



Follicular Population during the Oestrous Cycle in Nili-Ravi Buffaloes Undergoing Spontaneous and PGF_{2α} Induced Luteolysis*

H. M. Warriach** and N. Ahmad

Department of Theriogenology, Faculty of Veterinary Science,
University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT : The objective of this study was to compare the follicular population during spontaneous and PGF_{2α} induced oestrous cycles in Nili-Ravi buffaloes. In Exp.1, (n = 13 oestrous cycles) follicular population was monitored using ultrasonography on alternate days. Buffaloes were monitored for ovarian follicles from day 0 (first oestrus) until next oestrus. These animals were observed for oestrus twice daily using a teaser bull. Of 12 oestrous cycles, 9 (75%) had two waves of follicular activity and only 3 (25%) had three waves during the oestrous cycle. The mean number of small, medium and large follicles among various days of the oestrous cycle between two and three waves of follicular development were not significantly different (p>0.05). In Exp. 2, follicular population 3 days before oestrus was compared in buffaloes undergoing spontaneous (n = 12 oestrous cycles) and PGF_{2α} induced (n = 6) luteolysis. The mean number of small and large follicles increased (p<0.05) and the number of medium follicles decreased (p<0.05) during the 3 days before oestrus in buffaloes undergoing induced luteolysis as compared to those with spontaneous luteolysis. These results showed that the mean number of small, medium and large follicles among various days of the oestrous cycle were similar between the two and three waves of follicular development, and three days before oestrous the number of small, medium and large follicles altered due to induced luteolysis on day 9, compared to those with spontaneous luteolysis. (**Key Words :** Follicular Population, Oestrous Cycles, Buffaloes)

INTRODUCTION

In cattle, researchers utilizing repeated ultrasonic imaging to monitor ovarian follicles in different size categories (Pierson and Ginther, 1987) or individual follicles (Sirois and Fortune, 1988) over time have confirmed the postulate by Rajakoski (1960) that follicular growth occurs in a wave-like pattern. A follicular wave is characterized by the emergence of a cohort of follicles ≥4 mm in diameter (Knopf et al., 1989), from which one large follicle develops and subordinate follicles become atretic (Ginther et al., 1989). The largest follicle is anovulatory during the luteal phase. Commonly, two or three follicular waves occur within a single oestrous cycle (Ginther et al., 1989; Knopf et al., 1989; Ahmad et al., 1997).

Response to superstimulation appears to be related to

the number of follicles per follicular wave and could be predicted by a single ultrasound examination at wave emergence (Singh et al., 2004). High numbers of antral follicles have been reported positively associated with an increased responsiveness to gonadotropin treatments during superovulation (Cushman et al., 1999), with a larger number of normal oocytes recovered for *in vitro* fertilization (Taneja et al., 2000), up shot to a greater number of transferable embryos which eventually resulted in higher pregnancy rates following *in vitro* fertilization (Taneja et al., 2000). Whereas fertility was not affected by the numbers of antral follicles ≥4 mm in diameter in a single follicular wave (Starbuck-Clemmer et al., 2007). Taken together, these observations imply that number of follicles that develop during oestrous cycle may play an important role in regulation of fertility.

In buffaloes there are few reports on follicular development during oestrous cycle. The work of Danell (1987) in cyclic buffalo heifers, based on histological evaluations of the ovaries confirmed that pattern of follicular development in buffalo is similar to that earlier been documented in cows by Rajakoski (1960). Workers in the area of buffalo reproduction from India (Taneja et al.,

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** Corresponding Author: H. M. Warriach. Tel: +92-333-4315107, Fax: +92-42-9211461, E-mail: hassanwarriach71@yahoo.com

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1996), Brazil (Baruselli et al., 1997) and Pakistan (Warriach and Ahmad, 2007) confirmed that two or three follicular waves occur within a single oestrous cycle. Several studies indicate a low response to multiple ovulation and embryo transfer in buffalo (Madan, 1990; Misra et al., 1990; Schallenberger et al., 1990) and studies generally demonstrate the high degree of unpredictability in superovulatory responses resulted in decrease the efficiency and profitability of embryo transfer programs in the buffalo. To improve these responses, a better understanding of the mechanism controlling ovarian follicular growth and development.

The objectives of this study were to: i) examine and compare the numbers of small, medium and large follicles between two and three waves of follicular development during the oestrous cycle, and ii) evaluate and compare the effect of PGF_{2α} induced oestrous cycle on the number of follicles with spontaneous oestrous cycle.

MATERIALS AND METHODS

Animals and detection of oestrus

All experiments were conducted in spring 2005, at the University of Veterinary and Animal Sciences, Lahore, Pakistan (latitude 31° 34' N; longitude 74° 20' E). Experiments were based on eight multiparous, non-lactating, cyclic Nili-Ravi buffaloes, 4-10 years old and with a body weight in the range of 450-600 kg. Throughout the experimental period, the buffaloes were maintained as a group and were housed in semi-covered sheds under similar conditions of feeding and management. Each day, buffaloes were fed 30-40 kg of green fodder and 1-2 kg of concentrate mixture containing 15% crude protein and 65% total digestible nutrients. Animals were observed for oestrus, two times a day using a teaser bull.

Ovarian ultrasonography

Ultrasound examinations were done by a single operator under optimized conditions, as described by Pierson and Ginther (1988). The ultrasound scanner was a real time, B-mode instrument equipped with a 8 MHz linear array transducer (Falco Vet 100; Pie Med; Holland).

The day of standing heat was designated as day 0. Follicular diameters and the sequential identification of individual follicles ≥ 4 mm were done as described by Knopf et al. (1989). The total number of small (3-5 mm), medium (6-9 mm) and large (≥ 10 mm) follicles for each recorded day of the wave were evaluated. In Exp. 1, oestrous cycles (n = 13) in eight buffaloes were monitored. This experiment was conducted to compare the small, medium and large follicles in buffaloes with either two or three waves of follicular development during oestrous cycles. Ultrasonography was carried out on every other day

from day 0 = oestrus to the next oestrus.

Exp. 2, was conducted to compare the number of small, medium and large follicles during the 3 days before oestrus in buffaloes undergoing PGF_{2α} induced and spontaneous luteolysis. The data of spontaneous luteolysis were obtained from the ovulatory waves of oestrous cycles of experiment 1 (n = 12). Eight buffaloes used in experiment 1 were treated with two injections of 0.150 mg (2 ml of product) of PGF_{2α} (Dalmazine™ Fatro Co. Italy) i.m; 12 h apart on day 9 (day 0 = oestrus) in order to induce luteolysis (n = 9). Ultrasonography was done every other day from day 0 to the next oestrus and daily thereafter until ovulation.

Statistical analysis

In Exp.1, (n = 12 spontaneous oestrous cycles) number of small, medium and large follicles between two wave and three wave of oestrous cycles were analyzed using repeated measures ANOVA. One buffalo had one wave; therefore, data of this animal was excluded from all analyses. In Exp. 2, number of small, medium and large follicles 3 days before oestrus was compared between PGF_{2α} induced oestrous cycles (n = 6) and spontaneous oestrous cycles (n = 12 oestrous cycles of Exp. 1) using a paired *t*-test. In three buffaloes after PGF_{2α} administration second dominant follicle ovulated (follicular turnover). The data of these three animals was excluded from all analyses. Statistical analysis was carried out using SPSS (Version 10.0). $p < 0.05$ was regarded as significant. Data are presented as means \pm SEM.

RESULTS

In Exp. 1, 12 oestrous cycles, nine (75%) had two waves of follicular activity and only three (25%) had three waves during the oestrous cycle. The number of small, medium and large follicles among various days of oestrous cycle between two and three wave of follicular development were not significantly different ($p > 0.05$), as shown in Figure 1. In Exp. 2, nine buffaloes in which oestrus was induced by PGF_{2α} on day 9, six (67%) had first wave largest follicle activity till ovulation and in only three (33%) follicular turnover occurred. The number of small, medium and large follicles during the 3 days before oestrus in buffaloes undergoing PGF_{2α} induced and spontaneous luteolysis are presented in Figure 2. The number of small and large follicles increased ($p < 0.05$) and the number of medium follicles decreased ($p < 0.05$) in buffaloes undergoing induced luteolysis as compared to the ones with spontaneous luteolysis.

DISCUSSION

In buffaloes this is the first report to our knowledge

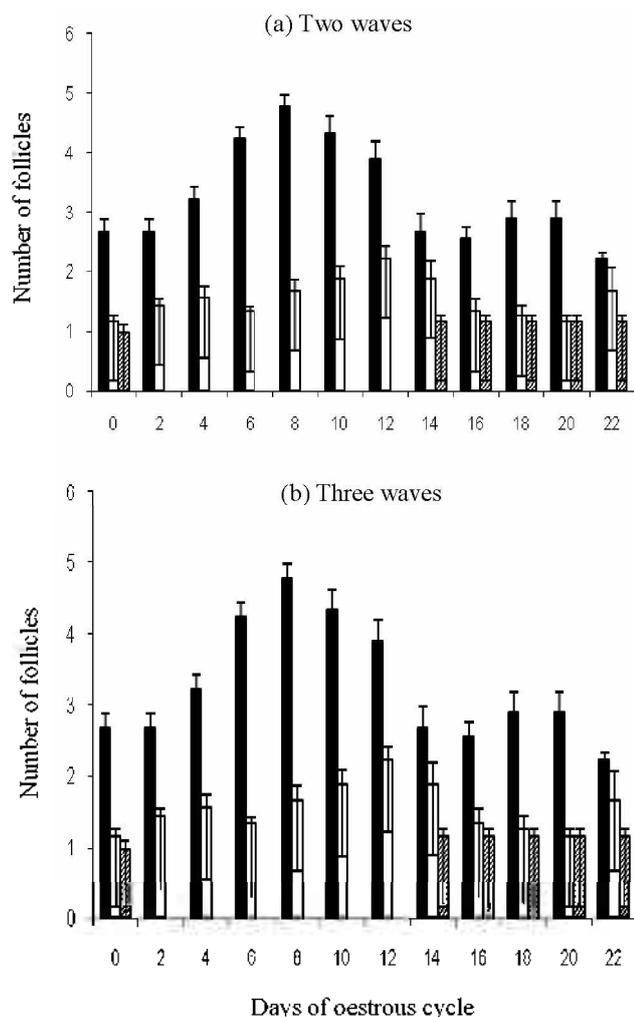


Figure 1. Mean (\pm SEM) number of small 3-5 mm (■), medium 6-9 mm (□) and large \geq 10 mm (▨) follicles in buffaloes with (a) two and (b) three waves during oestrous cycles.

which describes that the follicular population is altered due to induced follicular phase compared to those with spontaneous. In cattle, at this stage of oestrous cycle (day 10) follicular wave emergence is characterized by the sudden growth of 8-41 (average = 24) small follicles having diameter of 3-4 mm (Pierson and Ginther, 1987; Sirois and Fortune, 1988; Ginther et al., 1989). In the present study the number of medium follicles decreased or failed to grow during the three days before oestrus when large follicles were growing. This finding is consistent with earlier report in heifers where non-ovulatory follicles decreased in size or failed to grow during the 3 days before oestrus when preovulatory follicles are growing (Quirk et al., 1986). These results support the idea that the dominant follicle can inhibit the growth of smaller follicles as suggested by studies with heifers (Matton et al., 1981), and monkeys (Goodman et al., 1979).

In present study the mean number of small, medium and large follicles among various days of oestrous cycle was

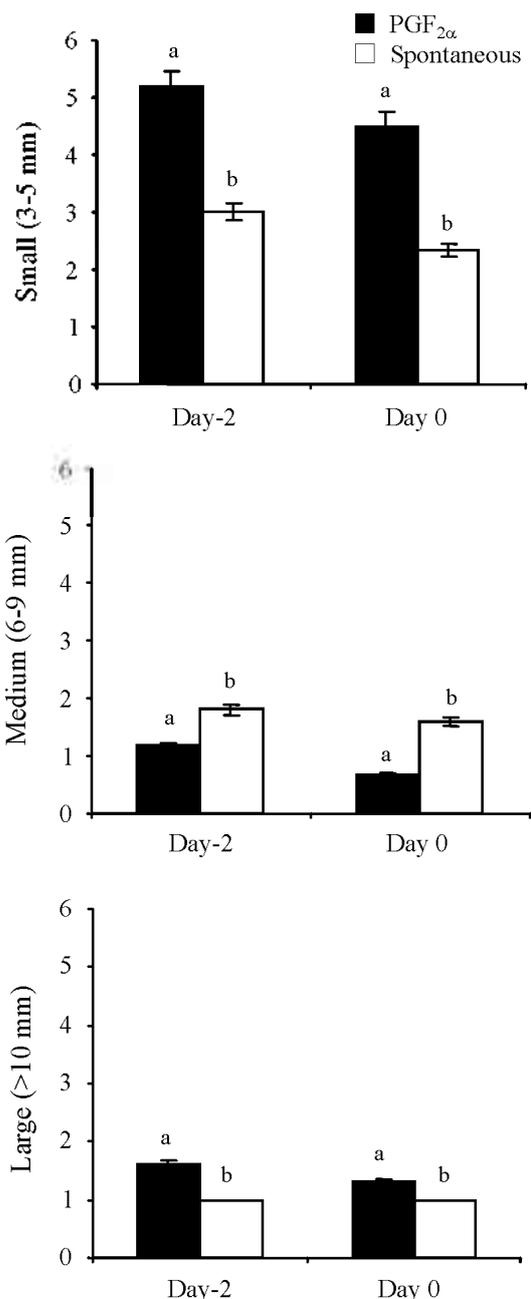


Figure 2. Mean number (\pm SEM) of follicles in each of three size classes during the follicular phase in 6 buffaloes treated with PGF_{2α} on day 9 of oestrous cycle to induced luteolysis or 12 buffaloes undergoing spontaneous luteolysis.

similar in buffaloes with two waves than in those with three waves of follicular development. This finding is supported by earlier report in buffaloes (Baruselli et al., 1997). In another study, repeated removal of follicles does not adversely affect the number of total follicles 3 or 5 days after transvaginal follicle aspiration (Akshey et al., 2005). There is a complete lack of information on the effects of these factors on follicular dynamics in this species. Such information could help in identifying inputs that are

required critically for improving management practices for rearing buffaloes.

In conclusion the mean number of small, medium and large follicles among various days of oestrous cycle were similar between the two waves and three wave of follicular development and three days before oestrous the number of small, medium and large follicles altered due to induced luteolysis on day 9, compared to those with spontaneous luteolysis.

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