THERAPEUTIC MANAGEMENT OF LUTEAL CYST IN DAIRY BUFFALOES

THESIS

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1. INTRODUCTION

Buffalo (*Bubalus bubalis*) is a premier milk producing animal contributing more than two third of total milk production in India. The buffaloes contribute around 21.23 per cent of the total livestock population in India. The population of buffaloes in India is 108.70 million, whereas, it is 7.53 million in Madhya Pradesh (19th Livestock census, 2012). In recent past Jabalpur has emerged as a dairy hub of Madhya Pradesh with a significantly large population of buffaloes.

There are several reproductive disorders viz. delayed puberty, silent oestrus, anoestrus, summer infertility and postpartum uterine disorders, adversely affecting the reproductive efficiency of buffaloes and thus their production potential. Cystic ovarian disease (COD) is one of the important causes of infertility in dairy animals. Bovine ovarian cysts are being investigated from more than sixty years (Casida *et al.*, 1944). Cystic ovarian disease in bovines is defined as the presence of large follicle-like structure at least on one of the ovaries having a diameter of 2.50 centimetre or greater, that persist for at least ten days, in the absence of luteal tissue (Jou *et al.*, 1999). There are three types of cysts that occur on the bovine ovary: follicular cysts, luteal cysts and cystic corpora lutea (Johnson, 2004). The incidence of the disease ranges between 5 to 8 per cent (Zduncyk *et al.*, 2002).

COD is more common in the early postpartum period (less than 60 days) at which cows are under great metabolic stress (Bartlett *et al.*, 1986). Many factors have been associated with COD although their exact mechanism of action is not known. These factors include, high milk production, a severe negative energy balance and ketosis, twinning and periparturient problems, genetic predisposition, season and nutritional disorders suggested that, COD results from the failure of the pituitary gland to release sufficient amounts of leutinizing hormone (LH) to induce ovulation.

Many treatment strategies have been used to resolve the cystic condition. These include manual rupture, progesterone, gonadotropic-releasing hormone (GnRH), human chorionic gonadotropin (hCG) and prostaglandin (PGF$_2$α) (Day, 1991). GnRH is currently the treatment of choice...
for undifferentiated COD (Stock and Fortune, 1993). PGF\textsubscript{2α} alone is the treatment of choice for luteal cysts (Augustin, 1997). A new protocol GnRH-PGF\textsubscript{2α}-GnRH (ovsynch) has also been used to treat COD (Bartolome \textit{et al.}, 2000). The use of progesterone for treatment of COD has also been well documented (Nanda \textit{et al.}, 1988).

It was also reported that melatonin through a complex action on hypothalamus-pituitary-ovarian axis, exerts stimulatory effect on GnRH in hypothalamus and follicle stimulating hormone (FSH) and luteinizing hormone (LH) in pituitary gland (Malpaux \textit{et al.}, 1997). Thus, it may also play a role in recovery of cyclicity, ovulation and conception in buffaloes.

COD remains an important cause of extension of the interval from calving to conception and increased number of inseminations per conception thus decreases the reproductive performance and subsequently the production from the animal. The contribution of buffaloes in dairy industry is remarkable. Thus it is necessary to manage the condition of cystic ovaries in dairy animals for increasing the reproductive performance of the animals and subsequently their production output. Despite the huge amount of literature, the exact pathogenesis of the disease is still not completely clear and therefore also the choice of treatment is still debated. Keeping in mind this economic point of view in dairy industry, this study was conducted with the following objectives:

1. Screening of anoestrus animals for the presence of luteal cyst on the ovaries.
2. Treatment of the animals having luteal cyst with different hormonal preparations.
3. Comparison of the efficacy of different therapeutic regimen.
2. REVIEW OF LITERATURE

The buffalo holds an important place and role in Indian rural economy. Buffalo is the premier animal in Indian dairy industry contributing about 60 per cent of total milk production in the country. Buffaloes are preferred over cattle in India because of their distinctive qualities such as better feed conversion efficiency, more resistance to diseases and higher milk fat percentage than in cows. However, they suffer from many reproductive issues; mainly delayed puberty, postpartum ovarian inactivity and seasonality which cause great economic loss to the farmer thus are the main obstacles in rearing this species.

2.1 Cystic ovarian disease

Cystic ovarian disease in bovines is defined as the presence of large follicle-like structure on one of the ovaries having a diameter of 2.50 centimetre or greater, that persist for at least ten days, in the absence of luteal tissue (Jou et al., 1999). There are three types of cysts that occur on the bovine ovary: follicular cysts, luteal cysts and cystic corpora lutea (Johnson, 2004).

Luteal cysts are thick-walled structures on the ovary that do not have an ovulation papilla, so the surface of the luteal cysts is smooth. They typically occur as a single dominant cyst, but have been reported to occur along with a follicular cyst (Brown et al., 1982). When compared to follicular cysts, luteinized cysts are more likely to persist over long periods of time and can lead to nymphomania in some animals (Ball and Peters, 2004).

Luteal cysts develop when ovulation fails to occur and the theca undergoes luteinisation (Schlafer, 2007). They are also often considered to be the later form of ovarian follicular cysts (Vanholder et al., 2006). The luteal cyst occurs when the cells of the follicular cyst (granulosa and theca) become luteinized and start producing progesterone (Peter et al., 2009).

2.1.1 Overall incidence of Cystic ovarian disease

Rao and Sreemannarayana (1982) in a study on clinical analysis of reproductive failure among female buffaloes analysed the clinical data of 20439 infertile graded Murrah female buffaloes and the analysis revealed that the incidence of cystic ovarian disease was 0.91 per cent.
Azawi et al. (2008) assessed the type and incidence of abnormalities occurring in the female reproductive tracts of 405 buffaloes, out of which 216 (53.30%) of the cases were observed to be suffering from various abnormalities with different degrees of severity and luteal cyst was one of the abnormalities observed among them.

Mittal et al. (2010) in a study on incidence of different pathological affections of ovaries in buffaloes reported that among 504 buffaloes examined for different affections of the ovaries, 33 animals displayed various pathological conditions of the ovary among which the incidence of follicular cyst was 1.78 per cent and that of luteal cyst was 0.19 per cent.

Modi et al. (2011) reported that the incidence of luteal cyst was 0.10 per cent and that of follicular cyst was 0.07 per cent in his study on incidence of common major reproductive problems in dairy buffalo in a total of 11209 infertile buffaloes.

Kumar et al. (2014) in a study on anoestrus in cattle and buffalo observed that in India, the incidence of follicular cysts has been reported to be 0.50-2.70 per cent in cattle, 1.26-3.40 per cent in buffaloes and 8.00-10.20 per cent in crossbred cattle, whereas incidence of luteal cysts were 1.45-3.00 per cent in cattle, 0.66-12.50 per cent in buffaloes and 9.00-13.30 per cent in crossbred cattle. The incidence of follicular and luteal cysts abroad in dairy cattle has been reported to be 6-19 per cent and 10-13 percent, respectively.

Purohit (2014) in his study on ovarian and oviductal pathologies in the buffaloes reported 0.50 to 1.40 per cent incidences of ovarian cysts among all the ovarian pathologies observed in buffaloes.

2.1.2 Month wise and season wise incidence of luteal cyst

Environmental stress (extreme cold and heat) affect the development of follicles and manifestation of oestrus both in cattle and buffaloes. Although buffaloes are well adopted in hot and humid climate, however, ovarian activity is greatly reduced by heat stress and manifested in the form of anoestrus (Singh et al., 2000). Heat stress affects folliculogenesis,
folicular fluid micro environment and oocyte quality. In buffaloes, decline in feed intake during summer results in reduced secretion of gonadotrophins (El-Sawaf et al., 1979). Besides this, high environmental temperature causes hyper-prolactinaemia and suppressing the secretion of gonadotrophin which leads to alteration in ovarian folliculogenesis and steroidogenesis.

Kumar et al. (2013) studied the incidence of postpartum anoestrus among buffaloes in and around Jabalpur (M.P.) and reported higher incidence of anoestrus in summer season (66.28%) followed by rainy (22.51%) and winter (11.25%) seasons. They also classified 60.58 per cent buffaloes as true anoestrus, 20.88 per cent as sub-oestrus and 18.52 per cent anoestrus due to other unknown conditions.

Singh (2013) studied the incidence of anoestrus in 2550 buffaloes and observed 37.85 per cent overall incidence of anoestrus. He examined a total of 542 buffaloes with a history of non-return to oestrus 60 days postpartum and observed higher incidence of true anoestrus (60.70%), followed by silent or un-observed oestrus (22.51%) and other pathophysiological conditions (16.79%). In the same study he also observed higher incidence of anoestrus during March (26.01%) and October (22.51%) as compared to other months of study i.e. November (14.94%), December (12.55%), January (10.89%) and February (13.10%).

2.1.3 Age wise and parity wise incidence of luteal cyst

The incidence of cystic ovarian disease is uncommon in animals in their first lactation. Animals of all ages were susceptible to cystic ovarian disease, but incidence was greater in animals during their fifth or sixth lactation, i.e. when milk yield is often greatest (Mukasa-Mugerwa, 1989). Several studies have linked the incidence of cystic ovaries to milk yield. Such a relationship was demonstrated by Marion and Gier (1968), who observed that the disease was more prevalent in high-yielding cows and that it occurs at the stage in the lactation curve when yield is at its peak.

Lee and Kim (2006) studied that advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. In their study they observed that the incidence of ovarian cysts was lower in cows with a parity of one than in cows with greater parities.
2.2 Progesterone (P₄) profile

Plasma steroid concentration is considered as an indicator of ovarian activity (Webb et al., 1980) and as a marker to predict response to hormonal treatment (Bulman and Lamming, 1978). Thus estimation of hormones before and during treatment is helpful in studying ovarian function and response to treatment.

2.2.1 During oestrus period

Kamonpatana et al. (1979) in a study to investigate the suitability of progesterone assay in blood plasma for oestrus control and pregnancy diagnosis in swamp buffalo reported that the normal progesterone value during oestrus was lower than 0.50 ng/ml in the buffaloes.

Takkar et al. (1982) studied the progesterone profile in buffaloes during various stages of oestrous cycle using radioimmunoassay technique in blood serum and observed that the progesterone level was 0.33 ng/ml on the day of oestrus in buffaloes.

Mondal et al. (2010) studied the endocrine profiles of oestrous cycle in buffalo and observed that the plasma progesterone concentration was 0.30 ng/ml during the perioestrus phase.

2.2.2 During luteal cyst condition

Leslie and Bosu (1983) studied the plasma progesterone concentrations in dairy cows with cystic ovaries and observed that rectal palpation accurately determined the presence of luteal cysts which was confirmed by plasma progesterone concentration of 3 ng/ml or more.

Carroll et al. (1990) studied the variability of ovarian structures and plasma progesterone profile in dairy cows with ovarian cysts and observed that the mean serum progesterone concentration in the cows having luteal cyst was more than 1 ng/ml.

Farin et al. (1990) in a study on diagnosis of luteal and follicular ovarian cysts in dairy cows by sector scan ultrasonography correlated the serum progesterone concentrations with the results of ultrasonography and observed that the mean serum progesterone concentration was 1.41 ng/ml for cows diagnosed as having luteal cyst.
Douthwaite and Dobson (2000) in a study on comparison of different methods of diagnosis of cystic ovarian disease in cattle and an assessment of its treatment with PRID (Progesterone releasing intra-vaginal device), reported that the mean plasma progesterone concentration was lower in the cows with follicular cyst (0.29 ng/ml) than in those with luteal cyst (3.90 ng/ml). In the same study, he also reported that at the time of PRID removal, plasma progesterone concentration had increased in the cows with follicular cysts to 1.59 ng/ml and decreased in the cows with luteal cysts to 0.87 ng/ml, although there was no change in original cyst structure in 45 per cent of the cases. However, new ovarian structures were frequently observed during the treatment.

Zulu et al. (2003) in a study on clinical response of ovarian cysts in dairy cows after PRID treatment reported that the plasma progesterone concentration in the animals having luteal cyst was more than 1 ng/ml.

Johnson (2004) in a study on cystic ovarian disease in cattle reported that the plasma progesterone concentration more than 1 ng/ml was considered as the minimum progesterone concentration for a cyst to be considered as luteal.

2.3 Therapeutic management of luteal cyst

The treatment of cystic ovarian disease in cattle has been reviewed in detail by Nanda et al. (1989). The earliest method of treating cysts was by manual rupture per rectum. Although rupture sometimes occurs inadvertently, it should not be done intentionally as it can cause trauma or haemorrhage, which might result in ovarobursal adhesions. Surgical removal of one chronically affected ovary or paracentesis using a long hypodermic needle through the sacrosciatic ligament might be worth considering in a limited number of cases where other treatments have failed.

Most cysts are now treated using reproductive hormones. The choice of hormonal treatment regimen depends largely upon the type of cyst that is present; follicular cysts are usually treated with either gonadotrophic hormones (i.e. hCG or GnRH) or progesterone, whereas luteinised cysts are normally treated with luteolytic substances.
2.3.1 Gonadotropic-releasing hormone (GnRH) and its analogue, alone or in combination with PGF$_2\alpha$

GnRH has been used successfully to treat ovarian cysts. It was thought at first that GnRH or hCG administration causes luteinisation of the cyst either by inducing an increase in endogenous LH secretion or by causing luteinisation directly. However, it is increasingly well recognised that GnRH has little direct effect upon the cyst itself but, instead, it causes ovulation of new follicles (Ribadu et al., 1994). These follicles develop into corpora lutea. Thus, whether GnRH induces luteinisation of the cyst or the formation of new corpora lutea, the result is an increase in progesterone concentration, usually within 10 days of treatment. Elevated progesterone concentrations cause a negative feedback-induced decline in endogenous LH secretion. A consequential decline in follicular steroid synthesis occurs, leading to declining oestradiol-17β concentrations. This is considered to be the most important factor in restoring normal cyclical activity (Kesler and Garverick, 1982). Doses of 100-250 μg of GnRH probably cause luteinisation of the cyst (Kesler et al., 1981).

Bierschwal et al. (1975) in a study on clinical response of dairy cows with ovarian cysts to GnRH performed the experiment on 114 cows diagnosed as having ovarian cysts via rectal palpation and treated them at random with a single intramuscular injection of either 50, 100 or 250 μg synthetic gonadotrophin-releasing-hormone (GnRH) or a sham injection of the carrier vehicle for GnRH (0 μg) and he reported that the positive response of cows in the 50, 100 and 250 μg groups was 18 of 28 (64%), 23 of 28 (82%) and 23 of 30 (77%), respectively, in contrast to 6 of 28 (21%) given 0 μg. For cows positively responding to treatment, the mean time in days from treatment to estrus, conception rates and services per conception for cows receiving 0, 50, 100 and 250 μg GnRH were 24.0, 4 of 6 (67%) and 1.50; 22.80, 13 of 18 (72%) and 1.60; 22.40, 20 of 23 (87%) and 1.60 and 22.20, 17 of 23 (74%) and 1.90, respectively.
Kesler *et al.* (1978) studied the clinical and endocrine response of dairy cows with ovarian cysts to GnRH and PGF$_{2\alpha}$ and reported that the luteinized ovarian cyst is responsive to the luteolytic effects of PGF$_{2\alpha}$ and PGF$_{2\alpha}$ 9 days following GnRH appeared to be the most desirable treatment for ovarian cysts.

Nakao *et al.* (1979) in a study on effects of systematic application of human chorionic gonadotrophin (hCG), gonadotrophin-releasing hormone (GnRH) analogue and bovine anterior pituitary gonadotrophin in cows with cystic ovarian disease reported that the therapeutic effect of hCG is disappointing and about half of the cases not responding to hCG were successfully treated with GnRH analogue.

Whitmore *et al.* (1979) in a large and detailed survey involving 225 cows with ovarian cysts and irregular oestrous cycles, 76 per cent responded to a single injection of 100 µg of GnRH and only four failed to respond to up to three injections; 83 per cent of the treated cows became pregnant, with a 49 per cent pregnancy rate to first service.

Ijaz *et al.* (1987) in a study on treatment and control of cystic ovarian disease in dairy cattle reported that gonadotrophin releasing hormone (GnRH), which stimulates the re-establishment of ovarian cycles within 28 to 30 days in 62 to 97 per cent of treated animals, has recently been recommended for ovarian cysts. In the same study he also reported that most cows that re-establish ovarian cycles subsequent to GnRH treatment exhibit oestrus 18 to 23 days after treatment, and conception rate at this first oestrus after GnRH treatment is 37 to 57 percent. The interval from GnRH treatment to oestrus has been reduced by administering prostaglandin F$_2$ alpha (PGF$_{2\alpha}$) 9 days after GnRH. With PGF$_{2\alpha}$ treatment, luteolysis of cysts occurs within 2 to 5 days in over 90 per cent of the cases with normal oestrous behaviour and fertility.

Probo *et al.* (2011) studied the reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin and reported the usefulness of GnRH to treat ovarian cysts regardless of their type, in relation to both recovery and conception rates. Intervals from treatment to resumption of ovarian activity were affected by the characteristics of ovarian cysts, with a faster recovery for the luteal type.
Rudowska et al. (2015) in a study on treatment of ovarian cysts in dairy cows with simultaneous administration of GnRH and PGF$_2$$\alpha$ and GnRH alone reported that the cows treated with GnRH and PGF$_2$$\alpha$ simultaneously displayed a good clinical response and slight improvement in reproductive performance as compared to the GnRH single-therapy.

2.3.2 Progesterone

Progesterone (P$_4$) has been used to treat cows with COD. Norgestomet implants, CIDR (Controlled Internal Drug Release) and PRID (Progesterone Releasing Intravaginal Device) have been used to deliver P$_4$ at a reasonably constant rate in cystic and chronically cystic cows. The premise for using exogenous P$_4$ therapy in cows with COD is to inhibit LH release and allow time for LH to be replenished in the pituitary. Therefore, when P$_4$ is removed, GnRH may stimulate a release of LH that would induce ovulation in a responsive follicle (Nakao et al., 1978).

Dobson et al. (1977) had a good response with eight of nine (88.88%) cows showing regression of the cyst, of which five (55.55%) conceived with a mean of 1.50 services per conception. They used a treatment regimen of 100 mg of progesterone in oil by intramuscular injection on three successive occasions at intervals of 48 hours.

Ijaz et al. (1987) in a study on treatment and control of cystic ovarian disease in dairy cattle reported that the use of progestational compounds as a single dose (750 to 1500 mg) or daily (200 to 500 mg) over a period of 10 to 17 days intramuscular or subcutaneous has produced recovery rate of 61 to 72 per cent and overall conception rate of 50 per cent.

Nanda et al. (1988) in a study reported that sixty-eight per cent (17/25) of the cystic cows that received a PRID had resolution of the cyst with or without a display of estrus. Of these 25 cows treated with PRID, 15 (60%) became pregnant to an average of 1.50 services per conception.

Todoroki et al. (1998) in cattle used CIDR as a treatment for COD and reported that the normal cyclicity was established for at least three cycles and normal follicular waves occurred while the CIDR was in place.
2.3.3 Melatonin (MLT)

MLT is a regulator of: seasonal reproduction in photoperiodic animals, synchronizing their reproductive activities with annual changes in photoperiod, temperature to give their offspring the best chance to survive and mature before onset of harsh environmental conditions. The timing of reproductive seasonality in most of the mammals is controlled by photoperiod and this photoperiodic information is conveyed to reproductive neuroendocrine system by circadian secretion of MLT from the pineal gland (Terek et al., 1976 and Karsch et al., 1984). Photoperiod and MLT are viewed as modulators of the GnRH pulse generator and the response to estrogen negative feedback (Karsch et al., 1984). As days become shorter, the exposure to MLT increases and through a complex action on the hypothalamo-pituitary-ovarian axis, it causes GnRH secretion from the hypothalamus in short-day breeders i.e. sheep and buffalo. On the contrary, in long-day breeders i.e., mare, increased MLT exposure has the opposite effect, inhibiting GnRH release by the hypothalamus. The mechanism of action of MLT appears not to be fully understood, however it is known to suppress prolactin secretion (Wuliji et al., 2003).

Ramadan et al. (2014) studied the effect of melatonin treatment on the resumption of ovarian activity during out-of-breeding season and reported that the implantation of melatonin (18 mg of melatonin per 50 kg of body weight subcutaneously for 45 days) successfully improved the diameter of largest follicles and the ability to maintain corpus luteum at 21 days after artificial insemination in buffalo heifers during out-of-breeding season under tropical conditions.

Kumar et al. (2016) in a study on supplementation of slow-release melatonin to improve recovery of ovarian cyclicity and conception in summer anoestrus buffaloes reported that melatonin-administered buffaloes showed significantly higher (90%) OIR (Mean oestrus induction rate) with OII (oestrus induction interval) of 18.06 ± 1.57 days. Results showed improvement in conception rate in buffaloes administered with post-insemination melatonin and they also concluded that the slow-release melatonin supplementation restored cyclicity in summer anoestrus animals resulting in improvement in conception rate in buffaloes.
Singh et al. (2016) studied the effect of melatonin implant (18 mg/50 kg body weight subcutaneously) on summer anoestrus buffaloes for induction of oestrus and reported that oestrus induction rate was higher in melatonin-implanted buffalo (65.8%) as compared to control (41.4%).
3. MATERIAL AND METHODS

3.1 Location and place of work

The work was carried out at organized dairy farm under private sector (Choubey Dairy Farm) situated at Kandra Kheda, Jabalpur; Livestock farm (LSF), Adhartal, Jabalpur and Department of Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur [M.P.].

3.2 Meteorological data and features of place

Jabalpur is situated at 23.17˚ latitude and 79.57˚ E longitudes at 410.87 MSL (meters above sea level) in the Southern part of second agro-climatic zone, including Satpura Plateau and Kymore hills. Jabalpur has a humid subtropical climate, typical of North-Central India. Summer starts in late March and last up to June. May is the hottest month with average temperature reaching up to and beyond 45˚C. They are followed by monsoon season, which last until early October, with a total precipitation of nearly 55 inches (1386 mm). Winter starts in late November and last until early March. Jabalpur gets moderate rainfall of 35 inches (889 mm) during July-September due to the South-West monsoon.

3.3 Experimental design

3.3.1 Selection of animals

The study was conducted on 24 postpartum (120 days onwards) anoestrous Murrah buffaloes belonging to LSF, Adhartal and private dairy farm having luteal cyst on either or both the ovaries. Post-pubertal buffaloes of all age groups having parity more than one were selected for the study. The anoestrous animals were selected on the basis of farm records and the luteal cyst was confirmed by two consecutive rectal examinations 10 days apart (Plate 01).

3.3.2 Materials used

A. Gonadotrophin Releasing Hormone (GnRH) analogue

Receptal VET (10 ml vial), Intervet India Private Limited, Each ml contains: Buserelin acetate 0.0042 mg equivalent to 0.004 mg Buserelin, 1% benzyl alcohol IP as preservative (Plate 02).
B. Prostaglandin F$_2$alpha (PGF$_2$α)

Clostenol (2 ml vial), Zydus Animal Health Limited, Each ml contains: 263 µg Cloprostenol Sodium B.P. Vet equivalent to Cloprostenol 250 µg (Plate 03).

C. Progesterone

Duraprogen (2 ml ampoule), Vetcare, Each ml contains: Hydroxyprogesterone caproate I.P. 0.25 g, as preservative: Benzyl alcohol I.P. 0.02 ml in an oily base (Plate 04).

D. Melatonin

Otto (25 g bottle), Biochemika Reagents, Each g contains: Melatonin 99 per cent (Molecular weight-232.28). 18 mg powder was dissolved in 1 ml of sterilized olive oil and used on the same day (Plate 05).

3.3.3 Treatment groups

A. Group 1 (n=6): Progesterone

Animals were administered Hydroxyprogesterone caproate (Progesterone) @ 4 ml=1000 mg/animal intramuscularly on day 0.

B. Group 2 (n=6): GnRH analogue and PGF$_2$α

Animals were administered Buserelin acetate (GnRH anlogue) @ 5 ml=20 µg/animal intramuscularly on day 0 then Cloprostenol (PGF$_2$α) @ 2 ml=500 µg/ml intramuscularly on day 9.

C. Group 3 (n=6): Melatonin

Animals were administered Melatonin @ 5 ml=90 mg/animal subcutaneously on day 0.

D. Group 4 (n=6): Control administered with 10 ml normal saline.

3.3.4 Treatment schedule

The selected animals were randomly divided equally (n=6) in four groups and treatment schedule in the present study was followed as per the given treatment protocol in table 01:
Table 01: Treatment protocol

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1</td>
<td>Progesterone (day-0) » ED and NS (day-1 to 21)</td>
</tr>
<tr>
<td>G 2</td>
<td>GnRH analogue (day-0) + PGF$_2$$\alpha$ (day-9) » ED and NS (day-10 to 21)</td>
</tr>
<tr>
<td>G 3</td>
<td>Melatonin (day 0) » ED and NS (day-1 to 21)</td>
</tr>
<tr>
<td>G 4 (Control)</td>
<td>Normal saline (day 0) » ED and NS (day-0 to 21)</td>
</tr>
</tbody>
</table>

Day-0: Start of treatment, ED: Oestrus detection, NS: Natural service

3.4 Managemental practices

The buffaloes were stall-fed and housed in pucca sheds with standard management norms of LSF and private dairies. The animals were let loose daily at least for 30 minutes during morning and evening hours in the paddock with good water splashing following milking. Clean drinking water was made available to the animals ad lib. During these hours oestrus behaviour of all the buffaloes was observed in the presence of accompanying breeding bull. Further confirmation of oestrus was done by rectal palpation of the genitalia. The animals in oestrus (heat) were bred by healthy fertile bull.

The herd was routinely vaccinated for the diseases, viz. Foot and Mouth Disease, Haemorrhagic Septicaemia, Black Quarter, and Brucellosis. The general health check-up and deworming of the animals were done routinely.

3.5 Blood collection and serum separation

The blood samples were collected aseptically from the jugular vein of all the animals before the start of treatment at day 0, post treatment on the day of oestrus in responded animals and at day 21 in non-responded animals.

About 5 ml of blood was collected in the test tube and it was kept in slanting position undisturbed for few hours until serum was separated. The serum samples were then stored in labelled vials and kept in a deep freezer at -20°C, till analyzed for progesterone concentration.
3.6 Serum progesterone assay

The quantitative determination of progesterone concentration in serum was made by Enzyme Linked Immuno Sorbent Assay (ELISA) using kits supplied by Calbiotech Inc., 1935 Cordell Ct, EL Cajon, CA 92020, USA (Plate 06).

3.6.1 Principle of assay

The CBI Progesterone is a solid phase competitive ELISA. The samples and Progesterone enzyme conjugate are added to the wells coated with anti-Progesterone monoclonal antibody. Progesterone in the patient's sample competes with a Progesterone enzyme conjugate for binding sites. Unbound Progesterone and Progesterone enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of colour is inversely proportional to the concentration of Progesterone in the samples. A standard curve is prepared relating colour intensity to the concentration of the Progesterone.

3.6.2 Procedure of assay

For ELISA, 10 µl of standards, controls and serum samples were taken into the appropriate wells. Then, 200 µl of Progesterone enzyme conjugate was added to all the wells and covered with a protective film and kept at room temperature for 60 minutes of incubation period. Three times washing was done with 300 ml of 1X wash buffer. Next, 100 µl of TMB reagent was added and incubated at room temperature for 15 minutes resulting in the development of blue colour (Plate 08). The colour development was stopped with the addition of 50 µl of stop solution (0.5 N H$_2$SO$_4$). Absorbance and concentration was measured using ELISA reader (Stat-fax-2100 Awareness Technology Inc.) (Plate 07) and the values were expressed in ng/ml. A standard curve was obtained by plotting the concentration of the standard versus the absorbance.

The validity tests and standardization of the ELISA was performed by preparing the standard curve and working out the sensitivity, intra-assay variation, and inter-assay variation for all the assays as depicted in table 02, before the use of ELISA kits for quantification of the hormones in the serum samples.
Table 02: ELISA validity test

<table>
<thead>
<tr>
<th>Validity criteria*</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (ng/ml)</td>
<td>0.22</td>
</tr>
<tr>
<td>Intra-assay variation (CV%)</td>
<td>5.36</td>
</tr>
<tr>
<td>Inter-assay variation (CV%)</td>
<td>9.68</td>
</tr>
</tbody>
</table>

(* As per literature supplied with the ELISA kit)

3.7 Fertility response

All the animals were examined per rectally for confirmation of pregnancy 60 days post service and fertility was calculated.

3.8 Statistical analysis

The results of conception rate were expressed in percentage. The data generated from progesterone profile before and after treatment were expressed as mean value in different treatment groups.
4. RESULTS

Reduced fertility, observed in high yielding dairy animals, is most likely due to the alterations at several consecutive steps in the reproductive process. One of the known causes of reduced fertility in dairy animals is ovarian dysfunction. Formation of cyst following ovulation failure is the most common ovarian dysfunctions during the postpartum period. Treatments for ovarian cysts are numerous and variable, also they have changed considerably over years. Economically, the decision to treat an animal is influenced by the costs and the expected treatment benefits, the cost of replacement and the breeding value of the animal. Many endocrine based treatments for cysts have been evaluated including steroids, gonadotrophin and gonadotrophin releasing hormone.

4.1 Incidence of luteal cyst at different location of work before the start of treatment (July, 2016)

To study the incidence of luteal cyst, 583 adult dairy buffaloes were screened at LSF, Adhartal and private dairy farm of Jabalpur before the start of treatment i.e. in July, 2016. At LSF, Adhartal, among 83 adult dairy buffaloes, 18 buffaloes were found to be suffering from postpartum anoestrus condition and on two consecutive per-rectal examinations of these 18 buffaloes, 12 buffaloes were found to have luteal cyst on their ovaries. Thus, the incidence of luteal cyst at LSF, Adhartal was observed to be 14.45 per cent contributing 66.66 per cent to anoestrus. Similarly, at private dairy farm, among 500 adult dairy buffaloes, 50 buffaloes were found to be suffering from postpartum anoestrus condition and on two consecutive per-rectal examinations of these 50 buffaloes, all of them were found to have luteal cyst on their ovaries. Thus, the incidence of luteal cyst at private dairy farm was observed to be 10 per cent contributing 100 per cent to anoestrus. The analysis of above data revealed that the overall incidence of luteal cyst in buffaloes before the start of treatment was 10.63 per cent and luteal cyst was contributing 91.17 per cent to anoestrus at that particular period.
Table 03: Incidence of luteal cyst before the start of treatment (July, 2016)

<table>
<thead>
<tr>
<th>Location</th>
<th>Total number of adult females in the herd</th>
<th>Number of anoestrus females</th>
<th>Number of females having luteal cyst</th>
<th>Incidence of luteal cyst (%)</th>
<th>Contribution of luteal cyst to anoestrus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSF, Adhartal, Jabalpur</td>
<td>83</td>
<td>18</td>
<td>12</td>
<td>14.45</td>
<td>66.66</td>
</tr>
<tr>
<td>Private (Choubey) Dairy Farm, Jabalpur</td>
<td>500</td>
<td>50</td>
<td>50</td>
<td>10.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>583</td>
<td>68</td>
<td>62</td>
<td>10.63</td>
<td>91.17</td>
</tr>
</tbody>
</table>

4.2 Month wise incidence of luteal cyst at Livestock Farm, Adhartal (July, 2016 to June, 2017)

Month wise incidence of luteal cyst in buffaloes at LSF, Adhartal was also studied during a period of twelve months (July, 2016 to June, 2017). The results are depicted in table 04. The incidence of luteal cyst from July, 2016 to June, 2017 were as follows: July, 2016-14.45 per cent, August, 2016-9.63 per cent, September, 2016-8.43 per cent, October, 2016-8.53 per cent, November, 2016-7.31 per cent, December, 2016-7.22, January, 2017-7.22 per cent, February, 2017-7.22 per cent, March, 2017-13.25 per cent, April, 2017-19.27 per cent, May, 2017-12.19 per cent and June, 2017-10.00 per cent. Analysis of the above results revealed higher incidence during April (19.27%), followed by July (14.45%), March (13.25%) and May (12.19%). The lowest incidence was observed to be 7.22 per cent during December, January and February.
Table 04: Month wise incidence of luteal cyst at Livestock Farm, Adhartal (July, 2016 to June, 2017)

<table>
<thead>
<tr>
<th>Month of year</th>
<th>Total number of adult females in the herd</th>
<th>Number of anoestrus females</th>
<th>Number of females having luteal cyst</th>
<th>Incidence of luteal cyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July, 2016</td>
<td>83</td>
<td>18</td>
<td>12</td>
<td>14.45</td>
</tr>
<tr>
<td>August, 2016</td>
<td>83</td>
<td>15</td>
<td>08</td>
<td>9.63</td>
</tr>
<tr>
<td>September, 2016</td>
<td>83</td>
<td>15</td>
<td>07</td>
<td>8.43</td>
</tr>
<tr>
<td>October, 2016</td>
<td>82</td>
<td>13</td>
<td>07</td>
<td>8.53</td>
</tr>
<tr>
<td>November, 2016</td>
<td>82</td>
<td>13</td>
<td>06</td>
<td>7.31</td>
</tr>
<tr>
<td>December, 2016</td>
<td>83</td>
<td>11</td>
<td>06</td>
<td>7.22</td>
</tr>
<tr>
<td>January, 2017</td>
<td>83</td>
<td>09</td>
<td>06</td>
<td>7.22</td>
</tr>
<tr>
<td>February, 2017</td>
<td>83</td>
<td>09</td>
<td>06</td>
<td>7.22</td>
</tr>
<tr>
<td>March, 2017</td>
<td>83</td>
<td>15</td>
<td>11</td>
<td>13.25</td>
</tr>
<tr>
<td>April, 2017</td>
<td>83</td>
<td>21</td>
<td>16</td>
<td>19.27</td>
</tr>
<tr>
<td>May, 2017</td>
<td>82</td>
<td>18</td>
<td>10</td>
<td>12.19</td>
</tr>
<tr>
<td>June, 2017</td>
<td>80</td>
<td>17</td>
<td>08</td>
<td>10.00</td>
</tr>
<tr>
<td>Total</td>
<td>990</td>
<td>174</td>
<td>103</td>
<td>10.40</td>
</tr>
</tbody>
</table>

4.3 Season wise incidence of luteal cyst at Livestock Farm, Adhartal (July, 2016 to June, 2017)

The data on month wise incidence of luteal cyst at LSF, Adhartal was also classified on the basis of season of the year (table 05) and the generated data revealed higher incidence of luteal cyst in buffaloes during summers (13.72%), followed by rainy (10.27%) and winter (7.25%) seasons.
Table 05: Season wise incidence of luteal cyst at Livestock Farm, Adhartal (July, 2016 to June, 2017)

<table>
<thead>
<tr>
<th>Season of year</th>
<th>Total number of adult females in the herd</th>
<th>Number of anoestrus females</th>
<th>Number of females having luteal cyst</th>
<th>Incidence of luteal cyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>328</td>
<td>71</td>
<td>45</td>
<td>13.72</td>
</tr>
<tr>
<td>Rainy</td>
<td>331</td>
<td>61</td>
<td>34</td>
<td>10.27</td>
</tr>
<tr>
<td>Winter</td>
<td>331</td>
<td>42</td>
<td>24</td>
<td>7.25</td>
</tr>
<tr>
<td>Total</td>
<td>990</td>
<td>174</td>
<td>103</td>
<td>10.4</td>
</tr>
</tbody>
</table>

4.4 Age wise incidence of luteal cyst at Livestock Farm, Adhartal

For the study of age wise incidence of luteal cyst at LSF, Adhartal, on a yearly basis, a total of 84 adult females present in the herd were classified into different age groups and in each age group number of females suffering from luteal cyst condition was screened out and incidence was calculated (table 06). The results were depicting an increasing trend of incidence of luteal cyst among the animals from younger to older age groups up to 12 years, followed by a decline in positive cases above 13 years of age. The higher incidence was observed among the animals belonging to the age group of 11 to 12 years (58.33%), followed by 30.76 per cent (9-10 years), 28.57 per cent (≥13), 21.62 per cent (7-8 years) and the lowest i.e. 12.50 per cent in the animals of age 6 years and below.

Table 06: Age wise incidence of luteal cyst at Livestock Farm, Adhartal

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of adult females in the respective age group</th>
<th>Number of females having luteal cyst</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6</td>
<td>08</td>
<td>01</td>
<td>12.50</td>
</tr>
<tr>
<td>7-8</td>
<td>37</td>
<td>08</td>
<td>21.62</td>
</tr>
<tr>
<td>9-10</td>
<td>13</td>
<td>04</td>
<td>30.76</td>
</tr>
<tr>
<td>11-12</td>
<td>12</td>
<td>07</td>
<td>58.33</td>
</tr>
<tr>
<td>≥13</td>
<td>14</td>
<td>04</td>
<td>28.57</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>24</td>
<td>28.57</td>
</tr>
</tbody>
</table>
4.5 Parity wise incidence of luteal cyst at Livestock Farm, Adhartal

For the study of parity wise incidence of luteal cyst at LSF, Adhartal, on a yearly basis, a total of 84 adult females present in the herd were classified into different groups based on their parity and in each group number of females suffering from luteal cyst condition was screened out and incidence was calculated (table 07). The results revealed higher incidence of luteal cyst among animals in fifth parity and above (34.48%), followed by fourth (27.27%), third (26.31%), second (26.66%) and first (20.00%) parities.

Table 07: Parity wise incidence of luteal cyst at Livestock Farm, Adhartal

<table>
<thead>
<tr>
<th>Parity</th>
<th>Number of adult females in respective parity</th>
<th>Number of females having luteal cyst</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>02</td>
<td>20.00</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>04</td>
<td>26.66</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>05</td>
<td>26.31</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>03</td>
<td>27.27</td>
</tr>
<tr>
<td>≥5</td>
<td>29</td>
<td>10</td>
<td>34.48</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>24</td>
<td>28.57</td>
</tr>
</tbody>
</table>

4.6 Fertility response to treatment in terms of conception rate

The fertility response to different treatment protocols and control group in terms of conception rate is summarized in table 08.

The conception rate after various treatments in different groups was recorded to be 83.33 per cent (group 1), 33.33 per cent (group 2), 0.0 per cent (group 3) and 0.0 per cent (group 4). Results revealed higher conception rate in group 1 (Hydroxyprogesterone), followed by group 2 (Buserelin+PGF$_{2\alpha}$). However, none of the animals responded in group 3 (Melatonin) and group 4 (Control).

Table 08: Fertility response to treatment in terms of conception rate

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>No. of animals Responded</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>05</td>
<td>83.33</td>
</tr>
<tr>
<td>G2</td>
<td>02</td>
<td>33.33</td>
</tr>
<tr>
<td>G3</td>
<td>00</td>
<td>0.00</td>
</tr>
<tr>
<td>G4</td>
<td>00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
4.7 Serum progesterone profile

The mean serum progesterone concentration in animals before treatment at day 0 and after treatment on the day of oestrus in responded animals and at day 21 in non-responded animals have been summarized in table 09.

It is apparent from the table that the mean serum progesterone concentration before treatment in all groups was above 1 ng/ml i.e. 1.42±0.17 and 1.26±0.32 in responded animals of group 1 and group 2 and 1.87±0.00, 1.21±0.08, 1.39±0.15 and 1.18±0.10 in non responded animals of group 1, group 2, group 3 and group 4, respectively.

The mean serum progesterone concentration (ng/ml) on the day of oestrus in responded animals was markedly reduced after treatment (below 0.50 ng/ml) as compared to the mean serum progesterone concentration in these animals before treatment (day 0) and was observed to be 0.49±0.38 (group 1) and 0.44±0.06 (group 2).

The mean serum progesterone concentration (ng/ml) at day 21 after treatment in non responded animals of all the groups was not considerably decreased after treatment as compared to the mean serum progesterone concentration (ng/ml) before treatment (day 0) in these animals and was observed to be 1.16±0.00, 0.98±0.11, 0.69±0.13 and 1.18±0.10 in group 1, group 2, group 3 and group 4, respectively.

Table 09: Mean serum progesterone concentration (ng/ml) before and after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Responded animals</th>
<th>Non responded animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At day 0</td>
<td>On the day of estrus</td>
</tr>
<tr>
<td>G1</td>
<td>1.42±0.17</td>
<td>0.49±0.38</td>
</tr>
<tr>
<td>G2</td>
<td>1.26±0.32</td>
<td>0.44±0.06</td>
</tr>
<tr>
<td>G3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
5. DISCUSSION

Cystic ovarian disease (COD) is an important ovarian dysfunction and a major cause of reproductive failure in dairy animals. Due to the complexity of the disorder and the heterogeneity of the clinical signs, a clear definition is lacking. A follicle becomes cystic when it fails to ovulate and persists on the ovary. Despite an abundance of literature on the subject, the exact pathogenesis of COD is unclear. It is generally accepted that disruption of the hypothalamo-pituitary-gonadal axis, by endogenous and/or exogenous factors, causes cyst formation. Secretion of GnRH/LH from the hypothalamus-pituitary is aberrant, which is attributed to insensitivity of the hypothalamus-pituitary to the positive feedback effect of oestrogens. In addition, several factors can influence GnRH/LH release at the hypothalamo-pituitary level. At the ovarian level, cellular and molecular changes in the growing follicle may contribute to anovulation and cyst formation, but studying follicular changes prior to cyst formation remains extremely difficult. Differences in receptor expression between cystic follicles and dominant follicles may be an indication of the pathways involved in cyst formation. The genotypic and phenotypic link of COD with milk yield may be attributed to negative energy balance and the associated metabolic and hormonal adaptations. Altered metabolite and hormone concentrations may influence follicle growth and cyst development, both at the level of the hypothalamus-pituitary and the ovarian level.

5.1 Incidence of luteal cyst

In the present study, out of surveyed 583 dairy buffaloes at LSF, Adhartal and private dairy farm of Jabalpur, the overall incidence of luteal cyst was recorded to be 10.63 per cent. The present observation was in accordance with the study of Kumar et al. (2014) who reported 0.66 to 12.50 per cent incidence of luteal cyst in buffaloes in India.

The incidence of cystic ovarian disease in buffaloes was reported within a range of 0.91 to 1.40 per cent by Rao and Sreemannarayana (1982) and Purohit (2014). The incidence of luteal cyst in buffaloes was reported in the range of 0.10 to 0.19 per cent (Mittal et al., 2010
and Modi et al., 2011) in the past. The incidence of luteal cyst in the present study was found to be higher as compared to the reports of above workers. The higher incidence of luteal cyst might be due to the fact that the present study for incidence was conducted during the transition month of the year i.e. in July when the animals were under environmental stress of humid climate and their reproductive ability might be compromised.

As per the literature available, the incidence of luteal cyst in abroad is comparable with the results of present study ranging from 10 to 13 per cent (Kumar et al., 2014).

5.1.1 Month wise and season wise incidence of luteal cyst

The literature on month wise and season wise prevalence of cystic ovarian disease is lacking, but it is well documented that cystic ovarian disease is one of the important causes of postpartum anoestrus in dairy animals (Kumar et al., 2014). Thus, we can compare the results of month wise and season wise incidence of luteal cyst in the present study with the month wise and season wise incidence of anoestrus in postpartum dairy animals in the past.

The present study revealed higher incidence of luteal cyst during April (19.27%), followed by July (14.45%), March (13.25%) and May (12.19%) and lowest incidence (7.22%) during December, January and February. The results of the present study were comparable with the findings of Singh (2013) who reported higher incidence of anoestrus during March (26.01%) as compared to other months i.e. November (14.94%), December (12.55%), January (10.89%) and February (13.10%).

The results on season wise incidence of luteal cyst in the present study were observed to be higher during summers (13.72%) followed by rainy (10.27%) and winter (7.25%) seasons. The results are in accordance with the study of Kumar et al. (2014) who reported higher incidence of anoestrus in summer season (66.28%) followed by rainy (22.51%) and winter (11.25%) seasons.
5.1.2 Age wise and parity wise incidence of luteal cyst

The present experiment also includes the age wise and parity wise study on incidence of luteal cyst in a total of 84 dairy buffaloes at LSF, Adhartal. The results revealed maximum prevalence of luteal cyst among the animals belonging to the age group of 11 to 12 years (58.33%) and in fifth parity and above (34.48%). The results of present study are comparable to the study of Mukasa-Mugerwa (1989) who reported that the incidence of cystic ovarian disease was greater in animals during their fifth or sixth lactation. The reason for higher prevalence of cystic ovarian disease during advanced stages of lactation can be correlated with the high milk yield in dairy animals in their advance lactation, which makes the animals susceptible to post-parturient disorders including ovarian dysfunction. The present findings are also in accordance with the study of Lee and Kim (2006) who observed that the incidence of ovarian cysts was lower in animals with a parity of one than in animals with higher parities.

5.2 Serum progesterone profile

The serum progesterone concentration was estimated before treatment (day 0) and the results of present study revealed that the mean serum progesterone concentration in all the animals was observed to be above 1 ng/ml and the results are in accordance with the previous study (Carroll et al., 1990; Farin et al., 1990; Zulu et al., 2003 and Johnson, 2004) where the progesterone concentration in animals having luteal cyst was observed to be above 1 ng/ml.

The progesterone concentration in the animals having luteal cyst was observed to be above 3 ng/ml (Leslie and Bosu, 1983 and Douthwaite and Dobson, 2000) in the past. The results of serum progesterone concentration in animals with luteal cyst condition in the present study were observed to be lower as compared to the findings of the above workers.

The serum progesterone concentration on the day of oestrus in animals responded to treatment was also estimated (<0.50 ng/ml) in the present study and the results were comparable to the findings of
Kamonpatana et al. (1979); Takkar et al. (1982) and Mondal et al. (2010) who observed that the progesterone concentration in the animals on the day of oestrus was below 0.50 ng/ml.

According to Douthwaite and Dobson (2000), at the time of PRID removal, plasma progesterone concentration had decreased in the cows with luteal cysts to 0.87 ng/ml, although there was no change in original cyst structure in 45 per cent of the cases. The results in the present study were comparable with the above findings where the serum progesterone concentration in the animals after treatment with progesterone had declined as compared to the progesterone concentration before treatment in these animals; however, there was no change in the original cyst structure.

5.3 Fertility response to treatment in terms of conception rate

Many treatment strategies have been used to resolve the cystic condition. These include manual rupture, progesterone, gonadotropin-releasing hormone (GnRH), human chorionic gonadotropin (hCG) and prostaglandin (PGF\(_2\alpha\)) (Day, 1991).

In the present study three hormonal therapies viz. Hydroxyprogesterone (group 1), Buserelin+PGF\(_2\alpha\) (group 2) and Melatonin (group 3) have been used for the treatment of luteal cyst and the recovery of ovarian cyclicity in dairy buffaloes. Group 4 was taken as control to compare the results of above hormonal treatment.

The results of fertility response to various treatments were studied in terms of conception rate following treatment.

The results of the study revealed highest conception rate in group 1 (83.33%) followed by group 2 (33.33%). However, none of the animals responded in group 3 and group 4.

In the present study the conception rate after the administration of slow release progesterone preparation @ 1000 mg intramuscularly as single dose was observed to be higher as compared to the studies of Dobson et al., (1977) and Ijaz et al., (1987) who reported 55.55 per cent and 50 per cent conception rate, respectively, with the use slow release progesterone
preparations. The exact reason behind this is difficult to explain, but probably 1000 mg of slow release hydroxyprogesterone caproate injected intramuscularly might have mimicked the action of PRID implant and would have allowed storage of sufficient amount of gonadotrophins, particularly, LH through negative feedback on hypothalamus and pituitary, and upon withdrawal of progesterone it resulted in LH release causing induction of oestrus and ovulation. However, the study should be carried out in large number of cystic buffaloes so that its utility in treatment of luteal cyst can be finally established.

The present study revealed 33.33 per cent conception rate with the use of Buserelin+PGF$_2$α and the results were in accordance with the studies of Kesler et al. (1978); Ijaz et al. (1987) and Rudowska et al. (2015) who reported satisfactory results with the use of GnRH+PGF$_2$α.

Results with the administration of slow release melatonin were observed to be satisfactory in restoration of cyclicity in anoestrous buffaloes (Ramadan et al., 2014; Kumar et al., 2015 and Singh et al., 2016). In the present study, conception rate was observed to be zero per cent with the use of slow release melatonin, the reason behind this may be the lower dose rate of melatonin (90 mg/animal) and lesser frequency of administration (once subcutaneously) in the present study.
6. SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

There are several reproductive disorders viz. delayed puberty, silent oestrus, anoestrus, summer infertility and postpartum uterine disorders, adversely affecting the reproductive efficiency of buffaloes and thus production potential. COD remains an important cause of extension of the interval from calving to conception and increased number of inseminations per conception thus decreases the reproductive performance and subsequently the production from the animal. Despite the huge amount of literature, the exact pathogenesis of the disease is still not completely clear and therefore also the choice of treatment is still debated. Many treatment strategies have been used to resolve the cystic condition. These include manual rupture, progesterone, gonadotropic-releasing hormone (GnRH), human chorionic gonadotropin (hCG) and prostaglandin (PGF2α).

The present study was conducted on 24 postpartum (120 days onwards) anoestrus Murrah buffaloes belonging to LSF, Adhartal and private dairy farm of Jabalpur having luteal cyst on either or both the ovaries. The anoestrus animals were selected on the basis of farm records and the luteal cyst was confirmed by two consecutive rectal examinations 10 days apart. The objectives of the study were to assess the incidence of luteal cyst in dairy buffaloes, effect of different treatment protocols on luteal cyst and comparison of efficacy of various therapeutic regimens.

To study the incidence of luteal cyst, 583 dairy buffaloes were screened at LSF, Adhartal and private dairy farm of Jabalpur before the start of treatment i.e. in July, 2016. A total of 68 animals were found anoestrus (120 days postpartum) during the particular period. After two consecutive per-rectal examinations of these 68 animals 10 days apart, a total of 62 animals were found suffering from luteal cyst. The analysis of surveyed data revealed that the overall incidence of luteal cyst in buffaloes before the start of treatment was 10.63 per cent and luteal cyst was contributing 91.17 per cent to anoestrus at that particular period.
The analysis of results on month wise incidence of luteal cyst revealed higher incidence during April (19.27%), followed by July (14.45%), March (13.25%) and May (12.19%). The lowest incidence was observed to be 7.22 per cent during December, January and February. The analysis of results on season wise incidence of luteal cyst revealed higher incidence of luteal cyst in buffaloes during summers (13.72%), followed by rainy (10.27%) and winter (7.25%) seasons. Age wise and parity wise incidence of luteal cyst was also studied in 84 buffaloes at LSF, Adhartal and the results revealed maximum incidence in age group of 11 to 12 years (58.33%) and in fifth parity (34.48%).

The conception rate after various treatments in different groups was recorded to be 83.33 per cent (group 1), 33.33 per cent (group 2), 0.0 per cent (group 3) and 0.0 per cent (group 4). Results revealed higher conception rate in group 1 (Hydroxyprogesterone), followed by group 2 (Buserelin+PGF\textsubscript{2}α). However, none of the animals responded in group 3 (Melatonin) and group 4 (Control).

The serum progesterone concentration (ng/ml) was estimated before and after the treatment and the results revealed that the mean serum progesterone concentration at day 0 in both responded and non-responded animals was above 1.0 ng/ml. The mean serum progesterone concentration (ng/ml) after treatment (on the day of estrus) in responded animals of group 1 (0.49±0.38) and group 2 (0.44±0.06) remarkably dropped to a level of below 0.50 ng/ml. The mean serum progesterone concentration (ng/ml) after treatment (at day 21) in non-responded animals of all groups was not considerably reduced as compared to the mean serum progesterone concentration at day 0 in these animals and was recorded to be in the range of 0.60-1.20 ng/ml.

Present study revealed higher incidence of luteal cyst in dairy buffaloes during the summer months \textit{i.e.} March, April and May, followed by the transition months \textit{i.e.} June and July. The results of fertility response were good in both the conventional treatment protocols \textit{i.e.} Progesterone and Buserelin+PGF\textsubscript{2}α but better results were observed with Progesterone, however, the results with the use of Melatonin were not satisfactory may be due to lower dose rate and lesser frequency of administration.
6.2 Conclusion

1. Present study revealed 10.63 per cent incidence of luteal cyst in dairy buffaloes. Higher incidence was observed during summer months *i.e.* March, April and May, followed by the transition months *i.e.* June and July.

2. The incidence of luteal cyst was observed to be higher in age group of 11-12 years and in fifth parity.

3. Serum progesterone was found as a marker for monitoring the functional status of ovarian follicles and corpus luteum and also as a diagnostic tool for identifying ovarian condition such as cystic ovaries in acyclic buffaloes.

4. The results of fertility response were good in both the conventional treatment protocols *i.e.* Progesterone and Buserelin+PGF$_{2}$$\alpha$ but better results were observed with Progesterone, however, the results with the use of Melatonin were not satisfactory may be due to lower dose rate and lesser frequency of administration.
6.3 Suggestions for further work

1. The detailed study of hormonal profile and Ultrasonography can be incorporated along with the per-rectal examination for further confirmation of cystic ovarian disease and accurate differentiation of follicular and luteal cysts.

2. The detailed study on causes of the disease can be conducted by the complete estimation of nutritional and reproductive status of the animals postpartum to reduce the occurrence of disease.

3. The study should be repeated in large number of animals before making any recommendation for field application.
7. REFERENCES


