DOES PRESENCE OF CL AND/OR FOLLICLE ON THE OVARY INFLUENCE THE SUBSEQUENT FOLLICULAR DYNAMICS IN TWO- AND THREE-WAVE CYCLES OF CATTLE?

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ABSTRACT

This investigation was undertaken on 24 unbred cyclic Gir and HF x K crossbred cattle of University farm for one cycle to see the influence of presence of CL and/or follicle on the subsequent development of dominant and ovulatory follicles in two- and three-follicular wave cycles using 7.5 MHz real time B-mode transrectal ultrasound scanning. The development of the first, second and third dominant follicle, and ovulatory follicle in relation to the position (ipsi- or contra-lateral) of the CL and the presence of morphologically dominant follicle were evaluated in both Gir and crossbred cattle, irrespective of parity. In both Gir and crossbred animals with 2-wave cycles, there was no relationship between the development of first dominant follicle and presence of CL in either of the ovaries, while in 3-wave cycle the first dominant follicle and the ovulatory follicle developed contralateral to the ovary bearing the CL, while the second dominant follicle developed on the same ovary bearing the CL. Similarly, no pattern was observed for the development of ovulatory follicle with respect to first dominant follicle on the ovaries in 2-wave cycle. However, the proportion of ovulatory follicles was higher on opposite side as compared to same side to the first dominant follicle in 2-wave cycle in both the breeds. In 3-wave cycle the second dominant follicle developed contralateral to first dominant follicle and the ovulatory follicle developed contralateral to second dominant follicle in all the animals. Thus, there was no difference in the pattern of this phenomenon between two breeds studied. In both Gir and Crossbred cows, the average CL size and plasma P_4 values were significantly higher ($P < 0.01$) while follicle size and plasma E_2 values were lower on day 7 and 14 than on day 0 and 21 post-estrus. Moreover, there were positive correlations between CL diameter and plasma P_4 values ($r = 0.82$ and 0.72), and follicle size and E_2 values ($r = 0.69$, 0.36) in two breeds studied.

KEY WORDS: Follicle size, CL size, Follicular dynamics, Cattle, Plasma Steroid profile.

INTRODUCTION

Reproductive inefficiency in lactating dairy cows is a source of frustration to the dairy producers and their consultants and it reduces dairy farm profitability. Since the ovary is one of the central organs of the reproductive system, its normal functioning is pivotal to the cow's breeding soundness and consequently profitability. Ultrasound technology has helped to delineate the bovine normal estrus cycle to have either two (Ginther *et al*., 1989[b]) or three (Savio *et al*., 1988) follicular waves (Satheshkumar *et al*., 2011). In each wave of follicular growth, one dominant follicle develops and suppresses other follicles. Dominant follicles grow and reach maximum diameter in the middle of the estrous cycle: when there are high levels of progesterone, there is no ovulation; regression starts allowing a new wave growth to occur. The dominant follicle that develops during the last wave of follicular growth in each estrous cycle is the ovulatory follicle. The mechanism that controls the follicular dynamics during estrous cycles needs to be understood to optimize the reproductive efficiency especially in zebu and crossbred cows. According to Son-Chang Ho *et al*. (1995) ultrasonographic assessment of the CL and follicle is a reliable method for estimating peripheral

P_4 and E_2 concentrations during the estrous cycle in cows. Therefore, the present study was aimed to know whether the presence of CL and/or follicle on the ovary influence the subsequent follicular dynamics in two- and three-wave cycles of cattle**?**

MATERIALS AND METHODS

Selection of Animals: The present study was carried out on 24 normal healthy cyclic Gir and HF x K crossbred cattle of University farm in Anand from January to June 2014. The animals were thoroughly screened for their genital health through gynaeco-clinical examinations. All the experimental animals were loosely housed in the same shed with free access to pure wholesome drinking water and were managed under identical nutritional conditions. Heat detection was done by visual observations twice daily, each lasting for an hour in the morning and in the evening. The cows in estrus were identified by palpation per rectum as well as well by transrectal ultrasound scanning, and were followed for follicular/luteal dynamics over entire cycle without AI/breeding.

Ultrasound Examination of Ovaries: The transrectal ultrasound examinations of all the cows were performed on alternate day over entire cycle between two consecutive estruses using a real-time B-mode ultrasound scanner (M-5 Vet, Mindray, China) equipped with a 7.5 MHz convex linear array transducer. The scanning of both the ovaries was accomplished in several planes through rectal wall to identify all the follicles greater than 4 mm in diameter and the corpus luteum. Desired images were frozen on the screen and the measurements were taken using a built in caliper system. The dominant follicle was characterized as the one, which grew at least 10 mm and exceeded the diameter of other follicles. Subordinate follicles (follicular cohort) were defined as those that appeared to originate from the same follicular pool as the dominant follicle (Ginther, 1995). A dominant follicle and its cohort were defined as a wave (Knopf *et al.,* 1989). The dominant follicle that ovulated was defined as ovulatory follicle.

Hormone Assay: The jugular blood samples were taken in heparinized vacutainers on day 0, 7, 14 and 21 of cycle for estimation of plasma profile of progesterone and estradiol-17β by employing standard Radio Immuno Assay (RIA) techniques of Kubasic *et al*. (1984) and Robertson *et al.* (1979), respectively. Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech-SAS, Marseille Cedex, 9, France. The values were calculated against standard curves of 0.1 to 60 ng/ml and of 10 to 2000 pg/ml, on the logit log papers for P_4 and E_2 hormones, respectively. The sensitivity of P_4 and E_2 assays was 0.1 ng/ml and 4 pg/ml. The intra-assay coefficients of variation were 5.4 and 15.1 per cent, while inter-assay variations were 9.1 and 14.4 per cent, respectively.

Statistical Analysis: The data on follicular and luteal dimensions as well as plasma P_4 and E_2 profile were analyzed statistically using ANOVA and critical difference tests as well as 't' test (Snedecor and Cochran, 1994). The correlations of average CL and follicle size and the relative plasma concentrations of progesterone and estradiol-17β observed in Gir and crossbred cattle under ultrasonographic monitoring for one unbred cycle each were worked out.

RESULTS AND DISCUSSION

The findings on the development of the first, second and third dominant follicles, and ovulatory follicle in relation to the position of the CL and the presence of morphologically dominant follicle evaluated in both Gir and crossbred cattle, irrespective of parity are presented in Tables 1 and 2

Effect of CL side on Development of next Dominant and Ovulatory Follicles

In 2-wave cycle of both Gir and crossbred cows, there was no relationship between the development of first dominant follicle and presence of CL in either of the ovaries, while in 3-wave cycles the first dominant follicles and the ovulatory follicles developed contralateral to the ovaries bearing the

CL, while the second dominant follicles developed on the same ovary bearing the CL (Table 1). This matter is still being debated for cattle. Ginther *et al.* (1989[a]) using ultrasonography did not find interovarian relationships between the ovaries containing the CL and the ovaries containing the dominant follicle (n=165) or between the location of CL and the characters of the dominant follicle.

Table 1: Effect of the CL side (left or right ovary) on development of dominant follicle during the next estrous cycle in Gir and crossbred cattle

Breed	Follicular wave	1st ovulation and CL formation	CL vs 1st dominant follicle	CL vs 2nd dominant follicle	CL vs 3rd dominant follicle
Gir	Two-wave (n=7)	-Right ovary 4 (57.14 %) -Left ovary 3 (42.86 %)	-Same side 4 (57.14 %) -Opp side 3 (42.86 %)	**Ovulatory follicle** -Same side 2 (28.57 %) -Opp. side 5 (71.42 %)	- -
	Three-wave (n=5)	-Right ovary 3 (60%) -Left ovary 2 (40%)	-Same side 0 (00%) -Opp. side 5 (100%)	-Same side 5 (100%) -Opp. side 0 (00%)	**Ovulatory follicle** -Same side 0 (00%) -Opp. side 5 (100%)
Cross-breds	Two-wave (n=8)	-Right ovary 6 (75.00 %) -Left ovary 2 (25.00 %)	-Same side 6 (75.00 %) -Opp side 2 (25.00 %)	**Ovulatory follicle** -Same side 2 (25.00 %) -Opp. side 6 (75.00 %)	- -
	Three-wave (n=4)	-Right ovary 2 (50 %) -Left ovary 2 (50 %)	-Same side 0 (00%) -Opp. side 4 (100%)	-Same side 4 (100%) -Opp. side 0 (00%)	**Ovulatory follicle** -Same side 0 (00%) -Opp. side 4 (100%)

Effect of Dominant Follicle on Development of next Dominant & Ovulatory Follicles

No clear pattern was observed for the development of ovulatory follicle with respect to first dominant follicles on both the ovaries in 2-wave cycles in any of the breeds studied. However, the proportion of ovulatory follicles were higher on contralateral ovary (5/7, 71.43 %) as compared to ipsilateral side (2/7, 28.57 %) to the first dominant follicle in 2-wave cycles. In 3-wave cycle the second dominant follicle developed opposite to first dominant follicles and the ovulatory follicles developed opposite to second dominant follicles in all the animals (Table 2). There was no difference in the pattern of this phenomenon between two breeds studied.

Almost similar pattern was observed in the development of second dominant follicles and ovulatory follicles with respect to side of development of first and second dominant follicles in 2-wave and 3-wave cycles of crossbred cattle. No clear clue could be seen in the literature reviewed regarding the effect of presence of first dominant follicle or CL on the development of subsequent follicular waves, either second or third, and ovulatory follicle on the contralateral or ipsilateral ovaries.

Table 2: Effect of the dominant follicles' side (left/right ovary) on development of next dominant follicle throughout cycle in Gir and crossbred cattle

Breeds	Follicular wave	1st dominant follicle	1st vs 2nd dominant follicle	2nd vs 3rd dominant follicle
Gir	Two-wave cycle (n=7)		**Ovulatory follicle**	
		RO 5 (71.43 %)	Same side 2 (28.57 %)	-
		LO 2 (28.57%)	Opp. Side 5 (71.43 %)	-
	Three-wave cycle (n=5)			**Ovulatory follicle**
		RO 5 (100 %)	Same side 0 (00 %)	Same side 0 (00 %)
		LO 0 (00 %)	Opp. side 5 (100 %)	Opp. Side 5 (100 %)
Cross-breds	Two-wave cycle (n=8)		**Ovulatory follicle**	
		RO 7 (87.5 %)	Same side 1 (12.5 %)	-
		LO 1 (12.5 %)	Opp. side 7 (87.5 %)	-
	Three-wave cycle (n=4)			**Ovulatory follicle**
		RO 3 (75 %)	Same side 0 (00 %)	Same side 0 (00 %)
		LO 1 (25 %)	Opp. side 4 (100 %)	Opp. Side 4 (100 %)

RO = Right ovary, LO = Left ovary

Mean Values and Correlations between CL and Follicle Size with Plasma Progesterone and Estradiol Profile in Gir and Crossbred Cows

In Gir cows, the average CL diameters on day 0 (estrus), 7, 14 and 21 of estrous cycle were observed to be 6.82 ± 0.71, 11.08 ± 1.88, 14.13 ± 0.57 and 7.70 ± 1.00 mm, respectively, and the corresponding plasma P4 levels were 0.14 ± 0.06, 3.67 ± 0.76, 5.08 ± 0.73 and 0.14 ± 0.04 ng/ml, respectively. Similarly, the average follicle diameters on day 0, 7, 14 and 21 of estrous cycle were 12.80 ± 1.10, 7.90 ± 0.63, 9.20 ± 1.00 and 13.33 ± 0.87 mm, respectively, and the corresponding plasma E2 concentrations were 51.66 ± 4.56, 20.66 ± 3.34, 20.66 ± 3.08 and 47.33 ± 7.08 pg/ml, respectively. There were positive correlations between CL diameter and plasma P4 values (r = 0.82), and follicle size and plasma E2 values (r = 0.69), as has been reported by Kastelic et al. (1990), Ribadu et al. (1994), Son-Chang Ho et al. (1995), Verones et al. (2002) and Chasombat et al. (2014). In the Crossbred cows, the average CL diameter on day 0, 7, 14 and 21 of estrous cycle was found to be 8.23 ± 1.04, 13.72 ± 0.44, 13.41 ± 0.26 and 10.63 ± 1.28 mm, respectively, and the corresponding plasma P4 levels were 0.82 ± 0.61, 3.53 ± 0.41, 5.11 ± 0.20 and 1.35 ± 0.37 ng/ml, respectively. Similarly, the average follicle diameters on day 0, 7, 14 and 21 of estrous cycle were 11.21 ± 0.87, 10.00 ± 2.50, 8.30 ± 1.06 and 12.58 ± 0.65 mm, respectively, and the corresponding plasma E2 concentrations were 51.16 ± 4.31, 22.83 ± 2.44, 19.33 ± 1.66 and 38.16 ± 3.68 pg/ml, respectively. Like Gir, there were also positive correlations between CL diameter and plasma P4 values (r = 0.72), and follicle size and plasma E2 values (r = 0.36).

The average P4 values were significantly higher ($P < 0.01$) and E2 values were lower on day 7 and 14 than on day 0 and 21 post-estrus, with corresponding larger CL and smaller follicular size in both Gir and crossbred animals. It is well-established fact that higher level of progesterone has a negative effect on the growth rate of ovarian follicle (Bergfelt et al., 1991; Baruselli et al., 1997).

Therefore, under higher plasma progesterone level the growth rate of dominant follicle is slower. This may be the reason for comparatively smaller preovulatory and ovulatory follicles seen in 3-wave as compared to 2-wave cycles in our study. Townson et al. (2002) also reported that luteal function was extended in cows with 3-wave cycle and the peak in plasma P4 occurred later in the cycle as compared to cows with 2-wave cycle.

In conclusion it can be said that the presence of CL and follicle on the ovary does not influence the emergence of next follicular waves on ispilateral or contralateral ovaries in either 2- or 3-wave cycles of cattle and that the CL size and plasma P4 as well as follicle size and plasma E2 concentrations are positively correlated.

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