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Occurrence of subclinical endometritis and oestrus characteristics in repeat breeder cows

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Abstract

The present research work was carried out to study the occurrence of subclinical endometritis, oestrus characteristics such as duration and intensity of oestrus and physical properties of oestrual mucus in repeat breeder (RB) cows and its association with age of the animal. Data on the occurrence of repeat breeding for the past three years were collected from breeding registers maintained at Livestock Research Station, Thiruvazhamkunnu. An overall occurrence of 21.08 per cent was noticed with a gradual reduction from 22.27 to 20 per cent. Further, the occurrence of various reproductive disorders for a period of 18 months was collected from the data available in the registers and by clinico-gynaecological examination and repeat breeding was observed to be having the highest occurrence (34.34 per cent). Subclinical endometritis (SCE) was screened using the Whiteside test and cytological endometritis was diagnosed using modified cytobrush technique (PMN cells ≥ 5 per cent) and the incidence of SCE and cytological endometritis in RB cows were 22.58 and 9.67per cent, respectively. There was no significant difference (P > 0.05) in the occurrence of SCE in RB cows below and above six years. The intensity of oestrus was assessed using a scorecard and classified into high, medium and low by observing behavioural, physiological signs and gynaecological examination. The oestrual mucus of 24 RB cows free of SCE was examined and physical characteristics like colour (transparent, cloudy, dirty), consistency (thick and thin) and volume (copious, moderate, low) were noted. Among 24 RB cows 54.16, 41.67 and 12.50 per cent exhibited high, medium and low intensity of oestrus, respectively and the mean duration of oestrus was found to be 27.50 ± 1.50 h. The majority of RB cows exhibited copious (50 per cent), transparent (83.33 per cent) thick consistency (66.67 per cent) oestrual mucus. There was no significant difference (P > 0.05) in the colour, consistency and volume of oestrual mucus in RB cows below and above six years.

Keywords: *Repeat breeding, subclinical endometritis, physical characteristics, oestrual mucus*

Reproductive efficiency in dairy cows is the foundation of profitable milk production and sustainable herd management. India, which contributes 24 per cent of global milk production (Economic Review, 2023), owes this achievement primarily to the vast population of dairy cattle rather than their high individual productivity. Hence, achieving optimal fertility in dairy cows is essential for ensuring profitability in dairy farm operations. In the Indian scenario, the

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predominant factors contributing to decreased productivity is the high incidence of reproductive disorders, notably endometritis, anoestrus and repeat breeding. Among these, repeat breeding accounts for 12 to 35 per cent of total infertility conditions and is considered one of the major reproductive disorders affecting the conception rate and reproductive efficiency of dairy cattle. Repeat breeding syndrome (RBS) in cows is characterised by a regular oestrous cycle and normal oestrus and has been bred three or more times to a fertile bull or semen yet failed to conceive (Roberts, 1986). Primary identified etiological factors of repeat breeding were genital tract infections (63 per cent), ovulatory defects alone (15 per cent), and a combination of ovulatory defect with genital tract infections (22 per cent), Azeez *et al*. (2017). Repeat breeding in particular as a reproductive disorder, has a severe economic implication, including increased cost of veterinary interventions, repeat inseminations and extended non-productive periods. Postpartum uterine disorders are a leading cause of repeat breeding in dairy cows. Lactating dairy cows had a greater occurrence of repeat breeding than heifer (Souza *et al*., 2016). The occurrence of repeat breeding was found to be higher in multiparous and aged animals (Bonneville-Herbert *et al*., 2011). Subclinical endometritis is a common yet undiagnosed condition which significantly impairs fertility in repeat breeders, making its early diagnosis and management essential. Furthermore, analysing the physical properties of oestrual mucus provides an effective non-invasive approach for assessing the reproductive status of the animals as it was found that the physical properties of the oestrual mucus will affect the survival and activity of spermatozoa and pregnancy rate (Sharma and Tripathi, 1987; Modi *et al*., 2011). Hence, the present study aimed to identify etiological factors causing repeat breeding in crossbred cows, to evaluate the oestrus characteristics and physical properties of oestrual mucus so that better treatment measures could be adopted resulting in good fertility response.

Materials and methods

The study was conducted in RB cows maintained under identical conditions of environment and management. The data regarding the occurrence of repeat breeding among crossbred cows for three years (January 2021 to December 2023) was collected from the breeding registers maintained in the farm and a total of 34 RB cows were identified. Data on the occurrence of reproductive disorders among the crossbred cattle during the study period (January 2023 to June 2024) were also collected. Clinico-gynaecological and ultrasonographic examination of 34 RB cows was performed to rule out the presence of any detectable anatomical abnormalities. Three RB cows with cervical defects and poor body condition were excluded from further study. Subclinical endometritis was screened in the remaining 31 RB cows using the white side test (Bhat *et al*., 2014) by mixing equal volumes of oestrual mucus and five per cent sodium hydroxide solution. The prepared mixture was heated to boiling point, then cooled and the colour change was observed. They were categorised into no colour or turbid (no infection), yellow (mild infection) and dark yellow (severe infection). Those animals positive for the white side test were subjected to endometrial cytology to rule out cytological endometritis using modified cytobrush technique (Kasimanickam *et al*., 2004). Cows with less than five per cent polymorphonuclear leucocytes (PMNL) were considered free from cytological endometritis (Pothmann *et al*., 2019). Twenty-four repeat breeder cows, free of detectable anatomical abnormalities and SCE, were further studied for the nature of oestrus and characteristics of cervico-vaginal mucus. The intensity of oestrus was determined based on the score card (Azeez, 2014) with slight modifications, which involved assessment of behavioural signs, physiological changes and gynaecological examination and classified as high (> 10 points), medium (5 to 10 points) and low (< 5 points). The duration of oestrus was measured in hours from the onset of visible behavioural symptoms to their disappearance, marking the end of the oestrus period. Physical traits of the oestrual mucus in RB cows were examined using the cervico-vaginal mucus collected on the day of oestrus. The colour of the oestrual mucus discharge was classified into transparent, cloudy and dirty. The consistency of the oestrual mucus was classified into thick and thin (Lim *et al*., 2014) which was determined by dropping a few drops of oestrual mucus in a clean, grease-free slide and tilting the slide to 45°. In thick consistency, mucus discharge flowed slower without breaking and wider and in thin consistency mucus flowed faster and less wide. The volume of the mucus was determined based on the quantity of the cervico-vaginal discharge during oestrus and was classified into copious, moderate and low. Statistical analysis of data regarding the occurrence of SCE and age groups (below and above six years) in RB cows and characteristics of oestrual mucus and age groups (below and above six years) was done using Fisher's Exact test.

Result and discussion

The intensity of oestrus, duration of oestrus and the physical properties of the oestrual mucus discharge was assessed on the day of oestrus.

Occurrence of repeat breeding and reproductive tract disorders

The occurrence of repeat breeding among crossbred dairy cows in 2021, 2022 and 2023 was 22.27, 20.73, and 20 per cent, respectively. The overall occurrence rate of repeat breeding was noted as 21.08 per cent (Table 1). The occurrence of repeat breeding in the present study was in accordance with the findings of Arun e*t al*. (2020) and Aruna *et al*. (2021) who reported an occurrence of 25.96 per cent and 23.60 per cent of repeat breeding respectively among the crossbred dairy cows

in Kerala. On the contrary, Harichandan *et al*. (2019) and Asaduzzaman *et al*. (2016) observed a higher and lower occurrence of repeat breeding, 51.94 and 11.60 per cent, respectively in crossbred dairy cows. The difference in the occurrence of repeat breeding may be due to differences in breed, age, location of study and sample size. The season of the year might also influence the occurrence of repeat breeding. The data on occurrence of various reproductive disorders for a period of 18 months among 255 cows and heifers maintained in the farm revealed an occurrence of 99 reproductive cases, which included anoestrus, retained foetal membrane, repeat breeding, SCE, endometritis, metritis, dystocia, abortion, cervicitis, cervico-vaginal prolapse and uterine prolapse (Table 2) with repeat breeding having highest occurrence rate of 34.34 per cent. These findings were in accordance with the observations of Maji and Samanta (2013), who had reported 32. 32 per cent occurrence rate of repeat breeding in dairy cattle. Retained foetal membrane had an occurrence rate of 19.44 per cent in cattle (Patel and Parmar, 2016). Contrary to these findings, Kumar and Singh (2018) reported a higher occurrence rate of 38.33 per cent and Kumaresan *et al*. (2009) reported a lower occurrence rate of 12.80 per cent of repeat breeding among crossbred cows. The variation in occurrence may be due to in breed of cow, production stress, nutritional status, age and parity of the cow. Zobel *et al*. (2011) noted that crossbred cows had the highest incidence of repeat breeding, followed by Holstein Friesian, Red Holstein and Simmental breeds of cattle. The gradual reduction in the occurrence of repeat breeding in the farm might be due to scientific feeding and management practices and consistent culling protocols adopted in the farm.

Occurrence of subclinical endometritis

White side test was used to evaluate the endometrial health of 31 RB cows. Out of these 22.58 per cent (7 out of 31) were diagnosed with SCE (Fig. 1). Using the modified cytobrush technique (Fig. 2), three cows out of seven cows were diagnosed with cytological endometritis (Fig. 3), with PMN cell level exceeding five per cent threshold. The overall incidence of subclinical endometritis was 22.58 per cent (7 out of 31), with cytological endometritis accounting for 9.67 per cent (3 out of 31). The RB cows were divided into two age groups (above six years and below six years) and the occurrence of subclinical endometritis was noted. In the below six years age group, three (23.1 per cent) out of 13 RB cows were positive for SCE. In. Above the six age group, four (22.6 per cent) out of 18 RB cows were found to be positive for SCE. There was no significant difference ($P > 0.05$) in the occurrence of SCE in RB cows below six years and above six years (Table 4). Out of 31 RB cows, 24 RB cows free of SCE were selected for assessment of intensity and duration of oestrus and assess the characteristics of oestrual mucus. Zarekar (2014) observed a similar occurrence of SCE of 20.62 per cent in RB cows diagnosed using the white side test. In a study by Savitha *et al*. (2005) involving 36 cows, a comparable incidence of SCE of 30.50 per cent was observed. Similarly, Pothmann *et al*. (2015) reported a prevalence of 12.70 per cent of cytological endometritis in RB cows with a threshold of PMN cells ≥ 5 per cent. Contrary to the above findings, a higher incidence of 52.70 per cent of cytological endometritis in RB cows was reported by Salasel *et al*. (2010). A higher occurrence of 71.25 per cent of SCE in RB cows was diagnosed using the white side test (Bhat *et al*., 2014). The reduced incidence of SCE in this study could be attributed to the lower prevalence of

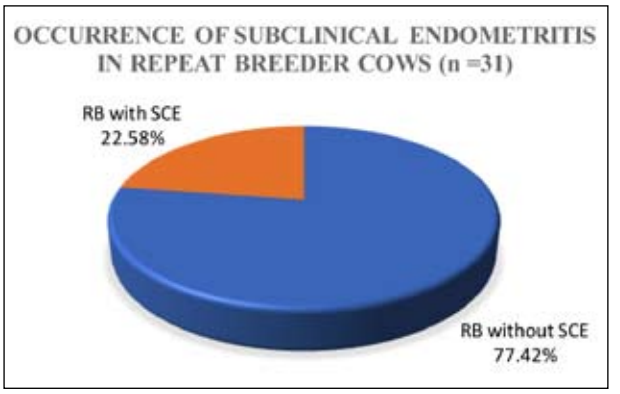

Fig. 1. Occurrence of subclinical endometritis in crossbred repeat breeder cows (n = 31)

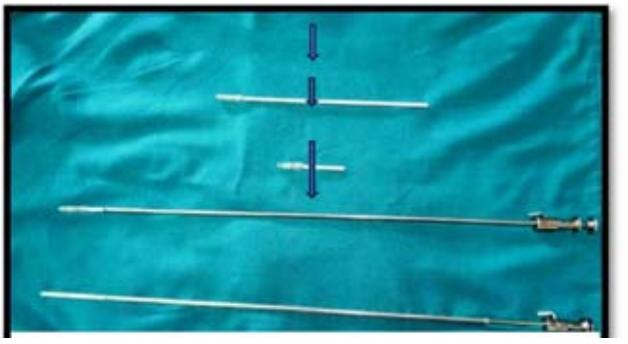

Fig. 2. Modified cytobrush assembly

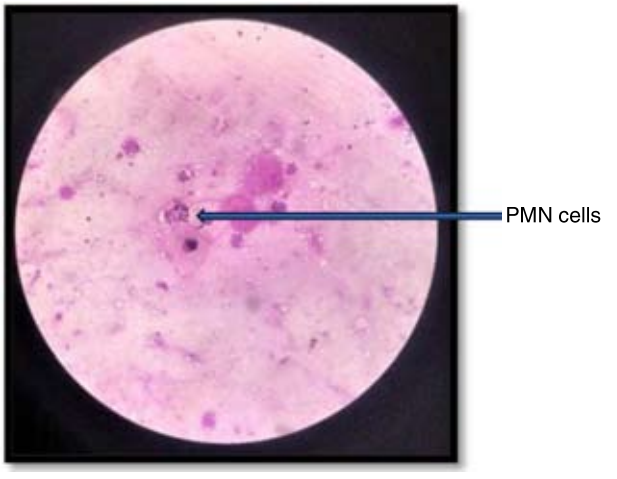

Fig. 3. Endometrial cytology; PMN cells, 400x

Table 1. Occurrence of repeat breeding among crossbred cows for a period of three years (2021 – 2023)

Year	Total number of cows	Number of repeat breeder cows	Per cent of repeat breeder cows (%)
2021	193	43	22.27
2022	164	34	20.73
2023	160	32	20.00
Total	517	109	21.08

Table 2. Occurrence of reproductive tract disorders over a period of 18 months

Total number of cows and heifers		255
Total number of reproductive disorder cases		99
Reproductive tract disorders	**Number of animals**	**Occurrence rate (%)**
Anoestrus	8	8.08
Retained foetal membrane	21	21.21
Repeat breeding syndrome	34	34.34
Subclinical-endometritis	7	7.07
Endometritis	7	7.07
Metritis	5	5.05
Dystocia	3	3.03
Abortion	8	8.08
Cervicitis	1	1.01
Cervico-vaginal prolapse	4	4.04
Uterine prolapse	1	1.01

reproductive disorders like metritis, cervicitis and dystocia (Adnane *et al*., 2017) and might be due to the better hygiene and optimal breeding management adopted in the farm, which might influence the incidence of SCE.

Intensity of oestrus

Out of 24 RB cows, 54.16, 41.67 and 4.17 per cent of cows exhibited high, medium and low intensity of oestrus, respectively (Fig. 7). This finding is in accordance with the observations of Velayudakumar 2003), who observed that high intensity of oestrus was exhibited by the majority of repeat breeder cows (77.50 per cent) with prolonged oestrus. Similarly, Bhat and Bhattacharya (2012) observed intense uterine tonicity for a longer period in RB cows when compared to normal cycling cows. Contrary to this, Perez-Marin and Espana (2007) reported that nearly half of the RB cows (n = 7) displayed delayed or silent oestrus. Sood *et al*. (2015) compared the intensity of oestrus in repeat breeder and normal cows and noted that the intensity of oestrus was higher than in normal cows. The higher intensity of oestrus may be due to longer periods of follicular growth and hormonal variations. The variation in the observation in the present study might be due to differences in environment, housing, management and sample size used for research work.

Duration of oestrus

The duration of oestrus in 24 RB cows ranged from 20 to 48 h with a mean of 27.50 ± 1.50 h. The above finding is similar to observations made by Kumar *et al*. (2008), (Selvaraju *et al*., 2008) and Bhat and Bhattacharya (2012) who recorded 28.90 ± 0.41 h, 29.25 ± 0.70 h and 26.33 ± 2.20 h duration of oestrus respectively, in RB cows. Contrary to this, Sood *et al*. (2015) noted that the duration of oestrus in RB cows was 21.40 h. The longer duration of oestrus in RB cows may be due to delayed ovulation of the dominant follicle, which can result in prolonged secretion of oestrogen, which might be responsible for the longer period of standing oestrus.

Physical properties of oestrual mucus

Physical properties of oestrual mucus like colour, consistency and volume were assessed. The colour of the oestrual mucus was classified into transparent, cloudy and dirty. Consistency of oestrual was classified into thick and thin and the volume into copious, moderate and low. There was no significant difference ($P > 0.05$) in the colour, volume and consistency of oestrual mucus in the age group below six years and above six years (Table 5).

Out of 24 RB cows, 83.33 per cent (20 out of 24) exhibited transparent oestrual mucus and the remaining 16.67 per cent (4 out of 24) exhibited cloudy discharge. In the below six years age group, transparent and cloudy discharge was shown by 90 (9 out of 10) and 10 per cent (1 out of 10) of RB cows, respectively. In the above six years age group, transparent and cloudy discharge was shown by 78.60 (11 out of 14) and 21.40 (3 out of 14) per cent of RB cows, respectively. There was no significant difference ($P < 0.05$) in the occurrence of transparent and cloudy discharge between the two age groups. None of the

Table 3. Scorecard for assessing the intensity of oestrus in repeat breeder cows

Sl.No	Oestrus characteristics	Points		
		Split up	Maximum	Remark
1	**Behavioural signs**			
	1) Standing to be mounted 2) Mounting on other animals 3) Restlessness and alertness 4) Bellowing 5) Chin resting and rubbing	3 1 1 1 1	7	Intense ≥ 5 Moderate 2 - 4 Mild < 2
2	**Physiological changes**			
	1. Vulval oedema ➢ High ➢ Medium ➢ Low	 2 1 0	2	6
	2. Hyperaemia of vestibular epithelium ➢ High ➢ Medium ➢ Low	 2 1 0	2	
	3. Genital discharge ➢ Copious ➢ Moderate ➢ Scant	 2 1 0	2	
3.	**Gynaecological observation**			
	Uterine tonicity ➢ High ➢ Medium ➢ Low	 2 1 0	2	2
	Total			**15**
	Grading of the intensity of oestrus High : More than 10 Points Medium : 5 to 10 Points Low : Less than 5 Points			

Table 4. Occurrence of repeat breeding in different age groups

Type of repeat breeding	Age group				Total	
	Below 6 years		Above 6 years			
	Number of animals	Per cent of animals	Number of animals	Per cent of animals	Number of animals	Per cent of animals
Repeat breeders with subclinical endometritis	3	23.1	4	22.2	7	22.6
Repeat breeding only	10	76.9	14	77.8	24	77.4
Total	13	100	18	100	31	100
Fisher's Exact p-value = 1.000^{ns}						

ns non-significant

RB cows exhibited dirty oestrual mucus discharge. This finding is in accordance with the observations of Singh *et al*. (2017), who reported that the majority of the repeat-breeding cows free of endometritis exhibited transparent oestrual mucus discharge. Similarly, Kumbhar *et al*. (2020) reported that clear cervical mucus discharge was shown by all (100 per cent) repeat-breeding buffaloes without uterine infections. Whereas, Zaman *et al*. (2013) reported that turbid oestrual mucus discharge was exhibited by 64.44 per cent of RB cows. The reason for the higher number of RB cows exhibiting transparent discharge might be due to the selection of RB cows without endometritis for study.

Among 24 RB cows 66.67 per cent (16 out of 24) and 33.33 per cent (8 out of 24) of RB cows exhibited thick and thin oestrual mucus, respectively. In the below six years age group, thick and thin consistency of oestrual discharge was shown by 60 (6 out of 10) and 40 per cent (4 out of 10) of RB cows, respectively. In the above six years age group, thick and thin consistency of oestrual discharge was shown by 71.4 (10 out of 14) and 28.6 (4

Table 5. Association of oestrual mucus characteristics with age

Characteristics of oestrual mucus	Age group				Total		Fisher's Exact p-value
	Below 6 years		Above 6 years				
	Number of animals	Per cent of animals	Number of animals	Per cent of animals	Number of animals	Per cent of animals	
Colour							
Transparent	9	81.8	11	78.6	20	80.0	0.615^{ns}
Cloudy	1	18.2	3	21.4	5	20.0	
Total	10	100	14	100	25	100	
Volume							
Copious	4	40.0	8	57.1	12	50.0	0.680^{ns}
Moderate and low	6	60.0	6	42.9	12	50.0	
Total	10	100	14	100	24	100	
Consistency							
Thick	6	60.0	10	71.4	16	66.7	0.673^{ns}
Thin	4	40.0	4	28.6	8	33.3	
Total	10	100	14	100	24	100	

ns non-significant

out of 14) per cent of RB cows, respectively. There was no significant difference (P < 0.05) in the occurrence of thick and thin consistency of oestrual mucus discharge between age groups. Similarly, Rangnekar *et al*. (2002) reported that the majority of RB cows had thick oestrual mucus discharge. Siregar *et al*. (2017) and Hanumant *et al.* (2019) reported that 100 and 87 per cent of repeat-breeding cows, respectively exhibited thick oestrual mucus discharge. But contrary to the above findings, Kumar *et al.* (2010) reported that 58 per cent of repeat breeder cows exhibited thin oestrual mucus discharge. The increased viscosity of oestrual mucus may be due to a reduced level of serum oestrogen, suprabasal progesterone level on the day of oestrus or due to increased levels of macrominerals like calcium, phosphorous, magnesium, sodium and potassium (Siregar *et al*., 2019).

Among 24 RB cows, copious, moderate and low volumes of oestrual mucus discharge were exhibited by 50 (12 out of 24), 41.67 (10 out of 24) and 8.33 (2 out of 24) per cent, respectively. In the below-six years age group, copious and moderate to low volume of oestrual mucus discharge was shown by 40 (4 out of 10) and 60 (6 out of 10) per cent of RB cows, respectively. In the above six years age group, 57.10 (8 out of 14) and 42.90 (6 out of 14) per cent of RB cows above six years of age. There was no significant difference (P < 0.05) in the occurrence of copious and moderate to low volumes of oestrual mucus discharge between age groups. These findings were similar to the observations of Bhat *et al*. (2015). They reported that an abundant volume of oestrual discharge was exhibited by the majority (77.02 per cent) of RB cows under study. Bernardi *et al*. (2016) reported that abundant (56.00) per cent) to moderate volume (31.00 per cent) of oestrual mucus discharge was exhibited by the majority of the cows. A similar finding was reported by Aruna (2021), who reported that the majority of RB cows exhibited abundant to moderate amounts of oestrual mucus discharge. Contrary to the above findings, Perez-Marin and Espana (2007) observed that 50 per cent of RB cows were not exhibiting external signs of oestrus.

Conclusion

The study revealed that the majority of the repeat breeder cows had high to moderate intensity of oestrus, duration of oestrus slightly higher (27.50 ± 1.50 h) than normal cycling cows. Most of these cows showed copious to moderate volumes of thick, transparent oestrual mucus discharge. It was also found that there was no significant difference in the occurrence of subclinical endometritis in two different age groups. Evaluation of the intensity of oestrus and physical characteristics of oestrual mucus is a non-invasive method of assessing the fertility of cows. Hence, the present study suggests that the identification of etiological factors, subclinical endometritis and evaluation of oestrual mucus characteristics has to be carried out for proper diagnosis of repeat breeding condition so that better treatment measures can be adopted.

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Conflict of interest

The authors declare that there is no conflict of interest.

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