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PROLONGED OESTRUS IN DAIRY CATTLE – THE CONSEQUENCE OF FAULTY OESTRUS DETECTION AND LATE INSEMINATION

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ABSTRACT

Increasing incidence of prolonged oestrus has been reported among dairy cattle especially after the popularization of artificial insemination (AI). The condition is often considered as a simple deviation of the physiological manifestation and the underlying causes and mechanisms have not been adequately explored. However, considerable impairment of fertility has been reported and various interventions are being carried out to restore normal fertility. Hence objective of the present communication is to describe the causative factors for prolonged oestrus with emphasis on human interventions in animal breeding such as oestrus detection and AI management. Lack of males in the herd, intensive system of management, minimal opportunity for social interactions and stress of high milk production have made the oestrus manifestations unusual and misleading. Hence, expulsion of mucus from the reproductive tract is highly relied upon for oestrus detection, which makes the distinction of oestrus from pro-oestrus very difficult and thus increasing the proportion of prolonged oestrus cases. Owing to the shorter fertilizable life of cryo-preserved spermatozoa, better synchrony of insemination with ovulation results from later AI adhering to AM:PM rule. However, the chance of infection increases considerably with late insemination contributed by inadequacy of tubular defense mechanisms and harmful effects of more anterior deposition. Thus, it appears that the major underlying reason for the increasing occurrence of prolonged oestrus is the use of cryo- preserved semen together with adherence to AM:PM rule for AI and the resultant indirect selection of animals having longer oestrus period as parents of the future generations.

Keywords: Cattle, Prolonged oestrus, Artificial insemination, AM:PM rule, Ovulation

INTRODUCTION

Incidence of prolonged oestrus is reported more in dairy cattle (Singh *et al.,* 2008) and the incidence is further high under intensive management situation, where the cows are restricted to barns (Forde *et al.,* 2010; Kumari and Pampana, 2015) and breeding is carried out exclusively through artificial insemination (AI) (Cutullic *et al.,* 2009; Singh *et al.,* 2012). Behavioral signs of oestrus are important as they form the means to alert male animals about the readiness to breed, attract them and to get the mating effected (Watts and Stookey, 2000; Roelofs *et al.,* 2010). However, the absence of male animals in the herd in farm household units as well as in commercial dairy farms has led to the lack of this male effect and a consequent abolition of the neuro endocrine mechanisms that make the oestrus signs pronounced together with enhancement of ovulation (Fiol *et al.,* 2010; Gokuldas *et al.,* 2010; Romano *et al.,* 2016). Thus, elimination of male animals from the herd for the convenience and other advantages of AI has initiated various aberrations in the reproductive physiology of bovines (Kutty, 2004) that include weakened behavioral signs of oestrus (Nasir and Kutty, 2005), ovulatory disturbances and hormonal imbalances leading to overall lowering of fertility (Kutty and Ramachandran, 2003).

Faulty detection of oestrus

Lack of opportunity for interaction between animals under the tied in barn system of farm management (Palmer *et al.,* 2010; Orihuela, 2000; Sumiyoshi *et al.,* 2014) and isolation in farmer homestead units has aggravated the abolition of behavioral signs of oestrus, (Kutty, 2004; Nasir and Kutty, 2005), making its detection and timely breeding very difficult. Lack of exercise, locomotor problems, stress of increased milk production, nutritional imbalances out of stall feeding, adverse weather and associated ill health are other factors complicating oestrus manifestations under intensive management (Yoshida and Nakao, 2005; Cutullic *et al.,* 2009; Forde *et al.,* 2010; Sumiyoshi *et al.,* 2014;). Various technological interventions for oestrus detection are being resorted in modern farms especially in developed countries (Diskin and Sreenan, 2000; Stevenson *et al.,* 2014). While absence of such facilities or suitable alternatives under intensive farming (Yoshida and Nakao, 2005; Selvam and Archunan, 2017) has made the detection of oestrus mostly based on observation of mucus flow from the reproductive tract along with various minor signs of oestrus. Ultimately, such a practice has considerably affected the reliability of oestrus detection and reproductive performance of dairy cows (Kutty and Ramachandran, 2000;

Nasir and Kutty, 2005; Sumiyoshi *et al.,* 2014).

Expulsion of cervical mucus in noticeable quantity from the genital tract of bovines occurs during oestrus as well as other stages, including pregnancy, mid cycle oestrus (dioestrus) and pathological conditions such as follicular cyst, reproductive tract infection and endocrine disturbances. The volume, consistency, colour, turbidity and duration of oestrual mucus flow varies between and within the same animal (Layek *et al.,* 2011; Selvam and Archunan, 2017). Thus, distinguishing mucus flow of oestrus from that of other stages becomes a difficult task. During the oestrous cycle, thin mucus flow is initiated during pro oestrus and the frequency of flow, volume and thickness of the discharge increases as the stage advances and arrests a few hours after oestrus (Sumiyoshi *et al.,* 2014). However, owing to a lot of variations as mentioned, it becomes difficult to distinguish between the pro-oestrus and oestrus stages and mucus flow of any stage will be reported as oestrus (Roelofs, 2005; Layek *et al.,* 2011). Thus, oestrus detected based on mucus flow becomes indistinguishable from that of pro-oestrus and will be considered together with oestrus and often reported as prolonged oestrus.

Consequence of late insemination:

Another major reason from a long-term perspective for the increased incidence of prolonged oestrus among AI bred animals is the wrong timing of AI. The AM:PM rule recommended for AI in bovines (Ravikumar, 2013) results in a major proportion of AI being done towards the end of oestrus and very often beyond the period of true oestrus. The recommendation was based on the fact that ovulation would occur around 12 hours after end of the oestrus period (Hagevoort and Garcia, 2013). Hence, later the insemination, greater the availability of sperms at the time of ovulation (Layek *et al.,* 2011). Natural mating, AI using fresh extended semen - and to some extent chilled semen - all facilitate the prolonged availability of active sperms for effecting fertilization (up to 48 hours or even more) and in such cases, there is no need to delay insemination to towards the time of ovulation (Hagevoort and Garcia, 2013). However, the aforementioned breeding practices are uncommon now a days, and even though AM:PM rule is being insisted in all cases, this has contributed to creating more chances for the elimination of animals with shorter / normal oestrus-ovulation intervals thus indirectly selecting those animals with delayed ovulation / prolonged oestrus, resulting in higher propagation of such animals/ traits into the future generations.

Indirect selection for prolonged oestrus:

While behavioral signs of oestrus are mainly for informing, attracting and getting bred by the male (Roelofs *et al.,* 2010), majority of the tubular tract changes of oestrus are intended to prevent infection inside the reproductive tract and to minimize the associated risk of infertility (Dhaliwal *et al.,* 2001; Subandrio *et al.,* 2000; Gilbert, 2013). Mating process *per se* causes invasion of the reproductive tract by a large number of microbes (Bas *et al.,* 2011; Meena *et al.,* 2015) and the elimination of these invaders is brought about by the oestrual enhancement of the defense mechanisms of the tubular reproductive tract (Ata *et al.,* 2010; Subandrio *et al.,* 2000; Singh *et al.,* 2018). Enhanced tubular defense mechanisms of oestrus includes increased vascularity, contractility and ciliary movements, infiltration of immune cells, profuse mucus secretion, pH changes, antimicrobial factors, and stratification and desquamation of epithelium and so on (Galvao, 2012). Such changes are mainly brought about by the action of oestrogen and these attain a maximum level during the peak of the oestrus period and weakens as the oestrogen level diminishes towards the end of oestrus (Subandrio *et al.,* 2000).

As insemination is delayed beyond the middle of oestrus (Stevenson *et al.,* 2014), weakening of the tubular defense mechanisms increases the chances for establishment of infection (Dhaliwal *et al.,* 2001; Beagley and Gockel, 2003; Hurley, 2014), affecting conception not only in the same cycle, but subsequent cycles as well. In nut-shell, even though the fertilization rate may increase by delaying insemination towards ovulation in those animals having a tendency for longer oestrus periods (Bombardelli *et al.,* 2016), there occurs a considerable reduction of conception and failure to establish pregnancy in animals of normal oestrus duration (Roelofs, 2005; Saraswat and Purohit, 2016). Many studies have already established reproductive tract infection as the major reason for infertility among AI bred animals (El-kader and Shehata, 2001; Kutty and Ramachandran, 2003; Salasel *et al.,* 2010), even though various aseptic practices are in force. Thus, late insemination as recommended by AM:PM rule (Ravikumar, 2013) could increase the chances of reproductive tract infection and thus form a major reason for high incidence of infertility among AI bred animals (Subandrio *et al.,* 2000).

Role of frozen semen AI

The occurrence of prolonged oestrus was further aggravated by the popularization of AI using cryo-preserved semen. Insemination nearer to the ovulation has become more relevant with cryo-preserved semen (Sales *et al.,* 2011) not only due to low count of sperms deposited (López-Gatius, 2012; Perez-Marin *et al.,* 2012), but also more importantly due to the shorter fertilizable life of cryo-preserved sperms inside the female reproductive tract (Bhattacharyya and Hafiz, 2009; Bombardelli *et al.,* 2016). The freeze-thaw cycle induces varying degrees of membrane changes on the sperms (comparable to capacitation), which in turn enhances their readiness for fertilization and at the same time considerably reduces their fertilizable life span after the insemination. Thus, the chance of fertilization by cryo-preserved sperms will be more if the insemination is carried out nearer to the time of ovulation and more anterior towards the site of fertilization. But such a practice of insemination causes minimal formation of sperm reserve, shortening the sperm availability, considerable reduction of the fertilizable life of sperms inside the female reproductive tract and further exacerbates the chance of infection. Thus, late insemination adhering to AM:PM rule was found promising for AI using cryo preserved semen (Laven, 2018), but the same practice has become a potential contributor to the phenomenon of a steady increase of prolonged oestrus in the herd, through elimination of animals with timely ovulation / shorter oestrus.

Formation of adequate sperm reserve within the female reproductive tract is essential to ensure prolonged availability of sperms so that possible variations of ovulation timing can be adjusted (Perez-Marin *et al.,* 2012). However, AI using cryo-preserved semen is forced to overlook such a phenomena for the reasons such as (1) Smaller dose of semen deposited that do not suffice for reserve formation (2) Major reserve forming sites (cervical folds) are totally bye passed by the anterior insemination, (3) Even if a larger dose (double dose as practiced by some inseminators) is deposited at two sites (cervix and uterine body), the benefit is negligible since the fertilizable life of sperms (already capacitated by the freezing – thawing process) is very limited. Thus, only very limited sperm reserve formation becomes possible within the fallopian tube and utero tubal junction) is possible by the AI as practiced for cryo-preserved semen. Ultimately, as the cause as well as consequence of prolonged oestrus, AI using cryo-preserved semen is necessitated more and more nearer to the time and site of ovulation for better results, and exaggerates the incidence of prolonged oestrus.

AM:PM rule as the major contributor:

As mentioned earlier, the recommendation of AM PM rule benefits the AI using cryo-preserved semen, but has considerably enhanced the selection phenomena for the occurrence of prolonged oestrus. Owing to the shorter fertilizable life of cryo-preserved spermatozoa, better conception may result from later insemination towards ovulation and at more anterior sites (Perez-Marin *et al.,* 2012). Elimination of animals having shorter / normal oestrus also occurs simultaneously through their predisposition to high incidence of infertility contributed by late insemination and more anterior sites of deposition. The chance of infection increases considerably with late insemination beyond the middle of standing oestrus (the point of maximum tubular manifestations of oestrus) attributable to the depletion of tubular defense mechanisms together with harmful effects of more anterior sites. Thus, prevalence of prolonged oestrus has become very high among dairy cattle after the popularization of AI and is still on the rise with respect to proportion of animals affected as well as duration of the oestrus. Even though various corrective interventions are being tried, none of them addresses the root cause. Hence a rational approach based on critical analysis of the contribution from the ongoing insemination practices is highly indicated.

Having stated the underlining philosophy that more later the insemination, more the chance of oestrus prolongation, adherence to AM:PM rule forms major contributor for occurrence of prolonged oestrus in AI bred animals. Owing to the shorter availability of cryo-preserved sperms for fertilization, strict adherence to AM:PM rule based on onset of oestrus signs should benefit by reducing further prolongation of oestrus beyond certain limits. That means inseminating animals within 12 hours of oestrus onset, those with more prolonged oestrus (beyond 36 hours) will have little chance of conception. Further, animals prone to have more extended duration of oestrus are also at risk of getting eliminated without propagation. However recommendations modified as a corrective measure for prolonged oestrus, to repeat the insemination at 24 hour interval until cessation of oestrus has further enhanced the selection for prolongation of oestrus. Thus AM:PM rule together with double insemination will enhance the elimination of those animals having shorter or normal oestrus, and causes enhanced propagation of others with longer and extra longer duration of oestrus.

It is well established that around 80.0 per cent cattle exhibit oestrus during night hours (between 6 PM to 6 AM) (Wattiaux, 2010) and if the oestrus ends within 12 hours, many of them goes unnoticed unless special care is exercised or some technological tools are utilized for night time detection of oestrus (Stevenson *et al.,* 2014). Out of those animals having onset of oestrus early during the night hours, only those showing extension of oestrus beyond 12 hours will be detected during the next morning (Diskin and Sreenan, 2000). If AM:PM rule is adhered to, most of them will be inseminated during afternoon hours ending up in late insemination. Thus, all the animals having shorter oestrus periods will miss being detected and even if some are detected and inseminated, suffer less chance of conception being late insemination, while those with prolonged oestrus will get propagated more. Thus, across generations bred exclusively through AI, especially with cryo-preserved semen, incidence of prolonged oestrus will be on the rise unless the existing recommendations are suitably modified.

Prolonged oestrus and post-oestrual bleeding

Manifestations of prolonged action of oestrogen on tubular tract of animals showing prolonged oestrus are evidenced by increased tonicity of uterus, prolonged mucus discharge and associated behavioral changes. Increased incidence of post-oestrual bleeding where in the discharge is profuse and thick, formed mainly of mucus mixed with blood, is one of the major consequences of prolonged oestrogen action on the reproductive tract. The term metoestrual bleeding is a misnomer for this type of bleeding and has to be distinguished from the physiological phenomena of slight bleeding during the metoestrual phase described as normal in cattle.

Interlinking the bleeding with prolonged oestrus, the uterus shows highest degree of tonicity during such phase of bleeding, the discharge predominantly of mucus mixed with blood, some animals continue to show behavioral signs of oestrus and most often there will be mature follicle persisting. Even though the ovulation may occur at a later stage, the fertility is impaired due to ageing of ovum, hostile uterine environment and infection favored by blood enriched uterine medium. Increased incidence of such a "bleeding" phenomena has been reported after popularization of AI. Further, there are reports of even conception for AI during the phase of such bleeding illustrating that such a discharge occurs during oestrus and is attributable to prolonged action of oestrogen on the reproductive tract. In other words, profuse muco-sanguneous discharge seen following oestrus signs might be one of the consequence of prolonged oestrus.

CONCLUSION

Increased occurrence of prolonged oestrus is the result of late insemination aggravated by (1) intensive system of tied in barn management under farms or isolation in household units denying opportunity for interaction among animals for expression of oestrus (2) abolition of behavioral signs necessitating more dependence on mucus discharge as the major criteria for detection of oestrus, (3) Lack of technological aids and limitations of human detection causing consideration of the pro-estrus together with oestrus (4) Consistently ongoing indirect selection for prolonged oestrus animals happening through late insemination, (5) Passive elimination of animals with normal / short duration of oestrus through fertility impairment out of insemination adhering to the AM:PM rule (6) AI using cryo-preserved semen necessitating more nearer towards the fallopian tube and time of ovulation and (7) Prolongation of oestrus aggravated by the corrective measure of repeating AI every 24 hours.

Owing to the shorter fertilizable life of cryo-preserved spermatozoa, insemination adhering to AM:PM rule provides better chances of fertilization compared to middle or early oestrus insemination. However increasing chances of infection caused by late insemination as per AM:PM rule and more anterior sites of semen deposition necessitated by the small dose of cryo-preserved semen adversely affects the conception especially in animals with normal or short duration of oestrus. Thus, indirect selection of animals having longer oestrus period and passive elimination of those with shorter or normal oestrus is taking place continuously in the herd and the same constitutes the underlying mechanism for increasing occurrence of prolonged oestrus among AI bred animals.

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CONFLICT OF INTEREST

The author has no conflict of interest to declare other than the differences of technical opinion expressed along with providing available documentary evidences.

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