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ENHANCEMENT OF OESTRUS DETECTION AND SUCCESS RATE OF ARTIFICIAL INSEMINATION USING SCORE CARD INCORPORATING SECONDARY SIGNS OF OESTRUS IN CROSS BRED DAIRY COWS HAVING REDUCED BEHAVIORAL SIGNS

Ibraheem Kutty Cholakkal*

Subject Matter Specialist, Krishi Vigyan Kendra, Malappuram,
Kerala Agricultural University, KCAET campus,
Tavanur P. O., Pin code 679573, Kerala
*Correspondence: ibraheemkutty50@gmail.com

ABSTRACT

Reduced behavioral signs and intensive management complicates oestrus detection in dairy cows. Hence, a score card incorporating more secondary signs was prepared, comprising sheet A and B for external findings and internal changes respectively. Based on ultrasonographic verification, animals getting at least score 20 (in sheet A) were indicated for internal examination and a cumulative score of at least 50 (sheets A&B) were ready for insemination. The score card was validated for oestrus intensity assessment in 60 postpartum (PP)cows (Gr. 1), closely watched for minor signs of oestrus followed by clinico gynecological examinationand 22 cows (Gr 2) under routine management formed the control group. Against 50.00 % in Gr. 2, 71.67 %cows of Gr. 1were detected in oestrus, including 9 animals reported twice, achieving oestrus detection rate (ODR) of 86.67 %. Despite non-significant variation ofAI success (60.42 versus 55.56 %) between Gr. 1 and 2, animals conceived during the study period was 48.33% and 22.73% respectively (P<.05), producing major enhancement of reproductive performance. Thus, the new score card for oestrus detection and insemination placing more focus on secondary changes will be highly promising to enhance oestrus detection and herd fertility under intensive management.

Key words: Oestrus detection, Score card, Behavioral sign, Insemination, cross-bred cattle

INTRODUCTION

Oestrus is the period of sexual receptivity in female animals characterized by the manifestations of behavioral signs to facilitate mating. In herds bred through artificial insemination (AI), detection of oestrus is very crucial in achieving adequate fertility rate. However, abolition /

weakening of the behavioral manifestations of the oestrus have become somewhat usual in dairy cattle herds continuously bred through AI across generations (Nasir and Kutty, 2004). Underlying reasons attributed for such a phenomenon include various stress factors out of increased milk production, adverse weather, chronic diseases, intensive management, and nutritional imbalances (Lopez-Gatius *et al*. 2005; Sakatani *et al.* 2012; Pedersen, 2014; Krishnan *et al*. 2017), aggravated by minimal opportunity for social interaction, absence of males, and denial of mating stimulus (Roelofs *et al.* 2010; Das *et al*. 2016). Reduced intensity and short duration of oestrus manifestations has become one of the major reasons for failure to detect oestrus and reduced fertility of dairy cows unless oestrus detection aids are being used (Krishnan *et al*. 2017). Thus, additional strategies are needed to improve oestrus detection in animals confined to barns / yards and to compensate the adverse effect of increased milk production and thermal stress affecting oestrus manifestation across different seasons and varied management situations (Sonmez *et al*. 2005).

In order to enhance oestrus detection rate (ODR), observation of various secondary signs were indicated either by manual observation or using adequate detection aids (Van Eerdenburg *et al.* 1996; Roelofs *et al.* 2010). Further, different methods of assessing the oestrus intensity are being utilizedfor the enhancement of detection efficiency. Van-Eerdenburg *et al.* (1996) suggested an oestrus intensity scoring system for dairy cattle reared freely under semi intensive management, wherein many factors associated with oestrus manifestations were taken into consideration even though major emphasis was placed on standing to be mounted.

Azeez (2014) used a scoring system for oestrus intensity in cross bred dairy cattle managed under intensive system with absolute lack of opportunity for the expression of major behavioural signs. Meenuja (2017) and Shakir (2018) also utilized the same scoring approach for oestrus detection under intensive management. However, the scoring criteria was very narrow giving emphasis only to very few physical and behavioral changes so that improvement of the oestrus detection could be achieved only in herds manifesting at least some of the prominent behavioral signs. Thus, need for improving the ODR incorporating more secondary/ minor changes was felt in cross bred dairy cows under intensive management and bred through AI across many generations, wherein most of the behavioral manifestations have become very weak or totally absent. Accordingly, objective of

the present study was to develop a suitable scoring system with more emphasis on minor signs of oestrus and validate the same for enhancing detection of oestrus and success rate of AI.

MATERIALS AND METHODS

The study was carried out at Livestock Research Station (LRS), Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University in India. The farm located at an altitude of 60-70 meters above the mean sea level, with latitude and longitude positioning denoted by 11°21' N and 76°21' E, respectively. Dairy farm of the station was maintaining around 300 cows belonging to cross-breds of Jersey and Holstein Fresian with local non-descript breeds. The animals were managed under intensive system with feeding as per standard recommendations (ICAR-NIANP 2013) and breeding was through AI routinely practiced from many years. Breedable animals were observed daily for oestrus signs, by herd-men of the respective barns as well as night watchmen, and those detected in oestrus were reported as per the routine practice.

a. Preparation of the score cards

As part of the study, trained workers were engaged for a period of three months, to watch the cows reported / suspected for oestrus daily, at least for 5 minutes at 6 hours intervals, with special attentionfor minor / secondary changes and behavioral alterations attributable to oestrus, and the findings were properly recorded. These animals were verified by the investigator based on previous records, observation of external signs, clinico-gynecological examination and B-mode ultrasonography (USG) for internal changes of oestrus. Besides those reported in oestrus, few anoestrus cows were also examined by USG at monthly interval to assess ovarian function and the occurrence of silent oestrus.

A score card having two separate sheets A and B respectively for oestrus reporting by the workers and recording the findings of internal verification was prepared. The scores were adequately modified considering the occurrence and relevance of various changes listed out to be used as indicators of oestrus and to assess the oestrus intensity for deciding insemination.Total score of sheet A was compared with the internal findings to assess usefulness of the score in predicting the oestrus and cumulative score from both the sheets were considered as criteria to confirm the oestrus and to decide on the time for insemination.

b. Validation of the scores

Validation of the score cards for oestrus detection and verification were

performed in 60 cows (Gr. 1) belonging second to fourth months PP, over a period of one year. Detection of oestrus and reporting was carried out day and night by the herd-men.All the animals reported to have at least some minor signs of oestrus were assigned scores using sheet A and those attained at least score 20 were verified based on history and clinico gynecological examination. Findings of verification were scored using sheet B and cows with a cumulative score of at least 50 from both the sheetswereinseminated. Further confirmation of proper oestrus at the time of AI was performed retrospectively based on the interval to subsequent oestrus reporting / pregnancy diagnosis beyond 45 days of AI.

Another 22 cows of the same PP stage was kept as control group (Gr. 2), wherein oestrus was detected as per the routine practice. Reported animals were verifiedbyhistoryandclinic-gynaecological examination and those at the right time of oestrus were inseminated. Reproductive management parameters such as number of oestrus detected, intensity of oestrus, number of AI done and conception rate of AI were compared in both the groupsto assess the effectiveness of the score card for detection of true oestrus and to decide on the time for insemination. Data were analyzed using excel sheet for descriptive details.

RESULTS AND DISCUSSION

A total of 103 oestrus periods were reported among 64 cows observed during three months of score card preparation. Oestrus scoring methods used by Azeez (2014) and Schuller *et al.* (2017) for intensive management situations were modified in this study incorporating various minor changes to develop the new score card. At least some expression of major behavioural signs were reported only in 18 (17.48 %) oestrus and detection of flow / remaining of mucous discharge was the major criteria in 32 (31.07 %) oestrus periods reported. However, inclusion of minor behavioral signs enabled reporting additional 59 (57.28 %) more oestrus enhancing the ODR to 1.60 instead of 0.53 per animal before observing minor signs of oestrus. Hence, final score card was prepared with two separate sheets as shown in Table 1 and 2,for oestrus reporting by the herd-men and further verification by the inseminators respectively.

a. Preparation of the score sheets

The score sheet A (40 points) intended for reporting oestrus detection by the farmers / care takers of the animals, incorporated more secondary behavioural signs and external manifestations attributable to oestrus, to enhance the detection especially when advanced oestrus detection devices

Table 1. Score sheet A for reporting oestrusassociated signs by the herd-men

Behavioural signs(Maximum 25 points)			Max Score	Given score
Specific signs (Maximum 10 points)	1	Trying to mount (animals / objects)	5	
	2	Standing for nudging / mounting	5	
	3	Characteristic vocalization	5	
		Total	0 to 15	
Non-specific signs (Maximum 15 points)	1	Standing up / not lying down	2	
	2	Sniffing nearby cows	2	
	3	Arching of back	2	
	4	Tail deviation	2	
	5	Excitement / anxious look	2	
	6	Intermittent urination	2	
	7	Disturbing nearby cows	2	
	8	Reduced milk yield	2	
	9	Reduced interest in feed	2	
	10	Exhibiting flehmen reaction	2	
		Total	0 to 20	
Changes of external genitals(Maximum 5 points)				
Vulvaloedema (Select one or Nil)	1	Mild degree	1	
	2	Moderate degree	2	
	3	Higher degree	3	
Vestibular hyperaemia (Select one or Nil)	1	Mild / Moderate degree	1	
	2	Higher degree	2	
		Total	0 to 5	
Mucous flow from the vagina (Maximum 10 points)				
Nature of discharge (Select one or Nil)	1	Thick and voluminous flow	10	
	2	Thin and scanty flow	8	
Location (Select one or Nil)	3	Smeared on the body / floor	5	
	4	Small volume persisting at vulva	2	
		Total	0 to 15	
		G. Total	40	

are not in use. Owing to the total absence / reduced intensity of most behavioral signs and lack of opportunity for the expression under intensive management, detection of oestrus has become tedious and many oestrus periods go undetected, extending the service period beyond satisfactory limits (Schuller *et al.* 2017). Similarly moderate elevation of ambient temperature (AbT) has been reported to affect the behavioural and physical manifestations of the oestrus so that as many as 80 per cent of oestrus

goes undetected (Roelofs *et al.* 2010; Das *et al.* 2016). Hence more emphasis has been placed on incorporating minor / secondary signs of oestrus in the score sheet A.

Based on repeated screening of animals with different scores in sheet A, those attaining at least 20 were indicated for clinico-gynaecological verification for oestrus and others having lesser scores were observed further for more changes on the subsequent days.

Verification through ultrasonography

Out of the 103 oestrus periods checked by USG, mature follicle(s) were detected in 87 (84.46 %) animals on the first day itself and in 10 (9.70 %) at rechecking on the subsequent day. Out of the 81 cows inseminated on the first day, oestrus persisted on the second day as well in 48 (59.26 %) with mature follicle detected upon USG verification and AI was repeated. Out of the 10 cows with larger follicles detected only

Table 2. Score sheet B for oestrus intensity and readiness to breed assessments by the inseminator

1. Reproductive history (Max 15 points)			Score	Given score
Heifer	1	Breedable age, Not yet bred	5	
Days from calving (Select one or Nil)	1	Less than 30 days	-10	
	2	More than 30 days	5	
Previous oestrus (Select one or Nil)	1	Oestrus 18-22 days back	15	
	2	Oestrus 38-44 days back	10	
	3	Oestrus 56-66 days back	5	
		Total	0 to 20	
2. Changes in the reproductive tract (Maximum 30 points)				
Cervical relaxation (Select one or Nil)	1	Mild degree	5	
	2	Mild to moderate	8	
	3	Moderate to high	10	
Appearance of mucous	1	Clear and voluminous flow	5	
Uterine tonicity (Select one or Nil)	1	Mild degree	5	
	2	Moderate	10	
	3	Higher degree	15	
	4	Extremely higher degree	-10	
		Total	0 to 30	
3. Ovarian structures (Maximum 15 points)				
Follicle (Select one or Nil)	1	Larger follicle palpable	10	
	2	Small follicles palpable	5	
Corpus luteum (Select one or Nil)	1	Recent RCL on any one ovary	10	
	2	Functional CL palpable	-15	
		Total	0 to 20	
		G. Total		

on the second day, eight were inseminated. Among 103oestrus periods, seven (6.79 %) revealed large functional corpus luteum indicated by structural features and profuse blood supply at colour doppler checking, together with large antral follicles in the same or contra-lateral ovary. These animals were skipped without AI, since the features were suggestive of mid cycle oestrus, and there was history of previous oestrus in five of them 12 to 14 days earlier. Among the 89 oestrus inseminated, 42 (47.19 %) were having regressing corpus luteum (CL) without history of oestrus or breeding in the previous cycle indicative of silent oestrus usually occurring in high yielding dairy cows.

Findings of tubular changes at palpation and ovarian structures detected through USG were comparable, except in five cows. In two of them, there was ovarian cyst together with mild tonicity of uterine horns and in other three, there was no large antral follicles, even though moderate tonicity was palpated. This could be due to individual variation or some abnormality of tubular manifestations. In 15 out of 17 anoestrus cows checked by u/s, the ovaries were not having recent CL or large antral follicles, attributable to ovarian non function of early lactation period concurring the report of Meenuja (2017). In other two cows, even though there were large antral follicles, there was no external signs and tubular manifestations of oestrus were also very less, attributable to improper secretion / imbalance of hormonal action. Overall, except in very few oestrus verified, internal changes detected through USG were comparable with the external manifestations, and assessment of the oestrus intensity based on the score cardenabled decision making on the time for breeding and to rule out improper oestrus. Relative weightage assigned for oestrus associated signs from both the score sheets to make breeding decision are shown in table 3.

On the basis of relevant history, repeated verification through clinico-gynaecological examination, USG and outcome of insemination, animals achieving a total score of 50 from both the sheets (20 + 30) were found to be ready for insemination on the same day, unless there is some abnormality of reproductive function detected. Cows with score less than 50 were postponed from breeding for reconsideration on subsequent days. The score card appears very useful in the current scenario since continuous adoption of AI and lack of male stimulus has resulted wide variations of tubular changes of oestrus, making assessment of the proper time for insemination very difficult (Kutty, 2019; Orihuela, 2000).

Table 3. Oestrus associated factors and weightage for assessing oestrus intensity

S. No	Oestrus signs	Item score	Category total
1	**Behavioural changes**		25
	a. Specific signs	10	
	b. Non-specific signs	15	
2	**Breeding history**	15	15
3	**Mucous flow**	10	10
4	**Changes of External genitals**		5
	a. Vulval oedema	2.5	
	b. Vestibular hyperaemia	2.5	
5	**Cervical & uterine changes**		30
	a. Cervical relaxation	10	
	b. Nature of cervical mucous	5	
	c. Uterine tonicity	15	
6	**Ovarian structures palpated**		15
	a. Medium /large Follicle	5	
	b. Corpus luteum (Regressing)	10	
7	**Total score**		100

Grading of oestrus intensity as suggested by Van-Eerdenburg*et al.* (1996) could not be adopted in the study herd since the animals were confined to the barns all the time and lack opportunity for expressing primary signs of oestrus such as mounting and standing to be mounted. Score cards used by Meenuja (2017) and Shakir (2018) under intensive management were based on very few parameters and needed modification for the current situation. Since maximum display of oestrusbehaviour was reported to occur in the morning and midnight, when the ambient temperature was low (Kumari and Pampana, 2015; Sonmez*et al*. 2005), more attention at these timings was necessitated to achieve better detection rates.

b. Validation of the score card

Validation of the score cardwas carried out during 28 to 120 days PP, being the most probable period of oestrusoccurrence. Among the 82 animalsstudied, a total of 63 oestrus periods were detected, 52 being in 43 animals of Gr. 1 with nine of them exhibiting oestrus more than once, and 17 (28.33 %) remained anoestrus. Among Group 2, 11 (50.00 %) cows were detected in oestrus only once. Total number of oestrus detected among the study group was less (Table 4) compared to overall ODR of the herd obtained from retrospective data and is attributed to the limited period of the study and focus on early PP animals wherein occurrence of oestrus becomes less with increase in milk production (Leroy *et al.* 2008).

Between the two groups,ODR was more in Gr.1 than Gr.2, which can be attributed to frequent watching and the focus on minor signs of oestrus enabling better detection, and stimulation of the reproductive tract through gynaecological examination and USG enhancing the occurrence and detection of oestrus (Orihuela 2000; Maurya *et al.,* 2017). However, the number of animals that showed oestrus (71.67 % versus 50.00 %) did not vary significantly (chi square statistic 3.36, *p-value* 0.066) between the two groups. Conception rate of AI was also better in Gr 1 (60.41 versus 55.56 %) attributable to improvement in oestrus assessment and timing of AI. Thus because of a 37 % increase in ODR and 5 % increase in conception rate of AI, the proportion of animals conceived was significantly higher (chi square statistic 4.3486, *p-value* 0.037) during the study period in Gr.1 than Gr 2 (48.33 versus 22.73 %), contributing considerable increase of post-partum reproductive performance of the herd.

Conclusion: Preparation and validation of the score card, with two separate sheets for oestrus detection and verification resulted 37 % increase in ODR and a 5 % increase of conception rate in early post-partum cows, which ultimately contributed 25.6 % more number of pregnancies in the study group than controls. Thus, the modified score card ensures better scope for improving oestrus detection and overall reproductive performance of the dairy cattle herds bred through AI under intensive management.

CONCLUSION

In order to enhance the oestrus detection rate, a modified score card was prepared with more focus on detection of minorsigns and internal changes associated with oestrus. The scores were assigned

Table 4. Comparison of oestrus detected, animals observed, proportion of oestrusanimals and success rate of AI among the study groups

Parameters	Gr. 1	Gr. 2	Total
Animals observed	60	22	82
Number of oestrusreported	52	11	63
Animals reported in oestrus	43	11	54
Proportion of animals in oestrus (%)	71.67	50	65.85
Number of oestrusconfirmed	49	9	58
Inseminations done	48	9	57
Conception rate of AI (%)	60.41	55.56	59.65
Animals conceived (%)	48.33*	22.73	41.46

*Significant at 5 % level (Chi square 4.3486, p value 0.037)

based on verification of the reported signs and associated internal changes through clinic-gynaecological examination and USG to confirm oestrus. The score card had two sheets, one for reporting the external findings by the herd-men and the other for verification of the internal findings. Validation of the score card was performed in a study involving 60 cross bred cows (Gr. 1) having weak manifestations of oestrus. Another 22 cows under the routine reproductive management was compared as control group. Animals achieving a score of 20 from Sheet A was indicated for internal verification and a total score of at least 50 from both the score sheets was found to be ready for insemination. The oestrus detection rate was 37 % higher in the study group with a 5 % increase in conception rate of AI as well, indicating effectiveness of the score cards for enhancing detection of oestrus and success rate of breeding contributing greatly to improve overall reproductive performance of the herd.

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