

EFFECTS OF MILKING FREQUENCY ON MILK PRODUCTION AND HISTOLOGICAL STRUCTURE OF UDDER IN ZARAIBI DAIRY GOATS

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ABSTRACT

This work was carried out on 32 Egyptian Nubian (Zaraibi) goats to investigate both the effect of the milking frequency on milk yield and milk composition and the effect of stage of lactation on histological structure and histochemistry of the secretory mammary cells and its relationship with milk production in Zaraibi goats. Biopsies were taken surgically from the mammary gland from 3 does milked once daily (1x) and 3 does milked twice daily (2x) at the three stages of lactation, early, mid and late, for histological and histochemical studies. The histological structure showed clear differences due to milking frequency and lactation stage, being more developed at early and mid stages and twice daily milking compared to late stage of lactation and once daily milking. The number of the alveolus secretory cells per alveolus increased from the early to the mid stage of lactation by 17.6% and then reduced at the late stage by 25% compared to mid stage, while no difference noticed between twice and once daily milking. Once daily milking (1x) reduced milk yield by 6%, and increased fat percentage to 4.0% compared to 3.67% in twice daily milking group (2x). Milk of once milking (1x) group contained higher percentage of total solids 11.38% than twice milking (10.93%), but milk protein and lactose did not differ between 1x and 2x milking. Lactation curve showed 32.2% increase in yield during mid stage than early stage, while late stage attained 61.3% reduction in milk yield compared to mid stage. Protein and lactose percentages did not change throughout different stages of lactation, while fat and total solids percentages showed the highest values at early stage of lactation (4.0 and 11.5%, respectively), and the lowest at mid and late stage of lactation. The total sectional areas (u/plate) of the alveoli were the smallest during late lactation (495399 u /plate) compared to that during early and mid stages of lactation (705206 and 759901u/plate, respectively).

Numerous loci of alkaline phosphatase (AP) were apparent on the outer surface of the alveolar secretory cells at the early and mid stages of lactation—reflecting high activity of this enzyme at these two stages. This was accompanied by a high level of milk secretion reaching 1778.2 ± 38.9 and 2351.4 ± 68.4 g/head/day, respectively.

In contrast, at the late stage of lactation, the size of alveoli was reduced and few alveoli showed weak AP activity. This coincided with the reduction in milk yield (910g/head/day). It could be concluded that stages of lactation influence the cell number and activity of mammary epithelial cells.

Key words: *Zaraibi goats, milking frequency, stage of lactation, mammary gland, alveoli histochemical*

INTRODUCTION

The mammary gland is a complex organ either in structure or function. Milk yield and performance of the lactation curve are determined by the number of mammary secretory cells and the secretion activity per cell (Anderson, 1974; Tucker, 1981 and El-sayed *et al.*, 2009). Knight and Wilde (1993) added that declining phase of lactation mainly due to loss in mammary cells. The highly significant correlation between number of the mammary epithelial cells and milk yield at the different stages of lactation indicates that the increase in milk production is related to increased cells number in the mammary parenchyma (Knight and Peaker, 1984 and El-sayed *et al.*, 2009). Number of daily milking / day is of great importance in determining milk yield in dairy animals. One milking compared to twice milking reduced milk yield by 7 to 38% in dairy cows (Stelwagen *et al.*, 1994 and Stelwagen and Knight, 1997), 15 to 48% in ewes (Knight and Wilde, 1993 and Negrao *et al.*, 2001) and 6 to 35% in dairy goats (Mocquot, 1978; Capote *et al.*, 1999 and Salama *et al.*, 2003).

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The aim of the present study was to investigate both the effect of the milking frequency on milk yield and milk composition and the effect of stage of lactation on histological structure and histochemistry of the secretory mammary cells in relation to production in Zaraibi goats.

MATERIALS AND METHODS

Animals, treatments and management condition

The present study was carried out at Sakha Experimental Farm, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Thirty-two multiparous Zaraibi healthy dairy goats (3 to 5 years old and 35 kg average body weight, BW) were used. Does were fed according to Nutrient Requirements of Dairy Goats (NRC, 2007, for production of 1-2 kg goat's milk/head/day). Kids were allowed to suckle dams until reach weaning age of 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, 0.75-1.1kw, and custom). Goats were divided randomly into 2 balanced groups, first group (n=16), milked once daily (1x) at 08:00 am and the second group (n=16) was milked twice daily (2x) at 08:00 am and 05:00 pm. Milk samples were taken weekly for chemical analysis. Milk yield was individually recorded once weekly until end of lactation at 255 days after parturition.

Mammary gland biopsies

Biopsies were taken from 3 does from 1x and 3 does from 2x at day 15th after parturition (early stage), at day 60th (mid lactation) and at day 240th (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 (0.2mg/kg by an intramuscular injection), then a small incision was made in the skin of the udder and a small piece of the parenchyma (0.25 cm³) was taken after dissection of

subcutaneous tissue and the gland capsule. The soft tissues were then sutured using chromic cat gut and the skin was closed using silk thread with simple interrupted sutures. 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 to mastlone injection into the teat. Mastlone treatment applied for 3 days. After surgery, does were separated for 7 days, milked manually and the produced milk was excluded.

Processing for light microscopy

The mammary gland tissue samples were fixed in 10 % neutral formal saline overnight at 4 °C then transferred to grades of ethyl alcohol for 24 h in each grade. Samples were cleared in xylene and embedded in hot paraffin (mp 55 °C), according to Junqueira and Carneiro (1980). These samples were sectioned using a microtome (4 µm thickness) and stained with hematoxylin and eosin then examined by light microscopy. The sections were viewed by light microscope (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 were then calculated. The light microscopy sections were used to describe the general features of the epithelial cells of mammary gland. The sectional areas of the alveoli were determined according to the equation recorded by Alan (2003). The number of alveoli was recorded.

Histochemical investigations

Histochemical investigations were executed to detect alkaline phosphatase (AP) enzyme activity, in mammary epithelial cells. AP activity was assessed by the method of Rutenburg *et al.* (1965) as follows: the sections were cleared by xylene for 10 min and grades of ethyl alcohol (100%, 90%, 70% and 50%) for 3–4 min. in each grade, washed in running tap water for 2–3 min, followed by fixation for 30 s by formol methanol at 4 °C and washing in running tap water for 2–3 min. These sections

were left to dry and then incubated at room temperature in a mixture of substrate Naphthol AS. Phosphate + Tris buffer for 15 min, followed by washing in running tap water for 2 min and left to dry. Lastly, counter staining was accomplished with sufranin for 57min, followed by dehydration and mounting in DPX and examined using a light microscope for denoting the sites of alkaline phosphatase enzymes on the secretory cells.

Statistical analyses

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

$$Y_{ijkl} = U + M_i + S_j + \text{Stages}_k + P_1 + (\text{stages } P)_{kl} + \text{SM}_{ij} + e_{ijkl}$$

Y_{ijkl} = Milk yield, milk composition, milk frequency (once or twice daily milking), stages of lactation (early, mid, and late), and interaction between stages and milk frequency

U = the overall mean.

M_i = Milk yield,

S_j = milk frequency (once or twice daily milking)

Stages_k = Stages of lactation (early, mid, and late)

P_1 = milk composition (Fat, protein, lactose and total slots)

$(\text{SM})_{ij}$ = interaction between lactation stage and milking frequency

e_{ijk} = residual error

$$Y_{ijkl} = U + \text{stages}_i + \text{pictures}_j + M_k + C_1 + e_{ijkl}$$

Y_{ijkl} : long diameter, short diameter and sectional area of alveoli

U = the overall mean.

stages_i = stages of lactation = (early, mid and late, respectively)

pictures_j = number of alveoli

C_1 = cell count

e_{ijk} = random error

RESULTS

Milk yield

Table 1 shows milk yield and composition for different milking frequencies at successive stages of lactation of Zaraibi goats. Once daily milking (1x) reduced milk yield by 6%, and

increased fat percentage to 4.0% compared to 3.67% in twice daily milking group. Milk of one milking (1x) group contained higher percentage of total solids (11.4%) than twice milking (10.93%), but milk protein and lactose did not differ between 1x and 2x milking (Table 1). Milk production is a function of the number and activity of mammary epithelial cells, regardless stage of lactation. Lactation curve showed 32.2% increase in yield during mid stage than that in early stage. While, late stage attained 61.3% reduction in milk yield compared to mid stage (Table 1). Protein and lactose percentages did not change throughout different stages of lactation, while fat and total solids percentages showed the highest values at early stage of lactation (4.0 and 11.5%, respectively), and the lowest at mid and late stage of lactation (Table 1). The fat percentage and total solids showed highest values at early stage of lactation (4.0 ± 0.06 and 11.5 ± 0.07 , respectively), and the lowest at mid and late stages (Table 1).

The interaction between stages of lactation and milking frequency was significant ($p \leq 0.001$) on fat, lactose and total solids percentages.

Histological and histochemical features

The histological sections of goats mammary tissues showed clear differences due to milking frequency and lactation stage, being more developed at early and mid stages and twice daily milking compared to late stage of lactation and once daily milking (plates 1-6).

The number of secretory cells per alveolus is the best indicator of mammary gland lactogenic activity (El-sayed *et al.*, 2009). It increased from the early to the mid stage of lactation by 17.6% and then reduced at the late stage by 25% compare to mid stage, while no significant difference noticed between twice and once daily milking (Table 2). The total sectional areas (u^2/plate) of the alveoli were the smallest during late lactation ($495399 u^2/\text{plate}$) compared to that during early and mid stages of lactation (705206 and $759901 u^2/\text{plate}$, respectively). Numerous loci of AP were apparent on the outer surface of the alveolar secretory cells at the early and mid

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Table 1. Means (\pm SE) of milk yield (ml) and milk composition (%) for different frequencies of milking at successive stages of lactation of Zaraibi goats.

Items	Milk yield	Fat	Protein	Lactose	Total solids
Once daily milking	1660.7 \pm 50.2 ^a	4.0 \pm 0.04 ^b	2.67 \pm 0.03 ^a	4.1 \pm 0.03 ^a	11.4 \pm 0.05 ^a
Twice daily milking	1760.7 \pm 47.4 ^a	3.67 \pm 0.03 ^a	2.7 \pm 0.02 ^a	4.03 \pm 0.02 ^a	10.9 \pm 0.05 ^b
Early lactation	1778.2 \pm 38.9 ^b	4.1 \pm 0.06 ^a	2.7 \pm 0.02 ^a	4.1 \pm 0.03 ^a	11.5 \pm 0.07 ^a
Mid lactation	2351.4 \pm 68.4 ^a	3.7 \pm 0.03 ^c	2.7 \pm 0.02 ^a	4.0 \pm 0.02 ^a	11.0 \pm 0.04 ^b
Late lactation	910 \pm 44.6 ^c	3.9 \pm 0.07 ^b	2.7 \pm 0.14 ^a	4.0 \pm 0.06 ^a	11.2 \pm 0.16 ^b

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

Table 2. Mean (\pm SE) of alveoli diameter (μ), sectional area (μ^2) and total sectional areas (/plate 111.2055 μ^2) at different types of milking and production at successive stages of lactation of Zaraibi goats.

Items	Number of Alveoli /plate	Cell count	Alveolus Diameter (μ)		Single alveolus sectional area (μ^2)	Total sectional areas of alveoli /plate (μ^2)
			Long	Short		
Twice daily milking	33.8 \pm 0.24 ^a	21.6 \pm 1.38 ^a	101.8 \pm 2.06 ^a	54.1 \pm 0.98 ^a	19946.9 \pm 741 ^a	674205.2 μ^2
Once daily milking	32.3 \pm 0.26 ^b	22.1 \pm 1.38 ^a	100.2 \pm 1.79 ^a	54.9 \pm 0.79 ^a	19683.7 \pm 655 ^a	635783.5 μ^2
Early lactation	32.2 \pm 0.18 ^b	21.1 \pm 0.96 ^{ab}	106.7 \pm 2.4 ^a	58.1 \pm 1.1 ^a	21900.8 \pm 857 ^a	705205.8 μ^2
Mid lactation	32.0 \pm 0.30 ^b	25.6 \pm 2.29 ^a	109.2 \pm 2.6 ^a	59.8 \pm 1.4 ^a	23746.9 \pm 1049 ^a	759900.8 μ^2
Late lactation	34.9 \pm 0.24 ^a	19.2 \pm 1.05 ^b	87.7 \pm 1.95 ^b	45.9 \pm 0.97 ^b	14211.0 \pm 577.4 ^b	495398.9 μ^2

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

stages of lactation—reflecting high activity of this enzyme at these two stages (Plates 7, 8, 9 and 10). During the last stage, a few alveoli showed weak staining for AP substrate, while most of them disappeared from membrane-associated AP, which reflects their weak enzyme activity (Plates 11 and 12).

DICUSSION

The number of secretory cells is maximal at the initiation of lactation, whereas the increase in milk production occurred in early lactation is due to cell differentiation (Knight and Wilde, 1993). After lactation peak, the differentiation state of the tissue is maintained constant throughout declining lactation, and the loss of secretory cells accounts resulted decrease in milk yield. It is well established in ruminants that losses in mammary cell during involution occurred through programmed cell death (apoptosis), Wild *et al.*, (1997). Early lactation and milking frequency influence the mammary gland capacity yet improve milk production efficiency, (Dahi *et al.*, 2004). Milking once daily resulted in 6% significant reduction in daily yield compared with milking

twice daily. This reduction was similar to the values previously reported in Canarian goats (6%) by Capote *et al.*, (1999), while smaller than that reported for Alpine goats (36%) by Mocquot (1978) for all lactation. Moreover, losses ranged between 6 and 7% in Damascus goats during middle and late lactation, respectively (Papachristoforou *et al.*, 1982). Stelwagen (2001) reported that increasing the frequency of milk removal increases milk production in cattle. Frequent milk removal is associated with reduction in somatic cells, which is a general indicator of mammary gland health (Dahi *et al.* 2004). Stewagen and Knight (1997) confirmed the inverse relationship between proportion of milk stored in the cistern or total udder capacity and milk yield reduction with milking once daily. The tight junction of the epithelium became leaky during 24h milking interval and the moment they became leaky (after approximately 20 h) coincided with the moment rate of milk secretion began to decline, suggesting that tight junction play a role in the milk reduction with one milking in goats (Stelwagen *et al.* 1994). The increase of milking frequency

enhances milk yield and reduces secretory cells loss during lactation in goats Knight and Wilde 1987). When less milking frequency is prolonged, the decrease in milk yield is sustained by sequential developmental adaptations, initially as a down-regulation of cellular differentiation (Wilde *et al.*, 1987), and later as a net loss in mammary cells number via apoptosis (Li *et al.*, 1999). Regulation of milk secretion is likely the culmination of a complex interaction of mechanisms operating at different levels within the mammary gland (i.e., gland anatomy); versus (inter) cellular processes (Stelwagen, 2001). Milking goats once daily (1x) produced concentrated milk than milking twice (2x). Milk had also higher concentration of total solids (+4.6%), fat (+9%), as indicated in Table 1. This could be expected as a consequence to concentration of milk components when milk yield decreased as well as a result of changes occurred in the synthesis of milk components. Salama *et al.*, (2003) found increase in dairy goats fat (+10%) and total solids (+6%) as a result of milking once daily. Changes in fat concentration in milk may be related to different regulatory mechanisms for secretion of milk fat globules that relative to components in the aqueous phase of milk and to the transfer between alveolar and cisternal compartments (Davis *et al.*, 1999, Mekusick *et al.*, 2002 and Salama *et al.*, 2003). The present study elucidated the enhanced activity of the epithelial cells of mammary tissue during early and mid lactation in conjunction with the hyperactivity of milk production during these stages (El Sayed *et al.*, 2009 and Hassan, 1997). During these stages, the epithelial cells showed an increase in the number of alveoli/plate, number of cell count and total sectional area of alveoli (Table 2), indicating an increase in alveolar activity. AP is a membrane-associated with glycoprotein enzyme which enhances hydrolysis of phosphates, a process widely distributed in the animal body (Goor *et al.*, 1989). This enzyme is located primarily on the cell membranes of tissues where active transport processes occur (Murray and Ewen, 1992). In accordance with our results, Silanikove and Shapiro (2007)

have shown that AP is located almost exclusively on the outside (milk facing) of mammary epithelial cells apical membrane. High activity of AP during early and mid stages of lactation, as found in this study, would delay and attenuate the response to factors such as stress and milk stasis (or reduced milking frequency) that induce down regulation of milk secretion through this mechanism (El- sayed *et al.*, 2009). Confirming to this proposition, it was shown that goats have particular refraction to the negative effect of stress on milk yield (Shamay *et al.*, 2000). At the late stage of milking, the wall of the disintegrating alveoli showed unclear loci of staining for AP, denoting that there is low activity coincided with declining milk processing (Hassan, 2004 and EL-sayed *et al.*, 2009). Numerous loci of AP were apparent on the outer surface of the alveolar secretory cells at the early and mid stages of lactation –denoting high activity of this enzyme at these two stages (Figs.7 to 9). The greatest density of AP staining was found in the thick-walled mammary alveoli with larger cells at early and mid stages of lactation (figs.7 and 9). During the late stage, few alveoli showed weak staining for AP and the connective tissue was increased with the regression of alveoli into smaller sizes (Figs 11 and 12). This was accompanied by high level of milk secretion reaching 1778 ± 39 and 2351 ± 68 ml/head/day, respectively, compared to late lactation (910 ± 44.6 ml/head/day), (Table (1). These histochemical evidences are in agreement with (Jeffrey *et al.*, 1989 and EL-Sayed *et al.*, 2009). The presence of high AP content of milk facing side of apical epithelium cell membrane at the early and mid lactation and the marked reduction in its presence at late lactation, suggest that these enzymes play an important regulatory role in controlling milk secretion, (EL-Sayed *et al.*, 2009).

It could be concluded that both stage of lactation and milking frequency influenced cell number and activity of mammary epithelial cells.

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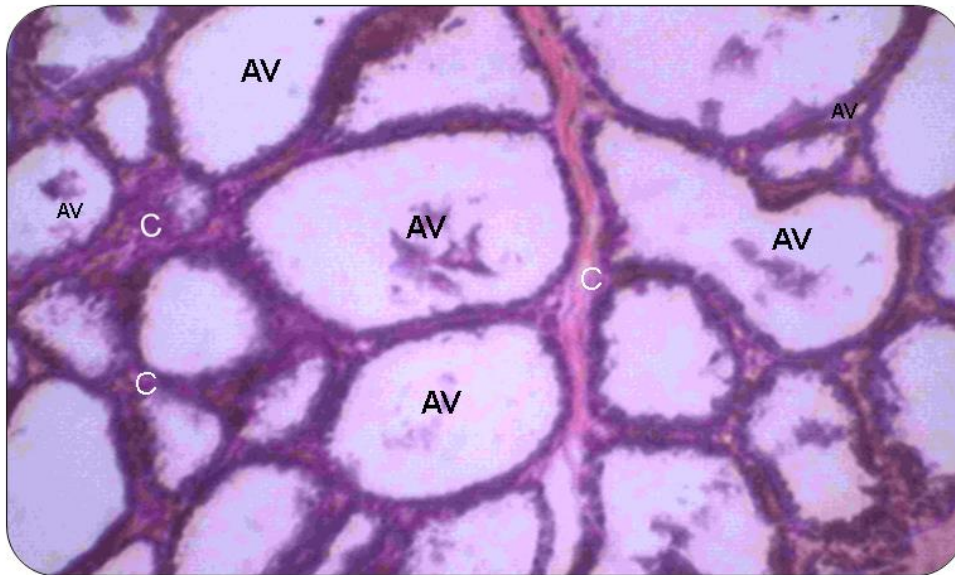


Plate 1. Section of the goat mammary tissue of the twice daily milking at early lactation showing secretory alveoli (AV) separated by thin trabeculate of connective tissue (c). 120 x.

AV

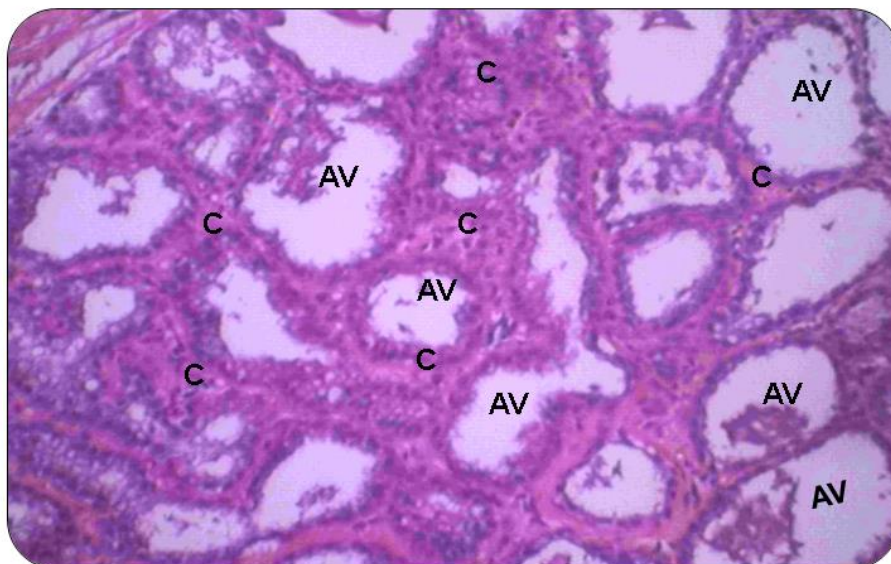


Plate 2. Section of the goat mammary tissue of once daily milking at early lactation showing decrease the size of the alveoli (AV) and increase of the area of connective tissue (c). 120x.

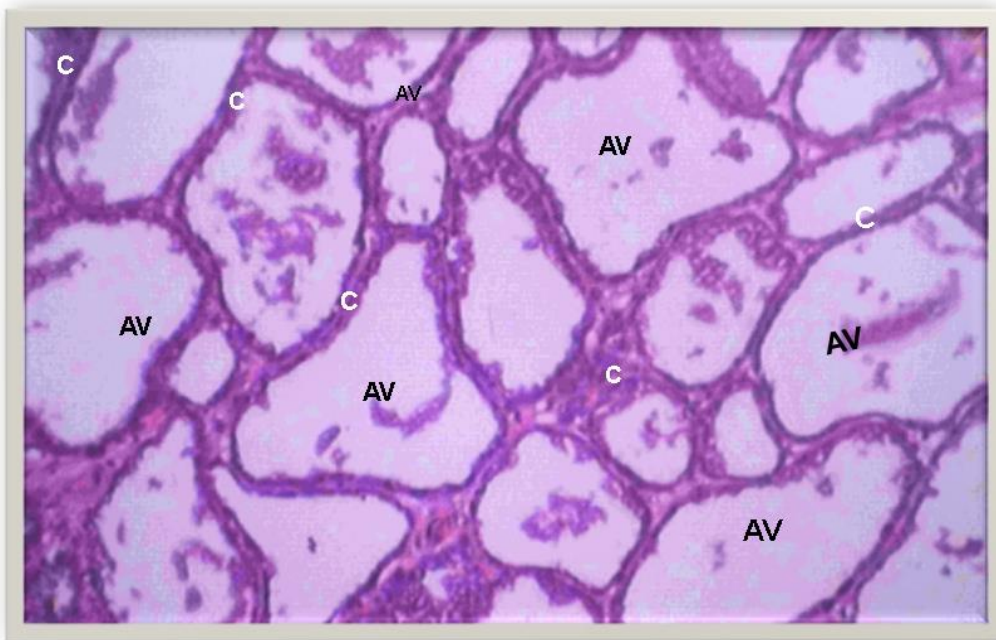


Plate 3. Section of the mammary tissue of the twice daily milking at mid lactation showing numerous alveoli (AV), separated by thin connective tissue (c). Alveoli are well-developed and distended with secretion. 120x.

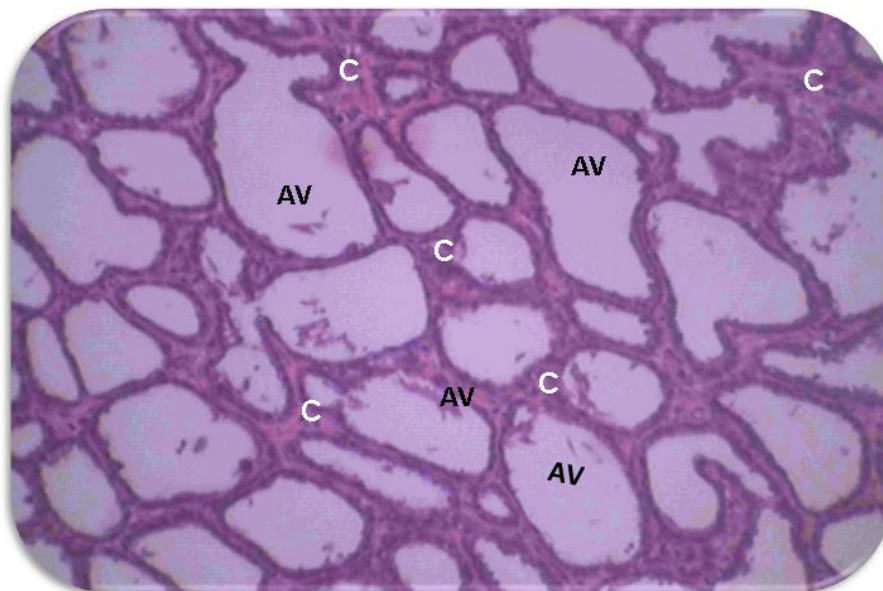


Plate 4. Section of the goat mammary tissue of the once daily milking at mid lactation showing numerous alveoli (AV) but less than twice daily milking. 120x.

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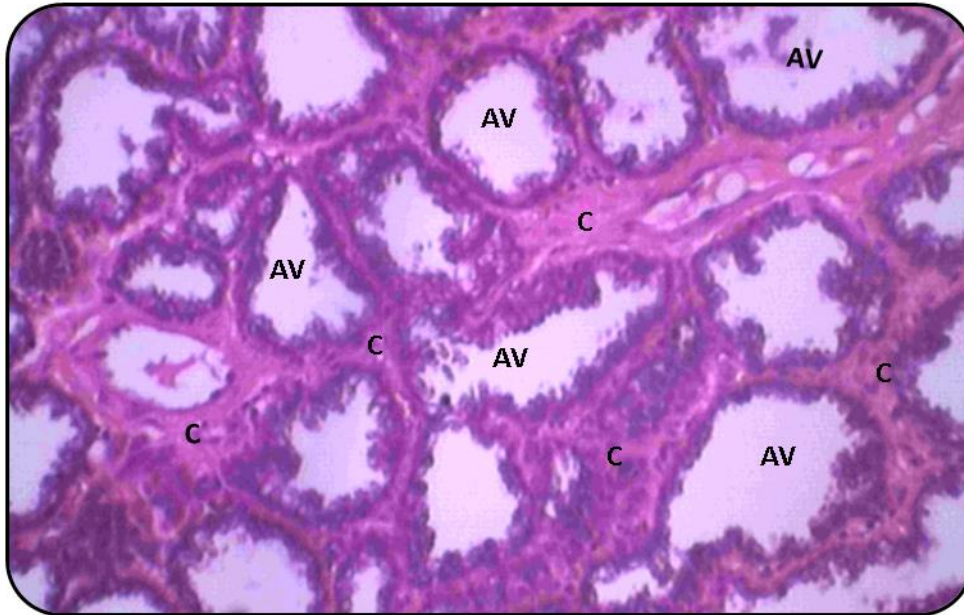


Plate 5. Section in the goat mammary tissue of the twice daily milking at late of lactation showing increased the area of connective tissue. 120x.

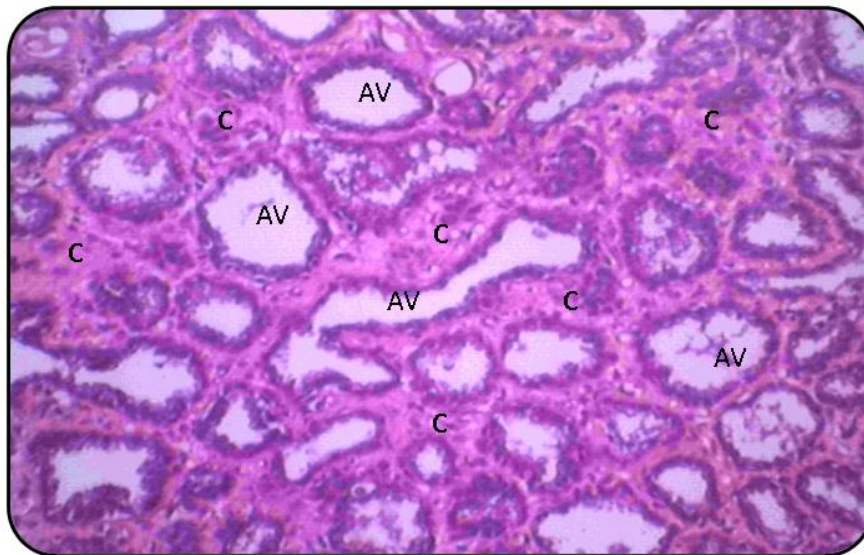


Plate 6. Section in the goat mammary tissue of the once daily milking at late of lactation showing several involuted alveoli (AV) separated by wide connective tissue (C). 120x.

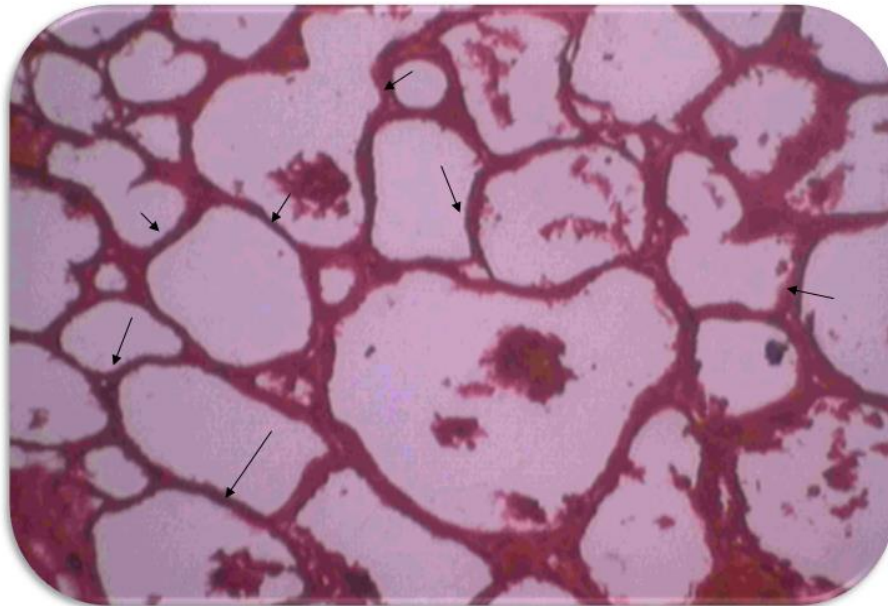


Plate 7. Section of the goat mammary tissue of the twice daily milking at early lactation. 120 x.

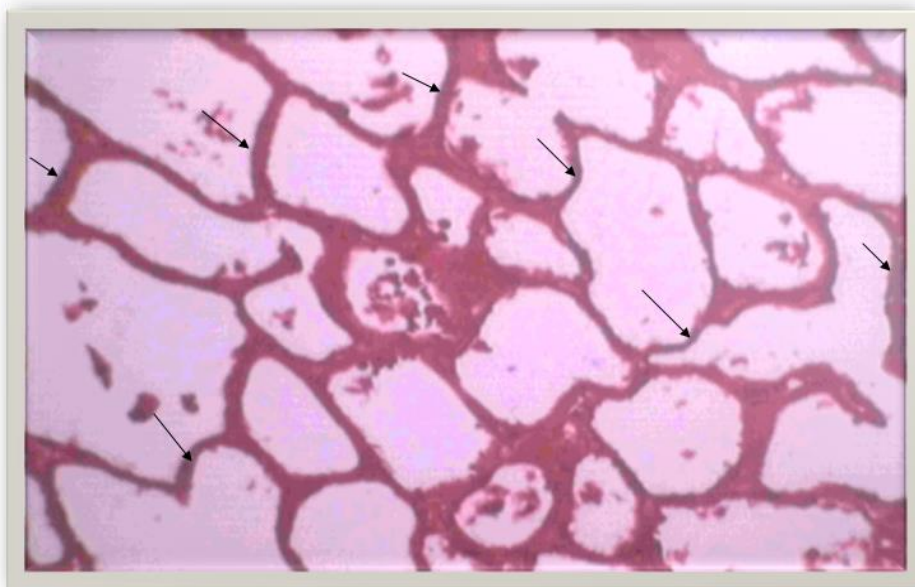


Plate 8. Section of the goat mammary tissue of the once daily milking at early lactation showing staining of epithelial secretory cells denoting activity of alkaline phosphatase enzyme on some sites than others (arrow) in two sections (twice and once daily milking) at early lactation. 120X.

EFFECTS OF MILKING FREQUENCY ON MILK PRODUCTION AND HISTOLOGICAL STRUCTURE OF UDDER IN ZARAIBI DAIRY GOATS

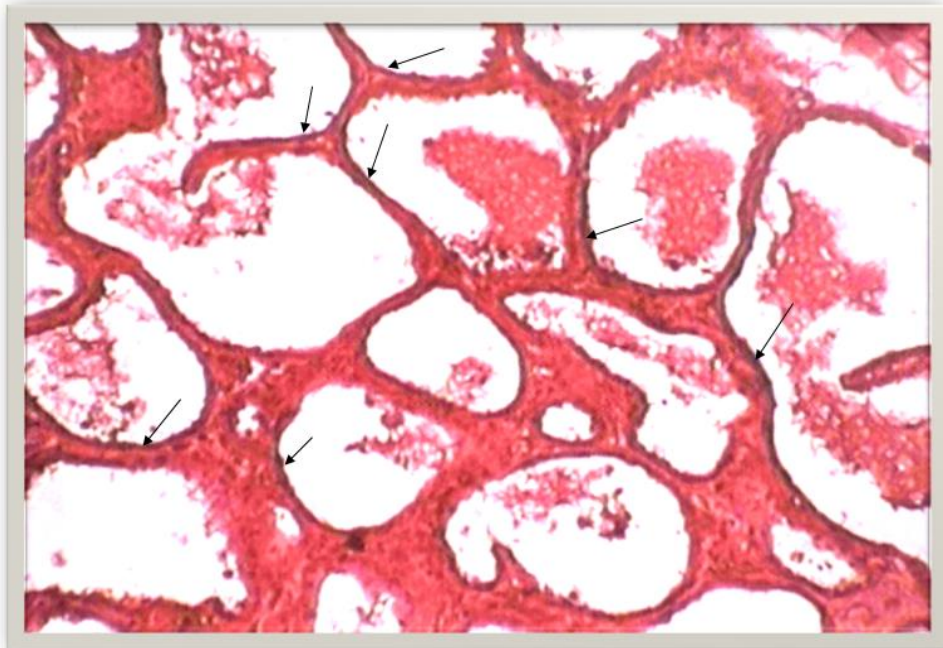


Plate 9. Section in the goat mammary tissue of the twice daily milking at mid of lactation 120X.

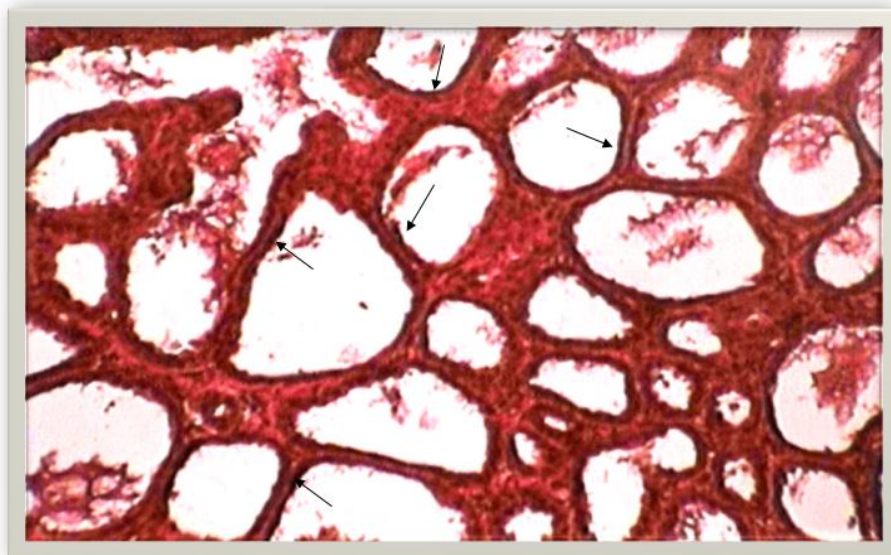


Fig.10. Section of the Zaraibi goat mammary tissue at mid lactation (twice and once daily milking) showing localization of the alkaline phosphatase on epithelial cells. High level of alkaline phosphatase activity expression (arrow). 120x.

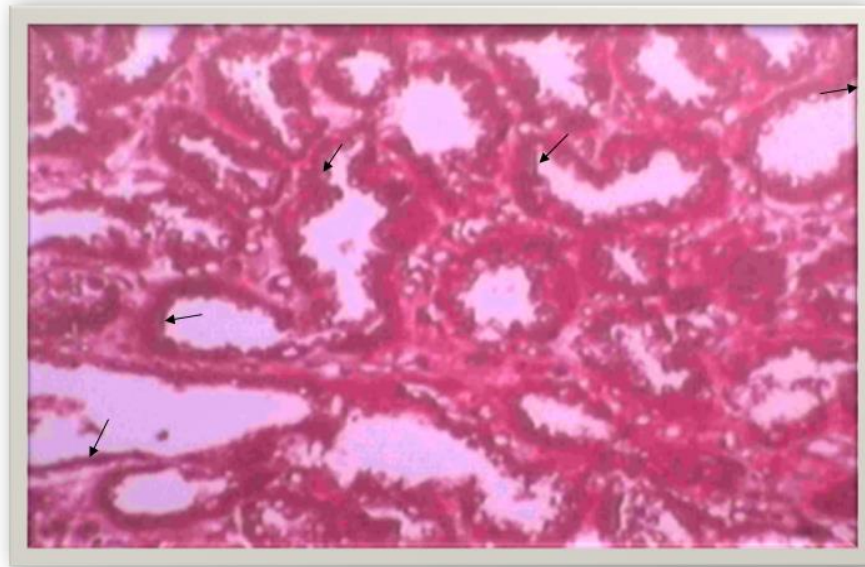


Plate 11. Section in the goat mammary tissue of the twice daily milking at late of lactation 120x.



Plate 12. Weak alkaline phosphatase activity (arrow) in the two sections (Twice and once daily milking) at late stage of lactation x120.