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Luteal Dysfunction: A Potential Cause of Repeat Breeding and the Strategies to Combat it

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Abstract

Luteal dysfunction, also called as luteal insufficiency or luteal inadequacy is a major endocrine etiology of repeat breeding syndrome in the cattle and buffalo leading to early embryonic mortality and decreased reproductive efficiency of the dairy herd. The luteal inadequacy as an independent component of repeat breeding acts as a sequela to either inadequate progesterone or the premature luteal regression that clinically manifest as delayed ovulation with an extended follicular phase, longer interval between luteolysis and ovulation, delayed postovulatory rise in progesterone concentration and presence of suprabasal progesterone concentration during follicular phase allowing follicular and oocyte ageing due to postponemet of LH surge. Luteal inadequacy can be addressed either at post breeding window of day 0-12 with different hormonal combinations including GnRH, hCG, progesterone or by non hormonal antiluteolytic strategies like ω-3 and ω-6 polyunstaturated fatty acid (PUFA) supplementation in the diet.

Keywords: Luteal inadequacy, repeat breeding, ovulation, progesterone, GnRH, hCG and PUFA.

Dairy sector is an integral component of Indian agriculture with a population of 190.9 million cattle and 108.7 million buffaloes. Although India ranks first in milk production with an annual production of 146.3 MT (GOI, 2014-15), the lactation yield of dairy animals in India is much less compared to developed countries (Joshi *et al.,* 2005). The major hindrance in the success of Indian dairy industry is the suboptimal reproductive rhythm of our dairy animals. A calving interval of 12 or 13 months is habitually recommended to be optimal for the annual high milk yield and reproductive efficiency of dairy animals, and is economically worth to the dairy producers (Murugavel, 2003). Repeat breeding (RB) syndrome continues to be a major problem in cattle and Buffalo breeding, leading to large substantial economic losses to the dairy producer (Lafi *et al.,* 1992). Being multifactorial aetiological concern, luteal deficiency is one of the predisposing factors causing low fertility due to early embryonic mortality (Diskin and Morris, 2008). Low plasma progesterone may affect reproductive processes before and after insemination. The situation has aggravated in recent years because rise in milk production is associated with a gradual decline in plasma progesterone, consistent with the negative relationship between milk production and progesterone concentrations (Lucy, 2001). High environmental temperatures (Wolfenson *et al.,* 2000), bacterial endometritis (Bouters, 1985), nutritional problems (Stevenson, 2001), high milk production, and excessive dry matter consumption (Vasconcelos, 1998) may cause accelerated metabolism of progesterone in liver,

thus leading to low plasma progesterone during luteal phase of estrous cycle (Stubbings and Walton, 1986), and hence increase in embryonic losses (Hommeida *et al.,* 2004).

Physiology of Corpus Luteum and Luteolysis

The corpus luteum (CL) develops from the residual follicular granulosa cells and theca cells after ovulation. It is a transitory endocrine gland and a principal source of progesterone. During a normal luteal phase, the CL increases in size along with its ability to secrete progesterone. Once it has obtained its mature size and reached its maximum potential for secretion of progesterone, luteal function is maintained for a few to several days depending on the species, and then if the animal does not become pregnant, luteal regression must occur to allow initiation of the next cycle (Niswender *et al.,* 2000). The preovulatory surge of gonadotropin (LH) induces ovulation and differentiation of residual follicular cells that form the corpus luteum and begins to produce progesterone at high rates (Niswender *et al.,* 2000). The mature corpus luteum is composed of at least two steroidogenic cell types based on morphological and biochemical criteria and on the follicular source of origin. Small luteal cells (SLC), which appear to be of thecal origin, contain most of the LH receptors and are more sensitive to LH stimulation, leading to increased progesterone production. Large luteal cells (LLC) are of granulosa cell origin and have few of the LH receptor sites and most of the $PGF_{2\alpha}$ receptors. LLC also produce and store oxytocin. Small and large luteal cells differ in their basal secretion rate of progesterone with LLC producing 2 to 40 fold more progesterone than unstimulated SLC. Granulosa and theca cells function coordinately to produce estradiol during follicular phase (Fortune and Quirk, 1991). Theca cells produce androgens from cholesterol, which is converted to estradiol by granulosa cells (Bao and Gaverick, 1998). The synthesis of progesterone is accomplished by increased expression of enzymes necessary for conversion of cholesterol to progesterone and decreased expression of enzymes that convert progesterone to estrogen (Niswender *et al.,* 2000).

Luteolysis is the demise of corpus luteum which is primarily caused in the bovines by $PGF_{2\alpha}$ (Senger, 1999). Degradation of CL tissue is known as structural luteolysis, while loss of steroidogenic activity is referred to as functional luteolysis (Pate, 1994). Luteal synthesis of progesterone is diminished prior to the start of structural luteolysis (Sawyer *et al.,* 1990). Estrogen from the developing dominant follicle activates oxytocin (OT) release from the posterior pituitary which in turn causes $PGF_{2\alpha}$ release from the uterus (McCracken *et al.,* 1996). Estrogen also lowers the action potential of uterine smooth muscle tissue, rendering it more sensitive to oxytocin. Prostaglandin $F_{2\alpha}$ reaches the ovaries through a counter-current exchange system from the uterine vein to the ovarian artery (Senger, 1999) where it acts to both initiate luteolysis and up-regulate further OT and $PGF_{2\alpha}$ release from luteal and uterine sources, respectively (Niswender *et al.,* 2000). Functional luteolysis begins primarily as a result of reduced blood flow to the CL through apoptosis of luteal tissues and corresponding diminutions in capillary density. In addition to release of oxytocin from luteal cells, prostaglandin $F_{2\alpha}$ also stimulates endothelin-1 production from luteal endothelial cells. Thus, at early stages $PGF_{2\alpha}$ initiates a positive feedback mechanism on oxytocin and subsequently itself (Flint *et al.,* 1990). Endothelin-1 acts to constrict ovarian capillaries and inhibit steroidogenic activity of luteal cells (Girsh *et al.,* 1996). Prostaglandin $F_{2\alpha}$ binds to G-protein coupled receptors on the surface of the large luteal cells initiating a cascade of events leading to the release of intracellular Ca^{2+} which stimulates the action of the catalyst protein kinase C. Protein kinase C in turn causes the modification of cellular proteins intrinsic to steroidogenesis, cholesterol availability, and maintenance of the luteal structural matrix (Niswender *et al.,* 2000). As blood flow is decreased and cellular

machinery stopped or destroyed, there is a marked increase in ovarian populations of leucocytes, T-lymphocytes, and macrophages which facilitate apoptosis of the luteal tissues (Penny *et al.,* 1999). As luteolysis progresses circulating progesterone is decreased, removing the negative feedback on the hypothalamus and anterior pituitary (McWilliams *et al.,* 1998). Estrogen is secreted from the developing preovulatory follicle and signals an increase in the release of GnRH and LH, resulting in ovulation.

Plasma Progesterone Profile during Estrous Cycle

Plasma progesterone concentrations directly reflect the functional status of CL. Plasma progesterone is at its nadir (0.1-0.3 ng/ml), during estrus, (Mondal *et al.,* 2005) followed by the first significant increase about 4-7 days after estrus (Ahmed *et al.,* 1977). In buffalo heifer and buffalo cow serum, plasma progesterone was around 1 ng/ml till day 6, followed by a gradual increase to 4.89+0.40 and 5.12+0.41 ng/ml, respectively on day 15 post-estrus (Takkar *et al.,* 1983). In another study, peak plasma progesterone was observed on day 16 of estrous cycle (3.47 ng/ml; Pahwa and Pandey, 1983). Infertile buffaloes had a combined pattern of delayed rise and low plasma progesterone indicative of insufficient luteal function (Kavani *et al.,* 2005). Moreover, in repeat breeding buffaloes, plasma progesterone was higher on day 0 than the regular breeding buffaloes (1.04+0.57 vs 0.14+0.01 ng/ml), whereas, on day 7 plasma progesterone was lower in repeat breeder buffaloes (2.63+0.95 vs 4.05+0.01 ng/ml (Venkatesan *et al.,* 2005). In Murrah buffaloes plasma progesterone during periestrus, early luteal, mid luteal and late luteal phase were observed 0.42±0.05, 0.66±0.12, 1.55±0.33 and 1.12±0.27 ng/ml, respectively in buffaloes with overt estrus, however, corresponding values in buffaloes with silent estrus were 0.38±0.06, 0.51±0.07,1.30 ±0.13 and 0.66±0.13 ng/ml, respectively (Mondal *et al.,* 2005). Plasma progesterone was significantly higher in fertile compared to infertile cycle of buffaloes on day 14 (2.14+0.21 vs 1.20+0.19 ng/ml) and day 21 (2.57+0.21 vs 0.42+0.04 ng/ml), but not at estrus (0.37+0.02 vs 0.39+0.05 ng/ml) or day 7 (1.29+0.08 vs 1.12+0.11 ng/ml) post-insemination (Kavani *et al.,* 2005). A trend for greater progesterone on the day of insemination (0.19+0.01 ng/ml vs 0.24+0.02 ng/ml, respectively) is likely to decrease the pregnancy rate (Lopes *et al.,* 2007).

Correlation of Preovulatory Follicle (POF) Diameter with Subsequent Spontaneous Corpus Luteum Development and Conception Rate

Around the onset of estrus, the preovulatory follicle produces substantial amounts of estradiol and a positive correlation exists between preovulatory follicle diameter and plasma concentrations of estradiol (Perry *et al.,* 2007). It is well established that, near the time of estrus, the preovulatory follicle under the pituitary gonadotrophic support grows continuously to a large size and produces substantial amounts of estradiol. Once plasma estradiol reaches a certain level, LH surge is induced, followed by ovulation about 24 to 32 h later. Corpus luteum is formed from the follicular cells followed by progressive increase in plasma progesterone with the attainment of maturity by the CL (Mann, 2009).

Optimum differentiation and growth rate of CL varies depending upon the duration and amplitude of LH surge (Quintal *et al.,* 1999). In fact, LH surge induces a chain of events in the ovulatory follicle that are essential for the formation of a normal CL. More specifically, LH surge induces a sharp decline in the production of P-450 aromatase, and a sharp increase in the production of P-450-scc and 3β-HSD in the granulosa cells, resulting in a drop in estradiol production and an increase in progesterone production (Zelinski-Wooten *et al.,* 1997). A slow rise in post-ovulatory progesterone concentrations could result from suboptimal luteinization of the CL due to low amplitude

LH (Ambrose *et al.,* 1998). Furthermore, the normal development of a CL depends upon preovulatory follicles having: (*i*) an adequate number of granulosa cells, (*ii*) an adequate number of LH receptors on granulosa and thecal cells, and (*iii*) granulosa cells capable of synthesizing adequate amounts of progesterone after luteinization (McNatty *et al.,* 1979).

Conception rate is influenced by preovulatory follicular size, the latter is important for the subsequent development of the spontaneous corpus luteum (SCL). A larger preovulatory follicle may generate a larger CL that will secrete more progesterone and hereby have a positive effect on pregnancy recognition and pregnancy rates (Binelli *et al.,* 2009). Cattle ovulating larger follicles had lower pregnancy rates and higher pregnancy loss between days 28 and 98 post-insemination (Vasconcelos *et al.,* 1999). An inverse relationship was recently reported between maximum diameter of ovulatory follicle and embryo survival (Lynch *et al.,* 2010). This report also suggests that neither CL diameter nor plasma progesterone were related to the size of the ovulatory follicle (Lynch *et al.,* 2010). Moreover, circulating concentrations of progesterone were not related to CL diameter (Lynch *et al.,* 2010). Effect of POF size on fertility in dairy cows has been observed (Bello *et al.,* 2006), suggesting that conception rates are greater after ovulation of a follicle of 16 mm in diameter and that conception rates declined if the POF size was either less or more than 16 mm. Recently, published report cows that ovulated follicles of 11 mm had lower fertility (17%) to TAI than that of cows that ovulated follicles of 15 mm (61%), no significant association between preovulatory follicle size and pregnancy rate to TAI (Colazo *et al.,* 2009).

Attributes for Luteal Dysfunction

1. **Nutritional Inadequacy**: Nutritional adequacies can result in deficiencies of growth hormone and or insulin with resultant low IGF-1 secretion (Chase *et al.,* 2000). Low IGF-1 can a possible account for poor development of a preovulatory follicle ultimately results to inadequate luteal tissue formation (Hunter, 1991).
2. **Lack or diminished luteotropic hormones**: Luteal inadequacy due to diminished response to the circulating luteotrophic hormones may contribute to embryonic mortality (Shelton *et al.,* 1990).
3. **Functional disturbances of progesterone production**: Serum progesterone is known to be altered in RB cows (Bage *et al.,* 2003) and buffaloes (Panchal *et al.,* 1993). In fact, low plasma progesterone during the early luteal phase is associated with poor embryo survival (Diskin and Morris, 2008). Progesterone is required not only to maintain a suitable uterine environment but also to facilitate the elongation of conceptus and consequently, the secretion of adequate interferone-tau (Mann, 2002). Early in the luteal phase progesterone down regulate the oxytocin receptors for atleast 10 days, thus preventing premature luteolysis. Low luteal progesterone concentration can cause poor embryo development and hence embryonic death due to a suboptimal uterine environment on account of low progesterone.
4. **Thermal Stress**: Thermal stress in cows during the days preceding AI and during early pregnancy is detrimental to fertility. High environmental temperature and humidity on day 2 prior to AI (Ingraham *et al.,* 1979), day 0 (Cavestany *et al.,* 1985) and d 1 after AI (Gwazdauskas *et al.,* 1974) were negatively correlated with conception rate. Low progesterone output of the CL with morphological changes due to possible alterations in uterine environment during thermal stress and its relationship to embryo survival.
5. **Miscellaneous**: High prolactin causes inadequate CL functioning results in low progesterone in buffaloes (Roy and Prakash,

2007). Oxytocin injections, uterine dilatation and intrauterine infusion of seminal and prepucial fluids causes luteal inhibition in bovine (Hansel and Wagner, 1960).

Strategies to Combat Luteal Dysfucntion

A. Hormonal Approaches

1. Administration of GnRH

The impact of GnRH in modifying reproductive efficiency of normal as well as repeat breeder dairy bovines has been investigated extensively. Gonadotropin releasing hormone (GnRH) has the primary effect at pituitary gonadotropes to stimulate the pulsatile release of gonadotropins viz. luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Martinez *et al.,* 2003). Luteinizing hormone is essential for the maintenance of progesterone production by luteal cells. Dependence of luteal functions on LH varies during different phases of the estrous cycle (Peters *et al.,* 1994). Preovulatory LH surge induces a chain of events in the ovulatory follicle that are essential for the formation of a normal CL. Episodic LH pulses are required to maintain CL development in cattle (Peters *et al.,* 1994).

(a) GnRH analogue on the day of estrus. Use of GnRH at the time of insemination as a treatment for repeat breeder animal has demonstrated an overall advantage in pregnancy rate of 18-25 per cent (Peters, 2005). In repeat breeding dairy cattle, after treatment with 10 µg GnRH at the time of insemination, the conception rate for treated and control groups was 48.1 and 31.0 per cent, respectively (Bon Durant *et al.,* 1991). About 54.2 per cent repeat breeding cattle conceived after GnRH treatment at the time of insemination (Rao, 1991). Similarly, an improvement in conception rate (50% vs 20%) was observed following the administration of 20 µg GnRH, on the day of insemination in repeat breeding cattle (Abhilash *et al.,* 2006).

(b) GnRH analogue on day 5 of estrous cycle. Post-ovulation plasma progesterone concentrations have been positively correlated to volume of uterine secretions (Garrett *et al.,* 1988), conceptus development (Mann and Lamming, 1999), ability of embryo to secrete IFN-τ (Starbuck *et al.,* 1999), embryo viability (Stronge *et al.,* 2005), and ultimately conception rates (Campanile *et al.,* 2008). Administration of a GnRH analogue on day 6 of estrous cycle induced accessory CL development in 75% cattle (Rusbridge *et al.,* 1992). Following treatment with GnRH (100 µg) on day 5 post-estrus, the number of accessory CL and the total CL tissue area present was greater on day 17 as well as treated cows had higher pregnancy rates (Willard *et al.,* 2003). Similarly, in another study, administration of GnRH on days 5 or 6 after estrus lead to higher luteal tissue development, and increased plasma progesterone, thus, resulting in a 45 per cent increase in pregnancy rate in comparison to controls (Arnett *et al.,* 2002). Buffaloes treated with 10 µg Buserelin acetate on day 5 exhibited first and third service conception rate as 37.5%, 50.0%, respectively as compared to controls (33.33%) (Mandal *et al.,* 2004).

(c) GnRH analogue on day 12 of estrous cycle. GnRH administration between days 11 and 13 post-insemination can boost the function of existing CL followed by an increase in plasma progesterone (Robertson *et al.,* 1993). This increase in plasma progesterone is due to luteotrophic response of small luteal cells to LH that is released in response to GnRH (Robertson *et al.,* 1993). However, no alteration in plasma progesterone was observed in buffaloes treated with GnRH on day 12 after insemination although pregnancy rate was increased by 17 per cent (Singh, 1997). Buffaloes treated with 10 µg Buserelin on day 12 post-estrus exhibit 70 percent first service conception rate (Mandal *et al.,* 2004). Improvement of fertility was also seen subsequent to the administration of GnRH analogues between days 11-14 in nulliparous beef heifers (Rettmer *et al.,* 1992) and lactating

dairy cows (López-Gatius *et al.,* 2006). A single injection of Buserelin acetate on day 12 post-insemination can reduce embryo mortality thus leading to 12 percent improvement in fertility in cattle (Peters *et al.,* 2000). Repeat breeding dairy cattle exhibited an increase in cycle length by 6 days (Bostedt and Okyere, 1988) and an increase in conception rate by 10 per cent (Lopez-Gatius *et al.,* 2006).

2. Administration of hCG

Human chorionic gonadotropin (hCG) hormone has biological activity similar to LH and hCG can bind to LH receptors on small luteal cells to activate a second messenger that enhances progesterone synthesis (Stevenson *et al.,* 2007).

(a) hCG on the day of estrus. Administration of hCG after insemination or at specific times coincident with the presence of the dominant follicle may stimulate CL function and increases plasma progesterone with a consequent positive effect on embryo survival (Thatcher *et al.,* 2003). First service conception rate was 62.50 and 18.75 per cent in hCG treated and non-treated repeat breeder cows, respectively (Selvaraju *et al.,* 2004). Administration of 1500 IU hCG on the day of insemination lead to 45% increases in conception rate (Abhilash *et al.,* 2006). When buffaloes were treated with hCG 16 h before insemination, plasma progesterone was 4.02±2.34 ng/ml on day 22 post-AI and conception rate was 50.8%, on day 40 (Carvalho *et al.,* 2007).

(b) hCG on day 5 of estrous cycle. On day 5 of estrous cycle, granulosa cells of the dominant follicle contain LH receptors, thus hCG may induce ovulation and formation of an accessory CL (Diaz *et al.,* 1998). Treatment with hCG (3300 IU) on day 5 after insemination lead to presence of more than one CL in 86.2% cattle in comparison to 23.2% in controls. hCG-induced formation of an accessory CL along with higher plasma progesterone may enhance embryo survival and increase pregnancy rate (Stevenson *et al.,* 2007). In fact, development of the conceptus is related to higher concentrations of plasma progesterone and ability of the conceptus to secrete IFN- τ (Mann and Lamming, 2001). On contrary, others suggested that following, hCG administration on day 7 of estrous cycle, there was no difference in CL diameter between the spontaneous- and treated-cattle up to day 12 of estrous cycle (Sianangama and Rajamahendran, 1996). Another reported absence of difference in plasma progesterone between cattle with and without hCG-induced CL (Santos-Valadez *et al.,* 1982).

(c) hCG on day 12 of estrous cycle. An increase in plasma progesterone subsequent to hCG treatment on day 12 post-insemination suggests that LH-like activity of hCG may provide luteotrophic stimulation to CL through conversion of small luteal cells to large luteal cells by an increase in the size of large luteal cells (Khan *et al.,* 2007). Conversion of stage-two luteal cells to stage-three luteal cells is reduced following GnRH-induced LH release or through LH-like activity of hCG. These stage-two luteal cells secrete more progesterone when compared with stage-three luteal cells (Schmitt *et al.,* 1996). Moreover, hCG administration (1000 IU) on day 14 post-insemination induced accessory CL and increased plasma progesterone, thus leading to 35% increase in conception rates in lactating dairy cows (Sianangama and Rajamahendran, 1992).

3. Progesterone supplementation between days 4 to 10 of estrous cycle

Progesterone secretion by the CL modulates the function of uterine glands, and thereby, creates a histotrophic environment for optimum nourishment and growth of the free-floating conceptus (Spencer *et al.,* 2004). Also, supplementation of progesterone may enhance pregnancy rate by blocking luteolysis due to embryotrophic effects. It is known that enhanced conceptus development favours IFN-τ secretion (Mann and Lamming, 2001).

B. Non Hormonal Antiluteolytic Strategies

1. Nutritional strategies

Nutrition plays a paramount role in animal reproduction. Majority of the reproductive disturbances could be alleviated by supplying the feed with balance of different nutrients. Fat is one of the essential nutrients, which have positive effect on the fertility in all the farm animals especially in diary cattle and buffaloes through minimising the negative energy balance. Fats are glyceride esters of fatty acids that may have a straight effect on the transcription of genes that encode proteins that are essential to reproductive events (Mattos *et al.,* 2000). Dietary fats increase concentrations of circulating cholesterol, the originator of progesterone (Grummer and Carroll, 1991). Fat supplementation has also been shown to stimulate programmed growth of a preovulatory follicle (Oldick *et al.,* 1997), total number of follicles (Lammoglia *et al.,* 1997).

Polyunsaturated fatt acids (PUFAs) such as omega-3 (ω-3) and omega-6 (ω-6) are component of essential fatty acids which need to b supplemented in the feed. ω-3 fatty acids play an important role in follicular development, oocyte maturation, maternal recoginition of pregnancy (MRP), embryonic survival and increasing the conception rate in female farm animals and improves semen freezing efficiency and motility in male farm animals (Karuppunaswamy *et al.,* 2016). ω-6 fatty acids have been associated with ovulation, parturition and postpartum ovarian rebound phenomenon, thus making feeding of essential fatty acids dietary essential in livestock. Cows receiving greater amounts of omega-3 fatty acids had the greatest average CL size (Petit *et al.,* 2002). Certain polyunsaturated fatty acids (PUFA) have the ability to inhibit the action cyclooxygenases (COX-1 and COX-2). Linoleic acid inhibited endometrial prostaglandin synthesis by inhibition of COX-2 activity *in vitro*. Linolenic acid competes with linoleic acid for desaturation and elongation enzymes for synthesis of prostanoids and their precursors (Petit *et al.,* 2002). Oral supplementation of Menhaden fish meal increases plasma concentrations of EPA in primiparous cows (Burns *et al.,* 2003). Eicosapentaenoic acid and DHA inhibited $PGF_{2\alpha}$ secretion by bovine endometrial cells *in vitro* (Mattos *et al.,* 2001). These fatty acids inhibit $PGF_{2\alpha}$ synthesis, thereby decreasing early embryonic mortality (Thatcher *et al.,* 2001). Cows fed any diet containing fishmeal had reduced plasma PGFM concentrations after the estrogen-oxytocin challenge than cows receiving no fishmeal (Mattos *et al.,* 2002).

2. Miscellaneous

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used for inhibition of prostaglandin release. This is accomplished through the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) involved in the formation of prostaglandins (Vane and Botting, 1998). So, prevention of prostaglandin release can be proficient through the administration of NSAIDs, such as flunixin meglumine (Smith *et al.,* 2000). During embryo transfer uterine manipulation occurs, which may cause the release of $PGF_{2\alpha}$ from the uterus and a subsequent loss of pregnancy. Flunixin meglumine injected at the time of embryo transfer (ET) may also improve pregnancy rates (Odensvik *et al.,* 1993). Average estrous cycle length increased in heifers receiving FM thrice daily from 19.8 to 22.5 day and four-times-daily from 19.5 to 26 day. Furthermore, luteolysis did not occur until after the treatment was terminated (day 23 or 24) in heifers receiving the four-times-daily dose. Those animals receiving four-times-daily FM injections had significantly decreased PGFM concentrations during the treatment period (Odensvik *et al.,* 1998). Injection of flunixin meglumine, on day 14 post-estrus resulted in higher pregnancy rates (84% treatment vs. 76% control; Merrill *et al.,* 2003). However, administration of flunixin meglumine @ 1.1 mg/kg twice i/m on the evening of day

15 and morning of day 16 after insemination in 26 Holstein heifers increased pregnancy rate by 23 per cent compared to control (69.2% *vs.* 46.2%) at day 65 of gestation (Guzeloglu *et al.,* 2007). They opined that two doses of the COX-inhibitor at a critical period inhibited /delayed synthesis of $PGF_{2\alpha}$ and luteolysis. The length of estrous cycle in buffaloes increased following administration of meloxicam at the dose rate of 0.5mg/kg body weight (I.M) once daily on day 15 and 16 of the cycle (Shukla *et al.,* 2007). In addition, administration on day 13 to 15 post-AI in buffalo increased first service conception rate by 20 per cent than the control (53.33 vs. 33.33%; Rajkumar, 2008).

CONCLUSION

Luteal insufficiency is one of the reasons for implantation failure and has been responsible for unsuccessful reproduction. Low progesterone environment is created iatrogenically due to interventions in reproduction. Use of gonadotrophin-releasing hormone analogs to prevent the LH surge and aspiration of granulosa cells during the oocyte retrieval may impair the ability of corpus luteum to produce progesterone. Treatment of the underlying disorder and use of progestational agents like progesterone/human chorionic gonadotrophin have been found to be effective.

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