

## Oestrus Duration and Status of Reproductive Organs in Repeat Breeding Cows

Research Article

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### ABSTRACT

The present study was conducted to see the effect of non-specific genital infections and ovulatory disturbances on clinical signs of oestrus namely oestrus duration, tonicity of uterus, relaxation of cervix and persistence of follicle. The mean values of oestrus duration, tonicity of uterus and relaxation of cervix varied significantly ( $P < 0.05$ ) among the group of impaired ovulation (anovulation and delayed ovulation) and normal ovulation. Following the onset of oestrus, follicle was palpated up to 144 hrs in all anovulation animals,  $72.00 \pm 4.17$  hrs in case of delayed ovulation,  $36.89 \pm 0.62$  hrs in case of normal ovulation with infection and  $36.71 \pm 1.61$  hrs in the control group (normal ovulation without infection). The study revealed that oestrus duration and status of reproductive organs are important criteria in the diagnosis of ovulatory disturbances in repeat breeding cattle.

**KEY WORDS** clinical signs of oestrus, crossbred cows, kashmir, ovulatory disturbances.

### INTRODUCTION

Repeat breeding is one of the most important causes of infertility in cattle that results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers (Ayalon, 1984; Bartlett *et al.* 1986; Lafi and Kaneene, 1992).

Economy of dairy industry largely depends on pregnancy rate following insemination, and achieving a 12 month calving interval is of great importance for maximum milk yield per cow per year with good economic return (Opsomer *et al.* 1996). Repeat breeding has multifactorial etiology such as infectious or inflammatory processes, nutritional deficiencies, managemental practices, ovulatory

disturbances *etc.* (Gunther, 1981). However, under rural conditions of Kashmir (a temperate region) ovulatory disturbances are one of the main causes of it. Anovulation and delayed ovulation characterized by fertilization failure and / or embryonic death are two other major causes of repeat breeding in high yielding crossbred dairy cows (Gustafsson and Larsson, 1985).

These conditions remain undiagnosed unless repeated per rectal examination of the reproductive tract is undertaken. The success of insemination in such cases depends not only on the oestrus detection, but also on ovulation timing / confirmation. Asynchrony in timing of insemination with ovulation results in fertilization failure and low pregnancy rate (Hunter, 1994). Efforts need to be made to enhance fertility by finding out the actual time of ovulation and then deciding the time of insemination.

Detailed gynaecological examination of the reproductive organs provides valuable information about the reproductive phases in individual animal. Therefore the present investigation was undertaken to study the status of reproductive organs during oestrus in repeat breeding animals.

## MATERIALS AND METHODS

The present study was conducted in repeat breeding cattle at clinical complex of the university during the period from October 2009 to December 2010. A total of 100 crossbred Jersey cows were selected for the study. Out of which, 80 repeat breeding cows presented for treatment and 20 normal cyclic cows presented for artificial insemination at their first service. Cows that failed to conceive in three or more regular services with apparently healthy genitalia were considered as repeat breeders (Kumar and Singh, 2009).

Detailed gynaeco-clinical examination was carried out to all the repeat breeding animals and those with gross genital pathology were excluded from the study (Ball, 1986; Settergren, 1986). Normal cyclic animals constituted the control group. Initially the repeat breeding animals were clinically examined for ovulatory disturbances.

They were examined per rectum at 12 hour interval up to 6 days from onset of behavioural oestrus for persistence or rupture of follicles and development of subsequent corpus luteum (CL) at the ruptured site. Another single per rectal examination was done at mid cycle (10-12 days) for ascertaining the presence of mature CL.

Animals with persistent follicle up to 4 days from onset of oestrus but not beyond that and a mature CL at mid cycle were considered as delayed ovulators; whereas, animals with persistent follicle beyond 4 days following oestrus and no CL at mid cycle were considered as anovulators. The repeat breeding animals that did not show any ovulatory disturbances were put into the category of normal ovulation with infection.

Thus the repeat breeding animals were initially divided into 3 groups i.e. anovulation, delayed ovulation and normal ovulation with infection. Repeated per rectal examination at 12 hour intervals were also conducted to the animals of control group and any animal showing ovulatory disturbances were excluded from the study. Then cervical mucus at oestrus that collected from all the repeat breeding animals and animals of control group were put to bacterial culture.

In our study, to compare different clinical signs of oestrus (oestrus duration, tonicity of uterus, relaxation of cervix, and persistence of follicle) among the four groups (i.e. anovulation, delayed ovulation and normal ovulation with infection and control group), animals with positive bacterial culture of oestral cervical mucus were excluded from the

group of anovulation, delayed ovulation and control group to avoid the technical error.

From the group of normal ovulation with infection, where animals were previously assumed to suffer from infection without any ovulatory disturbances, the animals that showed no bacterial growth in their oestral cervical mucus on bacterial culture were also excluded.

Thus four clear different groups were found that is anovulation (without infection), delayed ovulation (without infection), normal ovulation with infection and normal ovulation without infection (control group). The time that elapsed between the onset and completion of behavioural oestrus was taken as oestrus duration. Uterine tonicity and relaxation of cervix were recorded every 12 hour intervals (morning and evening) from the onset of the behavioural oestrus up to 6 days from the onset of oestrus.

### Tonicity of uterus was classified as:

- 1. Pronounced:** The period of extremely tonic uterus.
- 2. Moderate / Mild:** The period when uterus was found moderately tonic following pronounced uterine tone upto being atonic.

### Relaxation of cervix was classified as:

- 1. Completely dilated:** When cervix was completely dilated and insemination catheter passed easily through it. The time up to which this finding persisted was recorded.
- 2. Partially dilated:** When insemination catheter passed through the cervix with difficulty or hindrance due to partial closure of the cervix. The time up to which this finding was present was also recorded.

Both the findings of tonicity of uterus and relaxation of cervix were recorded during all examinations. The data obtained was subjected to statistical analysis by one-way ANOVA using SPSS, (1999). To test the significance of difference between means post-hoc analysis by LSD was done.

## RESULTS AND DISCUSSION

On the basis of repeated per rectal examination, out of 80 repeat breeding animals 8 and 18 were recorded to suffer from anovulation and delayed ovulation, respectively. Remaining 54 animals that did not show ovulatory disturbances were put to the category of normal ovulation with infection. On the other hand, not a single animal in the control group was found to suffer from ovulatory disturbances. The bacterial culture of oestral mucus collected from repeat breeding and control cows showed that 2, 4 and 3 animals from the group of anovulation, delayed ovulation and control group were found to have one or more than one of bacterial isolates.

However, all the 54 animals under the category of repeat breeding which were previously assumed to be suffered from bacterial infection, showed one or more than one type of bacterial growth. Thus anovulation, delayed ovulation, normal ovulation with infection and control group (normal ovulation without infection) comprised of 6, 14, 54 and 17 animals in each group, respectively.

The mean values for oestrus duration varied significantly among all the different groups ( $P < 0.05$ ) except between the animals of normal ovulation with infection and control group (Table 1, Figure 1).

Intense uterine tone varied significantly ( $P < 0.05$ ) among the different groups except between the animals of normal ovulation with infection and control group (Table 1, Figure 2). Moderate / mild uterine tonicity of animals of delayed ovulation group showed significant ( $P < 0.05$ ) differences with anovulation, normal ovulation with infection and control group (Table 1, Figure 2). Overall time up to what uterine tonicity present were varied significantly ( $P < 0.05$ ) among the groups except between normal ovulation with infection and control group.

The time up to what cervix was found completely relaxed varied significantly ( $P < 0.05$ ) among different groups except for normal ovulation with infection and control group animals (Table 1, Figure 3). Partial relaxation of cervix in animals of delayed ovulation group showed significant ( $P < 0.05$ ) differences with that of anovulation, normal ovulation with infection and control group (Table 1, Figure 3).

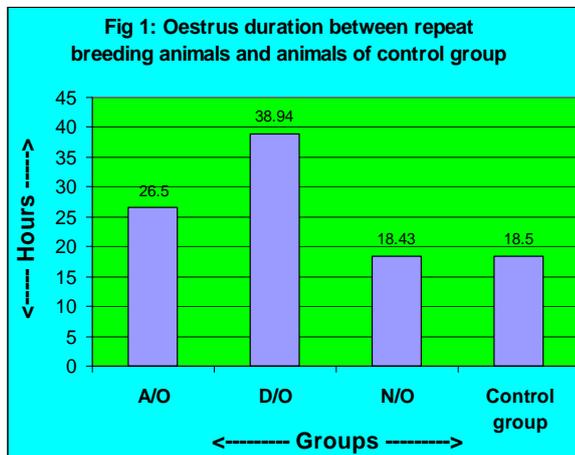
The overall time up to what cervix was found dilated following onset of oestrus was significantly ( $P < 0.05$ ) varied amongst the different groups except between normal ovulation with infection and control group. The time with respect to the persistence or intactness of follicle following onset of oestrus was found to be varied significantly ( $P < 0.05$ ) among the all groups except between the animals of normal ovulation with infection and control group (Table 1, Figure 4). The present finding with respect to oestrus duration in normal and impaired ovulatory animals simulated the findings of *Das et al. (2009)* who reported mean duration of oestrus as  $25.20 \pm 1.72$ ,  $33.27 \pm 1.56$  and  $18.90 \pm 1.05$  hours in anovulatory, delayed ovulatory and normal ovulatory animals, respectively. *Krishna Kumar et al. (2008)* reported that the duration of oestrus in repeat breeding cows suffering from ovulatory disturbances was  $28.90 \pm 0.41$  hours. Moreover, *Selvaraju et al. (2009)* reported duration of oestrus  $29.25 \pm 0.70$  hours in repeat breeding cows. Overall period of uterine tonicity was significantly ( $P < 0.05$ ) higher in the animals of delayed ovulation ( $104.57 \pm 4.25$ ) and anovulation ( $76.00 \pm 6.69$ ), than in the animals of both normal ovulation with infection ( $59.33 \pm 1.32$ ) and control group ( $57.88 \pm 2.12$ ). *Das et al. (2009)* reported that a higher proportion (68.18%) of animals with delayed ovulation revealed longer period of uterine tonicity i.e., up to 48 hours of oestrus than animals with anovulation (35.00%). On the other hand, the animals with normal ovulation did not show uterine tonicity after 36 hours of oestrus.

**Table 1** Comparison of oestrus duration, tonicity of uterus, relaxation of cervix and persistence of follicle between the animals of repeat breeding and control group

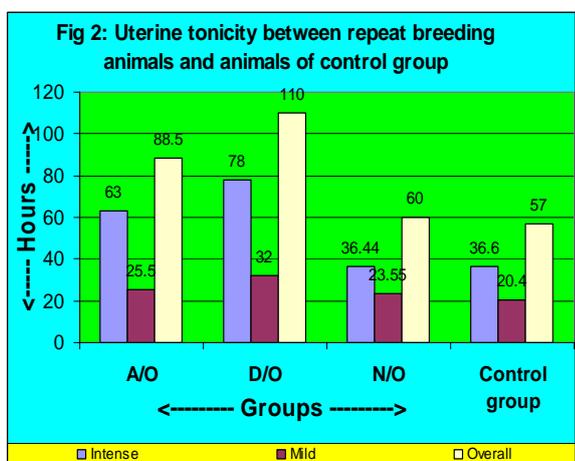
Parameters in hours (Mean±SE)		Repeat Breeder ( n=74)			Control group (n=17)
		Anovulation (n=6)	Delayed ovulation (n=14)	Normal ovulation with infection (n=54)	
Oestrus Duration		26.33±2.20 <sup>b</sup> (19-34)	34.43±3.67 <sup>c</sup> (19-68)	18.43±0.48 <sup>a</sup> (12-25)	18.71±1.40 <sup>a</sup> (12-28)
Tonicity of uterus (up to) following onset of oestrus	Intense uterine tone up to	54.00±5.14 <sup>b</sup> (36-72)	70.29±3.04 <sup>c</sup> (48-84)	36.89±0.83 <sup>a</sup> (24-60)	37.41±1.41 <sup>a</sup> (24-48)
	Mild / moderate tonicity following cessation of intense tone	22.00±2.00 <sup>ab</sup> (12-24)	34.29±2.13 <sup>b</sup> (24-48)	22.44±1.10 <sup>a</sup> (12-36)	20.47±1.37 <sup>a</sup> (12-24)
	Overall time up to what tonicity was present	76.00±6.69 <sup>b</sup> (48-96)	104.57±4.25 <sup>c</sup> (72-132)	59.33±1.32 <sup>a</sup> (36-72)	57.88±2.12 <sup>a</sup> (48-72)
Relaxation of cervix (up to) following the onset of oestrus	Completely relaxed cervix up to	56.00±5.06 <sup>b</sup> (36-72)	71.14±2.66 <sup>c</sup> (48-84)	36.44±0.77 <sup>a</sup> (24-48)	36.71±1.25 <sup>a</sup> (24-48)
	Partially relaxed cervix up to	22.00±3.69 <sup>ab</sup> (12-36)	32.57±2.93 <sup>b</sup> (12-48)	24.22±1.12 <sup>a</sup> (12-36)	23.29±1.16 <sup>a</sup> (12-36)
	Overall time up to what cervix was relaxed	78.00±7.43 <sup>b</sup> (48-96)	103.71±4.96 <sup>c</sup> (72-132)	60.67±1.43 <sup>a</sup> (36-72)	60.00±2.30 <sup>a</sup> (36-72)
Palpable follicle		144±0.00 <sup>c</sup>	72.00±4.17 <sup>b</sup> (60-120)	36.89±0.62 <sup>a</sup> (24-48)	36.71±1.61 <sup>a</sup> (24-48)

The means within the same row with at least one common letter, do not have significant difference ( $P > 0.05$ ).

The overall period up to which cervix was found dilated following onset of oestrus was significantly higher in the animals suffering from delayed ovulation ( $103.71 \pm 4.96$ ) and anovulation ( $78.00 \pm 7.43$ ) than in the animals of normal ovulation with infection ( $60.67 \pm 1.43$ ) and control group ( $60.00 \pm 2.30$ ), respectively.



**Figure 1** Oestrus duration between repeat breeding animals and animals of control group  
A / O: anovulation; D / O: delaye ovulation; N / O: normal ovulation with infection

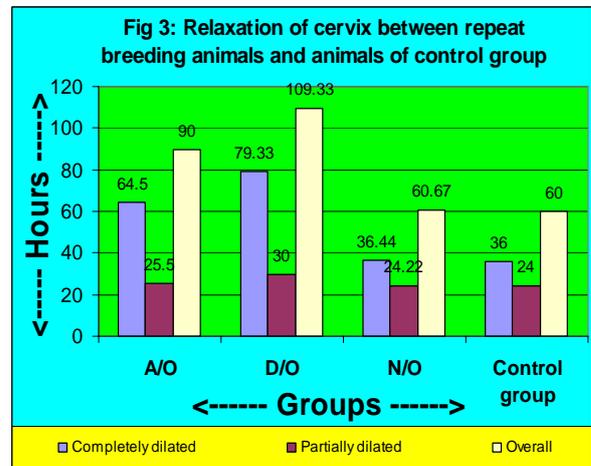


**Figure 2** Uterine tonicity between repeat breeding animals and animals of control group  
A / O: anovulation; D / O: delaye ovulation; N / O: normal ovulation with infection

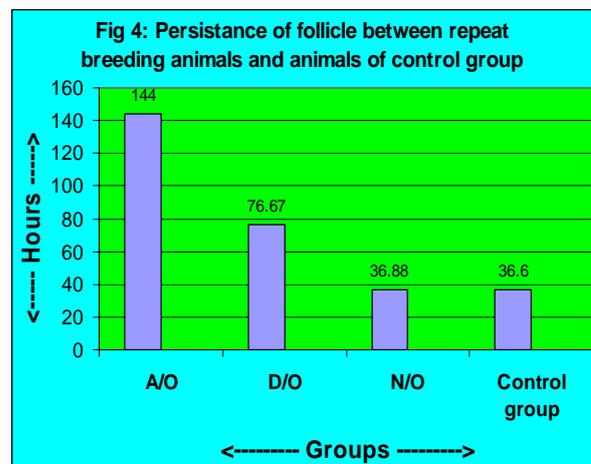
Das et al. (2009) reported that most of the animals with delayed ovulation (86.36%) and a small number of animals with anovulation (20%) had relaxed and open cervix upto 48 hours of oestrus, however, all animals with normal ovulation had firm and closed cervix at 48 hours of oestrus.

Follicle in the ovary was intact up to 144 hours in all the animals suffering from anovulation. In the animals suffering from delayed ovulation the follicle was palpable up to

72.00±4.17 hours following the onset of oestrus. In both the groups of normal ovulation with infection and control group the follicle had ruptured by 36 hours following onset of oestrus.



**Figure 3** Relaxation of cervix between repeat breeding animals and animals of control group  
A / O: anovulation; D / O: delaye ovulation; N / O: normal ovulation with infection



**Figure 4** Persistence of follicle between repeat breeding animals and animals of control group  
A / O: anovulation; D / O: delaye ovulation; N / O: normal ovulation with infection

Das et al. (2009) reported that follicle was palpated on the either of the ovaries till 48 hours of oestrus in all the animals (100%) with anovulation, and in 59.09% of the animals with delayed ovulation; however, Graafian follicle was palpated in all the delayed ovulation cases at 36 hours after onset of oestrus.

Repeat breeding due to anovulation or delayed ovulation primarily occurs because of either deficiency / delayed surge of luteinizing hormone or inadequate gonadotropin

releasing hormone priming. Presence of follicle for a long period of time in the animals with anovulation and delayed ovulation leads to persistent estrogen production resulting in prolonged oestrus duration and persistence of uterine tonicity and relaxation of cervix for a quite longer period of time. However, maximum size of a dominant anovulatory follicle is lesser than the maximum size of a preovulatory follicle and estrogen production is also lesser by the former one than the later one (Noseir, 2003). In the course of its development (during folliculogenesis) the dominant anovulatory follicle becomes static at certain stage and ultimately those anovulatory follicles naturally undergo atresia and hence estrogen level is also reduced or drops to basal level at an early period as compared to delayed ovulatory follicle. However, infection has no influence on duration and other clinical signs of estrus.

## CONCLUSION

In conclusion, oestrus duration, tonicity of uterus, relaxation of cervix and persistence of follicle adversely affected by impairment in ovulation leading to development of repeat breeding condition, but not affected by genital infections. Therefore under field conditions these four characteristics can be used as pivotal criteria for diagnosing repeat breeding condition in cattle.

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