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ORIGINAL ARTICLE

Estrus Behavior and Estrual Cervical Mucus Characteristics of Repeat Breeding Animal Subjected To Different Hormonal Protocols

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ABSTRACT
A study was conducted to compare the physical characteristics of cervico vaginal mucus and estrus behaviour of eighty repeat breeding animals subjected to different hormonal treatment protocol i.e. Group 1-control, Group 2-Area Specific Mineral Mixture, Group 3- ASMM+Doublesynch and Group 4-ASMM+Estra Doublesynch protocol. It was revealed that all the animals exhibited clear mucucs discharge. The consistency of mucus was thin, moderate and thick in 22.5, 52.5 and 25 per cent of cattle respectively. The moderate consistency nature was improved from group I (45%) to group II (50%), group III (55) and IV (60%). Typical and atypical ferning pattern of mucus was observed in 61.25 and 20 per cent of animal respectively. 18.75 per cent animals revealed no ferning pattern. Group IV animals exhibited highest per cent of typical fern pattern, followed by group II and III. The percentage of animal showing spinnbarkeit value ranging between 0-8, 8-16 and 16-24 cm were 21.25, 28.75 and 50 respectively. The number of repeat breeding animal with maximum spinnbarkeit value (16-24 cm) increased from control group (n=7) to group II (n=10), group III (n=11) and group IV (n=12). The pH value of 7-7.5, 7.5-8 and 8-8.5 was found in 26.25, 51.25 and 22.5 per cent of repeat breeding animal. Per cent of animals were having pH range within 7.5 to 8 with increased from group I to group II, group III and group IV respectively. In our study percentage of animals showing weak, moderate and intense estrus were 51.25, 30 and 18.75 per cent respectively. The percentage of animal showing intense estrus increased from group I (10%) to group II (20%) and group IV (30%). The grading of estrus was significantly higher in all the three treatment group than the control group, whereas no significant difference observed between group II and III. The significant value of chi-square test implied that all these estrus characteristics were depended upon the treatment protocols. There is significant difference between the estrus duration of all the four treatment group. The fertility (CR) also increased from control group (15%) towards group II (40%), group III (65%) and group IV (70%).
Key words: Doublesynch, Estra Doublesynch, Spinnbarkeit, Repeat breeding.

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INTRODUCTION

Cervical mucus is a visco elastic secretion of constantly secreting mucus producing cells of the endo-cervix [4] and acts as a mechanical barrier to prevent intruding organisms. The physical properties of cervico-vaginal mucus (CVM) have direct relationship with circulatory oestrogen-progesterone levels and fertility status of the animals [11] as it essentially undergoes certain changes during estrus phase for the passage of spermatozoa. There are consistent and definite gradual changes in the viscosity, clarity, stretchability and fern pattern of cervical mucus along with the cyclic rhythm of the reproductive phenomenon, hence are considered as effective laboratory tools to predict the infertility in bovines. In females, several interrelated factors such as estrous behavior and certain endocrine aspects have been

investigated in modern high-yielding repeat breeder cows. Prolonged and/or silent estrus has been observed in up to 50 per cent of repeat breeding cows [2]. Thus, the objectives of this study were to investigate the quantitative and sequential differences in estrous activity and CVM characteristics in repeat breeding animals subjected to different hormonal protocols around estrus as reason(s) for improving fertility in repeat breeding cows.

MATERIAL AND METHODS

Total eighty numbers of repeat breeding animal were divided into four groups. Group I (n=20) taken as control, Group II supplemented with 50gms of Area specific mineral mixture (ASMM)/day for 60 days, Group III supplied with ASMM and Doublesynch protocol and Group IV also supplied with ASMM and Estra Doublesynch hormonal protocols. Cervical mucus was collected on the day of estrus prior to the AI using sterile blue sheath fitted in a "Universal artificial insemination gun" and aspirating it from mid cervix by recto-vaginal method in a sterile tube and immediately taken to the laboratory for the examination of appearance. A few drops of mucus sample were placed into a grease free glass slide and slide was inclined to 45^0C for the detection of consistency of the mucus. For measuring the Spinnbarkeit value, 2-3 drops of collected mucus sample were taken on a grease free glass slide; The mucus was stretched between two slides by moving the second slide away from first one, until the mucus breaks. Two to three drops of well mixed cervical mucus spread uniformly over a grease free glass slide and air dried. The air dried slide was examined under microscope using low power objective (10X) for crystallization pattern of the mucus, known as arborisation pattern. pH of cervical mucus was measured using digital pH meter. Grading or rating was done after evaluating the oestrus behaviour of individual animal and graded from 4 to 1 (Mishra, 2011).

RESULT AND DISCUSSION

Appearance

In the present study clear cervico-vaginal mucus was observed in 100 per cent of experimental repeat breeding animals ruling out infectious cause of repeat breeding.

Consistency

About 52.5 (n=49) per cent of the total repeat breeding animal showed moderate consistency followed by 25 (n=20) per cent thick and 22.5 (n=18) per cent thin consistency. Present findings contradicts the findings of Rangnekar *et al*. (2002) who reported 35, 25 and 40 % occurrence of thin, medium and thick consistency of mucus, respectively, in repeat breeding cows, which may be due to the types (infectious/ non-infectious) of repeat breeders taken into consideration. Maximum number of animal showing moderate consistency (n=12, 60%) were found in group IV which contains least number of animal with thick consistency (20%) and thin consistency (25%). The moderate consistency nature was improved from group I (45%) to group II (50%), group III (55) and IV (60%).

Ferning Pattern

The fernning pattern of the total 80 cattle, 49, 16 and 15 number of repeat breeding cattle showed typical and atypical fern pattern i.e. 61.25, 20 per cent respectively similar to Kumaresan *et al*. (2001) who reported 57.97, 23.19 and 18.84 per cent of typical, atypical and nil fern pattern in buffaloes which might be due to increased peripheral estrogen concentration at the time of estrus indicating right time of insemination. Conception rate was highest in group IV (70%) showing highest (70%) per cent typical fern pattern, followed by group II and III (60%) is corroborates with the findings of Luktuke and Roy (1967) who observed better pregnancy rates in cows and buffaloes showing typical fern pattern and almost zero pregnancy rate, when there was absence of fern pattern in mucus. The atypical fern pattern was observed more in control (25%) group animal than cattle subjected to various treatment protocols (Gp II & III-20%, Gp IV-15%) which is similar to the findings of Modi *et al*. [9] who revealed that atypical fern pattern was observed more in repeat breeding cows than normal cows. Typical fern pattern is said to be indicative of ovulatory heat, whereas, in weak estrus atypical pattern is observed [3].

Spinnbarkeit Value

From the present study it was found that about 50 (n=40) per cent of the experimental animal were having spinnbarkeit value ranging from 16 to 24 cm, followed by 28.75 per cent having 8-16 cm and 21.25 per cent having 0-8 cm spinnbarkeit value. The number of repeat breeding animal with maximum spinnbarkeit value (16-24 cm) increased from control group (n=7) to group II (n=10), group III (n=11) and group IV (n=12) which is close to the study of Rangnekar *et al*. [11]who observed that elasticity of cervical mucus of normal breeder was superior than the repeat breeder cows. Mohanty *et al*. [10] also obtained a non-significantly higher spinnbarkeit value among fertile estrus than repeat breeding cows.

pH- value

Present study revealed that in 51.25 per cent of the repeat breeding animal the pH value was found to be within the range of 7.5 to 8 followed by 26.25 per cent within 7-7.5 and 22.5 per cent within 8-8.5 range. Among all experimental animals about 30, 50, 60 and 60 per cent of animals were having pH range within 7.5 to 8 in control, group I, group II, group III and group IV respectively, which corroborates with the findings of Tsiligianni *et al*. [15] who said that the pH values in cervical mucus collected from synchronized cows was slightly higher than spontaneous ovulators. Modi *et al*. [9] also stated that the normal breeders showed higher pH value as compared to the repeat breeders. Alkaline pH of cervical mucus was more favorable for sperms progressive motility hence increased fertility [11].

Estrus Intensity

Weak estrus was observed in 51.25 per cent, moderate estrus in 30 per cent and intense estrous in 18.75 per cent of the animals. The percentage of animal showing intense estrus increased from group I (10%) to group III (15%), group II (20%) and group IV (30%) whereas maximum number of animals (13) in group I (control) showed weak estrus as compared to other three groups which is similar to the findings of Senthilkumar and Chandrahasan [13]who stated that the normal and intense oestrus was higher and weak oestrus was lower in synchronized cows as compared to control cows. Maximum number of intense estrus was found in group IV because of estradiol benzoate inj. was given on 10^{th} day of protocol. The combination of estradioal cypionate in PGPE-1 protocol enhanced the expression of estrus and increased ovulation percentage. The administration of $PGF_2\alpha$ causes rapid regression of corpus luteum and brought down the blood progesterone level within 24 hr after injection [5], which might be the reason for more intense estrus in synchronized cows. In contradict to present study Sood *et al.* [14] said that estrus was more intense in repeat breeder than control with synchronization as indicated by numerically higher overall activity indexes and higher peak neck activity.

The univariate analysis of the characteristics and treatment groups revealed significant difference (λ^2 value in table 1) among each protocols.

Table 1. Univariate analysis for factor (Stage of animal*Treatment group) associated with characteristics of CVM of cattle (number) using chi-square (χ^2)test

Characteristics	Variable	Treatment group					**X^2-value**
		Gp1	Gp2	Gp3	Gp4	Total	
Consistency	Thin	5(25%)	5(25%)	4(20%)	4(20%)	22.5%	13.308*
	Moderate	9(45%)	10(50%)	11(55%	12(60%	25	
	Thick	6(30%)	5(25%)	5(25%)	4(20%)	52.5	
Ferning pattern	Typical	11(55)	12(60)	12(60)	14(70)	61.25	21.033*
	Atypical	5(25)	4(20)	4(20)	3(15)	20	
	Nil	4(20)	4(20)	4(20)	3(15)	18.75	
Spinnbarkiet value	0-8	6(30)	3(15)	4(20)	4(20)	21.25	14.643*
	8-16	7(35)	7(35)	5(25)	4(20)	28.75	
	16-24	7(35)	10(50)	11(55)	12(60)	50	
Ph value	7-7.5	8(40)	6(30)	3(15)	4(20)	26.25	13.356*
	7.5-8	7(35)	10(50)	12(60)	12(60)	51.25	
	8-8.5	5(25)	4(20)	5(25)	4(20)	22.5	
intensity	weak	13(65)	12(60)	12(60)	4(20)	51.25	13.653*
	moderate	5(25)	5(25)	4(20)	10(50)	30	
	intense	2(10)	3(15)	4(20)	6(30)	18.75	

Figures in parenthesis indicate percentage of animal.

Estrus Grading and duration of estrus

The duration of estrus and estrus grading of repeat breeding animals subjected to different treatment protocols is given in table 2.

The present study revealed that grading of estrus behaviour is significantly higher in animal subjected to different treatment protocol than the control group (1.98 ± 0.20). However, the other treatment groups more particularly, the group IV animals (2.95 ± 0.21) having been injected with estradiol benzoate showed highest grading which are certainly qualitatively better than all the groups including untreated control, where as no significant difference observed between group II and III. The duration of

estrus (hr) is also significantly varied between control and hormonal protocol treated groups but no significant difference is observed between group I and II. Non-significantly longer duration of estrus was observed in group IV than group III, might be due to the action of estradiol. Longer and shorter duration have also been reported by Sahu [12] and Chottray [1], which might be due to different infertility condition, species, hormonal protocols, season and nutrition etc.

Table 2: Estrus grading and duration of repeat breeding animals subjected to different treatment protocols

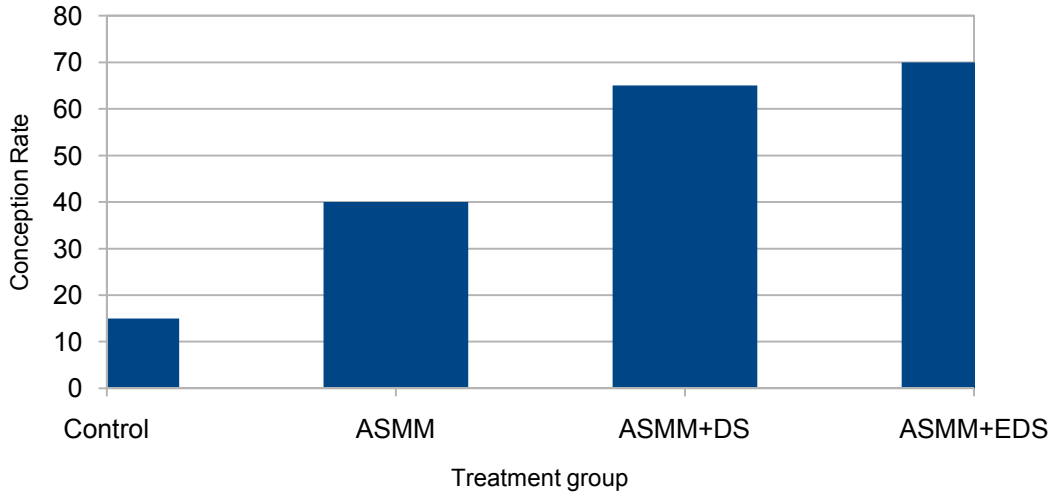
Treatment Groups / characteristics	Group I	Group II	Group III	Group IV	P value
Estrus Grading	$1.98^{a} \pm 0.20$	$2.45^{ab} \pm 0.23$	$2.50^{ab} \pm 0.19$	$2.95^{b} \pm 0.21$	0.02*
Estrus duration	$28.80^{a} \pm 3.87$	$23.20^{ab} \pm 1.89$	$20.10^{b} \pm 0.75$	$21.40^{b} \pm 0.85$	0.038*

Mean value having different superscript within the same row differ significantly, $p<0.05$

CONCLUSION

Overall duration of estrus and estrus grading was qualitatively and quantitatively better in animals belonging to treatment group than control group. Hence it was concluded that tones thus staging of estrus as well as cervico vaginal mucus can be used successfully and to measure the therapeutic efficacy of various treatment protocols which meant in turn implies the fertility of animals.

Fig: 1-Conception rate under different treatment protocols



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