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ULTRA-STRUCTURE STUDY OF MAMMARY GLAND IN ZARAIBI GOATS DURING DIFFERENT STAGES OF LACTATION

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ABSTRACT

This work was carried out on 32 Egyptian Nubian (Zaraibi) goats to investigate the effect of stage of lactation on the ultra-structure of secretory mammary cells and its relationship with milk production. Biopsies were taken surgically from the mammary gland from three goats for histological and fine structure studies. The histological structures of mammary gland showed clear differences between lactation stages, being more developed in the early and mid stages, compared to late stage of lactation. The number of alveolar secretory cells increased from early to mid stage of lactation by 17.6% then reduced at the late stage by 25% from that at mid stage. The milk yield increased by 32.2% from early to mid stage, and then reduced to 61.3% from mid to late stage. Ultra-structure features in mammary epithelial cells showed different features at successive stages of lactation. The nuclei of the active mammary epithelial cells were ovoid or nearly spheroid in shape and indentation in cells membranes. The stage of lactation had an effect on nucleus diameter and sectional area. The nucleus diameter was smallest during early and mid stages, while being greatest at late stage of lactation. The sectional area of the nucleus showed increased values in late stage of lactation than in other stages. The majority of cell organelles were distributed in the cytoplasm. These organelles showed different phenomenon during different stages of lactation.

The mitochondria showed a clear change in shape (ovoid or elongated), number and sectional area during different stages of lactation. The number of mitochondria per plate was maximal during mid-stage, followed by early stage (44.2 and 37.9, respectively). The total mitochondria sectional area at late stage was 27.9 um^2. This area increased at early and mid stage of lactation by 3.8 and 37.9%, respectively. A well- developed laminated endoplasmic reticulum (ER) was frequently found in the cytoplasm of the mammary epithelial cells. Stage of lactation influenced the diameter of ER. At early and mid stages of lactation, the ER was well developed to occupy large part of the cell, with diameters of 0.55 and 51 µm, respectively. This value was minimal during the late stage of lactation (0.36 um^2). Protein granules had an ovoidal shape and released in different sizes. The sectional area of the protein was the smallest during late lactation (0.05 μm^2) and increased during early and mid stages of lactation (0.1 μm^2). The lipid droplets were appeared in different sizes, the sectional area of the lipid droplets were the smallest during late lactation (1.46 μm^2), and increased during early and mid lactation (4.52 and 2.58 μm^2, respectively).

In conclusion, the results of the present study indicated that stage of lactation influence the activity of mammary epithelial cells. The activity increases at early and mid lactation which is reflected on ultrastructure of epithelial cells of mammary gland of Egyptian Nubian (Zaraibi) goats.

Keywords: *Zaraibi goat: milking frequency, stages of lactation, mammary gland, cytological structure*

INTRODUCTION

Mammary glands are unique organs developed for nursing newborn and offspring. They repeat cycles of development, lactation, and involution every pregnancy. The progress of lactation is characterized by major changes in the population of mammary gland secretory cells (Capuco and Akers, 1997). In order to function in an integrated manner, the individual cells composing epithelial and other organized tissues must adhere to one another. Moreover, integration of these tissues with surrounding cells and intercellular matrix has to secure the movement control of ions and small mechanism of membrane transportation molecules collectively (Capuco and Akers, 1997). In

goats, the number of secretory cells is maximal at the initiation of lactation, and the increase in milk production at early lactation is due to cell differentiation (Knight and Wilde, 1993). After peak lactation, the differentiation state of tissue is maintained constant throughout declining lactation, and the loss of secretory cells accounts for the decrease in milk yield. It is well established in rodents and ruminants that mammary cell loss during involution occurs through programmed cell death (apoptosis), Wilde *et al.* (1997). There was wide individual variation in the potency of milk yield and quality which denotes inherent differences in the secretory activity of the mammary gland between individual goats. Alongside this individual difference, shows change in rate of milk secretion and in the percentage of milk components through the stages of lactation period (Augsburger, 1988).

Egyptian Nubian (Zaraibi) goats, known to be a progenitor of the standard Anglo-Nubian, are raised as household dairy animal in North-East of Nile Delta and known for high prolificacy (Aboul-Naga *et al.*, 2012). Zaraibi goats is considered one of the best milk producers under Egyptian conditions, yielding 263 Kg milk in a 260 days lactation period, with an average litter size of 2.3 (Hamed, 2005). Few works was undertaken to study the microstructure of the goat mammary gland as related with its physiological activity.

The aim of the present study was to investigate the effect of the lactation stage on ultra-structure of the secretory mammary cells and its relationship with milk production in Zaraibi goats.

MATERIALS and METHODS

Animals, treatments and Management Conditions

The current study was carried out at Sakha Experimental Farm, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Thirty-two multiparous healthy Zaraibi dairy goats (3 to 5 years old and 35 kg average body weight, BW) were used in the present study.

At last month of pregnancy, does were fed according to (NRC, 2007, for production of 1-2 kg goat's milk/head/day). Kids weaned at age of 10-12 weeks.

Milking and Measurements

During suckling period, milk yield was measured weekly (hand milking) by using oxytocin technique (Doney *et al.*, 1979). After weaning, machine milking was used (Rotary vacuum pump, 0.75-1.1 kw, and custom). Does were milked twice daily at 08:00 am and 05:00 pm. Milk yield was recorded weekly for each doe in the early stage (from parturition till day 15), and mid stage of lactation (from day 15 to 90 from parturition) and the end of lactation was at days 255 of parturition (late stage). Milk yield was measured by using an electronic balance. Does were dried off when its daily milk declined to 100 g/day.

Mammary gland biopsies

Biopsies were taken from 3 does after 15 days from parturition (early stage), at 60 days (mid lactation), and at 240 days (late stage). The biopsies were taken from one half of the udder after milking through a minor surgical procedure. The animal was anaesthetized using xylaject (0.2 mg/kg by intramuscular injection), then a small incision was made in the skin of the udder and a small piece of the parenchyma ($0.25cm^3$) was taken after dissection of the subcutaneous tissue and the gland capsule. The soft tissues were then sutured using chromic catgut and the skin was closed using silk thread with simple interrupted sutures; and then the sutured was treated with antibiotic spray. The animals were injected intramuscularly with a systemic antibiotic (long-acting Terramycin, 1ml per 10 kg BW); in addition, mastlone was injected through the teat of the udder, (Mastlone produced by Pfizer animal Health). Mastlone treatment repeated for 3 days. After surgery, does were separated for 7 days, and milked manually and the produced milk was excluded.

Light microscopy

The mammary gland tissue samples were fixed in 10 % neutral formal saline overnight at 4 °C before being transferred to grades of ethyl alcohol, spending 24 h in each grade. Samples were cleared in Xylene and embedded in

paraffin (mp 55 °C), according to Junquerira and Carneiro (1980). These samples were sectioned (4 µm thickness) and stained with hematoxylin and eosin and then examined by light microscopy. The sections were viewed by light microscopy (Olympus XSZ-107BN, Olympus Corporation, Tokyo, Japan). For each case, five microscopic fields were detected randomly at 10x then the fields were examined at 40x to determine the number of cells per alveolus using a computer. The average numbers of cells for the five microscopic fields was then calculated.

Electron microscopy procedure

For electron microscopy, fresh mammary gland samples were perfused with a buffered glutaraldehyde 5% solution (1 glutaraldehyde 25%: 4 cacodylate buffer; pH 7.2) after washing in saline. The tissue samples were then cut into small pieces (1mm×1 mm), cleared in 1% osmic acid for 2 h and embedded in polyethylene capsules. Sections of 1 µm were stained with toluidine blue and examined by light microscopy (Bozzola and Russell, 1995). Ultra-thin sections were mounted on a copper grid, stained with uranyl acetate and lead citrate and examined using a JEOL-100 transmission electron microscope. The sectional areas of the available ovoid nuclei and mitochondria, lipid droplets, and protein granules were determined according to the equations reported by Alan (2003). The diameter of endoplasmic reticulum (rough and smooth) was measured.

The light microscopy sections were used to describe the general features of the epithelial cells of mammary gland. While, the electron microscope images were used to describe and measure the cellular organelles of the epithelial cells during different stages of lactation.

Statistical analyses

Data were analyzed by SAS (2002), using the general linear model (GLM) procedures, and Duncan's multiple range test. The statistical model was:

$$\mathbf{Y_{ij} = U + time_i + e_{ij}}$$

Where:

Y_{ij} = Cell number (CN), milk yield (MY),

U = overall mean

Timei = Stages of lactation ~~for~~ (early, mid, and late)

e_{ij} = random error

$Y_{ijk} = U + time_i + e_{ijk}$

Where:

Y_{ijk} = Long diameter, short diameter and sectional area of (nucleus, mitochondria, protein granules and lipid droplets).

U = overall mean

Timei = Stages of lactation ~~for~~ (early, mid, and late)

e_{ijk} = random error

RESULTS and DISCUSSION

Milk yield

The milk yield increased by 32.2% from the early to mid stage, and then reduced to 61.3% from the mid to late stage (Table 1).

Histological features

The histological structure of the mammary gland showed significant differences ($p \leq 0.01$) between lactation stages. The secretory alveoli were more developed at early and mid stages, compared to late stage of lactation (plates 1a, b and c). The number of the alveolar secretory cells per alveolus was increased from the early to mid stage by 17.6% and then reduced to 25% from the mid to late stage (Table 1).

Table 1: Milk yield (g), cell number per alveolus at the successive stages of lactation for Zaraibi goats (Mean ±S.E.)

Items	Milk yield (g)	Cell number per alveolus
Early lactation	1778 ± 38^{b}	$21.1 \pm 0.96^{a\ b}$
Mid lactation	2351 ± 68^{a}	25.6 ± 2.29^{a}
Late lactation	910 ± 44^{c}	19.2 ± 1.1^{b}

Means in columns with different superscript letters (a-c) are significantly different ($P \leq 0.01$).

Ultra-structural features

Ultra-structural features in the mammary epithelial cells showed different features at successive stages of lactation (plates 3, 5, 6 and 10). During - the successive stages of lactation (early and mid lactation), the mammary epithelial cells contained clearly developed - nucleus, endoplasmic reticulum (ER), mitochondria, lipid droplets and protein

granules (plates 3 and 6). While during late stage -, numerous small lipid droplets, few mitochondria and the endoplasmic reticulum was much less abundant (Plates 5, 9 and 10).

The nuclei of the active mammary epithelial cells were ovoidal or nearly spheroid shape. The nuclei showed indentations in cells-membranes (Plates 2, 3 and 4).

The sectional areas of the nuclei were the smallest during early and mid stage of lactation (71.4 μm^2 and 70.0 μm^2, respectively), and increased during late stage of lactation (84.4 um^2), Table (2). High values of long axes (6.5 um^2) was recorded at late stage of lactation and shows clear lost of nucleus regular shape to become irregular (Plate 10).

Numerous mitochondria were observed in the cytoplasm of the mammary epithelial cells, with an ovoidal or elongated shape. Mitochondria were distributed at random and at a higher frequency around the nuclei (Pates 2, 3 and 8).

The total mitochondria in a sectional areas of (μm^2/plate) was highest at early and mid lactation (37.1μm^2 and 38.5 μm^2, respectively), while being lowest at late lactation (27.9 μm^2), Table (3). This area increased 3.8% from the early to mid stage, and then reduced to 37.9% from the mid to late stage. The number of mitochondria per plate increased by 12.5% at early lactation and by 30.4% at mid lactation, compared to that at late lactation (Table 3).

Some organelles, such as endoplasmic reticulum (ER), appeared as numerous cisterna. These cisterna were greatly developed during early and mid stages of lactation (plate 4), but not at late stage of lactation (plate 5). A laminated ER surrounded the nucleus, was embedded in the middle and apical regions of the mammary epithelial cell (plate 2).

Table (4) demonstrates the effect of lactation stage on the diameter of ER, being the greatest at early and mid lactation (0.55 and 0.51 µm, respectively), which related with high milk production compared to that at late stage of lactation (0.36 µm).

Table 2: of nuclei diameters (µm) and nuclei sectional areas (μm^2) during the successive stages of lactation for Zaraibi goats (Mean ± SE)

Item	Diameter (µm)			Sectional area
	Number of tested nuclei	Long	Short	
Early lactation	43	5.8 ± 0.32 [b]	3.8 ± 0.18 [a]	71.4 ± 5.1[a]
Mid lactation	91	5.6 ± 0.17 [b]	3.7 ± 0.14 [a]	70.0 ± 3.9 [a]
Late lactation	30	6.5 ± 0.27 [a]	4.1 ± 0.16 [b]	84.4 ± 6.0 [b]

Means within column with different superscript letters (a-b) are significantly different (P≤0.01).

Table 3: Mitochondria diameter (µm) and mitochondria sectional area (μm^2) at successive stages of lactation for Zaraibi goats (Mean ± SE)

Item	Diameter (µm)			Single mitochondria Sectional area (μm^2)	Total sectional areas of mitochondria/ plate
	Number of tested mitochondria/ plate	Long	Short		
Early lactation	37.9	0.70 ± 0.02 [a]	0.38 ± 0.01 [b]	0.98 ± 0.04 [a]	37.2
Mid lactation	44.2	0.67 ± 0.02 [a]	0.37 ± 0.01 [b]	0.87 ± 0.03 [b]	38.5
Late lactation	33.7	0.61 ± 0.02 [b]	0.41 ± 0.01 [a]	0.83 ± 0.04 [b]	27.9

Means within columns with different superscript letters (a-b) are significantly different (P≤0.01).

Table 4: Number and diameter (µm) of endoplasmic reticulum (ER) in the mammary epithelial cells of Zaraibi goats during different stages of lactation

Item	Number of measurements/plate	Diameter (µm)
Early lactation	**48**	**0.55 ± 0.32[a]**
Mid lactation	**71**	**0.51 ± 0.10[a]**
Late lactation	**40**	**0.36 ± 0.03[b]**

Means within columns with different superscript letters (a-b) are significantly different (P≤0.01).

At the end of lactation, the endoplasmic reticulum loses has parallel arrangement and breaks into smaller pieces which minimize it's apparent, minimal abundant or even absent, and the channels showed smooth membranes (Plate 5).

In the active secretory cells, the channels' membranes are studded by granules (the ribosomes), thus denoted as rough ER, that receives its name from the many ribosomes, the site of synthesis of the polypeptide chains of the secreted proteins, that comprise the major source specific for milk proteins (Plate 4).

The density and diameter of protein granules were measured in the mammary epithelial cells. Protein granules had an ovoidal shape and released in different sizes, large diameter at early lactation (plate 6), while being of less diameter at mid lactation (plate 7).

The sectional area of the protein were the smallest during late lactation (0.05 µm^2) and increased during early and mid lactation (0.1 µm^2), denoting greater activity of the cells (Table 4).

The lipid droplets were appeared in different sizes, there were numerous large lipid droplets in the cytoplasm at early and mid lactation (plates 3, 6 and 8), while there were small at late lactation (plate 9). The sectional area of the lipid droplets were the smallest during late lactation (1.46 µm^2), and increased during early and mid lactation (4.52 µm^2 and 2.58 µm^2 respectively), (Table 5). The sectional area of the lipid droplets were reduced by 67.7% from early lactation to late lactation (Table 5).

The stages of lactation were significantly affecting all measurements of fat droplets (Table 6). At the end of lactation, the cytoplasmic organelles had been reduced by a process of auto digestion (Plates 10).

DISCUSSION

The present study elucidated the enhanced activity of the epithelial cells of mammary tissue during early and mid lactation in conjunction with the increasing activities of milk production during these stages (*El-sayed et al*., 2009 and Hassan, 1997). During early and

Table 5: Diameter of protein granules (µm) and protein granules sectional area (µm^2) during the successive stages of lactation for Zaraibi goats (Mean ± SE)

Item	Diameter (µm)		Sectional area
	Long	Short	
Early Lactation	0.15 ± 0.004 [a]	0.15 ± 0.004 [a]	0.10 ± 0.01 [a]
Mid Lactation	0.15 ± 0.01 [a]	0.15 ± 0.01 [a]	0.10 ± 0.01 [a]
Late Lactation	0.11 ± 0.01 [b]	0.11 ± 0.01 [b]	0.05 ±0.01 [b]

Means within columns with different superscript letters (a-b) are significantly different (P≤0.01).

Table 6.: Lipid droplets diameters (µm) and lipid droplets sectional area (µm^2) at the successive stages of lactation for Zaraibi goats (Mean ± SE)

Item	Diameter (µm)		Sectional area
	Long	Short	
Early lactation	0.95 ± 0.06 [a]	0.66 ± 0.04 [a]	4.52 ± 0.82 [a]
Mid lactation	0.88 ± 0.03 [a]	0.61 ± 0.02 [a]	2.58 ± 0.18[b]
Late lactation	0.59 ± 0.03 [b]	0.46 ± 0.02 [b]	1.46 ± 0.20 [b]

Means within columns with different superscript letters (a-b) are significantly different (P≤0.01).

mid stages of lactation, the epithelial cells showed an increase in the size of ER, increased number and size of mitochondria (plates 3, 4 and 6), lipid droplets and protein granules (plates 3, 4 and 6), indicating an increase in functional activity of mammary gland.

The number of the alveolar secretory cells per alveolus and milk yield was increased from early to mid stage of lactation. The number of the alveolar secretory cells per alveolus, is the best indicator of mammary gland lactogenic activity (*El-sayed et al*., 2009). At these stages, the lobuli of the mammary glands showed large numerous alveoli with wide lumina adjacent to the thin connective tissue. (*El-sayed et al*., 2009).

The number of secretory cells is maximal at the initiation of lactation, and the increase in milk production that occurs in early lactation is due to cell differentiation (Knight and Wilde, 1993).

After peak of lactation, the differentiation state of the tissue is maintained constant throughout declining of lactation, while the loss of secretory cells accounts for decrease in milk yield. It is well established in ruminants that mammary cell loss during involution occurs through programmed cell death (apoptosis), Wild *et al*. (1997).

The highly significant correlation between the number of mammary epithelial cells and milk yield at the stages of lactation, indicates that the increase in milk production is related to increased cellular number in the mammary parenchyma, which is consistent with the hypothesis of *El-sayed et.al*. (2009) whom attributed the increase in milk production to cell differentiation. While, indicators of metabolic activity increased during this period (Capuco *et al*., 2001).

The sectional areas of the nuclei were started to increase at late stage of lactation (84.4 μm^2) , denoting less activity of the cells (Table 2). Larson (1985) reported that at end of lactation the nucleus occupy a great part of the cell.

The endoplasmic reticulum (ER) had well-developed laminated cells during the early and mid stages of lactation (Plate 4 and Table 4). The ribosomes, arranged on the outer surface of laminated ER, formed the rough endoplasmic reticulum. The ribosomes, which is the site of synthesis of polypeptide chains of the secreted proteins, that comprise the major specific milk proteins (Larson, 1985). ER diameter was decreased markedly at the end of lactation (Plate 5 and Table 4), (Shikhov and Kudryashov, (1971), Uhrin and Kliment, (1983). In buffaloes, at the beginning of lactation, the relative size and area of ER had high values and ER diameter was decreased markedly at the end of lactation (Hassan, 1997). The ER in goat's remains poorly developed until 4 days ante-partum, while it is very prominent in the form at early lactation (Augsburger, 1988).

Mitochondria had increased in number and size during early and mid stages of lactation (Plates 3, 6 and 8 and Table 3). This result agree with Hassan (1997) who reported that the maximum density and size of mitochondria at early and peak of lactation, in Egyptian buffaloes, might related to the high activity of cells.

During lactogenesis, the number of large lipid droplets and protein granules were greater from the early to mid stages of lactation than during late stage (Plates 3, 6, 7 and 8). This case is in agreement with Uhrin and Kliment (1983), Augsburger (1988) and Khrus-taleva (1987) whom reported in cow that about 65% of the cells, at 1-2 months of lactation, contained great number of casein filled vacuoles and lipid droplets, indicating high functional activity of the cells. Hassan (1997) reported that the lipid droplets appear at early stage of lactation and increase in morphometric values through stages of lactation.

In conclusion, the results of the present study indicated that stages of lactation influence the activity of mammary epithelial cells. The activity increases at early and mid lactation which is reflected on ultrastructure of the epithelial cells of mammary gland of Egyptian Nubian (Zaraibi) goats.

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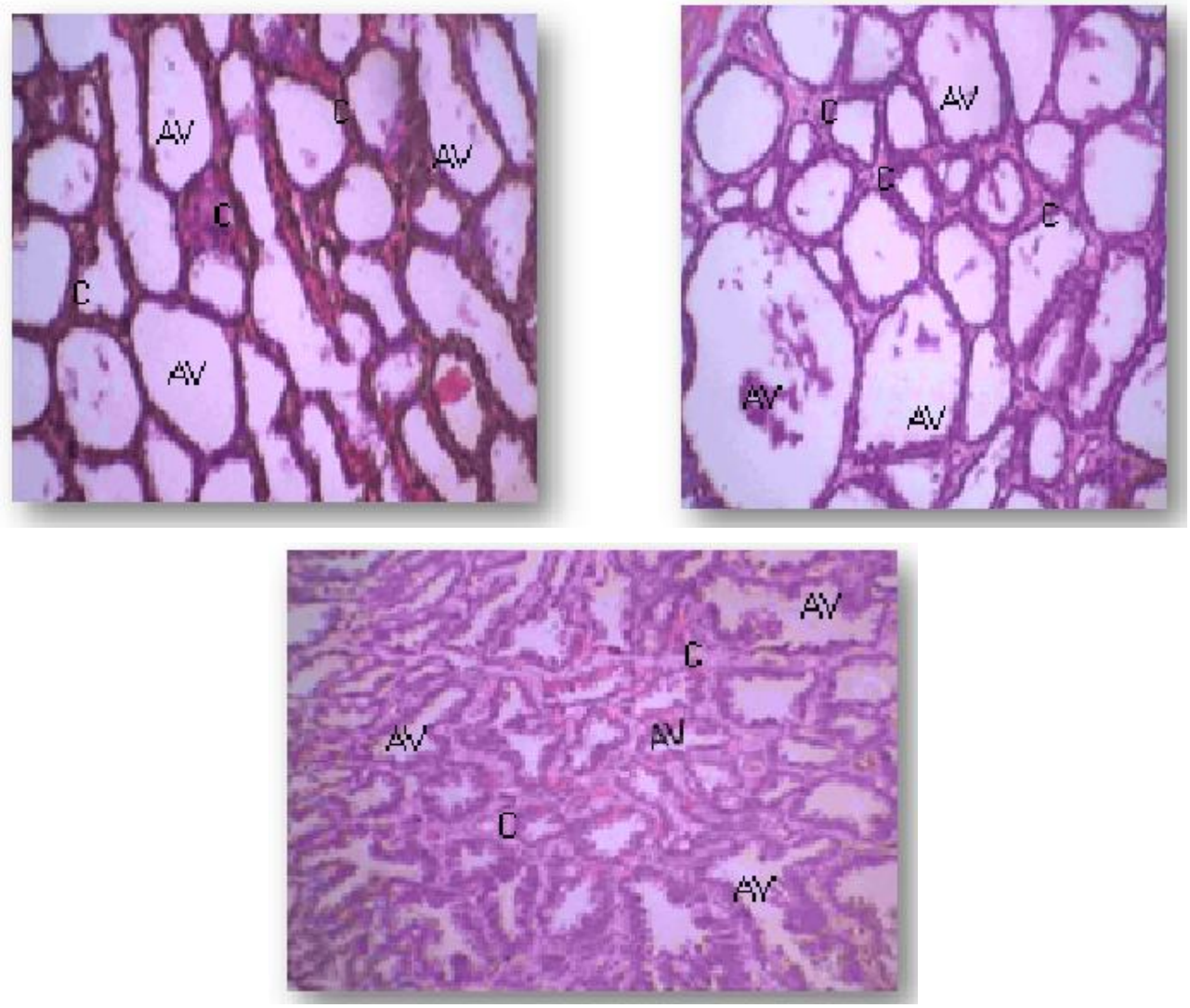

Figs. 1 (a & b) . Section in the goat mammary tissue at early and mid lactation showing secretory alveoli (AV), separated by thin trabeculate of connective tissue (C). Fig.c Section in the goat mammary tissue at late of lactation showing several alveoli (AV) separated by wide connective tissue (C).120x.

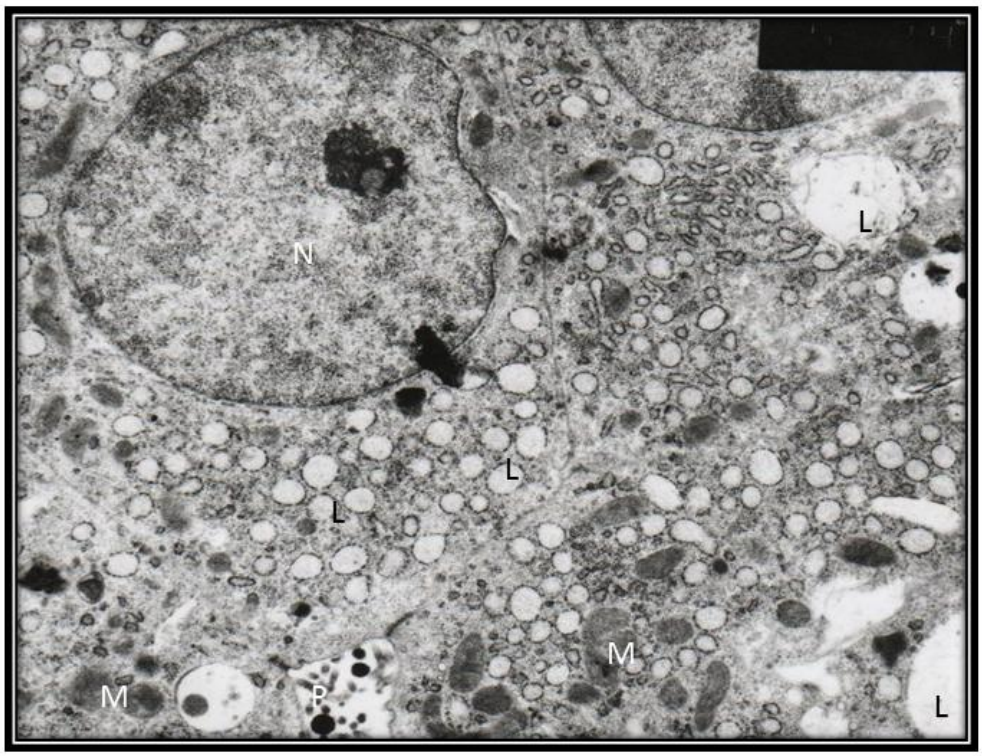

Plate 2. Mammary epithelial cells at early lactation, showing: nucleus (N), cytoplasm containing mitochondria different shapes and sizes (M), lipid droplets (L) and protein granules (P), (5000x).

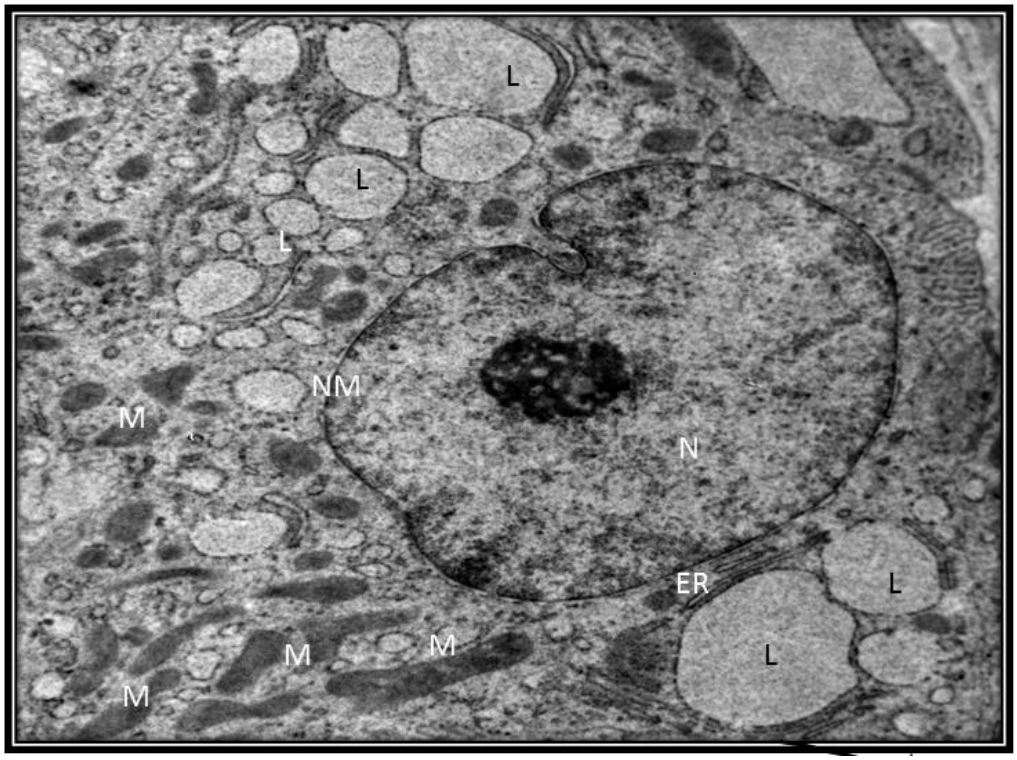

Plate 3. Ultra-structure of mammary epithelial cells at mid lactation showing, nucleus (N), double layered nuclear membrane (NM), cytoplasm containing mitochondria different shapes and sizes (M), and lipid droplets (L) (4800x).

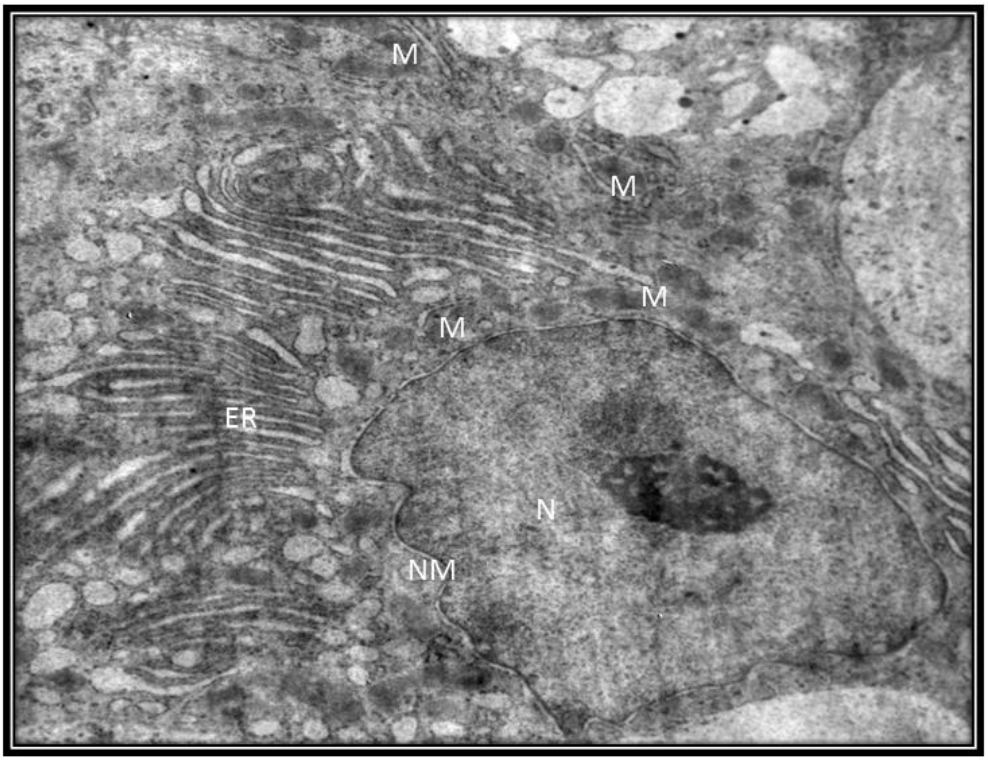

Plate 4. A well developed laminated endoplasmic reticulum (ER), nucleus (N), double layered nuclear membrane (NM), lipid droplets (L) and mitochondria (M) in mammary epithelial cells, during mid lactation, (4800x).

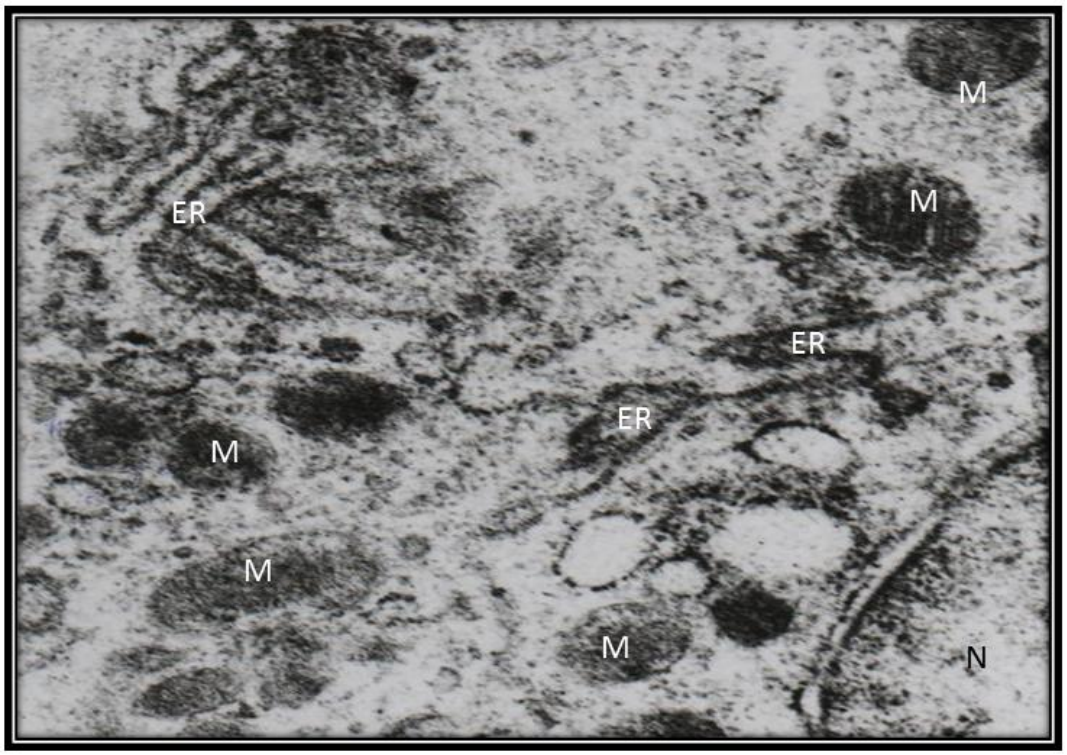

Plate 5. Thin endoplasmic reticulum (ER) decrease diameter at late lactation, mitochondria (M) (x14000).

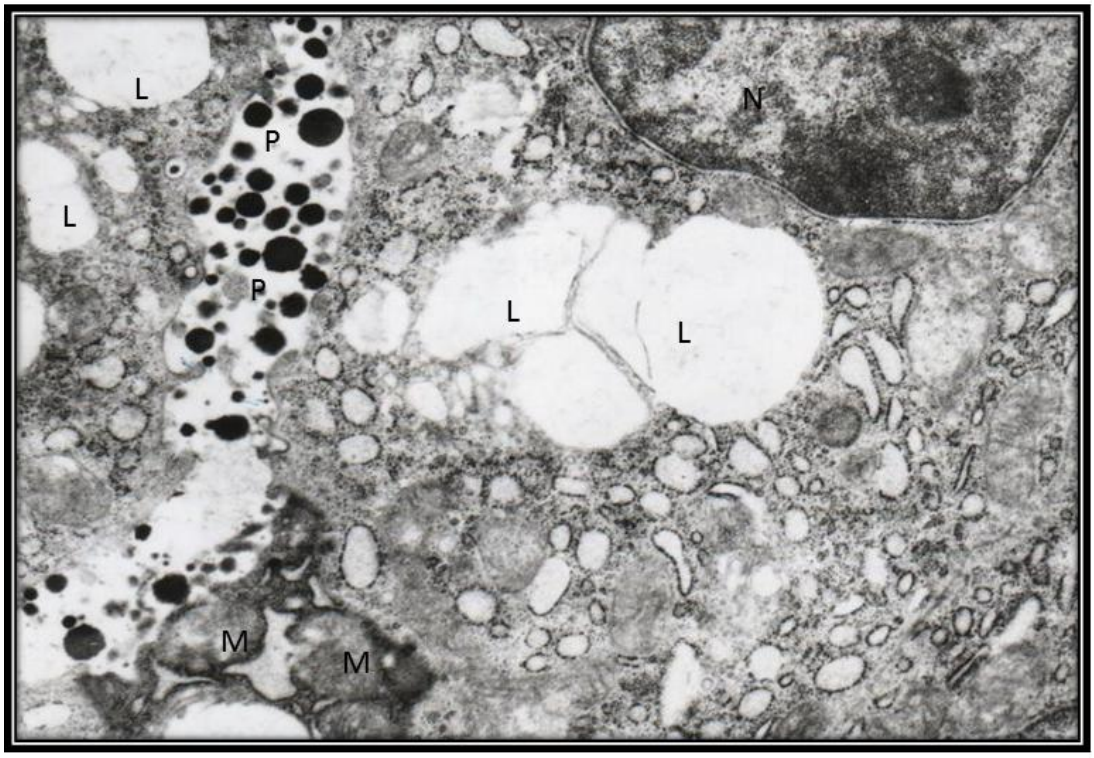

Plate 6. Structures of mammary epithelial cells at early lactation showing, large diameter of protein granules (P), nucleus (N), and large lipid droplets (L), and mitochondria (M), (5000x).

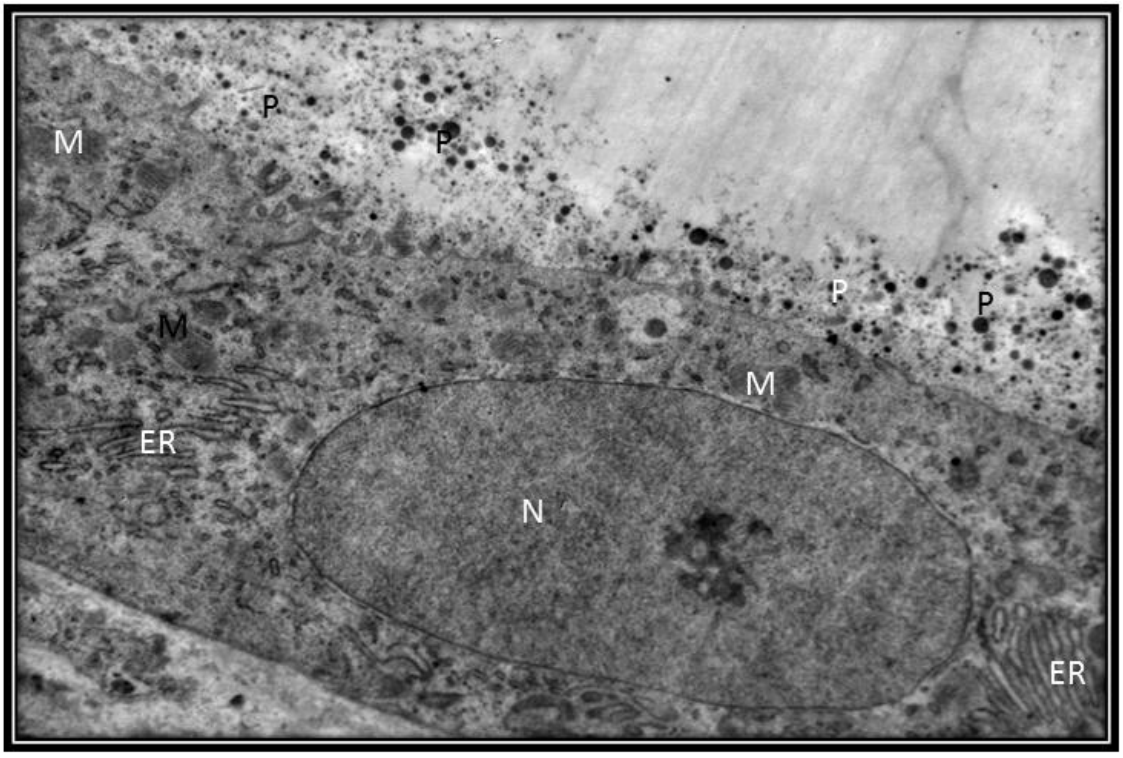

Plate 7. Low diameter of protein granules (P) at mid lactation, nucleus (N), mitochondria (M) and endoplasmic reticulum (ER), (4800x).

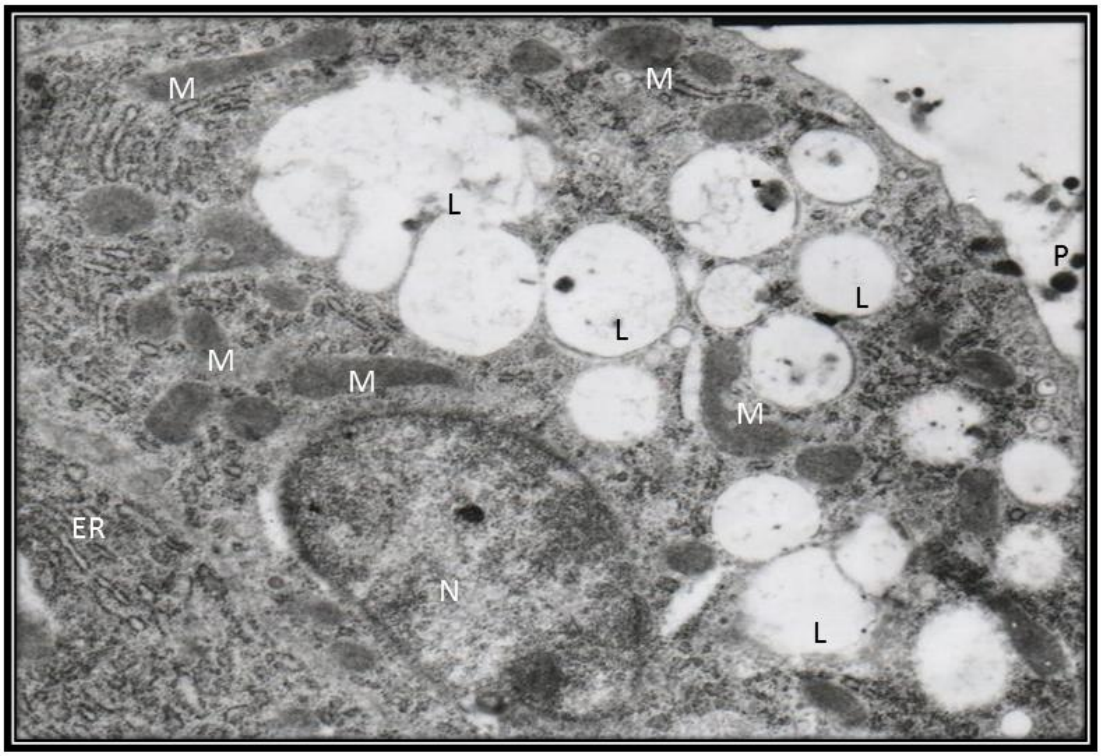

Plate 8. Ultrastructure of Zaraibi mammary epithelial at early showing, large lipid droplets (L) accumulated in the cytoplasm, different sizes and shapes of mitochondria (M) endoplasmic reticulum (ER), and nucleus (N) (6700x).

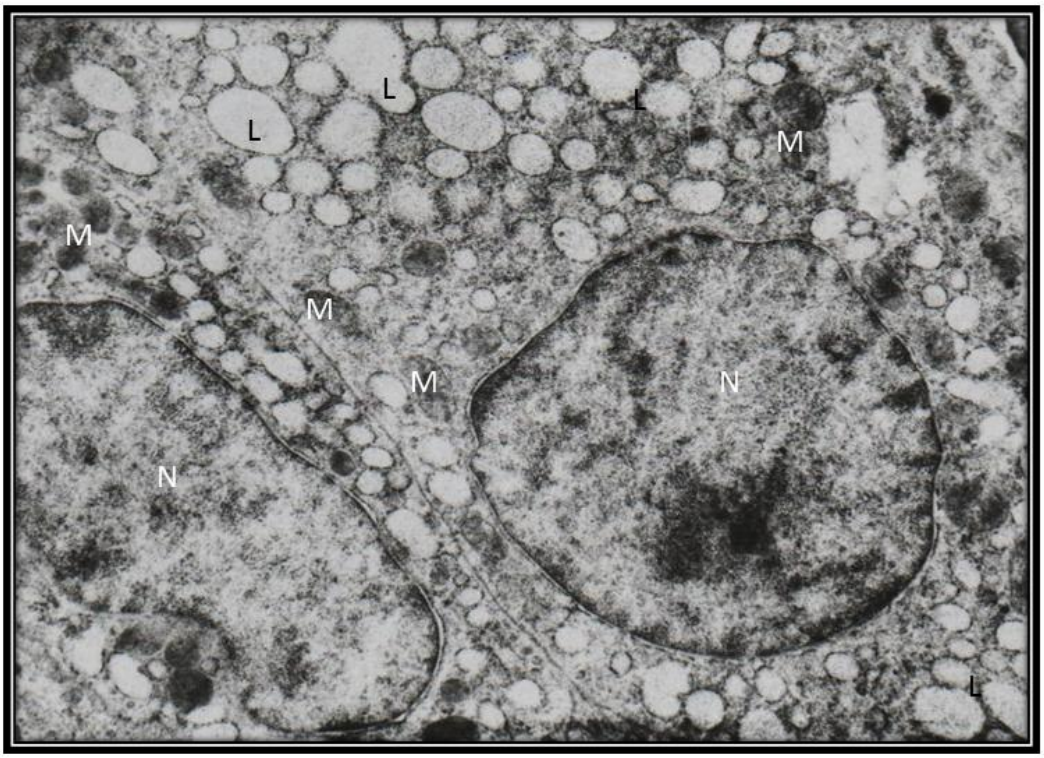

Plate 9. Mammary epithelial cells at late lactation showing, smaller lipid droplets (L) accumulated in the cytoplasm, mitochondria (M) and nucleus (N), (5000x).

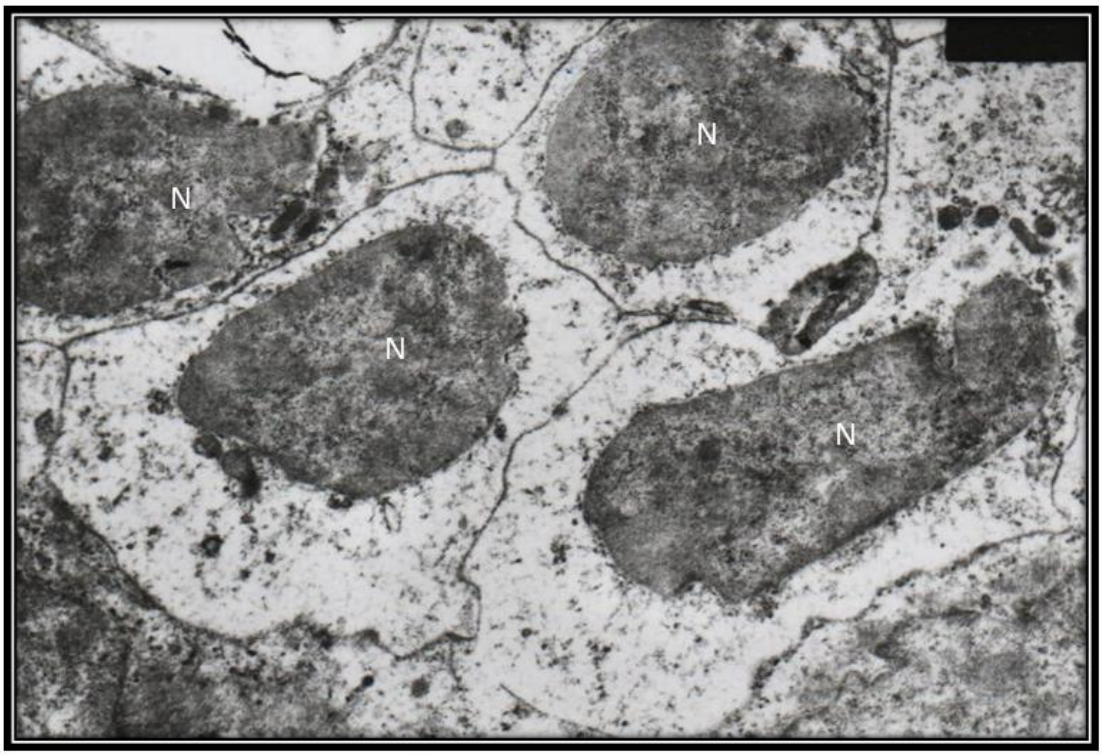

Plate 10. Involution mammary tissues at late of lactation showing, nucleus (N), the cells were shrinked and diminished in size, the cytoplasmic organelles become reduced by process of outodigestion (4000x).