Indian Journal of Animal Sciences **82** (5): 472–476, May 2012

Histomorphometry of ovarian follicular growth and atresia in summer and winter seasons in Iranian river buffalo

S HASANZADEH[1] and J SADEGHINEJAD[2]

Uromieh University, Uromieh, Iran

Received: 15 June 2011; Accepted: 5 October 2011

ABSTRACT

The ovaries of 24 adult and apparently healthy river buffaloes were collected in summer and winter of a year. The hitomorphometric study of ovaries revealed that, the mean dimension and weight of right ovary is significantly higher than the left ovary in both the seasons. The population of healthy follicles were significantly higher in winter than summer, but on the other hand the population of atretic follicles were significantly higher in the summer than winter. The populations of growing healthy follicles at follicular as well as luteal phases of the estrus cycle were significantly high in winter than that in summer. The populations of atretic primary and mature follicles were more than the populations of the secondary atretic follicles. It may be conclude that in the winter season the mean populations of the growing as well as healthy follicles are higher than the summer.

Key words: Buffalo, Ovary, Ovarian follicles, Summer, Winter

Many reports suggest seasonality of reproduction and ovarian activity (Parmer and Mehta 1992) in buffalo (*Bubalus bubalis*). Reduction in day length, and high temperature of summer may also have potential effects on seasonal breeding of the female buffalo (Bhosrekar *et al.* 1992, Hafez 1993). Controlled internal drug releasing based synchronization regimens improve fertility in anoestrous buffaloes (Naseer *et al*. 2011). Studies were made on primordial germ cells (Ranjbar *et al.* 1999), buffalo ovaries (Sadrkhanlou *et al.* 2000) and developmental study of ovary in fetus and new born (Ranjbar *et al.* 2003) native water buffaloes of Iran. Razzaque *et al.* (2008) studied biometry and follicular population on ovaries of Nagpuri buffaloes. The buffalo ovary has a smaller population of recruitable follicles at any given time than the ovary of the cow (Danell 1987). In Thai swamp buffaloes ovarian follicular activity showed wave-like pattern before the first ovulation leading to short oestrous cycles (Yindee *et al.* 2011).

The non growing follicles, primordial follicles, comprise 90–95% of ovarian follicles, whereas growing follicles are primary, secondary, tertiary (mature) or adult follicles (Hasanzadeh *et al.* 2000, Gao *et al*. 2001, Hasanzadeh *et al.* 2005). Razzaque *et al.* (2008) studied the average numbers of small, medium and large size surface follicles in the ovaries of cyclic adult Nugpuri river buffalo. Type A atresia, the degenerative changes first occur in the oocyte, whereas in type B atresia the primary changes starts in the follicular cell Ryane 1981).

Studies were made to examine all the visible surface follicles on the ovary to recover good quality oocyte (Bhardwaj 1996, Kumar *et al*. 1997), but the detailed studies on reproductive phase are still lacking. Bansal *et al*. (2004) divided the ovaries on the basis of gross morphological and hormonal studies. The literature lacks information on populations of different categories of preantral and antral healthy as well as atretic follicles during follicular and luteal phases of estrus cycle in summer (hot) and winter (cold). Thus histomorphometric study of the ovaries and populations of the different ovarian follicles during these 2 different climatic conditions would help us in identifying probable effects of temperature fluctuation on the pattern of follicular growth and atresia in the water buffalo.

MATERIALS AND METHODS

Ovaries of 24 adult (2 to 4 year age) apparently healthy, non pregnant and cyclic buffaloes, i.e. 12 specimens during summer (4 pairs in each month of season), and 12 specimens during winter (4 pairs in each month of season) were collected. By précised inspection for the presence of the

Present address: [1]Associate Professor (shasanzadehs @yahoo.com, s.hasanzadeh@mail.urmia.ac.ir), Department of Basic Science, College of Veterinary Medicine, Uromieh University, Uromieh-Iran.

[2]Assistant Professor (javadsadeghinejad@gmail.com), Department of Anatomy and Histology, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran.

corpus luteum or growing follicles on the surfaces of the ovaries, luteal and follicular phases of the animals were recorded. The specimens were put in physiologic formalin solution for the fixation.

Gross morphometry of the ovaries: The weights and dimensions ovaries were recorded. The specimens were processed through routine paraffin embedding, serial sections cut at 7 μm thickness and stained with hematoxylin- eosin staining technique (Gretenchen 1979).

Histomorphology and histomorphometry: Under the light microscope with the use of special lens device the healthy as well as atretic, including primary, secondary, and mature follicles were recorded.

Statistical analyses: By the use of SPSS computer software, ANOVA, and Duncan's tests were carried out on the dada and graphs were prepared with the use of Excel computer soft ware.

RESULTS AND DISCUSSION

This study revealed that the ovaries of this animal are ellipsoid in shape and weigh 3.5±0.2 g. A previous study on buffalo (Sadrkhanlou *et al*. 2000), reported its weight as 3.7g. The weight of ovary in the adult cyclic cow is 10–20 g (Nickel *et al*. 1979), thus the mean weight of this organ is lesser in buffalo than the cow. Our study revealed that, the mean length and width of the ovary in the buffalo is 2.8±0.16 cm and 1.08±0.2 cm respectively, and in comparison to dimensions of this organ in the cow, i.e. 4.00 cm and 2.5cm (Ranjbar *et al*. 1999), it is clear that in the buffalo this organ is smaller than in the cow.

The biometrical study revealed that there are no significant differences in widths of the right and left ovaries, but this was significant ($P<0.05$) in lengths as well as weights and the analyses showed that the right ovaries are significantly heavier and larger than the left ovaries in this animal. Ranjbar *et al.* (2003) reported that there is no significant differences in the biometrical values of the ovaries in these ages. Sadrkhanlou *et al*. (2000) did not report significant differences on the right and left ovaries. The studies suggested that differences in the dimensions and weights of the right and left ovaries appear after puberty and are significant only in adult buffaloes. The accelerated growth of the follicles and appearance of the corpora lutea in the ovaries may bring about these differences in the ovaries after puberty (Singh *et al*. 1987). According to Gao *et al.* (2001) the ovary holding large and active CL has great number of preantral follicles than the ovary lacking CL. Gao *et al*. (2004) observed that there were no significant differences in populations of the pre-antral follicles in ovaries with or without CL. They also observed that the population of primary follicles in the buffalo ovaries is lower than the cow's ovaries whereas, population of the secondary follicles was limited (Fig. 2). In some of these follicles, proliferation of the granulosa cells accelerated and the signs of hypertrophy were observed in cell layers.

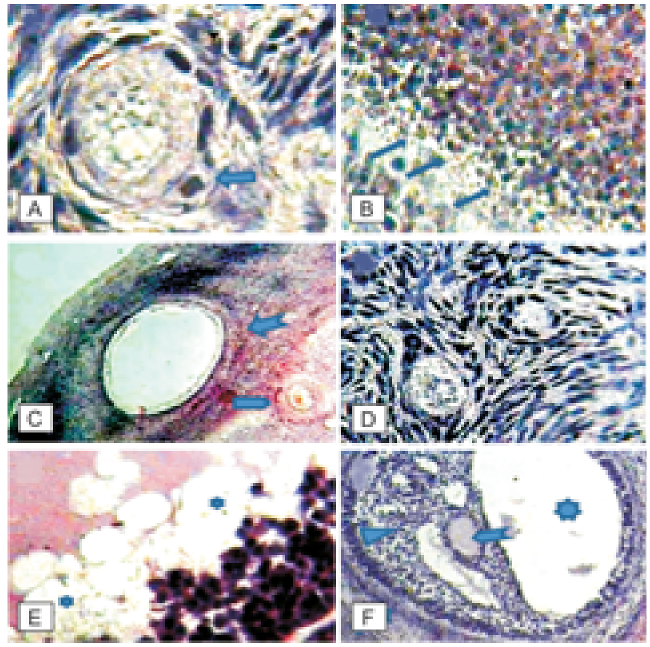


Fig. 1 (A–F). **A.** Primordial follicle H&E. ×400. **B.** Part of a tertiary follicle in advance stage of atresia. **C.** A cystic follicle (), secondary growing follicle (). H&E. ×40. **D.** The cortical region of follicle H&E. ×250. **E.** Part of a large tertiary follicle with advance degeneration of its granulosa cells. H&E. ×400. **F.** A tertiary follicle which is committed to atresia. Oocyte with abnormal ellipsoidal outline (), anntrum (), granulosa cell layer () and thickened theca layer H&E. ×100.

The basal granulosa cells were showing signs of nuclear pyknosis and degeneration (Fig. 1, C).

Sreejalekshmi *et al.* (2011) observed that the initial structural changes in atretic follicles occur primarily in the granulosa cells in adult water buffloes. In severely atretic follicles TUNEL positive granulosa cells along with theca cells have to be considered in assessing the rate and extent of atresia.

Our investigation on the tertiary follicles in these follicles, which are committed to the atresia, revealed granulosa cell layer invaded by the blood vessels and signs of nuclear pyknosis in the granulosa cells, hypertrophy of the theca cell layer, enlargement of the follicular antrum, breakdown of the basal membrane and invasion of blood vessels to the granulosa layer (Fig. 1, B).

In most of the ovaries, cystic follicles of different sizes were observed. In these follicles the antrum was enlarged and filled with fluid, but the granulosa cell layer reduced 1 or 2 layer (Fig.1, C). In the atretic and cystic follicles, in the border between the granulosa cells and antrum white bobbles (vacuoles) were seen, which may be expanded endoplasmic reticulum of the granulosa cells or absorbed vacuoles (Fig. 1, E).

The histomorphometric study of the ovarian follicles of the buffalo revealed that mean diameters of primary follicles in comparison to primordial follicles increased 3-fold, secondary follicles in comparison to primary follicles 4- fold, and in the mature follicles in comparison to the secondary this increase was once more than 5-fold. Earlier studies reported that in the cow diameters of primordial follicles, unilayer primary follicles, secondary follicles and mature follicles are 30–50 μm, 40–80 μm, 150 μm, 1000–3000 μm and > 3000 μm respectively (Braw *et al*. 1997,Hyttel *et al*. 1997, Telfer *et al.* 1996, Van Den Hurk et *al*. 1997). Comparisons of dimensions obtained in this study revealed that the diameters of the different follicular types are smaller in buffalo than that in the cow.

One of the major constraints in buffalo reproduction is the limited number of follicles and oocyte reserve in the ovary. The total number of primordial and graafian follicles is about 50% less than in cattle (Aboul-Ela 2000). In buffaloes, the rate of atresia ranged from 70% (Rajesha *et al.* 2001) to 90% (Palta *et al.* 1998) when compared with 65–70% in cattle (Grimes *et al.* 1987). In bovines, atresia occurs at all stages of follicular development but majority of the follicles undergo degeneration at early antral stage rather than the preantral and preovulatory stages (Chun *et al.* 1996). The accelerated rate of follicular atresia was reported to be one of the major causes for the reproductive failure in the buffalo (Rajesha *et al.* 2001).

The statistical analyses on the morphometric data in the summer and winter showed significant (P<0.05) and highly significant (P<0.01) differences in between healthy and atretic follicles (Fig. 4 A,B), thus the seasons (cold and hot climatic conditions) more likely exert significant effects on these parameters. In winter population of healthy follicles, especially in follicular phase increased and that of atretic follicles decreased.

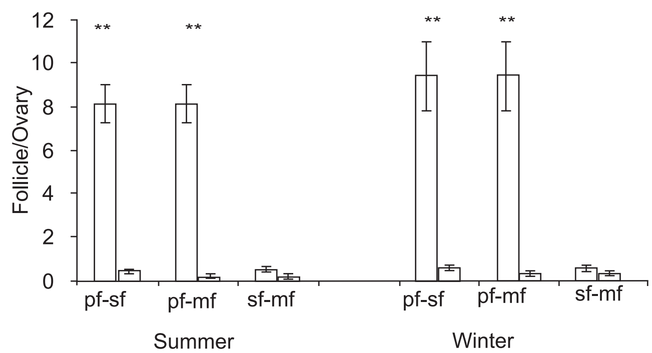
There was no significant (P> 0.05) differences in follicular growth in follicular phase of estrus cycle (Fig. 4 C), but in the luteal phase the differences in the population of the growing follicles in the summer and winter were highly significant (Fig. 4 D). From these result one may conclude that the internal conditions of the animals (follicular and luteal phases of the estrus cycle) have considerable effects on the security of the growing ovarian follicles.

It is revealed that, there is a significant (P<0.01) difference in the distribution of the healthy follicles in the follicular phase in between hot and cold seasons (Fig. 4 E). It is revealed that, there is significant differences (P<0.05) in mean distribution of healthy follicles in luteal phase of estrus cycle between summer and winter (Fig. 4 F). These results make an important concept that cold climatic condition is more suitable for increase in fertility of female buffaloes.

According to this study the differences in mean population of the atretic follicles at the follicular phase between summer and winter is highly significant (P<0.01) (Fig. 4G), and in the luteal phase mean population of the atretic follicles in between 2 seasons is also highly significant (P<0.01) (Fig. 4 H) and in both cases atretic follicles in summer was more than that in winter. It may be due to the fact that in winter the physiological condition is more suitable for the ovulation and consequently fertility in the buffalo.

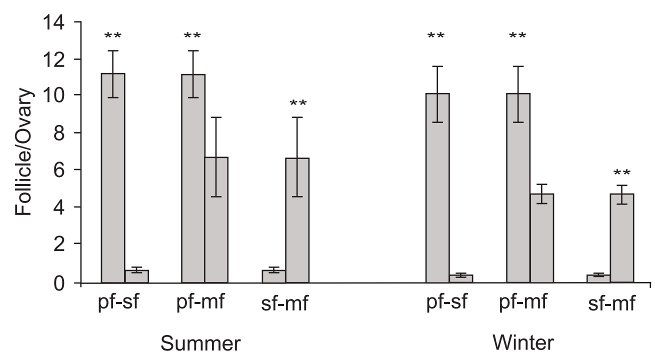
The results showed that in both seasons there is highly significant (P<0.01) differences in distributions of primary, secondary, and between primary and mature healthy follicles, but there were not significant (P>0.05) differences between secondary and mature healthy follicles (Fig. 2). In summer the differences between populations of the atretic primary and secondary follicles was highly significant (P<0.01), and between primary and mature atretic follicles significant (P<0.05), and between secondary and mature atretic follicles highly significant (P<0.01). In winter differences in populations of atretic primary and secondary, primary and tertiary, also secondary and mature follicles were highly (P<0.01) significant (Fig. 3).

Our study revealed that, in both seasons there is highly significant (P<0.01) differences in between populations of the atretic primary, secondary, and mature follicles. According to our results and previous reports (Byskov 1979),



**=P<0.001

Fig 2. Comparative representation of different types of healthy follicles populations (Mean ± SE) in the ovaries of the buffalo during summer and winter. Pf, primary follicle; sf, secondary follicle; mf, mature follicle.



**=P<0.001

Fig 3. Comparative representation of different types of atretic follicles populations (Mean ± SE) in the ovaries of the buffalo during summer and winter. Pf, primary follicle; sf, secondary follicle; mf, mature follicle.

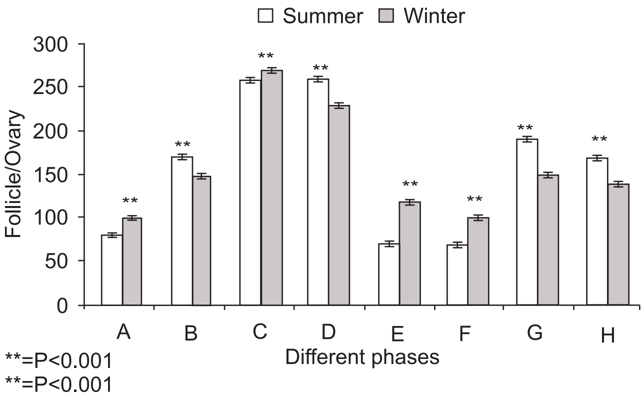


Fig 4. Comparative representation of parameters studied on the buffalo ovaries. A, Population of the healthy follicles; B, Population of the atretic follicles; C, Population of the growing follicles in the follicular phase; D, Population of the growing follicles in the luteal phase; E, Population of the healthy follicles in the follicular phase; F, Population of the healthy follicles in the luteal phase; G, Population of the atretic follicles in the follicular phase; H, Population of the atretic follicles in the luteal phase.

the follicles experience 2 critical situations in their growth, and in these circumstances most of them enter to phase of atresia and death, one is in the start of growth, during which great number of follicles in primary stage of growth phase committed to atresia and are removed from the ovaries. The second crisis occurs in the mature follicles (in the large follicles). From these follicles only restricted number (in some cases only one) are necessary for ovulation and rest of them are removed by atresia. According to our study, it is more likely that in winter physioendocrine condition is such that atresia and degeneration of growing follicles are reduced and population of healthy ovulatory follicles increases, and hence this season is appropriate for breeding of this animal.

According to previous reports the important factor which brings about differences in the activities of ovaries and consequently the reproductive behavior of buffalo in the summer and winter, is the differences in the temperature, but the length of the light and dark is also taken in to account as an important factor as well (Datta *et a*l. 1998, Gupta *et al.* 2007).

By knowing the fact that in buffaloes in hot season the populations of the different categories of healthy follicles are reduced, due to populations of the atretic follicles increase, and the rate of ovulation decline and the animal experienced a sort of anestrous, we can say that the hot environment has direct relation with anestrous of this animal.

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