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Dept. of Theriogenology,
Fac. Vet. Med., Assiut Univ., Assiut, Egypt

OVARIAN FOLLICULAR ATRESIA IN BUFFALO-COWS IN RELATION TO ITS SIZE AND STAGE OF ESTROUS CYCLE

(With 4 Tables and 3 Figures)

By

A. ALI; A. KH. ABDEL-RAZEK and S. KH.ABD ELGHAFFAR*

*: Dept. Pathology, Fac. Vet. Med., Assiut Univ., Assiut, Egypt

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اضمحلال جريبات المبيض في الجاموس وعلاقته بحجم الجريبة ومرحلة دورة الشبق

أحمد مصطفي على ، عبد الرازق خليفة ، سارى خليل

خطط هذا البحث بغرض ملاحظة معدل ودرجة اضمحلال الجريبات وعلاقة تلك التغيرات بحجم الجريبة ومرحلة دورة الشبق في الجاموس. تمت الدراسة على ٢١ جهاز تناسلي جمعت من السلخانة و تم تقسيم دورة الشبق طبقا للتركيبات المبيضية الموجودة إلى : مرحلة الجسم الأصفر المبكر و مرحلة الجسم الأصفر و مرحلة الجريبات. وتم فحص التركيب النسيجى المرضى(الهستوباثولوجى) لتلك التركيبات. وقسمت الجريبات حسب حجمها إلى صغيرة ومتوسطة و كبيرة. و كذلك قسمت طبقا لدرجة الاضمحلال إلى أربعة مجموعات : الغيرمضمحلة والخفيفة الاضمحلال و المتوسطة الاضمحلال وشديدة الاضمحلال. وقد أوضحت النتائج أن الجريبات الكبيرة (عددها٣١) كانت نسبة الاضمحلال فيها ٥٤,٨٤% و ٣٢,٢٦% و ٩,٦٨% و ٣,٢٣% وفي الجريبات متوسطة الحجم(عددها١٧) كانت ٢٩,٤١ % و ٤٧,٠٦% و ١١,٧٧% و ١١,٧٧% بينما كانت في الجريبات الصغيرة (عددها٧٩) ١٦,٤٦% و ١٠,١٣% و ٥٦,٩٦% و ١٦,٥% وذلك بالنسبة للجر يبات الغير مضمحلة وخفيفة و متوسطة وشديدة الاضمحلال على الترتيب. و أوضحت الدراسة أيضا أن نسبة الاضمحلال في الجريبات الكبيرة ٢٢,٢٢ كانت % و ٦٤,٧١% و ٢٠% أثناء مرحلة الجسم الأصفر المبكرة ومرحلة الجسم الأصفر ومرحلة الجريبات على الترتيب ، بينما كانت تلك النسبة في الجريبات المتوسطة ٦٢,٥% و٦٠% و١٠٠% خلال المراحل الثلاثة على الترتيب ، وفي الجريبات الصغيرة ٨٢,٥% و ٨٨% و ٧٨,٥٧% خلال نفس المراحل على الترتيب. و قد أوضحت النتائج أن مجموعه كبيرة من الجريبات المتوسطة و الصغيرة قد وجدت في حالة اضمحلال وأن تأثير دورة الشبق كان واضحا على معدل الاضمحلال في الجريبات الكبيرة دون الصغيرة.

SUMMARY

The purpose was to study the incidence and degree of follicular atresia in buffalo-cows in relation to follicular size and stage of the cycle. Genital tracts (n=21) were collected from local abattoir. Estrous cycle was classified into early luteal, luteal and follicular phases. Follicular populations were categorized into large, medium and small follicles. Ovaries were processed histopathologically. Their follicles were classified into non- (NA), light- (LA), moderate- (MA) or sever-atretic (SA). The results showed that, large follicles (n=31) were NA in 54.84%, LA in 32.26%, MA in 9.68% and SA in 3.23%, while medium follicles (n=17) were in 29.41, 47.06, 11.77 and 11.77% NA, LA, MA and SA respectively. The small follicles (n=79) were in 16.46, 10.13, 56.96 and 16.5% NA, LA, MA and SA, respectively. The incidence of atresia in large follicles was 22.22, 64.71and 20.0% during early luteal, luteal and follicular phases, while the incidence was 62.50, 60 and 100% in the medium follicles and 82.5, 88 and 78.57% in the small follicles, respectively. It was concluded that, 1) high proportion of small and medium follicles were found in atretic status, 2) stage of the cycle influences the incidence of atresia in large but not in the small follicles.

Key words: *ovary, follicle, atresia, estrus cycle, buffaloes*

INTRODUCTION

The water buffalo is used in many countries including Egypt as a source of milk and meat production. In spite of its usefulness, the reproductive capacity of buffalo is relatively poor in comparison to cattle (Dobson and Kamonpatana, 1986, Singh *et al.*, 2000). The low fertility in buffalo may be caused by intrinsic, extrinsic and environmental factors (Gordon, 1997 and Singh *et al.*, 2000).

Ovarian follicular atresia has been described as a normal phenomenon in the bovine ovary (Rajakoski, 1960 and Marion, *et al.*, 1968). However, the incidence of atresia in buffaloes was much higher than that recorded in cattle (Rajakoski, 1960, Aboul Fadle, *et al.*, 1974, Maurasse, *et al.*, 1985, Danell, 1987, Grimes, *et al.*, 1987, Bharadwaz and Roy, 1999 and Irving-Rodgers, *et al.*, 2001). Moreover, waves of degeneration were observed in the oogonia and oocytes of the buffalo fetal ovaries (El-Ghannam and El-Naggar, 1975). Also, the number of primordial follicles and Graafian follicles ≥1 mm in diameter in the ovaries of the buffalo heifers were much lower than in cattle (Erickson, 1966 and Samad and Nassari, 1979). Whether, the low number of follicles and the high incidence of atresia are related to the low fertility in buffalo, is questionable?.

Indeed, many factors have been reported to affect the frequency of ovarian follicular atresia. Diet (Maurasse, *et al.*, 1985), stage of the estrous cycle (Marion, *et al.*, 1968 and Guibault, et al, 1993), hormone application (Maracek, *et al.*, 1977 and 1983, Monniaux, *et al.*, 1984, Blondin *et al.*, 1996 and Yang and Rajamahendran, 2000) and days postpartum (Dufour and Roy, 1985) were noticed to affect the incidence of atresia in cattle.

Consequently, the purpose of this study was to observe the influence of the follicular size and the stage of estrous cycle on the frequency of follicular atresia in the Egyptian buffalo-cows.

MATERIALS and METHODS

The female genital tracts of twenty one healthy, mature (5-8 years) buffalo cows were collected from locally slaughtered buffaloes during spring season. The female genital tracts were collected within 15 minutes after slaughter and evisceration. The ovaries were dissected free along the meso-ovarium. According to the morphology of the corpus luteum ((Jainudeen, *et al.*, 1983) and the follicular dynamics (Ali *et al.*, 2002), the day of the estrus cycle was approximately determined. Accordingly the animals were classified into:

a) Animals in early luteal phase (d1 to d8, n=7): It is the interval between ovulation and the time when the corpus luteum is fully formed with vasculature visible around its periphery. The apex of the corpus luteum is red or brown and the remaining is grayish. Parallel to the corpus luteum, no follicles larger than 8 mm in diameter should be found. Later on in this phase, one follicle > 8 mm in diameter could be detected.
b) Animals in luteal phase (d 9 to d16, n=11): This phase begins when the red or brown color disappears leaving the entire corpus luteum bright red or gray. This phase is also characterized by the presence of 1-2 follicles larger than 8 mm in diameter.
c) Animals in follicular phase (d17 to d21, n=3): This phase is characterized by the presence of a regressing corpus luteum. The regressed corpus luteum appears small, hard, bright and with no

vasculature visible on its surface. Also, at least one follicle larger than 8 mm in diameter should be found during this phase.

The ovaries were fixed in Bouin's solution for 24h, thereafter dehydrated, paraffin embedded, sectioned and stained with Haematoxline and Eosine (Bancroft and Stevans, 1982).

For microscopic measuring and categorizing of the antral follicles, the method described by Rajakoski (1960) modified by Danell (1987) was followed. Accordingly, the follicular populations were categorized into:

a. Small sized follicle, 1-< 5 mm in diameter

b. Medium sized follicle, 5-8 mm in diameter

c. Large sized follicle, > 8 mm in diameter

For classification of the antral follicles as normal or atretic, methods and criteria described by Rajakoski (1960) were followed. The follicles were classified into:

a. Non atretic (NA) b. Light atretic (LA)

c. Moderate atretic (MA) d. Severe atretic (SA)

Frequencies were compared with Chi-square test using SPSS version 10. Data were considered to be significantly different at $p< 0.05$.

RESULTS

Histopathological pictures of follicular atresia:

A total of 31 large, 17 medium and 79 small sized follicles were examined microscopically for the incidence and degree of atresia.

The small non-atretic Graaffian follicle (Fig. 1a) was characterized by antrum formation, which was surrounded by 6-8 layers of granulosa cells resting on a basement membrane. A cluster of granulosa cells, which formed the cumulus oophorus, surrounded the oocyte. The theca layers, which surrounded the Graaffian follicle, were made of two parts, the inner theca interna and the outer theca externa. The theca interna consisted of two types of cells derived from connective tissue. The first were epitheliod cells with vesicular nuclei, while the outer was a loose net work of connective tissue. The outer layer, the theca externa, had a relatively low number of connective tissue cells and formed a fibrous sheath around the follicle.

In light atretic small Graaffian follicle (Fig. 1b) a number of pyknotic nuclei in the liquor folliculi and in the granulosa layer of the follicular wall could be seen. In sections where the cumulus oophorus could be seen, this was usually still intact. The moderate atretic follicle (Fig. 1c) was characterized by completely degenerated granulosa cell layer and no or very few pyknotic nuclei in the antral fluid. Later on the cumulus disappeared and only naked pyknotic oocytes remained. In severe atretic small follicle (Fig. 1d), the granulosa layer and the epitheliod cells disappeared and connective tissue cells predominated. Ingrowth of connective tissue took place in the antrum.

The wall of the non-atretic medium sized follicles (Fig. 2a) had more or less similar structure as that of small antral one. The light atretic one showed degeneration and pyknosis of some granulosa cell, which was sequestrated in the antrum (Fig. 2b). Moderate atretic follicle was characterized by disappearance of granulosa cell, and cumulus oophorus. The oocyte may appear naked in the antrum (Fig. 2c). In severe atretic follicle, there was ingrowth of connective tissue in the antrum (Fig. 2d).

In large sized normal follicle, the layer of granulosa cells were thinner, while the theca externa was rich in vasculature (Fig. 3a). The light atretic one showed pyknonsis and sequestration of the granulosa cells (Fig. 3b). The granulosa cell layer as well as the epitheliod cells of the theca interna were completely disappeared in the moderate atretic large follicle (Fig. 3c). Severe atretic large follicles showed marked ingrowth of connective tissue in the antrum (Fig. 3d).

Incidences of atresia in relation to follicular sizes:

The incidence of follicular atresia in relation to the follicular size is shown in Table (1). High proportion of the small and medium sized follicles were found in an atretic status, 83.54 and 70.59% receptively, in comparison to 45.16% for the large sized follicles, $p < 0.001$. The most common form of atresia in the large and medium sized follicle was the light form, 32.26 and 47.06% respectively, while moderate and severe forms were recorded mainly in the small sized follicles, 56.96 and 16.46% respectively, $p < 0.001$. In general, the incidence of atresia in all sizes of the antral follicles was 72.44%.

Effect of stages of estrous cycle on the incidence of atresia:

Effect of stages of estrus cycle on the incidence of atresia in shown in Tables 2, 3 and 4. The incidence of atresia in the large follicles was higher during the luteal phase (64.71%), than during the early luteal (22.22%) or during the follicular phase (20%), $p < 0.05$, (Table 2). On the other hand, this incidence did not differ statistically from one stage to the other of the estrus cycle either for the medium (Table 3) or the small (Table 4) sized follicles.

Table 1: The Incidence of follicular atresia in relation to the follicular size.

Follicular size	Nr. Follicles	Incidence of Atresia (n%)				
		NA*	LA	MA	SA	TA
Large sized follicle (>8mm Ø)	31	17 [a] 54.84%	10 [a] 32.26%	3 [a] 9.68%	1 [a] 3.22%	14 [a] 45.16%
Medium sized follicle (5-8 mm Ø)	17	5 [a] 29.41%	8 [a] 47.06%	2 [a] 11.77%	2 [a] 11.77%	12 [a] 70.59%
Small sized follicles (1-<5 mm Ø)	79	13 [b] 16.46%	8 [b] 10.13%	45 [b] 56.96%	13 [b] 16.46%	66 [b] 83.54%
Total	**127**	**35 27.56%**	**26 20.47%**	**50 39.37%**	**16 12.60%**	**92 72.44%**

*NA: non atretic, L: A light atretic, MA:Moderate atretic, SA: sever atretic, TA: totally atretic. [a-b] in the same column is significant, $p<0.05$

Table 2: Effect of stage of estrus cycle on the incidence of atresia in large sized follicles (>8mm in diameter, n=31)

Stage of estrus cycle	Examined follicles (n)	Incidence of atresia (n%)
Early luteal phase (d1-d8)	9	2 (22.22%) [a]
Luteal phase (d9-d16)	17	11 (64.71%) [b]
Follicular phase (d17-d21)	5	1 (20.00%) [a]

[a-b] in the same column is significant, $p<0.05$

Table 3: Effect of stage of estrus cycle on the incidence of atresia in medium sized Follicles (5-8mm in diameter, n=17)

Stage of estrus cycle	Examined follicles (n)	Incidence of aAtresia (n%)
Early luteal phase (d1-d8)	8	5 (62.50%) [a]
Luteal phase (d9-d16)	5	5 (60.00%) [a]
Follicular phase (d17-d21)	4	4 (100.00%) [a]

[a-a] in the same column is not significant

Table 4: Effect of stage of estrus cycle on the incidence of atresia in small sized follicles (1-< 5 mm in diameter, n=79)

Stage of estrus cycle	Examined follicles (n)	Incidence of atresia (n%)
Early luteal phase (d1-d8)	40	33 (82.50%) [a]
Luteal phase (d9-d16)	25	22 (88.00%) [a]
Follicular phase (d17-d21)	14	11 (78.57%) [a]

[a-a] in the same column is not significant

DISCUSSION

The histological picture of the ovarian follicular atresia, that recorded in the present study in the Egyptian buffalo-cows, is similar for a large extent to that reported in Surti buffalo in Indian (Danell, 1987) and to that recorded in cattle (Rajakoski, 1960 and Marion, *et al.*, 1968). Although, Irving-Rodgers, *et al.* (2001) indicated that, the original histological classifications of atresia were inaccurate. Many other authors confirmed that the conventional histological method as well as flow cytometry, ELISA, follicular fluid content of prorenin, estradiol, progesterone, testosterone and chondroitin sulphate are acceptable methods for analyzing follicular atresia in bovine granulosa cells (Blondin, *et al.*, 1996, Mukhopadhyay, *et al.*, 1991, Bellin and Ax, 1984).

The average frequency of atresia for all follicular sizes was about 72%, which is comparable to a frequency of 70%, which was recorded in Surti-buffalo by Danell (1987). On the other hand this incidence is much higher than that recorded in different breeds of cattle (Rajakoski, 1960, Maurasse, *et al.*, 1985 and Irving-Rodgers, *et al.*, 2001). The high rate of atresia in buffalo ovaries compared to cattle, meant that the available number of normal Graaffian follicles > = 1 mm in buffalo ovaries is much lower than that found in cattle ovaries. Consequently, this might influence the reproductive process and fertility in buffalo, and evoke equation about the possible causes of increased incidence of atresia in this species.

The present study indicated that high proportion of the small and medium sized follicles were present in an atretic status, which might be due to a suppressive effect of the large follicles on the subordinate ones. The same result was observed in buffalo heifers in India (Danell, 1987). In cattle, the dominate follicle suppressed the development of the subordinate follicles either through a local (Draincourt, 1991) and/or through a systemic (Ginther, *et al.*, 1989) pathways.

There was cyclical variation in the incidence of atresia in the large follicles during the sexual cycle. During d1-d8 most of the large follicles were normal. During the mid-cycle about half of the largest follicles were normal and half was atretic. During day 17-21 most of the largest follicles were non-atretic. This variation in the follicular system during the sexual cycle in buffalo could indicate the wave-pattern of follicular growth. This hypothesis was suggested previously in buffalo using rectal palpation (Singh, *et al.*, 1984), histological examination (Danell, 1987), histopathological and endocrinological studies (Ali, *et al.*, 2002) and ultrasonographic technique (Manik, *et al.*, 1994 and Taneja, *et al.*, 1996).

According to the current study on the Egyptian buffalo-cows, it could be concluded that, the incidence of ovarian follicular atresia is relatively high, which might be a cause of the low fertility in buffalo. The stage of estrous cycle affects the frequency of atresia in the large but not in the small or the medium follicles.

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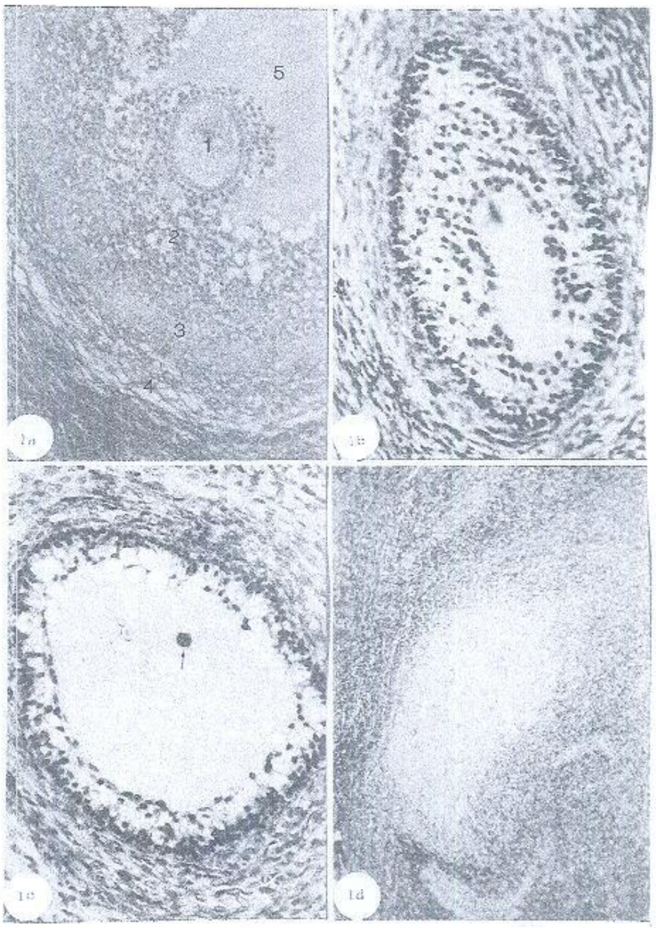

Fig. 1: Small sized follicle showing: a) Non-atretic follicle: 1: Oocyte, 2: Granulose cell layer, 3: Theca interna, 4: Theca externa, 5: liquor folliculi. b) Light atretic follicle with degeneration and pyknosis of the granulose cell. c) Moderate atretic follicle with necrosis in the granulose cells and advanced necrosis in the oocyte which appear naked in the antrum (arrow). d) Severe atretic follicle with ingrowth of connective tissue inside the antrum (H&E. 10x25).

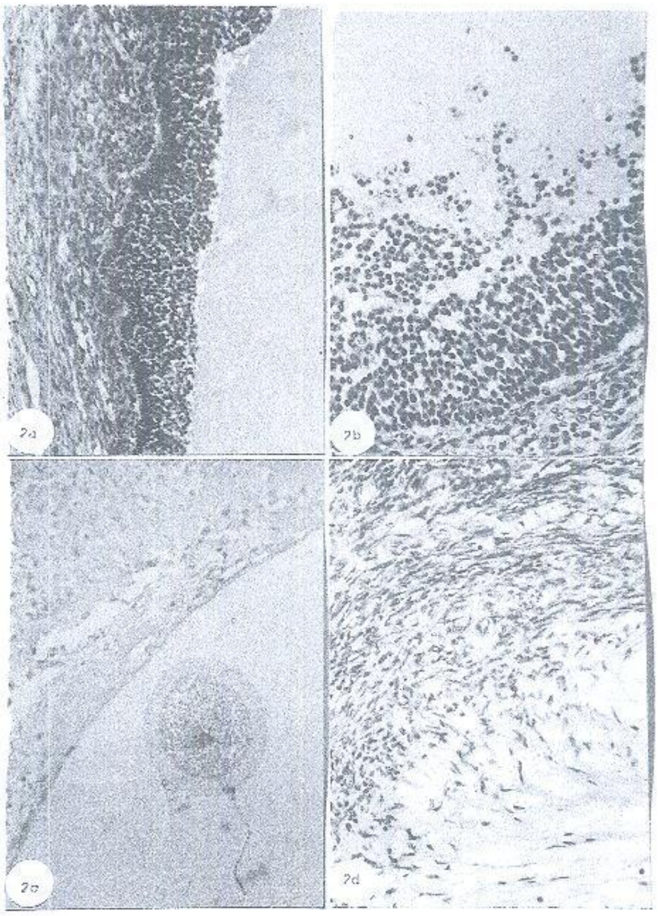

Fig. 2: Medium sized follicles showing: a) Non atretic follicle. b) Light atretic follicle with pyknosis and sequestration of the granulose cells. c) Moderate atretic follicle: cumulus disappeared, only naked oocyte remains with hyalinization of its zona pellucida. D) Severe atretic follicle with ingrowth of connective tissue inside the antrum (H&E.10x25).

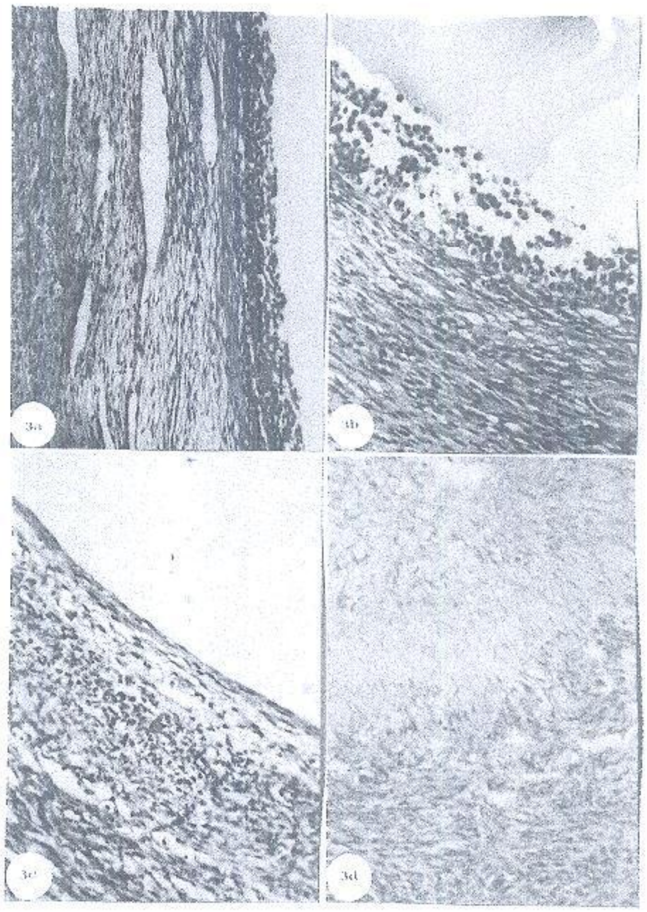

Fig. 3: Large sized follicles showing: a) Non atretic follicle. b) Light atretic follicle with pyknosis and sequestration of the the granulose cell in the antrum. c) Moderate atretic follicle showing disappearance of the cumulus and granulose cell layer. d) Severe atretic follicle with marked connective tissue ingrowth inside the antrum (H&E. 10x25).