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HISTOCHEMICAL ASSESSMENT OF MAMMARY GLAND CAPACITY PERTAINING TO MILK PRODUCTION IN BUFFALOES

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SUMMARY

This work was carried out on five Egyptian buffaloes, at successive stages of lactation. Biopsies were obtained from the mammary gland at three stages of lactation; onset (7-10 ds), peak (the 7th w.) and late, near to the end of lactation (during involution). Histochemical investigations were executed to determine the mammary alveoli density, density of cells per alveolus and the activity of the cells, at these successive stages of lactation. The highest expression of protein staining was found in the thick walled mammary alveoli coinciding with the greatest secretory activity at 7-10d. stage and peak stage of lactation than near to the end of lactation at which the adipose connective tissue was increased. During the lactogenesis from onset to peak of lactation the DNA concentration was greater than during involution (DNA was absent in several degraded cells). Numerous loci of Alkaline Phosphatase enzyme (AP) were apparent clearly on the outer surface of the alveolar secretory cells at onset and peak stages denoting high activity of this enzyme at these two stages. The milk yield at these two stages reached 8.54±0.60 and 10.09±0.44 kg/head/day, respectively. On contrast, at the late stage (involution of glands) few small alveoli showed only weak staining for AP substrate, while most of sections showed disappearance of staining associated with AP (negative staining) which reflects the weak enzymatic activity of the cells. The milk yield at this late stage was minimized, only 3.02±0.72 kg/head/day.

Keywords: Buffaloes, mammary gland, histochemistry

INTRODUCTION

The mammary gland is a complex organ both in structure and function. After pubertal mammogenesis it undergoes three physiological transition during a lactation cycle: from involution to colostrogenesis, to lactation, then back to involution. Marked changes occur in the mammary gland; size, structure and secretory activity as the gland progresses to or from state of active milk synthesis (Nakhasi and Qasba 1979 and Burditt *et al*., 1981).

Milk yield and the shape of lactation curve are determined by the number of mammary secretory cells and secretory activity per cell. Knight and Peaker (1984) found that, in dairy goats, mammary growth and differentiation during early lactation accounted for increasing milk yield during the ascending portion of the lactation curve, whereas after peak lactation loss of mammary cells largely, accounted for declining milk yield. On the other hand declining milk yield during extended lactation was primarily due to reduced secretory capacity per cell (Knight *et al*., 1984). Capuco *et al*. (1997) stated that, in dairy cows, a decline in mammary cell

number during lactation must, at least partially, account for the decline in milk yield after peak lactation.

The objective of the present investigation was to determine whether the decline in milk production during advancing lactation, in buffaloes, is due to a decline in mammary cells number or cellular activity per se or in combination.

MATERIALS AND METHODS

This work involved five lactating buffaloes at successive stages of lactation; onset (7-10 days), peak (the 7^{th} week) and late, near to the end of lactation (during involution).

Specimens of mammary tissue, approximately $2cm^3$ with 1cm thickness were obtained, by biopsy, from each buffalo at the mentioned stages of lactation.

The daily milk yield was recorded within a week at each stage of lactation for each buffalo as recorded in mean values in the following (Table 1).

Table 1. Mean values of daily milk yield (kg/head/day) of buffaloes at each stage of lactation

Stage	Mean ± S.E
Onset (7-10d.)	8.54 ± 0.60
Peak (7^{th} w)	10.09 ± 0.44
Late (Involution)	3.02 ± 0.72

Basic technical approaches:

Histochemical investigations were executed to detect the mammary alveoli density and the density of cells per alveolus alongside the activity of the cells, at these stages of lactation.

The protein staining density-in the microscopic sections-against the connective tissue, in particular the adipose tissue, was adopted to detect the sizes and density of alveoli per volume unit of the mammary tissue, indicated by pictures in sections. The cells density was assessed from the spreading of DNA in the secretory cells of the alveoli. The activity of the secretory cells was determined by the frequency of the Alkaline phosphatase enzyme loci on the outer surface of the alveoli cells.

Histological procedure:

The mammary gland samples were fixed in 10% neutral formol saline for 24hr. Each sample was then cut into small pecies treated successively by; dehydration in grades of ethyl alcohol (50-100%), clearing in xylene and embedding in paraffin wax. The embedded pecies were sectioned at thickness of 4-5 μ.

Histochemical technique:

A-Total protein:

Total protein was determined by the Mercury-Bromphenol blue method, according to Bonahag (1955) as follows: - Sections were deparaffinized by xylene and ethyl alcohol (5min in each), then brought to water, followed by: Staining in Mercury-Bromphenol blue solution for 2 hours at room temperature. Differentiating in 0.5 percent acetic acid for 5 min. Transferring directly into tertiary butyl alcohol. Clearing in xylene and mounting in Canada balsam.

B- DNA:

Feulgen reaction for DNA demonstration (Feulgen and Rossenbeck, 1924) was applied as follows: -

Sections were deparaffinized by xylene and ethyl alcohol (5min. in each), then brought to water, followed by: Rinsing for 10 min. in 1-N HCl, at 60°c, followed by rinsing in water at room temperature. Transferring directly into Schiff's reagent for 30-60 min. Washing three times with 0.5% sodium metabisulphite (2 min. each time) then washing in water. Dehydration through graded ethyl alcohol. Clearing was accomplished in xylene followed by mounting in Canada balsam.

***C- Alkaline phosphatase (AP) assessment**:*

Alkaline phosphatase activity was assessed by the method of Rutenburg *et al*., (1965) as follows: -

Sections were deparaffinized by xylene and ethyl alcohol (5min. in each), then brought to water, followed by: Fixation for 30 sec. by formol methanol at (0-4°C). Washing in tap water and leaving to dry. Incubation in a mixture of substrate (Naphthyl Phosphate) + tris buffer for 15 min. Washing in tap water and leaving to dry. Lastly counter staining was accomplished with sufranin for 7 min. followed by dehydration and mounting in Canada balsam.

RESULTS

A-Total protein:

The greatest expression of protein staining was found (plates 1&2) in the thick walled mammary alveoli with larger cells, at the early and peak stages. This denotes greater activity of the secretory cells.

At these early stages the lobuli of the mammary glands showed large numerous alveoli with wide lumina containing surplus milk secretion (Plate 3). Hassan (1994) reported that at the early stage of lactation the alveoli were closely adjacent with thin intervening connective tissue.

During the late stage (involution) the fat content deposited in the connective tissue was increased concomitant with the regression of the alveoli into the smallest size, and reduced frequency, moreover with few secretion in their lumen (Plate 4).

B-DNA:

During lactogenesis development from onset to peak of lactation (Plate 5), the DNA concentration was greater than during involution, this case is in agreement with Capuco *et al*. (2001) who reported, in cows, that the total parenchymal DNA was the greatest at 14d. of lactation and declined to a low level at 240d.

The high content of DNA at early stage of lactation indicates a high correlation between mammary cell number and milk yield. The highest amount of DNA at peak of lactation (Plate 5), indicates the greatest number of secretory cells with high activity. Hassan (1997) reported that the high development of the cellular organelles was observed at the peak stage of lactation in the mammary gland cells of buffaloes.

During involution, the size of alveoli was reduced and the DNA was absent in several loci on the alveoli sections. (Plate 6). This is due to autolytic processes in the cells.

C- Alkaline phosphatase activity (AP):

At the onset stage, the cells of the alveoli showed prominent sites of alkaline phosphatase enzyme imposed on the outer surfaces of the cells. Plate (7) shows numerous loci of AP at 7-10 days and peak of lactation in all cells denoting high activity of this enzyme at these two stages. This case was accompanied with high level of milk secretion, reaching 8.54±0.60 and 10.09±0.44 kg/head/day at these

stages, respectively (Table 1). However, it is clear in Plate (8,a&b) that this enzyme was apparent on some cells than on others, even in some alveoli than others denoting stunted activity of secretion in some dispersed sites. These histochemical evidences are in agreement with Jeffrey *et al*., (1989). These authors identified a significant disparity in the levels of AP activity in a growth medium collected from cultured cells, which indicates that such a difference can be directly correlated with the presence of the enzyme on the plasma membrane of these cells.

During the last stage (involution of glands) few of the small alveoli showed only weak staining for AP substrate, while most of the section showed disappearance of membrane associated alkaline phosphatase (negative staining) which reflects their weak enzyme activity (Plate 9). This case coincided with reduction of milk yield in this stage of lactation (3.02±0.72 kg/head/day, Table 1).

DISCUSSION

The wide spread of staining for protein in the mammary gland sections at early and peak lactation is a good evidence of augmented alveoli growth disrupting the surrounding connective tissue (Plates 1&2). On the contrary the space of protein staining in sections of involuting glands was apparent only in some shrinked alveoli (Plate 4). This decomposition of parenchyma, encompassing the active cellular protein, was substituted by spreading connective tissue, particularly adipose tissue (Plate 4).

The DNA concentration in mammary tissue is an excellent indicator of cell number, the greater frequency of DNA stained loci at the early and peak stages of lactation (Plate 5-) indicates a high correlation between cells frequency and milk production which reached the maximum production at the peak stage.

Alkaline phosphatase (AP), a membrane associated glyco-protein enzyme which enhances the hydrolysis, prosses is widely distributed in animal tissues (Goor *et al*., 1989). On the other hand AP increases markedly at sites of matrix mineralization (Lewinson *et al*., 1982 and Safadi *et al*., 1991).

This enzyme is located primarily on cell membranes of tissues where active transport processes occur (Murray and Ewen 1992). In Plates (7&8) the staining pattern of AP substrate varied with the development and activity of the cells of mammary gland alveoli. In Plate (7) the highly staining for this enzyme indicates high exchange of the blood precursors during the biosynthesis of milk. It is interesting that complete filling of the alveolus with milk is accompanied with reduced loci of AP assessment (Plate 8b). Near to the end of lactation (involution) the late stage, the wall of the disintegrating alveoli showed no clear loci of staining for AP denoting that there are low activity (Plate 9), this coincided with declining milk processing.

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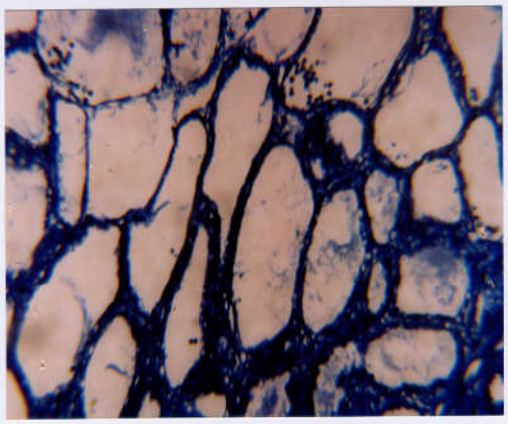

Plate (1): Section at 7-10 day of lactation.

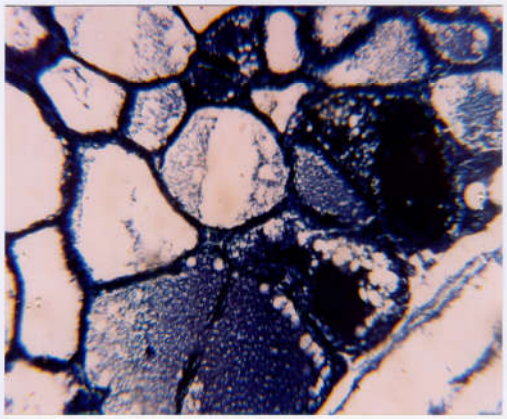

Plate (2) : Section at peak stage of lactation.

Plates (1&2) : Show high expression of protein staining.

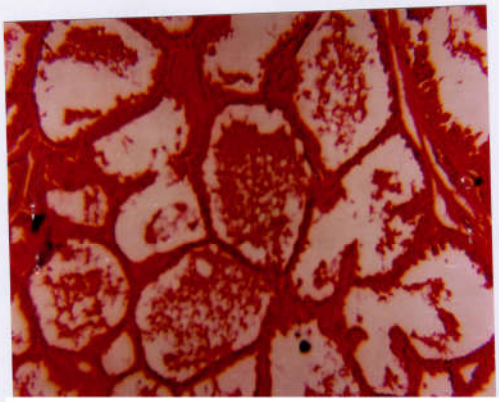

Plate (3) : The lobuli of mammary gland, with numerous alveoli, separated by thin trabiculae of connective tissue. X200.

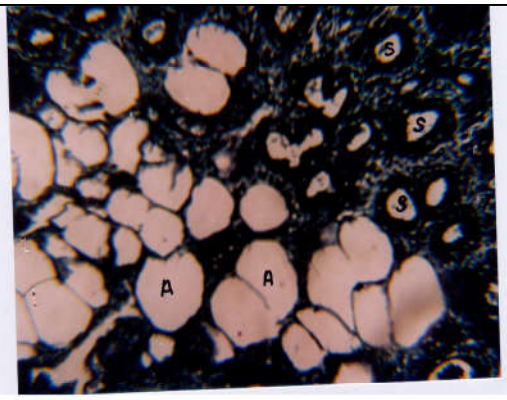

Plate (4) : Near to the end of lactation, showing some shrinked alveoli (s) and large amount of adipose tissue (A). X200.

Plate 5. At the peak of lactation, the highest amount of DNA. X 400

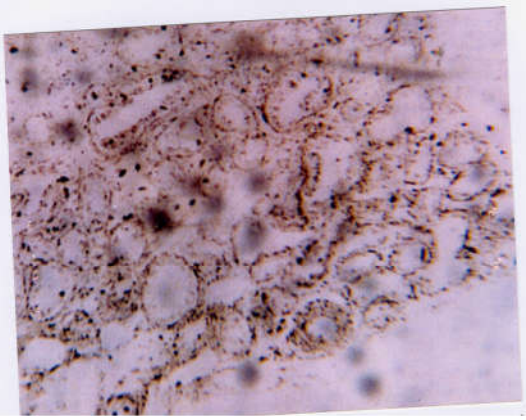

Plate 6. The lowest amount of DNA at near end of lactation. X200.

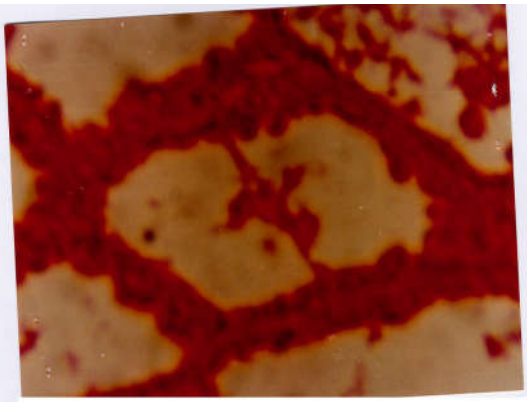

Plate 7. High level of Alkaline phosphatase activity expression. X400.

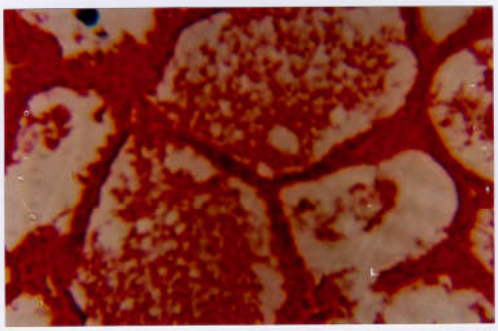

Plate 8a

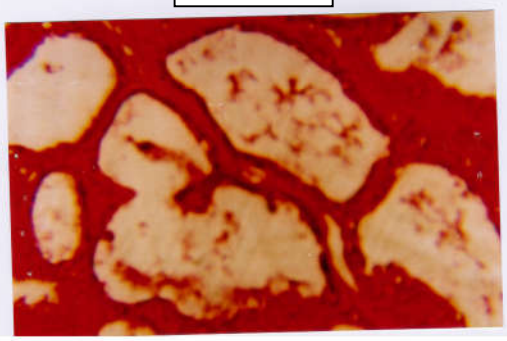

Plate 8b

Plate (8a & 8b) . The expression of AP enzyme in some sites than another. X400.

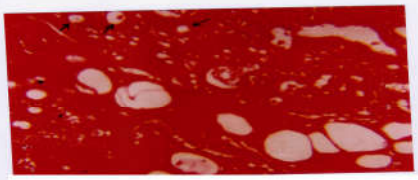

Plate 9. Near the end of lactation, very weak staining of AP some activity in small alveolis X200.

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قياسات هستوكيميائية لنشاط الغدة اللبنية وعلاقتها بإنتاج اللبن في الجاموس

ليلى رشاد حسن

معهد بحوث الإنتاج الحيواني – الدقي

تم إجراء هذا البحث على ٥ جاموسات على مراحل الحليب المتتالية , أخذت عينات الضرع على ثلاث مراحل متتالية من الحليب (في بداية الحليب ٧ – ١٠ أيام , وخلال مرحلة ال peak اى عند الأسبوع السابع من موسم الحليب وعند قرب نهاية الموسم) , وكان الهدف من هذه الدراسة هو تحديد كثافة الحويصلات وكذلك كثافة الخلايا داخل الحويصلات وتحديد نشاط الخلايا خلال مراحل الحليب المتتالية بعدة تقديرات هستوكيميائية .

وأوضحت الدراسة أن أعلى كثافة لصبغة البروتين في النسيج كان قد سجل خلال بداية الحليب (٧ – ١٠ أيام) حتى مرحلة ال peak بعكس عند قرب نهاية مرحلة الحليب حيث ازداد ظهور نسبة النسيج الدهنى في قطاع الغدة.

وأوضحت الدراسة أن أعلى تركيز لمحتوى الDNA في القطاع كان من بداية الحليب حتى الـ Peak بالمقارنة عند قرب نهاية موسم الحليب حيث غاب الكثير من ال DNA في الخلايا المتحللة .

أما بالنسبة للنشاط الانزيمى قد شوهد العديد من مواقع نشاط إنزيم الالكالين فوسفاتيز بوضوح على الجدار الخارجي للخلايا المفرزة من الحويصلات اللبنية من بداية الحليب أيضا حتى ال peak , حيث شوهد في هاتين المرحلتين أعلى نشاط انزيمى , يدل على أعلى نشاط للغدة , وكان محصول اللبن أثناء هاتين المرحلتين قد وصل إلى ٨.٥٤ ± ٠,٦٠ و١٠.٠٩±٠,٤٤ كيلو جرام \ راس \ اليوم على التوالي أما في أخر الموسم قد شوهد في القطاع القليل جدا من الحويصلات ولوحظ عليها ضعف الصبغة لإنزيم الكالين فوسفاتيز بينما اختفى تماما من باقي القطاع (صبغة سالبة) , ويشير هذا إلى ضعف النشاط الانزيمى في الخلايا في هذه المرحلة المتأخرة من الحليب , وكان إنتاج اللبن خلال هذه المرحلة قليل (٣.٠٢ ± ٠.٧٢ كيلو جرام اراس \ اليوم).