestrus then duration</b> (Tick)	2-4	4-6	6-8	>8
<b>If repeat breeder then duration</b> (Tick)	2-4	4-6	6-8	>8
<b>Season in which protocol applied</b> (Tick)	Summer		Winter	
<b>Fate of protocol</b> (Tick)	Success		Failure	
<b>If failure of protocol then fate of animal</b> (Tick)	Sold		Kept as it is	

**3.2.4 Observations**

Impact analysis regarding farmer's acceptability and economic impact of hormone-based therapeutic strategies to overcome anestrus in buffalo by recording of responses (frequency %) of rural smallholder dairy farmers.

**3.2.5 Statistical analysis**

The data collected using questionnaire was entered in an excel spread sheet (Microsoft office excel 2013). The statistical analysis was conducted using GIMMIX procedure of SAS version 9.3(SAS/STAT). Frequency percentage for all categories was calculated individually. Statistical evidence for significance ( $P < 0.05$ ) for a select categorical variable compared with other categorical variable (in response to a categorized question) was generated using the chi-squared statistical procedure.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 To select a hormone-based therapeutic strategy to reduce non-productive period in anestrus buffalo under field conditions

The present study was carried out on lactating buffaloes having the history of anestrus since three months with the aim to evaluate the success rate of two hormone-based therapeutic strategies during the breeding (winter) and non-breeding (summer) season. The success rate was evaluated based upon estrus induction response and the conception rate at induced as well as subsequent two spontaneous estruses. In addition, the interval between start of each hormonal strategy to fertile estrus was recorded.

##### 4.1.1 Estrus induction response

The use of estrus induction protocols viz., progesterone (P<sub>4</sub>) + estrogen (E<sub>2</sub>) + equine chorionic gonadotropin (eCG) protocol and estradoublesynch protocol in anestrus lactating buffaloes during breeding and non-breeding seasons revealed a variable estrus induction response ( $p > 0.05$ ; Table 1 and Fig. 3). The estrus period score, used to calculate estrus induction response, was based upon the estrus behavior and genital tract alterations recorded in the buffaloes on the day of fixed-time AI. The buffaloes exhibiting estrus period score  $> 50$  over two consecutive observations were designated positive for estrus induction (Table 2). In the previous studies, the investigations were undertaken to document estrus period score based on the estrus specific signs such as restlessness, frequent urination, sniffing of vagina, chin resting, chasing, bellowing, mounted by others, mounting others and tail raising in Surti buffaloes (Gamit *et al* 2015) and in dairy cattle (Ghuman *et al* 2014, Kumar *et al* 2019). The estrus detection was also done by visual observation combined with parading a teaser bull at every 6 h interval (Parmer *et al* 2014, Chaudhary *et al* 2015). Moreover, the intensity of estrus was also measured by estrus period score to a specific set of behavioral expression by the buffaloes and their interaction with teaser bull (Dhali *et al* 2008).

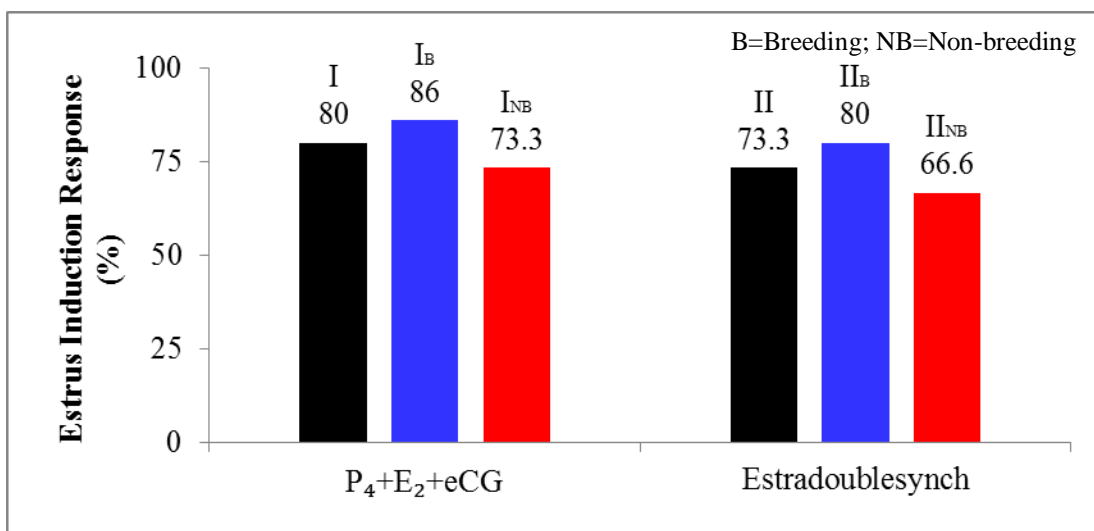
The buffaloes subjected to P<sub>4</sub>+E<sub>2</sub>+eCG protocol or estradoublesynch protocol during breeding season had higher ( $p > 0.05$ ) estrus induction response (86.0 and 80.0%, respectively) compared to their counterparts during non-breeding season (73.3

and 66.6%, respectively; Table 1). In previous studies, a better estrus induction response was reported following the use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol in anestrus buffalo during breeding season (98.0%; Khan *et al* 2018) compared to non-breeding season (83.3%; Mungad *et al* 2016). Furthermore, estrus induction response following the use of estradoublesynch and doublesynch protocols in anestrus Gir cattle during non-breeding season was reported as 95.0 and 85.0%, respectively (Chaudhary *et al* 2018).

**Table 1: Estrus induction response following the use of estrus induction protocols during breeding (winter) and non-breeding (summer) season in anestrus buffaloes.** P<sub>4</sub>: Progesterone; E<sub>2</sub>: Estrogen; eCG: Equine chorionic gonadotropin; B=Breeding; NB=Non-breeding

Protocol	Group	Estrus induction response (%)
P <sub>4</sub> +E <sub>2</sub> +eCG	Gp I (n=30)	80.0 (24)
	Sub-gp I <sub>B</sub> (n=15)	86.0 (13)
	Sub-gp I <sub>NB</sub> (n=15)	73.3 (11)
Estradoublesynch	Gp II (n=30)	73.3 (22)
	Sub-gp II <sub>B</sub> (n=15)	80.0 (12)
	Sub-gp II <sub>NB</sub> (n=15)	66.6 (10)

p>0.05: between and within groups, Figures in parenthesis indicate number of buffaloes



**Fig. 3: Estrus induction response following the use of estrus induction protocols during breeding (winter) and non-breeding (summer) season in anestrus buffaloes.**

In the present study, the buffaloes subjected to P<sub>4</sub>+E<sub>2</sub>+eCG protocol had better (p>0.05) estrus induction response compared to their counterparts subjected to estradoublesynch protocol (Table 1). The administration of a small dose of eCG in P<sub>4</sub>+E<sub>2</sub>+eCG protocol about 3-4 days before the expected ovulation might have lead to competitive binding along with FSH to the FSH receptors of granulosa cells (Butnev *et al* 1996). This response might have resulted in a decreased rate of granulosa apoptosis (Tilly *et al* 1995), increased IGF1 (Sun *et al* 2013) and estrogen production, thus, resulting in an increased diameter of the pre-ovulatory follicle and ultimately better estrus response (De Rensis and Lopez-Gatius 2014). In comparison to our results, others have found a better (90-100%) estrus induction response in anestrous buffaloes subjected to protocols like progesterone releasing intravaginal device (PRID) + pregnant mare serum gonadotropin (PMSG) protocol and estradoublesynch protocol (Patel *et al* 2018). In addition to the use of eCG, the presence of favorable climatic conditions during the breeding season as well as treatment with eCG along with estradiol could enhance the estrus response (Khan *et al* 2018). The buffaloes during the non-breeding season fail to exhibit estrus due to alterations in the endocrine profiles leading to anovulatory condition (Zicarelli *et al* 2010).

Nevertheless, the detailed analysis of estrus period score at induced estrus in buffaloes subjected to P<sub>4</sub>+E<sub>2</sub>+eCG or estradoublesynch protocol in breeding or non-breeding seasons revealed that their estrus period score at induced estrus was not different (p>0.05), despite the fact that the proportion of buffaloes showing estrus induction response was higher (p>0.05) during breeding season as well as following the use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol (Table 1 and 2). In addition, the estrus period score of buffaloes failing to conceive at induced estrus and subsequently returning to spontaneous estrus was not different (p>0.05) between seasons or protocol used (Table 2). On the other hand, the estrus period score of buffalo at induced estrus was higher (p>0.05) compared to the score exhibited by buffalo at spontaneous estrus (Table 2). In fact, in the buffaloes at spontaneous estrus, the expression of estrus was low due to the consistent failure of buffaloes to exhibit 'standing estrus', thus leading to a mean estrus score of <100 during most of the spontaneous estruses. However, in the present study, buffaloes at spontaneous estrus exhibited estrus behavioral signs like sniffing, chin resting, mounting but not standing, mounting on other buffalo except standing estrus. In addition, the genital tract signs like vulva swelling, moist

**Table 2: Estrus period score (AV±SE) of induced and spontaneous estrus following the use of estrus induction protocols during breeding (winter) and non-breeding (summer) season in anestrus buffaloes. P<sub>4</sub>: Progesterone; E<sub>2</sub>: Estrogen; eCG: Equine chorionic gonadotropin; B=Breeding; NB=Non-breeding**

Protocol	Group	Induced estrus			Spontaneous estrus	
		<50	>50	Overall	First	Second
<b>Estrus behaviour score</b>						
<b>P<sub>4</sub>+E<sub>2</sub>+eCG</b>	<b>Gp I (n=30)</b>	<b>27.8±2.3 (n=6)</b>	<b>106.0±5.7 (n=24)</b>	<b>90.9±7.6 (n=30)</b>	<b>79.2±5.8 (n=5/10)</b>	<b>63.0±0.0 (n=2/8)</b>
	Sub-gp I <sub>B</sub> (n=15)	29.0±4.0 (2)	107.2±8.3 (13)	96.8±10.8 (15)	86.5±11.5 (2/4)	63.0±0.0 (1/3)
	Sub-gp I <sub>NB</sub> (n=15)	27.2±3.2 (4)	106.0±8.2 (11)	85.0±10.5 (15)	74.3±6.3 (3/6)	63.0±0.0 (1/5)
<b>Estradoublesynch</b>	<b>Gp II (n=30)</b>	<b>32.4±3.1 (8)</b>	<b>110.7±5.9 (22)</b>	<b>89.8±7.6 (30)</b>	<b>84.0±6.5 (8/14)</b>	<b>84.0±6.8 (5/13)</b>
	Sub-gp II <sub>B</sub> (n=15)	31.6±4.4 (3)	111.0±6.2 (12)	95.1±11.1 (15)	80.3±7.4 (4/6)	90.0±10.4 (3/5)
	Sub-gp II <sub>NB</sub> (n=15)	32.8±4.6 (5)	110.3±11.1 (10)	84.4±11.1 (15)	87.2±11.6 (4/8)	75.0±0.0 (2/8)
<b>Genital tract score</b>						
<b>P<sub>4</sub>+E<sub>2</sub>+eCG</b>	<b>Gp I (n=30)</b>	<b>22.8±5.6 (n=7)</b>	<b>105.6±10.0 (n=23)</b>	<b>86.3±10.1 (n=30)</b>	<b>74.0±4.0 (n=5/10)</b>	<b>60.0±0.0 (n=2/8)</b>
	Sub-gp I <sub>B</sub> (n=15)	23.0±5.0 (2)	96.1±10.6 (13)	86.6±14.3 (15)	75.0±5.0 (2/4)	60.0±0.0 (1/3)
	Sub-gp I <sub>NB</sub> (n=15)	26.0±7.5 (5)	111.0±15.7 (10)	96.0±14.3 (15)	73.3±6.7 (3/6)	60.0±0.0 (1/5)
<b>Estradoublesynch</b>	<b>Gp II (n=30)</b>	<b>23.6±3.9 (11)</b>	<b>107.9±10.5 (19)</b>	<b>77.0±10.1 (30)</b>	<b>82.5±8.0 (8/14)</b>	<b>84.0±11.7 (5/13)</b>
	Sub-gp II <sub>B</sub> (n=15)	30.0±5.5 (5)	101.0±14.2 (10)	77.3±14.5 (15)	75.0±6.4 (4/6)	93.3±18.4 (3/5)
	Sub-gp II <sub>NB</sub> (n=15)	18.3±4.8 (6)	115.5±16.0 (9)	76.6±14.5 (15)	90.0±14.7 (4/8)	70.0±0.0 (2/8)

p>0.05: between and within groups, Figures in parenthesis indicate number of buffaloes

vulva and uterine tone was better at spontaneous estrus as compared to induced estrus. Others have suggested that in dairy cattle with suprabasal plasma progesterone, animals would not stand to be mounted, but the secondary signs of estrus were observed for an extended period due to prolonged growth of ovulatory follicle and increased release of estradiol (Duchens *et al* 1994, 1995b).

#### 4.1.2 Pregnancy rate

The use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol in buffaloes lead to better (p>0.05) pregnancy rate at induced estrus (66.6%) compared to estradoublesynch protocol (53.3%; Table 3). Moreover, the overall pregnancy rate was better (p>0.05) using the P<sub>4</sub>+E<sub>2</sub>+eCG protocol compared to estradoublesynch protocol (73.3 vs 63.3%; Table 3). In previous studies, the pregnancy rate using P<sub>4</sub>+E<sub>2</sub>+eCG protocol was recorded as 45.4% in anestrus Surti buffaloes (Patel *et al* 2018) and 40.6% in anestrus Murrah buffaloes (Murugavel *et al* 2009). Further, following the use of Estradoublesynch protocol, the pregnancy rate was recorded 27 to 40% in anestrus buffalo (Patel *et al* 2017, Prajapati *et al* 2018).

**Table 3: Pregnancy rate (%) following induced and spontaneous estrus in buffaloes subjected to estrus induction protocols during breeding (winter) and non-breeding (summer) season.** P<sub>4</sub>: Progesterone; E<sub>2</sub>: Estrogen; eCG: Equine chorionic gonadotropin; B=Breeding; NB=Non-breeding

Protocol	Group	Induced estrus	Spontaneous estrus		Overall Pregnancy rate
			First	Second	
P <sub>4</sub> +E <sub>2</sub> +eCG	<b>Gp I (n=30)</b>	<b>66.6</b> <b>(n=20)</b>	<b>40.0</b> <b>(n=2/5)</b>	<b>0.0</b> <b>(n=0/2)</b>	<b>73.3</b> <b>(n=22)</b>
	Sub-gp I <sub>B</sub> (n=15)	73.0 (11)	50.0 (1/2)	0.0 (0/1)	80.0 (12)
	Sub-gp I <sub>NB</sub> (n=15)	60.0 (9)	33.3 (1/3)	0.0 (0/1)	66.7 (10)
Estradoublesynch	<b>Gp II (n=30)</b>	<b>53.3</b> <b>(16)</b>	<b>12.5</b> <b>(1/8)</b>	<b>40.0</b> <b>(2/5)</b>	<b>63.3</b> <b>(19)</b>
	Sub-gp II <sub>B</sub> (n=15)	60.0 (9)	25.0 (1/4)	33.3 (1/3)	73.3 (11)
	Sub-gp II <sub>NB</sub> (n=15)	46.0 (7)	0.0 (0/4)	50.0 (1/2)	53.3 (8)

p>0.05: between and within groups, Figures in parenthesis indicate number of buffaloes

In the present study, the higher pregnancy rate with P<sub>4</sub>+E<sub>2</sub>+eCG protocol could be due to the administration of eCG at removal of the P<sub>4</sub> device which increases the diameter of the dominant follicle and ovulation rate (Nunez-Olivera *et al* 2014). Furthermore, P<sub>4</sub>+E<sub>2</sub>+eCG protocol also increases the corpus luteum (CL) growth, early luteal phase P<sub>4</sub> concentrations and pregnancy rate (Carvalho *et al* 2013).

The present study targeted to decide a better estrus induction protocol out of the two protocols used for buffaloes during the breeding as well as non-breeding season. Our results suggested that the overall pregnancy rate including the pregnancy rate at induced and spontaneous estrus was higher ( $p>0.05$ ) during the breeding as well as non-breeding season following the use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol compared their counterparts subjected to estradoublesynch protocol during the respective seasons (Table 3). In earlier studies, pregnancy rate in anestrus Brazilian Murrah buffaloes subjected to P<sub>4</sub>+E<sub>2</sub>+eCG protocol was 66.7 and 62.7% in breeding and non-breeding season, respectively (Carvalho *et al* 2016). Also, the crossbred Murrah buffaloes had pregnancy rate of 64.2 and 60.2% in breeding and non-breeding season, respectively (Mouteiro *et al* 2018). Using estradoublesynch protocol, the pregnancy rate was recorded as 60 and 64% in anestrus crossbred cattle and buffalo, respectively (Sahoo *et al* 2017). The pregnancy rate in cattle which were subjected to estradoublesynch or doublesynch protocols during non-breeding season was 55.0 and 45.0%, respectively (Chaudhary *et al* 2018).

#### **4.1.3 The fate of buffaloes failing to conceive at induced estrus**

Out of thirty buffalo subjected each to P<sub>4</sub>+E<sub>2</sub>+eCG protocol or estradoublesynch protocol, a comparatively less (33.3%;  $p>0.05$ ) proportion of buffaloes failed to conceive at estrus induced with P<sub>4</sub>+E<sub>2</sub>+eCG protocol compared to their estradoublesynch protocol counterparts (46.7%; Table 4). Similarly, in previous studies, about 54.6% buffalo failed to conceive following P<sub>4</sub>+E<sub>2</sub>+eCG protocol and this proportion was 72.7% following estradoublesynch protocol (Patel *et al* 2018).

In the present study, following the use of two estrus induction protocols, it was revealed that there is a marginal difference ( $p>0.05$ ; 50 vs 57%) in buffaloes returning to spontaneous estrus following the failure of conception at induced estrus (Table 4). This suggested that either of protocol can be used in buffaloes with respect to the proportion of buffalo returning to spontaneous estrus after the failure of conception at

induced estrus. It was suggested that the failure of buffalo to return to spontaneous estrus following use of estrus induction/synchronization protocol is a major drawback in popularity of these hormonal protocols in field conditions (Ghuman and Dhami 2017). Moreover, in present study, the proportion of buffalo conceiving at spontaneous estrus was almost similar ( $p>0.05$ ) in both the protocol groups (Table 4).

**Table 4: The fate of buffaloes failing to conceive at induced estrus during breeding (winter) and non-breeding (summer) season.** P<sub>4</sub>: Progesterone; E<sub>2</sub>: Estrogen; eCG: Equine chorionic gonadotropin; B=Breeding; NB=Non-breeding

Protocol	Group	Buffalo failing to conceive at induced estrus	Buffalo returning to spontaneous estrus	Buffalo conceiving at spontaneous estrus
<b>P<sub>4</sub>+E<sub>2</sub>+eCG</b>	<b>Gp I (n=30)</b>	<b>33.3% (n=10)</b>	<b>50.0% (n=5/10)</b>	<b>28.5% (n=2/7)</b>
	Sub-gp I <sub>B</sub> (n=15)	26.6% (4)	50.0% (2/4)	33.3% (1/3)
	Sub-gp I <sub>NB</sub> (n=15)	40.0% (6)	50.0% (3/6)	25.0% (1/4)
<b>Estradoublesynch</b>	<b>Gp II (n=30)</b>	<b>46.7% (14)</b>	<b>57.1% (8/14)</b>	<b>23.0% (3/13)</b>
	Sub-gp II <sub>B</sub> (n=15)	40.0% (6)	66.7% (4/6)	28.5% (2/7)
	Sub-gp II <sub>NB</sub> (n=15)	53.3% (8)	50.0% (4/8)	16.6% (1/6)

$p>0.05$ : between and within groups, Figures in parenthesis indicate number of buffaloes

Nevertheless, in present study, the pregnancy rate at spontaneous estrus was less in comparison to the pregnancy rate at induced estrus (Table 3 and 4). Also, in the previous studies, the pregnancy rate at induced and subsequent spontaneous estrus following P<sub>4</sub>+E<sub>2</sub>+eCG protocol was 80.3 and 61.6%, respectively (Ghuman and Dhami 2017). Similar results in non-breeding season using the same protocol were reported in Brazilian Murrah buffalo (Carvalho *et al* 2016). This suggests that there is need to make better strategies to increase the pregnancy rate in buffalo at spontaneous estrus following the use of a hormonal protocol. Some researchers have used resynchronization strategies in buffalo failing to conceive at first induced estrus (Neglia *et al* 2018), while others have used ovsynch and CIDR-GnRH protocol to resynchronize buffalo failing to conceive at first induced estrus (Arshad *et al* 2017).

The detailed analysis of data in the present study with regard to the fate of buffalo failing to conceive at induced estrus in breeding and non-breeding season revealed that P<sub>4</sub>+E<sub>2</sub>+eCG protocol had edge over estradoublesynch protocol in terms of better pregnancy rate at spontaneous estrus in breeding as well as non-breeding seasons (Table 4). The use of eCG hormone in P<sub>4</sub>+E<sub>2</sub>+eCG protocol might have alleviated the adverse impact of season by improving the ovulatory follicular growth rate and hence better follicle diameter and better luteal profile subsequently (Safilho *et al* 2010).

#### 4.1.4 The interval between start of an estrus induction protocol and conception

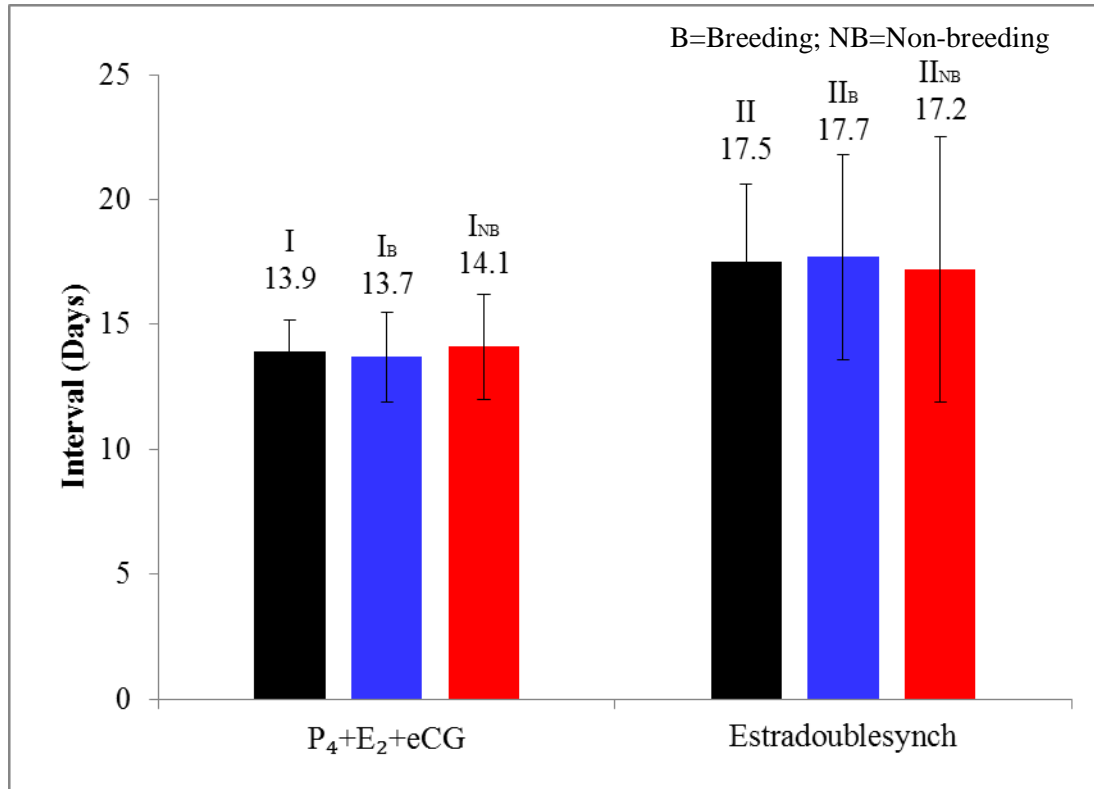
Another part of the present study was to evaluate the efficacy of P<sub>4</sub>+E<sub>2</sub>+eCG and estradoublesynch protocol in terms of the interval (days) between the start of protocol and conception during breeding and non-breeding season. The lesser the number of days between start of protocol and conception, the better is the protocol in terms of economics of the dairy herd (Mungad *et al* 2016).

The results of present study suggested that the interval between start of a protocol and conception in P<sub>4</sub>+E<sub>2</sub>+eCG protocol was 13.9±1.3 days as compared to 17.5±3.1 days for estradoublesynch (p>0.05; Table 5 and Fig. 4). This interval pattern was same in both the protocols in breeding and non-breeding season (Table 5).

**Table 5: The interval (days; AV±SE) between start of an estrus induction protocol and conception during breeding (winter) and non-breeding (summer) season.** P<sub>4</sub>: Progesterone; E<sub>2</sub>: Estrogen; eCG: Equine chorionic gonadotropin; B=Breeding; NB=Non-breeding

Protocol	Group	Interval (days)
P <sub>4</sub> +E <sub>2</sub> +eCG	Gp I (n=22)	13.9±1.3
	Sub-gp I <sub>B</sub> (n=10)	13.7±1.8
	Sub-gp I <sub>NB</sub> (n=12)	14.1±2.1
Estradoublesynch	Gp II (n=19)	17.5±3.1
	Sub-gp II <sub>B</sub> (n=8)	17.7±4.1
	Sub-gp II <sub>NB</sub> (n=11)	17.2±5.3

p>0.05: between and within groups, Figures in parenthesis indicate number of buffaloes



**Fig. 4: The interval (days; AV±SE) between start of an estrus induction protocol and conception during breeding (winter) and non-breeding (summer) season**

Thus, it can be inferred that P<sub>4</sub>+E<sub>2</sub>+eCG protocol has an edge over estradoublesynch protocol in terms of getting the animal pregnant at less interval. In previous studies, the interval between start of protocol to pregnancy was 15.0±3.0, 15.6±3.6 and 24.0±5.7 days for doublesynch, estradoublesynch and ovsynch, respectively (Prajapati *et al* 2018).

#### **4.1.5 The relation between estrus period score and pregnancy rate**

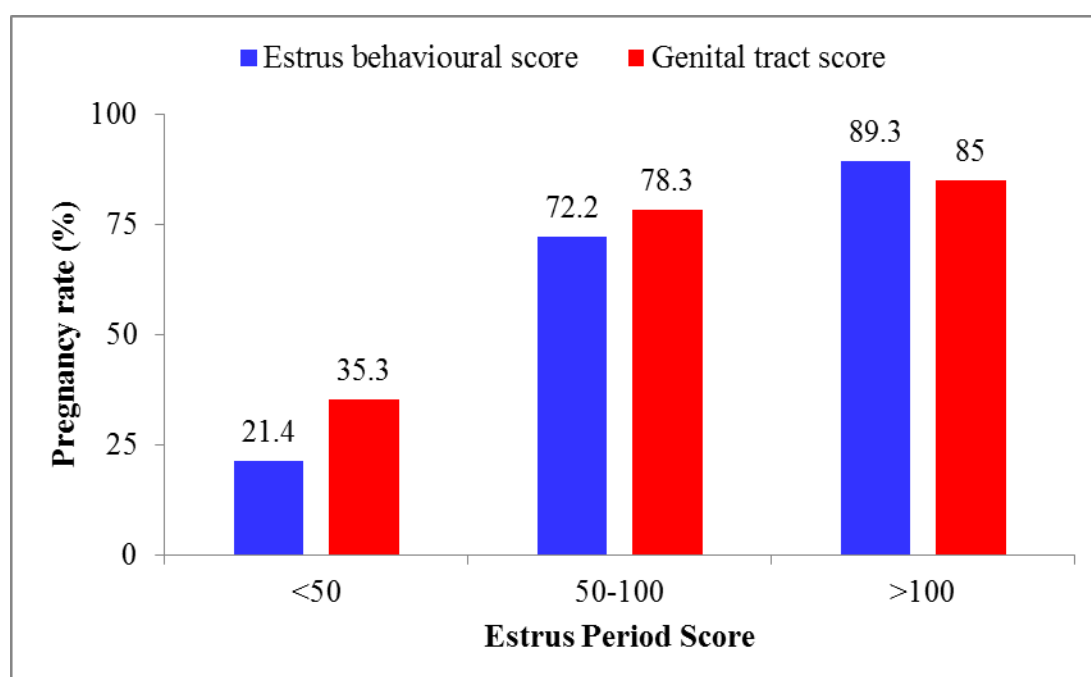
The buffaloes exhibiting better signs of overt estrus had better chance of getting conceived was suggested earlier (Kumar *et al* 2016). This is due to the fact that the estrus behavioral and genital tract alteration on the day of estrus are related to circulatory estrogen concentration (Saxena *et al* 2017). A better developed ovulatory follicle will have higher steroidogenic capacity and subsequently highly steroidogenic corpus luteum, which are prerequisite for successful conception (Nayak *et al* 2009, Parmer *et al* 2014). In the present study, the pregnancy rate was lower in buffalo exhibiting <50 estrus period score (estrus behavior and genital tract score) in comparison to their counterparts exhibiting estrus period score in the range of 50-100

or >100 ( $p < 0.05$ ; Table 6 and Fig. 5). This suggested that estrus period score even at induced estrus is related to subsequent chances of successful conception. In previous studies, pregnancy rate based on the intensity of estrus response viz., intense or moderate estrus response, was 80 vs 33% with cosynch and 71.4 vs 66.6% with cosynch plus protocol, respectively (Nayak *et al* 2009, Kumar *et al* 2016).

**Table 6: The relation between estrus period score (induced and spontaneous estrus) and pregnancy rate in buffaloes subjected to estrus induction protocol in breeding (winter) and non-breeding (summer) season.**

Estrus period score	Pregnancy rate (%) based upon	
	Estrus behaviour score	Genital tract score
<50	21.4 <sup>a</sup> (3/14)	35.3 <sup>d</sup> (6/17)
50-100	72.2 <sup>b</sup> (13/18)	78.3 <sup>e</sup> (18/23)
>100	89.3 <sup>c</sup> (25/28)	85.0 <sup>f</sup> (17/20)

$p < 0.05$ : values with different superscripts within a column differ from each other, Figures in parenthesis indicate number of buffaloes



**Fig. 5: The relation between estrus period score (induced and spontaneous estrus) and pregnancy rate in buffaloes subjected to estrus induction protocol in breeding (winter) and non-breeding (summer) season.**

## **4.2 To assess the acceptability and economic impact of hormone-based therapeutic strategies to overcome anestrus in buffaloes**

### **4.2.1 Dairy household survey outcome regarding the application of hormonal (estrus induction/synchronization) protocols in dairy animals**

It is well known that there is compromised breeding activity in dairy animals especially buffaloes during hot and humid summer season, in tropical countries like India (Marai and Haebe 2010, Kumar *et al* 2014). With the advancement in ovarian ultrasonography, it was observed that the dairy animals keep on showing regular ovarian activity with the availability of a ovulatory size dominant follicle (Ghuman *et al* 2010). This lead to a lot of research on availability of suitable hormonal protocols (estrus induction/synchronization) in terms of their cost, ease of use and success rate. The researchers have revealed the success rate in the range of 31.0 to 56.0% in buffaloes during breeding season (Khan *et al* 2018) and 23.0 to 34.5% in non-breeding season (Sharma *et al* 2017). Ovsynch and ovsynch related protocols are better advocated for use in breeding season (Jabeen *et al* 2014) and progesterone based protocols for non-breeding season (Patil *et al* 2017, Neglia *et al* 2018).

However, the studies on acceptability of these estrus induction/synchronization protocols by the dairy farmers in terms of regular and repeated use at their farms (small or large) remains unknown. The present survey conducted on 500 dairy households regarding the use of hormonal protocols in their dairy animals revealed that 81.8% dairy household are not aware about any of these protocols (Table 7). Further analysis of data with respect to <5, 6-15 or >15 dairy animal per household revealed that out of dairy households unaware about hormonal protocols, the majority (57.9%) belonged to household with <5 animals (Table 7). On the other hand, out of dairy households which were aware about hormonal protocol use in dairy animals, the majority (74.7%) belonged to households with >15 animal (Table 7). This pattern remained same for the dairy household with crossbred cattle, indigenous cattle or buffalo (Table 7). Thus, it can be suggested that the dairy farmers with large dairy herd are more aware about the availability and usefulness of hormonal protocols compared to small dairy herd. This could be due to the education level of farmers, as the present study further revealed that out of the dairy farmers using hormonal protocols, the proportion of illiterate was only 14.8% whereas the proportion of illiterate was 52.3%, who were not aware about hormonal protocols (Table 7). Nevertheless, about 24.7% dairy household who were aware about hormonal

protocols for dairy animals were not using the protocol (Table 7). This strengthens the requirement of not only education of farmers but also regular use of extension activities for pursuing dairy farmers to take benefits associated with the use of hormonal protocols. The same observation were suggested in a recent study (Purohit *et al* 2019).

**Table 7: Dairy household (HH) response (yes/no) with regard to field application of hormonal estrus induction/synchronization protocols in crossbred/ indigenous cattle and buffaloes.**

Response whether protocols used or not	Animals per household		
	<5	6-15	>15
<b>(p&lt;0.05); Dairy animal per HH (Total HH =500) vs Response</b>			
<b>Yes, HH=91 (18.2%)</b>	12 (13.1%)	11 (12.0%)	68 (74.7%)
<b>No, HH=409 (81.8%)</b>	237 (57.9%)	55 (13.4%)	117 (28.6%)
<b>(p&lt;0.05); Crossbred cattle per HH (HH=213/500) vs Response</b>			
<b>Yes, HH=44 (20.6%)</b>	32 (72.7%)	4 (9.0%)	8 (18.1%)
<b>No, HH=169 (79.3%)</b>	166 (98.2%)	1 (0.6%)	2 (1.8%)
<b>(p&lt;0.05); Indigenous cattle per HH (HH=102/500) vs Response</b>			
<b>Yes, HH=25 (24.5%)</b>	21 (84.0%)	0	4 (16.0%)
<b>No, HH=77 (75.5%)</b>	77 (100.0%)	0	0
<b>(p&lt;0.05); Buffalo per HH (HH=500) vs Response</b>			
<b>Yes, HH=91 (18.2%)</b>	20 (21.9%)	18 (19.7%)	53 (58.2%)
<b>No, HH=409 (81.8%)</b>	268 (65.5%)	97 (23.7%)	44 (10.7%)
<b>(p&lt;0.05); Farmer education vs Response</b>			
----	<b>Illiterate</b>	<b>10<sup>th</sup> Pass</b>	<b>Graduate</b>
<b>Yes, HH=91 (18.2%)</b>	13 (14.8%)	55 (60.4%)	23 (25.3%)
<b>No, HH=409 (81.8%)</b>	214 (52.3%)	147(35.9%)	48 (11.7%)
<b>(p&lt;0.05); Awareness about protocols vs Response</b>			
----	<b>Yes</b>	<b>No</b>	
<b>Yes, HH=91 (18.2%)</b>	91 (100.0%)	0	
<b>No, HH=409 (81.8%)</b>	101 (24.7%)	308 (75.3%)	

#### 4.2.2 Dairy household response following the use of hormonal protocols in dairy animals

A detailed analysis of 91 dairy households who had used hormonal protocols in dairy animals was carried out. About 65.9% revealed that the protocols were used

only in summer season, whereas only 9.8% dairy households used protocol in dairy animals during winter season (Table 8). This could be due to the inability of dairy animal to express estrus and get inseminated during summer season (De Rensis and Lopez-Gatius 2007, Qayyum *et al* 2018). Furthermore, the present study suggested that 82% dairy household had used protocol due to anestrus condition of animals and only 18% used for making profits (Table 8). Thus, estrus induction/synchronization protocol is a tool for farmers to reduce the economic loss occurring due to non-productive buffaloes.

**Table 8: Dairy household (HH) response following the use of estrus synchronization protocols in dairy animals.**

Survey	Response			
	Season in which protocol used, HH=91	Summer	Winter	Both
60 (65.9%)		9 (9.8%)	22 (24.1%)	
Reason for regular use of protocols, HH=50	Anestrus	Less labour cost	Profit	
	41 (82.0%)	0	9 (18.0%)	
Reason for not regular use of protocols, HH=73	Less success rate	Costly	Doctor non availability	
	15 (20.5%)	37 (50.6%)	22 (30.1%)	
Protocol cost beared first time, HH=91	Farmer	GADVASU	Others	
	68 (74.7%)	23 (25.2%)	0	
Protocol cost beared subsequently, HH=45	37 (82.2%)	8 (17.7%)	0	
Number of times protocol used, HH=91	Once	Twice	Thrice	If required
	47 (51.6%)	22 (24.1%)	8 (8.7%)	14 (15.3%)
Farmers suggestion for increasing acceptability of protocols, HH=91	Increase success rate	Decrease cost	Cost bearing by govt	
	22 (24.1%)	19 (20.8%)	50 (54.9%)	

The dairy households also suggested less success rate (20.2%), high cost (50.0%) and non-availability of doctor (29.7%) as the reasons for the irregular use of hormonal protocols (Table 8). About 74.7 to 81.8% dairy households had beared the cost of hormonal protocol when used first time or subsequently (Table 8). In fact, about 75.7% households had used hormonal protocols once or twice. About 54.9% of households were of the opinion that the hormones used in protocols should be made

available free of cost through Govt schemes (Table 8). Further research should be carried out to increase the success rate of hormonal protocols and to decrease the cost was suggested by 24.1% and 20.8% dairy households, respectively (Table 8). It was inferred from the present study that in addition to requirement of research to improve the efficacy of protocols, the measures should also be taken to improve farmer education and make free availability of hormones which will help to increase acceptability of hormonal protocols for use in dairy animals.

#### **4.2.3 Dairy household response related to the use of hormonal protocols in buffaloes**

Dairy household survey with respect to buffalo rearing and field application of hormonal protocols suggested that majority (52.3%) of animal subjected to these protocols were anestrus, followed by repeat breeder (31%) and cyclic buffalo (16.5%; Table 9). The duration of anestrus or repeat breeding was up to 6 month in 78.6% and 70.3% buffalo, respectively (Table 9), which were subjected to hormonal protocols.

**Table 9: Dairy household survey regarding the outcome of dairy buffaloes (n=380) subjected to estrus induction/synchronization protocols.**

<b>Parameter</b>	<b>Observation</b>	<b>Protocol applied (%)</b>
<b>Reproductive status of animal</b>	<b>Anestrus</b>	199 (52.3)
	<b>Repeat breeding</b>	118 (31.0)
	<b>Cycling</b>	63 (16.5)
<b>Duration of anestrus</b>	<b>2-4 mo</b>	69 (34.9)
	<b>4-6 mo</b>	87 (43.7)
	<b>6-8 mo</b>	21 (10.5)
	<b>&gt; 8 mo</b>	22 (11.0)
<b>Duration of repeat breeder</b>	<b>2-4 mo</b>	0
	<b>4-6 mo</b>	83 (70.3)
	<b>6-8 mo</b>	21 (17.7)
	<b>&gt; 8 mo</b>	14 (11.8)
<b>Season in which protocol applied</b>	<b>Summer</b>	230 (60.5)
	<b>Winter</b>	150 (39.5)
<b>Fate of protocol</b>	<b>Success</b>	179 (47.1)
	<b>Failure</b>	201 (52.8)
<b>Fate of animal (If failure of protocol)</b>	<b>Sold</b>	56 (27.8)
	<b>Kept as it is</b>	145 (72.1)

Previous studies have also suggested that estrus induction/synchronization protocols were used in anestrus (Savans *et al* 2017) or repeat breeder (Biradar *et al* 2016) buffalo. The timely application of these protocol can minimize the loss due to unproductive period in the life span of a buffalo (Dhindsa *et al* 2016).

Furthermore, about 60.5% of buffalo which were subjected to hormonal protocol belonged to summer season (Table 9). It is well known that anestrus in summer season is the major issue which warrants the application of these protocol in buffaloes (Warriach *et al* 2015). Dairy household survey for buffaloes further revealed that the success rate of use of protocol in terms of conception was 47.1% in field conditions (Table 9). Here, it is important to consider the fact that if a buffalo fails to conceive after the end of voluntary waiting period, a farmer has to bear a loss of ₹300/day in terms of loss of milk and other managerial expenses whereas the cost of using a P<sub>4</sub>+E<sub>2</sub>+eCG protocol is ₹1850 (Ghuman and Dhami 2017). In the present survey, majority of (52.3%) of buffaloes subjected to protocol were anestrus (Table 9) and 82% dairy household used protocols due to anestrus condition (Table 8). Thus, even 47.1% success rate of protocols recorded in present study can have huge impact on economics of dairy farming. Others have suggested that success rate of ovsynch protocol was 30% (Savalia *et al* 2013) and 58% (Francesco *et al* 2012). The success rate of 50-60% was achieved following the use of progesterone based protocols in buffaloes (Rathour *et al* 2005, Naseer *et al* 2013). The present survey also suggested that following the failure of hormonal protocols, about 27.8% buffalo were sold out by the dairy farmers since most of farmers were not ready to bare economic losses due to low milk yield of non-pregnant buffaloes.

## CHAPTER – V

### SUMMARY AND CONCLUSIONS

Buffalo (*Bubalus bubalis*) is an important livestock resource and occupies a critical niche in many agricultural systems, providing milk, meat and work power. Despite having a high productive potential, buffalo is often blamed for poor reproductive performance reflected by various inherent problems like delayed sexual maturity, silent estrus, reproductive seasonality, prolonged postpartum anestrus and longer inter-calving intervals. Reproductive seasonality is evidenced by 74–86% buffalo exhibiting anestrus during non-breeding (summer) compared to only 22-26% in breeding (winter) season. Hormonal strategies (estrus induction/ synchronization) in anestrus buffaloes during breeding and non-breeding season provide a potential alternative for increasing their productive period as these strategies do not warrant estrus detection and animals could be inseminated at a fixed-time.

The phase-I of present study was to select a hormone-based therapeutic strategy ( $P_4+E_2+eCG$  or estradoublesynch protocol) to reduce non-productive period in anestrus buffalo under field conditions. The phase-II of study was a questionnaire based survey conducted to assess the acceptability of hormone-based therapeutic strategies to overcome anestrus in buffalo.

Under phase-I, 60 lactating Murrah buffalo were selected with the history of anestrus for at least three months. The selected anestrus buffaloes were subjected to  $P_4+E_2+eCG$  protocol (Breeding season = 15; Non-breeding season = 15) or estradoublesynch protocol (Breeding season = 15; Non-breeding season = 15). The estrus induction response of each protocol was measured by estrus period score based on estrus behavioral and genital tract signs. Estrus period score of induced estrus and subsequent spontaneous estruses in non-pregnant buffaloes were recorded. Estrus induction response (%) and the interval (days) between start of hormonal protocol to fertile estrus was recorded. Pregnancy diagnosis was done by rectal palpation at day 60 post AI. Pregnancy rate (%) of induced estrus, spontaneous estrus and overall pregnancy rate was recorded.

The buffaloes subjected to  $P_4+E_2+eCG$  protocol or estradoublesynch protocol during breeding season had higher ( $p>0.05$ ) estrus induction response (86.0 and 80.0%, respectively) compared to their counterparts during non-breeding season (73.3

and 66.6%, respectively). Further, the detailed analysis of estrus period score at induced estrus in buffalo subjected to P<sub>4</sub>+E<sub>2</sub>+eCG or estradoublesynch protocol in breeding or non-breeding seasons revealed that their estrus period score at induced estrus was not different (p>0.05), despite the fact that the proportion of buffalo showing estrus induction response was higher (p>0.05) during breeding season as well as following the use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol. In addition, the estrus period score of buffalo failing to conceive at induced estrus and subsequently returning to spontaneous estrus was not different (p>0.05) between seasons or protocol used. On the other hand, the estrus period score of buffalo at induced estrus was higher (p>0.05) compared to the score exhibited by buffalo at spontaneous estrus.

The use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol in buffaloes lead to better (p>0.05) pregnancy rate at induced estrus (66.6%) compared to estradoublesynch protocol (53.3%). Moreover, the overall pregnancy rate was better (p>0.05) using P<sub>4</sub>+E<sub>2</sub>+eCG compared to estradoublesynch protocol (73.3 vs 63.3%). The present study targeted to decide a better estrus induction protocol out of the two protocols used for buffaloes during breeding as well as non-breeding season. Our results suggested that the overall pregnancy rate including the pregnancy rate at induced and spontaneous estrus was higher (p>0.05) during the breeding as well as non-breeding season following the use of P<sub>4</sub>+E<sub>2</sub>+eCG protocol compared their counterparts subjected to estradoublesynch protocol during the respective seasons.

The present study suggested that the interval between start of a protocol and conception in P<sub>4</sub>+E<sub>2</sub>+eCG protocol was 13.9±1.3 days as compared to 17.5±3.1 days for estradoublesynch (p>0.05). This interval pattern was same in both protocols in the breeding and non-breeding season. The pregnancy rate was lower in buffalo exhibiting <50 estrus period score (estrus behavior and genital tract score) in comparison to their counterparts exhibiting estrus period score in the range of 50-100 or >100 (p<0.05).

Under phase-II, a personal interview was conducted at 500 dairy households in rural areas of Punjab and data collected regarding the application of hormonal (estrus induction/synchronization) protocol in dairy animal. It was revealed that 81.8% dairy household had no awareness about hormonal protocols. Further analysis of data with respect to <5, 6-15 or >15 dairy animals per household revealed that out of dairy

household unaware about hormonal protocols, the majority (57.9%) belonged to households with <5 animals. On the other hand, out of dairy household which were aware about hormonal protocol use in dairy animal, the majority (74.7%) belonged to households with >15 animal. This pattern remained same for the dairy household with crossbred cattle, indigenous cattle or buffalo. The present study further revealed that out of the dairy farmers using hormonal protocols, the proportion of illiterate was only 14.8% whereas the proportion of illiterate was 52.3% who were not aware about hormonal protocols. About 24.7% dairy households who were aware about hormonal protocols for dairy animals were not using the protocols.

Further, a detailed analysis of 91 dairy households of who had used hormonal protocols in dairy animals was carried out. About 65.9% revealed that the protocols were used only in summer season, whereas only 9.8% dairy households used protocol in dairy animals during winter season. The present study suggested that 82% dairy household had used protocol due to anestrus condition of animal and only 18% used for making profits. Furthermore, dairy households also suggested less success rate (20.2%), high cost (50.0%) and non-availability of doctor (29.7%) as the reasons for the irregular use of hormonal protocols. About 74.7 to 81.8% dairy households had beared the cost of hormonal protocol when used first time or subsequently. In fact, about 75.7% households had used hormonal protocols once or twice. About 54.9% of households were of the opinion that the hormones used in protocol should be made available free of cost through Govt schemes. Further research should be carried out to increase the success rate of hormonal protocols and to decrease the cost was suggested by 24.1% and 20.8% dairy households, respectively.

Dairy household survey with respect to buffalo rearing and field application of hormonal protocols suggested that majority (52.3%) of animals subjected to these protocols were anestrus, followed by repeat breeder (31%) and cyclic buffaloes (16.5%). The duration of anestrus or repeat breeding was up to 6 month in 78.6% and 70.3% buffalo, respectively, which were subjected to hormonal protocols. The success rate of use of hormonal protocols under field condition in terms of conception was 47.1%.

The following conclusions were drawn from the present study:

1. Under field conditions, Progesterone + Estrogen + equine chorionic gonadotropin (P<sub>4</sub>+E<sub>2</sub>+eCG) protocol has an edge over estradoublesynch protocol for treatment of anestrus buffalo during breeding as well as non-breeding season, however, data need to be validated further with a field study on large buffalo population.
2. Pregnancy rate subsequent to insemination at induced or spontaneous estrus was associated with estrus period score in buffaloes.
3. The major bottlenecks in the acceptability of hormone-based therapeutics strategies are lack of awareness in 81.8% dairy household especially in household with <5 animal, illiteracy and high cost.
4. Use of hormone based therapeutic strategies under field conditions have economic impact on dairy households as 82% households used these strategies due to anestrus condition of dairy animals and 41% success rate was recorded.

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