Indian J Dairy Sci 74(5): 379-386
https://doi.org/10.33785/IJDS.2021.v74i05.001

INVITED REVIEW

Prolonged oestrus as a cause of infertility in dairy cattle – A review

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Received: 13 August 2021 / Accepted: 18 August 2021 / Published online: 31 October 2021

Abstract: Prolonged oestrus is one of the conditions associated with perturbed follicular dynamics that culminates into substantial economic losses to dairy industries through low success rate of artificial insemination, increased inter-calving period, reducing total milk yield, lowering calf production, shortening reproductive life of the animals, lowering fertilisation rate and abnormal embryonic development. Prolonged oestrus is characterized by the exhibition of extended duration of oestrus in various breeds of cattle. Cows having prolonged oestrus need to be examined at frequent intervals for determining the correct time of artificial insemination. This makes it challenging to inseminate the animals at the correct time, which is a major inconvenience for the field veterinarians and farmers. Since the follicular dynamics in cows is intricate to comprehend, the disorders in which also often go unnoticed and not treated/managed wisely in the field conditions. Therefore, it is of paramount importance to understand the aetiology of prolonged oestrus so that strategies to improve fertility could be designed. Alterations in hypothalamo-hypophyseal-gonadal axis, which is also influenced by some other extrinsic factors like stress, nutrition, age, parity, breed etc., apart from the endocrine alterations could cause the prolonged dominance of follicle. The presence of suprabasal progesterone and luteal insufficiency are considered as the main cause of this condition among cattle. In this review, we attempted to analyse the prior art on the topic and to delineate the possible causes, and to suggest managemental strategies to mitigate the prolonged oestrus condition in dairy cows

Keywords: Cattle, Follicular dynamics, Fertility, Suprabasal progesterone, Prolonged oestrus, Repeat breeding

Introduction

The profitability of dairying depends to a large extent on the efficiency of reproduction. Maximizing reproductive efficiency requires the matching of genotypes to the production environment in order to ensure that the calving intervals are short and the rates of conception to artificial breeding are high. However, when the reproductive efficiency of the dairy cows are analysed, based on the records, it was observed that the conception rates are low (country's average is 35%) and the calving intervals are quite high. One of the important reasons for extended calving interval is repeat breeding. It has been reported that the incidence of repeat breeding in cattle ranged from 5.5 to 33%. It is also reported that one of the most significant reason for repeat breeding in high producing crossbred cows is prolonged oestrus and associated alterations in the endocrine profile.

Prolonged oestrus is a condition in which the duration of oestrus and the interval between the onset of oestrus and ovulation interval both are exceeding the normal range. In some cases, the duration of oestrus has been reported to be greater than 36 hrs, which can vary from 2-7 days (Bage et al. 2002; Bloch et al. 2006). At field conditions, the inseminators/veterinarians are baffled to precisely time the insemination. Prolonged oestrus condition can increase the number of services per conception to more than 4 against the optimum of <2. In addition, it also increases the inter-calving period and decreases the total milk yield, fertilisation rate, embryo development and calf production (Government of Kerala-Economic Review, 2015). Prolonged oestrus has been reported to be observed in 50% of repeat breeder cows (Cummins et al. 2012; Ghuman et al. 2014; Arun et al. 2020). The conception rate in cows showing prolonged oestrus decreased drastically as the duration of oestrus increased (Nebel et al. 2000). The

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conception rate for the animals exhibiting prolonged oestrus was reported as 70%, 80%, 41.66% in two, three- and four-days duration, respectively (Nath 2014). Although several treatment protocols have been evaluated for their effectiveness in treating the condition, their effect is variable and inconsistent (Nakao et al. 1984; Shelar et al. 2002; Shakir 2018). To develop a suitable management and therapeutic strategy for management of prolonged oestrus condition and to achieve high conception rates in cows affected with the condition, it is essential to understand the underlying aetiology. However, systematically organized and analysed reviews are not available on the topic; therefore, in this review we attempted to analyse the prior art on the topic and to delineate the possible causes, and to suggest managemental strategies to mitigate the prolonged oestrus condition in dairy cows.

Incidence and duration of prolonged oestrus

The incidence of prolonged oestrus in cattle of different regions are shown table 1. has been reported as 26.6 and 21.62% among crossbred cattle in Kerala maintained under field and farm conditions, respectively (Jeba-Sujana, 2005). Nearly, 30-40% of repeat breeder crossbred cows displayed prolonged oestrus ie, about 37-60 hrs against the normal duration of 24-36 hrs. Moreover, 70% of these repeat breeder animals had suprabasal plasma progesterone (>1ng/ml) at oestrus (Bage et al. 2002; Singh 2003, Dadarwal et al. 2005 and Singh et al. 2009). The low progesterone (1ng/ml) is vital for conception and favours typical fern pattern in cervical mucus, but when progesterone increases, the typical pattern changes to atypical fern pattern or nil pattern (Kumaresan et al. 2001). Parvathy (2015) reported that 21.78% of prolonged oestrus conditions among the crossbred cows reared under farm conditions. Bedi et al. (2007) observed that out of 1332 oestrus in crossbred cows, 21.9% of crossbred cows exhibited oestrus period for less than 24hrs, whereas 48.9% of cows exhibited oestrus period for 24-36 hrs and 30.05% of cows exhibited oestrus period for more than 36 hrs. Study conducted on crossbred cattle by Das (2017) recorded that the incidence of prolonged oestrus was about 14.24%, and 7.60, 8.04 and 1.06% of cows showed oestrus for 2-, 3- and 4-days duration, respectively. The duration of prolonged oestrus in cattle of different regions are shown **table 2.** The variation in the incidence of prolonged oestrus in crossbred cows may be due to differences in the breeds of cattle and their environment, level of nutrition and stress factor affecting the animals**.**

Suprabasal progesterone concentration and prolonged oestrus

Elevated progesterone concentrations during oestrus extended the secondary signs of oestrus due to persistence of preovulatory follicle (Duchens et al. 1995a). The marginal rise in serum progesterone level during oestrus affected the expression of normal oestrus signs, hormonal synchrony around oestrus and disturbances in ovulation leading to poor fertility in dairy cows. In addition, delay in ovulation due to increased plasma progesterone in oestrus leads to ageing of oocyte and reduced conception rate (Duchens et al. 1995a, Meier and Bruke 2010). An abnormal serum progesterone concentration was observed in repeat breeder cows with prolonged oestrus (0.5-1.0 nmol/L) against the normal basal value of <0.5 nmol/L during oestrus (Albihn et al. 1991; Båge et al. 1997; Gustafsson et al. 1986; Singh et al. 2009). Layek et al. (2013) stated an extended interval from oestrus to ovulation in cows, which had progesterone level greater than 1ng/ml, compared to the cows that had normal progesterone concentration (less than 1ng/ml) during oestrus. Parvathy (2015) observed that the animals exhibited prolonged oestrus had increased progesterone level during oestrus and luteal insufficiency during mid luteal stage.

Table 1 Incidence of prolonged oestrus in cattle of different region

Incidence	Breed	Place	Reference
30-40% repeat breeder cows	Swedish Red & White Breed	Sweden	Bage et al. 2002
46.1% delayed ovulation		Germany	Braun and Sarmento 2004
26.6 and 21.62%	Crossbred cows	India (Kerala)	Jeba-Sujana 2005
30-40% repeat breeder cows	HF x Sahiwal	India (Punjab)	Dadarwal et al. 2005
			Bedi et al. 2007
29.33% delayed ovulation in repeat breeder	Crossbred cows	India (Assam)	Das 2017
20-30%	Crossbred cows	India	Nanda and Singh, 2008
51.47% repeat breeder	Crossbred cows	India (Kerala)	Parvathy 2015
31.82%	Crossbred cows	Croatia	Zobel et al. 2009
19.86%	HF		
5.88%	Simmental		
50% repeat breeder	Crossbred cows	India	Ghuman et al. 2014
59.64%	Crossbred cows	India (Kerala)	Mathew et al. 2014
25.86%	Crossbred cows	India (Kerala)	Shakir 2018
14.24%	Crossbred cows	India (Guwahati)	Das 2017
16.75%	Crossbred cows	India (Assam)	Nath et al. 2019
25.96%	Crossbred cows	India (Kerala)	Arun et al. 2020

One of the foremost reasons for prolonged oestrus exhibition by the cattle is the abnormal prolongation of the life of dominant follicle, which ultimately leads to delayed ovulation. Normally the oestrus to ovulation interval in cows is about 25-35 hours; the onset of oestrus to LH surge occurs at about 9 hr i.e., about 18-26 hrs before ovulation (Saumande and Humblot, 2005). As far as when the chronology of different events is concerned, peak concentrations of estradiol-17â is observed at 6.8 hr, the LH surge occurs at 9.1 hr and the ovulation occurs at 29.4 hr after the onset of oestrus (Stevenson et al. 1998, Saumande and Humbolt, 2005). A follicle that persists beyond its normal time is called as prolonged dominant follicle and is the main reason for the prolonged oestrus condition. Delayed ovulation of the follicle can be assessed using three parameters, which include (i) the interval from oestrus to LH surge (ii) the interval from LH surge to ovulation and (iii) the interval from oestrus to ovulation (Saumande and Humbolt, 2005; Bloch et al. 2006; Meier & Bruke 2010).

In several studies, it has been stated that the interval between the onset of oestrus and ovulation was more variable than the interval between the LH peak and ovulation (Rajamahendran et al. 1989; Saumande and Humbolt, 2005; Niyas et al. 2019). The endocrine milieu in which the preovulatory follicle grows determines its persistency. The problem may lie at the level of hypothalamus to release normal GnRH, which in turn can delay the pituitary LH surge leading to delayed LH surge or untimely LH surge (Pursley et al. 1995; Saumande and Humbolt, 2005). In addition, the increase in the time period between the LH surge and the time of ovulation can be due to problems at the ovarian cellular level and its microenvironment, which may occur due to effect of suprabasal progesterone (Duchens et al. 1995a, Duchens et al. 1996).

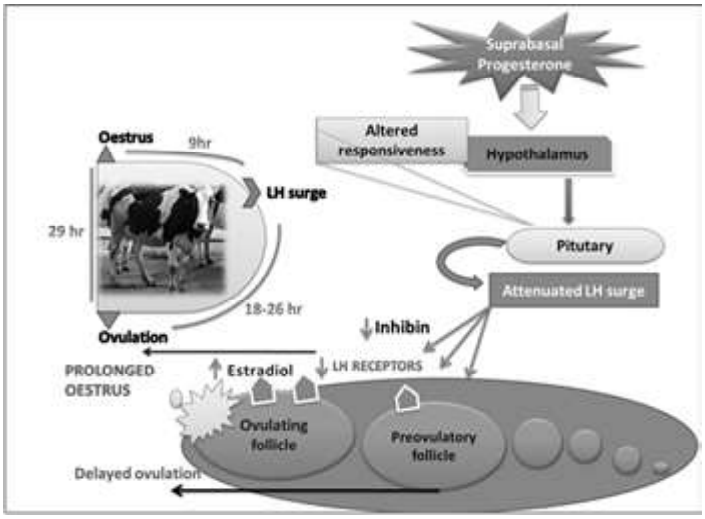

Fig. 1 Hypothetical model on the effect of suprabasal progesterone concentrations on hypothalamo-hypophyseal-ovarian axis and oestrus duration

The most accepted cause for prolonged oestrus in cattle is the presence of suprabasal progesterone of about >0.5ng/ml or the delayed ovulation (Singh et al. 2009). The elevated level of progesterone in turn results in altered LH pulse frequency (Bridges and Fortune, 2003). It is reported that suprabasal progesterone is due to the result of incomplete luteolysis and some extra-gonadal source like adrenal which can elevate the basal progesterone level when the cows are exposed to stress (Honparke et al. 2010). The effect of suprabasal progesterone on the hypothalamo-hypophyseal-ovarian axis, which alters interval between i) oestrus to LH surge, ii) LH surge to ovulation and iii) oestrus to ovulation to prolong the duration of oestrus by

Table 2 Duration of prolonged oestrus in cattle

Duration of prolonged oestrus	Breed	Place	Reference
65.3±15.3 hr	Swedish Red & White breed	Sweden	Bage et al. 2002
42.66±2.74 hr	Crossbred cattle	India (Punjab)	Singh, 2003
90.48±20 hr in repeat breeder cows	HF×Sahiwal	India (Punjab)	Dadarwal et al. 2005
58.0 ± 5.29 hr	Swedish Red and White breed	Sweden	Singh et al. 2005
65.65±2.57 hr	Crossbred cows	India (Kerala)	Jeba-Sujana, 2005
>36 hr	Crossbred cows	India (Punjab)	Bedi et al. 2007
33.27±1.56 hr in delayed ovulation	Crossbred cows	India (Guwahati)	Das et al. 2009
72.00±4.17 hr in delayed ovulation	Crossbred Jersey cows	India (Kashmir)	Bhat and Bhattacharyya 2012
36-80 hr Repeat breeder cows	Crossbred cows	India (Punjab)	Singh et al. 2012
37-80 hr repeat breeder cows	Crossbred cattle	India (Punjab)	Ghuman et al. 2014
>96 hr	HF crossbred cattle	India (Kerala)	Mathew et al. 2016
73.36±3.14 hr repeat breeder cows	Crossbred cattle	India (Tamilnadu)	Senthilkumar et al. 2017
84.0±6.26 hr	Crossbred cows	India (Tirupathi)	Radhika 2017

affecting the ovarian microenvironment is shown in figure 1. Nanda and Singh, (2008) stated that the factors like lactational and nutritional stress were the major reasons for the occurrence of suprabasal progesterone and prolonged oestrus among dairy cattle. This elevated suprabasal progesterone inhibits IGF-1 secretion and reduces responsiveness of follicular cells to LH (Omari et al. 2020). The maintenance of prolonged dominant follicle may cause premature maturation of the oocyte which causes the chromosomes to condense and the meiosis to progress to metaphase II before LH surge. It is also stated that embryos obtained from cows that ovulated from persistent follicles were compromised and could not reach the 16- cell stage (Ahmad et al. 1995).

In a study, it was reported that relative risk of a cow becoming repeat breeder was 58% and 42%, when the AI is performed at suprabasal progesterone level and basal progesterone level, respectively (Bage 2003). Delayed LH surge and extended LH pulse frequency delays ovulation and ultimately results in preovulatory ageing of the oocyte (Singh et al. 2005). The estrogen: progesterone ratio should be greater than 1 for a normal ovulation to occur. The presence of suprabasal progesterone reduces the tubal contractility and impairs transport of sperm from sperm reservoir (Bloch et al. 2006). Recently, a study conducted on the differential abundance of proteins in follicular fluids from preovulatory follicles of less fertile dairy cows, reported the increased abundance of TIMP2, which can disrupt the tissue remodelling necessary for ovulation which leads to delayed ovulation and suggested to have a prolonged heat onset to ovulation interval in low fertile cows (Zachut et al. 2016).

Predisposing factors for prolonged oestrus in cattle

Stress factors

Stress can be of several types which includes environmental, managemental, physical or social stress. These kind of stresses were stated to be the causative agent for sustained stimulation of adrenal glands, which could be a factor for occurrence of suprabasal progesterone during prolonged oestrus (Bage et al. 2000). Generally, heat stress is associated with a lot of physiological changes that cause immediate and delayed negative effects on secretion of gonadotropins, follicular dynamics, ovulation, corpus luteum development, steroidogenesis, oocyte developmental competence, embryonic survival, utero-placental function, lactation and post-natal development. Heat stress particularly reduces follicular dominance by inducing multiple large follicles as well as prolonged dominant follicles (Hansen 2009). Alteration in high tonic FSH availability disturbs normal follicular selection and dominance. Altered LH surge and negative energy balance during heat stress can disrupt the normal oocyte maturation (eg; premature meiosis) and reduces developmental competence (Mihm et al. 1994). Heat stress will depress follicular oestradiol due to reduced theca cell androstenedione production associated with low 17-alpha-hydroxylase expression and reduced aromatase activity in granulosa cells. Thereby, heat stress reduces follicular dominance by inducing multiple large follicles as well as prolonged dominant follicles (Wolfenson et al. 2000, Hansen 2009).

Heat stress causes some direct effects on oocytes like, oxidative damage, apoptotic cell death, irreversible changes on cytoskeleton and meiotic spindle, which will interfere with cell division, reduced mRNA and protein reserves for early embryonic development and altered membrane integrity which effects both the signal transduction and protein transport (Hansen 2009). In addition, heat stress causes major differences in gene transcript levels of DNA binding proteins, transcription factors, Erg-1, progesterone, and prostaglandin receptors ultimately leads to reduced oocyte competence which results in poor fertility rate after insemination (Wakayo et al. 2015). During hot season, in order to maintain body temperature, imbalances in energy metabolism occurs. This would lead to suppressed activity of aromatase enzyme in granulosa cells and retard the functionality of dominant follicles (Wolfenson et al. 2000). Roth et al. (2000) stated that follicular microenvironment was adversely affected by elevated exposure of temperature and will lead to deterioration of functioning dominant follicles. Thus, the ovulatory follicle might require more time and a larger size for attaining ovulation under heat stress and such follicles would be of inferior quality and lead to yield poor quality oocytes and subsequent infertility (Hansen 2009). Satheshkumar et al. (2015) studied natural influence of season on Indian crossbred cows and found that the ovulatory wave emerged significantly earlier (Day 11.5) and the dominant follicle of that wave had a prolonged growth phase (11days) during summer compared to the cold season (days 14.8 and 5.8 respectively). They concluded that increased incidence of two follicular wave cycles which accounted for the persistence and aging of the follicle at the time of ovulation and altered luteal endocrine activity might be the reasons for the diminished fertility in crossbred cattle during hot season. Lactational and nutritional stresses are also considered to be the major reasons for the occurrence of suprabasal progesterone and prolonged oestrus among dairy cattle (Nanda and Singh, 2008).

Age and Parity

Occurrence of prolonged oestrus among repeat breeder cows were also influenced by age and parity of the cows, mainly because of underlying endocrine disturbances, nutritional and production stress as the age and parity increases. In several studies, it was reported that, cows of parity four and above in an age group of 8-12 years were more prone to be repeat breeder exhibiting prolonged oestrus. These may occur due to negative energy balance which alters the insulin responsiveness of the follicle, altered prostaglandin synthesis, CL function, anatomical defects due to increased parity and age (Singh et al. 2012, Asaduzzaman et al. 2016, Arun et al. 2020).

Nutritional causes

Negative energy balance is also a cause for alteration of function, development, and maintenance of follicles. It is strongly correlated with low concentrations of glucose, insulin, IGF-1, and secretion of gonadotropins, which ultimately leads to low FSH and LH peaks and hence results in anovulation. IGF-1 has a key role in follicular development, reduction in its concentration during negative energy balance reduces ovarian responsiveness to LH stimulation. Decreased insulin can cause anovulation by interfering normal LH pulses and FSH pulses. Low IGF-1 and insulin together reduces the responsiveness of follicles towards LH and thereby suppress follicular oestradiol production (Omari et al. 2020).

The dairy cattle reared in small holder production system are deficit in most of minerals (Kumaresan et al. 2010), which can impact reproductive performance (Kumaresan et al. 2009). In addition, the minerals and their deficiency were area specific, especially the correlation between calcium level in soil and in cattle were reported (Kumaresan et al. 2010). Calcium-Calmodulin system participates in the regulation of steroidogenesis at different stages of granulosa cell differentiation, thereby affecting the growth of preovulatory follicles (Kendell et al. 2003). It has effects on gonadotropin regulation, independent of stage of follicular maturation and cellular differentiation. Calcium also has a role in influencing delivery of cholesterol by mitochondria or by stimulating conversion of pregnenolone to progesterone in the adrenal gland and ovaries (Wiederkehr et al. 2011). Calcium dependent mechanism has a role in the luteinising hormone release from the pituitary gland. Calcium also plays a key role in increasing the number and size of ovarian preovulatory follicles as well as ovulation rate (El-Shahat and Maaty 2010). Marginal deficiency of phosphorus could cause disturbances in the pituitary-ovarian axis including ovulation. Inorganic phosphorus is essential for normal phospholipid metabolism, cAMP synthesis, energy transformation at cellular level, and integral part of many coenzymes may be a key to its effect on reproduction.

The effect of dietary supplementation with calcium salts of long chain fatty acids with or without of L-carnithine on ovarian activity was studied by El-Shahat and Maaty (2010). They found that calcium played a key part in improving the number and size of ovarian preovulatory follicles as well as ovulation rate. Significantly ($p<0.01$) lower level of inorganic phosphorus (3.73 ± 0.29mg/dL) in infertile repeat breeder cows than normal cyclic cows (5.06 ± 1.19 mg/dL) was reported by Awasthi and Kharche, (1987). Das et al. (2009) observed that the serum inorganic phosphorus concentration was significantly higher in the animals with normal ovulation (5.45 ± 0.15mg/dl) than the animals with anovulation (4.45 ± 0.14mg/dl). However, Nath et al. (2014; 2019) recorded the mean serum calcium and phosphorus levels in prolonged oestrus cows did not differ among different days of prolonged oestrus.

Deficiency of zinc can cause reduction in GnRH secretion by hypothalamus and decreases the levels of FSH and LH and results in anovulation (Karaca et al. 2007). It is a co-factor for more than 300 metalloenzymes in DNA synthesis and gene transcription. Zinc in proteins can either participate directly in chemical catalysis or maintain protein structure and stability. Zinc finger proteins implicated in gene expression of receptors of the steroid hormones which are involved in reproduction. Das et al. (2009) reported the concentration of Zn (ppm) was significantly ($p<0.01$) lower in the animals with anovulation (0.85 ± 0.03) and delayed ovulation (1.41 ± 0.04) compared to the animals with normal ovulation (1.78 ± 0.02). Ahmed *et al*., 2017 reported that the plasma concentration of zinc (µg/dl) was significantly higher ($p<0.05$) in normal cyclic cows (26.4 ± 17.4) than in repeat breeder (18.8 ± 17.7) crossbred cows.

Strategies for improving fertility in cows exhibiting prolonged oestrus

The regulation of follicular dynamics by inducing the occurrence of three follicular wave cycles might be a potential target for therapeutic intervention of summer infertility syndrome in crossbred cows (Satheshkumar et al. 2012). The conception rate in prolonged oestrus crossbred cows was found to be 70%, 80%, 41.66% in two, three- and four-days duration of oestrus, respectively (Nath et al. 2014). The prolonged oestrus exhibited by the repeat breeder cows amplified the risk of poorly timed AI, which resulted in reduced conception rate (Nebel et al. 2000). A higher pregnancy rate was achieved when the period of dominance was restricted to 1-4 days, whereas dominance of >10 days was associated with no pregnancies and concluded that pregnancy rate decreases with the increase in duration of dominance (Viñoles et al. 2001). The duration of dominance for optimum fertility is less than 8 days. Reducing the period of follicle dominance by optimizing the ovulatory response to the initial GnRH injection of synchronization protocol improved early embryo development (Cerri et al. 2009). Reduction in embryo quality was observed even when concurrent extension of follicle dominance was of only 1.5 – 2 days (Cerri et al. 2009). Period of dominance is more important for early embryo quality in high producing lactating dairy cows than the endocrine steroidal milieu in which the ovulatory follicle develops. High producing cows with extended interval between follicle deviation to oestrus have reduced fertility because embryo quality is compromised when dominance of the ovulatory follicle is increased by as few as 1.5 days (Cerri et al. 2009). Pregnancy rate of the cows that ovulates 24 hr after the oestrus onset can be optimized through repeated insemination every 24 hr till the occurrence of ovulation (Van-Eerdenburg et al. 2002). However, the oocytes from delayed ovulating follicles are abnormal due to the extended growth period and therefore repeated AIs may be unsuccessful (Duchens et al. 1995b).

In crossbred cows the mean duration of oestrus before treatment with 1500 IU hCG was 70.50±4.20 hrs and after the treatment it was 47.25±2.39. The mean duration of oestrus was 48.62±1.84 hr when treated with 3000 IU hCG as against 69±4.39 hrs in the control (Mathew et al. 2016). Senthilkumar et al. (2017) studied 60 repeat breeder cows with the history of prolonged oestrus and noticed that the mean duration of oestrus before hormonal therapy was 75±3.71 hr. However, the duration was reduced to 48.21±3.12 hr after the treatment with 1500 IU hCG or 10µg GnRH analogue at the time of insemination. The slow ovulatory follicle growth and corpus luteum regression could be associated with the persistence of oestrus characters which can be optimised following PGF2á administration during early luteal phase of cows followed by timely insemination and improved conception rate (Ghuman et al. 2014; Shakir 2018).

GnRH or its analogues are indicated for inducing ovulation close to the time of insemination to enhance conception rates as they stimulate acute release of LH and FSH from anterior pituitary (Shaw 1999). Fertility improved in some studies when GnRH was administered between days 11 and 14 in lactating dairy cows (Drew and Peters, 1994) but not in other studies (Jubb et al. 1990; Stevenson et al. 1993, Bartolome et al. 2005). GnRH can be used in the prolonged oestrus cows as they are affected with delayed ovulation and/or suboptimal functioning of corpus luteum (Bedi et al. 2007). The conception rate in repeat breeder crossbred cattle having prolonged oestrus was 50% and 42.80% following intramuscular injection of 2.5 mL of Buserelin acetate after single and double insemination, respectively (Sharma et al. 2006). The double injection of Buserelin (GnRH) is efficient in improving the conception rates in prolonged oestrus repeat breeding crossbred cattle. The conception rate in the cows those were administered Buserelin (20µg) 6 hrs before AI and again on day 12 were significantly higher (52%) than those were given single Buserelin (20µg) 6 hrs before AI (34%) (Dadarwali et al. 2007).

Conclusions

Prolonged oestrus in dairy cattle baffles field veterinarians in deciding the appropriate time of insemination ultimately leading to reduced conception rates and repeat breeding. As a managemental measure the animal should be maintained under normal energy balance and minerals like calcium, phosphorus and zinc level should be maintained at adequate levels in the feed of cows. In cows showing prolonged oestrus, reducing the period of follicle dominance by administration of GnRH/LH will be advantageous. Pregnancy rate of the cows that ovulates 24 hr after the oestrus onset can be improved through repeated insemination every 24 hr till the occurrence of ovulation. Use of different regimens of GnRH shots were reported to have more significant improvement in the prolonged oestrus condition, by its action to induce ovulation and prevent luteal insufficiency. The 1500 IU hCG or 10µg GnRH analogue at the time of insemination can be tried to reduce the duration of oestrus in the cows having prolonged oestrus. However, further studies needed to determine the oocyte quality, probably in terms of genomics, in prolonged follicle and other risk factors associated with prolonged follicular dominance.

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