FATE OF DOMINANT FOLLICLE IN SUMMER ANESTRUS BUFFALOES

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ABSTRACT

During peak summer for a period of 24 days, anestrus (n=6) and cycling (n=5) buffaloes were subjected to transrectal ovarian ultrasonography and jugular vein blood sampling on alternate days. During this period, anestrus buffaloes exhibited successive development of follicles which reached dominant phase (19 follicles, 12.6±0.3 mm diameter) but remained non-ovulatory. The diameter of ovulatory follicles (6 follicles) in cycling buffaloes was 13.1±0.8 mm. Plasma progesterone in anestrus and cycling buffaloes was 0.1±0.0 and 0.71±0.03 ng/ml ($p<0.05$), respectively. In brief, with the help of ultrasonography, it has been revealed for the first time that in the buffaloes displaying summer anestrus, ovarian follicular activity is going on although the dominant follicles that were able to attain ovulatory size failed to ovulate and regressed.

Key words: Anestrus, Buffalo, Follicle, Progesterone, Summer

INTRODUCTION

Although buffaloes are classified as polyestrus, the percentage of buffaloes exhibiting anestrus during the summer is appreciably greater than that during the winter (74-86% *versus* 22-26%, respectively; Singh *et al.,* 1989, Tailor *et al.,* 1990). This enigma of seasonal variation in ovarian activity in buffaloes remains unsolved (El-Wishy, 2007), probably due to lack of information about ovarian activity in the buffaloes displaying anestrus during summer. Some reports of the buffaloes displaying true anestrus revealed that these animals never ovulated and had inactive ovaries with small to medium follicles of <10 mm diameter (Arya and Madan, 2001). However, in the ewes displaying anestrus during summer season, the follicles as large as those found during the luteal phase of the estrous cycle were present (Webb and Gauld, 1985). Within this framework, the objective of the present study was to monitor the fate of dominant follicle (DF) in summer anestrus buffaloes.

MATERIALS AND METHODS

This study was conducted for a period of 24 days on buffaloes (pluriparous) having history of normal estrous cycles (cycling, n=5) or having failed to exhibit estrus (anestrus, n=6). Buffaloes were housed at the dairy farm of the University (latitude: 30° 56' North, longitude: 75° 52' East), during the hot-humid month of June to August, referred to as the "peak summer season" with maximum ambient temperatures and relative humidity ranging between 36 to 45°C and 30 to 80%, respectively. Buffaloes were confined for the entire period of the study to a barn with access to an open sheltered space. The daily feed consisted of adequate chaffed green fodder, wheat straw, concentrates (maize or wheat 60%, groundnut cake 25%, wheat bran 10%, rice bran 5% and common salt 1%), mineral mixture and water available *ad lib*. The animals were allowed to wallow in a pond of water twice in a day for at least 30-60 min. All the buffaloes were subjected to gynecological examination before inclusion in the study, and the buffaloes diagnosed with any pathological condition of the reproductive tract were not included.

Ovarian ultrasonography of cycling and anestrus buffaloes was carried out with a battery operated B-mode ultrasound scanner (Agroscan AL, ECM, Angouleme, France) equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM, Angouleme, France) on every second day for 24 days. In cycling buffaloes, the start

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of ultrasonography (day 0) was not necessarily on the day of estrus. Ovaries were systematically examined and images were recorded on a diagram of the ovary by carefully sketching the size and relative location of all follicles of >4 mm diameter and size and relative location of visible corpus luteum (CL) (Ghuman *et al.,* 2010). Optimal scan images were frozen and the size of the follicles / CL was determined by measurement of the diameter of the follicles / CL at their widest poles. All measurements were made using the built-in, on-screen calipers. Blood was sampled from the jugular vein in a heparinized vial after each ultrasonography. Plasma was separated immediately and frozen at -20°C until analysis.

The emergence of DF was the day when the DF was, retrospectively, identified at a diameter of 4-5 mm (Ghuman *et al.,* 2010). The DF was the largest ovarian follicle in a follicular wave that was 2 mm greater than the second-largest follicle (Ghuman *et al.*, 2010). The buffaloes were considered to have ovulated or not based upon the disappearance of DF and subsequent emergence of the CL and with plasma progesterone reaching concentrations of 0.50 ng/ml during post-ovulation period (Ghuman *et al.,* 2010). The day when ovulatory follicle disappeared was considered as the day of ovulation. The DF was considered non-ovulatory when the maximal diameter of DF apparently decreased and was no longer detectable and plasma progesterone was persisting to <0.50 ng/ml. The diameter of ovulatory or dominant non-ovulatory follicle was defined as the diameter recorded on day of ovulation or maximal diameter attained by follicle before regression, respectively (Ghuman *et al.,* 2010).

Plasma progesterone was assayed by solid-phase radioimmunoassay using an indigenous progesterone antibody (Ghuman *et al.,* 2009). Assay sensitivity was 0.1 ng/ml; intra- and inter-assay variation coefficients were 5.5% and 9.5%, respectively.

Numerical data are represented as mean ± S.E.M., and differences were considered to be significant at P<0.05. Chi-square ($\chi 2$) test (Dytham, 1999) was employed for the number of buffaloes that ovulated between cycling and anestrous groups. Two sample Student's *t*-test (Dytham, 1999) was employed for the diameter of ovulatory and dominant non-ovulatory follicles as well as mean plasma progesterone between cycling and anestrus groups. Statistical analyses were performed using MINITAB release 13.2 statistical software (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

The present study is the first one to demonstrate that follicles in summer anestrus buffaloes attain ovulatory size (>10 mm diameter) but failed to ovulate followed by their regression.

During the study period of 24 days, all the cycling but none of the anestrus buffaloes ovulated (P<0.05, Table 1). Preliminary assessment of ovarian sketches of all anestrus buffaloes revealed that a follicle larger (=10 mm diameter) than any other follicle within the pair of ovaries was detected on 90 per cent of the days of ultrasonographic monitoring. This was attributed to the days (2-4 days) spent by a selected follicle (~10 mm) to attain maximum diameter and, thereafter about 2-4 days spent by a DF in static phase. This study was not planned to observe the exact time period spent by a selected follicle to attain maximum diameter or the time span of static phase of each DF. Nevertheless, by retrospectively tracing the large follicles, the fate of a DF was determined. A representative pattern of the ovulatory follicle in a cycling buffalo and the successive dominant non-ovulatory follicles in an anestrus buffalo is shown in Fig 1A and B, respectively. Dominant non-ovulatory follicles (8 follicles) as well as ovulatory follicles (6 follicles) were seen in all the cycling buffaloes (n=5), whereas all the anestrus buffaloes (n=6) only expressed dominant non-ovulatory follicles (19 follicles). Comparable scenario exists in anestrus ewes during summer period where follicular development does not stop, periods of follicle growth are taking place but DF never ovulate (Souza *et al.,* 1997). Moreover, some reports of the buffaloes displaying true anestrus during the post-partum period suggested that these animals never ovulated and had active ovaries in which ovulatory size (12–14 mm) was attained by the DF (Presicce *et al.,* 2005). In the present study, the diameters of dominant non-ovulatory follicles in anestrus buffaloes (12.57±0.34 mm) were identical (P>0.05) to that attained by ovulatory follicles in cycling buffaloes (13.14±0.82 mm, Table). The later diameter of ovulatory follicles was in corroboration with the previous findings in buffaloes (Baruselli *et al.,* 1997). Thus, this study has revealed that although follicular diameter required for ovulation was attained by the anestrus buffaloes, but merely attaining this diameter may not be enough for ovulation to occur during summer season.

In fact, the concentration and frequency of the pulses of GnRH and gonadotrophins (follicle stimulating hormone, FSH and luteinizing hormone, LH) required

to induce the growth of follicles are different from that required for ovulation (Rensis and Scaramuzzi, 2003). In summer anestrus buffaloes, gonadotrophins required for the development of small follicles to ovulatory size may be present but the failure of DFs to ovulate can be attributed to lack of sufficient gonadotrophin stimulus. Low plasma gonadotrophins in buffaloes and ewes during summer (Janakiraman *et al.*, 1980, Robinson *et al.*, 1985) could have disturbed the development of LH

Table : Ovarian activity in cycling and anestrus buffaloes during the study period of 24 days.

Variables	Anestrus (n=6)		Cycling (n=5)
Buffaloes ovulated	0^a		5^b
Number of dominant follicles (mm)	Ovulatory follicles	0^a	6^b
	Non-ovulatory follicles	19^a	8^b
Diameter of dominant follicles (mm)	Non-ovulatory follicles: 12.6±0.3^a [10.2-15.1]		Ovulatory follicles: 13.1±0.8^a [10.6-15.6]
Diameter of corpus luteum (mm)	0.0^a		14.1±0.3^b [8.5-20.1]
Plasma progesterone (ng/ml)	0.11±0.00^a [0.0-0.37]		0.71±0.03^b [0.00-2.15]

$^{a\ vs\ b}$P<0.05: within row of a parameter, Figures in parenthesis indicate range

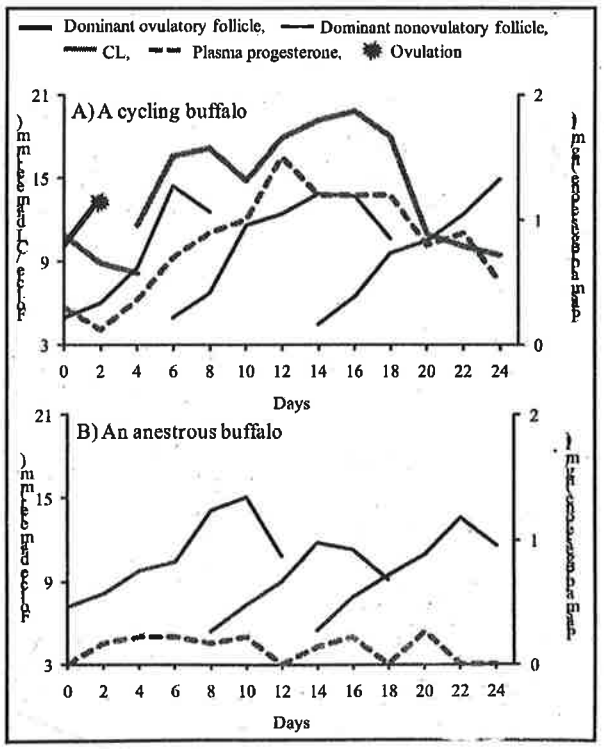

Figure 1: Representative patterns of ovarian activity in a cycling (A) and an anestrus (B) buffalo during a study period of 24 days. Cycling buffalo shows ovulation of a dominant follicle, subsequent corpus luteum (CL) formation and rise in plasma progesterone. Anestrus buffalo shows successive emergence of dominant nonovulatory follicles, absence of CL and presence of negligible plasma progesterone. Start of study (day 0) was on any arbitrary day without taking into consideration the day of estrus cycle in cycling buffalo or the day of start of a follicle wave in an anestrus buffalo.

receptors and the synthesis of adequate estradiol in DF (Parmar and Mehta, 1994, Rensis and Scaramuzzi, 2003). Consequently, low estradiol might have failed to induce ovulatory surge of gonadotrophins (Alam and Dobson, 1987). Hence, none of the DFs ovulated in the summer anestrus buffaloes.

In cycling buffaloes, ovulation was confirmed by the presence of active CL (plasma progesterone =0.50ng/ml, Fig 1A, Table), whereas in anestrus buffaloes, nonovulatory condition was confirmed by the absence of CL and presence of consistently low (<0.50 ng/ml) plasma progesterone (Fig 1B, Table). Between cycling and anestrus buffaloes, higher ($P<0.05$) plasma progesterone was recorded in the former group (0.71±0.03 ng/ml) on different days of study period compared to the latter group (0.11±0.0 ng/ml, Table). This confirmation of ovulation based upon the appearance of CL and plasma progesterone in the ovulatory (cycling) and non-ovulatory (anestrus) buffaloes were in accordance with the established findings (Presicce *et al.,* 2005).

In conclusion, ovaries of summer anestrus buffaloes were displaying some degree of follicular activity. In these buffaloes, DF attained ovulatory size but failed to ovulate and regressed. Treatment of summer anestrus buffaloes with exogenous gonadotrophins could be highly effective for inducing ovulation of ovulatory size DFs and hence ovarian cyclicity.

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