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Short Communication

Ovarian follicular dynamics in normal and repeat breeder cows

R.K. SHARMA[1], C.V.S. RAWAL, M. HOQUE, M.R. ANSARI AND V.P. VARSHNEY

Division of Animal Reproduction
Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 (UP)

ABSTRACT

Follicular growth pattern was studied in normal and repeat breeder cross bred cows. Ultrasonic studies revealed that bovine oestrous cycle is characterized by two or three-wave of follicular growth commonly referred as anovulatory and ovulatory wave. The length of oestrous cycle was significantly higher ($P < 0.01$) in cows having three-wave of follicular growth compared to those having two-wave of follicular growth. The study revealed that in two-wave cycle repeat breeder cows have altered pattern of follicular growth compared with normal cows. The maximum diameter of anovulatory dominant follicle was significantly higher ($P < 0.05$) in repeat breeder cows as compared to those in normal cows. Similarly, the ovulatory wave emerged earlier in repeat breeder cows ($P < 0.05$). The diameter of corpus luteum on day 8 of cycle was significantly smaller ($P < 0.05$) in repeat breeders compared to that in normal cows. However, progesterone concentrations did not differ significantly in normal and repeat breeder cows.

Key words: Cattle, Repeat breeder, Follicular waves, Oestrous cycle

The prerequisites for high fertility in females are timely ovulation of a good quality oocyte, optimal fertilization environment, proper hormonal milieu and maintenance of the corpus luteum (CL). Failure of these inter-related events often lead to return to oestrus at regular intervals post insemination. With the advent of ultrasound it has also become possible to monitor the development of antral follicles in which oocyte reside. The present study has therefore been designed to compare the difference, if any, between the follicular growth pattern of normal and repeat breeder cross bred cattle.

Twelve regular cyclic Holstein-Friesian cross bred cows, aged 4-7 years, weighing 350-500 kg were selected on the basis of their breeding records and gynaeco-clinical examinations. Animals were divided into normal (n=6) and repeat breeding (n=6) group. Cows which failed to conceive on three or more inseminations at a regular oestrus interval without any detectable reproductive abnormalities were considered as repeat breeders. In the repeat breeder group, one cow became anoestrus and one became cystic during period of study, hence excluded.

In all animals, oestrus was synchronized with two injections of prostaglandin F2α administered i.m. at 11 days apart (Iliren, 750 µg Tiaprost, Hoechst Roussel Vet). From the day of synchronized oestrus (Day 0), two successive oestrous cycles were monitored using a real time, B-mode diagnostic ultrasound scanner equipped with a linear array 6.0 MHz rectal transducer. Ultrasonographic examinations were made on alternate days of the oestrous cycle. During each examination, ovarian maps were drawn to record size and relative position of follicles and presence of CL to facilitate sequential evaluation of follicles turnover (Savio *et al.*, 1988). The parameters used for characterization of ovarian dynamics were: diameter of dominant follicle (DF), subordinate follicles (SF) and their growth rates, duration of follicular wave, emergence of follicular wave and diameter of corpus luteum.

[1] Corresponding author & Senior Scientist, BPR Division CIRB, Sirsa Road Hisar

In all animals, blood samples were collected on day 0 (oestrus), 4, 8, 12, 16 and 18 days of the cycle for one cycles. Serum was separated and stored at -20°C till progesterone assay. Progesterone was measured with RIA Kit (ImmuChemTM, ICN Pharmaceuticals Inc., Costa Mesa, California) as per standard procedure. The data were analysed by using suitable statistical methods (Snedecor and Cochran, 1989).

In normal and repeat breeder cows both, two-wave and three-wave of follicular activity was observed. Of the twenty interovulatory periods studied in all animals, sixteen (80%) had two-waves and four (20%) had three-waves of follicle growth. Of the four interovulatory periods of three-waves, majority were appeared in normal group cows (75%). As related to fertility an assumption is that animals having three-waves of follicular growth might have better conception rate as they ovulate a freshly recruited oocyte. Lower fertility of dairy cows exhibiting two-wave pattern of follicular growth has been reported by Driancourt (2001). In all animals, three-wave pattern was not observed in subsequent cycle indicating that two or three wave pattern is not animal specific. The length of oestrous cycle was significantly higher ($p<0.01$) in cows having three-wave cycle (23.0±0.58 days) as compared to those having two-waves (21.12±0.25 days).

The emergence and duration of anovulatory wave did not vary significantly and normal in repeat breeders. However, maximum diameter attained by DF was earlier ($p<0.1$) in normal than repeat breeder cows. Analysis of wave characteristics revealed that this difference probably was due to two reasons: first slightly longer growth phase duration in repeat breeder cows (7.00±1.13 vs 5.78±0.32 days) and second, a significantly larger ($P < 0.05$) diameter of the DF in the repeat breeder cows (10.00±0.53 vs 8.33±0.29 mm). The increased size of DF and increased dominance of first anovulatory follicle may lead to repeat breeding as it exposes the embryo and reproductive tract to elevated levels of oestradiol. Follicular fluid oestradiol concentrations in the largest follicle were reported to be elevated when its mean diameter was more than 8 mm (Guilbault *et al.*, 1993).

Ovulatory wave emerged significantly earlier ($p<0.05$) in repeat breeders than in normal cows. The interval between wave emergences to oestrus was slightly shorter ($p<0.1$) in normal than in repeat breeder cows (8.00±0.65 vs 10.28±1.30 days). Prolongation of wave emergence to oestrus interval might adversely affect the quality of ovulating oocyte leading to repeat breeding condition. Stock and Fortune (1993) reported that prolonged follicular dominance is associated with increased LH pulse frequency and higher peripheral levels of 17β oestradiol. Ovulation of an aged oocyte into suboptimal oviductal environment (Binelli *et al.*, 1999) may reduce the fertility of animal following insemination. Furthermore, fertility has been found to be low when the ovulatory wave is experimentally extended by subluteal concentration of progesterone (Ahmad *et al.*, 1995). Conception rate has been reported to be lower in cows having longer interval from ovulatory follicle emergence to oestrus (Bleach *et al.*, 1998). Bage *et al.* (2000) also reported that the growth of ovulatory follicle from its deviation to ovulation was longer in repeat breeder heifers than in control animals.

The corpus luteum was distinctly visible on day 4 of the cycle. The overall diameter of CL on a particular day of the cycle was similar between normal and repeat breeder cows except day 8 (Table 2). On day 8 of the cycle, CL diameter was significantly smaller ($p<0.05$) in repeat breeders compared to normal cows. Furthermore, on a particular day of cycle progesterone concentration was same between normal and repeat breeder cows. Lesser diameter of CL on day 8 of cycle in repeat breeder cows probably reflects its slower growth rate during the period. Inadequate functioning of the CL often referred to as luteal dysfunction or luteal phase deficiency has been reported as one of the important causes of repeat breeding in dairy cows (Kimura *et al.*, 1987). Therefore, other factors associated with smaller CL also need to be elucidated that leads to repeat breeding syndrome.

Table 1: Anovulatory and Ovulatory follicular wave growth pattern in normal and repeat breeding cows having two-wave cycle

End points	Anovulatory wave		Ovulatory wave	
	Normal cows (n=9)	Repeat breeder (n=7)	Normal Cows (n=9)	Repeat breeder (n=7)
1. Wave emergence (day)	2.22 ±0.32	2.67 ±0.56	13.33[a] ±0.67	10.71[b] ±1.28
2. Wave duration (days)	11.22 ±0.72	9.86 ±1.40	8.00[a*] ±0.65	10.28[b*] ±1.30
3. Maximum diameter of DF (mm)	8.33[a] ±0.29	10.00[b] ±0.53	10.22 ±0.36	10.85 ±0.34
4. Day of attainment of maximum diameter (day)	7.78[a*] ±0.40	9.14[b*] ±0.96	-	-
5. Growth phase duration of DF (days)5.78	7.00 ±0.32	±1.13	-	-
6. Growth rate of DF (mm/day)	0.78 ±0.03	0.96 ±0.15	0.83 ±0.12	0.72 ±0.09
7. Static phase duration of DF (days) 3.71	3.33 ±0.52	±1.33	-	-
8. Beginning of regression of DF (day)10.44	10.57 ±0.65	±1.21	-	-
9. End of regression of DF (day)	14.33 ±0.65	15.00 ±1.17	-	-
10. Regression rate of DF (mm/day)	1.26 ±0.17	1.38 ±0.12	-	-
11. SF emergence (day)	3.22 ±0.36	3.43 ±0.89	16.78 ±0.88	15.28 ±1.36
12. SF maximum diameter (mm)	5.22 ±0.22	6.00 ±0.49	5.22 ±0.36	5.14 ±0.26
13. Day of attainment of maximum diameter of SF (day)	5.55 ±0.80	6.28 ±0.68	18.67 ±0.67	16.71 ±1.20
14. Wave duration of SF (days)	5.44 ±1.04	4.86 ±1.08	2.89 ±0.79	4.28 ±1.34

Mean values bearing different superscripts in a row differ significantly
a,b Significant at $P < 0.05$
a*b* Significant at $P < 0.1$

Table 2. Progesterone concentrations and Diameter of CL at different days of oestrous cycle in normal and repeat breeder cows
(n=12)

Day	0	4	8	12	16	18
Progesterone (ng/ml)						
Normal (n=6)	0.31±0.04	0.81±0.05	1.72±0.21	2.66±0.23	2.71±0.26	1.19±0.19
Repeater (n = 4)	0.36±0.03	0.76±0.03	1.59±0.10	2.48±0.18	2.37±0.17	0.94±0.11
Diameter of CL (mm)						
Normal	-	4.83±0.27	10.50±0.54[a]	13.91±0.53	13.83±0.53	11.58±0.74
Repeater (n = 8)	-	4.75±0.25	8.12±0.44[b]	12.37±0.62	12.37±0.59	11.37±0.68

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)

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