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Full Length Research Paper

Ultrastructural study of ovarian follicles in growth (Algerian rumbi sheep)

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The present study is a contribution to the knowledge of the ultrastructure of ovarian follicles in growth of the sheep of breed Algerian Rumbi. This last is characterized by parameters of reproductions very valuable and puberty seems to occur between 10 and 12 months with the onset of the first heat among lambs. The aim of the present study was to characterize the ultrastructural of Rumbi sheep preantral and antral follicle. Secondary follicle consisted of an oocyte surrounded by a variable number of layers of cuboidal granulosa cells. At this follicular stage, the zona pellucida with transzonales projections was beginning to form around the oocyte. The secondary follicle is characterized by the presence of a large nucleus; the endoplasmic reticulum is in the form of citernea dilated. The zona pellucida was totally developed around the oocyte. Several granulosa cell projections could be detected that were encroaching into the zona pellucida and protruding towards the oocyte, where gap junctions were observed between oocyte and granulosa cell membranes. Organelles within the oocyte were located at the periphery of the cytoplasm. Our results show that the tertiary and Graffian follicles are characterized by ultrastructural changes of their cellular organelles including the mitochondria and Golgi complex.

Key words: Follicles, preantral follicle, antral follicle, ovary, zona pellucida, sheep, rumbi sheep, Algeria.

INTRODUCTION

In the sheep as in the other mammalians, the ovary is the seat of the formation and maturation of oocytes, cellular differentiation, hormones synthesis, apoptosis and follicular atresia. The reserve of germ cells formed during embryonic development represents a very important stock of follicles and oocytes at pubertal age (Saumande, 1991).

The folliculogenesis represents the transformation of primordial follicle in Graffian follicle, which frees the breach and the oocyte II turns into the corpus luteum. The primordial follicle is formed just before birth and that when the primary oocyte surrounded by a layer of flattened somatic cells (Land, 1970). When the primordial follicle begins to evolve, the cells in the pregranulosa become cuboides (Sawandji et al., 1997; Fortune, 2003). The growth period of primordial follicles in the provulatory stage than six months (Cahill and Mauleon, 1980). In the sheep, the population of primordial and primary follicles estimated at between 40,000 to 300,000 follicles (Driancourt et al., 1991; Cahill and Mauleon, 1981), constitute the reserve for its period of reproduction. Only a small number of follicles will arrive to maturity (Graffian follicle) (Caroll et al., 1990) and the majority is vowed to the involution or atresia (Gordon, 1994).

Algerian Rumbi breeds are resulting from crossing the muffled rams of “Djebel Amour”, and “Ouled Djellal” sheep, this ovine breed is characterized by noticeable reproduction parameters: fertility (80%), fecundity (95%) and prolificacy (110%). The puberty among this race appears between 10 and 12 months, and appearance of first heats in young sheep does not mean that they assured their puberty, and can be fertilized (Chellig, 1992); however, few studies were devoted to the ovary function of this breed whose livestock is estimated at 3,355,169 which 2,440,123 are females.

MATERIALS AND METHODS

The ovary of lambs (n = 60), grouped by age (4 – 12 months) were

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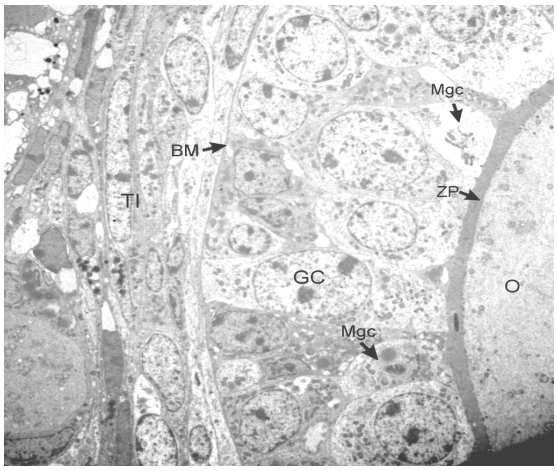

Figure 1. Part of secondary follicle. O: oocyte, ZP: zona pellucida, Mgc: granulosa cell in mitosis, GC : granulosa cell, BM : basement membrane, TI : theca interna (Glutaraldehyde/ osmium tetroxide, 6500X).

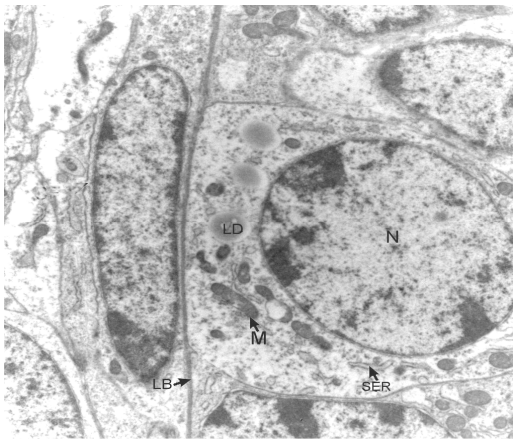

Figure 2. Detail of peripheral cell of the granulosa. N: nucleus, M: mitochondria, SER: smooth endoplasmic reticulum, LD: lipid droplet, LB: lamina basal (Glutaraldehyde/ osmium tetroxide, 10000X).

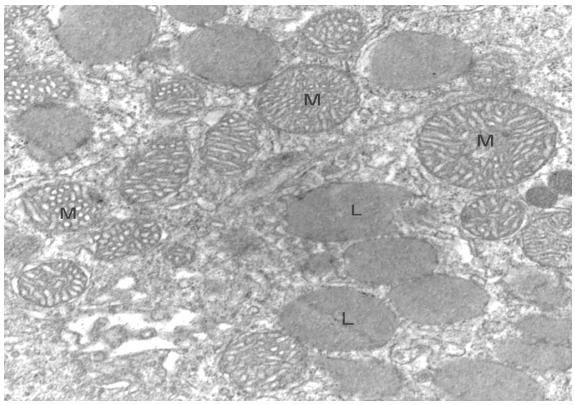

Figure 3. Part of granulosa cell :M: mitochondria, L: lipid droplets (Glutaraldehyde/ osmium tetroxide, 50000X).

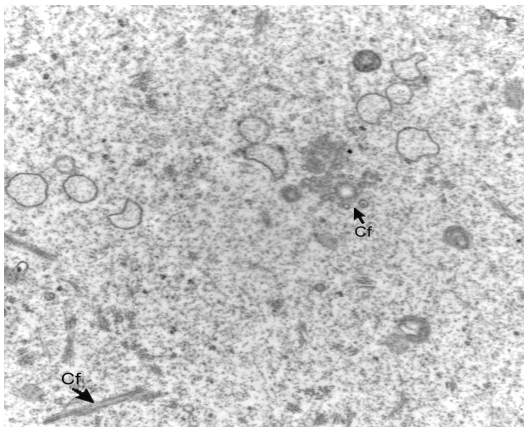

Figure 4. Presence of microtubules in the cytoplasm of the oocyte. Mt: microtubules (Glutaraldehyde/ osmium tetroxide, 15000X).

collected at Tiaret commercial slaughter-house during the period from Mars to June 2005.

For transmission electron microscopy, small pieces of ovary were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 1 h and post fixed in 1% osmium tetroxid in the same buffer for 1 h (Reynolds, 1963).. Fixation was carried out at 4°C. They were then dehydrated in graded alcohol and embebbed in Epon. Before thin sectioning, the preselected cortical blocks are sectioned (1 µm) serially, and successive groups of five serial, thick sections are examined with light microscopy to search for the presence of primary and secondary follicles. When the edge of a follicle is identified, the block is trimmed, and serial, thin sections are cut and mounted on 100 mesh grids. The ultrathin sections were contrasted with uranyl acetate and lead citrate and observed with a JEOL transmission electron microscope.

RESULTS

The follicle in growth presents more than two layers of follicular cells. The whole is surrounded by the basal lamina, theca interna and theca externa.

The secondary follicle is characterized by the absence of spaces between follicular cells also and by the presence of two layers of follicular cells, which are surrounded by the basal lamina (Figure 1).The granulosa cells present a nucleus more or less rounded and chromatin attached to the nuclear envelope (Figure 2).

The cytoplasm contains more cellular organelles such as lipoid droplets, the smooth endoplasmic reticulum, mitochondria to cristea laminated (Figure 3).

At this stage of follicular development, the oocyte observed contains:

• Most cellular organelles represented by the smooth endoplasmic reticulum with cisternae dilated, a Golgi apparatus and the microtubules (Figure 4).
• The zona pellucida becomes important. The microvilli of the plasma membrane of the oocyte enter in this area. They establish connections with cells of the corona radiata (Figure 5).

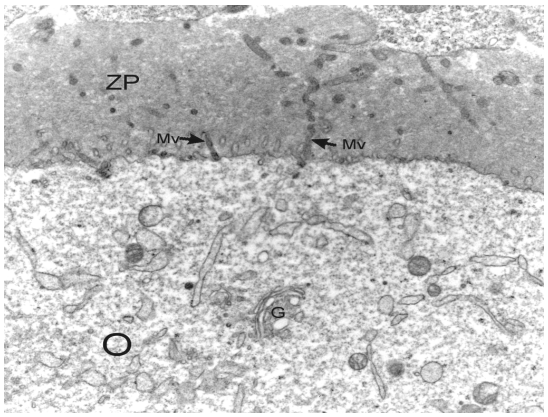

Figure 5. Aspect of cytoplasm of oocyte and zona pellucida. O: oocyte, G: Golgi apparatus, Mv: microvilli, ZP: zona pellucida (Glutaraldehyde/ osmium tetroxide, 15000X).

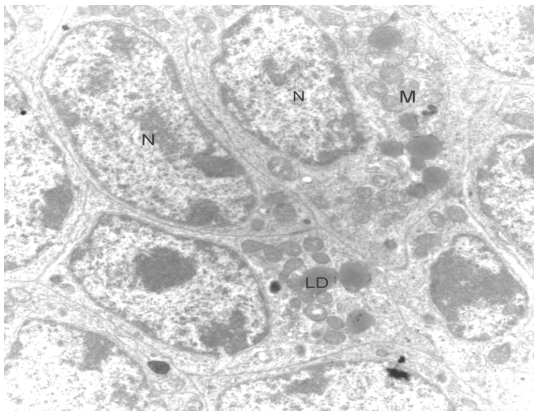

Figure 8. Arrangement and shape of the granulosa cells. N: nucleus, M: mitochondria, LD: lipid droplet (Glutaraldehyde/ osmium tetroxide, 10000X).

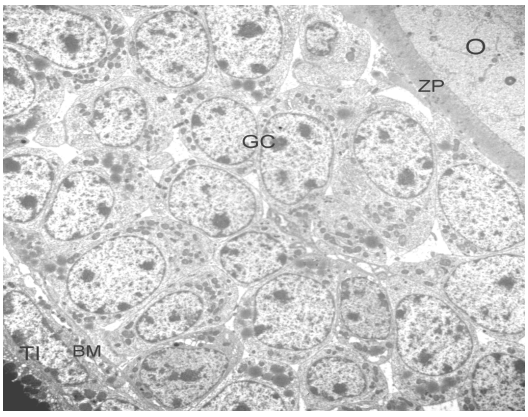

Figure 6. Tertiary follicle. O: oocyte, ZP: zona pellucida, GC: granulosa cell, BM: basement membrane, TI: theca interna (Glutaraldehyde/ osmium tetroxide, 2500X).

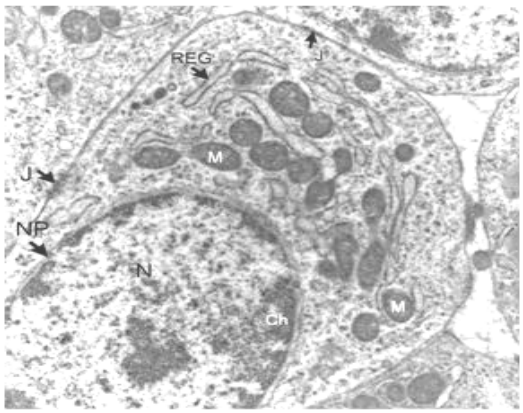

Figure 9. Detail of granulosa cell of tertiary follicle. N: nucleus, NP: nuclear pore, J: junction, REG: rough endoplasmic reticulum, M: mitochondria, Ch: chromatin (Glutaraldehyde/ osmium tetroxide, 20000X).

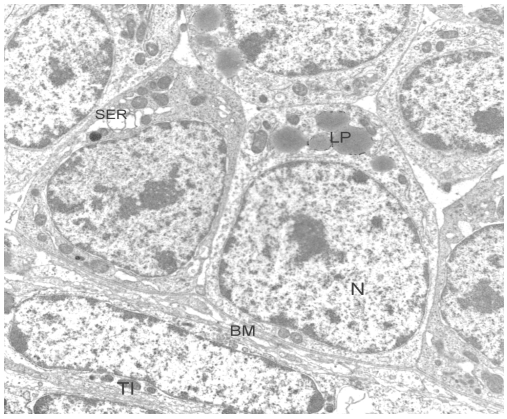

Figure 7. Detail of peripheral cell of the granulosa. N: nucleus, SER: smooth endoplasmic reticulum, LP: lipid droplet, BM: basement membrane, TI: theca interna (Glutaraldehyde/ osmium tetroxide, 7500X).

Tertiary follicle is distinguished by the appearance space between follicular cells which are, arranged in stratified concentric layers (Figure 6).

a) The granulosa cells in contact with the basement membrane form a compact layer. They have large lipoid droplets in the cytoplasm with a wealth of mitochondria, endoplasmic reticulum smooth and rough (Figure 7).

These cells have a round or polyedrique form (Figure 8). The nucleus is voluminous and presents a large chromatin distributed under the nuclear envelope and arranged in clusters. The nucleolus occupying a central position. The cytoplasm contains the organelles implicated in the synthesis of proteins and lipids: mitochondria, endoplasmic reticulum rough, smooth endoplasmic reticulum in the form of citernea dilated and lipoid droplet. We also noted that the nuclear membrane is bisected by nuclear pores (Figure 9). The follicle cells are connected by com-

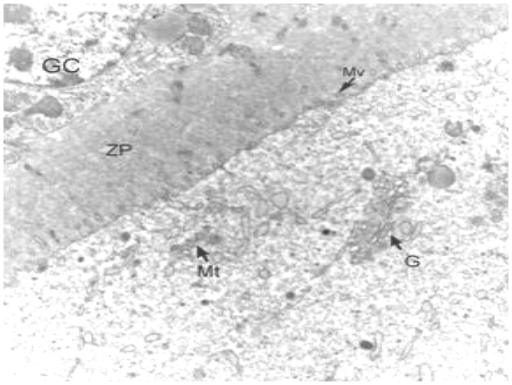

Figure 10. Aspect of oocyte in tertiary follicle. GC: granulose cell, ZP: zona pellucid, Mv Microvilli, Mt: microtubules, G: Golgi apparatus (Glutaraldehyde/ osmium tetroxide, 12000X).

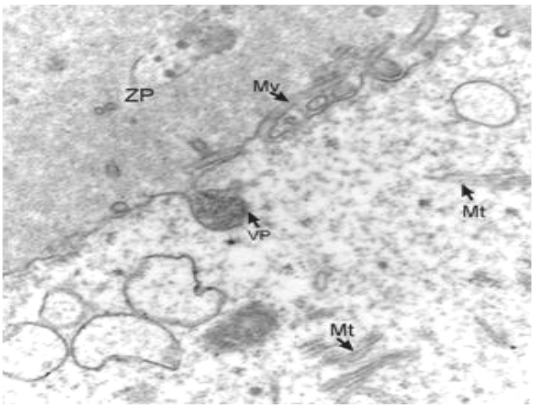

Figure 11. Observation of blister on pinocytosis in the plasma membrane of the oocyte. Mt: microtubules, VP: vesicle on pinocytosis, Mv: microvilli, ZP: zona pellucida (Glutaraldehyde/ osmium tetroxide, 40000X).

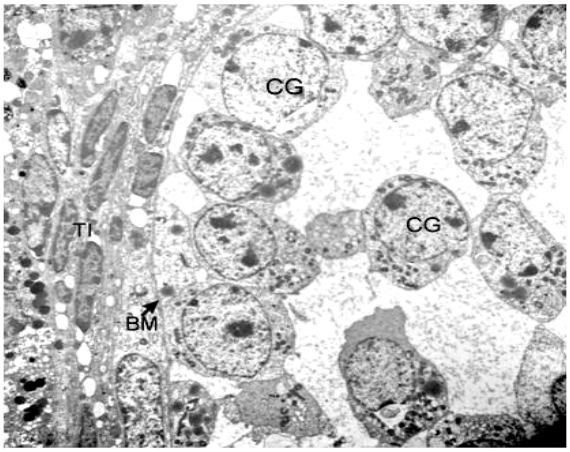

Figure 12. Aspect on antral follicle. CG: granulosa cells, BM: basement membrane, TI: theca interna (Glutaraldehyde/ osmium tetroxide, 3000X).

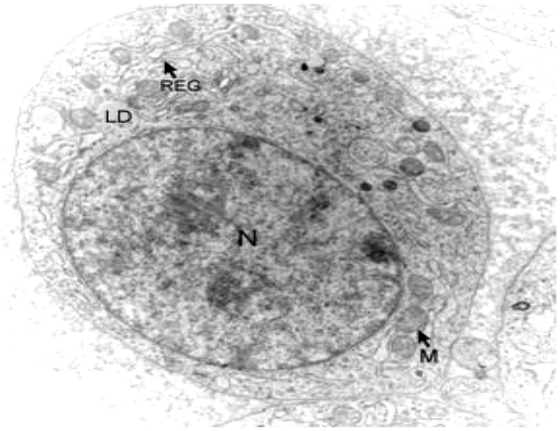

Figure 13. Granulosa cell of antral follicle. N: nucleus, M: mitochondria, REG: rough endoplasmic reticulum, LD: lipid droplet (Glutaraldehyde/ osmium tetroxide, 12000X).

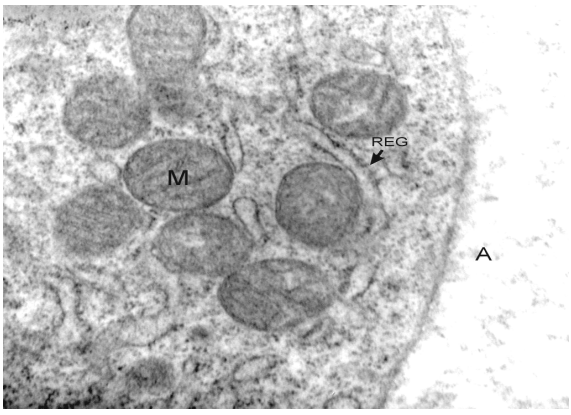

Figure 14. Cytoplasm of granulosa cell in antral follicle. REG: rough endoplasmic reticulum, M: mitochondria, A: antrum (Glutaraldehyde/ osmium tetroxide, 50000X).

municating junctions.

b) The diameter of the oocyte has greatly increased and the peripheral band containing the organelles is proportionately smaller (Figure 10). In the cytoplasm there are few mitochondria and vesicular bodies, but abundant ribosomes and polyribosomes. The plasma membrane of the oocyte has blisters on pinocytosis as well as microvilli (Figure 11). The oocyte is surrounded by zona pellucida.

Antral follicle is characterized by the appearance of a cavity resulting of the fusion of small gaps containing follicular fluid (Figure 12). The gaps will eventually occupy most of the follicle leading to the disappearance of most of the granulosa cells. It will remain only two to three layers of cells located in the basal lamina.

The few granulosa cells that remain present a nucleus rounded up in position eccentric and a chromatin scattered in clusters in the nucleoplasm (Figure 13). The cytoplasm contains mitochondria in laminated cristea, granular endoplasmic reticulum and lipoid droplets (Figure 14).

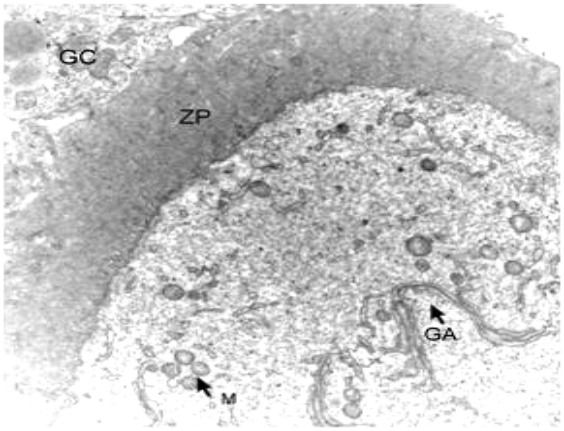

Figure 15. Oocyte of tertiary follicle and its zona pellucida. M: mitochondria, GA: Golgi apparatus, ZP: zona pellucida, GC: granulose cell (Glutaraldehyde/ osmium tetroxide, 10000X).

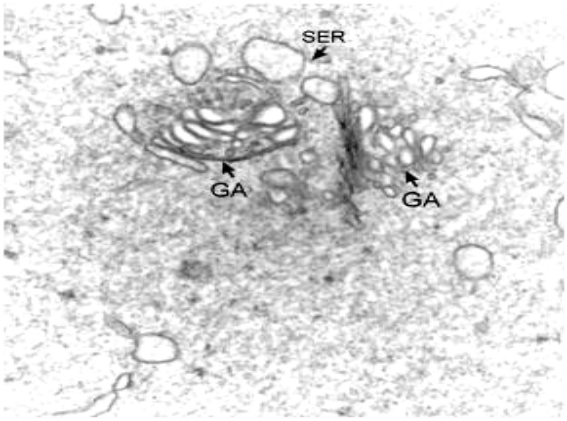

Figure 16. Cytoplasm of oocyte. GA: Golgi apparatus, SER: smooth endoplasmic reticulum (Glutaraldehyde/ osmium tetroxide, 40000X).

The oocyte observed in antral follicle has a plasma membrane presented projections that penetrated between adjacent granulosa cells and a few short microvilli lying parallel to the oocyte surface (Figure 15).

The cytoplasm of the oocyte contains mitochondria clustered or dispersed, smooth endoplasmic reticulum and large Golgi complex (Figure 16 and 17).

DISCUSSION

The results of ultrastructural study describe changes which affect follicles during their evolution. These results bring some information on the cellular structures of ovarian follicles of Algerian rumbi sheep.

It appears that the ultrastructure of preantral and antral follicle of Algerian Rumbi sheep is similar of mammalians species such as the cattle (Van Wezel and Rodgers,

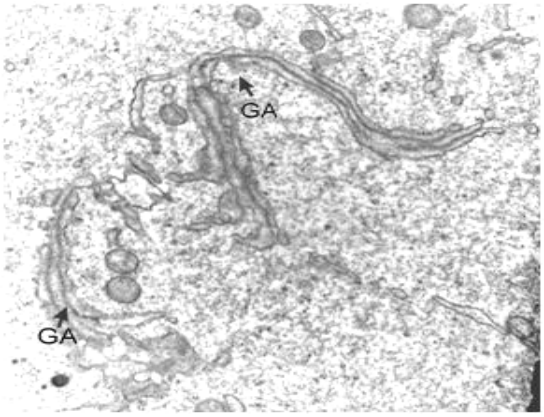

Figure 17. Oocyte of tertiary follicle. GA: Golgi apparatus (Glutaraldehyde/ osmium tetroxide, 20000X).

1996; Fair et al., 1997; Hyttel et al., 1997), the cat (Jewgenow et Stolte, 1996), the goat (Lucci et al., 2001) and the human (Oktay et al., 1997) during the follicular development.

The zona pellucida which begins to emerge from secondary follicle is a major transformation that is suffering the follicles to grow. The zona pellucida is secreted mainly by the oocyte there by creating a barrier between him and cumulus cells (Shimizu et al., 1983; Maresh et al., 1990). In our observations electron microscopy highlighted microvilli issued by the plasma membrane of the oocyte and through the zona pellucida.

These structures were observed by Albertini and Barret (2003) and have been named transzonales projections. According to these authors, they form a broad contacts gap junction. The density and structure of this zone change during the folliculogenesis, which indicates that there is a constant communication between the oocyte and cumulus cells (Gougeon, 1996).

In our study, the formation of the zona pellucida is similar to that observed in the cow (Braw-Tal and Yossefi, 1997), goat (Lucci et al., 2001) and sheep (Lyndy et al., 1999). First appearance took place in secondary follicles. However, this area is different from other species such as the mouse (Oakberg, 1979), guinea pigs (Adam and Herting, 1964), rabbit (Nicosia et al., 1975), cats (Jewgenow et Stolte, 1996), Monkey (Zamboni, 1974) and humans (Himselstein-Braw et al., 1976). Among these species, the zona pellucida appears in the primary follicles in the form of small plates.

Regarding the mitochondria, their location and their morphology changes during the development of follicles. Indeed, in the primary and primary follicles, there are available around the nucleus and have a rounded shape. Mitochondria are elongate shape characteristics of growing follicles. They are scattered throughout the cytoplasm, their movement is facilitated by the microtubules. The morphology of mitochondria indicates a significant

metabolic activity. There proliferation is important for the production of energy needed to increase the oocyte which will acquire the competence of meiosis.

With the regard to the junctions macula adherens also observed by different authors, they only ensure the cohesion between the cells. These junctions disappear at the phenomenon of atresia causing a disruption of follicular cells.

We met with cellular junctions similar to those found in rabbits (Nicosia et al., 1975), monkey (Zamboni, 1974) and cats (Jewgenow and Stolte, 1996). These joints are of two types: the macula adherens and gaps junctions.

The presence of these junctions between the cumulus cells and the oocyte indicates that the highest rate of oocyte maturation is accompanied by the persistence of these communications permeable (Modina et al., 2001). However, according to these authors, the permeability of gaps junctions, the expansion of cumulus and a maturation of quality have been correlated with high levels of cAMP.

The ultrastructural features of the granulosa cells of ovarian follicles sheep race Rumbi are similar to those described in the goat (Lucci et al., 2001) and monkeys (Zamboni, 1974).

Conclusion

The presents study allowed describing for the first time the ultrastructure of primary and secondary follicles observed in the ovaires of local rumbi sheep. For some differences in the structure of ultra preantral follicles, it would be judicious to study the molecular aspects of the follicular growths to allow the understanding of their development in vitro.

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