



# Ultrasonographic studies on ovarian characteristics in cows exhibiting prolonged and normal oestrus<sup>#</sup>

R. S. Athira<sup>1</sup>, C. P. Abdul Azeez<sup>2\*</sup>, K. Promod<sup>3</sup>, Leeba Chacko<sup>4</sup>,

S. Pramod<sup>5</sup> and Renjith Sebastian<sup>6</sup>

Department of Animal Reproduction, Gynaecology and Obstetrics  
College of Veterinary and Animal Sciences  
Pookode, Wayanad-673576  
Kerala Veterinary and Animal Sciences University  
Kerala, India

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## Abstract

Crossbred cows exhibiting prolonged oestrus (oestrus duration > 36 h, n=6, Group I) and normal oestrus duration (n=6, Group II) which are negative for cytological endometritis, were selected for the present study. Ovaries were subjected to ultrasonographic examination on day 0 (day of oestrus) at, 12 h intervals until ovulation, day 10, 15, and 20 of the oestrus cycle to study the changes in the ovarian structure (follicles and corpus luteum (CL)). On day 0, no significant difference ( $p > 0.05$ ) was observed in the largest follicle size between groups I and II ( $11.02 \pm 0.73$  vs  $10.94 \pm 1.03$  mm). In contrast, a significant difference ( $p < 0.05$ ) was noticed in the diameter of the largest follicle on day 1 ( $11.7 \pm 0.68$  vs  $5.48 \pm 2.55$  mm). In cows exhibiting prolonged oestrus persistence of pre-ovulatory follicle was observed between 36-48 h in three cows (50 %), between 48-60 h in two cows (33.33%) and between 60-72 h in one cow (16.66%) whereas in animals exhibiting normal oestrus, the pre-ovulatory follicle persisted for < 24 h. The mean diameter of CL on days 10, 15 and 20 in cows exhibiting prolonged oestrus and normal oestrus were  $20.53 \pm 1.43$ ,  $17.34 \pm 0.96$ ,  $14.78 \pm 1.21$  mm and  $21.62 \pm 1.62$ ,  $19.20 \pm 1.26$ ,  $13.7 \pm 91.1$ mm, respectively. A decrease in the rate of CL regression was observed on day 20 of the oestrus cycle in animals exhibiting prolonged oestrus

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1. MVSc Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode
2. Associate Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Pookode
3. Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Pookode
4. Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Pookode
5. Assistant Professor, Livestock Research Station, Thiruvizhamkunnu.
6. Assistant Professor, Department of Veterinary Biochemistry, CVAS, Pookode

\*Corresponding author: [azeezargo@gmail.com](mailto:azeezargo@gmail.com), Ph. 9847701028

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compared to normal oestrus. Hence, it can be inferred that B-mode ultrasonography could be a reliable non-invasive tool to study the ovarian characteristics in cows exhibiting prolonged oestrus.

**Keywords:** *Crossbred cow, prolonged oestrus, normal oestrus, ultrasonography, ovarian characteristics*

The profitability of dairying depends to a large extent on the efficiency of reproduction. Enhancement of reproductive efficiency requires ensuring shorter calving intervals and higher rates of conception to artificial breeding. One of the important reasons for extended calving interval is repeat breeding and the incidence in cattle ranged from 5.5 to 33 per cent. Prolonged oestrus in cows is a reproductive disorder characterised by a normal oestrous cycle, but with an extended duration of oestrus lasting for more than 36 hours (Dadarwal *et al.*, 2005). About 30 to 40 per cent of repeat breeder cows display prolonged duration of oestrus of which 15 per cent were reported due to ovulatory defects (Abdul *et al.*, 2016). This condition can increase the number of services per conception to more than four against the optimum of less than two.

The hormonal asynchrony associated with prolonged oestrus can lead to repeat breeding syndrome in crossbred cows (Arun *et al.*, 2019). This could be due to delayed ovulation following the prolonged oestrus affecting the oocyte quality and ultimately resulting in poor fertility (Sing *et al.*, 2012). Abnormal prolongation of the dominant follicle, delayed ovulation and follicular characteristics can be more precisely studied by ultrasonographic examination of the ovaries. The current study aimed to compare ovarian follicular and luteal changes by ultrasonographic examination in crossbred cows exhibiting prolonged and normal oestrus during various days of the oestrous cycle.

## Materials and methods

The study was conducted in apparently healthy postpartum crossbred cows, with parity between 2 to 5 and having a body condition score of 2.5 to 3.5, maintained at Livestock

Research Station, Thiruvizhamkundu. The cows were closely observed for the visual/physiological signs of oestrus and clinico-gynaecological examinations were conducted for the confirmation and onset of oestrus. The cows were ruled out for cytological/subclinical endometritis on the day of oestrus using modified cytobrush technique (Kasimanickam *et al.*, 2004) and cows with less than five per cent polymorphonuclear leukocytes (PMN) were considered free from cytological endometritis (Pascottini *et al.*, 2015) and among these, cows exhibiting prolonged oestrus of duration > 36 h (n=6, Group I) and cows with normal oestrus (18-24 h) duration (n=6, Group II) were selected for the study. The ultrasonographic examination of the ovaries was done using B mode Esaote Veterinary Scanner equipped with a 5 MHz transrectal linear probe on the day of oestrus (day 0) at 12 h intervals until ovulation and on days 10, 15 and 20. The data obtained were subjected to statistical analysis through the application of a t-test.

## Results and discussion

### ***Number and size of follicles on the day of oestrus (day 0) and day 1 in cows exhibiting prolonged and normal oestrus***

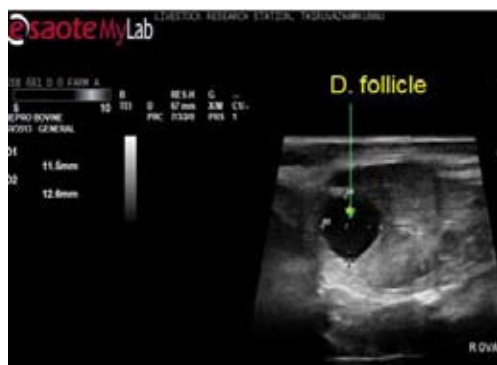
The details of the number and size of follicles on day 0 and day 1 of oestrus are presented in Table 1. The total number of follicles present on day 0 in animals exhibiting prolonged and normal oestrus were  $4.99 \pm 1.36$  and  $4.66 \pm 0.97$ , respectively whereas on the next day of oestrus (day 1) the number of follicles was  $4.71 \pm 0.60$  and  $3.28 \pm 0.42$ , respectively. A decrease in follicle number was noticed in cows exhibiting normal oestrus on day 1 and it was due to ovulation of the largest follicle (Fig. 1) which was confirmed by the absence of anechoic fluid-filled structure and appearance of hypoechoic structure (corpus haemorrhagicum, Fig. 2) on ultrasonographic examination, whereas the largest follicle was persisted on day 1 in cows exhibiting prolonged oestrus.

In cows exhibiting normal oestrus duration, the dominant follicle (pre-ovulatory follicle) ovulated within 24 hours, whereas in animals exhibiting prolonged oestrus, the

**Table 1.** Number and size of ovarian follicles on day 0 and day 1 of oestrus in cows exhibiting prolonged and normal oestrus

Day of oestrus	Parameters	No. of animals Group I (n=6)	No. of animals Group II (n=6)
Day 0	Largest follicle on Rt ovary	5	4
	Largest follicle on Lt ovary	1	2
	Total number of follicles	4.99±1.36	4.66±0.97
	Size of the largest follicle (mm)	11.02±0.73	10.94±1.03
t- value	0.059 <sup>ns</sup>		
p-value	0.954		
Day 1	Largest follicle on Rt ovary	5	1
	Largest follicle on Lt ovary	1	1
	Total number of follicles	4.71±0.60	3.28±0.42
	Size of the largest follicle (mm)	11.7±0.68	5.48±2.55
t- value	2.351*		
p-value	0.041		

\* Significant at 0.05 level; ns- non-significant



**Fig. 1.** Transrectal ultrasonographic image of dominant follicle on the day of oestrus (day 0)



**Fig. 2.** Transrectal ultrasonographic image of Corpus haemorrhagicum (CH) after ovulation (day 1)



**Fig. 3.** Transrectal ultrasonographic image of corpus luteum with cavity on day 10 of oestrus cycle



**Fig. 4.** Transrectal ultrasonographic image of corpus luteum (CL) on day 15 of the oestrus cycle

dominant follicle persisted for more than 24 hours. Out of six cows that exhibited prolonged oestrus, the dominant follicle persisted between

36-48 h in three cows (50 %), between 48-60 h in two cows (33.33%) and between 60-72 h in one cow (16.66%). This was consistent with the

**Table 2.** Number and types of follicles present during different days of the oestrous cycle in cows exhibiting prolonged and normal oestrus (n=6)

Days	Number of follicles								
	Small (<4mm)			Medium (4-8mm)			Large (>8mm)		
	Group I	Group II	p-value	Group I	Group II	p-value	Group I	Group II	p-value
Day 0	2.33± 0.61	2.50 ±0.56	0.845	1.33 ±0.42	1 ±0.25	0.515	1.33 ±0.33	1.16 ±0.16	0.664
Day10	2.83±0.40	3.16± 0.60	0.654	0.66± 0.33	0.83± 0.47	0.780	1±0.25	0.83±0.16	0.599
Day 15	2.33 ±0.66	2.83 ±0.90	0.673	1±0.25	1.16 ±0.16	0.599	0.66 ±0.33	0.83 ±0.16	0.664
Day 20	1.5 ±0.34	1.66 ±0.21	0.686	0.83 ±0.47	1 ±0.25	0.765	1.16 ±0.16	1.33 ±0.21	0.549

**Table 3.** Mean ± SE diameter of medium and large follicles during different days of oestrus cycle (n=6)

Types of Follicles	Days	Diameter (Mean±SE) of follicles (mm)			
		Group I	Group II	t- value	p-value
<b>Medium (4-8mm)</b>	Day 0	6.29±0.37	5.69±0.52	0.936 <sup>ns</sup>	0.377
	Day 10	4.98±0.31	4.91±0.40	0.147 <sup>ns</sup>	0.888
	Day 15	7.18±0.35	7.80±0.17	-1.381 <sup>ns</sup>	0.226
	Day 20	7.12±0.46	6.88±0.46	0.349 <sup>ns</sup>	0.738
<b>Large (&gt;8mm)</b>	Day 0	11.0±10.73	9.77 ±0.53	1.368 <sup>ns</sup>	0.201
	Day 10	13.28±1.40	10.55±0.51	1.826 <sup>ns</sup>	0.105
	Day 15	12.60 ±1.28	9.28 ±0.41	2.105 <sup>ns</sup>	0.062
	Day 20	11.04±0.71	10.55±0.52	0.509 <sup>ns</sup>	0.620

*ns*- non-significant

observations of Niyas *et al.* (2019) who reported the persistence of ovulatory follicle (>24 h, 70%) in animals exhibiting prolonged oestrus and in animals exhibiting normal oestrus the ovulatory follicle persisted for < 24 h. Singh (2003) also reported an increase in oestrus to ovulation interval (53.3 h) in cows exhibiting prolonged oestrus. Bage *et al.* (2002) and Shaji *et al.* (2021) opined that the extended growth of the preovulatory follicle in repeat breeder was due to the suprabasal progesterone concentration after luteolysis which delayed preovulatory LH surge and caused delayed ovulation.

On day 0, no significant difference ( $p>0.05$ ) was observed in the largest follicle size between groups I and II ( $11.02 \pm 0.73$  and  $10.94 \pm 1.03$  mm). This was consistent with Bloch *et al.* (2006) who reported statistically no significant difference ( $p>0.05$ ) in the average size of preovulatory follicle on the day of oestrus

in animals having normal and longer oestrus, to ovulation interval. However, in the present study, a significant, difference ( $p < 0.05$ ) was noticed in the diameter of the largest follicle on day 1 ( $11.7 \pm 0.68$  vs  $5.48 \pm 2.55$  mm).

The right ovary was found to be more active bearing the largest size follicle (preovulatory follicle) than the left ovary in both groups. Niyas *et al.* (2019) reported that the average diameter of dominant follicle (DF) before ovulation in animals exhibiting normal and prolonged, oestrus were  $13.57 \pm 0.96$  and  $14.92 \pm 0.74$  mm, respectively. Perez *et al.* (2003) reported that the average diameter of preovulatory follicles in repeat breeder (RB) cows was slightly larger ( $17.8 \pm 0.38$  mm) than the control group with a considerable variation in the range (12.5mm to 25mm) and this was not linked to the occurrence of RB syndrome.

**Table 4.** Diameter of corpus luteum in crossbred cows exhibiting prolonged and normal oestrus (Mean  $\pm$  SE) (n=6)

Groups	Diameter of corpus luteum (Mean $\pm$ SE) (mm)		
	Day 10	Day 15	Day 20
Group I	20.5 $\pm$ 1.43	17.34 $\pm$ 0.96	14.78 $\pm$ 1.21
Group II	21.62 $\pm$ 1.62	19.20 $\pm$ 1.26	13.79 $\pm$ 1.10
t value	-0.479 <sup>ns</sup>	-1.173 <sup>ns</sup>	0.578 <sup>ns</sup>
P value	0.642	0.268	0.576

#### **Number and size of follicles on different days of oestrus cycle**

The number and size of follicles present during various days of the oestrous cycle (days 0, 10, 15 and 20) in group I and II animals are depicted in Tables 2 and 3. The number of small (<4mm), medium (4-8mm) and large (>8mm) follicles did not vary significantly ( $p > 0.05$ ) between the animals exhibiting normal and prolonged oestrus on days 0, 10, 15 and 20 of the oestrus cycle and also the medium and large follicles diameter did not vary significantly ( $p > 0.05$ ). The diameters of all the small-sized follicles were difficult to obtain because it was unmeasurable. Savio *et al.* (1998) reported that the number of median as well as large follicles varied on most days of the oestrous cycle.

#### **Diameter of the corpus luteum at various days of oestrus cycle (mean $\pm$ SE)**

The corpus luteum diameter during various days of the oestrus cycle is depicted in Table 4. The mean  $\pm$ SE diameter of CL during days 10, 15, and 20 in animals exhibiting prolonged oestrus were 20.53  $\pm$  1.43, 17.34  $\pm$  0.96, 14.78  $\pm$  1.21 mm, respectively and in animals exhibiting normal oestrus were 21.62  $\pm$  1.62, 19.20  $\pm$  1.26, 13.7  $\pm$  1.1mm, respectively. The diameter of CL did not differ significantly ( $p > 0.05$ ) between groups I and II, despite animals that displayed prolonged oestrus had a slightly smaller mean maximum corpus luteum diameter on days 10 and 15.

On day 20, the mean corpus luteum diameter in cows exhibiting normal oestrus was lesser as compared to animals exhibiting prolonged oestrus. This might be due to the slow regression of the corpus luteum in animals exhibiting prolonged oestrus. Cavitory corpus luteum was observed in 50 per cent of

the selected animals (Fig. 3). The cavitation of CL was noticed as a non-echogenic area surrounded by greyish echogenic luteal structure on day 10. The disappearance of these cavities was observed on day 15 (Fig. 4) and this finding was consistent with Jyoti *et al.* (2019) they reported that within the initial 10 days following ovulation, approximately 30-50 per cent of CL structures developed a cavity and subsequently underwent a gradual regression process and reverted to a compact state. Herzog *et al.* (2010) studied the corpus luteum regression pattern using B mode and colour doppler ultrasonography in cows exhibiting prolonged oestrus and reported a slower rate of CL regression in cows when compared with cows exhibiting normal oestrous cycles leading to higher level of progesterone on day of oestrus.

#### **Conclusion**

B-mode ultrasonographic studies of the ovaries were performed for the assessment of ovarian changes and patterns of luteal regression in cows exhibiting normal and prolonged oestrus. A decline in follicle number was observed in cows with normal oestrus on the second day of oestrus (day 1), while cows with prolonged oestrus exhibited the persistence of the pre-ovulatory follicle for more than 36 hours, confirming delayed ovulation. Furthermore, cows with prolonged oestrus displayed a slower rate of corpus luteum regression on day 20 of their oestrus cycle when compared with cows exhibiting normal oestrus phase. The result obtained in the particular study was consistent with the external signs of exhibition of oestrus in these cows. Hence, the present study concluded that B mode ultrasonography is a reliable non-invasive diagnostic tool to evaluate the ovarian status in cows displaying prolonged oestrus so that suitable treatment

measures can be adopted for the improvement of the conception rate.

### Conflict of interest

The authors declare that they have no conflict of interest.

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