Chapter 13

Regulation of Mammary Development as It Relates to Changes in Milk Production Efficiency

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1. Introduction

1.1. Mammary development and function

The development and function of the mammary gland occurs through a cyclical process that changes during the physiological states of pregnancy, lactation, and involution. During pregnancy, maternal hormones in the circulation are responsible for stimulation of mammary gland development and this ensures a sufficient number of mammary cells to produce milk during lactation. Immediately prior to parturition, lactogenic hormones stimulate differentiation of mammary cells, and they adopt a secretory phenotype. In addition to the influence of hormones, the mammary gland itself is thought to be involved in the regulation of mammary cell proliferation, differentiation, and response to hormones. Ultimately, milk yield is dependent on the number and metabolic activity of secretory cells; however, both of these factors are tightly regulated by the endocrine system and physiological state, and also by local factors including the uptake of nutrients by the mammary gland, the connective tissue surrounding the epithelium, and the frequency and degree of milk removal. The following sections will briefly discuss each of the above areas of regulation during lactogenesis and lactation, with emphasis on ruminant species.

1.2. The ultimate determinants of milk production potential: mammary cell number and activity

Milk production potential is a function of the number of mammary epithelial cells in the gland, as well as the secretory activity of those cells (Akers, 2002; Capuco et al., 2003; Boutinaud et al., 2004). Therefore, improved lactation performance can be achieved under conditions that enhance mammary cell proliferation (or decrease apoptosis), biochemical and structural differentiation of mammary epithelium, and synthesis and secretion of milk

components. Moreover, any factors involved in the regulation of these processes can directly impact mammary function and milk yield (Akers, 2002).

The majority (~80%) of mammary cells are formed during pregnancy and prior to lactation; however, cell proliferation during established lactation has been observed in both rodents (Tucker, 1969) and ruminants (Knight & Peaker, 1984; Capuco & Akers, 1990). Because the DNA content per mammary cell nucleus remains relatively constant during pregnancy and lactation, total DNA is considered an accurate indicator of mammary cell number (Tucker, 1987). Mammary cell secretory activity can be estimated by quantification of mammary RNA, and the ratio of RNA to DNA (Paape & Tucker, 1969). Measurements of both DNA and RNA content have provided insight into the relationship between mammary cell number, secretory activity, and milk yield.

It is well established that total mammary cell numbers and milk yield are positively correlated in both ruminants (Linzell, 1966; Keys et al., 1989) and rodents (Tucker, 1969; Nagai & Sarkar, 1978). The secretory activity of these cells is also an important factor involved in determining milk production potential. During lactogenesis, the mammary epithelium becomes highly differentiated. This period is associated with an overall increase in the size and metabolic activity of each cell, closure of tight junctions between cells, an increase in mitochondrial size, and development of the endoplasmic reticulum (Nickerson & Akers, 1984). During established lactation, any new cells that are formed are thought to become differentiated almost immediately (Tucker, 1969). The increase in milk yield during early lactation is associated with an increase in mammary DNA, followed by an increase in mammary cell secretory activity (Knight & Peaker, 1984). In addition, enhanced milk production potential is associated with increases in both mammary epithelial cell number and secretory activity (Keys et al., 1989). In rodents, successful rearing of pups and high rates of litter weight gain are both associated with an increase in mammary DNA, RNA, and ratio of RNA to DNA (Hackett & Tucker, 1969; Paape & Tucker, 1969). Consistent with the observed effects of mammary cell number and secretory activity on milk yield, the declining phase of lactation has been associated with losses in both mammary cell number and metabolic activity (Tucker, 1969; Knight & Peaker, 1984).

Taken together, these observations illustrate the importance of mammary cell number and secretory activity in determining milk yield. Therefore, to improve lactational performance of dairy cows, it is critical to understand the factors involved in the regulation of mammary development and differentiation. Indeed, novel management strategies based on discoveries in mammary gland biology have proven highly successful for use in improving milk production efficiency (Dunlap et al., 2000; Dahl et al., 2004). Some of these techniques will be discussed in more detail later in this chapter.

2. Hormonal regulation of mammary function

One of the major roles of the endocrine system is to coordinate mammary function with the reproductive state of the animal. This physiological synchronization is a very complex process that involves the action and interaction of multiple hormones, as well as the

interplay between hormones in the circulation and local regulation of the mammary response to these hormones. Although much of this chapter will be focused on local regulation of mammary function, it is important to appreciate the role of hormones in regulating mammary function and milk yield. During lactation, several key hormones are involved in the regulation of mammary cell number, secretory activity, and consequent milk production potential.

2.1. Hormones involved in lactogenesis and lactation

2.1.1. Prolactin

As the name indicates, prolactin (**PRL**) is known as the hormone of lactation. Accordingly, it has received much attention from lactation biologists studying the hormonal regulation of mammary function. In ruminants, PRL and glucocorticoids provide the primary stimulus for lactogenesis (Akers, 1985). A role for PRL in the onset of lactation was indicated by a peak in concentrations of the hormone in circulation immediately prior to parturition (Ingalls et al., 1973). Akers et al. (1981a; 1981b) used a dopamine agonist to inhibit periparturient PRL secretion in dairy cows, and this resulted in failure of the mammary epithelium to reach complete structural differentiation. The inhibition of cellular differentiation was accompanied with a 35% reduction in mammary RNA content, a decrease in rates of lactose and fatty acid biosynthesis, and a 40% reduction in milk yield (Akers et al., 1981a). In addition, cytological analysis revealed that inhibition of PRL secretion resulted in a decrease in the size of the metabolic machinery of the cell, including the rough endoplasmic reticulum and the Golgi Apparatus (Akers et al., 1981b). These effects were reversed by treatment with exogenous PRL; therefore, periparturient PRL secretion is essential for complete biochemical and structural differentiation of the mammary gland.

During established lactation, PRL is released during milking or suckling, indicating a role for the hormone in the maintenance of milk production (Koprowski & Tucker, 1973b; Akers, 1985). Indeed, PRL has been shown to maintain both the structural integrity and the functional activity of the mammary epithelium during lactation in rodents (Tucker, 1969; Flint & Gardner, 1994). In addition, PRL maintains and enhances lactation performance in rabbits (Cowie, 1969). Reports on the effect of PRL on milk yield in dairy cows, however, have been inconsistent (Plaut et al., 1987; Wall et al., 2006; Lacasse et al., 2008; Titus et al., 2008). It is generally accepted that PRL is not involved in galactopoiesis (the maintenance of milk production) in ruminant species (Tucker, 2000; Akers, 2006).

As mentioned previously, PRL and glucocorticoids are the major mediators of lactogenesis in many species. In addition to a well-established role in the structural differentiation of the mammary gland, PRL initiates lactogenesis by stimulating the mammary expression and secretion of milk proteins. Using explant culture, Guyette et al. (1979) reported that PRL and glucocorticoids stimulated the expression of casein mRNA within 1 hr of treatment. Similar observations were subsequently made for α-lactalbumin gene expression (Goodman et al., 1983). Subsequent research has confirmed that indeed, PRL and glucocorticoids elicit an

increase in mRNA and protein expression, as well as a decrease in the degradation of milk protein gene transcripts (Vonderhaar, 1987; Rosen et al., 1999). The ability of PRL and glucocorticoids to regulate casein gene expression is due to the presence of response elements in the promoter region of the casein gene (Rosen et al., 1986).

The action of PRL in the mammary gland is mediated through its receptor, which activates the Janus kinase/signal transducers and activators of transcription (**JAK/STAT**) pathway (Hennighausen et al., 1997a). Stimulation of casein and α-lactalbumin gene expression by PRL is mediated mainly by STAT5a, which is essential for both mammary gland development and lactation (Hennighausen et al., 1997b; Hynes et al., 1997; Horseman, 1999). Expression of the PRL receptor is also critical for normal mammary development and differentiation. In rodents, the number of PRL receptors in the mammary gland is positively correlated with milk yield and litter weight gain (Sakai et al., 1985). Additional evidence supporting a direct role for PRL receptor in the mammary gland was reviewed by Ormandy et al. (2003). The results of knockout experiments have revealed that there is a minimum requirement for PRL receptor expression in the mammary epithelium of mice, and this is critical for normal mammary development, lactogenesis, and lactation.

2.1.2. Glucocorticoids

Cortisol is the main glucocorticoid in cattle, and, as mentioned previously, its major function is to enhance the action of PRL in stimulating differentiation of the epithelium and milk protein gene expression in the mammary gland during lactogenesis (Akers, 2002). In addition, glucocorticoids are involved in the regulation of tight junction closure (Stelwagen et al., 1998) and uptake of glucose by the mammary gland (Paterson & Linzell, 1974) during lactogenesis. In pregnant dairy cows, administration of exogenous glucocorticoids resulted in parturition and subsequent induction of lactation (Tucker & Meites, 1965).

During established lactation, glucocorticoids are released during milking or suckling in both rodents and ruminants (Koprowski & Tucker, 1973a; Ota et al., 1974). Interestingly, however, treatment with exogenous glucocorticoids is galactopoietic in rodents (Thatcher & Tucker, 1970) but not dairy cattle (Braun et al., 1970). It is thought that the galactopoietic effect of glucocorticoids in rodents is mediated via an increase in mammary cell secretory activity (Akers, 2002).

The action of glucocorticoids is mediated by its receptor, which is located in the cytosol of the mammary epithelial cell (Gorewit & Tucker, 1976). Upon binding to its receptor, the complex is translocated to the nucleus of the mammary epithelial cell, where it stimulates milk protein gene expression (Tucker, 1985; Li & Rosen, 1994). In addition, the glucocorticoid receptor has been observed to interact with PRL-activated STAT5 molecules to enhance the action of PRL in inducing β-casein gene expression (Wyszomierski et al., 1999). Surprisingly, however, local expression of glucocorticoid receptor was not critical for normal function during lactogenesis and lactation of mice (Kingsley-Kallesen et al., 2002). Therefore, although glucocorticoids enhance the action of PRL during lactogenesis, their direct action on the mammary gland is not essential for normal lactation in rodents. It is

unknown whether expression of glucocorticoid receptor is required for normal mammary function during lactation of ruminants.

2.1.3. Growth hormone

Growth hormone (**GH**) is widely known for its galactopoietic effect in lactating dairy cattle. The first evidence of this was reported by Asimov and Krouze (1937), who observed that injections of dairy cows with pituitary extracts was associated with increased milk production. Although these findings represented an opportunity for increasing milk production efficiency of dairy cows, it was not practical to harvest and purify pituitary GH for commercial use until the 1980's, when the discovery of recombinant DNA technology made it possible to synthesize large quantities of GH. The recombinantly-derived bovine GH (**rbGH**) was subsequently used extensively for research, and was eventually approved for commercial use on dairy operations (Bauman, 1999). A galactopoietic effect of GH in rodents has not been observed (Tucker, 1985; Hadsell et al., 2007); however, Allan et al. (2002) suggested that GH is involved in maintaining mammary cell number during lactation of mice.

In ruminants, the action of GH on the mammary gland is thought to be mediated mainly by the insulin-like growth factor (**IGF**) signaling axis (Etherton, 2004). Treatment with exogenous GH increases the concentrations of IGF-1 in the circulation (Purup et al., 1993), which acts directly on the mammary gland (Shamay et al., 1988; Baumrucker & Stemberger, 1989). In addition to systemic IGF, locally produced IGF, as well as mammary expression of IGF receptor may influence mammary function and the response of the mammary gland to GH (Plath-Gabler et al., 2001; Akers, 2002). Indeed, the effect of GH on the mammary gland of ruminants varied with physiological state. During early lactation, treatment with exogenous GH had no effect on mammary cell proliferation in goats (Sejrsen et al., 1999). When administered during mid-lactation, however, GH was associated with an increase in mammary cell proliferation in cows (Capuco et al., 2001) and an increase in total volume of secretory tissue in goats (Knight et al., 1990). Because local production of IGF, as well as expression of IGF receptors are also physiologically regulated (Sinowatz et al., 2000; Plath-Gabler et al., 2001), this may explain the differences in the response to GH across physiological states.

Although the action of GH is mediated mainly through the IGF axis, there is evidence that GH may act independently of IGF-I to stimulate milk production (Hadsell et al., 2008). In addition, expression of GH receptor has been detected in mammary tissue (Knabel et al., 1998; Sinowatz et al., 2000; Plath-Gabler et al., 2001). The GH receptor belongs to a superfamily of transmembrane receptors, of which PRL receptor is a member (Postel-Vinay & Kelly, 1996). Therefore, the signaling pathway of GH is very similar to that of PRL: binding of GH to its receptor leads to activation of the JAK/STAT signaling pathway, which stimulates changes in gene expression in target tissues (Postel-Vinay & Kelly, 1996). Unlike PRL receptor, however, expression of GH receptor in mammary epithelium is not required for normal mammary development and function in rodents (Kelly et al., 2002). Instead,

expression of GH receptor in the mammary stroma is critical for normal mammary development, supporting the concept that the action of GH on the mammary epithelium is indirect and may be mediated by locally-produced IGF from the stroma (Kelly et al., 2002).

2.2. Other hormones

2.2.1. Leptin

Leptin is a hormone produced mainly by adipose tissue and is involved in appetite regulation. Although it is primarily associated with appetite regulation, leptin and its receptors are expressed in the mammary gland so it is thought to act locally to influence mammary development (Laud et al., 1999; Chilliard et al., 2001). Indeed, treatment of human mammary epithelial cells with leptin elicited a marked increase in cell proliferation (Hu et al., 2002). In contrast, treatment of bovine (Silva et al., 2002) or mouse (Baratta et al., 2003) mammary epithelial cells with leptin was associated with a decrease in proliferation. In fact, it is thought that leptin mediates the negative effects of a high-energy diet on mammary development of dairy heifers (Silva et al., 2002). In addition to the involvement of leptin in mammary development, it has also been proposed to work synergistically with prolactin to regulate mammary function and inflammation (Motta et al., 2004). More recently, it has been reported that leptin specifically induces expression of its long form receptor in goat mammary gland, and influences mammary development and function through several distinct JAK pathways (Li et al., 2010). Therefore, although the action of leptin in the mammary gland is not fully understood, it clearly plays a role in development and function and may directly influence changes in lactation efficiency by acting locally within the gland.

2.2.2. Melatonin

Melatonin is secreted by the pineal gland during exposure to dark and is involved in the circadian rhythm of many biological functions. For over 30 years, melatonin has had an implicated role in mammary development due to its association with the incidence of breast cancer (Cohen et al., 1978). Indeed, a direct negative relationship between melatonin treatment or presence of the pineal gland and the development of mammary cancer was reported long ago (Tamarkin et al., 1981), and it has subsequently been well documented that melatonin inhibits mammary cancer (For reviews see Cos & Sanchez-Barcelo, 2000; Sanchez-Barcelo et al., 2003; Sahar & Sassone-Corsi, 2007; Pandi-Perumal et al., 2008). Because melatonin is secreted during the dark, and has a negative effect on breast cancer risk, the incidence of breast cancer is increased in night-shift workers and people with sleep disturbances (Stevens, 2006; Blask, 2009), and decreased in the blind (Feychting et al., 1998). It is thought that melatonin exerts its effects on breast cancer possibly by modulating estrogen receptor binding activity (Danforth et al., 1983; Cos et al., 2006; Hill et al., 2009).

Melatonin has also been observed to act directly on the mammary gland to inhibit growth in both rodents (Sanchez-Barcelo et al., 1990) and ruminants (Sanchez-Barcelo et al., 1991; Asher et al., 1994). As will be discussed in a later section of this chapter, exposure of

lactating dairy cows to long day photoperiod (16h light; 8h dark) increases milk production, and exposure of late-pregnant cows to short day photoperiod (8h light; 16h dark) increases milk production in the subsequent lactation (Dahl et al., 2000; Dahl & Petitclerc, 2003). It was initially thought that this effect was mediated by melatonin. Because feeding melatonin did not mimic the effect, however (Petitclerc et al., 1998), alternative mechanisms have been proposed (Dahl, 2008). Nevertheless, melatonin plays a clear role in mammary development and function, and it may work together with other hormones, such as prolactin, to mediate the effects of varying daylength on milk production efficiency.

2.2.3. Oxytocin

Oxytocin is a peptide hormone that is secreted as part of the neuroendocrine response to milking or suckling (Goodman & Grosvenor, 1983). Once secreted into the bloodstream, oxytocin acts on the mammary gland to elicit the ejection of milk from the alveolar tissue so that it can be removed by the offspring or by the milking machine. Although it is mainly associated with milk ejection, treatment with exogenous oxytocin was associated with increased milk production of both dairy cows (Nostrand et al., 1991; Ballou et al., 1993; Lollivier & Marnet, 2005) and sheep (Zamiri et al., 2001). During milk stasis in lactating mice, treatment with exogenous oxytocin delays the onset of apoptosis and subsequent involution of the mammary gland (Akers, 1985). The action of oxytocin is mediated by its receptor, which is located on the membrane of myoepithelial cells in the mammary gland (Soloff, 1982; Reversi et al., 2005).

Local regulation of the response of the mammary gland to oxytocin has been observed. In lactating rats, milk stasis was associated with a decrease in the response of the mammary gland to exogenous oxytocin (Kuhn et al., 1973). Similarly in dairy cattle, Linnerud et al. (1966) observed that treatment with exogenous oxytocin did not increase milk yield in the absence of milk removal. In addition to the effects of mammary fill with milk, locally produced hormones are thought to influence the effects of oxytocin on the mammary gland of ruminants (Peaker et al., 1995).

2.2.4. Ovarian hormones

Estrogen and progesterone are both secreted by the ovary, as well as the placenta of pregnant animals, and these hormones are mainly involved in the growth and development of the mammary gland during puberty and pregnancy (Erb, 1977; Schams et al., 1984; Tucker, 1985). Both hormones, however, have additional roles during lactogenesis and lactation. Prior to parturition, estrogen is one of the first hormones to increase in circulation, indicating a role for estrogen in lactogenesis (Akers, 2002). Indeed, administration of exogenous estrogen has been used to induce lactation in both pregnant and non-pregnant dairy cattle (Meites, 1961; Smith & Schanbacher, 1973; Howe et al., 1975; Collier et al., 1977). Estrogen also stimulates the anterior pituitary gland to secrete PRL, and it increases the expression of PRL receptors in the mammary epithelium (Tucker, 2000). During established lactation, estrogen decreases milk yield by interfering with milk ejection (Bruce & Ramirez,

1970), and by inducing mammary involution (Athie et al., 1996; Bachman, 2002). Similar to GH, the action of estrogen on the mammary epithelium is thought to mediated locally by the mammary stroma and by local production of growth factors (Imagawa et al., 2002; Cunha et al., 2004; Parmar & Cunha, 2004).

Prior to parturition in dairy cattle, progesterone inhibits the synthesis of α-lactalbumin, casein, and lactose and consequently inhibits the onset of lactogenesis (Goodman et al., 1983; Wilde et al., 1984; Akers, 1985; Tucker, 2000). Once lactation has been established, however, progesterone has no effect on mammary function or milk production, probably because expression of progesterone receptor in lactating mammary gland is very low (Tucker, 2000).

2.2.5. Relaxin

Relaxin is a protein hormone that is involved in relaxing the pelvic ligaments around the time of parturition of several species (Sherwood et al., 1993). Although not classically considered to be involved in mammary development, research has shown that it is critical for normal mammary development in rodents (Bani et al., 1986), ruminants (Cowie et al., 1965), and pigs (Hurley et al., 1991; Bagnell et al., 1993). Relaxin is also thought to be involved in the inhibition of lactation prior to parturition (Harness & Anderson, 1975). Wahab and Anderson (1989) suggested that relaxin works synergistically with estrogen and progesterone to stimulate mammary growth in pregnant rats, and similar observations have been made in pigs (Winn et al., 1994). In mice, however, relaxin appears to work independently of sex hormones to stimulate nipple development (Kuenzi et al., 1995). Of particular relevance to milk production efficiency, the stimulus of suckling by piglets appears to overcome any effects of relaxin deficiency on lactation performance of lactating pigs (Zaleski et al., 1996). In mice, however, deletion of the relaxin gene resulted in death of pups due to insufficient nipple development and the inability of the pups to suckle (Zhao et al., 1999). Therefore, although relaxin clearly plays a role in mammary development and function, it is still unclear what role, if any, it plays during lactation. In addition, there are clear differences in the role of relaxin across species.

2.2.6. Thyroid hormone

Thyroid hormones have no clearly established role in mammogenesis (Tucker, 2000), but they are galactopoietic in dairy cows. In addition, they may enhance the effect of other lactogenic and galactopoietic hormones such as PRL and GH (Capuco et al., 1989; Akers, 2002). Leech (1950) investigated the effects of exogenous thyroxine on milk yield of dairy cows, and reported that the hormone was galactopoietic in a dose-dependent fashion. The author speculated that thyroxine functions to increase mammary cell secretory activity; however, the treatment only transiently increased milk yield and upon cessation of treatment, milk yield decreased below pre-treatment levels (Leech, 1950). Consequently, although the milk yield response was investigated further (Stanley & Morita, 1967; Schmidt et al., 1971), treatment with exogenous thyroid hormone to increase milk production of dairy cows was never adopted by the dairy industry.

Hormone	Role in Mammary Gland During Lactation
Prolactin	Lactogenesis; cellular differentiation; galactopoiesis (rodents)
Glucocorticoids	Lactogenesis; cellular differentiation; galactopoiesis (rodents)
Growth Hormone	Mammary development; galactopoiesis (ruminants)
Leptin	Mammary development and function
Melatonin	Inhibition of mammary development
Oxytocin	Milk ejection; cellular differentiation; galactopoiesis
Estrogen	Lactogenesis; involution
Progesterone	None
Relaxin	Mammary development; suppression of lactation
Thyroid Hormone	Galactopoiesis (ruminants)

Table 1. The role of various hormones on mammary function during lactation

Clearly, the endocrine system plays an important role in the regulation of mammary function and milk yield across many species. In addition, there is substantial evidence for local regulation of the response of the mammary gland to the endocrine system. This local regulation includes changes in the expression of specific hormone receptors in the gland, as well as the local production of growth factors that mediate or enhance hormonal effects on mammary function. Moreover, there are regulatory mechanisms in the mammary gland that are thought to act separately from, and may sometimes interact with, the effects of the endocrine system.

3. Local regulation of mammary function

In addition to the influence of hormones, mammary function is also under the regulation of local factors. It is essential that sufficient nutrients are taken up by the mammary gland to support the synthesis of milk components. Although the mammary epithelium is the site of milk synthesis, it is highly responsive to and largely dependent on the dynamics of the surrounding connective tissue and extracellular matrix. Finally, removal of milk from the gland is involved in the regulation of mammary cell number, secretory activity, and milk yield.

3.1. Mammary blood flow

An extensive vascular system provides the mammary gland with the nutrients required for milk synthesis. Uptake of nutrients, and subsequent synthesis of milk components, is dependent on the rate of blood flow through the capillaries surrounding each alveolus, and also the exchange of nutrients across the capillary wall (Prosser et al., 1996). During pregnancy, the number of blood vessels in the mammary gland gradually increases as the gland prepares for copious milk production (Yasugi et al., 1989; Matsumoto et al., 1992; Djonov et al., 2001). During established lactation, the vasculature is maintained and then slowly regresses with advancing lactation and involution. Consequently, changes in milk yield are usually associated with changes in blood flow to the mammary gland (Prosser et al., 1996).

Local regulation of mammary blood flow in rodents was originally observed by Silver (1956). He observed that within 100 h of sealing selected teats and subsequent engorgement with milk, mammary involution had taken place, and capillaries were empty and collapsed. This occurred even when contralateral glands were suckled, indicating that mammary blood flow is indeed under the control of local factors and not hormones (Silver, 1956). When pups were allowed to resume suckling of the previously sealed teats, the capillary bed was promptly re-filled with blood and mammary function was restored (Silver, 1956). In agreement with those observations, Mao and Caruolo (1973) reported that mammary blood flow was inversely related to the amount of milk accumulated in the gland, and that decreased milk secretion during milk stasis may be mediated by a decrease in availability of nutrients to the mammary gland. Similarly, during extended milk stasis in lactating goats, blood flow to the mammary gland decreased linearly over 36 h (Stelwagen et al., 1994). Stelwagen et al. (1994) suggested that during milk stasis, the decline in mammary blood flow may be the result of negative feedback from the gland due to a reduction in demand for milk precursors. Farr et al. (2000) reported that extended milk stasis in lactating goats resulted in a 50 to 75% decrease in mammary blood flow and capillary permeability, as well as a marked regression of the vasculature, in agreement with previous observations in mice (Silver, 1956). The results of this research support the concept that during milk stasis, blood flow to and metabolic capacity of the mammary gland is impaired (Farr et al., 2000).

In contrast to the negative effect of milk stasis on mammary blood flow, a positive relationship has been observed between mammary blood flow and frequent milk removal. During hourly milking of lactating goats, blood flow to the mammary gland increased (Farr et al., 2000). In addition, milk yield of lactating goats increased within 2 h of an experimental increase in mammary blood flow via vasodilatation (Prosser et al., 1990). After the treatments stopped, however, milk yield decreased to pre-treatment levels. Despite these observations, frequent milk removal does not always stimulate an increase in mammary blood flow (Maltz et al., 1984), and an increase in mammary blood flow does not always elicit an increase in milk yield (Prosser et al., 1994; Lacasse & Prosser, 2003). Therefore, although mammary blood flow and milk yield are closely associated, they are not always causally linked. This indicates that although mammary blood flow sometimes influences milk yield, other limiting factors are involved.

3.2. Extracellular matrix

As discussed above, the development and differentiation of the mammary gland requires stimulation by hormones. However, an important local mediator of cellular function is the environment surrounding the epithelium. This surrounding environment contains the extracellular matrix (**ECM**), which acts directly on the mammary epithelium to regulate cell differentiation, growth, gene expression, and response to hormones (Wilde et al., 1984; Lee et al., 1985; Streuli et al., 1991).

Suard et al. (1983) reported that the nature of substratum used in culture had a marked effect on both proliferation and differentiation of primary epithelial cells harvested from rabbit

mammary glands. Whereas cells cultured on a floating collagen gel were able to synthesize and secrete caseins in response to PRL, cells embedded in collagen were observed to secrete caseins in response to PRL and also to proliferate (Suard et al., 1983). In contrast, cells cultured on an attached collagen gel were able to proliferate only and did not synthesize or secrete caseins in response to PRL. Moreover, the cells cultured on the attached collagen gel did not express casein mRNA. The authors concluded that cell surface conditions, as well as cell-ECM interactions regulate cellular proliferation and differentiation (Suard et al., 1983). Similar conclusions had been previously made based on work with primary mouse mammary cells (Shannon & Pitelka, 1981). They speculated that the function of a mammary cell is directly linked to its shape, which is dictated by the nature and flexibility of the substratum. Subsequent work using mouse mammary cells has confirmed that the nature and physical state of ECM regulates cell shape, as well as the mRNA expression, synthesis, degradation, and secretion of caseins (Lee et al., 1984; Lee et al., 1985; Bissell & Hall, 1987; Schmidhauser et al., 1990; Streuli & Bissell, 1990). Mouse mammary cells cultured on floating collagen gels expressed up to ten-fold more casein mRNA than those cultured on plastic. In addition, caseins that were synthesized by cells cultured on plastic were degraded intracellularly, whereas those synthesized by cells on floating gels were secreted into the culture media (Lee et al., 1985). This research also provided evidence that milk proteins are differentially regulated, because synthesis and secretion of some non-milk proteins was not affected by the culture substratum (Lee et al., 1984). In no culture conditions has there been any significant expression of α-lactalbumin (and, consequently, there is no synthesis of lactose), indicating that expression of this protein is regulated by an alternative mechanism within the mammary gland (Lee et al., 1984; Wilde et al., 1984; Bissell & Hall, 1987). More recently, it has been observed that in addition to the presence of ECM, adhesion of the mammary epithelium to ECM is critical for cellular differentiation (Zoubiane et al., 2004). Based on their observations, these authors suggested a role for the integrins in coordinating the cytoskeleton and regulating the induction of cellular differentiation by PRL. To regulate cellular proliferation and mammary growth, ECM influences the cellular response to steroid hormones (Wilde et al., 1984), and also appears to interact with local growth factor signaling pathways (Berry et al., 2003). In addition, remodeling of ECM and mammary involution is induced by decreased milking frequency and milk stasis in ruminants (Weng et al., 2008).

The local environment and ECM surrounding the mammary epithelium play a critical role in regulating cellular development and function. As discussed above, the action of ECM on the epithelium influences the mammary response to hormones, and this interaction has a marked effect on the number and secretory activity of mammary cells in the gland. This, in turn, determines the milk production potential of the animal. A deeper understanding of stromal-cell interactions and how they influence and limit milk production may provide the means to promote a desirable local environment to improve milk production efficiency.

3.3. Milk removal

Across many species, regular removal of milk from the mammary gland during established lactation is critical to maintaining mammary cell number, activity, and consequent milk

production. The local mechanisms regulating the mammary response to milk removal are poorly understood, although several have been proposed. In addition to the effect of milk removal on mammary blood flow and uptake of nutrients for milk synthesis, the mammary response to milk removal may involve changes in the extracellular matrix, negative feedback by factors present in the milk or milk fat, and changes in intramammary pressure.

3.3.1. The effect of milk removal on mammary cell number and activity

Local regulation of mammary cell number and secretory activity was originally observed in experiments using teat ligation in lactating rats. In those experiments, selected teats were ligated and pups were allowed to continue suckling intact glands. Tucker and Reece (1963b) observed that coincident with 24h of milk stasis, the ratio of RNA to DNA in ligated glands decreased by 31%, indicating a decrease in mammary cell activity. The authors suggested that during milk stasis, intact (suckled) glands were able to take up more nutrients and hormones from the circulation than the sealed glands, and that this may explain the observed increase in mammary cell secretory activity (Tucker, 1966; Tucker et al., 1967). In contrast to the effect of milk stasis on mammary cell activity, increased suckling frequency was linearly associated with an increase in mammary cell number, activity, and litter weight gain (Tucker, 1966; Tucker et al., 1967; Tucker & Thatcher, 1968). The increase in mammary cell secretory activity was observed within 24 h of increased suckling intensity, indicating rapid local regulation in response to increased demand of the offspring (Tucker, 1966). Similar experiments have revealed local regulation of lactogenesis and cellular differentiation in ruminants (Akers et al., 1977; Guy et al., 1994), as well as mammary involution and cellular apoptosis (Goodman & Schanbacher, 1991; Quarrie et al., 1994). Taken together, these observations support the concept that mammary cell proliferation and differentiation can be regulated locally by factors within the mammary gland. Moreover, milk removal from the gland can elicit a stimulatory effect on these processes. The mechanisms underlying this response, however, are unclear.

3.3.2. Local regulation of mammary function by factors in milk

It has been hypothesized that a chemical in milk negatively regulates milk secretion in the absence of milk removal (Linzell & Peaker, 1971). Subsequently, a small glycoprotein in milk was reported to reversibly inhibit casein and lactose synthesis in a dose-dependent manner (Wilde et al., 1987). This glycoprotein has been named feedback inhibitor of lactation (**FIL**). It is both synthesized and secreted by mammary epithelial cells, and is located in the whey fraction of milk. Therefore, it is thought that FIL may be involved in autocrine regulation of milk secretion and the adjustment of milk production to meet (but not exceed) the nutritional demands of the offspring (Peaker & Wilde, 1987). Similar observations have been made in lactating women (Daly et al., 1993; Daly & Hartmann, 1995; Wilde et al., 1995). The mechanisms underlying this regulation are unclear; however, it has been suggested that FIL inhibits milk production by interfering with the casein secretory pathway (Rennison et al., 1993; Burgoyne & Wilde, 1994). In addition, Peaker and Wilde (1987) proposed that the mammary gland responds to removal of FIL in a sequential manner consisting of an

immediate response that increases milk secretion within hours of milk removal; an acute response that increases mammary cell differentiation after several days of frequent milk removal; and finally a long-term response that increases mammary cell proliferation after several weeks or months of frequent milk removal. Unfortunately, no experiments have been conducted to determine the mechanism by which FIL inhibits milk secretion. To the contrary, research on this protein has not been pursued since the 1990's; therefore, the identity of this protein and its role in the mammary gland have yet to be confirmed.

3.3.3. Negative feedback on milk fat synthesis

Before the reports on FIL, it was observed that the synthesis of fatty acids by the mammary gland was regulated by a factor within the milk fat itself (Levy, 1963, 1964). This research, however, received much less attention than the FIL literature. Levy (1964) observed an accumulation of fat within 12 h of weaning and a consequent diminution of fatty acid synthesis in the mammary gland of lactating rats. By 24 h, fatty acid synthesis was inhibited by 90%, and lactose was reabsorbed into the bloodstream. The synthesis of fatty acids was restored, however, when pups were returned to the mother to suckle (Levy, 1964). Teat-ligation experiments showed that the regulation occurred at the level of the individual mammary gland, since intact (suckled) glands continued to synthesize milk and milk fat (Levy, 1964). In an attempt to identify the factors involved in the inhibition of fatty acid synthesis, Levy (1964) used tissue explants from rat mammary gland and observed that whole milk markedly inhibited the synthesis of fatty acids in a dose-dependent response. Subsequent analysis revealed that the inhibitory activity was acting on acetyl CoA carboxylase, and was not associated with milk fat itself but was located in the particulate fraction of milk (Figure 1; Levy, 1964). Levy (1964) speculated that the inhibitor was bound to microsomes in the milk.

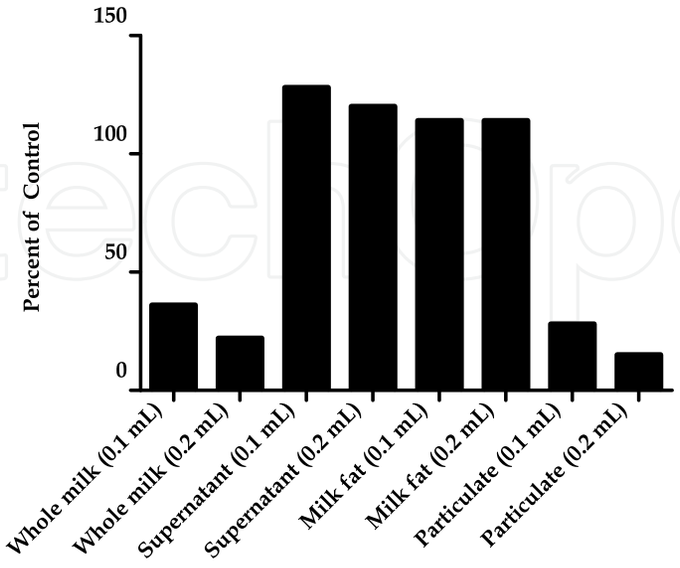

Figure 1. Inhibition of fatty acid synthesis by milk fractions. Mammary glands were removed from lactating rats, incubated with various milk fractions, and assayed for incorporation of $^{14}CO2$ as described by Levy (1964).

More recently, inhibition of mammary lipogenesis by medium chain fatty acids has been observed (Agius & Williamson, 1980; Heesom et al., 1992). Heesom et al. (1992) suggested that FIL may regulate lactose and casein synthesis, whereas fat synthesis may be regulated by a negative feedback mechanism involving medium chain fatty acids. To test this hypothesis, Peaker and Taylor (1994) investigated the effect of milk fat on litter weight gain in mice. Infusion of whole milk (which contains milk fat globules) into the mammary glands of lactating mice inhibited litter growth, whereas skim milk (which contains FIL) or fractions of milk fat globules alone had no effect. The authors concluded that there is no negative feedback mechanism located in the milk fat (1994). This conclusion, however, seemed particularly dismissive, as their results did not prompt them to question a role for FIL, which had no apparent effect on litter weight gain in this experiment. Perhaps coincidentally, that report was one of the last published primary research articles investigating a role for FIL in the mammary gland.

Certainly, there is substantial evidence for the existence of at least two types of chemical negative feedback mechanisms involved in the regulation of milk synthesis and secretion. Moreover, it is probable that there are other feedback mechanisms that have yet to be discovered. These factors may act on distinct components of milk, or there may be some redundancy in their activity. It makes biological sense that a costly metabolic process such as lactation would be tightly regulated by a variety of local mechanisms to prevent overproduction in the absence of milk removal.

3.3.4. Intramammary pressure

Because accumulation of milk elicits an increase in pressure within the mammary gland, it is not surprising that intramammary pressure has been investigated as a potential regulator of mammary blood flow and milk secretion. Infusion of air or milk into the mammary glands of goats was associated with an increase in intramammary pressure and a linear decrease in mammary blood flow (Pearl et al., 1973). The infusion of only one udder half revealed that this response is regulated locally within the gland, as adjacent glands were unaffected (Pearl et al., 1973). Peaker (1980) reported that loss of mammary cell secretory activity during milk stasis of lactating goats was caused by an increase in intramammary pressure, and not to a decrease in mammary blood flow. An increase in intramammary pressure, however, did not always result in a decrease in milk production (Henderson & Peaker, 1984). Therefore, the relationship between intramammary pressure, mammary blood flow, and milk removal remains unclear. It is possible that intramammary pressure may indeed be a local mediator of mammary function, but its role may change with physiological state, metabolic status, and stage of lactation.

Interestingly, fur seals do not undergo inhibition of milk secretion or mammary involution during prolonged absence of milk removal (reviewed by Sharp et al., 2006). During lactation, these animals go through cycles of suckling their young on land, and foraging for food for up to 30 d at a time. During foraging, milk secretion continues and mammary function is maintained so that the seals can suckle their young when they return to shore. It

has been suggested that fur seals have adapted to override the influence of local negative feedback mechanisms to accommodate their foraging cycles and continue to rear their offspring successfully (Sharp et al., 2006). Moreover, this adaptation is thought to be regulated at the transcriptional level (Sharp et al., 2008). This is an exciting and active area of study. Once the mechanisms of local regulation and negative feedback are understood, and the genes involved are identified, there may be an opportunity to identify limits on milk secretion and improve milk production efficiency of dairy animals.

4. Lactation persistency

The performance of lactating animals is assessed by examination of the lactation curve, which depicts milk production over time during a complete lactation. A typical lactation curve consists of 3 phases: a phase of increasing milk yield during early lactation, followed by a phase of peak milk production, and finally a phase of gradually decreasing milk yield which occurs post-peak and continues throughout the remainder of lactation (Wood, 1967). One of the key aspects of the lactation curve, and a general indicator of lactation performance, is lactation persistency. Lactation persistency is defined as the degree to which peak milk yield is maintained throughout lactation. As expected, animals with persistent lactations are highly desirable, as they have the ability to attain exceptional milk production efficiency.

The shape of the lactation curve and lactation persistency is influenced by many factors, including mammary cell number and secretory activity, hormones, and nutritional status (McFadden, 1997; Sorensen & Knight, 2002; Capuco et al., 2003; Hadsell et al., 2007). As mentioned previously, milk yield is ultimately a function of the number of secretory cells in the mammary gland, and the metabolic activity of these cells. In lactating goats, the increase in milk yield during early lactation was associated with an increase in mammary cell number, followed later by an increase in mammary cell activity (Knight & Peaker, 1984). In dairy cattle, however, the increase in milk yield during early lactation appears to be a result of increased secretory activity of mammary cells, and not an increase in cell number (Capuco et al., 2001). In both species, the decrease in milk production during the declining phase of lactation was associated with a decrease in mammary cell number only (Knight & Peaker, 1984; Capuco et al., 2001). If the lactating animal was pregnant, however, a decrease in secretory cell activity was also observed (Knight & Peaker, 1984). Similar results have been observed in rodents, such that mammary cell number and activity decline during advancing lactation despite continued milk removal (Tucker & Reece, 1963a; Thatcher & Tucker, 1968; Hadsell et al., 2007). Therefore, several researchers have suggested that lactation is a transient process, and that the declining phase of lactation is a programmed response (McFadden, 1997; Capuco et al., 2003; Hadsell et al., 2007).

4.1. Hormonal regulation of lactation persistency

The role of hormones in regulating persistency of lactation is not thoroughly understood, but some hormones are clearly involved. Whereas concentrations of PRL, glucocorticoids,

and GH are high during early lactation and decrease with advancing lactation, concentrations of oxytocin are low during early lactation and increase with advancing lactation (Koprowski & Tucker, 1973a, b; Tucker, 1985). In addition, glucocorticoids, oxytocin, and prolactin are released during milking (Tucker et al., 1975; Carruthers & Hafs, 1980; Akers & Lefcourt, 1982). Treatment with exogenous PRL increased milk yield in rabbits (Cowie, 1969), but reported effects of PRL on milk yield of dairy cattle have been inconsistent (Plaut et al., 1987; Wall et al., 2006; Lacasse et al., 2008; Titus et al., 2008). Oxytocin was galactopoietic in cattle (Nostrand et al., 1991; Ballou et al., 1993; Lollivier & Marnet, 2005) and sheep (Zamiri et al., 2001), but had no effect on milk yield of rodents (Thatcher & Tucker, 1970). As mentioned previously, GH is galactopoietic in ruminants (Knight, 1992; Etherton & Bauman, 1998; Baldi, 1999). Estrogen also plays a role in lactation persistency: during established lactation in pregnant dairy cows, increasing estrogen concentrations are associated with the onset of declining lactation and mammary involution (Akers, 2002; Bachman, 2002; Capuco et al., 2003).

4.2. Effect of parity on lactation persistency

The lactation curve of primiparous dairy cows is more persistent than that of multiparous cows; however the mechanisms involved are unknown (McFadden, 1997). Miller et al. (2006) compared mammary cell dynamics, milk yield and milk composition of primi- vs. multiparous cows and reported that the extent of mammary cell differentiation was lower in primiparous than multiparous cows. Mammary cell renewal, however, was greater in primiparous cows (Miller et al., 2006). Because loss of mammary cells is associated with the declining phase of lactation, the authors concluded that primiparous cows are more persistent than multiparous cows because they maintain the population of secretory cells (Miller et al., 2006). Concentrations of IGF-1 in circulation were approximately 20% higher in primiparous cows, and this may have elicited a mitogenic effect on the mammary gland. Interestingly, the percentage of lactose in milk was constant during the lactation of primiparous cows, whereas it decreased during lactation of multiparous cows (Miller et al., 2006). A similar relationship between maintained lactose concentration in milk and lactation persistency was reported by Sorensen et al. (2008). The synthesis of lactose by the mammary gland is a marker for cellular differentiation during lactogenesis (Akers, 2002). Taken together, these observations support the concept that to increase lactation persistency, the population of functionally active secretory cells must be maintained. Clarification of the mechanisms involved in maintenance of secretory cell number and metabolic activity could lead to improved lactation persistency in multiparous animals and consequent enhancement of lactation efficiency.

4.3. The effect of pregnancy on lactation persistency

Dairy cows are typically pregnant for most of their lactation. This is a standard management practice to optimize generation of replacement animals, and also to ensure that the cow will continue to lactate. Unfortunately, however, lactation persistency is decreased by concurrent

pregnancy (McFadden, 1997; Sorensen et al., 2008). After about the 5th month of pregnancy, concentrations of estrogen in the circulation increase, and this is associated with a decline in milk yield (Bachman, 2002), as well as reductions in both mammary cell number and secretory activity (Capuco et al., 2003). Similar observations have been made in rodents, that pregnancy decreases mammary function and milk yield (Paape & Tucker, 1969). Therefore, despite continued milk removal, mammary involution does occur as the gland prepares for the next lactation.

4.4. Manipulation of lactation persistency

Management interventions such as long-day photoperiod, supplementation with rbGH, and increased milking frequency have been used to change the shape of the lactation curve and increase milk production efficiency (Bauman, 1999; Dunlap et al., 2000; Stelwagen, 2001). It has been suggested that these management practices stimulate an incremental increase in milk yield (Erdman & Varner, 1995; Stockdale, 2006). Whether these interventions actually increase lactation persistency, however, is questionable (McFadden, 1997).

4.4.1. Manipulation of photoperiod

Seasonal effects on mammary development and function have been extensively studied, and it is well established that manipulation of day length influences mammary development and milk production in dairy cattle (reviewed in Dahl et al., 2000). Exposure of dairy cows to long day photoperiod (16 h light: 8 h dark) during established lactation was associated with increased milk production (Peters et al., 1981; Evans & Hacker, 1989; Miller et al., 1999). In contrast, exposure to short day photoperiod (8h light: 16h dark) during the last 2 mo of pregnancy was associated with an increase in milk yield in the subsequent lactation (Miller et al., 2000). Because serum PRL concentrations change with photoperiod, researchers have focused on PRL signaling as a potential mediator of the milk yield response (Auchtung et al., 2005; Wall et al., 2005; Dahl, 2008). The results of this research have indicated that although PRL signaling may be involved in the effects of photoperiod on non-lactating cows, the IGF-1 axis may be more important during established lactation. In addition to changes in the concentration of hormones in the circulation, manipulation of photoperiod is also associated with changes in mammary gene expression (Auchtung et al., 2005; Wall et al., 2005; Dahl, 2008). This indicates that local regulation of mammary function is involved in the response of the mammary gland to changes in photoperiod.

During established lactation, manipulation of photoperiod appears to influence lactation persistency (Peters et al., 1981; Evans & Hacker, 1989; Miller et al., 1999), whereas manipulation of photoperiod during the non-lactating period had no effect on subsequent lactation persistency (Miller et al., 2000). Despite the uncertainty with respect to the underlying mechanisms, and whether there is truly an effect on lactation persistency, manipulation of photoperiod has emerged as an effective management strategy to improve milk production efficiency of dairy cows (Dahl & Petitclerc, 2003).

4.4.2. rbGH

As mentioned previously, GH is galactopoietic in dairy cows (Bauman et al., 1985). Since it became commercially available for use on dairy operations, the effects of rbGH on lactation performance and animal health have been extensively studied (Crooker & Otterby, 1991; Bauman, 1999; Dohoo et al., 2003). Treatment of dairy cattle with rbGH is associated with a decrease in lipogenesis and an increase in gluconeogenesis by the liver, which increases the availability of fatty acids and glucose to the mammary gland for the synthesis of milk fat and lactose (Akers, 2002). This shift in metabolism and nutrient utilization is thought to be mediated by interactions between GH, insulin, and the IGF axis (Molento et al., 2002). In addition to altered nutrient metabolism to support increased milk production, treatment with rbGH is associated with an increase in blood flow to the mammary gland (Breier et al., 1991; Prosser et al., 1996). The mechanisms underlying the milk yield response, however, remain unclear. Capuco et al. (2001; 2003) suggested that bGH increased lactation performance by increasing the population of mammary epithelial cells, possibly via the IGF signaling axis, and similar observations have been made in rodents (Allan et al., 2002). Because the milk yield response to bGH is acute and disappears upon cessation of treatment, however, it seems more likely that the mechanism works to enhance the milk synthetic activity, rather than the number, of secretory cells (Akers, 2002; Yang et al., 2005).

Intervention	**Timing of implementation**
Manipulation of photoperiod	Lactation; dry period
Increased milking frequency	Early lactation; full lactation
Suckling (with or without machine milking)	Early lactation; full lactation
rBST	Lactation
Genetic selection	Selection of cows for breeding; purchase of semen

Table 2. Various management interventions that can increase milk production efficiency.

4.4.3. Frequent milking

Another management strategy that increases milk production is frequent removal of milk from the mammary gland by either suckling or increased milking frequency. Frequent milking (3 or more times daily) has been adopted on many dairy farms and has proven to be a highly successful approach to increase milk production efficiency. Reports on the effects of frequent milking on lactation persistency, however, are inconsistent. Whereas several researchers have observed an increase in persistency in response to frequent milking (Pearson et al., 1979; Poole, 1982; Amos et al., 1985; Hillerton et al., 1990; Sorensen & Knight, 2002), others have reported no effect (Allen et al., 1986; Gisi et al., 1986). The discrepancy in results may be due to differences in the definition or measure of persistency, the nature and duration of frequent milking treatment, or to the physiological state of the animals (pregnancy status, stage of lactation). Like photoperiod treatment and rbGH, the mechanisms underlying the milk yield response to increased milking frequency are unknown. It has been suggested, however, that use of the three interventions combined will elicit additive effects on milk production (Dunlap et al., 2000). This indicates that distinct mechanisms may be involved in each of the responses.

5. Conclusions

Milk production potential is dependent on the number of secretory cells in the mammary gland, as well as the metabolic activity of those cells. Both of these factors are greatly influenced by the endocrine system, by local regulatory mechanisms within the mammary gland, and by the interaction between endocrine and local regulation. Moreover, interventions that perturb the endocrine system or the local mammary environment can result in changes in mammary cell number, secretory activity, and consequent milk production potential. The mechanisms underlying the response of the mammary gland to those interventions are unknown. Research focused on determining the mechanisms involved will improve the knowledge of mammary gland biology and regulation of mammary function, and could lead to novel management strategies to further optimize milk production efficiency. Our companion chapter provides an extensive review of the literature on frequent milking or suckling as they influence milk production and mammary function in dairy animals.

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