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Follicular Dynamics during Estrous Cycle in Murrah Buffaloes

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ABSTRACT

An experiment was conducted to study the follicular dynamics during estrous cycle in Murrah buffaloes. Seven animals of 5 to 10 years were selected for the study. All the animals were synchronized as per the ovsync protocol and the pair of ovary of each animal was scanned by ultrasonography machine on 0, 3rd, 7th, 11th, 14th, 17th and 21st day of estrous cycle. The non-significant difference was observed in antral follicle count of 3 to 5 mm, 5 to 8 mm and follicles count (> 3 mm). Whereas, significantly ($p<0.05$) lower mean values were recorded on the day of estrus (0.14 + 0.14) as compared to 3rd, 7th, 11th, 14th, 17th and 21st day of estrous cycle. The significant individual variation ($p<0.05$) was recorded in average mean of 3 to 5 mm and total antral follicle count (>3). However, the difference for 5 to 8 mm and >8 mm was non significant. The positive correlation of low and high antral follicle count was observed in the present study. Two animals of low antral follicle count remained non pregnant and two animals showed three follicular waves during estrous cycle.

Keywords: Antral follicle count, Buffaloes, Follicular dynamics, Estrous cycle

Buffalo are predominantly dairy animals and is distributed in different regions of India. Recent studies indicated that the antral follicle population may be of paramount importance to improve reproductive performance in cows. There is already an agreement that the antral follicle count (AFC; follicles ≥3 mm in diameter) is a highly variable trait among animals, but with high repeatability in the same individual. Thus, females can be classified into low, intermediate or high AFC (Morotti *et al.,* 2015). The buffalo has been regarded as poor reproductive efficiency and characterized by low conception rates. Estimating ovarian reserves can be a tool to determine the buffalo of high reproductive potential and a criterion for selection. There are many methods of determining ovarian reserves in both animals and human beings but antral follicle count (AFC) is considered as one of the most reliable, non-invasive method of defining ovarian reserve (Hansen *et al.*, 2011). There are waves like pattern of follicular growth and a single follicle becomes dominant and ovulates in cattle (Ginther *et al.*, 1996). There is positive association between follicular count and fertility in cattle (Singh *et al.*, 2004). However, poor reproductive management of dairy buffalo has resulted in various reproductive problems including unknown reproductive disorders causing infertility and thus economic losses in the milk industry.

Use of ultrasound technology in animal reproduction has played an important role in the collection of data regarding ovarian follicular dynamics. Cattle can be phenotyped on the basis of antral follicular count, but similar kind of studies are lacking in Murrah buffaloes, so aim of the present study was to study follicular dynamics during estrous cycle for selection of high reproductive potential buffaloes.

MATERIALS AND METHODS

Experimental animals

All the animals selected in this group were postpartum lactating buffaloes, having the history of calving 60 days

before were roughly selected for the present study. Total 9 animals were synchronized as per the protocol for the present study. All the animals were injected with the hormone GnRH and PGF2α as per the standard ovisync protocol GnRH - PGF2α - GnRH (G-P-G protocol) as mentioned in Table 1.

The buffaloes were kept on observation from day 11 onwards. The animal showing discharge from vagina and/or estrous behavior was considered as estrus day i.e. '0' day. Total 7 out of 9 buffaloes showed the sign of estrus, therefore, 7 (seven) animals were continued for the study. All the buffaloes were examined by a real-time ultrasonographic machine (SonoScape S2V - SN/3242090) with a probe of 7 to 8 MHz frequency, linear array.

Pair of the ovaries of each animal was scanned by probe on day 0, 3, 7, 11, 14, 17 and day 21 of estrous cycle and images were saved for further analysis and record. The follicles were counted of both the ovaries of each animal during various stages of estrous cycle. The follicles > 3 mm in diameter were counted and categorized into three groups- small (3 to 5 mm), medium (5 to 8 mm) and large (8 mm and above).

All the animals of the present study were again synchronized as per the above protocol of G-P-G. The artificial insemination (AI) of all the seven animals was done on the day of estrus to know the fertility and compare with the present study. All the inseminated animals were checked per rectally and ultrasound sonography after 45 days to confirm the pregnancy.

Management and feeding of buffaloes

All the animals were housed in a shed having concrete floor with asbestos roof. Balanced concentrate mixture was offered to the experimental buffaloes as per the thumb rule. As the experimental animals were lactating buffaloes, 2 kg balanced concentrate mixture was offered for maintanance. The production requirement was calculated as for every 2 kg milk yield, 1 kg balanced concentrate was offered, over and above the maintance requirement. The fodder; green as well as dry including para grass, Dharwad Hybrid Napier (DHN - 6), paddy straw was offered *adlibitum* to meet out their dry matter requirement as well as to satisfy their bulk. The animals had free access to drink water, and all the animals were dewormed regularly.

RESULTS AND DISCUSSION

The mean values of small (3 to 5 mm), medium (5 to 8 mm), large (>8 mm) and total follicle count during estrous cycle are presented in Table 2 and Fig. 1 to 3. Non significant difference was observed in total follicle count (> 3 mm) during estrous cycle, the follicle count was highest on the day of estrous (0 day) and follicular phase (17^{th} and 21^{st} day) as compared to other days of estrous cycle. Whereas, the difference between 3-5 mm and 5-8 mm follicles was also non significant on day 0, 3^{rd}, 7^{th}, 11^{th}, 14^{th}, 17^{th} and day 21^{st} of estrous cycle. The number of small follicles (3-5 mm) were higher during metestrous (day 1 to day 3), early diestrous (day 11^{th}) and follicular phase (day 17to 21) and lower during early diestrous (day 5 to day 10) and late diestrous phase (day 11 to day 17). Fewer follicles between 5 to 8 and >8 mm were recorded during estrous cycle. The result obtained in the present study are agreement with the findings reported by Starbuck-Clemmer *et al*. (2007) in beef cattle, Warriach and Ahmad (2009) in Nili Ravi buffaloes, Cushman *et al.* (2009) in beef cows and Shahzad *et al.* (2014) in buffaloes. However, significantly ($P < 0.05$) lower mean value for large follicular count was recorded on day 0, supports the finding recorded by Yilmaz *et al.,* (2014) in buffaloes.

High variability and repeatability of maximum numbers of antral follicles > 3mm in diameter during follicular wave was recorded during estrous cycle in the present study, supports the findings of Burns *et al*. (2005). Two buffaloes in the present study showed three follicular waves and five buffaloes show two follicular waves. The dominant follicle attained its maximum diameter on day 7 and 17 in two wave cycles, whereas, in three wave cycles, the second dominant follicle began to regress on day 19 when it was replaced by a third large dominant follicle which was the ovulatory follicle in all three wave cycles, which are comparable to the finding reported by Taylor and Rajamahendran (1991). Several studies had shown the prevalence of 2-wave follicular activity during an estrous cycle in cattle. This could be attributed due to the high incidence of the 2 or 3 waves of follicular activity might be based on presence of 2 or 3 peaks of gonadotrophic hormones, particularly FSH. The peaks of FSH were also related to lower estrogen concentration, which in turn depended on regression in follicular size (Ginther *et al.,* 1996). Genetic predisposition or uncontrolled environmental conditions may play an important role

Table 1: Synchronization protocol for onset of estrous cycle in buffaloes

0 Day	7th Day	9 th Day	10th Day onwards
GnRH - 5 ml (I/M)	PGF2α - 2 ml I/M	GnRH- 5 ml (I/M)	Observation of estrus (Three times a day)

Table 2: Mean + SE of Small, medium and large follicle count during estrous cycle

Follicle count	Day of estrous cycle						
	0	3	7	11	14	17	21
3-5 mm	6.29 ± 0.52	4.71 ± 0.36	4.14 ± 0.67	5.00 ± 0.44	4.00 ± 0.31	4.71± 0.68	4.57 ± 0.61
5-8 mm	1.00 ± 0.22	1.71 ± 0.42	1.43 ± 0.48	2.14 ± 0.40	1.43 ± 0.30	1.71 ± 0.52	2.57± 0.37
>8 mm	0.14 ±0.14[b]	1.00 ± 0.22[a]	1.14 ± 0.14[a]	1.14 ± 0.26[a]	0.86 ± 0.26[a]	1.43 ± 0.30[a]	1.28± 0.29[a]
Total	**7.43 ± 0.53**	**7.42 ± 0.65**	**6.71 ± 0.87**	**8.28 ± 0.68**	**6.29 ±0.71**	**7.85 ± .51**	**8.42 ± 0.90**

[a,b] Values within a row with no common superscript differed (P<0.05).

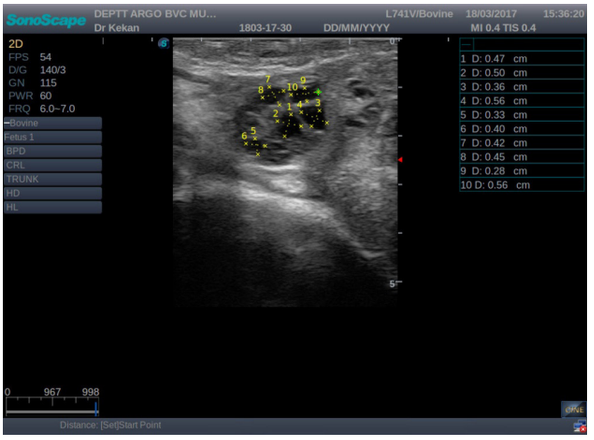

Fig. 1: Ultrasonograms of 3 to 5 mm follicles

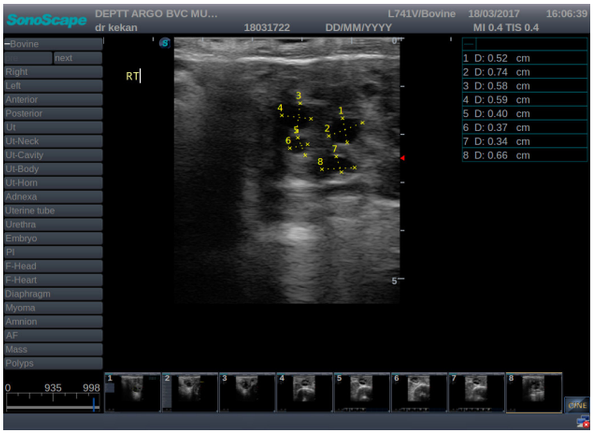

Fig. 2: Ultrasonograms of 5 to 8 mm follicles

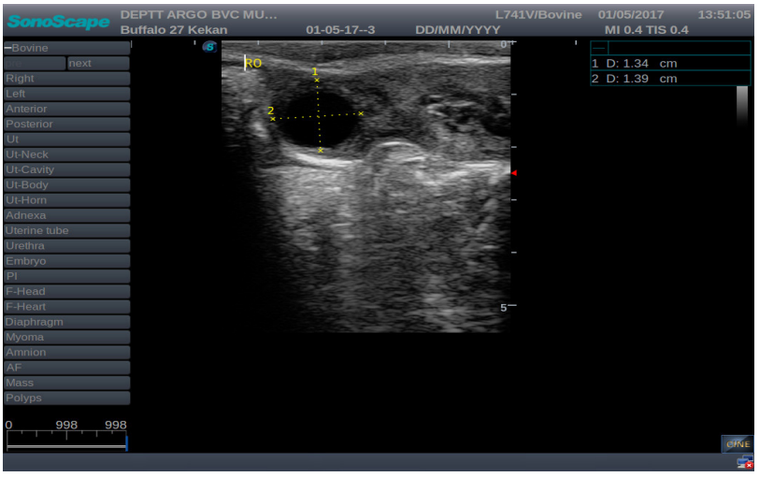

Fig. 3: Ultrasonograms of > 8 mm follicle

in regulation of incidence of the 2 or 3 follicular waves within one estrous cycle, through influences on follicular development and the level of their estrogen secretion (Ginther *et al.,* 1996; Nosier 2003).

It is observed that, significant ($p<0.05$) individual variation from animal to animal in total follicle count and 3 to 5 mm count was recorded. Whereas, non significant difference was recorded in 5 to 8 mm and <8 mm follicle count. The positive correlation of antral follicular count and fertility was observed in the present study. It was observed that buffaloes having low number of small follicles (3 to 5 mm) did not conceive and the buffaloes having significantly higher follicle counts remained pregnant. Two buffaloes in the present study were not pregnant and five remained pregnant. This may probably be due to association between low AFC, enhanced FSH secretion, and decreased progesterone production may result in increased rates of embryo mortality (Mossa *et al.,* 2012).

CONCLUSION

The positive correlation of antral follicle count was observed in between pregnant and non pregnant animals. The buffaloes which are having 2 or 3 follicular waves did not affect the conception rate. Whereas, the individual variation of antral follicle count was recorded.

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