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Study of Ovarian Follicular Development During Summer and Winter Seasons in River Buffalo

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Abstract: Buffalo is one of the farm animals with prominent economical importance and in Iran its population is in noticeablenumber. Twenty two pairs of buffalo ovaries were collected from slaughter houseduring summer and winter seasons and transported to histology laboratory within appropriate fixative. After paraffin embedded, semiserially sectioned and stained with HandE, histological and morphometerical investigations are carried out. Histological observation showed that a layer of cuboidal cells on the surface of ovary resting on the thick layer of connective tissue tunica albuginea and different types of ovarian follicles were embedded in loose connective tissue of the cortical stroma. By using a calibrated light microscopic lens device the follicle diameter was measured and data showed that the non-growing follicles had diameter of 26.4±.31 μm, on the other hand, the full grown follicles showed a diameter of 1905.7±279.8 μm. There were highly significant ($P<0.01$) differences in mean distribution of healthy and atretic follicles during summer, whereas this was only significant($P<0.05$) during winter., During both seasons, number of atretic follicles is higher than healthy ones. Differences in mean distribution of healthy and atretic follicles during Summer was highly significant ($P<0.01$) during the luteal phase and only significant ($P<0.05$) during the follicular phase. During winter difference was not significant. These results suggested that, during winter because of the reduced rate of follicular atresia, differences between healthy and atretic follicles is insignificant. Differences between mean distribution of healthy and atretic follicles in one year were highly significant ($P<0.01$). It is to be noted that the highest number of healthy follicles were noticed in winter, while the highest number of atretic follicles were seen in summer. The results of this study clearly showed seasonality of buffalo reproduction and ovarian activity. In conclusion, ovarian activity in summer because of hot stress is lower than in winter.

Key words: buffalo · Ovary · Ovarian follicles · Summer · Winter

INTRODUCTION

The buffalo is an important livestock resource in many parts of the world and Iran is home of noticeable numbers of buffalo in its various provinces.

The indigenous river buffalo is the primary dairy animal, the major contributor of meat, milk production and draft-work. It is considered the animal of future with ability to survive under unfavorable conditions as compared to cattle. In spite of being the major contributor for the dairy industry, delayed maturity, poor conception rate, silent estrus, low germ cell reserve, higher rates of follicular atresia, longer inter-calving period are major constraints to the reproductive performance and productivity in buffaloes [1, 2].

The ovary of buffalo contains hundreds of oocytes enclosed in follicles. In fact, there are two major follicular groups: growing follicles consisted of primary, secondary and Graafian follicles and the non-growing follicles which act as follicular reserve for growing follicles and constitute 90-95 percent of ovarian follicles [3, 4].

Among these follicles only few get chances to become mature and reach to the ovulatory stage and the reset become atretic in various growing stages [5].

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Hence, the present study was carried out to evaluate the effect of season on distribution of growing follicles of buffalo ovary in hot condition (summer) and cold condition (winter).

MATERIALS AND METHODS

Twenty two pairs of buffalo ovaries were collected from slaughter house during summer and winter seasons. The specimens were fixed in 10% formaldehyde solution and processed through routine paraffin embedding, cut at 5-7μ semiserially and stained with HandE and then histological and morphometrical investigations are carried out. The results were analyzed by student œ t-test. P values of 0.05 or lower were considered significant.

RESULTS

Histological observation showed a layer of cuboidal cells on the surface of ovary resting on a thick layer of connective tissue termed tunica albuginea and different types of ovarian follicles were embedded in loose connective tissue of the cortical stroma.

By using a calibrated light microscopic lens device, the follicle diameter was measured and data showed that the non-growing follicles had diameter of 26.4±31μm, on the other hand, the full grown follicles showed a diameter of 1905.7±279.8 μm (Table 1).

There were highly significant (P<0.01) difference in mean distribution of healthy and atretic follicles during summer, while it was only significant (P<0.05) during winter. Differences between mean distribution of healthy and atretic follicles in luteal phase during summer was highly significant (P<0.01), whereas it is only significant (P<0.05) in the follicular phase. In winter, difference was not significant (Table 2). Differences between mean distribution of healthy and atretic follicles in one year was highly significant (P<0.01). It is to be noted that highest number of healthy follicles were in winter, whereas the highest number of atretic follicles were seen in summer (Table 3).

Table 1: The follicle diameter in various ovarian follicles (Mean±SD)

		Primary F.			
Follicle type	Primordial F.	Simple layer multiple layers		Secondary F.	Graafian.
Size (μ)	26.4±0.31	33.6±0.4	84±18.6	373.7±56.7	1905.7±279.8

Table 2: Deference in mean distribution of various ovarian follicles in summer and winter (Mean±SD)

Parameter/ season	Summer	Winter
Mean distribution of healthy follicles	8.5±0.87	10.37±1.63
	**	*
Mean distribution of atretic follicles	17.95±1.5	15.16±1.81
Mean distribution of follicles in follicular phase	26.58±2.9	27.9±4.36
Mean distribution of follicles in luteal phase	26.16±3.11	23.4±3.17
Mean distribution of healthy follicles in follicular phase	7.5±0.91	12.1±3.5
Mean distribution of healthy follicles in luteal phase	9.5±1.47	9.8±1.38
Mean distribution of healthy follicles in follicular phase	7.5±0.91	10.7
	*	
Mean distribution of atretic follicles in follicular phase	19.7±2.47	15.57±2.12
Mean distribution of healthy follicles in luteal phase	9.9±1.5	9.8±1.38
	**	
Mean distribution of atretic follicles in luteal phase	17.09±2.19	14.6±3.3
Mean distribution of atretic follicles in follicular phase	19±2.19	14.8±1.47
Mean distribution of atretic follicles in luteal phase	17.09±2.19	14.6±3.3

* significant difference between the two groups(P<0.05)

** significant difference between the two groups(P<0.01)

Table 3: Deference in mean distribution of various ovarian follicles in one year (Mean± SD)

Parameter	one year (summer and winter)
Mean distribution of healthy follicle	9.43±0.92
	**
Mean distribution of atretic follicles	16.56±1.2
Mean distribution of follicles in follicular phase	26.18±2.64
Mean distribution of follicles in luteal phase	24.9±2.19
Mean distribution of healthy follicles in follicular phase	9.54±1.75
Mean distribution of healthy follicles in luteal phase	9.63±0.99
Mean distribution of atretic follicles in follicular phase	16.47±1.74
Mean distribution of atretic follicles in luteal phase	15.9±1.91

** significant difference between the two groups (P<0.01)

DISCUSSION

Histological observation showed that ovaries consist of an outer dense zona paremnchymatosa (cortex) and a central less dense vascular zona vasculosa (medulla). The epithelium that covers the ovary consists of a single layer of cuboidal cells and deep to the epithelium is the tunica albuginea, a layer of distinct dense connective tissue (Fig. 1).

Different classes of ovarian follicles were embedded in loose connective tissue of the cortex stroma.

The primordial follicles which their oocytes are surrounded by single layer of squamous follicular cells, are the source of the growing follicles, therefore they are the basic productive units in ovary [6].

The present study besides above mentioned notes, showed that primordial follicles are sparse and the cluster gather is rare in buffalo ovary (Fig. 2), but growing follicles which consist of primary, secondary and graffian follicles have the general histological features of other ovaries of domestic mammals.

According to histological characteristics [7], atretic signs in different classes of follicles were observed in ovaries of buffalo in this study (Fig. 3a-d).

In the most specimens, cystic follicles with various sizes were observed. They had specific features such as reduction of stratum granulosum and theca folliculi with a uniform of follicular fluid (Fig. 4).

The histomorphometeric study showed that the diameter of primary follicles is three times larger than primordial follicles and secondary follicles four times than primary and Graafian follicles five times than secondary ones (Table 1) and all of them are lesser than ovarian follicles of cow [8, 9].

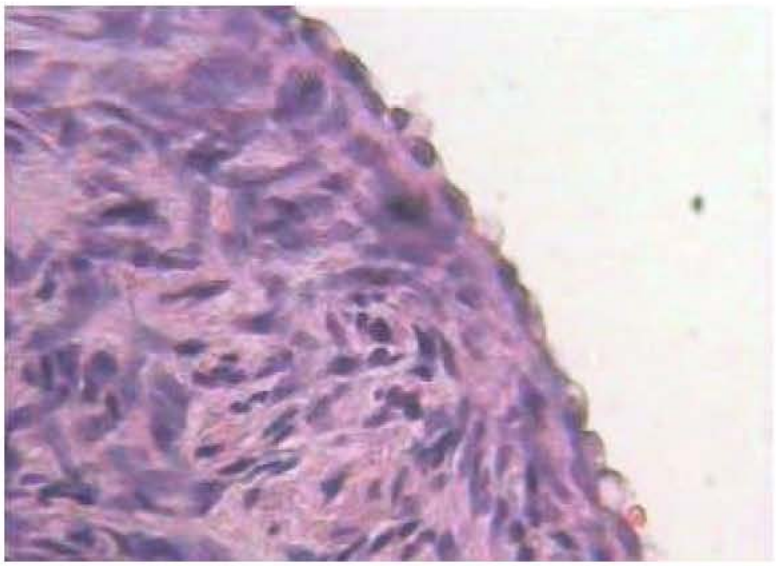

Fig. 1: Cortex of ovary with Simple cuboidal epithelium that covers it (HandE, X400).

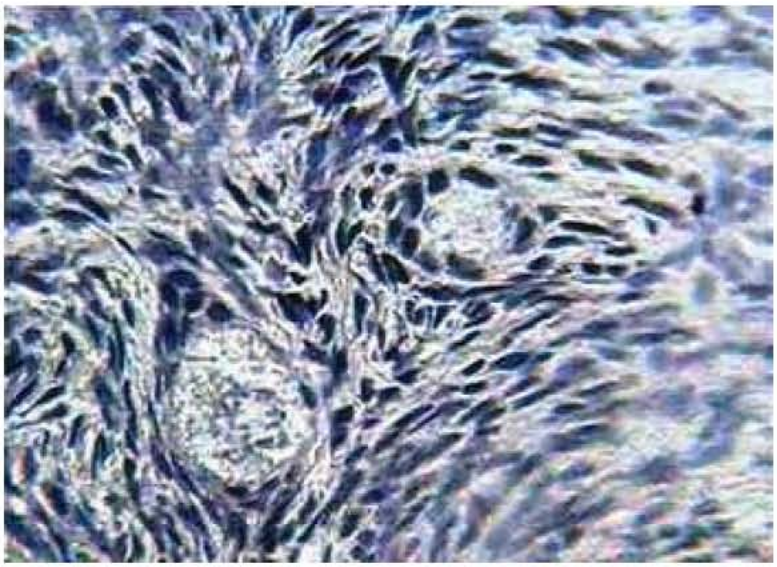

Fig. 2: Two healthy primordial follicles in cortex stroma of ovary (HandE, X400).

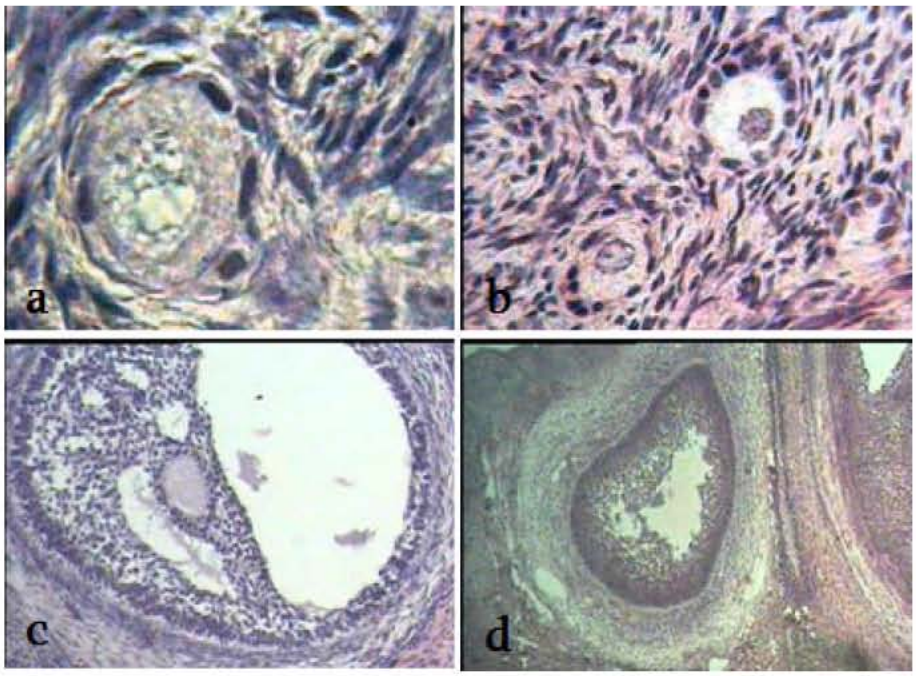

Fig. 3a-d: Atretic follicles: 3a:primordial follicle (HandE, X1000), 3b: primary follicle (HandE, X400), 3c:secondary follicle(HandE, X100), 3d: graffian follicle(HandE, X40)

There were highly significant difference in mean distribution of healthy and atretic follicles during summer, whereas this difference was only significant ($P<0.05$) in winter. This result suggested that, in both seasons, number of atretic follicles is higher than healthy ones. Therefore the atresia is ordinary in ovarian follicles in buffalo and this is the same as other animals whereas more than 99% of ovarian follicles go into process of atresia [4].

Deferences in mean distribution of healthy and atretic follicles in luteal phase during Summer was highly significant, whereas it was only significant during the follicular phase, while during winter difference were not significant. In relation to this condition, it seems that in luteal phase, because of endocrinological condition of buffalo, the growth of follicles is decreased and atresia increased, thus difference between healthy and atretic follicles increases. On the other hand,

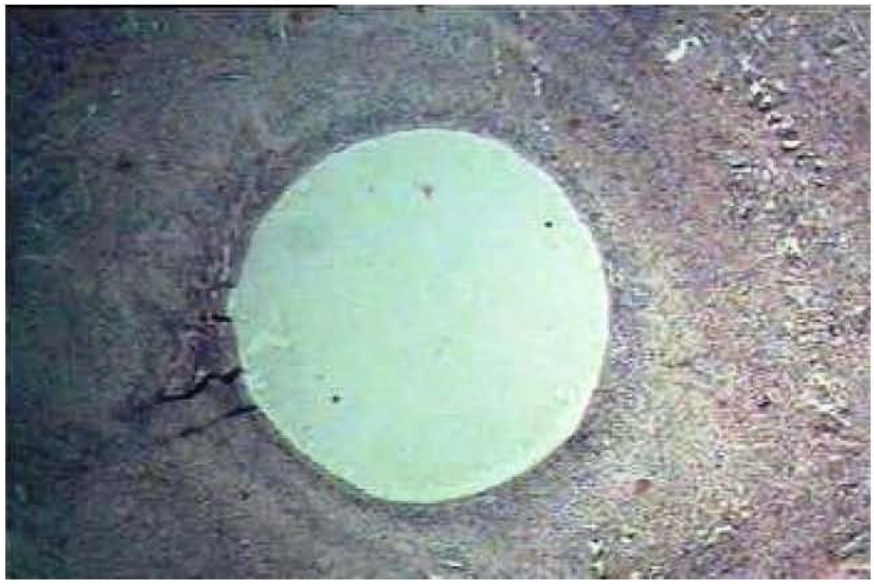

Fig. 4: Cystic follicle (HandE, X40)

the difference between healthy and atretic follicles in both follicular and luteal phases exists during summer, but not during winter. This indicated that during winter more follicles escape from atresia and the reproductive condition becomes better thgan during summer.

It was concluded that season (summer or winter) is more important than sexual phase (follicular or luteal), in mean differences between healthy and atretic follicles.The temperature was positively correlated to anoestrous in buffaloes[10], which indicated that during hotter period of the year development of primordial follicles to the large follicles and ovulations are less leading to anoestrous condition.The observation made in the present study supports the reports on low reproductive performance of buffalo during summer [2, 11].

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