Controlled Breeding of Buffalo on Government and Small-holder Dairy Farms in Pakistan

Mohammad Riaz Khan

Master of Veterinary Medicine (Bio-Security)
DVM (Doctor of Veterinary Medicine)
B.Sc. (Honours) Animal Husbandry
Bachelor of Arts (Political Sciences)

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I Mohammad Riaz Khan hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

I agree that the thesis be accessible for the purpose of study and research in accordance with the normal conditions established by the University Librarian for the care, loan and reproduction of the thesis.*

Signature                     Date                     9th, August 2013

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I dedicate my thesis to my wife, daughter and all the suppressed and oppressed women of Pakistan tribal areas.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AI</td>
<td>Artificial Insemination</td>
</tr>
<tr>
<td>CIDR</td>
<td>Control Internal Drug Release</td>
</tr>
<tr>
<td>CL</td>
<td>Corpus Luteum</td>
</tr>
<tr>
<td>DF</td>
<td>Dominant Follicle</td>
</tr>
<tr>
<td>e.g.</td>
<td>For example</td>
</tr>
<tr>
<td>eCG</td>
<td>Equine chorionic gonadotropin</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>hCG</td>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>hr</td>
<td>Hours</td>
</tr>
<tr>
<td>i.m</td>
<td>Intra muscular</td>
</tr>
<tr>
<td>inj.</td>
<td>Injection</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
<tr>
<td>NARC</td>
<td>National Agriculture Research Centre</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram ($10^{-9}$ gram)</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>P4</td>
<td>Progesterone</td>
</tr>
<tr>
<td>PGF2α</td>
<td>Prostaglandin F2α</td>
</tr>
<tr>
<td>PRID</td>
<td>Progesterone Releasing Intra-uterine Device</td>
</tr>
<tr>
<td>PRL</td>
<td>Prolactin</td>
</tr>
<tr>
<td>TAI</td>
<td>Timed Artificial Insemination</td>
</tr>
<tr>
<td>vs</td>
<td>Versus</td>
</tr>
</tbody>
</table>
Abstract

The reproductive efficiency of buffalo is greatly affected by late age at first calving (47 months), seasonality of calving, long postpartum anoestrus, weak display of oestrus signs, low conception rate and long inter calving intervals (24 months). So far in buffaloes, various hormones (GnRH, oestradiol etc) are being used in combination with or without CIDR (Controlled Internal Drug Release). Progesterone is reported to have a good impact on inactive ovaries. Prostaglandin F2α is extensively used in Pakistan as a drug of choice for anoestrus dairy buffaloes. The PG group in this study was considered as control. In this study the effect of CIDR and prostaglandin (PGF2α) was studied during the low breeding summer season to synchronise oestrus.

Materials and Methods:

Experimental design:

Buffaloes were randomly allocated to two treatment groups (n=60 per group). Group one was treated with a CIDR for 7 days and prostaglandin (PG) on day 6 with the CIDR being removed on day 7. Group two was treated with PG injection on day 0 and day 11. Pregnancy diagnosis was carried out after 40 days using transrectal ultrasonography (Honda; Model: HS-1500; 7.0MHZ).

Statistical analysis:

Data for oestrus response and comparisons of pregnancy rate between the two treatments, the impact of management (tied and untied) and the presence of a calf was compared using a Chi square analysis. The effect of body condition score (BCS) and parity on the oestrus response and pregnancy rate was confirmed with Logistic Model SPSS (version 20). The effect of age and daily milk production on the oestrus response, and the duration of anoestrus post-partum in days were compared using Anova and a t-test. A probability level with P value <0.05 was considered significant.

Results:

When the CIDR was used with PGF2α (Treatment 1) oestrus was detected in 84.5% of animals, but only in 23.3% of animals receiving a PGF2α regime only. This did not lead to a higher pregnancy rate (CIDR 19.6%; PGF2α regime 30.8%; P = 0.296)
Discussion:

This study demonstrated that oestrus expression was higher in animals receiving a protracted regimen of progesterone release using a CIDR device. The PG is only effective in the presence of a functional CL, while in summer the buffalo have small inactive ovaries without any palpable structures and so they do not respond to PG. Therefore the oestrus response was lower in PG receiving animals.

The pregnancy percentage was lower in the CIDR group than the PG group animals and there were no significant differences across treatments.

Conclusion:

This study shows that the CIDR device is more effective in synchronizing oestrus than the use of PG. The high oestrus response and low pregnancy rate in CIDR group requires more research on the use of TAI techniques with the CIDR device to optimize the timing of AI.
Chapter 1. Introduction

1.1. Role of livestock in Pakistan’s economy:

Agriculture plays a major role in Pakistan economy. According to the economic survey of 2009-10, agriculture accounts for over 21 percent of gross domestic products (GDP) and provides the largest number of jobs (45 percent) amongst all of the sectors of the economy. Although the contribution of Agriculture to national GDP is decreasing (21.8% in 2008-09 and 21% in 2009-10), the contribution of livestock to value added agriculture increased from 51.8 to 53.2% and to national GDP from 11.3 to 11.4% in 2008-09 and 2009-10 respectively (Ministry of Finance Government of Pakistan, 2011-12).

Pakistan is the third or fourth largest milk producing country (Afzal, 2010; Lateef, Faraz, Mustafa, Akhtar, & Bashir, 2009; Luthra, Khar, & Singh, 1994) and has the second largest buffalo population (M. A. Khan, 2009) in the world. The main products from livestock are milk and meat, and cattle and buffaloes are the main contributors. The cattle and buffaloes populations are 38.3 and 33.7 million respectively providing a gross total of 49.5 million tonnes of milk out of which 39.9 million tonnes of milk is available for human consumption (subtracting 20% (transportation (15%) and calving (5%)) wastage from total gross production). The buffaloes contribute around 30.5 million tonnes of milk to the total national milk production (Ministry of Finance, 2012-13). In Pakistan, Punjab provides 73% of total milk production, with 76% of the total buffalo population, while the remaining 24% of buffaloes are found in other parts of Pakistan (Borghese, 2005).

The majority (85%) of livestock is reared in rural areas having 5.5 million small production units consisting of 1 to 5 animals, and together with landless farmers providing 65 to 70% of total milk production from cattle and buffaloes (Borghese, 2005; K. Singh & Pundir, 2003). According to Raza et al. (2000) cited by Borghese (2005) there are 5.5 million landless farmers contributing 71% percent out of 73% of the total milk production supplied in Punjab, the largest state of Pakistan in terms of both human and buffalo population.

1.2. Importance of buffaloes:

The buffalo (Bubalus bubalis) population is distributed in Asia, Africa, Egypt, South America and Australia. The highest population is in Asia (95.83% (Bhat, 1992)) with India having the largest population with a total number of 98.7 million buffaloes (FAO, 2007).
According to the FAO (2007), Pakistan is second with a total number of 28.2 million head. Swamp buffalo and river buffalo are the two main types of domestic buffalo found everywhere in the world. River buffaloes are reared for milk production, while the swamp buffaloes are for draught and meat purpose. River buffalo are found in many parts of the world. The poor developing countries are populated with buffalo and this may be the reason for the lack of research on this species.

Buffaloes are a versatile animal found in 50 countries of the world having temperate to tropical environments where daily temperature ranges from below zero to 45 degree Celsius in various season of the year. The river buffaloes are found in India, Pakistan, Bangladesh, Sri Lanka, Iran, Thailand, Indonesia, China, Brazil, Turkmenistan, Iraq, Syria, Egypt, Azerbaijan, Turkey, Greece and Bulgaria with small numbers of imported animals found in Australia and South America as well (FAO, 2007). The river type milk buffalo have various local names including Nili-Ravi, Kundi and Azakhely in Pakistan, Murrah in India, Beheri and Saidi in Egypt and Shuman and Italian in Europe (Borghese, 2005; Rosati & Van Vleck, 2002). The buffalo has natural ability to resist many diseases and has the ability to adapt to hot and humid climates and harsh tropical environments than cattle. The buffalo has many advantages like conversion of low quality roughages, suitability for grazing on swamp areas. (Abayawansa, Prabhakar, Singh, & Brar, 2011; Ahmad, Chaudhry, & Khan, 1981; Bashir, Khan, Bhatti, & Iqbal, 2007; Beaton, 1975; Bilal, Suleman, & Raziq, 2006; Borghese, 2005; Borghese & Moioli, 2011; Razdan, 1988). Therefore more research is needed to develop buffalo as a better milk breed which will strengthen the economies of developing countries.

The buffaloes are the main contributors to the Pakistan dairy system, providing 62-70% of the total milk: they are called the “black gold” or “black beauty” of Pakistan (Afzal, 2010; M. A. Khan, 2009). Buffalo milk is preferred to cow’s milk due to its high fat (6-8%) and solid not fat (9-10.5%) content: there is therefore a greater market demand for buffalo milk commanding a higher price when compared with cow (Afzal, 2010; Bilal et al., 2006). Buffaloes are important as they have the potential to convert low quality roughages into milk and meat (Bilal et al., 2006).

If properly managed, buffaloes have a longer productive life than cattle. The Italian buffalo have 25 years productive life with 15-17 lactations (M. A. Khan, 2009). This is far longer than the life-span of Pakistani buffalo which normally have 3.5 years of productive life and live to an age of to 11 years (Bashir et al., 2007). In India the average life span of buffaloes is 10.5 years while the productive life is 6.9 years (Dutta, Singh, & Desai, 1965).
In spite of the buffalo’s contribution to the national economy, the policy makers consistently ignore this “black beauty” of Pakistan. After independence in 1947 the cattle were greater in number when compared with buffaloes. But later on, due to its ability to survive in harsh environments, resistance to diseases, consumer preference for its milk and conversion of low quality roughage into milk and meat, their numbers have grown to outnumber cattle. In 1965 and onward, due to lack of research facilities and trained man power the government spent a lot of money on the introduction of cross bred exotic cattle from European countries. They consider buffaloes as ugly, high cost and slow reproducing animals without concentrating and working on the problems of buffalo reproduction. The population of buffaloes has increased during this time from 6.7 million in 1956 to 29 million in 2010 (M. A. Khan, 2009).

There are two main types of buffaloes, the Swamp and River type for draught and dairy purpose respectively.

Figure 1 shows the classification and distribution of buffaloes across the world (Borghese, 2005; M. A. Khan, 2009).
Figure 1 Classification and distribution of buffalo species.

(Source: Borghese, 2005; M. A. Khan, 2009)
1.3. Reproduction in buffaloes:

Buffaloes are found in developing countries and therefore the research work on buffaloes is scarce and of low quality due to lack of resources. So far not much work has been done on the basics of reproduction in buffaloes. The major constraints reported in buffalo reproduction are high calf mortality, delayed age at maturity in both the sexes, seasonal breeding pattern (primarily due difficulties and failure in detection of oestrus during summer), delay in resumption of ovarian cyclicity after calving (resulting in long inter-calving intervals) seasonal effect on reproductive routine and semen quality in males, poor conception rates from artificial insemination with frozen semen, and sensitivity to the physical environment (especially direct solar radiation and poor thermo regulation). These problems are now drawing the attention of research workers (Acharya & Bhat, 1989).

The structure and location of female reproductive tracts are similar in cattle and buffaloes. The cervix in the buffalo is less conspicuous and the uterine horns are more coiled. During oestrus the uterine horns are turgid and coiled and have marked tone in buffaloes.

The length of the oestrous cycle is 21 days ranging from 17 to 26 days and length of oestrus is 10 hours (Range: 5-27h). The ovulation occurs after 34 hours after the onset of oestrus (Range: 24-48) and after 14 hours (Range: 6-21) at the end of oestrus. Length of gestation is 310 days in river type (Range: 300-340 days) and 330 days in swamp (Range: 320-340 days) type buffaloes. Birth weight of calves is recorded as 26 kg (Range: 22-36) and involution of uterus take place after 30 days (Range: 25-35 days) in both type of buffaloes (Bmao Perera, 2008; B. Perera, De Silva, Kuruwita, & Karunaratne, 1987; Presicce, 2007).

Buffaloes are considered poor breeders and their reproductive efficiency is affected by delayed puberty, seasonality of calving, long postpartum anoestrus, weak oestrus signs, low conception rate and long calving intervals (Barile, 2005). Low reproductive efficiency provides a major limitation for commercial dairy production from buffaloes (Shah, Willemse, Van De Wiel, & Engel, 1989b).

According to Shah et al. (1989b) reproductive efficiency of dairy animals is the outcome of the interaction of various factors. These factors include environment, physiology, and management such as oestrus detection, mating procedures (natural or artificial techniques) fertility of the bull or semen quality and herd health policies.
Several researchers from countries with high buffalo populations (India, Pakistan and Egypt) have concluded that the seasonality of breeding and parity influence the reproductive efficiency of dairy buffaloes (Ahmad et al., 1981; Cady, Shah, Schermerhorn, & McDowell, 1983; Gangwar, 1980; Sharma, Gandotra, Prabhakar, & Nanda, 2010). These authors have concluded that those animals calving in winter and spring (November to April) have significantly longer inter-calving intervals than those calving over late summer and autumn.

The long calving interval in winter and spring (November to April) may be due to summer starting in May and ending in September. The shorter inter-calving interval in summer and autumn (May to November) calving animals has been confirmed in a number of studies (Ahmad et al., 1981; Cady et al., 1983; Gangwar, 1980; Sharma et al., 2010). Many authors have also found that an increase in parity has a positive effect on reproductive efficiency (Cady et al., 1983; Naqvi, 2000; Shah et al., 1989b; Vale, 2010).

Shah et al. (1989b) studied the effect of parity and season on various reproductive parameters in 600 Nili-Ravi buffaloes in the Punjab region and concluded that even in the presence of a strong relation between parity and season, reproductive efficiency is better in those buffaloes calving in summer and autumn. The reason for better reproductive efficiency in summer calving animals might be related to the decreasing photoperiod associated with increased melatonin secretion from the pineal gland and its effect on the hypothalamic-pituitary-gonadal axis (Shah, Van De Wiel, Willemse, & Engel, 1989a; Shah et al., 1989b).

1.4. Inter calving interval and Economics:

In Pakistan the majority (85%) of livestock is reared in rural areas by landless (Borghese, 2005; K. Singh & Pundir, 2003) farmers under closed management conditions. The animals are tied and kept in closed sheds. The farmers take them out during day time and tie them under the shade of a tree in summer and without shade in winter. In winter during night time when temperatures drop to zero Celsius the farmers keep their sheds closed, sometimes being completely air sealed (personal experience).

Shah et al. (1989b) have also reported a long inter calving interval and inter service interval for winter calved buffaloes. Hurnik, King, and Robertson (1975) and Esslemont and Bryant (1976) studied postpartum oestrus behaviour in Holstein and Friesian cows and they reported that animals in close confinement show more oestrus signs during night time. The situation is
further aggravated with a high percentage of calving occurring in autumn and winter (Shah et al., 1989b) while Ahmad et al. (1981) reported that the maximum incidence of calving in buffaloes occurs between July and September.

In winter the animals are confined in closed spaces with limited observation by the farmers especially during night time when they show maximum signs of heat. Thus, these factors combine in buffaloes to cause long inter calving intervals.

The optimal inter-calving interval for buffaloes is 360-390 days (Shah, 1990; Shah et al., 1989b). A calving interval less than 400 days is generally considered ideal. The inter-calving intervals reported so far in buffaloes in Pakistan and from other buffalo populated countries are shown in the following tables (1.1).

**Table 1** Inter-calving interval in buffaloes reported from Pakistan and other countries where buffaloes are found. (CI: Calving Interval)

<table>
<thead>
<tr>
<th>Species (country)</th>
<th>Mean CI in Days (Range)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo (Pakistan)</td>
<td>552.78 (445-685)</td>
<td>(Ahmad et al., 1981; Ashfaq &amp; Mason, 1954; Cady et al., 1983; M. S. Khan, Ahmad, &amp; Khan, 2007; Shah et al., 1989b; Zafar, 1985)</td>
</tr>
<tr>
<td>Buffalo (Sri Lanka)</td>
<td>530.00 (N/A)</td>
<td>(Lundström, Abeygunawardena, De Silva, &amp; Perera, 1982)</td>
</tr>
<tr>
<td>Buffalo (India)</td>
<td>512.34 (337-611)</td>
<td>(Kandasamy, Lagaithan, &amp; Krishnan, 1993; B. Yadav, Yadav, Singh, &amp; Khan, 2007)</td>
</tr>
<tr>
<td>Buffalo (Egypt)</td>
<td>460.75 (345-584)</td>
<td>(Aziz, Schoeman, Jordaan, El-Chafie, &amp; Mahdy, 2001; Marai, Daader, Soliman, &amp; El-Menshawy, 2009)</td>
</tr>
<tr>
<td>Buffalo (Italian)</td>
<td>440.70 (N/A)</td>
<td>(Rosati &amp; Van Vleck, 2002)</td>
</tr>
</tbody>
</table>

Details of some of the Pakistani studies are provided here. Ashfaq and Mason (1954) studied a herd of Nili-Ravi buffaloes from 1947 to 1951 and reported that calving interval can be decreased from 685 days to 385 days with improved reproductive management. These
workers showed that continuous close observation of animals for oestrous behaviour during winter was an important aspect of management.

Another peculiar characteristic of buffaloes is the expression of silent heat and summer anoestrus. Due to all these factors only 17.2% buffaloes have a calving interval of less than a 400 days (Shah et al., 1989b).

Summer infertility associated with low oestrus frequency in buffaloes is due to the regression of size and activity of ovaries associated with environmental stress (Roy & Prakash, 2007; Shah, 2010; Shah et al., 1989a; C. Singh, 2003; G. Singh, Dhaliwal, Sharma, & Biswas, 1988). The long photoperiod inhibits the pineal gland which secretes melatonin over a shorter time period and this in turn controls the change from light to dark (Carcangiu et al., 2011).

Cady et al. (1983) conducted a study on two herds of Nili-Ravi buffaloes at the Livestock Research Institute (LRS) at Bahadurnagar (Okara) Punjab Pakistan. They studied 6054 calving of 1531 cows from 1951 to 1978. They reported calving intervals ranging from 497 to 525 days and days open ranging from 206 to 235 days.

Along with summer anoestrous according to Usmani and Mirza (2000) the animals calving from February to May had the highest interval to first postpartum observed oestrus (PPIE) ranging from 170.8 ± 16.8 days in February to 114.8 ± 11.3 days in May (Average: 139.78 ± 15.25 days). Therefore special care and attention is required for winter and spring calving animals before they enter the summer season. However breeding the entire herd in one season will cause problems in the continuous supply of milk to the market across the year. To overcome hurdles in continuous supply of milk to the market, modern management techniques such as oestrus synchronization and detection can be used as tools for improving herd fertility. In the case of summer anoestrous and the expression of silent heat, we need to find an efficient way or technique to solve these problems in Pakistan to ensure that positive results are achieved with artificial insemination and natural mating.

In Pakistan the farmers in rural areas are less educated and their level of understanding of farm economics is very poor. There is also a scarcity of research on farm economics to generate basic data for economic policy formulation targeting the dairy sector. In Pakistan very few people (Shah, Dijkhuizen, Willems, & Van de Wiel, 1991; Teufel, 2007; Teufel & Gall, 1999; Zafar, 1985) have worked on this in the dairy sector. This may be due to the lack of availability of farm records, the lack of farmer education, and the skills provided by dairy
extension workers. The high inflation rate makes the comparison with past data very difficult also.

Ishaq (1961) had reported an annual loss of 251,000 tonnes of milk and 800,000 calves as a result of delayed and repeat breeding in buffaloes of Pakistan. Shah et al. (1991) reported a loss of 9 to 14 Pakistani rupees (PKR) for each days increase in calving interval. Farmers are often forced to replace their stock due to poor reproductive efficiency and this costs them an average of PKR 133 per buffalo on the farm (Shah et al., 1991). In 1991 the milk price was PKR 4 when the Pakistani rupee/US dollar conversion was 25. The present milk price varies from 60 to more than 80 rupees per litre and the dollar conversion rate is round 100 rupees per US dollar (Historical Exchange Rate Regime of Asian Countries, 2000; Shah et al., 1991). Furthermore, Jalil, Rehman, Sial, and Hussain (2009) reported that the average spending of a farmer is PKR16,370 per month and PKR3274 per day. The farmer’s daily spending includes PKR 140 on labour, PKR 2906 on feed, PKR 18 on vaccination, PKR 36 on medicine, PKR 56 on utility bills and PKR 119 on transportation of milk.

Therefore, with a herd of almost 32.7 million buffaloes (Ministry of Finance Government of Pakistan, 2011-12), predominantly (85%) in rural areas (Borghese, 2005; K. Singh & Pundir, 2003), with an agricultural based economy, high inflation and a relatively poor economic condition of rural farmers, a slight decrease/increase in calving interval can have a drastic effect on the well-being of farmers and the national economy.
Chapter 2. Literature Review

2.1. Reproduction Problems in buffaloes:

According to the Pakistan economic survey (Ministry of Finance Government of Pakistan, 2011-12) the population of buffaloes is 32.7 million providing 29,565 thousand tonnes of milk annually. Buffaloes however suffer from reproductive problems including long prepubertal period, seasonal breeding and silent oestrus. Furthermore, low fertility due to wrong timing of insemination, failure in heat detection and environmental stress in summer further aggravate the problem (Bhosrekar, Phadnis, Gokhale, & Mang, 1994; Shah, Willemse, & Van de Wiel, 1990). These problems alone or collectively contribute towards prolonged inter calving intervals (Shah et al., 1989b).

Buffaloes are polyoestrus, but the oestrus expression, conception and calving is seasonal. The expression of heat is greater in winter and therefore domestic buffaloes have an inclination towards seasonal breeding (M. Qureshi, Habib, Samad, Lodhi, & Usmani, 1999a) with a suspension of sexual activity during summer in many parts of the world (E. Hafez, 1955; Shah, 1990).

Buffalo body temperature is lower than cattle. Buffaloes are heat absorbent due to their black skin with less hairs and the dissipation of heat is also poor due to a lower density of sweat glands. In comparison with cattle, buffalo skin has one-sixth the density of sweat glands of cattle skin (Muhammad, 1973).

Therefore buffaloes are very prone to thermal stress (Pandey & Roy, 1966) causing hyperprolactinemia and low luteinizing hormone (LH) concentrations. The fewer sweat glands further delay heat dissipation and cause thermal stress (Acharya, 1990; Cockrill, 1993; Heranjal, Sheth, Wadadekar, Desai, & Rao, 1979). The hyperprolactinemia induced by high ambient temperature is also associated with increased photoperiod causing changes in pineal gland secretary activity. Day length controls the function of the pineal gland. High levels of prolactin (2 to 6 fold high) have been reported in summer months. It has been assumed that high levels of prolactin interfere with oestrus cycle and fertility. Prolactin interferes with ovarian steriodogenesis by decreasing the number of LH receptors. Furthermore it is also reported that prolactin blocks the hypothalamic mechanism responsible for episodic release
of LH and blocks the positive feedback of oestrogen on LH secretion. This causes problems in successful reproduction (Sheth, Wadadekar, Moodbidri, Janakiraman, & Parameswaran, 1978; Wettemann & Tucker, 1974). In temperate regions where buffalo are found such as Italy where feeding management is very good, a distinct seasonal reproductive pattern also prevails and according to Borghese (2005) and Zicarelli (1997b) seasonality is influenced by photoperiod through changing melatonin secretion which reduces fertility in a seasonal manner (Morgan & Williams, 1989). This effect of light on the animal is termed as ‘sexual photoperiodicity’ (EL-Battawy K, 2002).

Seasonality is one of the prominent factors affecting the pattern of reproductive activity (Barile, 2005). In summer 74-86% animals show anoestrus, while in winter the percentage is only 22-26% (G Singh, Singh, & Dhaliwal, 1989; Tailor, Jain, Gupta, & Bhatia, 1990). Previously, high ambient temperature and low fodder availability was considered as a reason for anoestrous in buffaloes.

The reason behind the occurrence of anoestrous in buffaloes during summer was anticipated to be the collective effect of high ambient temperature and low fodder availability (Shah et al., 1989a). However, this reason is not greatly significant because buffaloes managed on a balanced diet with a continuous supply of green fodder still exhibit a seasonal reproductive pattern (Zicarelli, 1992). Also only buffaloes showed seasonal reproductive behaviour when reared along with cattle in similar conditions (Shah, 1988). Although, the period of day length showed a negative correlation with the number of buffaloes showing oestrus, the provision of experimental darkness reduced the problem of anoestrus during periods of long day length (J. Singh, Nanda, & Adams, 2000).

In photoperiod sensitive seasonal species (ewe and mare) GnRH and LH are regulated by melatonin and this may be similar to the seasonal activation and regulation of ovarian cyclicity in buffaloes (Borghese, Barile, Terzano, Pilla, & Parmeggiani, 1995; Zicarelli, 1997b).

Melatonin is secreted by pineal gland during darkness. Thus a decrease in day length is associated with longer pattern of melatonin secretion. This in turn has a stimulatory effect on short day breeder (ewe) reproduction. This is achieved by increasing GnRH/LH pulses due to a change in the pattern of melatonin release from one major pulse in 6 hours during long days to 10 pulses each 6 hours in short days in ewes. Further studies of the neuroendocrine system
in ewes suggest that melatonin targets the hypothalamic and pituitary axis and thus enhances reproduction (Malpaux, Viguié, Skinner, Thiéry, & Chemineau, 1997).

The impact of melatonin administration has not been investigated much in buffaloes; few attempts have been made to understand the relationship between plasma melatonin concentrations and seasonal reproductive pattern. The Mediterranean buffaloes having seasonal reproductive patterns showed high plasma melatonin concentration in winter and low in summer (Parmeggiani et al., 1994; Parmeggiani et al., 1993). In another study on buffaloes (heifers and cows) there was season dependent difference in melatonin. In summer melatonin production was low due to short periods of darkness overnight, while in winter there was high plasma melatonin concentration corresponding to the activation of hypothalamus-pituitary-ovarian axis (Borghese et al., 1995).

Low plasma melatonin is also associated with ovulation failure in long summer days even in the presence of ovulatory size (12-14 m) follicles in nulliparous and multiparous Mediterranean buffaloes (Parmeggiani et al., 1994; Presicce, Senatore, De Santis, & Bella, 2005; Zicarelli, 1997b).

European countries use subcutaneous melatonin implants widely in ewes for the activation of the hypothalamic-pituitary GnRH/LH axis. Through slow release, this implant imitates the effect of short days by extending melatonin presence in the circulation (Gomez, Balasch, Gomez, Martino, & Fernandez, 2006; Misztal, Romanowicz, & Barcikowski, 2002). However, to cause oestrous in anoestrous ewes several weeks of exposure to melatonin implants are required. This exposure causes crucial change in the feedback action of oestradiol on GnRH/LH. This change plays an important role in the activation of gonadotropic system in anoestrous ewes (Viguie, Caraty, Locatelli, & Malpaux, 1995).

Furthermore, daily melatonin injection can induce oestrus in summer anoestrous buffaloes (Hassan, Battaway, Menoufy, Younis, & Khattab, 2000). But its short half life (<30 min) provides a narrow window for its therapeutic use (Nowak & Zawilska, 1998). Therefore further research is needed on the use of melatonin in summer anoestrous buffaloes.

Hyper-prolactinemia is a significant cause of sub fertility and ovarian inactivity by suppressing gonadotropin secretion and ovarian steroid synthesis thereby decreasing progesterone in the blood supply (E. Hafez, Jainudeen, & Rosnina, 2000; Razdan, Kaker, & Galhotra, 1981; Reiter, 1991, 1998; Roy & Prakash, 2007; Wettemann & Tucker, 1974). Low levels of circulating LH due to high ambient temperature and photoperiod cause poor follicle
maturation and thus a low level of oestradiol (Heranjal et al., 1979; Palta, Mondal, Prakash, & Madan, 1997).

Management and nutrition are also important contributory factors for summer anoestrous (Kaker, Razdan, & Galhotra, 1982; M. Qureshi et al., 1999a; M. Qureshi, Samad, Habib, Usmani, & Siddiqui, 1999b; Zicarelli, 1997b). The reason for 36.5% of breeding Egyptian buffaloes not being pregnant in the study of Heranjal et al. (1979) was nutritional deficiency. In summer the hot ambient temperature decreases feed resources (Mudgal, 1979) and intake (M.R Jainudeen, 1988), thus causing loss in body condition score and therefore reproductive condition. Management plays an important role in heat detection. Buffaloes are not renowned for detectable oestrous expression (M.R Jainudeen, 1988), which is often silent (Chaudhry, 1988) and mostly occurs during night time: the problem is exacerbated by hot summers.

During silent heat, animals only show physiological symptoms and no behavioural signs of oestrus. There are similarities in the general pattern of sexual behaviour in both cattle and buffaloes. The major reason for anoestrus relates to the lack of an efficient system of thermoregulation in buffaloes during high environmental temperature and relative humidity (Drost, 2007). Thus this summer stress compels the animals to suspend behavioural signs. Furthermore, the problem of silent heat is more pronounced and common in close confined animals tied to an object on concrete floors without the provision of any free space for exercise and any protection from the extreme of the weathers (Suthar & Dhami, 2010).

There are some other factors that affect the length of oestrous cycle and heat expression in buffaloes.

The major role of hormones in the expression of oestrus and the fact that buffaloes respond well to exogenous hormones (De Rensis & López-Gatius, 2007) suggest that the use of hormones to control fertility and decrease inter calving intervals (Madan, Naqvi, Tri, & Prakash, 1983; BMAO Perera, 2011) may be a viable way of minimising the problem of long inter calving interval. Various hormones are used in different combinations in cattle and buffalo so far: progesterone based protocols are considered to be the most effective in controlling summer anoestrus (Barile et al., 2001; Naseer, Ahmad, Singh, & Ahmad, 2011; Neglia et al., 2003; G. Singh, Singh, Sharma, & Nanda, 1984). High circulating progesterone levels increase ovarian follicle turnover and also sensitize the hypothalamus and pituitary to gonadal activity (Baruselli, 2001).
Reproductive problems in Buffaloes are most commonly related to nutritional imbalance, milk yield, parity, health status and history, season, failure to detect oestrus and incorrect timing for artificial insemination.

Inadequate nutrition is the single largest factor affecting reproduction. In developing countries like Pakistan under feeding is very common in rural areas due to the poor economic condition of the farmers and a lack of knowledge. Hot weather and poor management strategies along with under feeding cause further drastic effects on reproduction. The hot summer seasons lead to disturbances in the hormonal balance of the animals. It is possible to correct these imbalances through the injection of exogenous hormones (Shah et al., 1990).

Data from India, Pakistan and Egypt show that 31 to 42 percent of buffaloes remain in anoestrous 150 days post partum (El-Wishy, 2007). Balanced nutrition is very important and any deficiency will cause problems: for example a deficiency of vitamin A and specific minerals in the feed prevent maturation of graafian follicles (J. Singh et al., 2000). Along with nutrition, various types of environmental stresses are responsible for abnormal cyclicity and lack of prominent oestrus (M. Qureshi et al., 1999a; M. Qureshi et al., 1999b). Although nutrition is the major limiting factor in buffalo reproduction, there is a very poor, understanding of the correct way to feed animals in developing countries like Pakistan, where 62% of the population live in rural areas.

The target of this study is to identify resources that can be used to improve the reproductive efficiency of the 64-75% of animals with varying degrees of ovarian activity and the 30% of cycling animals expressing silent or no oestrus in summer season. This will also solve the question of synchronization efficacy in cycling animals. Among the various oestrus synchronization methods, progesterone based protocols are reported to have higher efficacy as compared with other protocols used during the low breeding season (De Rensis & López-Galtius, 2007; C. Singh, 2003).

2.2. The problem of summer anoestrus:

The summer anoestrus is a major issue in the growth of dairy industry across the world. The seasonal breeding in buffaloes (either a complete suspension or very low level of sexual activity during summer) has been reported from many countries including Pakistan, India and Egypt (Barile, 2004; M. Qureshi et al., 1999a; Shah, 1990; Shah et al., 1989b). This condition
of sexual inactivity is popularly known as summer anoestrus characterized by small inactive
smooth ovaries and abnormal hormonal profiles (Das & Khan, 2010). A similar condition is
observed in Mediterranean countries during spring, called spring anoestrus (Das & Khan,
2010). The summer anoestrus causes a delay in postpartum conception resulting in long inter
calving intervals. Inter-calving interval is long in animals calving before the start of summer
season and short in animals calving at the end of summer season.

The incidence of summer anoestrus was reported to be higher in nomadic buffaloes (83.0%)
than household rural ones (63.0%). This may be attributed to a high frequency of exposure to
direct sun light in nomadic herds (Das & Khan, 2010). Thermal stress plays a major role,
potentially with low thyroid activity contributing to the ovarian inactivity. Also seasonality in
buffalo reproduction is mainly related to environmental factors and not to genetic factors
Environmental factors such as photoperiod or day length, temperature, relative humidity and
rainfall alone or in combination play very important roles (Ribeiro, Vale, Andrade, &
Marques, 2003).

Furthermore Zicarelli (2010) stated that the reason behind summer anoestrus may relate to
the combination of long photoperiod and poor body condition score through under-feeding.
He compared the environmental temperatures in Pakistan and Italy during anoestrus seasons
in his review. The temperature in Italy remains in the range between 13.5 to 23.5°C, but the
longer photoperiod causes anoestrus, while in Pakistan the temperature remains very high
(above 35°C). He further stated that an improvement in body condition score in autumn
initiated the breeding season in buffaloes. In Pakistan maximum calving in buffaloes occurs
between July and September (Ahmad et al., 1981). The long photoperiod initiates the pineal
gland which secretes melatonin, and this in turn controls reproductive responses associated
with changes in the periodicity of the daily light and dark phases (Carcangiu et al., 2011).

Along with summer anoestrous according to Usmani and Mirza (2000), the animals calving
from February to May have the highest interval to first postpartum observed oestrus (PPIE)
ranging from 170.8 ± 16.8 days in February to 114.8 ± 11.3 days in May (Average: 139.78 ±
15.25 days). The average postpartum interval to first observed oestrus (PPIE) for the whole
year was 104.7 days. They also reported a similar pattern for postpartum interval to
conception. The month of calving had a significant effect on postpartum interval to oestrus.
The animals calving in February to May had a longer postpartum interval to conception
ranging from 144.7 to 211.7 days and those calving in the breeding season from August to
December had a short postpartum interval to conception ranging from 51.8 to 71.7 days. The average postpartum interval to conception or service period year-round was 104.7 days in their study. These results are quite different from Naqvi (2000), who reported a postpartum interval to conception ranging from 210 to 243 days. Usmani and Mirza (2000) conducted their experiment on the Pakistan Agriculture Research Council (PARC) dairy farm on 103 buffaloes under good feeding and management conditions. This might be the reason that the postpartum interval to conception is less. The postpartum interval to conception is long on small holder rural farms, where management and husbandry practices are poor along with poor nutrition. In contrast Naqvi (2000) conducted his study on military farms located across the country. He studied service period records of 1921 animals maintained at Military dairy farms at Peshawar, Rawalpindi, Khuber, Okara, Punjab and the Livestock Research Station (LRS) of the National Agricultural Research Centre (NARC) Islamabad Pakistan. Furthermore, on the basis of sample size and data from across the country, the results of Naqvi (2000) came from a more effectively designed study compared with the report of Usmani and Mirza (2000). But on these government and military farms animals were kept unrestrained with free access to adequate drinking water. These conditions are not available on the majority (85%) of the small rural farms contributing 65 to 70% of total national milk production of Pakistan (Borghese, 2005; K. Singh & Pundir, 2003). Therefore there is a need to investigate these issues on small scale rural farms, where a lack of facilities and poor management can further exacerbate the problem of summer postpartum anoestrous. There is no reliable data available for small rural farms and the situation might be worse than expected. Keeping in view the importance of small rural dairy systems in Pakistan, the current study is designed to include both large government and small rural farms having 1-10 animals.

2.3. History of Oestrus Synchronization:

The concept of oestrus synchronization and use of hormones to pharmacologically control the oestrous cycle was first based on blocking ovulation. Progestogens were first used for blocking ovulation. This resulted in reasonable oestrus synchrony but the pregnancy rates were depressed and lower especially with exogenous progesterone administered over longer periods (Christian & Casida, 1948; Odde, 1990). The hormones used for oestrus synchronization were either analogs or identical to the native reproductive hormones e.g. oestradiol, progesterone, prostaglandin F2α (PGF2α) and GnRH.
In 1970 the discovery of PGF2α by Pharriss (1971) gave hope for new methods of oestrus synchronization. The role of PGF2α as a luteolytic agent was influential in the development of new synchronization protocols. In 1990s with an improvement in our understanding of follicular dynamics it was revealed that persistent dominant follicles were the reason for lower pregnancy rates. (Lucy, McDougall, & Nation, 2004). With these new developments various research programs were designed targeting follicular development, ovulation and various phases of the oestrous cycle.

2.4. Oestrus synchronization in Buffaloes:

Synchronization of oestrus and ovulation is based on controlling the two phases of the oestrous cycle, the luteal and the follicular phases. This control and manipulation can be achieved through using external hormones. This is necessary to achieve acceptable pregnancy rates (Presicce, 2007). The buffalo has many similarities with cattle and the introduction of new techniques in cattle is often followed in buffaloes either as such or with slight modification. The reason behind this may be that the higher population of Buffalo is in developing countries of South Asia. These countries are lacking the modern research facilities found in developed countries (Bhosrekar et al., 1994).

Hormonal treatments and oestrus synchronization were initially used to facilitate artificial insemination and save time, labour and solve difficulties associated with the detection of heat in large herds of cattle. Furthermore, these techniques were also used to improve fertility in the low breeding season and to advance puberty in order to reduce the age at first calving (Barile, 2004).

The most common approaches used for oestrus synchronization are as follows (Busch et al., 2007; Cavalieri, Rabiee, Hepworth, & Macmillan, 2005; Lamb et al., 2006; Patterson, Kojima, & Smith, 2003; Schafer et al., 2007).

- Shortening of luteal phase using a luteolytic agent (prostaglandin F2α or its analogues)
- Prolonging luteal phase using progesterone treatments.
- Synchronizing or controlling follicular waves using progesterone along with oestradiol (E$_2$), GnRH.
Mixing of two or more protocols e.g. synchronizing follicular waves followed by luteolysis.

The four phases of buffalo oestrous cycles are similar to those of cattle. Much research work has been done on the reproduction of both swamp and riverine buffaloes. McCool and Entwistle (1989) found an oestrous cycle length of 18-24 days and the duration of oestrus of 11-23 hours.
<table>
<thead>
<tr>
<th>Buffalo type</th>
<th>Oestrous cycle length (Days)</th>
<th>Oestrus duration (Hours)</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swamp</td>
<td>18-24</td>
<td>11-23</td>
<td>Australia</td>
<td>(C. J. McCool &amp; Entwistle, 1989)</td>
</tr>
<tr>
<td>Riverine (Nili-Ravi)</td>
<td>21 to 22</td>
<td>6-16</td>
<td>Pakistan</td>
<td>(Warriach, Channa, &amp; Ahmad, 2008)</td>
</tr>
</tbody>
</table>

In the past the reason for the long inter-calving interval in buffaloes was considered to be the delay in involution of the uterus, delayed resumption of ovarian activity and poor reproductive performance of the bull. Later on B. Perera et al. (1987) reported that the uterine involution is completed by about 40 days after calving, while Usmani and Mirza (1985) reported a mean interval of 25.6 days for complete uterine involution for Nili-Ravi buffaloes in Pakistan. Rajamahendran and Thamotharam (1983) stated that libido and seminal characteristics did not vary significantly from month to month and according to Perera et al. (1980) the ovarian activity is delayed even under optimum management conditions. In Pakistan, according to some researchers the month of calving and age has no influence on ovarian activity, especially follicular recruitment and first postpartum oestrus in Nili-Ravi buffaloes (Usmani et al., 1985; Usmani & Mirza, 2000).

These findings indicate that the long calving interval may be due to delay or failure in ovarian activity and can be corrected with the injection of exogenous hormones into the blood supply. According to De Rensis and Lopez-Gatius (2007) buffaloes respond well to exogenous hormones. Therefore various researchers have worked on the correction of this problem, either targeting the luteal phase or follicular phase of the oestrous cycle.(De Rensis & López-Gatius, 2007).

Reproductive efficiency can be increased with the artificial control of the oestrous cycle. For this purpose several protocols targeting either the luteal or follicular phase have been developed. These protocols either induce luteolysis or prolong the luteal phase by using PGF2α or progestagens respectively (Senger, 2005).

However to achieve tighter synchronization of ovulation and improve fertility, a more precise control over the oestrous cycle is required. Therefore various hormones are been used to
control the luteal phase using control internal drug release (CIDR) mechanisms with either
prostaglandins or progesterone analogues (norgestomate, progesterone releasing intravaginal
device (PRID) (De Rensis & López-Gatius, 2007; De Rensis et al., 2005; Simoneit,
Heuwieser, & Arlt, 2011).

In conclusion, whatever techniques are used, the aim should not only to get tighter synchrony
but also to increase and achieve acceptable levels of fertility.

Different protocols and hormones have been used for oestrus synchronization. The following
is a list of protocols used so far in buffaloes.

2.5. Prostaglandin:

Prostaglandins were initially considered as a product of the prostate gland. Ulf von Euler of
Sweden first discovered and isolated prostaglandin from human semen in the 1930s (O'Neil
& Grisham, 2003). Therefore prostaglandin was thought to originate from the prostate gland.
After further research it was determined that prostaglandins exist and are synthesized in
virtually every cell of the body (O'Neil & Grisham, 2003; Rowson, Tervit, & Brand, 1972)

2.5.1. Prostaglandin Structure:

Prostaglandins are unsaturated carboxylic acids, consisting of a 20 carbon skeleton that also
contains a five member ring which is based upon the fatty acid, arachidonic acid. There are a
variety of structures one, two, or three double bonds. Attached to the five member ring there
may also be double bonds, a ketone, or alcohol groups (O'Neil & Grisham, 2003).

2.5.2. Functions of Prostaglandins:

Prostaglandins have a variety of physiological effects some of which are mentioned below:

1. They activate the inflammatory response and initiate production of pain and fever.
   When a tissue is damaged, white blood cells are redirected to the affected site to
   minimize tissue destruction, with prostaglandin being produced as a result (O'Neil &
   Grisham, 2003; Rowson et al., 1972).

2. They also play a role in blood clotting. The type of prostaglandin responsible is called
   thromboxane. Thromboxanes stimulate constriction and clotting of platelets. There is
   another prostaglandin 12 (PG12) having an opposite effect on the walls of blood
   vessels in minimizing clotting (O'Neil & Grisham, 2003; Rowson et al., 1972).
3. They also are involved in the induction of labour and other reproductive processes. For example PGE2 causes uterine contractions and has been used to induce labour. PGF2α helps in the regression of the corpus luteum (CL). The regression of the CL is important for termination of pregnancy, initiation of labour and oestrous cycle (O’Neil & Grisham, 2003; Rowson et al., 1972).

4. They play an important role in digestion by inhibiting acid synthesis and supporting the secretion of mucus to protect the gastrointestinal tract from acids (O’Neil & Grisham, 2003; Rowson et al., 1972).

After the discovery of the luteolytic activity of PG and its analogues in cattle (Rowson et al., 1972) it was combined with progestins for controlling the oestrous cycle. The effect and use of PGF2α in buffaloes is very similar to that in cattle. Injection of PGF2α on day 5 of the oestrous cycle causes regression of the corpus luteum (CL) and progesterone levels to decline within 24 hours to basal levels, resulting in initiation of oestrus and ovulation. In the breeding season single or double injection treatment with PGF2α induces about 60-80% of animals to display oestrus and ovulation in buffaloes (De Rensis & López-Gatius, 2007).

Exogenous PGF2α causes luteolysis and is effective in about 60% of the cycle in most species. Its effect starts from day 6 of the oestrous cycle (after the formation of CL) in the cow and a similar effect is assumed in buffalo as well. Due to its luteolytic activity PGF2α is used for oestrus synchronization, abortion and to initiate parturition (Senger, 2005).

This protocol forms a part of my study and the reason behind selection is its extensive use in Pakistan where many practitioners use PGF2α without proper timing and often administer only one injection (3 years Personal experience). The limitation with prostaglandin use as a single injection is that a functional CL is required for its success. Therefore the standard protocol consists of two PGF2α injections at an interval of 11-14 days. So if the first injection misses the CL, the second injection 11 days later will synchronize the animal (De Rensis & López-Gatius, 2007).

Barselli (1994a) found a variation of 36-96 hours in the onset of oestrus after prostaglandin injection. Porto Filho et al. (1999) observed the cow by radiotelemetry injected before and after day 10 of their oestrous cycle. Animals injected before day 10 showed oestrus within 40.4+/-2.1 hours and ovulation within 56.3+/-3.3 hours. Animals injected after day 10 showed longer periods to oestrus and ovulation (70+/-11.3 and 87.2 +/-12.9 hours respectively) (P<0.01). Perera et al. (1987) conducted two trials on buffalo heifers using a
two PG shot protocol 11 days apart on two groups of animals comparing natural mating versus fresh semen AI and reported pregnancy rates of 33% and 30% respectively. Khattab, Ibrahim, Mohsen, and El-Shamaa (1996) conducted a trial on four different analogues of prostaglandin on Egyptian buffaloes and recorded significant variation in response rate and conception over a five year period. The animals not responding were re-injected with a second injection. The oestrus response and conception rate is given in table 3. There was no significant difference in oestrous response between seasons, but conception rate was lower in summer (39.1%) than in winter (64.0%).

Table 3 Responses in oestrus and conception to four commercial prostaglandin analogues administered to Egyptian buffaloes. (Ibrahim, 1987; Khattab et al., 1996)

<table>
<thead>
<tr>
<th>Market Name</th>
<th>Dose rate (ml)</th>
<th>Oestrus response (%)</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzaprost®</td>
<td>2.5</td>
<td>57</td>
<td>69.0</td>
</tr>
<tr>
<td>Lutalyse®</td>
<td>5</td>
<td>73.2</td>
<td>41.5</td>
</tr>
<tr>
<td>Prosolvin®</td>
<td>2</td>
<td>70.7</td>
<td>70.7</td>
</tr>
<tr>
<td>Estrumate®</td>
<td>2</td>
<td>79.5</td>
<td>72.9</td>
</tr>
</tbody>
</table>

2.5.3. Gonadotropin releasing hormone (GnRH):

Neurons in the hypothalamus produce hormones. The basic role of the hypothalamic hormones is to cause the release of other hormones from the anterior lobe of the pituitary. GnRH is the primary releasing hormone of reproduction released from terminals neurons in the surge and tonic centres of the hypothalamus. GnRH, a decapeptide causes the release of two important reproductive hormones from the anterior lobe of the pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH). Oxytocin is also produced from the pituitary but from the posterior lobe. Along with hypothalamic and pituitary hormones, reproductive hormones also originate from gonads (ovary in the female produce oestrogens, progesterone, inhibin, some testosterone, oxytocin and relaxin, while testes in the male produce testosterone and other androgens, inhibin and oestrogen), the uterus (PGF2α) and placenta (progesterone, oestrogen, equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG)) (B. Hafez & Hafez, 2000; Senger, 2005).

Rhythms of GnRH occur in small quantities spontaneously from tonic centre. GnRH is released after 1.5 to 2 hours and 4 to 8 hours during the follicular phase and luteal phase respectively. The small amount of GnRH releases a small quantity of LH from the anterior lobe of the pituitary. This small quantity of LH helps in recruiting of follicles. High
progesterone levels in the blood supply have negative feedback effects on GnRH release: therefore the episodes of GnRH occur at 4 to 8 hour intervals in the luteal phase (B. Hafez & Hafez, 2000; Senger, 2005).

For successful ovulation a high dose of GnRH is required for the release of the preovulatory LH surge. This surge of GnRH followed by a surge of LH occurs when the oestradiol level secreted by development follicle increases in the blood supply during the late follicular stage. This is also called positive feedback. On the other hand low oestradiol in the presence of high level of progesterone suppresses the GnRH preovulatory surge centre.

In summary the tonic release of GnRH followed by FSH and LH helps in growth and development of the follicle while the surge centre helps in ovulation by releasing a surge of GnRH followed by FSH and LH (Senger, 2005)

**Oestrogens:**

Oestrogen is an 18-carbon steroid released from theca interna of the ovarian follicle. The primary oestrogen is oestradiol. Oestrone and oestriol are other metabolically active oestrogens. Oestrogens are steroids and carried by a binding protein in the systemic circulation to the target site. Oestrogens have widest broad spectrum of physiological functions as compared with other steroid hormones. Some of these functions are listed below.

1. Expression of oestrus behaviour in the female by acting on the CNS. However, in some species like the cow and ewe a small amount of progesterone is needed to induce oestrus.

2. Increase frequency of uterine contraction.

3. Changes in the physiology of female secondary sex characteristics.


5. Cause negative and positive feedback on LH and FSH release (B. Hafez & Hafez, 2000; Senger, 2005).

**2.5.4. Placental hormones: Equine Chorionic Gonadotropin (eCG) and Human chorionic gonadotropin (hCG)):**

Equine Chorionic Gonadotropin (eCG) is also called pregnant mare’s gonadotropin (PMSG) and produced in the early embryonic life by the endometrial cups in equine uterus, formed at around days 35 and 60 of pregnancy and are sloughed into the uterine lumen after day 60. A
glycoprotein with beta subunits, eCG is similar to LH and FSH in structure, but has more carbohydrate content (especially sialic acid). This extra carbohydrate helps in maintaining the long half life of this hormone in the circulation. Therefore a single injection of eCG can have its biologic activity for more than a week on the target tissue.

The biological action of eCG is similar to FSH and LH (luteotropic): the FSH action is stronger as compare to LH action. In luteotropic action eCG helps in formation of primary and secondary corpus luteum (CL) in the mare. The CL produces progesterone, thus eCG enhances progesterone production from the ovary. In addition to LH-like actions, eCG has a strong FSH-like function when injected into the female of other species, stimulates follicles and is mostly used for super ovulation in cows, sheep and rabbits during embryo transfer. Human chorionic gonadotropin (hCG) is found in human and many other primates. It originates from trophoblastic cells of the chorion and is available in pregnant women’s urine as early as day 8 to 10 of gestation. It helps in CL formation and has more LH-like activity. It causes ovulation when injected to non-primates females and is also used for super ovulation (Cole, 2009; B. Hafez & Hafez, 2000; Senger, 2005).

2.5.5. Ovsynch:

This protocol is widely used in cattle across the world with various refinements (pre-synch, Co-synch, double synch etc) and success. The Ovsynch protocol consists of a GnRH injection on day 0, PGF2α on day 7 and second GnRH on day 9 followed by timed artificial insemination after 16 and 20 hours. The presence of a preovulatory follicle is necessary after the second GnRH treatment for successful synchronization and high pregnancy rate. In summer the buffaloes have small inactive ovaries without any palpable structure; still people have worked on its efficacy in summer in buffaloes. In Pakistan Warriach et al. (2008) used Ovsynch in Nili-Ravi buffaloes and reported a pregnancy rate of 36.3% and 30.4% in high and low breeding seasons respectively. He compared Ovsynch with prostaglandin administration and reported a higher pregnancy rate in the prostaglandin group in the high breeding season (62.5% vs 36.3%). He also tested Ovsynch protocol in breeding and non breeding seasons in buffaloes. The resulted pregnancies 36.3% versus 30.4% were not significantly different in breeding and non breeding seasons respectively ($P > 0.05$). The animals in this study were grouped on the basis of ovarian status. All the animals having a corpus luteum were in one group and those having follicles in another group. Therefore there was a lack of randomization in this study and the division of animals on the basis of ovarian
status makes the efficacy and comparison of these protocols inconclusive. Zain, Abdel- Razek, and Anwar (2001) studied the effect of Ovsynch in three groups of animals with single GnRH, Saline injection (no GnRH) and full Ovsynch protocols and reported pregnancy rates of 31.3%, 58.8% and 68.8% for G1, G2 and G3 respectively and this difference was significant (p<0.05). All the animals (n=58) in this study were bearing luteal structures on ovaries. The author recommended a protocol consisting of GnRH and PGF2alpha for optimum pregnancy, but the success may have been due to the selection of cycling animals having a CL on ovaries.

De Araujo Berber (2002) compared the effect of substituting the second injection of GnRH with LH on 305 buffaloes after 60 days post partum. The resulting pregnancies after double insemination were 66.9% and 75.5% for GnRH and LH respectively (P>0.05). These results showed that Ovsynch produces good results, though the study was conducted in the breeding season on cycling buffaloes in Brazil. This might be a good option in the breeding season in Pakistan as well. But the real problem is in the low breeding summer season. Camelo et al. (2002) studied pregnancy rates in 89 lactating buffaloes with fixed time artificial insemination (AI) after 12 (G1) and 24 (G2) hours with Ovsynch and natural oestrus detection with AI after 24 hours in the control group (G3). The pregnancy rate was 46.7%, 27.6 and 36.7% for the three groups respectively. According to the author, body condition score (BCS) was a limiting factor and the animals with better body condition score (BCS) showed first oestrus and pregnancy early in their life as compared with low BCS animals (P<0.05). Body condition score is a reflection of feeding regime before and during treatment and there is a need for data on feeding of these treatments groups. The best result recorded in group one may be due to optimum feeding.

Barile (2004) compared two protocols on 186 lactating buffaloes on 9 farms. The group one was treated with progesterone releasing intra-uterine device (PRID) containing 1.55 G P$_4$ and a capsule having 10mg oestradiol benzoate for 10 days and PGF2alpha (1.5mg) and pregnant mare serum gonadotrophin (PMSG) on day 7. The second group was treated with the standard Ovsynch protocol. The animals were inseminated at 72-96 and 60-84 hours after PRID removal and PGF2alpha injection in group one and two respectively. The cyclicity was determined from progesterone concentration in milk samples. The mean pregnancy rate for group one and two was 47.8% and 42.6% respectively (P>0.05).

The problem with Ovsynch is that the protocol is less effective in low or out of breeding season or summer breeding season and is also less effective in primiparous animals (Camelo
et al., 2002; De Rensis & López-Gatius, 2007). The results of Warriach et al. (2008) discussed previously further makes it less applicable in Pakistan. Recently in Bulgaria Yotov, Atanasov, and Ilieva (2012) compared Ovsynch with PRID and they reported ovulation and pregnancy rates (PR) in PRID treated animals (Ovulation rate: 72.2% vs. 55.6%, PR: 56.6% vs. 38.8%) (P<0.05). This shows the higher efficacy of progesterone impregnated devices in buffaloes.

2.5.6. Progestogen:

The most prevalent and naturally occurring progestogen is progesterone (P4). The P4 is secreted by the corpus luteum, placenta and adrenal gland. Some of the important functions of P4 are given below.

- Prepares uterus (endometrium) for implantation by increasing secretion from endometrium and inhibits myometrial contractions.
- Plays a role with estrogens in the expression of behavioural oestrus.
- Helps in the development of alveoli, the secretory structure of the mammary gland.
- Negative feedback on GnRH, FSH and LH.

Synthetic progesterone is available for oestrus synchronization. It is either fed to animals or inserted in the body as implants or intra vaginal devices (B. Hafez & Hafez, 2000).

The progestogens are available in various forms for oestrus synchronization. They are used in implants, injections, given orally (oral contraceptives) or in intra vaginal devices.

2.5.6.1. Ear Implants:

“CRESTAR™ (Previously Syncromate B, Intervet, Boxmeer, The Netherlands) consists of 2 components: an injection of oestradiol valerate (5 mg) with the synthetic progestogen norgestomet (3 mg) and a silicone ear implant containing norgestomet (17a-acetoxy-11β-methyl-19-nor-pregn-4-ene-3, 20-dione). The injection was administered intramuscularly and at the same time the implant was inserted SC at the outer edge of the ear in all animals. After 9 to 10 d the norgestomet implants were removed.” Norgestomet is another synthetic progestogen that has progestational effects similar to progesterone. The protocols that have been used in buffaloes were similar to cattle; the implant is also accompanied by an injection of pregnant mare serum gonadotrophin on day 9 and fixed timed artificial insemination 56 hours after the implant was removed for cows and 48 hours for heifers. Numerous studies
have been conducted to evaluate the potential of using a norgestomet-oestradiol treatment for
oestrus synchronization in cyclic and anoestrous cattle and buffaloes. The role of the
synthetic progestin is to suppress oestrus while progesterone also suppresses oestrus along
with other physiological functions. The major limiting factor is the variable rate of follicular
development after the decline in plasma progesterone levels causing failure in achieving
synchrony of oestrus and ovulation (Hattab, Kadoom, Palme, & Bamberg, 2000; Gurdial

Bó, Baruselli, and Mapletoft (2012) recommended a slightly different protocol with time
specification for buffaloes. According to his recommendation a progesterone auricular
implant (PAI) implantation was given on day 0 at 1800h along with oestradiol benzoate
(2mg). The protocol then involved removal of PAI on day 9 and injection of PGF2alfa and
eCG (400IU) on the same day at 1800h and GnRH injection on day 11 at 1800h followed by
fixed time AI on day 12 at 0800h.

N. Yadav, Lohan, Singal, and Dhanda (1994) studied the oestrus response in 100 Murrah
buffalo cows in rural India. The animals were divided into 6 groups and treated with Lugol’s
iodine solution, Oestradiol valerate, PMSG, GNRH or synchronate B, while the control
group went without any treatment. The resultant oestrus percent response was 42.3, 50.0,
66.7, 53.8, 75.0 and 30.8 respectively: the synchronate B gave the best result. Virakal and
Chantaraprateep et al. (1992) studied 24 Swamp buffaloes and induced 3 twins using the
synchronate B thereby showing positive impact on ovulation. Although this is positive, there
is a need for further research on a large sample size along with other factors to confirm any
significant relationship.

Luthra et al. (1994) used this implant on 7 cattle and 7 buffalo cows. The implant was
accompanied by oestradiol valerate (5mg) on day 0 and removed on day 9. An injection of
PMSG (400IU) was given on day 8. The oestrus response was 100% in cattle and 86% in
buffaloes. The sample size is very small and on the basis of this sample size, the external
validity is very poor.

Diaz et al. (1994) studied fixed time artificial insemination with 15 cows in group one and 19
cows in group two. Group one without CL group was treated with a progesterone implant for
12 days along with norgestomet (3mg) and oestradiol (5mg) injections. The animals in group
two having a CL were treated with two PGF2alfa injections 12 days apart only. The animals
were fixed time inseminated: group one was inseminated at 68 hours after implant removal
and group two was inseminated at an interval of 68 (2A) and 65-74 (2B) hours. The oestrous
signs were visible in 92-100% animals and the conception rate was 43%, 67%, 83% for group
1, 2A and 2B respectively. In this study there was a lack of randomization: the animal with a
CL were injected with prostaglandin and those without a CL were ear implanted with
synchromate-B (3mg norgestomet) and an injection of 3mg norgestomet and 5mg oestradiol
valerate. This is not always possible in the low breeding season, where there are inactive
ovaries and a lack of cyclicity.

In contrast to the above studies Hattab et al. (2000) concluded that the use of the
CRESTAR™ progesterone ear implant was not effective in synchronizing oestrus in buffalo
cows. He studied its effect in 6 cyclic and 3 anoestrous buffalo cows. One out of six cycling
buffalo showed oestrus two days after implant withdrawal while there was no response from
the non cycling group.

CIDR on the other hand gave better results by inducing 4 of the 6 cycling animals on the next
cycle in yet another report (Naseer et al., 2011).

Patel, Sarvaiya, Patel, Parmar, and Dugwekar (2003) studied the impact of a progesterone
implant plus oestradiol valerate injection on 10 cows and 10 heifers in the breeding and non
breeding season. The oestrus response was 100% in cows and 70% in heifers and the total
response was 90% and 80% in breeding and non breeding seasons respectively. The sample
size in this study is very small and on the basis of ten animals, the external and internal
validity of this study is very poor. The author also reported an elevation in levels of
progesterone and oestradiol but this elevation in the level of these hormones may be due to
other reasons, such as milking status, environmental, feeding and external injections.

Therefore there is need for further research using these protocols with a larger sample size.

Martins et al. (2002) divided female Murrah buffalo according to the stage of oestrous cycle
and treated them with progesterone ear implants and PMSG for oestrus induction and
reported that this protocol is efficient in the induction of oestrus synchronization. In the low
breeding season there is less or even no activity in ovaries, so there are practical difficulties
in detecting animals that are cycling. Furthermore Crestar is no more used routinely in cattle
Theriogenology.

2.5.6.2. **Control Internal Drug Release Inserts:**
The developments of intravaginal devices for controlled release of progesterone were started in early 1970s and have been successfully used for oestrus synchronization in cattle. These progesterone releasing devices regulate pituitary release of luteinizing hormone (Mauer, Webel, & Brown, 1975; Rathbone et al., 2001). They are now commercially available and called EAZI-BREED™ control internal drug release (CIDR) inserts and inserted in vagina of the animals rather than uterus. Insertion in the vagina is safer with less chances of infecting intra-uterine environment. They consist of a T-shaped or Y-shaped nylon spine with collapsible, flexible wings for insertion into the vagina of the animals and are coated with an inert matrix, usually silicon rubber and impregnated or loaded with 1.38g of progesterone evenly dispersed (Lamb et al., 2006; K. L. Macmillan & Peterson, 1993). The CIDR device continuously secretes progesterone which is absorbed in the vaginal wall. The absorption of the progesterone hormone is rapid through the vaginal wall and quickly reaches the systemic circulation. Due to the speed of absorption the very high levels of progesterone of 1ng mL\(^{-1}\) are reached in a short time (Rathbone et al., 2002). There is a challenge of keeping the synchronization between the hormone release from the device and accumulation in the body and excretion from the body in such a way as to maintain progesterone concentrations greater than 2 ng mL\(^{-1}\). This level is required for the drug intervention procedure (Mariano et al., 2010).

After device insertion the blood progesterone concentration reaches its peak within 1 hour. The decline is also quick and within 12 to 24 hour after CIDR removal (Lamb et al., 2006; Perry, Smith, & Geary, 2004). There is not much data on its absorption in buffaloes. In ovariectomized beef heifers, plasma progesterone concentration reaches its peak of 8.7 ng mL\(^{-1}\) in 6 hours after CIDR insertion. The plasma progesterone level decreased to 2.5 ng mL\(^{-1}\) after CIDR removal. In a 15 day treatment program the concentration of progesterone was reported to be 5.7 ng mL\(^{-1}\) during the CIDR device insertion period (K. Macmillan, Taufa, Barnes, & Day, 1991). In 4 to 15 day treatment protocols the average retention rate of CIDR is 96 to 99% (Lucy et al., 2001; K. Macmillan et al., 1991; Naseer et al., 2011).

In comparison with MGA feed supplements, the CIDR has been reported to have quick clearance rate along with better synchrony in beef cows and dairy buffaloes. The better results as a device of oestrus synchronization are attributed to CIDR’s ability to release a consistent amount of progesterone in the vagina and into the systemic circulation (Perry et al., 2004; Tauck & Berardinelli, 2007).
2.5.6.3. Development of 7 day CIDR protocol:

CIDR’s were initially inserted for various days starting from 12 and up to 21 days. In a set of studies conducted in New Zealand by Macmillan and Peterson (1993), the animals were divided in 3 groups. Animals in group 1 (n=234) were treated with a 7 day CIDR insert followed by a PGF2α injection upon CIDR removal. Animals in group 2 (n=243) and group 3 (n=247) were treated with 14 day and 21 day CIDR insert protocols without a luteolytic treatment respectively. Animals treated with the longer 21day CIDR insert showed higher oestrus responses (96%) as compared to the other two treatments. The oestrus response in the 7 day treatment group was 52.6% and 74.5% in the 14 day treatment. After 96h post treatment 95.3 and 99.6% of the animals in the two groups were detected in oestrus and were inseminated. The pregnancy for the treatment was opposite to the oestrus response. The pregnancy rate was higher for the shortest treatment (7d CIDR) and was 57.7%, while it was 44.4% and 39.7% for group 2 (14d CIDR) and 3 (21d CIDR) respectively. The reason for low pregnancy in the longer treatment group may be associated with the persistent ovarian follicle.

Therefore in the current study 7day treatment followed by a PGF2α injection 24hour before CIDR removal was selected to determine the rate of oestrus response and pregnancy to a CIDR 7 day treatment.

PRID (Progesterone Releasing Internal Device) and CIDR (Control Internal Drug Releasing Device) are the most common and well known commercially available intravaginal P4 devices with their own unique features. “PRID has a triangular shape, while CIDR is a T-shape device. Surface area and progesterone content between the two devices used herein is also different (CIDR ~ 120 cm² and 1.38 g of P4 vs PRID-Delta ~ 155 cm² and 1.55 g of P4)” (Barile, 2004; Barile et al., 2001). In standard protocols the device is implanted for 7 days. However C. Singh (2003) reported high pregnancy rates by increasing the duration of CIDR implantation to 11 days. The sample size in this study was very small (n=6) and it was possible that the high percentage of pregnant animals in this study was due to the presence of a palpatable CL which indicated that these animals were cycling.

Vale (2007) stated that use of protocols with PRID is recommended for out of season anoestrous buffaloes in Brazil. The latest recommended protocol for Brazil is to insert PRID with an oestradiol benzoate injection (2mg) at 1800h on day 0, PRID removal plus a 2ml injection of PGF2alfa and eCG (400IU) at 1800h on day 9, GnRH (10ug) injected at 2200h.
on day 11 and fixed time artificial insemination on day 12 at 0000h. However this four
injection protocol along with PRID or CIDR insertion is difficult to adopt in Pakistan due to
its high cost and need for intensive management.

Rajamahendran et al. (1980) conducted two trials on buffaloes. They injected 18 buffaloes
having a CL with a single PGF2α injection out of which three exhibited oestrus and two of
them became pregnant after natural mating. In the second trial a PRID was inserted along
with injection of oestradiol in 16 animals selected randomly: 13 animals retained the device
for 12 days. Out of these 13 animals 10 showed signs of oestrus between days four and five.
The pregnancy rate was 50% in the PRID group and 11% in the initial trial with PGF2α. The
animals in this study were fed adequately with green fodder, concentrate, mineral mixture
and free access to water. This excellent feeding regime may be the reason for the oestrus
response and is not always available under field conditions even on government farms.

De Santis, Senatore, and Presicce (2004) conducted a study on the comparison of Ovsynch
with PRID+PMSG in Italy in the low breeding season from February to May. The animals
were divided into two groups. Group one consisted of cycling heifers and cycling mixed
parity cows and group two consisted of non cycling heifers and non cycling mixed parity
cows. Group one was treated with the Ovsynch protocol and group two was treated with
PRID+PMSG (PRIDE for 9days with a PMSG (1000IU) injection on day 7. The resultant
conception rates were 36.6% and 42.8% in group one heifers and mixed parity cows
respectively and 70.5% in group two non cycling mixed parity cows. The total animals in
group one was 44 (heifers 30 and cows 14) and 17 in group two. The authors claimed a direct
comparison between cycling and non-cycling animal groups, but assigning cyclic animals to
the Ovsynch group and none cycling to the PRID group questions the validity of these
protocols in terms of a lack of randomization of animals. The results may have been different
if the animals were grouped randomly.

Neglia et al. (2003) compared PRID (n=111) and Ovsynch (n=117) protocols in buffalo and
reported that Ovsynch and PRID were more effective in cycling and non cycling animals
while in the low breeding season treatment with progesterone is more effective than Ovsynch.

Bhosrekar et al. (1994) compared three different protocols on 403 buffaloes with two
inseminations. The data is given in table 4.
Table 4 The effect of different hormonal treatment protocols on the synchronization of oestrus in Nili Ravi buffalo (Bhosrekar et al., 1994; Lemcke, 2008)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Showed visual oestrus signs %</td>
<td>72.4</td>
<td>82.6</td>
<td>82.5</td>
</tr>
<tr>
<td>RP of oestrus %</td>
<td>94.4</td>
<td>98.1</td>
<td>97.6</td>
</tr>
<tr>
<td>CR non-cycling (NC) summer %</td>
<td>29.3</td>
<td>34.0</td>
<td>31.1</td>
</tr>
<tr>
<td>CR cycling summer %</td>
<td>25.8</td>
<td>32.5</td>
<td>38.1</td>
</tr>
<tr>
<td>CR NC rainy season %</td>
<td>50.5</td>
<td>57.0</td>
<td>55.0</td>
</tr>
<tr>
<td>CR Cycling rainy season %</td>
<td>52.1</td>
<td>68.7</td>
<td>61.3</td>
</tr>
<tr>
<td>CR NC Winter %</td>
<td>42.3</td>
<td>54.3</td>
<td>50.5</td>
</tr>
<tr>
<td>CR cycling winter %</td>
<td>57.6</td>
<td>39.7</td>
<td>42.9</td>
</tr>
</tbody>
</table>

There was a variation between seasons and also a relationship between body condition score and expression of oestrous behaviour. The study time was very long and overall results were positive with a recommendation for the use of progesterone for synchronization of oestrus in buffaloes.

Subramaniam and Devarajan (1991) conducted a trial on the comparison of CIDR along with PGF2α and without PGF2α and they reported a higher response to insemination at 12 hours after the onset of oestrous with animals receiving CIDR + PGF2α injection. The pregnancy rates were 50.00, 57.14 and 50 in CIDR, CIDR+ PGF2α and PGF2alpha treatment groups respectively. The sample size was very low though (n=24) and each treatment group consisted of only 8 animals, which are too few for a reproductive trial of this nature.

In Pakistan Nasser et al. (2011) conducted a trial in the high and low breeding seasons on buffaloes. They used new CIDR and CIDR used once previously in a trial. They reported 37% pregnancy rate in both groups in the low breeding season. In their second trial CIDR’s were implanted for 7 days and PGF2α administered on the day of withdrawal. Animals were then randomly assigned into three groups. Animals in group one (n=17) received oestradiol benzoate, group two (n=18) received GnRH and group three (n=20) received the control CIDR and PGF2α only. The results are given in the following table.
The effect of different hormonal regimes given with CIDR on ovulation and pregnancy rates in Nili-Ravi buffaloes (p < 0.1) (Naseer et al. (2011))

<table>
<thead>
<tr>
<th></th>
<th>CIDR + GnRH n=18</th>
<th>CIDR+EB n=17</th>
<th>CIDR n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulatory follicle size (mm)</td>
<td>10.7±0.3</td>
<td>9.4±0.4</td>
<td>10.9±0.3</td>
</tr>
<tr>
<td>Ovulation rate (%)</td>
<td>16/18 (89)</td>
<td>16/17 (94)</td>
<td>14/20 (70)</td>
</tr>
<tr>
<td>Interval to ovulation after CIDR removal (h)</td>
<td>64.9±1.8</td>
<td>61.3±0.8</td>
<td>65.1±16.7</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>11/18 (61)</td>
<td>10/17 (59)</td>
<td>6/20 (30)</td>
</tr>
</tbody>
</table>

In the proposed study the efficacy of a controlled internal drug release (CIDR) device was studied along with a PGF2α administration protocol in summer season. PGF2α is extensively used in Pakistan. These methods were used without any addition of GnRH and oestradiol in the interest of keeping the protocol simple and cheap for small-holder farmers to adopt.

2.5.6.4. GnRH with CIDR:

According to some authors (Gimenes et al., 2010; Yotov et al., 2012) follicles less than 8mm in diameter fail to ovulate in response to exogenous GnRH administration. We also know that in summer when buffaloes fail to breed their ovaries are small and ovarian structures are not palpable (Das & Khan, 2010; Francesco et al., 2011; Prakash et al., 2005; Shah, 2010; C. Singh, 2003; G. Singh et al., 1988). With this information in mind GnRH was not included in the experimental protocols evaluated in the present studies.

Previously GnRH was considered to increase ovulation and pregnancy rate. In one experiment Naseer et al. (2011) compared CIDR-GnRH, CIDR-oestradiol benzoate and CIDR alone and reported ovulation rates of 64.9 ±1, 61.3 ± 0.8, 65.1 ± 16.7 h and pregnancy rates of 61%, 58%, and 30% respectively. The differences in ovulation and pregnancy rates were not significant; however there were some drawbacks to this work. The study was conducted in the high breeding season, while the problem of anoestrus is very common in the low breeding summer season (April to August) (Warriach et al., 2008) and the number of animals or sample size used was also very low (20± 3). The animals in these trials, however, were kept under good management conditions and optimal feeding, which is not the case.
everywhere in Pakistan. Furthermore, in Pakistan 85% of animals are kept in small herds of 1 to 10 animals (Borghese, 2005; K. Singh & Pundir, 2003), under harsh climatic and feeding conditions. The similarity of responses to CIDR-GnRH and CIDR-oestradiol benzoate and the recent report of (Leitman et al., 2009) that the use of GnRH with CIDR protocol did not improve pregnancy rate confirmed our belief that GnRH should not be included in synchronization protocols for buffalo. Jyothi, Naidu, Bramhaiah, and Padmaja (2012) reported that oestradiol esters may give better results if used in Ovsynch protocols in place of GnRH.

2.6. Oestrous synchronization in Pakistan:

The present study was designed keeping in view the dairy system of Pakistan where 85% of livestock are reared in small units of 1 to 5 animals by landless farmers (Borghese, 2005; K. Singh & Pundir, 2003). Many authors have conducted studies on oestrus synchronization in Pakistan. Warriach et al. (2008) reported a 36.3 and 30.4% pregnancy rate in high and low breeding season using the Ovsynch protocol in Pakistan. Other researchers (Camelo et al., 2002; De Rensis & López-Gatius, 2007) reported problems with Ovsynch due to its low efficacy when breeding out of season in summer or when using primiparous animals (Camelo et al., 2002; De Rensis & López-Gatius, 2007). In a recent study in Pakistan, Naseer at al. (2011) reported similar results for oestrus expression (88.5% vs 96.6%) and pregnancy rate (37.1% vs 36.6%) in groups receiving once used and new CIDR’s respectively. The efficacy of the double use of CIDR’s shows their durability in buffaloes and therefore their economic value for use with landless poor farmers.

2.7. Study Design:

Pakistan’s population is 173.965 million and Punjab is the largest province having 96.676 million people (Pakistan, 2012). Punjab is not only the largest human populated province but also the largest buffalo habituated area. Lahore is the capital and the densest populated area of Punjab province. The population of Lahore division is 12.016 million while that of Lahore district is 6.319 million (Pakistan, 2012). The high urban population therefore provides a great market for milk and meat. The neighbouring districts are thus very important for producing and selling their milk in the larger urban market of Lahore city.

Kasur (31° 7’3.40”N, 74°26’59.82”E, altitude 610 feet) and Okara (31° N, 74° east, altitude 500 feet) are adjacent districts situated on the South West of Lahore sandwiched between the
two rivers Ravi on the West and Sutlag on the East side. Both these rivers originate from India. Data on annual rain fall is not available (P&D Department Punjab).

The population of the districts of Kasur and Okara is 2.376 and 2.232 million respectively (Pakistan, 2012). The famous canal irrigation system of Pakistan fed from the two rivers provides an efficient source of irrigation water for the fertile land of these two districts. With the availability of fertile land, a good irrigation system and large urban market of Lahore, Kasur and Okara are ideal districts for dairy farming.

The government of Pakistan also established the Buffalo Research Station (BRI) in Pattoki district Kasur and Livestock Production and Research Institute (LPRI) Bahadurnagar district Okara to conduct applied research for the benefit of farmers. In this study, the effectiveness of the use of CIDR’s in comparison with a PGF2α injection protocol was studied on government and small private dairy farms in Okara and Kasur districts of Pakistan during the low breeding season (June to August).
In the proposed study the traditional two prostaglandin injections protocol was studied with CIDR based synchronization protocols on oestrus and fertility in Nili Ravi buffaloes in the low breeding season in Pakistan. The reason for using prostaglandin was its intensive use in Pakistan. CIDR has been reported to be effective in low breeding summer season in cattle. In Pakistan, CIDR was used in combination with other hormones and from the results it is not clear where the resultant oestrus response and pregnancy was due to the use of CIDR only or whether the use of other hormones contributed to the response. Furthermore use of too many injections was not encouraged for farmers in rural areas due to their low level of education and therefore understanding and replication of the protocol. On the other hand CIDR is simple to use with only a single injection of PG. The economics or the cost of using any protocol along with management is important factor in developed world, where labour is expensive and market prices for milk and meat are very competitive. But in developing world where labour is cheap and management is easy due to small herds of 1 to 10 animals these factors are not very important to consider. Furthermore, the long inter-calving interval and late age at puberty in buffaloes cost farmer a great deal, postpartum anoestrus decrease the market value of the animal. To avoid this resale loss, the farmer therefore prefers to keep this...
animal even without any production. Therefore, even more expensive oestrus synchronization protocols will be accepted by the farmers if properly convey through efficient extensions program.

In this study the animals were divided randomly into two groups (n=60): one group was treated with CIDR for 7 days and prostaglandin on day 6 and the second group was treated with two prostaglandin injection 11 days apart. The pregnancy was checked after 35 days using an ultrasound machine.

Group 1:

1. CIDR Insertion
2. PG Injection
3. CIDR removal
4. Twice breeding at 48 & 60 Hours
5. Ultrasound Scan for pregnancy

Day 0  Day 06  Day 07  Day 9  Day 35+

Group 2:

1. PG Injection
2. Breeding on heat detection
3. PG Injection
4. Breeding on heat detection
5. Ultrasound Scan for pregnancy

Day 0  Day 1-5  Day 11  Day 12-15  Day 35+

Figure 3: Oestrus synchronization treatment protocols for buffalo receiving a CIDR- based synchronization protocol (group1) and a prostaglandin protocol (group2)

2.7. **Outcome Variables:**

The animals were observed closely three times a day for five days after CIDR removal and after first and second injection of PGF2α, for detection of oestrus and timing of artificial insemination. The semen, which were obtained from semen processing unit (SPU) Qadirabad
were of high quality and of known genetics. The pregnancy was checked on day 35.

2.8. Data recording:

Buffaloes are lower in reproductive efficiency when compared with cows. Various factors influencing the reproductive efficiency in buffaloes have been reported. These include demographic location or environmental temperature (Naqvi, 2000), calf suckling (M. S. Qureshi & Ahmad, 2008; Usmani, Dailey, & Inskeep, 1990), parity and season of calving (Naqvi, 2000). Daily milk production will be recorded in each treatment group. On Government farms animals are maintained in a loose management system while on small rural farms the animals are tied 24 hours. Therefore in the current study data on the management system (tied or untied) will also be collected.

2.9. Role of body condition score (BCS) on fertility and oestrus response:

Body condition score reflecting nutritional status is also very important (Alapati, Kapa, Jeepalyam, Rangappa, & Yemireddy, 2010) and was recorded visually on a scale from 1-5 (1=emaciated, 5 fat) with the help of the scale and charts of Alapati (2010) and Negretti (2008). The body condition score is a reflection of the plane of nutrition the animals are currently fed and also reflects the body weight of the animal. The age at puberty, oestrous response and fertility are linked with body condition score. The genotype of the animal strongly influence the age at puberty. The swamp buffaloes gain puberty at a body weight of 200 to 300 kg, while the river type of buffaloes attains puberty at body weight of 250 to 400 kg (Borghese, 2005; BMAO Perera, 2011). In the current study data on body condition score was recorded and will be analysed for oestrus response and pregnancy rate.

2.10. Effect of age on fertility and oestrus response:

Age and parity are among the various non-genetic factors which can influence reproductive efficiency and cyclicity. Buffaloes are relatively slow attaining peak reproductive activity. They achieve puberty very late. Naqvi (2000) reported that buffaloes gain puberty at the age of 2.73 years in Pakistan. The age at puberty is highly variable. M. Jainudeen and Hafez (1993) also reported that puberty is quite highly variable ranging from 18 to 46 months.
Borghese (2005), through his review of studies from buffalo studies in many countries reported that under favourable conditions some breeds particularly the river type show oestrus at an early age ranging from 15 to 18 months. He concluded that swamp buffaloes showed their first oestrus at the age ranging from 21 to 24 months. Buffalo compensate this delay in puberty with longer reproductive life as compared to cattle which have shorter reproductive lives (BMAO Perera, 2011). According to Perera (2011) the reasons for delay in puberty are genotype, nutrition, management social environment, climate, year or season of birth and diseases.

The exact reasons for late puberty and the influence of parity on reproductive efficiency are not known and there are conflicting reports in the literature. Shafiq et al. (1997) did not find any effect of parity on postpartum interval to conception up to the 6th lactation. Whereas Chaudhry (1988) reported a negative correlation between postpartum interval to conception and lactation number in Nili-Ravi buffaloes. Cady et al. (1983) reported that parity only affects days open or postpartum interval to oestrus and calving interval. He reported a positive correlation between reproductive efficiency and parity. He also reported that age was not a significant factor. This shows that with advancement in age and parity buffaloes improve their reproductive efficiency and can better cope with their environment. Naqvi (2000) analysed 1921 records from military farms and reported a significant decrease (p<0.01) in service period as parity increased. He reported a significant (p<0.001) lowest mean service period in parity number eight of 107.95±19.72 days.

All these reports show that parity and age can have an effect on calving to conception interval and can be an important parameter for assessing reproductive efficiency of buffaloes. Furthermore on the data shows the importance of further research on buffalo reproduction. If nutritional means of advancing the age at puberty can be found, buffalo will be a very efficient dairy animal.

In the current study we will collect date on parity and age and will study the effect of parity and age on the oestrous response and fertility.

In order to measure the effect of these parameters on oestrous response and fertility the following data was collected during these trials. The animals were also checked physically and rectally for the presence of any disease or reproductive disorder. The impact of this data were analysed relative to the oestrous response and fertility.

- Age
• Suckling (presence of calf)
• Days postpartum
• Parity
• Body condition score
• Management system (tied or untied)
• Milk production (per day)
• Oestrous behaviour
• Pregnancy

2.11. Objectives of this study:

The objectives of this study were as follows:

• To study the effect of two oestrus synchronization protocols on the oestrus response and pregnancy rate in buffaloes receiving CIDR (seven day insertion and PGF2α on day six) and PGF2α (two injections of PGF2α 11 days apart).
• To determine the effect of CIDR on oestrus and pregnancy in anoestrous post partum dairy buffaloes. Also to study the use of CIDR as a future synchronization device for buffaloes.
• To monitor the impact of body condition score, parity, age and milk production on the oestrus response and fertility in buffaloes.
Chapter 3. Materials and Methods:

3.1. Animals:

Non pregnant multiparous Nili Ravi buffaloes (*Bubalus bubalis*) that had not showed any signs of oestrus over the past two months or at least calved 3 months earlier (n=120) were randomly selected from government (n=70) and small nearby rural dairy farms (n=50). The animals were divided in two groups using simple randomized technique. In this technique the first animal was assigned to one of the treatment groups by flipping a coin and subsequent animals were allocated alternatively to the 2 treatment groups. The average period of anoestrus was of 9 months *postpartum* with a range of 2.5 to 24 months *postpartum*. The animals ranged in age from 4 to 8.6 years and were fed under normal small-holder farming conditions.

The normal feeding system consisted of concentrates, green fodder and wheat straw. The small rural farmers are less aware of balance feeding. The farmer’s financial position decides the level of feeding; a rich farmer will feed his animal on a better ration as compared with a poor small-holder farmer. The concept of cost per litre of production is not adopted by these farmers and they rarely calculate their budgets. This is due to lack of awareness and low literacy rate in rural areas. The animals are managed under close confinement and are tied to a fixed object with provision of water twice a day. Rectal palpation was carried out to determine pregnancy status and the animal’s history of any reproductive disorders was collected from records collected by the farmer. Buffaloes are less cyclic during summer. The animals clear of any disease were judged as reproductively sound and there were no detectable reproductive abnormalities in their genital tracts. All the animals were in moderate to good body condition with the objective of selecting animals with a BCS of 3 or above. BCS was measured visually as described by Alapati et al. (2010). Animals were kept in open and closed management systems. In closed management systems the animals were tied to an object. On the government farm they were kept in a loose management system, while on the small holder farm they are kept tied.

The animals were offered concentrate comprising of cotton seed cake (2kg) and wheat bran (1kg) mixed with wheat straw at the rate of 2kg per animal per day and were also offered green fodder at 40kg per animal per day. Milking animals were milked twice daily and those animals with a calf were suckled for 5 minutes before milking.
3.2. Treatments/pharmacological reagents and experimental design:

Buffaloes were randomly divided into two treatment groups (n=60 per group). Each group consisted of 60 animals randomly selected from small rural communities and adjacent government farms. A standard CIDR implant protocol was followed in group 1; The CIDR device was inserted for 7 days. The method for this implantation is illustrated in Figure 3.1. The animals were injected with PGF2α injection (10ml Lutalyse) on day 6 and the CIDR was removed on day 7. The CIDR devices were sourced from Pfizer Australia containing 1.38mg progesterone and the PGF2α source was Lutalyse, manufactured by Pfizer.

Animals in group two were injected with PGF2α. PGF2α (10ml) was injected twice (5ml morning and evening) 11 days apart in group 2. On each of these days the animals were injected twice with 5ml of PGF2α (dinoprost tromethamine, Lutalyse, Zoetus Kalamazoo Michigan) at 0900h and 1700h daily. Warriach and Ahmad (2007) used two injections of Dalmazin™ 2ml (0.150mg/2ml) 12 hour apart and reported an earlier luteal regression (p<0.05) in buffaloes in Pakistan. Therefore the same technique of two injections 12 hours apart was used in this study. Two injections helped in maintaining higher serum level of PGF2α. The animals were watched for oestrus for three to four days after the first injection. Animals were inseminated if detected to be on heat. The unresponsive animals were reinjected on day 11 with a similar dose of Lutalyse (PGF2α). The animals were again watched for 3 to 4 days for expression of oestrus after the second injection. Again animals detected to be on heat were inseminated by a trained inseminator.

Behavioural changes in buffaloes are largely variable. This is because high percentage of ovulations is associated with a low intensity of oestrus. The intensity of oestrus is weak as compared with cattle. Almost half of the buffalo population suitable for breeding show weak or silent oestru and silent oestrus is the main reason for 80% of recorded long oestrus cycle. The buffalo breed in the cooler part of the day early morning and evening while some time during the night. In 96% of Nili-Ravi buffaloes there are mucus discharge and swollen vulva (M. A. Khan, 2009; Muhammad, 1973; Usmani et al., 1985). In the current study animals were closely watched two times a day for oestrus signs. The animals with suspicious signs and symptoms for oestrus were separated for further checking. The separated animals were rectally palpated for uterine tone. Oestrus was confirmed with uterine tone 2+ on a scale of 1-5, the uterine tone along with mucus discharge and vulvar swelling were used as confirmatory signs for oestrus. Ultrasonography was not use due to power problems and lack
of resources. The animals with a uterine tone of more than 2 along with mucus discharge and vulvur swelling were considered on heat only.

Animals in the CIDR group were also checked for their oestrus response and were rectally palpated. All the animals in group 1 receiving the CIDR which retained the device for 7 days were inseminated using the TAI technique. Part of the protocol for all animals in group 1 was that all animals were inseminated 48 to 60 hours after removal of the CIDR even in animals not responding to the CIDR. The oestrus response was recorded in the CIDR group as well using the same procedure of assessing uterine tone 2+ on a scale of 1-5 based on the presence of mucus and vulvular swelling.

After 40 days animals were rectally scanned by trans-rectal ultrasonography (Honda; Model: HS-1500; 7.0MHZ) for pregnancy. Pregnancy records were maintained for the calculation of pregnancy rate.

To see the effect of various parameters on pregnancy and oestrus response the following data was collected: age, parity, body condition score (BCS), daily milk production and presence of a calf. BCS was assessed visually.

Figure 4 CIDR device, applicator and insertion method

3.3. Oestrus confirmation:

Animals were checked twice a day for heat detection at 0900 and 1700h daily. The animals showing any visual sign like swelling of vulva, presence of mucus, micturition and restlessness were separated from the herd daily for further confirmation. All animals suspected of displaying oestrus were rectally palpated.
For the confirmation of oestrus visual signs and uterine tone were taken into account. The uterine tone was calculated on a scale of 1-5, with 5 showing the highest uterine tone. Oestrous response rate was calculated by confirmation of oestrus. Animals with a uterine tone of 2 or more on a scale of 1-5 and displaying mucus and swelling of the vulva were considered to be on heat. Restlessness and vocalisation/bellowing were considered additional signs for oestrus confirmation. Buffaloes are famous for the display of silent heat and being docile in nature; therefore bellowing and restlessness were not taken as the primary signs for oestrus confirmation (Andurkar & Kadu, 1995; O. I. Azawi, Omran, & Hadad, 2008; Bashir et al., 2007; Cady et al., 1983; Carcangiu et al., 2011; Das & Khan, 2010; Gangwar, 1980; Gokuldas et al., 2010; M.R Jainudeen, 1988; Shah, 2010).

All the animals in the group receiving a CIDR implant were inseminated as part of the protocol including animals with no signs of oestrus. The pregnancy rate was calculated by determining the proportion of inseminated animals that became pregnant. Two animals out of 60 lost their CIDR’s and the CIDR device retention rate was therefore 96.7%. Three animals died (2 in the CIDR and one in the PGF2α group) after insemination due to bloat after grazing accidently on fresh fodder overnight. The reason for death was not related to the experimental treatments.

### 3.4. Statistical analysis:

Data for oestrus response and pregnancy rate between the two treatments, the impact of management (tied and untied) and the presence of a calf was compared using a Chi square analysis. The effect of age and daily milk production on the oestrus response, and the duration of anoestrus post-partum in days were compared using Anova and a t-test. A probability level with P value <0.05 was considered to be significant.

The data for the effect of parity and BCS on pregnancy success and oestrus response was analysed using a logistic regression model in SPSS (version 20). Data for these dependent variables (Pregnancy and Oestrus response) were binomial data (i.e. 0, 1), therefore a simple linear regression is not appropriate (due to the non-normality of the data) and instead the logistic regression is required. The details of the analysis to assess the effect of parity and BCS on pregnancy and oestrus status through a Logistic Model in SPSS (version 20) ("SPSS," 2015) are given below.
\[ \ln \left( \frac{p}{1-p} \right) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \epsilon \] \hspace{1cm} 1

Where as

\[ \frac{p}{1-p} = \text{Odd ratio (Pregnant=1, non pregnant=0)} \] \hspace{1cm} 3

\[ \ln \left( \frac{p}{1-p} \right) = \text{Dependent variable (Pregnant=1, non pregnant=0)} \] \hspace{1cm} 4

\[ \beta_1 - \beta_2 = \text{Coefficients, } X_1= \text{Parity, } X_2= \text{BCS} \] \hspace{1cm} 5

\[ \ln \left( \frac{p}{1-p} \right) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \epsilon \] \hspace{1cm} 7

Where as

\[ \frac{p}{1-p} = \text{Odd ratio (Oestrus expression=1, Oestrus absence=0)} \] \hspace{1cm} 9

\[ \ln \left( \frac{p}{1-p} \right) = \text{Dependent variable (Oestrus expression=1, Oestrus absence=0)} \] \hspace{1cm} 10

\[ \beta_1 - \beta_2 = \text{Coefficients, } X_1= \text{Parity, } X_2= \text{BCS} \] \hspace{1cm} 11

---

**Figure 5** Time Line for Prostaglandin F2α protocol

**Figure 6** Time Line for CIDR protocol
Chapter 4.  Results

4.1. Results:

This study provides a pilot study, since the management of animals on small-holder private farms is hard to control. On government farms the animal were checked twice daily for oestrus expression with the help of a teaser bull. On both government farms there were only two attendants who could recognize the animals. The ear tags were faded and due to black colour and physical similarities, it is always hard to differentiate buffaloes from each other without ear tags. We were not allowed to tag them again. Therefore we always needed those two persons to identify and separate our trial animals from the herd of 300 animals. Our trial animals were fifty selected out of more than 300 on the first government farm and 20 out of more than 200 on the second government farm. We marked them with paint spray but it faded within a few days due to the buffaloes’ nature of lying in the mud and water. The animals after separation were additionally confirmed from both attendants.

Initially we had 60 animals in each group. Two animals (3.3%) in CIDR group lost their CIDR which is an acceptable level according to the literature, where a loss of 2 to 3% is acceptable. (Andurkar & Kadu, 1995; K. L. Macmillan & Peterson, 1993). Therefore we had 58 animals in the CIDR group. We lost 3 more animals after insemination due to deaths, one in the prostaglandin and 2 in the CIDR treated groups. The pregnancy is therefore calculated on the basis of the availability of 56 animals. The reasons for the losses of animals were independent of the treatment.

Availability of electric power is a major issue in Pakistan and even worse in rural areas and villages. The power source for the ultrasound machine and restraint of animals were two limitations to the study. On occasion we had to wait for the electric power for hours to conduct ultrasound scanning after 40 days. In some villages where the power supply was not available at all, we used car batteries along with UPS (Uninterruptible Power Supply or uninterruptible power source) to provide required power for using the ultrasound machine.
The local farms were scattered in 7 villages and there was a need for many follow up visits to meet with farmers on site for data recording. Without the farmers presence it was hard to enter the houses and check animals for oestrous expression. One of the advantages of using local small farms was that the animals were under frequent observation and therefore oestrous expression was detected more efficiently than on government farms. The oestrus detection was very low on government farms. In the prostaglandin group only one animal out of 35 was detected on heat. While on private farms 13 out of 25 animals were detected on heat in the same treatment group.

The overall oestrus response was higher in animals treated with the CIDR device as compared to prostaglandin injection. When the CIDR was used with PGF2α (Treatment 1) oestrus was detected in 84.5% of animals, but only in 23.3% of animals receiving a PGF2α regime only (Table 6). This did not lead to a higher pregnancy rate (CIDR 19.6%; PGF2α regime 30.8%; P = 0.296). Body condition score did not vary significantly between animals expressing oestrus and those that did not irrespective of treatment with a P-value .703 (Table 9). However animals that expressed a calf were in significantly (P=0.009) better condition than those that did not at the time of joining (Table 8).

Similarly there were no differences in age between animals expressing oestrus and those that did not (Table 7).

However animals tended to perform well and have a high pregnancy percentage with an increase in age as compared to when they are young (p=0.057). There was also no difference...
in the parity of animals expressing oestrus nor was there an association with success at
delivery of a calf (Table 8, 9).

The animals on government farms were kept under lose management system, while animals
on small private farm are reared inside house or outside in a small space. The small private
farmers in the village tie their animals to an object due to limited space and lack of technical
knowledge. Therefore we collected data on animal management systems (lose and tied). The
restraint of animals by tying them to a specific object resulting in less than optimal water and
feed availability did not affect either oestrus expression or success with delivery of a calf.

Similarly the farmers in the villages delay to rebreed their animals after calving. There is a
myth that there will be a decrease in milk production if they become pregnant soon after 60
days postpartum. Therefore we collected data on animal’s milk productions status during the
treatment period and analysed the data to see the impact of milk production on oestrus
response and pregnancy. The milk production was not associated with either oestrus
expression or a successful pregnancy (Table 7).

The results for oestrous behaviour and pregnancy rates for the two groups of animals are
given below in table 6.

Table 6 The relationships between reproductive success (oestrus expression and
pregnancy success) and treatment group (CIDR versus prostaglandin only) in Nili Ravi
buffalo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oestrus expression (%)</th>
<th>P-value</th>
<th>Pregnancy (%)</th>
<th>P-value</th>
<th>Test used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment1 (CIDR)</td>
<td>84.5</td>
<td>0.000</td>
<td>19.6</td>
<td>0.296</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Treatment2 (PGF2α)</td>
<td>23.30</td>
<td>0.000</td>
<td>30.8</td>
<td>0.296</td>
<td>Chi-Square</td>
</tr>
</tbody>
</table>

Table 7 The effect of Milk production and age of animals on oestrus expression and
pregnancy success in Nili Ravi buffalo.

<table>
<thead>
<tr>
<th>Parameter treatments</th>
<th>across Oestrus Expression</th>
<th>P-value</th>
<th>Pregnancy success</th>
<th>P-value</th>
<th>Test used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.30</td>
<td>0.253</td>
<td>9.80</td>
<td>0.057</td>
<td>Anova or T-test same results</td>
</tr>
<tr>
<td>Milk production (Kg)</td>
<td>5.10</td>
<td>0.160</td>
<td>5.20</td>
<td>0.986</td>
<td></td>
</tr>
</tbody>
</table>

Page 48
Table 8 Dependent Variables associated with pregnancy success.

<table>
<thead>
<tr>
<th>Name of variables</th>
<th>Coefficient</th>
<th>S.E.</th>
<th>t-ratio</th>
<th>Sig.</th>
<th>Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>0.329</td>
<td>0.241</td>
<td>1.865</td>
<td>0.172</td>
<td>1.390</td>
</tr>
<tr>
<td>BCS</td>
<td>1.768</td>
<td>0.724</td>
<td>5.970</td>
<td>0.015</td>
<td>5.861</td>
</tr>
<tr>
<td>Constant</td>
<td>-8.005</td>
<td>2.564</td>
<td>3.751</td>
<td>0.002</td>
<td>0.000</td>
</tr>
</tbody>
</table>

CH² = 9.526, with P-value .009, likelihood 45.518

Table 9 Dependent Variables associated with the Oestrus response

<table>
<thead>
<tr>
<th>Name of variables</th>
<th>Coefficient</th>
<th>S.E.</th>
<th>t-ratio</th>
<th>Sig.</th>
<th>Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>0.206</td>
<td>0.257</td>
<td>0.642</td>
<td>0.423</td>
<td>1.228</td>
</tr>
<tr>
<td>BCS</td>
<td>-0.212</td>
<td>0.714</td>
<td>0.088</td>
<td>0.767</td>
<td>0.809</td>
</tr>
<tr>
<td>Constant</td>
<td>1.789</td>
<td>2.213</td>
<td>0.654</td>
<td>0.419</td>
<td>5.983</td>
</tr>
</tbody>
</table>

CH² = 0.705, with P-value 0.703, likelihood 44.917

The data obtained from private farms were analyzed separately to determine the impact of
prostaglandin and CIDR protocols on private farms using the same analysis and tests.
The feed on private farms were also sampled and analyzed to see if there were any marked
differences. The farmers in villages fed their animals according to their resources. We were
not allowed to analyze the feed on government farms as they were producing their own
commercial feed and this was analyzed in their own laboratories. The green feed consisted of
a mix of various green fodders including maize, sorghum and millets. The feed samples were
collected from all farms. The similar samples based on constituents were mixed and a
representative sample was taken for analysis. Most of the farmers were feeding similar types
of concentrates sample so representative samples were presented for analysis. The feed was
analyzed at the Nutrition laboratory of Buffalo Research Institute Pattoki in district Kasur.
The concentrate feed consisted of cotton seed cake, maize oil seed cake, sunflower seed cake,
wheat bran, maize grains, dry bread, molasses and commercial feed formulations. Wheat
straw was often mixed with green fodder or concentrate such as crushed grains along with wheat bran which was sprinkled on wheat straw with some water.

Table 10  The analysis of green fodder samples from selected private farms for fat (ether extract), moisture, protein, fiber ash and phosphorus content

<table>
<thead>
<tr>
<th>Farmers details</th>
<th>Feed Analysis (Sample composition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample composition %</td>
<td>Fat</td>
</tr>
<tr>
<td>M. Hussain Sample 1</td>
<td>0.560</td>
</tr>
<tr>
<td>Dawood 5</td>
<td>0.510</td>
</tr>
<tr>
<td>Shaukat 2 Fodder</td>
<td>0.970</td>
</tr>
<tr>
<td>Younus 3 Fodder</td>
<td>0.350</td>
</tr>
<tr>
<td>BRI Fodder</td>
<td>0.200</td>
</tr>
<tr>
<td>Bao Amjad</td>
<td>0.050</td>
</tr>
<tr>
<td>Rana Yonus</td>
<td>0.030</td>
</tr>
<tr>
<td>Manzoor 22GD</td>
<td>1.520</td>
</tr>
<tr>
<td>Bao Amjad</td>
<td>0.600</td>
</tr>
<tr>
<td>Ch Akhlaq 43 GD</td>
<td>0.300</td>
</tr>
<tr>
<td>Average</td>
<td>0.509</td>
</tr>
</tbody>
</table>

Table 11 The analysis of concentrate samples from selected private farms for fat (ether extract), moisture, protein, fibre ash and phosphorus content concentrate

<table>
<thead>
<tr>
<th>Farmers Details</th>
<th>Feed Analysis (Sample composition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>Shaukat2 Feed</td>
<td>8.260</td>
</tr>
<tr>
<td>Younus 3 Feed</td>
<td>4.890</td>
</tr>
<tr>
<td>Ch Akhlaq 43 GD</td>
<td>6.040</td>
</tr>
<tr>
<td>Cotton seed cake</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>6.397</td>
</tr>
</tbody>
</table>
Chapter 5. Discussion

5.1. Oestrus response in both treatments:

Postpartum anoestrus is a major cause of infertility and long inter-calving intervals. This study demonstrated that oestrus expression was higher in animals receiving a protracted regimen of progesterone release using a CIDR device. This is because high progesterone in the blood supply suppresses GnRH neurons in the tonic centre of the hypothalamus with a flow-on to suppression of both LH and FSH from the pituitary. However LH secretion and tonic FSH release remain at levels high enough to maintain the development of a cohort of follicles. However the high progesterone prevents follicles from reaching the preovulatory stage. The high progesterone also primes the brain for the actions of oestrogen. Upon CIDR removal the rapid drop in circulating concentrations of progesterone promotes the release of a surge of GnRH, followed by FSH and LH from the anterior pituitary. The surge of GnRH followed by FSH and LH causes the resumption of ovarian cyclicity (O I Azawi et al., 2012; Zaabel, Hegab, Montasser, & El-Sheikh, 2009). The increase in the size of the follicle due to FSH and LH release also increased oestrogen production (Senger, 2005). Therefore the CIDR causes a high percentage (84.5%) of animals displaying an oestrus response relative to those receiving PGF2α only. The PGF2α is only effective in the presence of a functional CL, while in summer the buffalo have small inactive ovaries without any palpable structures and so they do not respond to PGF2α. Therefore the oestrus response was lower (23.30) in the PGF2α treatment group.

5.2. Pregnancy response in both treatments:

The pregnancy rate was calculated on the basis of the number of animals pregnant divided by the number of animals inseminated, which numbered 56 in the CIDR treatment group and 13 in the PGF2α group. The deaths of 3 animals in the study were not related to treatments according to the autopsy reports. The pregnancy percentage was lower in CIDR group than PGF2α group animals (19.6% in CIDR vs. 30.8% in PGF2α) and there were no significant differences across treatments. This may be due to the failure of ovulation after the GnRH surge, or an inappropriate timing of AI following a delay in ovulation by 30 hours after standing heat was observed. Therefore further research is needed on timed AI in buffaloes. Similar findings were reported by (Kanai & Shimizu, 1983; Warriach et al., 2008). The result
and work reported in these studies suggested that the insertion of CIDR’s for longer than 7
days may be sufficiently long for the low levels of LH to recruit an ovulatory follicle (C. Singh, 2003). In another study using CIDR’s in Pakistani Nili Ravi buffaloes during the low
breeding season (June, July) by new and used CIDR devices were able to induce oestrus
responses of 88.5% and 96.6% (Naseer et al., 2011). The low oestrus response in the PGF2α
group in the present study suggests the absence of a CL on ovaries since PGF2α is only
effective in the presence of a functional CL. This was also indicative of a low number of
animals (14 out of 60 or 23.30%) having active ovaries with a functional CL. Naseer, et al.
(2011) reported a pregnancy rate of 37.1% and 36.6% using once recycled and new CIDR’s
respectively. They used 35 and 30 animals in their two groups respectively. The current study
used the largest number (n=120) of animals in CIDR trials in Pakistan so far. Another
possible reason for low pregnancy rate in CIDR might be aging of gametes. The long P4 level
prevents ovulation and cause aging of gametes. Such gamete can be successfully fertilized
but there are reports of early embryonic deaths (Rathbone et al., 2001).

The relatively high pregnancy percentage in the PGF2α group suggested that heat detection
was accurate especially on small rural farms. This is an important observation suggesting that
if the nutritional regime used on these farms to feed animals was of sufficient quality and
quantity, high reproductive efficiencies could be achieved consistently. Only one animal
(1/35) in the PGF2α group was detected on heat on government farms where a teaser bull was
used twice a day for heat detection along with visual observation. However workers on the
government farms are most likely not motivated in any way to observe animals closely as
there is no financial incentive for them to do so. In contrast on small-holder farms both the
farmer and his wife were available to observe animals closely around the clock, whereas
government employed veterinarians left their place of work at a regular time late in the
afternoon, leaving animals unobserved. We can conclude from this study; however that
PGF2α is effective in cycling animals with a functional CL. Another reason might be the
variation in the feeding profile between Government and the small-holders private farms.
This is in contrast to the previous reports, where improved feeding enhances oestrus detection
and conception. The feeding on government farms was better as compared to small-holder
private farms, but the oestrus expression especially in PG group was better on small private
farms. There might be some potential reason on government farms for not detecting animal
on heat where they rely heavily on a teaser bull. The ability of a teaser bull to detect heat
needs to be checked at regular intervals. On government farms the stored feed and storage
need to be checked on regular intervals to monitor levels of aflatoxin.

Suspected animals were rectally palpated for confirmation of heat. Therefore the current 48
and 60 hours TAI, which was originally estimated for cattle/cows, needs further refinement
for use with buffaloes. There is also a need for further investigation on detecting heat and
timing of AI following CIDR device withdrawal rather than using a standardized timed
artificial insemination in small-holder rural dairy systems where close observation of animals
is maintained around the clock. The farmer can detect animals on heat without any additional
time and cost. The use of fixed time AI is more effective in large herds.

5.3. Effect of body condition score (BCS) on fertility and oestrus response:

In the current study body condition score had no influence on the oestrus response but did
significantly affect pregnancy rate. This further accentuated the need for an improvement in
the dietary regime offered these animals to facilitate successful reproductive management
irrespective of treatment.

5.4. Effect of age on fertility and oestrus response:

In the current study we collected date on parity and age and studied the effect of parity and
age on oestrous response and fertility. According to results there was no difference in age
between animals expressing oestrus (8.3 years) and those that did not (9.0 years) with a p-
value of 0.253 (Table 7).

In contrast there was a distinct trend for animals achieving a successful pregnancy (9.8 yr) to
be older than those which did not (8yr) (Table 7). This indicated that there was an
improvement in pregnancy with an increase in age. It also illustrates the longevity of buffalo
productive and reproductive life. The effect of parity however was not consistent as poor
lifetime nutritional status most likely compromised the timing of puberty and inter calving
intervals. Therefore the age of the animal can provide at best a rough indication of the
reproductive efficiency of the animal. Thus in our study with animals from small-holder
farms it was likely that some animals of 7 years of age may have been at the same
reproductive age as 10 year old animals that had not been as well fed during their growth and
development.
In the present study there was no difference in parity between oestrus (3.0) and non oestrus (3.1) expressing animals with a P value 0.712 (Tables 9). Similarly there was no significant difference in parity between pregnant (3.6) versus not pregnant (2.9) P value 0.141. Though there was a positive trend towards an increase pregnancy with increase in parity (Tables 8).

Two animals out of 60 lost their CIDR and the CIDR device retention rate was 98.7 which was near the figure of 98.4% reported by (K. L. Macmillan & Peterson, 1993).
Chapter 6. Conclusion

It is clear that buffalo species suffer from a variety of reproductive problems. These problems include a long prepubertal period, seasonal breeding and silent oestrus. These problems alone or collectively contribute towards prolonged inter calving intervals, which can extend to 522 days, whereas the ideal period should less than 400 days.

The use of the CIDR device for oestrus synchronization in Pakistani dairy buffaloes and its effect on oestrus response, ovulation rate and fertility will help to decrease this inter-calving interval. The device is reported to be very successful in cattle but the physiology of buffaloes is different from cows. Therefore there is a need for refinements in the protocols developed for cattle to ensure their effective use in buffalo. Progesterone hormones are very effective in the induction of reproductive activity in non cycling cows and buffaloes. Traditionally in cows the CIDR is inserted for 7 days, but in buffaloes the oestrus response with the 7day protocol appears not to be ideal and there is a need to extend this period to ensure that the endocrine physiology of the hypothalamic-pituitary reproductive axis is functionally tuned for ovulation once the source of progesterone is withdrawn. The greater likelihood of the recruitment of an ovulatory follicle in this phase will most likely lead to higher conception rates.

In Pakistan 85% of livestock are reared in rural areas, where women are mainly responsible for all the labour (cleaning, feeding and milking). The long inter calving intervals without any reproductive outcomes adds significantly to the workloads of women who are required to detect oestrus behaviour among with all of their other household duties. This results in social issues or tensions within families that have seldom been investigated and of course are beyond the scope of the current study.

If they are able to breed their animals on time this is likely to alleviate some of this social pressure. Oestrus synchronization can play a vital role to decrease the inter-calving interval from the current 500 days to the ideal duration of 360 to 390 days.

This study has identified the key problems associated with the field research on small-holder farming operations at the village level. Providing farmers with a simple but effective reproductive technology well proven for use in buffalo must be a high priority for research in Pakistan. This must be combined with the nutritional research that needs to go hand in hand with these studies to ensure the technology is adopted successfully. It is clear that financial subsidies will not be an effective mechanism to provide aid for these farmers. The current
study shows that CIDR can induce a higher oestrus response but lower pregnancy rate.

Careful studies of the timing of each operation in the use of CIDR’s in buffalo need to be conducted both at the experimental level and then on farm among the small-holder dairy communities throughout the country.

Success here will provide more than simple financial benefits to farmers: clearly the social implications for farming communities need to be included in this complex equation. The need to adjust feeding regimes is a difficult subject to tackle since the resources available to these farmers are limited.

However recent studies (unpublished data) have shown that high levels of mycotoxins are found in the most used cotton-seed concentrate feeds available to these farming communities. Mycotoxins are known to compromise ovarian activity, although these effects have not been reported in buffalo in Pakistan. This is definitely another topic requiring close attention in future research programs. These toxins may also be responsible for the expression of silent heat in this species; however we have no knowledge of this interaction at present.

The high preference for buffalo milk in Pakistan will ensure that there will be a significant demand for research of the reproductive inefficiency in Pakistan’s buffalo herd. The study reported in this thesis identifies just some of the areas requiring further investigation.
References:


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