The evolution of mammary glands at different stages in Sarda dairy ewes: preliminary results

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ABSTRACT - The significance of cytological evolution of alveolar cells explains differences in milk yield that resulted in a different shape of lactation curve. In this paper, investigation of cellular background for this pattern was studied morphometrically in mammary gland of dairy ewes prior to lambing to involution. The ratio of epithelial to the luminal area was greatest at 7 days from lambing, it diminished on day 30 and 60 of lactation and it increased thereafter on day 150 of lactation and on day 7 of involution. The evolution of mammary tissues shows a clear decrease in stromal mass from prepartum until 60 DIM. Structural changes of mammary tissues during late pregnancy, lactation and dry off, can be used as a basis to estimate the evolution of the number of secretory cells in dairy ewes.

Key words: Mammary gland, Tissue morphology, Ewes, Milk.

Introduction - The mammary gland is one of few organs in the body that undergoes repeated cycles of structural development, functional differentiation and regression. The major process involved in the evolution of milk yield in dairy species is the number of active secretory cells and their rate of secretion throughout lactation (Cappio-Borlino *et al.*, 2004). Most of mathematical mechanistic models of lactation curve are based on this simple assumption to simulate the behavior of a mammary system (Dimauro *et al.*, 2007). Those mechanistic models are used to input an estimate of the number of secretory cells and their secretion rate because experimental paper reported only the concentration of DNA as measure of cell number and the DNA/RNA ratio as measure of their specific activity. In order to provide a first attempt for a future estimate of the number of cells and their activity in various stages of lactation (prepartum, lactation and dry periods), changes at the morphological level in the mammary glands have been studied in Sarda dairy ewes. This paper reports some preliminary results.

Material and methods - Twenty-five Sarda dairy sheep, managed under identical condition were used to evaluate changes in tissue morphology of mammary glands. Five sheep were slauthered approximatively 7 days before lambing; five ewes 7 d after dry off (7IN), and the other (5 per group) at 30, 60, 150 days of lactation (30, 60, 150 DIM). Individual milk yield was measured for 4 days before slaughter. Samples of mammary tissue were collected from the middle area of the caudal part of both right and left udders (about 50 mg). Weight and volume of whole mammary gland without skin were measured. Slides were prepared from each tissue sample and the section was evaluated microscopi-

cally to determine the proportion of tissue area occupied by epithelium, stroma and lumen. The percentage of the area occupied by epithelium, stroma and lumen on the slide was considered, with large approximation, as an estimation of the volumetric percentage of the tissues. Milk yield for volume (g/ml) of epithelium (used as an estimate of secretory tissue) and lumen was calculated. Data were analyzed with one-way ANOVA using time as the only main effect.

Results and conclusions - Structural changes from immature, prepartum - to terminally differentiated alveoli - at 30 and 60 DIM - were observed, consistent with the observations reported in studies on cows (Akers, 2002). In fact, in the late pregnancy group, mammary glands consisted of immature alveoli with a small lumen, surrounded by cuboidal luminal cells with nuclei that accounted for most of the cellular area. However, large alveoli filled with milk and lipid droplets, were also observed in this group. In the mammary glands at 30 DIM, alveoli were fully differentiated, constituted by polarised epithelial cells with vacuoles at the apical membrane and nuclei with a central or basal location. In groups at 60 and 150 DIM, the alveolar lumen was larger, secretory cells showed a lacy appearance at the apical ends with a high protrusion, indicating the presence of secretory vesicles and darkly stained basolateral areas. At 7 IN, alveoli were shrunken, characterized by a small lumen area and closely packed cells; the interalveolar space contained large bundles of connective tissue.

Table 1. Proportion of tissue area occupied by epithelium, lumen and stroma in mammary glands of ewes at different physiological stages.

	% Epithelium area		% Lumen area		% Stromal area	
group	mean	se	mean	se	mean	se
-7 from lambing	24.83^{AB}	±2.33	21.48^{AB}	±6.33	53.68^{AB}	±7.74
30DIM	21.68^{B}	±2.58	32.75^{A}	±3.28	45.57^{B}	±3.15
60DIM	29.49^{A}	±0.92	36.51^{A}	±3.22	35.03^{B}	±3.87
150DIM	31.23^{A}	±2.88	22.89^{AB}	±2.52	47.46^{B}	±1.95
+7 dry off	18.29^{B}	±2.39	11.77^{B}	±2.28	69.93^{A}	±4.20

^{A,B}*=P<0.01.*

Morphometric analysis showed that the percentage of epithelial area reached its height at day 60 and day 150 of lactation whilst at day 7 of involution it was lower than at 60 and 150 DIM (P<0.01; Table 1). The percentage of lumen area remains constant from day 7 before lambing to day 150 of lactation, but it significantly decreased 7 days after dry off (P<0.01). The area occupied by stroma was higher at day 7 of involution than during lactation, and at day 60 and 150 of lactation was opposite to that measured for the epithelial area percentage (P<0.01; Table 1). The ratio of epithelial to the lumenal area was greatest at 7 days from lambing (2.21±2.36; mean ±sd), it diminished on day 30 and 60 of lactation (0.70±0.29 and 0.83±0.14, respectively) and it increased thereafter on day 150 of lactation and on day 7 of involution (1.54±0.89 and 1.85±1.06, respectively). The high variability in the first group, where the ratio of epithelial to the lumen area was numerically higher, was consistent with the different structural aspects of the alveoli observed in mammary glands at that time. These changes were also reflected in the proportion of alveolar area occupied by the epithelium, which was higher at dry off.

The volume of epithelium and stroma significantly decreases (P≤0.05) from the 7 days before lambing to 60 DIM, in concomitance with the decrease of the total udder volume (Figure 1). The volume increases thereafter and most of all for the stroma component during dry off, when the volume of epithelium and lumen were at their lowest (Figure 1).

Figure 1. Volume (ml) of epithelium, stroma and lumen components of mammary glands in udders of dairy ewes at different physiological stages ($^{a,b}P<0.05$).

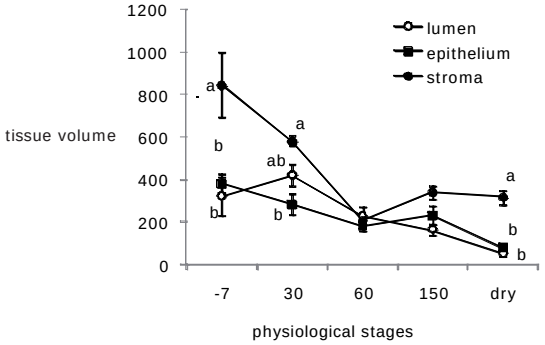
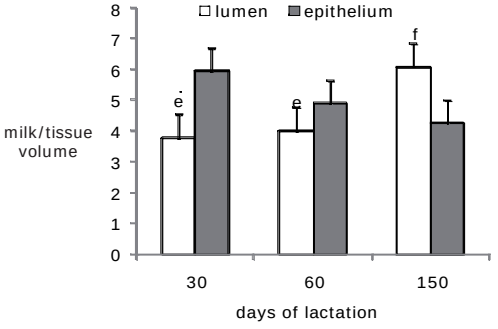


Figure 2. Milk yield per volume of epithelium and lumen (g/ml) in udders of dairy ewes at different days of lactation ($^{e,f}P=0.10$).



Milk yield per units of volume of alveolar lumen (Figure 2) tended to increase during lactation whereas the epithelium, which represents the secretory tissue, produced the same amount of milk.

Following the end of pregnancy, mammary tissues underwent large structural modifications because of the onset of milk synthesis. During involution, alveoli showed a reduced size, and large vacuoles appeared in epithelial cells as it was observed in a previous paper (Colitti *et al.*, 1999). At 7 days from lambing, the epithelial area percentage was greater than the lumen area percentage, but this proportion decreased after lambing until 30 days of lactation as a consequence of the increased size of the glandular cells. The only comparable data in literature are those by Smith *et al.* (1989), which described the percentages of the epithelium and lumen areas in ewes at 140 days of gestation. At 60 and 150 DIM, as reported also in cows (Akers *et al.*, 2006), the relative areas occupied by the epithelial and luminal spaces were quite similar and they decreased significantly at 7 days after dry off. Differently, the percentage of stromal area diminished from late gestation to lactation and peaked at 8 days of involution, as it had previously been observed (Colitti *et al.*, 1999).

The evolution of mammary tissues shows a clear decrease in stromal mass from prepartum until 60 DIM. The milk production around the lactation peak is sustained by the higher epithelium volume and higher, even if not significantly different, milk secretion rate per unit of tissue compared to 60 DIM. Structural changes of mammary tissues during late pregnancy, lactation and dry off, can be used as a basis to estimate the evolution of the number of secretory cells in dairy ewes.

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