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Regulation of cell number in the mammary gland by controlling the exfoliation process in milk in ruminants

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ABSTRACT

Milk yield is partly influenced by the number of mammary epithelial cells (MEC) in the mammary gland. It is well known that variations in MEC number are due to cell proliferation and apoptosis. The exfoliation of MEC from the mammary epithelium into milk is another process that might influence MEC number in the mammary tissue. The rate of MEC exfoliation can be assessed by measuring the milk MEC content through light microscopy, flow cytometry analysis, or an immuno-magnetic method for MEC purification. Various experimental models have been used to affect milk yield and study the rate of MEC exfoliation. Reducing milking frequency from twice to once daily did not seem to have any effect on MEC loss in goat and cow milk after 7 d, but increased MEC loss per day in goats when applied for a longer period. An increase in MEC exfoliation was also observed during short days as compared with long days, or in response to an endotoxin-induced mastitis in cows. Other animal models were designed to investigate the endocrine control of the exfoliation process and its link with milk production. Suppression of ovarian steroids by ovariectomy resulted in a greater persistency of lactation and a decrease in MEC exfoliation. Administering prolactin inhibitors during lactation or at dry-off enhanced MEC exfoliation, whereas exogenous prolactin during lactation tended to prevent the negative effect of prolactin inhibitors. These findings suggest that prolactin could regulate MEC exfoliation. In most of these studies, variations of MEC exfoliation were associated with variations in milk yield and changes in mammary epithelium integrity. Exfoliation of MEC could thus influence milk yield by regulating MEC number in mammary tissue.

Key words: dairy cow, dairy goat, milk yield, mammary epithelial cell, exfoliation

INTRODUCTION

The mammary glands of ruminants are made up of a heterogeneous tissue containing various cell populations, including mammary epithelial cells (**MEC**), myoepithelial cells, fibroblasts, and adipocytes. Mammary epithelial cells are the main cells present in the lactating mammary gland and are responsible for milk synthesis. The number of MEC in the mammary gland and their secretory activity are key factors regulating milk yield. The number of mammary cells in the mammary gland varies throughout lactation, along with variations in milk yield, and is regulated by the balance between cell proliferation and apoptosis. In most species, MEC number increases during early lactation up to peak lactation. After peak lactation, the gradual decrease in milk yield is associated with a decrease in cell number in the mammary gland, due to a greater rate of apoptosis compared with proliferation (Knight and Peaker, 1984; Capuco et al., 2001). Mammary epithelial cell exfoliation can also occur through shedding from the mammary epithelium into milk during lactation. Capuco et al. (2003) suggested that this exfoliation process did not significantly participate in the declining number of mammary secretory cells after peak lactation. From our experiments, however, we estimated that on average 390×10^6 MEC are lost each day by exfoliation in dairy cows. This loss represents 1.6% of the total MEC number in the udder (M. Boutinaud, unpublished data). Moreover, as will be shown in this review, several physiological, breeding, or environmental factors affect both milk yield and the MEC exfoliation process. Thus, this phenomenon cannot be neglected, as we suspect that the number of MEC in the mammary gland partly depends on the regulation of the exfoliation process. This review summarizes the current knowledge pertaining to MEC exfoliation into milk in ruminants, with emphasis on the factors that influence daily rates of MEC exfoliation.

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THE PROCESS OF MEC EXFOLIATION IN MILK

MEC in Milk

Different somatic cell types including leukocytes (lymphocytes, macrophages, neutrophils, and eosinophils) and nonimmune cellular types are present in mammary secretions of all mammals, even those with healthy mammary glands. In human milk, it was shown that the nonimmune cellular types include mature and differentiated epithelial cells and myoepithelial cells, as well as mammary progenitors and stem cells. Differentiated cells originate from the ducts and alveoli of the lactating mammary gland. Stem cells are multipotent and have the ability to differentiate both main mammary epithelial lineages, namely luminal and myoepithelial cells (reviewed by Hassiotou and Hartmann, 2014).

In contrast with human milk where MEC account for more than 90% of milk somatic cells (Ho et al., 1979), MEC represent a minority of the somatic cell content in ruminant milk. The concentration and proportion of MEC in milk vary between species (Table 1). The concentration of MEC is lower in bovine than in caprine milk (Boutinaud and Jammes, 2002). In ewes, milk MEC represent less than 2 to 3% of the total somatic cells (reviewed by Bergonier et al., 2003). The presence of MEC has also recently been reported in buffalo (Yadav et al., 2015) and zebu (Janjanam et al., 2013) milk.

Some of the MEC exfoliated into milk are apoptotic cells, but on average 60 to 70% of milk MEC are viable and exhibit characteristics of fully differentiated alveolar cells (Boutinaud et al., 2013c). Moreover, exfoliated epithelial cells recovered from milk can grow in culture (Ben Chedly et al., 2010; Sorg et al., 2012). The proportion of dying and viable MEC in milk varies between species and experimental models. The MEC exfoliation process thus makes it possible to evacuate both living and dying MEC simultaneously and in various proportions. In humans, it has been hypothesized that MEC were exfoliated from the ductal or luminal layers as a consequence of either a turnover of the secretory tissue or the pressures associated with the continued filling and emptying cycle associated with breastmilk synthesis and breastfeeding (Cregan et al., 2007). Suckling or milking induces a myoepithelial contraction in response to oxytocin release, which in turn, could participate in the exfoliation of MEC. The role of myoepithelial contraction on the MEC exfoliation process has yet to be investigated further.

How to Assess MEC Exfoliation Rate

The rate of MEC exfoliation can be defined as the number of MEC lost in milk per day. This rate is estimated by measuring daily milk yield and milk MEC content. Mammary epithelial cell content can be determined through light microscopy, flow cytometry analysis, or an immuno-magnetic method for MEC purification. Regardless of the method chosen, to determine MEC content in milk, the milk must first be defatted and the cells must be pelleted. In the light microscopy method, cells are usually stained to observe the different cellular components (such as the nucleus, cytoplasm, or cytosolic granules) and to recognize the various cellular types (Leitner et al., 2000). For the flow cytometry procedure, cells are incubated with a selective set of monoclonal antibodies to identify each cell type (Leitner et al., 2000), or with an anti-cytokeratin antibody to specifically mark MEC in bovine milk (Wagner et al., 2009). In the immuno-magnetic method, total milk cell suspension is incubated with magnetic beads coated with a specific anti-MEC antibody directed against cytokeratin. Specifically bound cells are collected by placing the sample vials in a magnetic particle concentrator and by aspiration of the supernatant containing leukocytes. Purified milk MEC can be directly counted in a hematocytometer (Ben Chedly et al., 2011) or with a cell counter (Boutinaud et al., 2012). The latter was originally used to purify MEC from human milk (Alcorn et al., 2002) and was

Table 1. Concentration and percentage of mammary epithelial cells (MEC) in ruminant milk

Species	MEC percentage in milk (% of total somatic cells)	MEC concentration in milk (10^3 cells/mL)	Counting method	Reference
Cow	36 ± 8	—	Light microscopy	Benic et al. (2012)
	2	—	Light microscopy	Feng et al. (2007)
	45 ± 25	—	Light microscopy	Leitner et al. (2000)
	2.7 ± 2	—	Flow cytometry	Wagner et al. (2009)
	9.3 ± 1.9	14.7 ± 4.1	Immuno-magnetic purification	Boutinaud et al. (2012)
	2.2 ± 0.1	1.5 ± 0.9	Immuno-magnetic purification	Boutinaud et al. (2008)
Goat	4.6 ± 0.6	23 ± 4	Immuno-magnetic purification	Ben Chedly et al. (2011)
	27.2 ± 1.5	11.2 ± 1.2	Light microscopy	Boutinaud and Jammes (2002)
Ewe	2–3	—	—	Bergonier et al. (2003)
	2.2 ± 1.6	1.1 ± 1.3	Light microscopy	Morgante et al. (1996)