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Risk factors for prolonged estrus in crossbred dairy cattle

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ABSTRACT

The present study involving 40 crossbred dairy cattle from various private dairy farms in Punjab assessed various risk factors associated with prolonged duration of estrus (63.30±2.5 h). About 62.5% cattle had regressed corpus luteum (CL) at estrus, 17.5% cattle were over-conditioned and 30% had daily milk yield >30L. Mean duration of estrus in 60% cattle was 52.5±1.2 h, whereas, remaining 40% exhibited 79.5±2.87 h duration. Plasma progesterone at estrus in the former group was 0.48±0.04 ng/ml compared to 0.59±0.04 ng/ml in the latter group. In both these groups, duration of estrus was not influenced by body condition score (BCS) and regressed CL at estrus. About 75.7% cattle with normal BCS exhibited regressed CL at estrus. Moreover, 75% cattle with 19.11±1.30 litre per day milk yield had regressed CL at estrus in comparison to 33.3% cattle producing 39.67±1.98 litre. Plasma progesterone on the day of estrus was similar in cattle either with or without regressed CL. Luteal deficiency during the phase subsequent to prolonged estrus was recorded in 72.5% cattle. About 40% cattle without regressed CL and 92% cattle with regressed CL at estrus exhibited subsequent luteal inadequacy. About 70% cattle had per day milk yield <30 litre and 82% of these exhibited luteal deficiency at subsequent estrus. In brief, plasma progesterone at estrus is associated with prolonged duration of estrus. Regressed CL may not be the source of suprabasal plasma progesterone, but could be responsible for subsequent luteal inadequacy. High milk yield is associated and over-conditioning is not associated with occurrence of regressed CL at estrus, prolonged duration of estrus and subsequent luteal deficiency.

Key words: Body condition score, Corpus luteum, Milk yield, Progesterone, Prolonged estrus

Repeat-breeding is among the major problems associated with decreased fertility in dairy cattle as about 30–40% repeat-breeder crossbred cattle display prolonged estrus (37–60 h vs 24–36 h; Dadarwal *et al*. 2005). Out of these repeat-breeder cattle, 70% record marginally elevated plasma progesterone (e”0.35ng/ml) during estrus, so-called suprabasal progesterone, and is believed to impair fertility (Bage *et al*. 2002). Nevertheless, the origin of excessive plasma-progesterone at estrus in prolonged estrus exhibiting repeat-breeding cattle remains unidentified. Suprabasal progesterone could arise either due to an incomplete luteolysis of corpus luteum (CL) or could be of extra-gonadal origin (Bage *et al*. 2000, Honparkhe *et al*. 2010). A sustained adrenal stimulation associated with environmental, managemental or physical stress could be one factor for existence of suprabasal progesterone at estrus (Bage *et al*. 2000). Moreover, progesterone profiles have altered over the years with the intensive selection for higher milk production (Royal *et al*. 2000). Suprabasal progesterone plays a role in follicular cyst formation in cattle with high lactation yield (Vanholder *et al.* 2005). Not even a single study is available in literature to show the involvement of various risk factors in the occurrence of prolonged estrus in cattle. Hence, the aim of this study is to investigate the association of various factors such as body condition, milk yield, plasma progesterone at estrus, regressed CL at estrus and duration of estrus in prolonged estrus exhibiting repeat-breeder crossbred dairy cattle.

MATERIALS AND METHODS

The present study was conducted on 40 lactating repeat-breeder crossbred cattle (Age, 3–7 years; Parity, 1–5; Milk production: 25.9±1.8 l/day) reared at private farms of Punjab state. The enrolled repeat-breeder cattle had normal estrous cycles but had failed to conceive during at least three previous services. All these cattle were exhibiting prolonged duration of estrus (>36 h) with no abnormality in the estrual cervico-vaginal mucus and the reproductive tract.

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Plan of work

Duration of estrus: Cattle were observed for various visual signs of estrus starting from two days before expected onset of estrus, at least twice daily for 30 min, by a same person. Scoring scale for observed symptoms of estrus was: cajoling (3 points), restlessness (5), mounted but not standing (10), sniffing the vagina of another cattle (10), chin resting (15), mounting other cattle (35), standing estrus (100) and mounting head-side of other cattle (45). Each time a symptom was observed and if the sum of points were more than 50 during 2 consecutive observation periods, cattle was considered to be in estrus. Termination of estrus was defined as first period in which no further estrus behavior was observed (Van Eerdenburg *et al.* 2002).

Body condition score (BCS) and milk yield: Body condition scores (normal condition or over-conditioned/ fatty) were estimated by the same observer. Cattle were categorized as over-conditioned when the bone structures of the backbone, short ribs and pin bone region was not apparent. Tail head was surrounded with fat deposits (Edmonson *et al.* 1989). Per day milk yield was recorded by the same person.

Transrectal ovarian ultrasonography and blood sampling: Ovaries were systematically examined and the size of regressed CL was determined by measuring the diameter at the widest poles (Ghuman *et al.* 2010). Jugular vein blood samples were collected at standing estrus and on day 10 after the end of estrus. Plasma was separated immediately and was frozen at –20°C until analysis.

Hormone analysis: Plasma progesterone was assayed with solid-phase radioimmunoassay using a progesterone antibody (Ghuman *et al*. 2009). Sensitivity of the assay was 0.1 ng/ ml, intra- assay and inter-assay variation coefficients were 6.5 and 9.1%, respectively. Cattle was said to be suffering from luteal deficiency when progesterone failed to cross 1.0 ng/ml in the plasma sample collected at day 10 after the end of estrus (Honparkhe *et al.* 2010).

Statistical analysis: Various parameters at estrus (duration of estrus and plasma progesterone) and milk yield were compared by *t*-test. Moreover, relationships were calculated between various parameters through Pearson's correlations.

RESULTS AND DISCUSSION

This is the first study to demonstrate the association of various risk factors in prolonged estrus exhibiting crossbred dairy cattle. Repeat-breeder cattle enrolled in the present study had prolonged duration of estrus (63.30±2.5 h) that was proposed to be mainly due to presence of suprabasal progesterone (e" ≥ 0.35ng/ml) at estrus (Bage *et al.* 2002). Similarly, in cattle of present study, plasma progesterone concentrations at estrus were 0.52±0.03 ng/ml. Suprabasal progesterone at estrus have an inhibitory effect on positive feedback of high estradiol on the hypothalamus resulting in high LH pulse frequency (Stock and Fortune 1993). These hormonal asynchronies at estrus lead to delayed ovulation, degenerative cell changes in the follicular wall, impaired developmental capacity of the oocyte and, thereby, poor conception rate (Duchens *et al.* 1996). The origin of excessive plasma progesterone at estrus in prolonged estrus exhibiting repeat-breeding cattle remains unidentified, but it could release from partially lysed CL or from sources other than ovary (Bage *et al*. 2002).

Moreover, in present study, 62.5% cattle had regressed CL at estrus, 17.5% cattle were over-conditioned and 30% cattle had daily milk yield above 30 litre (39.67±1.98 l/day, Table 1). It is highly plausible that incompletely degenerated CL of the previous cycle, high milk yield and obesity in cattle can somehow affect fertility through disturbed endocrine profile at estrus (Honparkhe *et al*. 2010, Humblot *et al*. 1998). Progesterone, being a fat-soluble steroid, is stored and

Table 1. Risk factors associated with prolonged estrus in crossbred repeat-breeder dairy cattle

Parameters	Categories	Duration of estrus (h)	Plasma progesterone at estrus (ng/ml)	Per day milk yield (l)	Cattle with luteal deficiency at diestrus (%)	Over-conditioned cattle (%)	Cattle with regressed CL at estrus (%)
Duration of estrus	36–60 h (n=24)	52.5±1.21*	0.48±0.04*	22.38±2.39	70.8	12.5	66.7
	>60–96 h (n=16)	79.5±2.87	0.59±0.04	29.63±2.65	75.0	25.0	56.2
Body condition	Normal body condition (n=33)	63.27±2.92	0.51±0.03	24.03±2.10	78.8*	–	75.8*
	Over-conditioned (n=7)	63.43±4.31	0.59±0.08	31.14±3.05	28.6	–	0.0
Per day milk yield	<29.9 L (19.11±1.30 L, n=28)	61.71±2.94	0.53±0.04	–	82.0*	14.3	75.0*
	30–55 L (39.67±1.98 L, n=12)	67.00±4.78	0.50±0.05	–	50.0	25.0	33.3
Regressed corpus luteum (CL) at estrus	Without regressed CL (n=15)	64.00±3.45	0.54±0.05	31.40±3.35	40.0*	46.7*	–
	With regressed CL (n=25)	62.88±3.48	0.51±0.04	21.60±1.87	92.0	0.0	–
Luteal deficiency at diestrus	Without luteal deficiency (n=11)	61.33±3.71	0.55±0.06	34.78±3.13	–	45.5*	18.2*
	With luteal deficiency (n=29)	64.97±3.18	0.52±0.04	23.38±2.00	–	6.9	79.3

*P<0.05, within column of the parameter categories.

released from adipose tissue depending on the metabolic condition particularly during stress of high milk yield (Bage *et al*. 2000).

Categorization of prolonged estrus exhibiting cattle based upon the duration of estrus revealed that 60% cattle had mean duration of estrus around 52.5±1.21 h (36–60 h), whereas, remaining 40% cattle exhibited estrus for 79.5±2.87 h (>60–96 h, Table 1). Plasma progesterone at estrus in the former group was 0.48±0.04 ng/ml in comparison to 0.59±0.04 ng/ml in the latter group (P<0.05, Table 1). This observation further emphasized our previous observations that higher plasma progesterone at estrus is related to extended duration of estrus (Honparkhe *et al*. 2010). Nevertheless, the % of cattle either with or without over-conditioned body score, with or without regressed CL and with or without subsequent luteal deficiency were similar (P>0.05) in cattle that exhibited estrus duration of 36–60 h or >60–96 h (Table 1). These observations in prolonged estrus exhibiting cattle suggested that: (a) duration of estrus is not influenced by their BCS and by the presence of regressed CL at estrus, and (b) duration of estrus is not associated with subsequent luteal adequacy.

Further analysis of data revealed that none of the over-conditioned cattle exhibited regressed CL at estrus, whereas, 75.7% cattle with normal BCS exhibited regressed CL at estrus (P<0.05, Table 1). The reason behind occurrence of regressed CL at estrus may be related with milk yield because 75% cattle with 19.11±1.30 l/day milk yield had regressed CL at estrus in comparison (P<0.05) to 33.3% cattle with 39.67±1.98 l/day milk yield (Table 1). Alterations in insulin metabolism in high milk yielders interferes with normal follicle development and hence subsequent CL that may not respond properly to luteolytic doses of endogenous prostaglandin (Vanholder *et al*. 2005, Honparkhe *et al.* 2010).

Nevertheless, regressed CL may not be the source of suprabasal progesterone at estrus because plasma progesterone was similar (P>0.05) in cattle either with or without regressed CL (Table 1). However, it has been reported that adrenal cortex can secrete substantial amount of progesterone in response to exogenous adrenocorticotropin hormone (Bage *et al*. 2000). During day-to-day herd management, stressors such as high milk production and improper energy balance have been addressed as potential causes of impaired reproductive efficiency (Dobson *et al*. 2001). Cortisol and progesterone produced by the adrenal glands in response to stress might, independently or together, have direct inhibitory effects on the preovulatory LH surge (Bage *et al*. 2000, Dobson *et al*. 2001). However, in present study, besides the presence of regressed CL at estrus in higher percentage of cattle with normal BCS, the plasma progesterone at estrus were similar (P<0.05) in cattle with normal or over-condition body score (Table 1). Furthermore, no differences (P>0.05) were observed in plasma progesterone at estrus in cattle with low or high per day milk yield (Table 1). As the impact of BCS and milk yield in not apparent on suprabasal plasma progesterone concentrations at estrus, it becomes more difficult to find the explanation for the occurrence of plasma-progesterone in prolonged estrus exhibiting repeat breeding cattle. Many other factors in modern dairy farming were identified as potential stressors, e.g., postpartum disorders, inflammations and infections, lameness, social factors, transport and heat stress (Dobson *et al*. 2001). However, cattle enrolled in present study were well managed and seemingly normal in estrous cyclicity and behaviour, without abnormalities in the genital tract.

In this study, during luteal phase subsequent to prolonged estrus, occurrences of luteal deficiency were noted in 72.5% cattle (Table 1). In fact, 78.8% cattle with normal body condition had luteal deficiency in comparison to 28.6% cattle with over-condition body score (P<0.05, Table 1). The reason behind luteal deficiency in cattle with normal BCS could be the presence of regressed CL in higher percentage of cattle with normal BCS. Thus, it can be observed from this study that the presence of regressed CL at estrus may have significant impact on subsequent luteal phase activity. This is further supported by the fact that 40% cattle without regressed CL and 92% cattle with regressed CL at estrus exhibited subsequent luteal inadequacy (Table 1). Moreover, 79.3% cattle with luteal deficiency had regressed CL, whereas only 18.2% cattle without luteal deficiency exhibited regressed CL (Table 1). However, no impact of plasma progesterone at estrus was observed on subsequent luteal adequacy.

Milk yield may also play a role in disturbed luteal profile a strong unfavorable genetic relationship exists between milk production and fertility (Royal *et al*. 2000). In present study, 70% cattle were low yielders and 82% of these had luteal deficiency at subsequent estrus (Table 1). In comparison, out of the 30% high yielders of present study about 50% had luteal insufficiency (Table 1).

Plasma-progesterone at estrus is associated with duration of prolonged estrus. Body condition and milk yield are not related to suprabasal plasma progesterone at estrus. Regressed CL may not be the source of suprabasal plasma progesterone and could be responsible for subsequent luteal inadequacy. Over-conditioning is not associated with occurrence of regressed CL at estrus, duration of prolonged estrus and subsequent luteal deficiency. High milk yield is associated with occurrence of regressed CL at estrus and subsequent luteal inadequacy. Further studies are required to delineate the other stressful risk factors associated with occurrence of suprabasal progesterone at estrus and hence prolonged estrus in repeat-breeder cattle.

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