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REPEATABILITY OF ANTRAL FOLLICULAR COUNT AND ITS CORRELATION WITH SUPER STIMULATION IN NILI-RAVI BUFFALOES

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ABSTRACT

Small ovarian size and low number of follicles are major causes of low reproductive potential in buffalo (*Bubalus bubalis*). There is no tool through which reproductive performance of buffalo can be phenotyped. Therefore, aim of the present study was to develop a method on the basis of which reproductive performance of buffaloes can be phenotypically assessed. For this purpose two experiments were designed. In first experiment, ten Nili-Ravi buffalo heifers were used in the study to measure repeatability of follicular count. Each heifer was scanned on alternate day, from day 1 to day 9 of the successive estrous cycle. Antral follicles and repeatability was assessed in different follicular waves. In 2nd experiment, 4 lactating buffaloes were selected to count antral follicles from day 1 to day 10 of the estrous cycle and super-stimulated by using FSH hormone. Follicular count after FSH injection was examined by ultrasound. Follicular count is highly repeatable in different follicular waves within (r = 0.83) and between (r = 0.85) different estrous cycles. Follicular count and super stimulatory response are positively correlated (r = 0.95). In conclusion, animals with high antral follicular count can be selected for super stimulation and embryo transfer to get optimum response.

Key words: Antral follicular count, Super-stimulation, Repeatability, Buffalo.

INTRODUCTION

There is no phenotypic characterization to determine the buffalo of high reproductive potential. Estimating ovarian reserves can be a phenotypic character 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 human (Baerwald *et al*, 2003) and cattle (Ginther *et al*, 1996a). There is positive association between follicular count and fertility in human (Scheffer *et al*, 2003) and cattle (Singh *et al*, 2004). Consequently, the well characterized bovine dominant follicle model (Ireland *et al*, 2000) was recently used to establish the degree of variation both within and between animals in the number of antral follicles (Burns *et al*, 2005) during the follicular waves of estrous cycles in dairy cattle.

In buffaloes, the follicular system has not been studied as much as in cattle. The use of ultrasound technology in animal reproduction has played an important role in the collection of data regarding ovarian follicular dynamics and related hormonal profiles in domestic animals as well as other species (Radcliffe *et al*, 2001). Nili-Ravi buffaloes typically show two follicular waves (67 percent) and three follicular waves (33 percent) during an oestrus cycle (Warriach and Ahmad, 2007), with the first wave beginning around day 0 (day of ovulation).

The numbers of follicles ≥3 mm in diameter recruited in each wave have been counted in beef, dairy heifers and postpartum dairy cows (Singh *et al*, 2004). There is high variation in antral follicular count among animals but there is high repeatability (0.85 to 0.95) within individuals (Mossa *et al*, 2010). Due to this reason cattle can be phenotyped on the basis of counting of antral follicles.

Cattle having low ovarian size have lower follicular count. Cattle having low number of antral follicles show reduced response to superovulation (Ireland *et al*, 2007) and much lower circulating concentrations of progesterone and reduced endometrial thickness from day 0 to 6 of the estrous cycle (Jimenez-Krassel *et al*, 2009) compared with cattle of similar age with a higher antral follicular count. In cattle there has been high embryonic mortality due to lower progesterone concentration (Diskin and Morris, 2008). Consequently, cattle having lower number of antral follicles results in lower progesterone concentration and reduced endometrial development and this might lead to high embryonic mortality.

Cattle can be phenotyped on the basis of antral follicular count, but similar kind of studies are lacking in Nili-Ravi buffaloes, so aim of the present study was to develop a phenotypic character for selection of high reproductive potential buffaloes. It was hypothesized in the study that there is high repeatability of antral follicular count in Nili-Ravi buffaloes between the different waves of estrous cycle and animals having high follicular count have higher super-stimulatory response.

MATERIALS AND METHODS

Animals: Nili-Ravi adult buffalo heifers were kept at Livestock Experiment Station, Buffalo Research Institute Pattoki. Heifers were housed free in the stall. All the heifers were provided balanced feed and water ad libitum. Our study was conducted in spring season March-April 2013.

Ultrasound Scanning Procedure to Count Antral Follicles: Ovaries were scanned using trans-rectal probe (7.5 - MHz transducer; Honda Ultrasound, HS 1500). To standardize the counting, ovaries were scanned end to end and positions of antral follicles were drawn on ovarian map. To take diameter of antral follicles each follicle was measured twice and averaged. To confirm that each follicle is exactly on the ovarian map, scanning procedure was repeated. Total number of antral follicles was counted by counting the follicles ≥ 3mm in diameter on ovaries.

Repeatability of Antral Follicles during Follicular Waves: To determine repeatability of antral follicles 10 heifers, 2-3 years of age were used. Repeatability of antral follicles in different follicular waves of the same and consecutive estrous cycles was determined by using ultrasound examination of both the ovaries. To synchronize heifers for estrus, animals with confirmed corpus luteum on the ovaries were treated with prostaglandin injection (2cc, Dalmazine). After that animals were observed for heat by using teaser bull. Each animal was subjected to ultrasound scanning on alternating days until the end of last follicular wave of same estrous cycle and up till end of first follicular wave of subsequent estrous cycle. All the antral follicles present on both the ovaries were counted.

Relationship between number of Antral follicles and Super-stimulatory Response: To determine relationship between the numbers of antral follicles with superstimulatory response, four normally cyclic lactating buffaloes were used. To synchronize, animals with corpus luteum were treated with prostaglandin injection (2cc, Dalmazine). Animals were observed for heat through teaser bull. Animals were examined for ovulation through ultrasound. After ovulation animals were scanned for counting antral follicles on alternating days. From day 10-13 after heat all the animals were treated with follicle stimulating hormone (follitropin), 2cc each animal in morning and evening. Ultrasonography was performed daily in morning and evening and the total numbers of follicles were counted.

RESULTS AND DISCUSSION

Number of follicles for 10 representative heifers during the different follicular waves in an estrous cycle is depicted in Figure 1. Total number of follicles during different follicular waves of estrous cycle among different animals were consistently low (1-4), intermediate (5-8) and high (9 or above). These results showed that there is remarkable similarity in the total number of follicles during different follicular waves of individual animals and the great variation among different animals. Animals had great variation in maximum numbers of follicles; they were in range from 4-12. Average no. of follicles was 7.6±1.02.We studied 22 follicular waves of same estrous cycles and 32 follicular waves of different estrous cycles of different animals. Repeatability of maximum numbers of antral follicles was very high in follicular waves of same (0.83) and different (0.85) estrous cycles. P-Value for the repeatability of maximum numbers of follicles in same (0.008) and different (0.0006) estrous cycles was low ($P<0.05$). This depicts that our data is significant and maximum numbers of antral follicles in same and different estrous cycles are highly repeatable.

In Figure (1, 2, 3), follicular waves of different groups (low, intermediate and high) can be seen. From the data of follicular waves it can be summarized that first follicular wave starts on day 1 of ovulation. Follicles grow by the day 6-7 of estrous cycle. As one of the follicles attains dominancy other follicles start to regress. First follicular wave ends on day 9 to 10 in multi follicular waved animals. Second follicular wave starts on day 10-11 and ends in ovulation in animals having two follicular waves. In case of animalswith three follicular wave pattern, the second follicular wave ends on day 15-16 and 3rd follicular wave ends in ovulation. Results of animals having different follicular count and their superstimulatory response can be seen from Table 2. There is high positive correlation ($r = 0.95$) between follicular count and superstimulatory response.

High repeatability of maximum numbers of antral follicles ≥ 3mm in diameter in different follicular waves of same estrous cycle and different estrous cycle was observed in Nili-Ravi buffalo heifers in the present study. In case of follicular waves of same estrous cycles and different estrous cycles, repeatability of follicular count was found to be 0.83 and 0.85 respectively. The results endorse the studies done by other scientist in other species like cattle. In cattle (Mossa *et al*, 2012) a study on repeatability of antral follicles was conducted. It was

observed that there is high repeatability of maximum numbers of follicular count during different follicular waves of different estrous cycles of individual cattle (0.95).

In a study in beef cattle there was high positive correlation (0.77) among 2-6 mm follicles at emergence (Singh *et al*, 2004) of two successive follicular waves of an estrous cycle. Repeatability was lower in this experiment but it might be due to size of follicles (2-6mm). We counted all the follicles ≥ 3mm in diameter. In another study individual cattle numbers of growing follicles aspirated through (Boni *et al*, 1997) ultrasound guided needle after every 7-10 days was highly repeatable (0.58). It had great difference with our results, this might be because they did not observe follicular waves, they did it only for aspiration propose after every 7-10 days.

In human being maximum numbers of follicles are moderately repeatable during two successive menstrual cycles (Scheffer *et al*, 1999). In ours result in buffaloes it was highly repeatable. Human beings and buffaloes are physiologically much different. They both have their distinct heat pattern as in human beings they have menstrual cycle while in cattle and buffalo its estrous cycle. It was reported that numbers of antral follicles remains constant in cattle until 8-10 years of age (Erickson, 1966; Katska and Smorag, 1984). Before these 8-10 years of age they are highly repeatable as ours experiment was on heifers age was no problem. In human beings it remains constant at 35-40 years of age (Scheffer *et al*, 1999).

In a study total numbers of antral follicles were greater in 10-12 months old heifer than by nearly 2 years old lactating cow, which suggests that milking has a negative effect on follicular dynamics of cattle. Although there is high repeatability in maximum numbers of antral follicular count but different physiological states affect maximum numbers of antral follicles like inadequate nutrition, lactation (Lucy *et al*, 1991), heat stress (Wolfenson *et al*, 1995) and pregnancy (Ginther *et al*, 1996b). In ours study there was no such a limiting factor like nutrition, it was properly provided according to the body weight of heifers, there was no heat stress as this study was done in spring season.

In the second experiment there was high positive correlation (r = 0.95) between follicular count and superstimulatory response. The results supported the hypotheses that superstimulatory response is related to intrinsic number of follicles recruited into a follicular wave. Results of the present study are supported by other scientists. It was observed that there is high correlation (r = 0.55) between follicular count (Singh *et al*, 2004) and super-stimulatory response.

Table 1. Repeatability of follicular waves in same and consecutive estrous cycle in buffalo heifers.

Estrous cycle	No. of animals	No. of Follicular waves	Range of Antral Follicles count	Repeatability	P-Value
Same	10	22	4-12	0.83	0.008
Consecutive	10	32	4-12	0.85	0.0006

Table 2. Superstimulatory response of Buffaloes having different Follicular Count.

Antral Follicular Count	Follicular Count after Superstimulation	Correlation
3	4	r = 0.95
6	12	
9	14	
10	15	

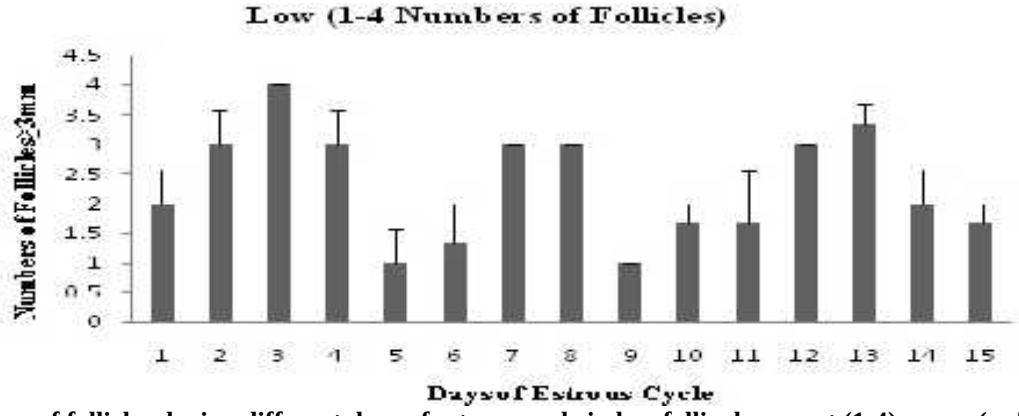

Fig.1. Number of follicles during different days of estrous cycle in low follicular count (1-4) group (n=3).

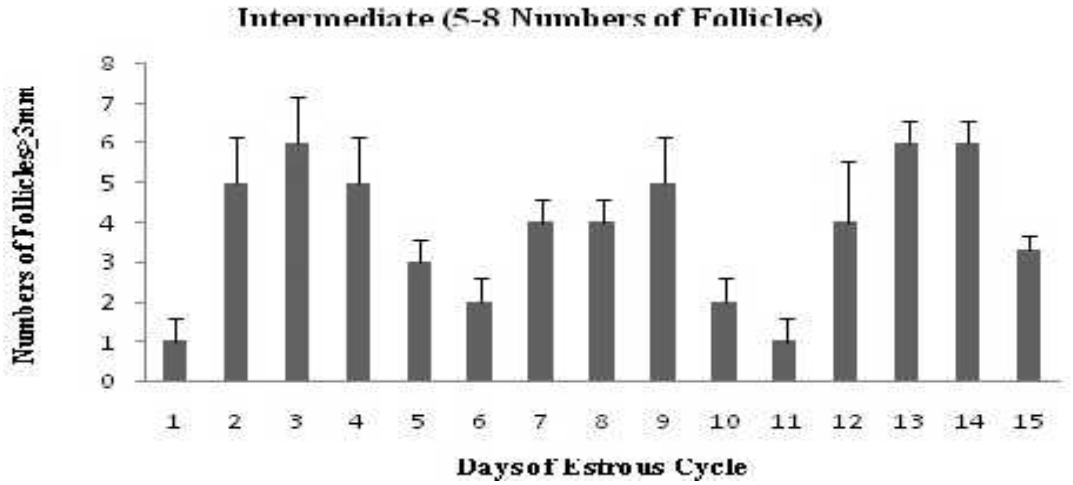

Fig. 2. Number of follicles during different days of estrous cycle in intermediate follicular count (5-8) group (n=3).

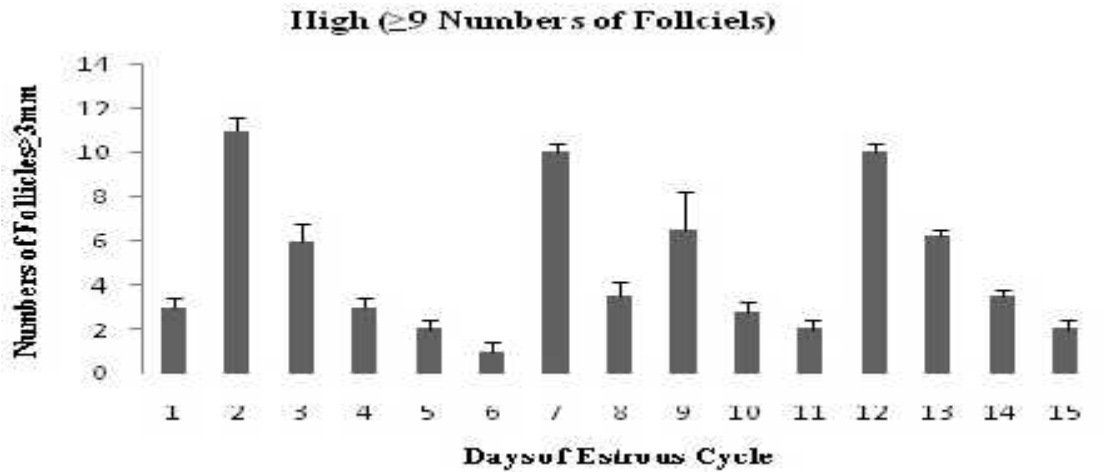

Fig. 3. Number of follicles during different days of estrous cycle in high follicular count (≥ 9) group (n=4).

Conclusion: In conclusion, buffalo can be phenotyped on the basis of antral follicular count. Antral follicular count is highly repeatable within individual animal. Follicular count is positively correlated with super stimulatory response. So it is suggested from this study that animals with high antral follicular count should be selected for super stimulation to get optimum response.

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