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MICROSCOPICAL STUDIES ON FOLLICULAR ATRESIA DURING OOGENESIS IN UROMASTYX AEGYPTIA

BY

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ABSTRACT

The process of follicular atresia is quite common in U. aegyptia, especially in previtellogenic follicles. It seems similar to that reported for other reptiles in that the granulosa cells apparently play a role in phagocytosis of the ooplasm. These become, later, dagenerated whereas the theca interna becomes hypertrophied and remain as a stromal tissue. No follicular fluid has been observed during follicular atresia.

INTRODUCTION

Follicular atresia is common in most reptiles and has been reported in Seps [1], in Terrapene [2], in Anguis, Lacerta, Natrix, Vipera, Terrapene. Testudo, Crocodilus [3], in Natrix, Thamnophis [4,5], in Xantusia [6], in Terrapene [7], in Natrix rhombifere [8], in Naja tripudians and Bungarus coeruleus [9] and Calotes versicolor [10]. However, it occurs less frequently in Platydactylus [3] and Hoplodactylus [11].

Mingazini [1] was the first to describe the atretic follicles and corpora lutea in reptiles and considered them as false and true corpora lutea respectively. The observation of Dubisson [2] and Boyd [11] is in agreement with Bragdon [4] who stated that the active participation of the granulosa cells in the removal of follicular debris during atresia results in marked granulosa hypertrophy. Betz [8] found that the rugae of the granulosa of atretic follicle probably result from the shrinkage of the follicle, caused by a decrease in the amount of yolk. Also the increase in the size of the cells "due to the increased number of intracytoplasmic vacuoles" would tend to increase the intercellular pressure enough so that formation of rugae would be favored.

The follicular cells probably play an important role in the digestion and removal of ooplasmic contents [8-10]. Weekes [12], Altland [7], Bragdon [4], Betz [8] and Roger [13], have observed that the theca interna cells appear to play a minor role in the phagocytosis of yolk during atresia.

The interstitial cells have been denied in the ovaries of reptiles [8,14]. However, Guraya [15] has reported their presence in the ovary of lizard (Hemidactylus). Moreover, Guraya [9] and Varma [10] stated that the hypertrophied theca interna cells of degenerating follicles may constitute ovarian interstitial gland cells.

MATERIAL AND METHODS

The animals in the present investigation were the desert lizard, Uromastyx aegyptia. Adult female specimens of U. aegyptia were collected monthly from Saudi Arabian desert (Khoreis road, about 58 kilometers east to Riyad). The total specimens examined in this work allover the year were 70 females.

The ovaries were dissected out immediately from the narcotised animals and were fixed in Bouins fluid, neutral buffered formalin (N B F) solution, formol-calcium, and the dichromate sublimate fixative of Elftman [16]. Tissues were dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin wax. Cross sections of 4-6 microns in thickness were cut, mounted and stained with haematoxylin and eosin.

RESULTS

Atresia may occur in the follicle at any stage of development(Fig. 1). It is more frequently seen in the non-vitellogenic follicles with a polymorphic granulosa, or in early stages of vitellogenesis. Up to 9 follicles may be found at different stages of atresia in one ovary.

It has been found that atresia in U aegyptia may be divided into different stages.

Follicular atresia stage 1:

Macroscopically, the atretic follicle is not appreciably different from the surrounding follicles especislly at early stages of atresia. Microscopically, The first sign of atresia is the slight vacuolar appearance of the oocyte. The ooplasm shows some degree of disorganization (Fig. 2). The cytoplasm of the granulosa cells is filled with variously-sized vacuoles. Intermediate and pyriform cells are reduced in size and are similar enough that they can not be distinguished from each other. Their nuclei are smaller than those of normal intermediate or pyriform cells. In the periphery of the granulosa, the small cells typically form a single layer, two or three cells deep. Nuclei of some cells are somewhat pycnotic and stain intensely.

Follicular atresia stage II:

The folliole appears shrunken and vascular (Fig. 3). The small granulosa cells have much increased in number, whereas the remaining cells, formerly known as intermediate and pyriform cells, show a marked decrease in size and number. The follicular cells become now monomorphic instead of the characteristic polymorphic appearance and they possess deeply stained nuclei and vacuolated cytoplasm. The vacuoles between these granulosa cells become numerous (Fig. 4). This stage is characterized by a very disorganized ooplasmic mass which is reduced in quantity (especially at the periphery of the oocyte and becomes yet deeply stained . The zona pellucida as well as the radiata disappeared so that there is no separation between the granulosa cells and the ooplasm. A large number of granulosa cells migrates towards the center of the oocyte, where some of them are located. Migration of the granulosa cells occurs from almost all the circumference of the oocyte. Therefore, the granulosa cells, located at their original places, become fewer in number resulting in the decrease of the layer's thickness certain regions. On the other hand the theca interna has considerably increased in thickness and its cells possess round nuclei.

Follicular atresia stage III:

The aggregations of ooplasmic substance either in the center or at the periphery of the oocyte are no longer observed (Fig. 5). They have become completely phagocytozed and the interior of the follicle is invaded with numerous granulosa cells which are usually seen concentrated at one side. These granulosa cells, wherever located, are undergoing degeneration. This is evidenced by cell autolysis (appearance of vacuoles within the cytoplasm and chromatolysis of the nuclei). In areas of granulosal coalescence there are occasional patches of connective tissue (Fig. 6). The theca externa is much thinner and thecal cell becomes more numerous.

Follicular atresia stage IV:

The cells of the granulosa layer grow in size due to ingestion of the ooplasm. These cells are arranged in a radial manner and become characteristically elongated. Their bulging free ends protrude into the follicular cavity as cytoplasmic patches surrounded by phagocytes, apparently from the granulosa layer (Fig. 7). The cytoplasm of the granulosa layer appears highly granular. The theca becomes very highly vascular and no distinction of theca interna and externa could be seen. Through some clear areas in the granulosa layer, strands of fibrous tissue are uaually seen, with occasional fibroblasts insinuated between the columnar granulosa cells.

Follicular atresia stage V:

In this stage the atretic follicle becomes strongly collapsed and its'follicular cavity is markedly reduced. The columnar granulosa epithelial cells undergo degeneration and resorption and finally they disappear, with a concomitant invasion of the follicle by connective tissue elements (Fig. 8). Finally, the follicle is reduced to a small variously persisting scar of dense connective tissue in the ovarian stroma.

DISCUSSION

Follicular atresia, in reptiles, results in substantial changes and modifications of the granulosa cells of the regressed follicle. The process of follicular atresia in U aegyptia appears to be similar to that reported for other reptiles [4-6,11,17-19] in that the granulosa cells apparently play a role in phagocytosis of the ooplasm and removal of follicular debris.

The follicular granulosa cells of U aegyptia are involved in follicular atresia, as well as luteal tissue formation and they may play a role in yolk deposition.

In the case of previtellogenic atretic follicles, phagocytosis was found more evident. Many macrophages were observed in the ooplasm which is not the case in the vitellogenic follicles. According to Betz [8] and Varma [10], the phagocytes are probably transformed follicular cells.

There are conflicting reports on the frequency of follicular atresia in different lizards and snakes. Boyd [11] reported that it is a rarity in Hoplodactylus, but it is of common occurrence in the Thamnophis sirtalis and Natrix sipedon, Bragdon [5]. In Xantusia vigilis, Miller [6] found that while 20 to 40 follicles begin development every year all but one or two undergo atresia. Betz [8] reported that atresia may occur in the follicles of the water snake

Natrix rhombifera at any stage of development but it is more frequently seen in follicles with polymorphic granulosa (previtellogenic) or in mature follicles which have not been ovulated. Follicular atresia in U. aegyptia of the present investigation and in Chalcides [18], is fairly common in nonvitellogenic eggs and during early stages of yolk deposition, but the regression of late vitellogenic eggs is only occasional. The frequency of atresia in U. aegyptia increases in postovulatory ovaries and ovaries out of the breeding activity. Varma [10] suggested that this may be due to low levels of gonadotrophins after the breeding period is over.

Fig.1 Portion of C.S. of ovary with different stages of atresia.
(Hx. and E.) X 88.

Fig. 2 Portion of C. S. of ovary with an early atretic follicle. Note the vacuolar appearance (V.) of the ooplasm.
(Hx. and E.) X 244.
G. = Granulosa
Oc. = Oocyte
Th. = Theca
Z.p.= Zona pellucida.

Fig. 3 Portion of C.S. of ovary with advanced stage of atretic follicle. Note a very disorganized ooplasmic mass (O.m.) which is reduced in quantity and becomes yet deeply staining. (Hx. and E.) x 247.

Fig. 4 An enlarged portion of Fig. 3, showing phagocytic granulosa cells (Ph.g.c.) migrating toward the centre of the oocyte (Oc.) and the appearance of vacuoles (V.) between and withen the granulosa cells (G.)
(Hx. and E.) X 1440

Fig. 5 More advanced stage of atretic follicle, showing the disappearance of ooplasmic substance which is replaced by phagocytic cells (Ph. g. c.) (Hx. and E) X 228.

Fig. 6 An enlarged portion of Fig. 5 showing Phagocytic granulosa cells (Ph. g.c.) almost degenerating and having vacuolated cytoplasm and chromatolysed nuclei (Ch.n.) Note the patches of connective tissue (C.t.) in the areas of granulosal coalescence. (Hx. and E) X 576.

Fig. 7 Late stage of atretic follicle, showing the growth of granulosa cells from the ingestion of the ooplasm They are now columnar in shape (Hx. and E.) X1400.

Fig. 8 Atertic follicle about to come to an end. Notice the reduction of the follicular cavity which is filled with degenerating cells (Dg.c.), and the complete disappearance of the granulosa layer. Also notice the beginning of connective tissue invasion in its way to transform the follicle to a stromal tissue elements (Hx. and e.) X560.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

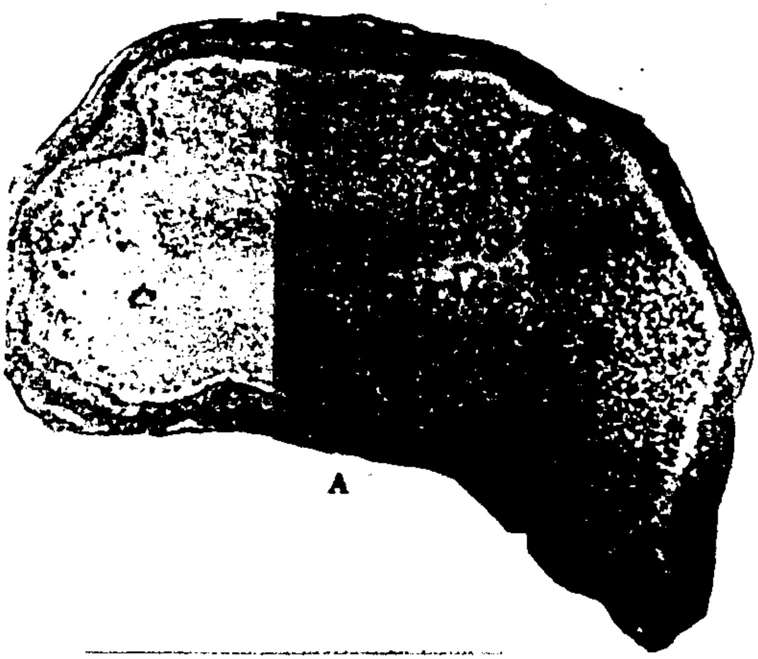

Fig. 5

Fig. 6

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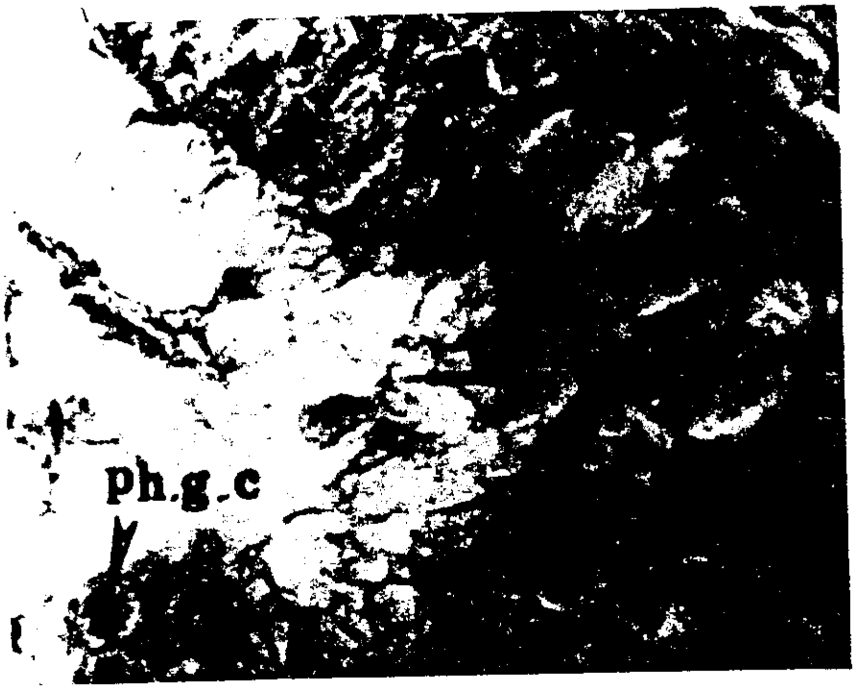

Fig.7

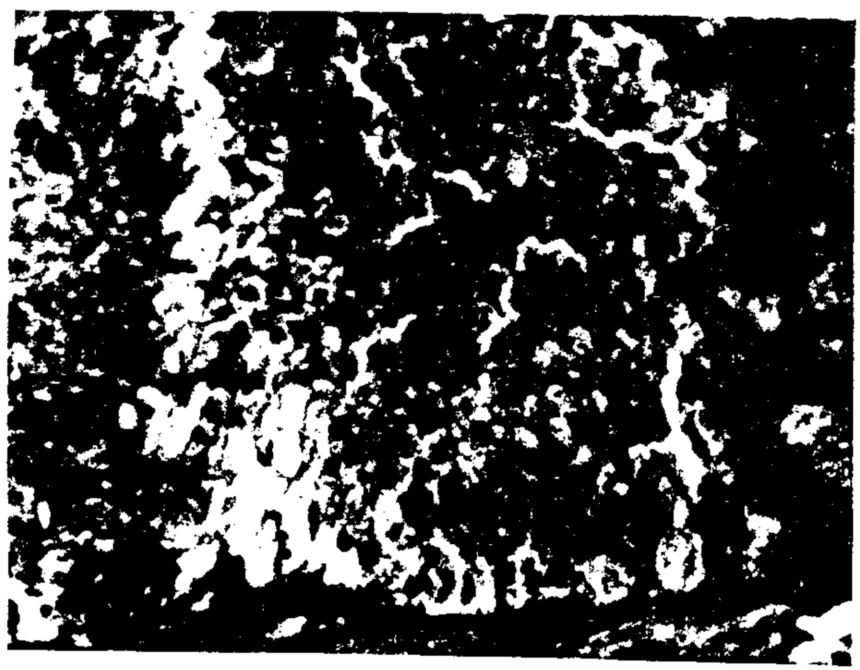

Fig.8

دراسات ميكروسكوبية على الحويصلات المندثرة
خلال التبويض فى الضب المصرى

السيد محمد حمودة ، زكى توفيق زكى ، ابراهيم جمعة ابراهيم ، محمد عثمان على

قسم علم الحيوان ـ كلية العلوم

جامعة الازهر

تم دراسة التغيرات التى تطرأ على بعض الحويصلات المبيضيه قبل تكوين المح فيها والتى يتم اندثارها فى مبيض الضب المصرى وقد وجد انها تشابه مثيلاتها فى بعض الزواحف الاخرى. ودلت الدراسه على ان خلايا الطبقه المحببه الاكوله تقوم بالتهام محتويات البويضة حتى تستهلكها عن آخرها .
وتتداعى جميع خلايا الطبقه المحببة ، بينما يتضخم الغلاف الحويصلى الداخلى ويبقى كسدى للمبيض . واثناء اندثار هذه الحويصلات لا يتكون سائل حويصلى ابدا .