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FOLLICULOGENESIS AND FOLLICULAR ATRESIA IN ALGERIAN RUMBI EWES

(With One Table and 17 Figures)

By

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تكوين وتراجع جريبات المبيض عند نعاج سلالة الرمبى الجزائرية

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أجريت هذه الدراسة للمساهمة فى التعرف على سن البلوغ الجنسى عند نعاج سلالة "الرمبى" الجزائرية، وذلك عبر دراسة نمو وتراجع جريبات المبيض. لقد تبين لنا من خلال النتائج أن سن البلوغ الجنسى فى هذه السلالة من الغنم، يبدأ من سن عشرة (١٠) إلى إثنى عشر(١٢) شهراً، مع بداية فترات الشبق عند الإناث الصغار. يبدأ المبيض بتكوين عدد من الجريبات البدائية والجريبات الأولية. عدد هذه الجريبات يبدأ فى التراجع مع التقدم فى السن. يرجع هذا التناقص فى عدد الجريبات إلى التراجع فى تكوين الجريبات نفسها على مستويات متعدده من نموها. هذا التراجع فى نمو الجريبات يمس خاصة الجريبات فى المرحلة النهائية وما قبل النهائية. تم ملاحظة نمو جريبات كاملة الخصائص وجسيمات صفراء فى مبايض النعاج التى بلغ سنها الثمانية (٨) أشهر. هذا يدل على قابلية هذه النعاج على التلقيح، إذا كان وزنها مناسب.

SUMMARY

This work is a contribution to the knowledge of the age of entry in puberty, by the study of folliculogenesis and follicular atresia in Algerian Rumbi ewes which is characterized by very appreciable reproduction parameters. It seem that puberty in this ovine breed appear between 10 and 12 months, with the onset of first heats in pubertal ewes. The present results show that the ovaries present a germinative area, constituted with a great number of primordial and primary follicles. Their number decrease gradually with the advancement of the ewes. This regression is due to follicular atresia at various stages of their development. This atresia is more marked in the pre-antral and antral follicles. Presence of corpora luteum and graafian follicles without any structural anomaly was observed on young ewe's ovaries, of 8 months old, meaning that they can be fertilized if their body weight is satisfied.

***Key words**: Folliculogenesis, atretic follicle, ovary, algerian rumbi ewes, puberty.*

INTRODUCTION

As in other mammals, ewe's ovary is the seat of formation and oocytes maturation, cellular differentiation, hormone synthesis, apoptosis, folliculogenesis and follicular atresia. The germinal cells reserve formed during embryonic development represents a very significant stock of follicles and oocytes at pubertal age (Saumande, 1991).

During folliculogenesis, primordial follicles growth into Graafian follicle, which ovulate a mature oocyte, and there after give rise to a corpus luteum. Primordial follicles are formed just before or immediately after birth, when primary oocytes are surrounded by a layer of flattened somatic cells (Land, 1970). When primary follicles begin their development, pre granulosa cells became cubical (Wandji *et al.* 1997; Fortune, 2003). The total period of growth of a primary follicle to reach the pre ovulatory stage exceeds six months (Cahill and Mauleon, 1980). In the ewe, the stock of primordial and primary follicles is estimated to be between 40.000 and 300.000 follicles (Cahill and Mauleon, 1981; Driancourt *et al.* 1991). Only a small number of themes became Graafian follicles Carroll *et al.* 1990, and the majority is dedicated to involution or follicular atresia Gordon, 1994. Atresia represents the follicular regression until its disappearance in the ovarian stroma (Thibault and Levasseur, 2001). It occurs by apoptosis or follicular cells and oocyte death (Chun and Hsueh, 1998). Algerian Rumbi breeds are resulting from crossing the muffled rams of "Djebel Amour", and "Ouled Djellal" ewes; this ovine breed is characterized by noticeable reproduction parameters: fertility (80%), fecundity (95%) and prolificacy (110%). Puberty appears between 10 and 12 months of age, and appearance of first heats in young ewes does not mean that they assumed their puberty, and can be fertilized (Chelling, 1992); however, few studies were devoted to the ovary function.

This work is a contribution to the knowledge of puberty entering age in Algerian Rumbi ewes, by studying folliculogenesis and follicular atresia.

MATERIALS and METHODS

Ovaries of young ewe (n = 60) aged from 2 to 12 months, were collected at Tiaret commercial slaughter-house, during the period from March to June 2003. Ovaries were divided in the equatorial plan, after removing their ligaments and peripheral fascia, and then fixed in 10% formaldehyde. Ovaries fragments were washed with running water, and then dehydrated in increasing degrees of ethanol and embedded in paraffin. Sections of 5µm thickness were (Leica RM2135) spread out over gelatined slides, are stained with the Hematein-Eosine and trichrome at a fixed time (Martoja and Martoja, 1967). Microscopic observations were carried out by means of a stereomicroscope (SV11 ZEISS) coupled to a microcomputer.

RESULTS

Macroscopical observations showed an ovoid form for the whole examined ovaries in this species. The average diameter of ovaries is reported in Table I, which shows the direct relationship between ovarian diameter and age. Examined ovaries showed at microscopic observations (Fig. 1), a structural similar to that reported by many authors (Cahill and Mauleon, 1980; Driancourt *et al.* 1991). Ovaries are covered by a simple cubic epithelium, under which there is a layer of connective tissue rich in fibres called "tunica albugina". Just following this layer, lies the cortex, site of follicular activity, which is constituted with support tissues, such as fibroblasts and collagen fibres. The central part of the ovary, the medulla contains blood vessels, fibres and nerves. Some modifications in ovarian histological structure were observed, particularly, those concerning cellular organoides during folliculogenesis and atresia.

Folliculogenesis:

At the periphery of ovaries of young ewes aged from 2 to 3 months (Fig. 2), there is a great number of primordial follicles, of an average diameter of 17.09 ± 0.38µm, containing each one an ovocyte surrounded by some peri oocyte cells. Oocyte diameter is directly proportional to the size of follicles (Table 1). Primary follicles are also present at this age and are larger than the preceding (19± 0.65µm), containing an oocyte surrounded by a layer of cubical cells whose nucleus are in a central position and presents a dispersed chromatin. This unit is separated from conjunctive tissue by a basal layer. At this stage, zona pellucida is not yet formed (Fig. 2). Secondary and tertiary follicles present at this age (Fig. 3 and 4), have a respective average diameters of 24.2±2.84µm and 92.1±1.59µm. In these follicles, ovocytes are surrounded by two or several layers of follicular cells constituting the granulosa. The latter is separated from the oocyte by zona pellucida, which appears well individualized. In ovaries of 6 months old ewes, we have observed many growing follicles at advanced stages, representing some of primarily follicles, tertiary follicles, small antral follicles and Graafian follicles which present signs of atresia. Antral follicles (Fig. 5) present small cavities delimited by several layers of granulosa cells, resting on a basal layer. This unit is surrounded by internal and external theca cells. In ovaries of 8 months and more aged ewes, Graafian follicles (Fig. 6) are characterized by a large antral and central, delimited by some layers of granulosa cells, and with an emerging oocyte. This one present a good delimited zona pellucida, surrounded by the specialized cells of cumulus oophorus (Fig. 7), with a round nucleus, presenting a dense chromatin, joined with the nuclear envelope. This unit is surrounded by 2 to 3 layers of granulosa cells which constitute the "Cumulus Oophorus". The basal cellular layer, constituted with internal and external theca cells, surrounds this unit. Presence of corpora luteum and their scars left by their degeneration constitutes an important characteristic of these ovaries (Fig. 8 and 9).

Follicular atresia:

In growing follicles, atresia is characterized by some modifications of oocyte nucleus, indeed of aspect and cohesion of granulosa cells. These modifications are more marked in the follicular cavity. Follicular cells do not present any organization and a oocyte nucleus became pycnotic and retracted; that's characteristic of primary follicle atresia (Fig. 10).

*/ In secondary and tertiary follicles:

- Oocyte cytoplasm seems to be contracted and vacuolated with nucleus condensation; this is typical for early stages of atresia (Fig. 11).
- Oocyte degeneration, accompanied by nucleus disappearance and grouped granulosa cells detachment from the basal layer, is typical for late stages of atresia (Fig. 12).

Inside the follicular cavity, penetration of internal theca cells inside granulosa cells, involves rupture of the basal layer (Fig. 13 and 14).

*/ Granulosa cells:

- They loose their cohesion, are detached from the basal layer, and can be found in the antral cavity; cumulus oophorus cells disappears, leaving only some pycnotic nuclei, around a degenerating oocyte, typical of late stages of atresia (Fig. 15).
- The cumulus oophorus is dissolved, and then disappears completely; oocytes lose their spherical form and internal theca cells lose their polyhydric form and became globulous, typical of a final atresia (Fig. 16).

The antrum is gradually occupied by fibres, some pycnotic nucleus remains and internal theca cells becomes hyaline and are completely dissociated, typical of final stages of atresia (Fig. 17).

Table 1: Oocyte diameter according to the follicular type.

Follicle type	Primordial	Primary	Secondary	Tertiary
Oocyte diameter (µm)	17.09 ± 0.38	19 ± 0.65	24.2 ± 2.84	92.1 ± 1.59

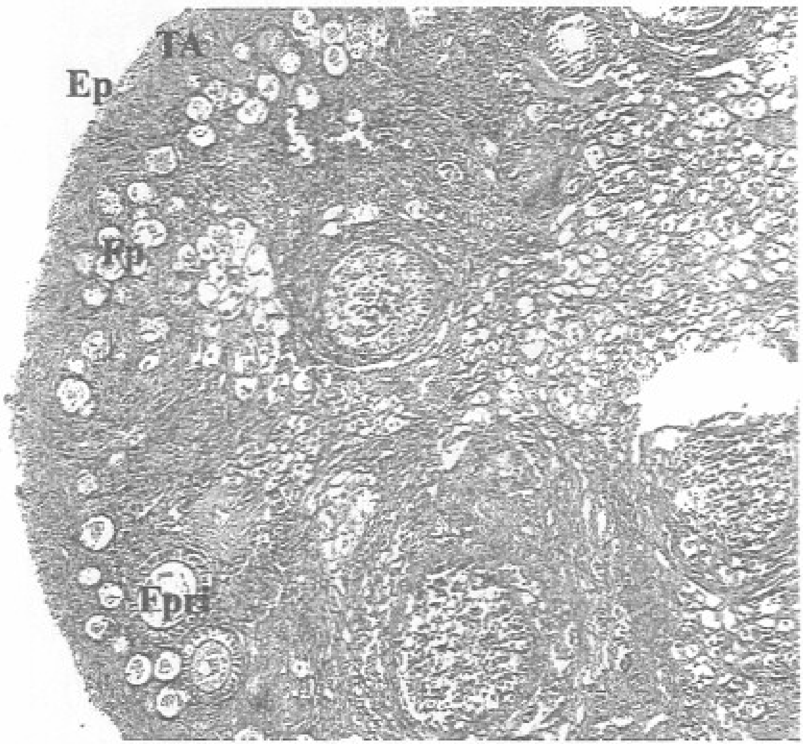
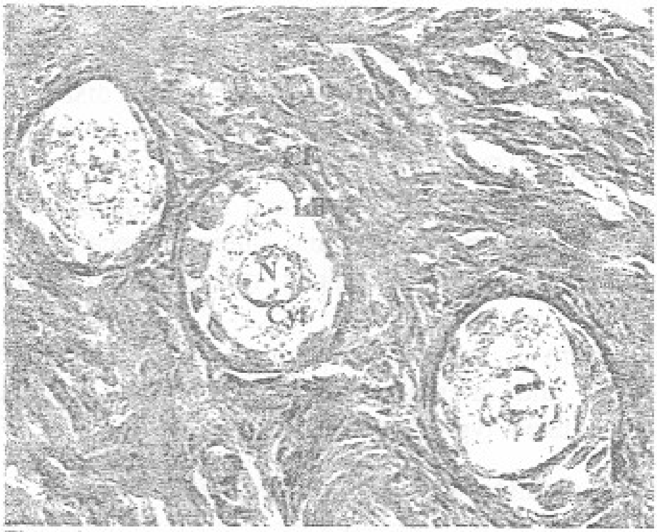


Figure 1 :



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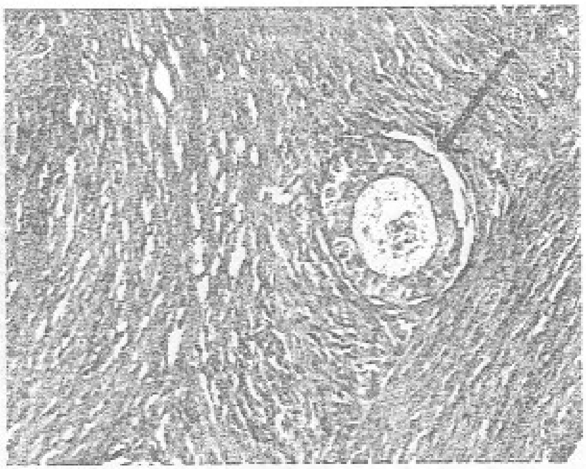

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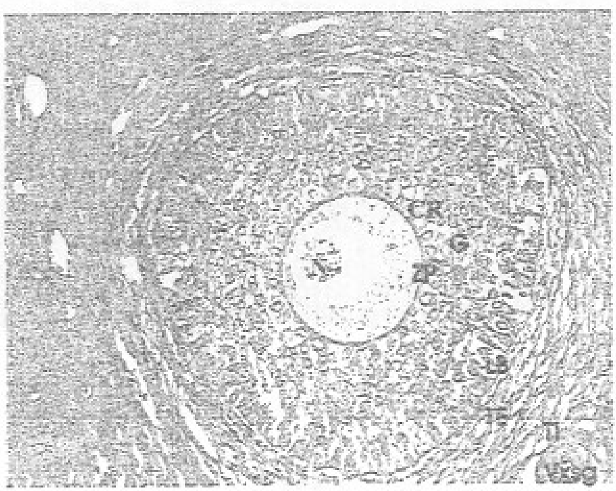

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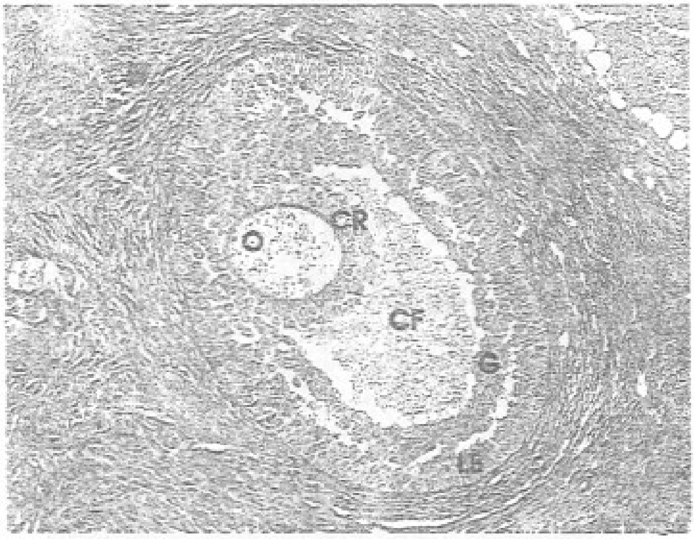

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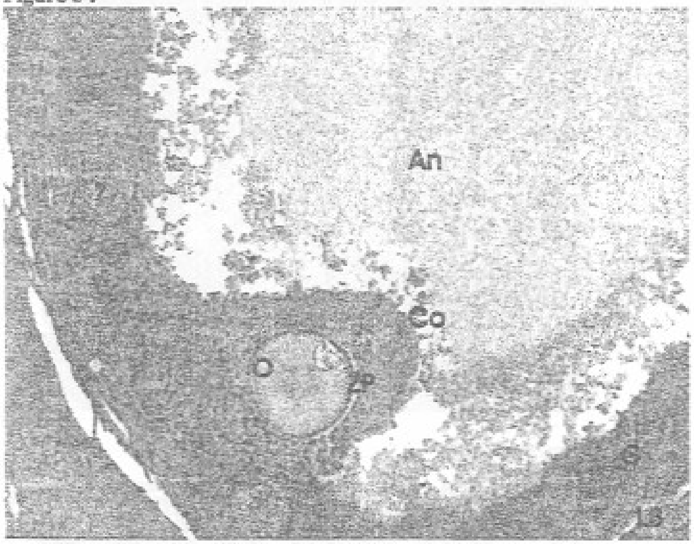

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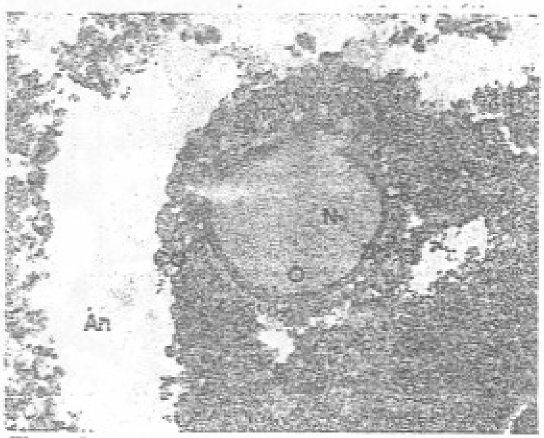

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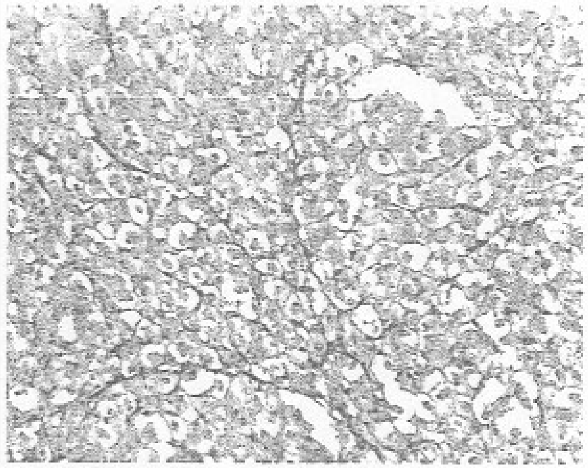

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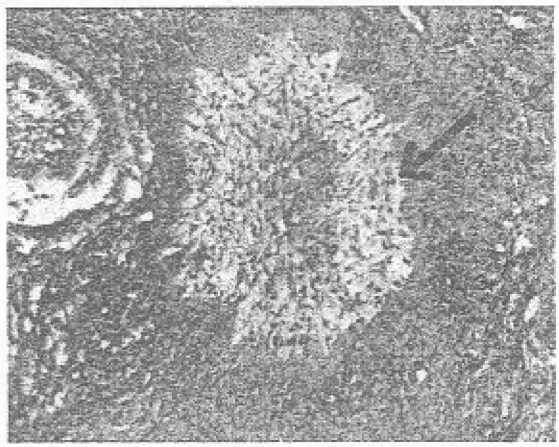

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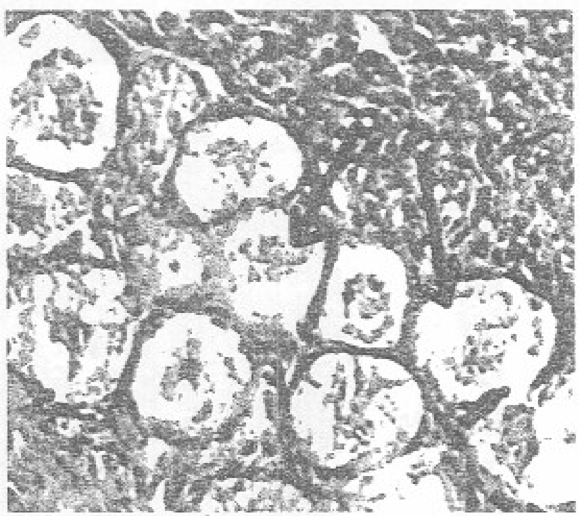

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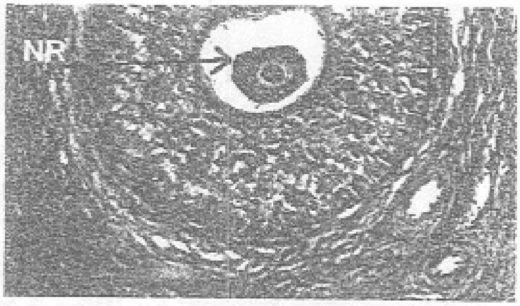

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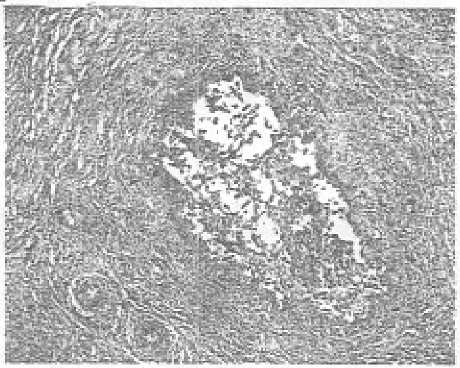

Figure 12 :

Fig. 13 :
H & E (10X)

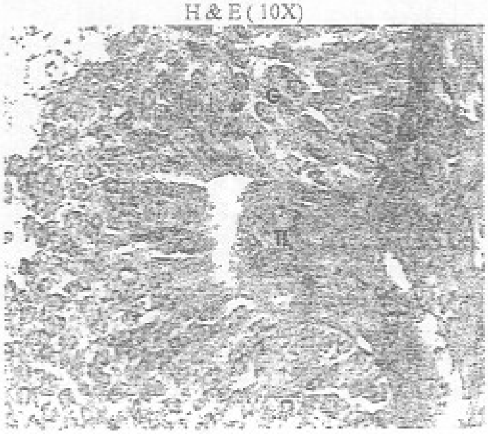

Fig. 14 :
H & E (40X)

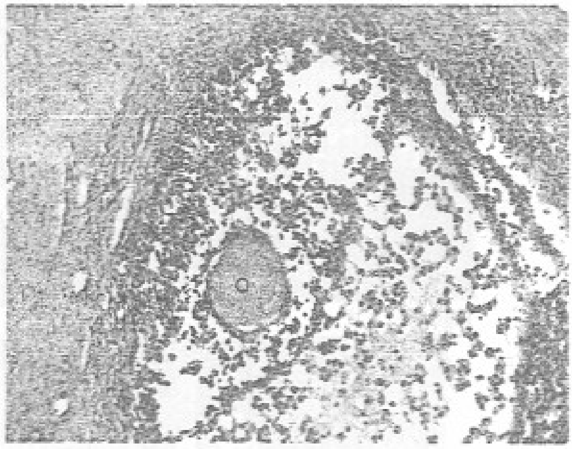

Fig. 15 :
H & E (10X)

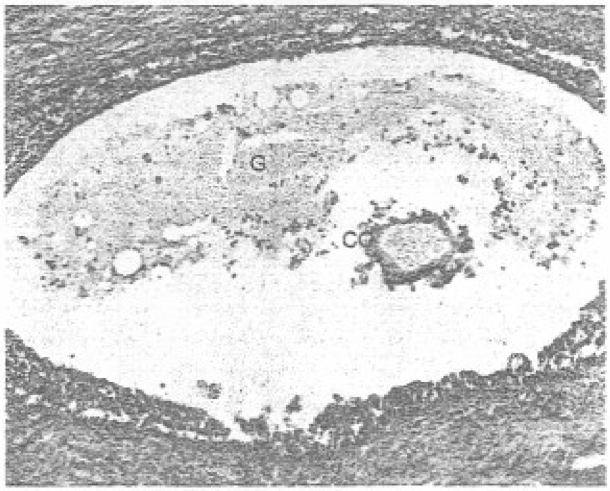

Fig. 16 :
H & E (10X)

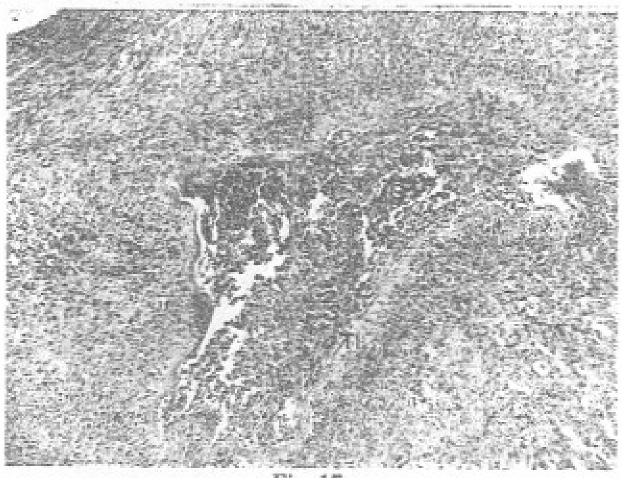

Fig. 17:
H & E (10X)

LEGENDS

Fig. 1: Cortex of ovary at 3 month. Covering epithelium (CE), with simple epithelial cells, tunica albugina (TA). Presence of primordial follicular (Fp) and primary follicle (Fpri).

Fig 2: Primary follicles in which appears nucleus (N), cytoplasm (Cyt) and basal layer (LB).

Fig. 3: Cortex of ovary at 4 month with presence of young secondary follicle.

Fig. 4: A Tertiary follicle in which appear oocyte nucleus (N), zone pellucida (ZP), cumulus oophorus (CO), granulosa cells (G), basal layer (LB), theca interna (TI) and theca external (TE).

Fig. 5: Young Graafian follicle with an oocyte (O). A distinct zone pellucida (ZP) is present. The cylindrical granulosa cells (G) next to the layer basal are arranged orientally.

Fig. 6: Portion of a mature follicle in which the oocyte (O) has a spherical nucleus (N), cumulus oophorus (CO), granulosa (G).

Fig. 7: Cumulus oophorus in mature follicle in which the oocyte (O) has a spherical nucleus (N) surrounded by a zone pellucida (ZP).

Fig. 8: Corpus luteum.

Fig. 9: Cortex of ovary at 8 month with presence of corpus albicans (arrow). Trichrome stain (10X).

Fig. 10: Atresia of primary follicle (PF) in which we noted disorganized nucleus.

Fig. 11: Early stage of atresia in secondary and tertiary follicles characterised by contracted cytoplasm (C) with nucleus condensation (N).

Fig. 12: Late stage of atresia in secondary and tertiary follicles characterised by grouped granulosa cells detachment from the basal layer.

Fig. 13: Particularities of antral follicle atresia: Penetration of theca interna cells into grunulosa. antrum (An), granulosa (G), basal layer (LB), theca interna (TI).

Fig. 14: One fold of collapsing in early atresia, fibrocytes from the tip of theca interna (TI), folds have penetrate into the granulosa layer, the basal layer (LB) has definitely broken.

Fig. 15: Part of follicle with definite atresia in which the granulosa (G) has degenerated and cumulus is in the early stage of dissolution, while the oocyte (O) appears almost normal.

Fig. 16: Both granulosa (G) and cumulus oophorus (CO) are in the late stage of dissolution, the cumulus has disappeared and the oocyte is vacuolated and shrivelled.

Fig. 17: Part of a follicle in the late atresia where the theca interna (TI) is hyalinized and the antrum completely filled with necrotic fibrous remnants of granulosa (G). The follicle has become an atretic corpus.

DISCUSSION

Ovaries of young Rumbi ewes present macroscopically an ovoid form whose average diameter shows a directly proportional evolution with age. Microscopically, it presents two distinct areas: a medulla rich in blood vessels, fibroblasts and fibres as well as a cortex delimited by a superficial epithelium comprising an albugineous tunica and a stroma. Ovaries of two months old ewes, shows a germinative zone which presents significant amounts of primordial and primary follicles, disseminated in a dense connective tissue. We have also observed primary follicles, and their number decrease gradually according to the age of ewes, similar to those described in the yak (Cui and Yu, 1999) and goat (Silva *et al.*, 2001; Lucci *et al.*, 2001). Cahill *et al*, (1979) and Sonjaya *et al.* (1987) estimate that the stock of primordial follicles in the pre pubertal animals is significant and regresses with age. This follicular stock at rest represents the major part of ovarian population (Mariana *et al.* 1991). Formed during the foetal period, they are not renewable during animal lives, and will not reach ovulatory stage because they will undergo atresia at various stages of their development (Land, 1970).

The important population of antral follicles observed was also brought back by Tassel *et al.* (1978) on one month old ewes ovaries. According to Kennedy *et al.* (1974), follicle number is high during the first two months, then decreases and is stabilized around period of first ovulation. The variability of follicular size and number according to age was highlighted by transrectal ultrasonography on youngest ewes aged from 1 to 6 months. The number of antral follicles, whose diameter is equal to 3 mm, is high between 4 and 16 weeks, decreases between 16 and 18 weeks and then increase between 22 and 24 weeks (Bartlewski *et al.*, 2002). Moreover, work of Cahill *et al.* (1979) showed that growing follicles (pre antral and antral) of youngest ewes of one month old are three times more than in older ewes.

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