Small Ruminant Research 85 (2009) 11–17

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Histological and histochemical study on mammary gland of Damascus goats through stages of lactation

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ARTICLE INFO

Article history:
Received 3 November 2008
Received in revised form 16 June 2009
Accepted 16 June 2009

Keywords:
Damascus goat
Stage of lactation
Mammary gland
Alveoli
Histochemical

ABSTRACT

This research was carried out on seven Damascus goats, to study the relationship between milk production, during advancing lactation, and the changes in secretory mammary cells frequency and cellular activity. Biopsies were obtained from the mammary gland at the three stages of lactation, early, mid and late, for histological and histochemical studies. The histological structures of the mammary gland showed clear differences between lactation stages—being more developed in the early and the mid stages, compared to the late stage of lactation. The number of the alveolar secretory cells increased from the early to the mid stage of lactation by 12.9% and then was reduced at the late stage by 35.9% from that at the mid stage. The milk yield increased by 51.3% from the early to the mid stage, and then was reduced at the late stage by 71.4% from that of the mid stage. The total sectional areas of plate equal to 1.22 mm^2/plate of the alveoli were the smallest during late lactation (0.36 mm^2/plate) compared to that during the early and the mid stage of lactation (0.50 and 1.17 mm^2/plate, respectively). Numerous loci of alkaline phosphatase enzyme (AP) were apparent on the outer surface of the alveolar secretory cells at the early and the mid stages of lactation—suggesting that this enzyme plays an important physiological role in the apical membrane of the alveolar epithelial cells during lactation. Dense protein staining of these cells as well as increased frequency of DNA expression denote great development and increased numbers of these cells at early and mid stages of lactation. This was accompanied by a high level of milk secretion reaching 939.3 ± 130 and 1421.4 ± 123.4 ml/head/day, respectively. In contrast, at the late stage of lactation, the size of alveoli was reduced and few alveoli showed weak AP activity, weak protein manifestation and the lowest frequency of DNA loci. This coincided with the reduction in milk yield (407 ml/head/day). It could be concluded that the stages of the lactation influence the cell number and activity of the mammary parenchyma.

1. Introduction

The mammary gland is a complex organ in both structure and function. Synthesis of milk by the mammary secretory cells is under the complex control of local and systemic hormones and other factors that influence milk output (Neville and Daniel, 1987; Silanikove et al., 2006). Marked changes occur in the mammary gland in its size, structure and secretory activity as the gland progresses to or from the state of active milk synthesis (Nakhasi and Qasba, 1979; Burditt et al., 1981).

Milk yield and the shape of the lactation curve are determined by the number of mammary secretary cells and the secretory activity per cell (Anderson, 1974; Tucker, 1981). In dairy goats, mammary growth and differentiation during early lactation account for increasing milk yield during the ascending portion of the lactation curve, whereas after peak lactation, loss of mammary cells, accounts largely for

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doi:10.1016/j.smallrumres.2009.06.019

declining milk yield (Knight and Peaker, 1984). In dairy cows, a decline in number of mammary cells during lactation must, at least partially, account for the decline in milk yield after peak lactation (Capuco et al., 1997).

Milk protein synthesis in the mammary gland is controlled by multiple interactions of several peptide and steroid hormones (Topper, 1970; Denamur, 1974). With the onset of gestation, the gland proliferates and development of the endoplasmic reticulum occurs (Tucker and Reece, 1970 and Oka and Topper, 1971), resulting in the increased protein synthetic activity of the gland. The actual secretion of proteins and other organic substances is under the regulation of milk-borne negative feedback regulatory system (Silanikove et al., 2006).

The aim of the present study was to investigate the relationship between milk production throughout the lactation stages and the changes in secretory mammary cells frequency and cellular activity in Damascus goats.

2. Materials and methods

The experimental work was conducted at the Gemaiza Experimental Station, Animal Production Research Institute, Sheep and Goats Department, Agriculture Research Center, Egypt. Seven Damascus goats aged 2–3 years, with body weight ranging between 45 and 50 kg were used to determine the changes in structure and activity of their mammary glands concomitant with changes in milk production. During November to April, the goats were fed Egyptian clover (*Trifolium alexandrinum*), rice straw and concentrate feed mixture. For the rest of the year, a similar diet was fed, but the green clover was replaced by its hay. The does had free access to water throughout the day.

The milk yield was recorded weekly in the early stage (from birth to 15 days), and mid stage of lactation (from 15 days to 60 days of birth), and then it was recorded daily to near the end of lactation at 240 days of birth (late stage) for each dose. The milk yield was recorded in millilitres as the routine work at the Gemizah research station.

3. Mammary gland biopsies

To study the changes in the histological features of the mammary gland, biopsies were obtained from each doe at 15 days from birth (early stage); at 28 days (mid lactation), and at 240 days from the birth (late stage). The biopsies were obtained from one-half of the udder – after milking – through a minor surgical procedure. The animal was anaesthetised using xylaject (0.2 mg kg^{-1} by an intramuscular injection), and then a small incision was made in the skin of the udder and a small piece of the parenchyma (0.25 cm^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 antibiotic (long-acting terramycin at 1 ml per 10 kg body weight), in addition to mastlon injected through the teat of the udder. The biopsies were fixed in 10% neutral formol saline overnight at 4 °C before being transferred to grades of ethyl alcohol (50%, 70%, 90% and 100%) for 24 h in each grade. Samples of each biopsy were cleared in xylene and embedded in paraffin wax (m.p. 55 °C), according to Junquerira and Carneiro (1980). These samples were sectioned at 4 μm thickness (four slides per doe, each slide containing four sections) and stained with haematoxylin and eosin. The sections were viewed by light microscopy (Olympus XSZ-107BN, Olympus Corporation, Tokyo, Japan). For each case, five microscopic fields were detected randomly at 4× and then the fields were examined at 100× to determine the number of cells per alveolus using a computer. The average numbers of cells for the five microscopic fields was then calculated.

The sectional areas of the alveoli were determined according to the equation recorded by Alan (2003). The number of alveoli was recorded.

4. Histochemical investigations

Histochemical investigations were executed to detect AP enzyme activity, protein expression and DNA frequency in mammary epithelial cells.

AP activity was assessed by the method of Rutenburg et al. (1965) as follows: the sections were deparaffinised by xylene 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 57 min, 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.

The staining density of secretory cell protein in the sections against the connective tissue, in particular, the adipose tissue, was adopted to detect the sizes and density of alveoli in the biopsies of the mammary tissue as indicated by protein loci in the section. Protein loci were determined by the mercury–bromophenol blue method, according to Bonahag (1955) as follows: the sections were deparaffinised by xylene for 10 min and also processed by grades of ethyl alcohol (100%, 90%, 70% and 50%) for 3–4 min in each grade, then brought to tap water for 5 min, followed by staining in mercury–bromophenol blue solution for 2 h at room temperature, then differentiating in 0.5% acetic acid for 5 min and transferring directly into tertiary butyl alcohol for 5 min, then cleared in xylene for 5 min and mounted in DPX. The sections were examined using light microscope to detect the density of protein staining as denoted by the expansion of the alveoli secretory cells.

The density of the secretory cells was assessed from the amount of DNA spreading in the secretory cells of the alveoli using the Feulgen reaction for DNA demonstration (Feulgen and Rossenbeck, 1924). The sections were deparaffinised by xylene for 10 min and processed in grades of ethyl alcohol (100%, 90%, 70% and 50%) for 2–3 min in each grade, then washed in running tap water for 10 min. Sections were immersed in 1-N HCl for 2 min at room temperature, then for 10 min at 60 °C, returned to 1-N HCl for 2 min at room temperature and transferred directly into Schiff's reagent for 60 min, followed by washing thrice with 0.5% sodium metabisulphite for 2 min each time and then washed in distilled water for 2 min. Dehydration was executed through graded ethyl alcohol for 2 min in each grade after which clearing was accomplished in xylene for 2 min,