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STRUCTURAL CHANGES OF SHEEP MAMMARY GLAND DURING INVOLUTION

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Key words: Sheep, mammary gland, involution, electron microscopy

ABSTRACT:

Involution in ovine mammary gland was studied by light and electron microscopy at the last day of lactation and after eight days from drying off. Morphological and structural changes in epithelial secretory cells were described. The ultra-structural features and differences of alveolar cells of the mammary gland at the two days considered during drying-off was emphasised.

INTRODUCTION

During puberty, pregnancy and lactation, mammary gland is characterised by a proliferation of epithelial cells which are mostly removed during drying-off. Dramatic morphological changes occur during involution and the elimination of secretory epithelial cells takes place before an extensive reconstructing process. Mammary involution is regulated by apoptosis [1, 2], an active physiological process of cellular self-destruction of multicellular organisms with distinct morphological and biochemical features such as membrane blebbing, nuclear fragmentation and swelling of endoplasmic reticulum [3]. The process is similar in various species, but the time course and extent of cell death depends on the nature of the stimulus and the reproductive state of the animal [4]. In rodents, the involution of mammary gland is described as a two steps process: the first, reversible within 30-36 h after weaning, has not essential morphological changes, and the second-one, irreversible, is characterised by a lobulo-alveolar structure remodelling, DNA fragmentation and basement membrane degradation. In mice, apoptosis is most active during the first stage, starts at about 1day after weaning, peaks at day 3 and decreases thereafter [5, 6, 7, 8, 9]. In goats, mammary cell number declines before drying off and DNA content decreased significantly between peak lactation and drying off [10,11], but, as in bovine mammary tissue, there was not a complete loss of secretory activity throughout the nonlactating period [12, 13, 14]. Histological examinations of bovine mammary gland showed that there was a progressive increase in the interalveolar area and a concurrent decrease in the alveolar lumen area as the involution progressed; moreover, in alveolar epithelial cells, the formation of large intracellular vacuoles was observed [12]. In sheep Tatarczuch et al., [15] demonstrated that the apoptosis was most active 2-4 d after weaning and that the gland was completely involuted within 30 days.

In a recent study of lactating bovine mammary gland of African elephant it is observed that programmed cell death occurred for entire group of alve-

oli among actively secreting lobules, probably correlated with the long duration of the lactation [16]. This is not observed in a electron microscopic study of lactating bovine mammary gland where just the differences among alveolar cells are described, but apoptotic cells were not observed [17].

Since in literature there are not many papers focused on ultrastructural observation changes during the involution of mammary gland in sheep, this study emphasises the changes of ovine mammary tissue during the early stages of involution observing the ultrastructural features that occur in the epithelial cells of mammary gland.

Material and Methods

Tissues

Eight multiparous sheep with single lamb were selected at lambing and kept during whole lactation with lambs in paddocks, with free access to feedstuffs and water. Daily ration was constituted of barley s.e. soybean meal, barley straw and vitamin and mineral supplement according to the requirements of NRC [18]. The amount of each diet component was adjusted to meet the decreasing nutrient demand of sheep and increasing nutrient requirements of lambs. The eight sheep used in this study were healthy and their mammary glands were found to be free of abnormality and not pregnant at the time of sampling. Mammary gland tissue was obtained from the same sheep after one day of the end of lactation (1S), and after 8 days (4S). Two samples of tissue were collected by biopsy (Magnum, Bardt, USA) after subcutaneous anaesthesia and little skin cut.

Light microscopy

The tissue was fixed in 10% neutral formalin, embedded in paraffin wax using standard histological procedures. Sections were cut at 5μm, stained with haematoxylin-eosin and methyl green pyronin (MGP).

Electron microscopy

Samples of tissue were fixed in glutaraldehyde 2% at 4°C for 2 hours, postfixed in 1% osmium tetroxide for 1 hour at room temperature, washed in distilled water and then dehydrated in a series of graded alcohol solution before embedded in a mixture of Epon-Araldite. The blocks were polymerised at 60°C for 72 h. Semithin sections (1μm) were mounted on glass microscope slides and stained with a solution of 1% toluidine blue. Ultrathin sections were collected on uncoated 200 mesh copper grids, stained with an aqueous solution of 5% uranyl acetate for 20 min at 40°C and then with a lead citrate solution for 10 min at room temperature according with Reynolds procedure [19]. The sections were examined under a Philips CM100 electron microscope.

RESULTS

Light microscopy

Repeated bioptic samples from the same animal did not result in apparent damages of the mammary gland structure. As described in a previous work [20], only a small amount of interalveolar connective tissue was shown at 1S (first day of dry-off) with numerous vessels and the alveolar lumen occupied most of the tissue area (Fig. 1) and apoptotic cells were not found in the alveolar epithelium. The epithelial cells of mammary gland defined a very compact layer around the lumen and presented the apical half with numerous small vacuoles which was spread into the lumen. The shape of the cells was cuboidal and large; the cytoplasm contained numerous secretory vesicles with various size, nuclei were round and occupied the middle or the basal part of the cell (Fig. 2). The alveoli lumina were filled of casein micelles and lipid drops.

After 8 days from the end of lactation, the epithelial layer of alveoli changed completely: Cytoplasm was confined to a very thin layer at the border of the cells by very large vacuoles which occupy a great proportion of the intracellular area (Fig. 3). In these samples the alveoli appeared to collapse so the lumen area was very low, whereas the intralveolar space was filled with large bundles of connective tissue and the apoptotic cells increased significantly [20] (Fig. 4). Cell outline was not well defined and the cytoplasm condensed, so nuclei lost their position and appeared to be spread into the cytoplasm, often being distorted. At this stage the cytoplasm of alveolar cells contained numerous stasis vacuoles and others with dense material.

Transmission electron microscopy

After one day from dry off (1S) minimal stromal area with large proportions of epithelium and distended lumina occupying the tissue area was observed. Epithelial cells appeared polarized and secretory vesicles and small fat droplets accumulated in the apical cytoplasm, abundant rough endoplasmic reticulum (RER), Golgi apparatus and numerous mitochondria scattered around the nucleus and in the apical part of the cells were observed (Figs. 5, 6). Well defined microvilli on the apical surface protruded into the alveolar lumen and tight junctions between cells, near the apicex, were intact (Figs. 7, 8); basement membrane was present (inset Fig. 8). Nuclei were round and there was a large cytoplasm to nucleus ratio.

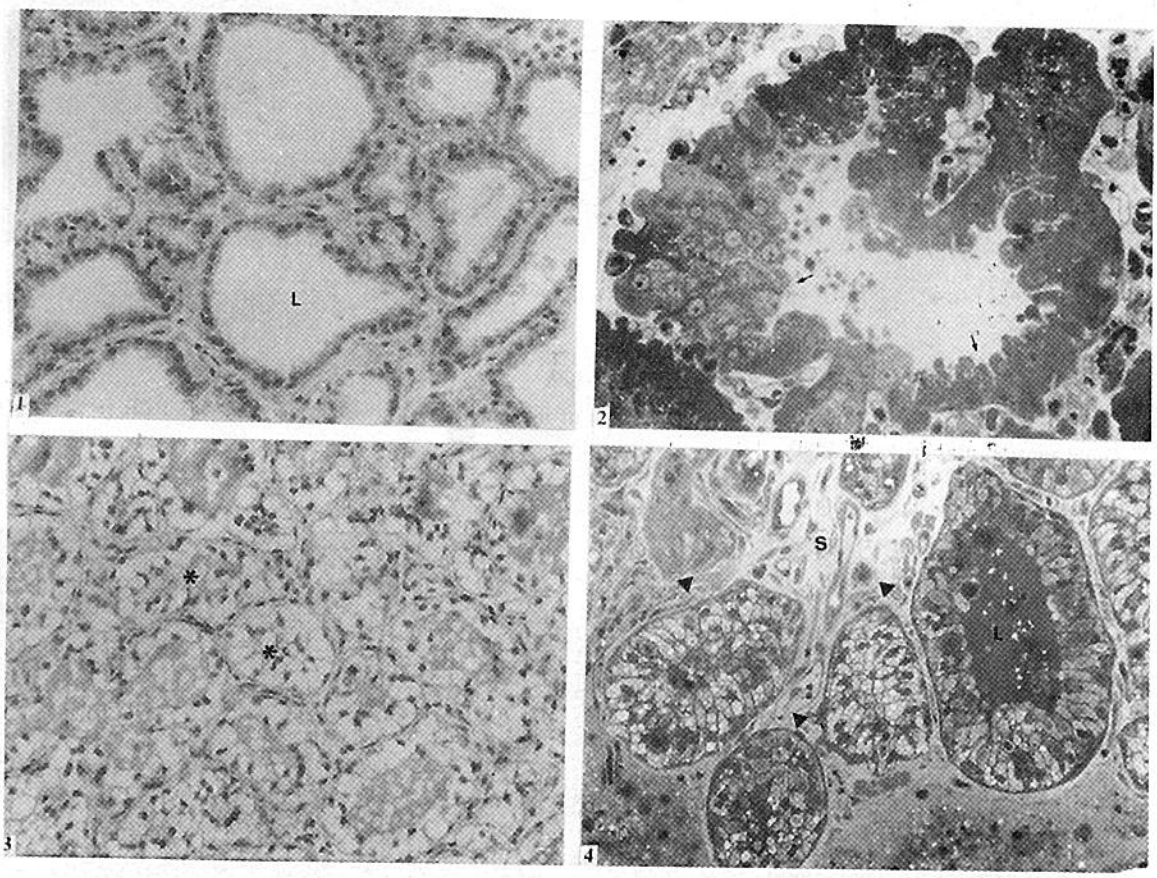


Fig. 1- 4:
1- One day after dry off (1S). Sheep mammary tissue showing large lumina (L) and well defined alveoli. Em-eos. x 80.
2. (1S). Semithin section of sheep mammary tissue showing tall cells with prominent apical protrusions (arrows). Toluidine-blue. x 144.
3- Eight days after dry off (4S). Sheep mammary tissue. At uniform magnification note the reduction of alveoli diameter (asterisks) with progression of involution. Em-eos. x 80.
4- (4S). Semithin section of sheep mammary tissue showing shrunken alveoli (arrowsheads), large stromal area (S) and limited lumen stained deeply basophilic (L). Toluidine-blue. x 90.

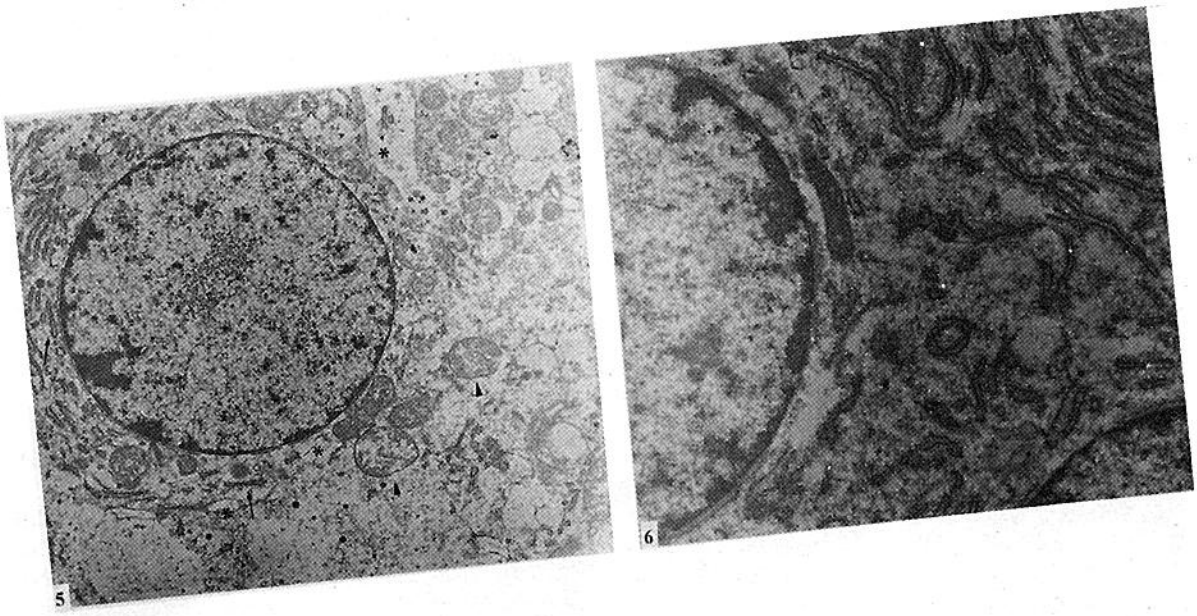


Fig. 5. 6:
5- (1S) Transmission electron microscopy; note presence of mitochondria (arrowsheads), extensive RER (arrows) and Golgi apparatus (asterisks). x 5500.
6. Particular. x 11500.

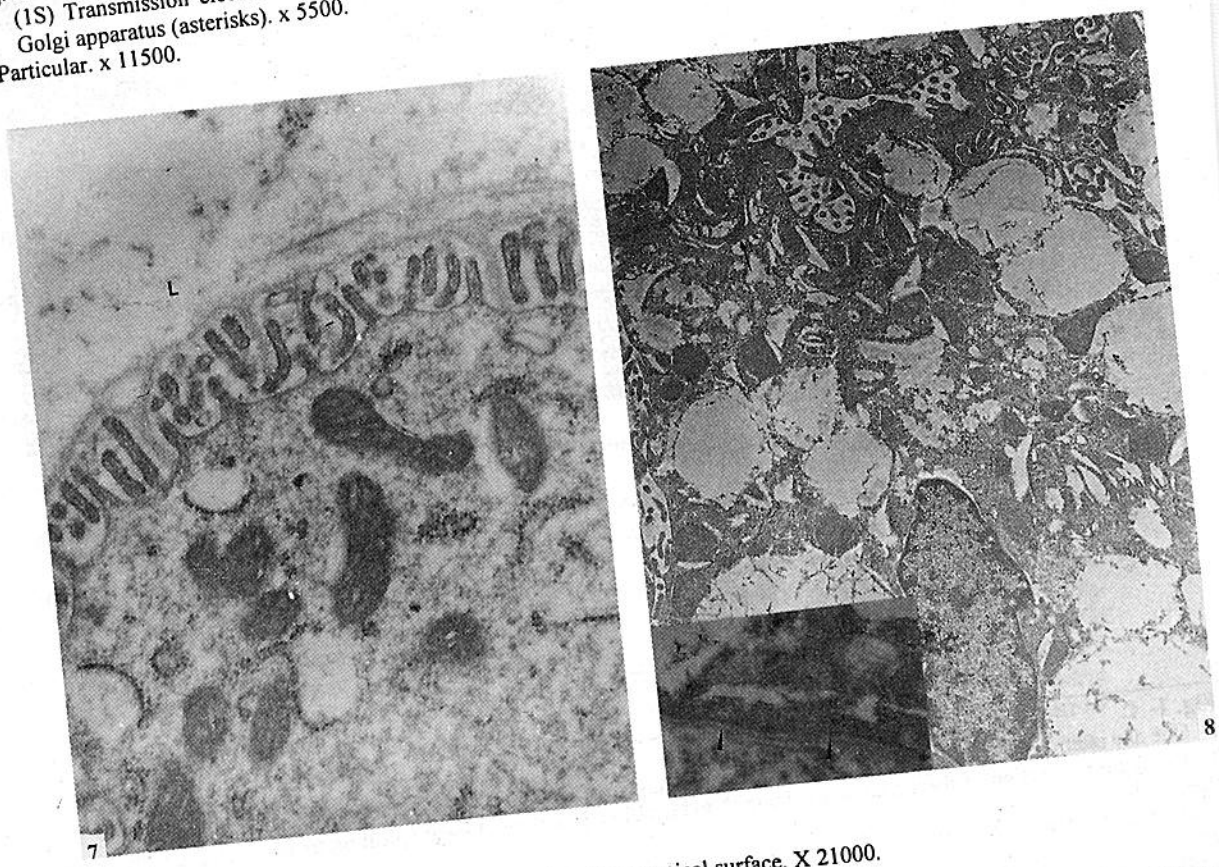


Fig. 7: (1S). Transmission electron microscopy; microvilli on apical surface. X 21000.

Fig. 8: (1S). Transmission electron microscopy; low-power magnification of alveolar epithelial cells; note the presence of tight juction (arrow) x3800. **Inset**: basement membrane (arrowshead) x 11200.

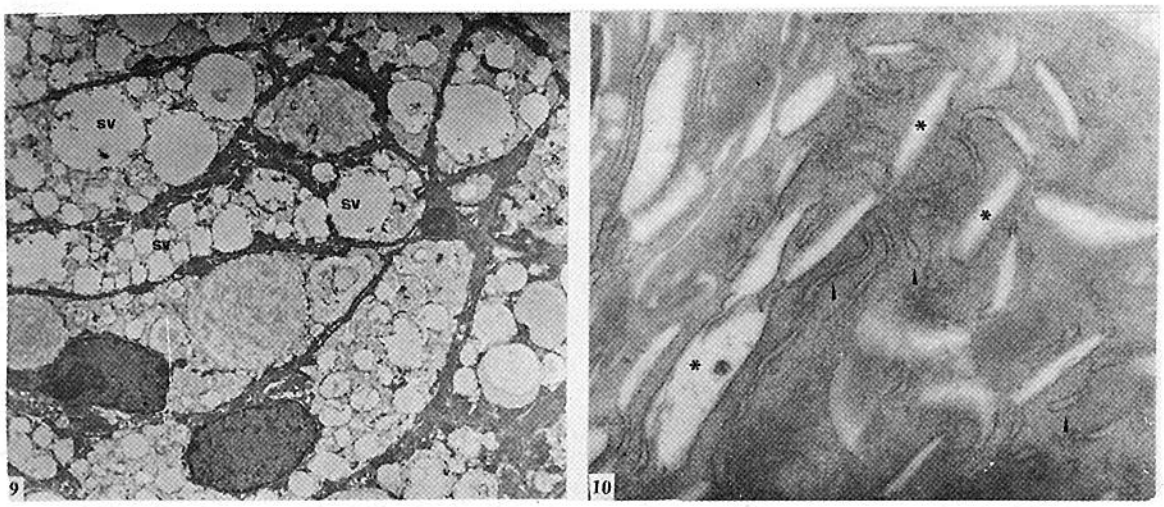


Fig. 9. - (4S). Transmission electron microscopy; low-power magnification of alveolar epithelial cells containing stasis vacuoles (sv). X 2200.

Fig. 10. - (4S). Transmission electron microscopy; note loss of microvilli (arrowshead). X 6300.

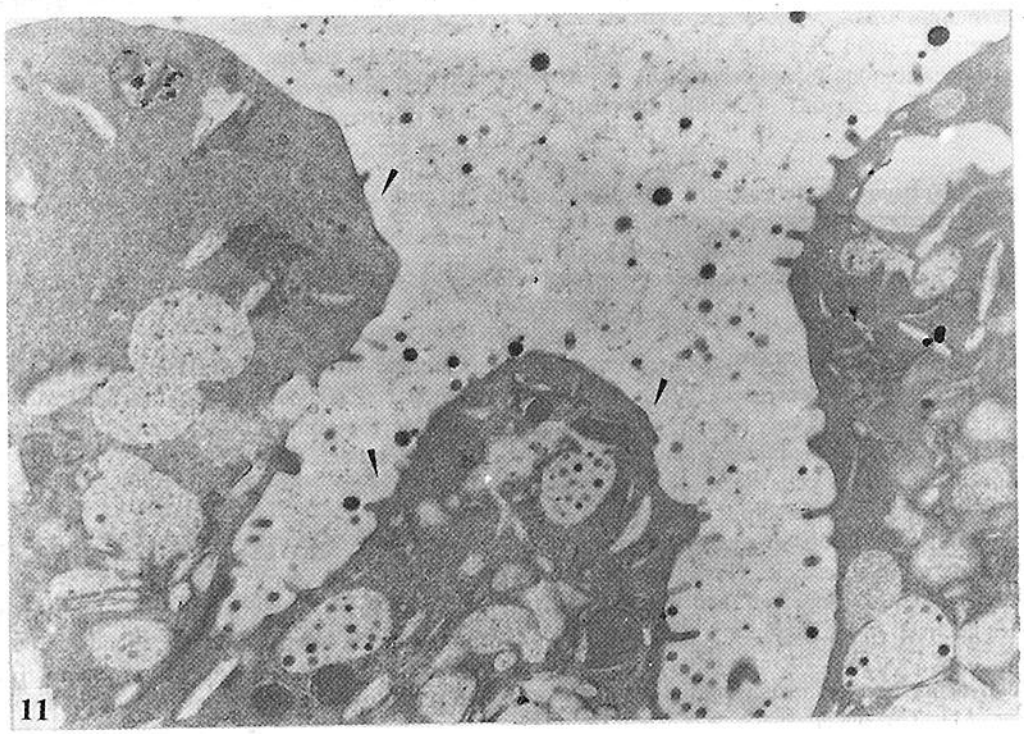
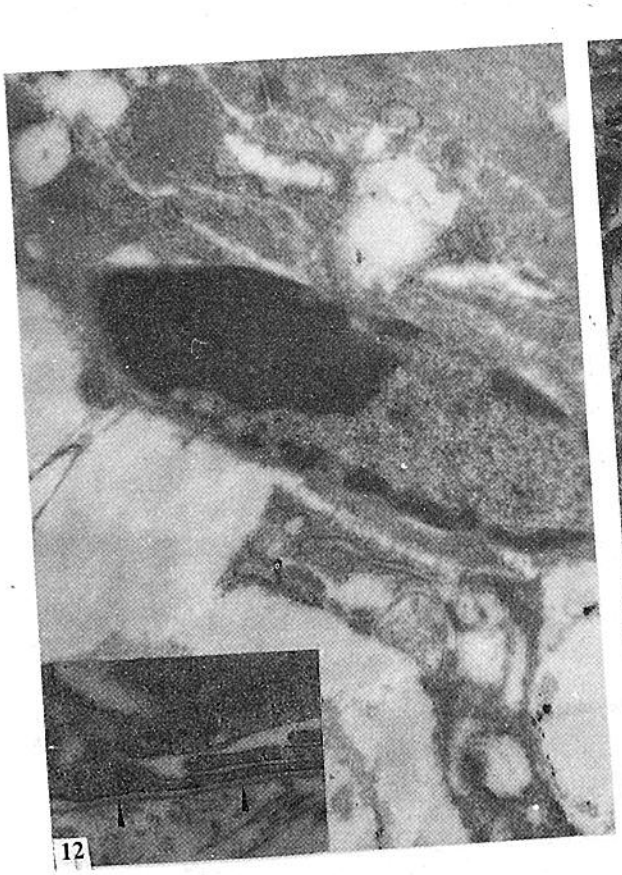


Fig. 11: (4S). Transmission electron microscopy; higher magnification of residual RER (arrowshead) and coalescent vesicles (asterisks) in the condensed cytoplasm. x 15500.



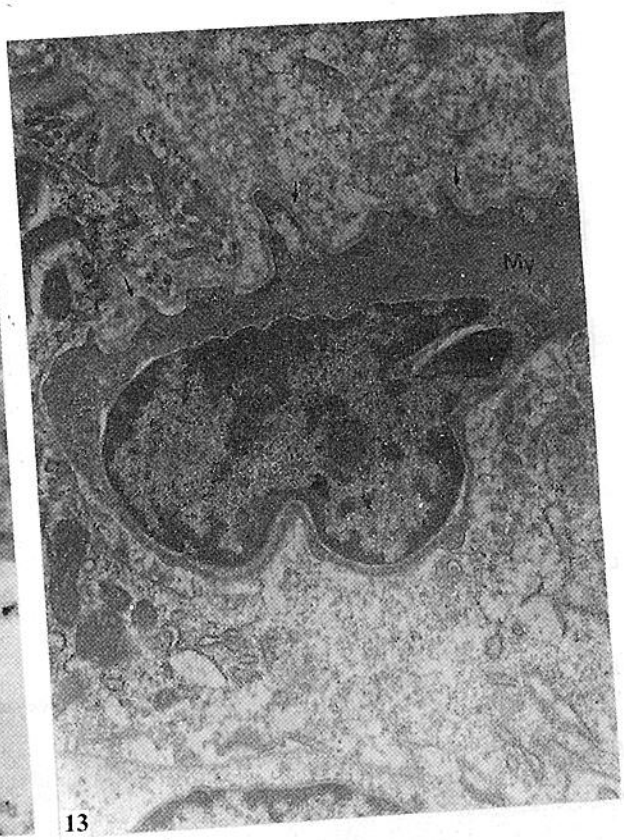


Fig. 12: (4S). Transmission electron microscopy. Nucleus with chromatin condensed at the border of nuclear membrane x 15500. **Inset**: basement membrane (arrowshead). x 6300.

Fig. 13: (4S). Transmission electron microscopy. Myoepithelial cell (My) with cytoplasmatic protrusions (arrows) into alveolar cells. x 8900.

After 8 days from dry off (4S) the proportions of stromal with lumen area decreased, the alveoli were characterized by a layer of very closely packed cells. The epithelium of sheep mammary gland lost its secretive structures and the cells were filled with large stasis vacuoles and large fat drops (Fig. 9). At this stage it was possible to observe just residual microvilli and some protein vescicles (Fig. 10). The cytoplasm organisation disappeared and cytoplasmatic organelles declined in the involuting cells. The residual RER was spread, whereas mitochondria and Golgi dictyosomal components were not distinguishable (Fig. 11). The nuclei shape was irregular and they showed characteristic changes of apoptosis as compacting chromatin at the margins of nuclear membrane (Fig. 12) but the integrity of basement membrane was conserved (insert Fig. 12). The myoepithelial cells become irregular and extended numerous cytoplasmic process into the connective tissue and alveolar cells (Fig. 13).

DISCUSSION

The involution of ovine mammary gland presents morphological changes of the glandular epithelium that are quite similar to those of rodents. However, the nature and the extent of mammary involution is very different and it is quite difficult to compare the mammary remodelling which occurs in rats or mice to that of cows and sheep. In bovine the extent of

lactation and drying-off does not lead to a complete loss of the alveolar structure, that is maintained for 42 days after cessation of milk [13, 21], until next calving. This is in marked contrast to that occurs in rats where degenerative cells were shed into alveolar lumens by 48-72 h after weaning [22].

The deletion of basement membrane, observed in mice [2, 7, 23], was not noticed in the present and previous studies on sheep mammary gland during involution, maybe due to undefined, protease-independent mechanism [15, 20]. This could confirm that the alveolar structure of the gland is not lost completely between successive lactations. In the reported study the attention has been focused to the first week after the end of lactation and repeated collections of mammary tissue from the same animal, allowing to observe which morphological changes occur. The alveolar structure begins to alter after 2 days from the end of lactation and the apoptosis is very active at 8th day [20], then, as reported by Tatarczuch et al., [15], the apoptotic cells number declines quickly. Similarly to the mammary tissue of cows and goats, the epithelial cells in sheep show a good preservation of morphology that is lost as the involution course when the cells undergo swelling and lysis. For the reported period, there is not an extensive shedding of alveolar epithelial cells rather a massive structural change. Large intracellular vacuoles form within the first 3 days of dry-off and seem to arise by fusion of secretory vescicles and lipid droplets, this could be consequent to the rearrangement of cytoskeletal component during cytoplasm condensation. Tight junctions between epithelial cells are observed just on the 1st day from the end of lactation and this is consistent with the appearance of skrunken alveoli in the last period. Although at 4S the protein synthesis and consequent secretory activity are breakdown, the involuting epithelial cells maintain fewer organelles involved in metabolic and secretory function. Free ribosomes and segments of remaining RER are more sparse and appeared stifle in the condensed cytoplasm, Golgi apparatus is not distinguishable after 8 days from the end of lactation. The nuclei appear larger and the notable feature of these nuclei is their identations which seem to divide the nuclei into several fragments. These ultrastructural observations point out which changes occur during mammary involution and agree with previous works carried out on sheep [15], goat [24] and cow [12, 25, 26, 27] and further confirm that the sheep mammary gland does not loose completely its arrangement.

The turnover of ovine mammary epithelial cells during dry period suggests that it is important for replacement of senescent and exhausted cells prior a subsequent lactation.

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