UNIVERSITI PUTRA MALAYSIA

LACTATIONAL FAILURE IN SAHIWAL FRIESIAN COWS

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By

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Sahiwal Friesian cows have been bred specifically for dairying in Malaysia for the past 20 years but there still exist a proportion of these cows having a lactation period less than 60 days of milk production. The aim of this project was to determine the physiological incidence and characteristics of lactation persistency and to identify factors that might be responsible for cessation of milk secretion in lactational failure cows (LF).

The study on the milking characteristics exhibited a high residual milk volume in early lactation, which increased with stage of lactation in the LF cows. Milk storage studies in the udder indicated there was no significant difference in the characteristics of milk distribution between alveolar lumina and gland cistern with time after milking between the normal and LF cows. This indicated that retention of residual milking after milking was more likely to be a consequence of a defective neuroendocrine letdown reflex, perhaps involving insufficient systemic oxytocin or mammary insensitivity to oxytocin.

Analysis of the metabolic activity of the mammary epithelial (milk secreting) cells and estimation of mammary cell numbers suggested that the lactational failure in the Sahiwal Friesian cows was due, at a cellular level, to loss of a proportion of the secretory epithelial cell population. This finding indicates premature involution of the mammary gland in the LF cows.

Prolactin measurements during the early lactation showed that there was no evidence of differences between normal and LF cows. It was only after the fourth week onwards of lactation, that there was a steady decrease in prolactin levels in the lactational failure cows. Therefore, it appears unlikely that an inherent deficiency in prolactin secretion in LF cows, apparent from the first weeks *post partum*, had compromised mammary development and so precipitated lactational failure.

In conclusion, the results obtained in this project suggest that a principal cause of lactation failure in Sahiwal Friesian cows is a progressive increase in the proportion of milk left in the gland after milking. Oxytocin treatment may alleviate the problem if residual milk is primarily a consequence of restricted oxytocin release at milking. The roles of other galactopoietic hormones remain to be established.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Dokotor Falsafah

KEGAGALAN MENGELUAR SUSU PADA LEMBU SAHIWAL FRIESIAN

Oleh

MURUGAIYAH MARIMUTHU

Jun 2000

Pengerusi: Prof. Madya Dr.Aziz Sharee.

Fakulti : Perubatan Veterinar

Lembu betina Sahiwal Friesian telah dibiakkan khusus untuk tenusu di Malaysia sejak 20 tahun lalu tetapi masih terdapat sebahagian besar lembu ini yang mempunyai tempoh pengeluaran susu kurang daripada 60 hari. Tujuan projek ini ialah untuk menentukan keadaan fisiologi dan ciri-ciri kemantapan penyusuan serta menentukan faktor-faktor yang mungkin bertanggungjawab menahan pengeluaran susu pada lembu Sahiwal Friesian yang gagal mengeluarkan susu (LF).

Lembu LF menunjukkan isipadu susu baki yang tinggi di awal penyusuan dan meningkat naik pada tahap penyusuan seterusnya.

Penyimpanan susu dalam ambing lembu normal dan LF menentukan tidak terdapat perbezaan antara lumina alveol dan kelenjar sistern dengan masa selepas susu diperah.

Kajian analisis aktiviti metabolisma, sel epitelium mama (sel pengeluar susu) dan menganggar bilangan sel mama mencadangkan bahawa kegagalan

penyusuan lembu Sahiwal Friesian adalah disebabkan oleh kehilangan sebahagian sel epitelium pengeluar susu, dan pembezaan separa sel-sel yang lain.

Ukuran prolaktin semasa penyusuan awal menunjukan tiada bukti yang membezakan lembu normal dan LF. Hanya selepas empat minggu penyusuan terdapat kadar prolaktin yang berkurangan pada lembu LF. Oleh itu, hal ini menunjukkan bahawa tidak ada kekurangan yang nyata dalam perembesan hormon prolaktin pada lembu LF, yang ketara pada minggu pertama selepas beranak, yang merencat pertumbuhan mama dan seterusnya menyebabkan LF.

Keputusan yang diperolehi dari semua ujikaji di atas menunjukkan bahawa sebab utama kegagalan penyusuan pada lembu Sahiwal Friesian adalah disebabkan oleh peningkatan kadar susu baki dalam kelenjar selepas diperah. Rawatan oksitosin boleh mengurangkan masalah ini jika susu baki adalah disebabkan oleh pengeluaran osksitosin yang terhad semasa susu diperah. Peranan hormon galaktopoietik yang lain masih perlu dikaji lagi.

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I certify that an Examination Committee met on 5th June 2000 to conduct the final examination of Murugaiyah Marimuthu on his Doctor of Philosophy thesis entitled "Lactational Failure in Sahiwal Friesian Cows" in accordance with Universiti Putra Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.

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DECLARATION

I hereby declare that the thesis is based on my oringinal work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

(MURUGAIYAH MARIMUTHU)

Date: 7/6/2000.

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CHAPTER 1

REVIEW OF LITERATURE

Introduction

Mammals are distinguished from other vertebrates by the presence of mammary glands which provide nutrition for the young after parturition (Mepham, 1987). The milk produced may be the primary if not the sole energy source for the young. Supply of milk from mother to offspring improves their immunity and the close contact of the young aids maternal bonding (Peaker, 1989). Lactation itself usually follows a distinct pattern in a species in terms of milk secretion and production. In cows, lactation commences after nine months of pregnancy, followed by approximately ten months of lactation, with peak production occurring about six weeks *post partum* (Linzell, 1973; Shinde, 1978), and steadily declining over the remaining period. Milk secretion is then intentionally stopped (drying off) when the animal approaches the late stages of its next pregnancy.

The mammary gland is unique among the body tissues, in the sense that mammary development takes place before and during puberty. Simplistically, mammary development, which prepares and maintains the lactating tissue involves massive cell numbers and the creation of a remarkable ductal network which acts to drain the secretory tissue in which the milk is synthesied (Turner, 1988). Mammary development and process of milk secretion are both subjected to control by a complex interaction of systemic and local factors. Local mechanisms, growth inhibitors and other factors, moderate the systemic control by peptide and steroid hormones during tissue development, and as will become apparent in the course of

this thesis, act within the tissue during lactation to influence its performance. The endocrine system regulates mammary function directly and also acts to alter nutrient partitioning and physiological parameters such as cardiac output and voluntary food intake (Vernon, 1988; Smith and Walsh, 1984; Bauman *et al.* 1980, Hart and Morant, 1980).

Mammary Development

The process of growth and development of the mammary gland determines its potential secretory capacity, and the secretion of milk during lactation is itself influenced by a variety of other factors.

During pregnancy, the sequence of mammary growth is similar in different species and only their course varies with the length of gestation. (Rillema, 1994). The parenchyma of the differentiated mammary gland consists of ductal epithelium, alveolar epithelium and myoepithelium (Rudland and Hughes, 1989; Streuli, 1995). Alveoli are developed and the lobuloalveloar system progressively takes over most of the space occupied by stroma so that the gland becomes a compact mass of lobules of alveoli, which are composed of alveolar cells that are responsible for the production and eventual secretion of milk (Vorherr, 1974).

The Lactating Mammary Gland

Mammary glands are paired structures, located external to the body cavity. The ruminant mammary gland consists of an udder which is composed of two or four glands, each drained by a single teat. The secretory portion of the gland is the lobulo-alveolar tissue, composed of specialised epithelial cells arranged in spherical sacs or

alveoli which are surrounded intimately by myoepithelial cells and blood vessels. Fine ducts drain the alveoli; these lead into progressively larger ducts which drain into a common storage space, the cistern (Schmidt, 1971) (Fig 1.1). Collectively, lobulo-alveolar tissue and ducts form the parenchyma, with connective and fat within the gland, constituting the mammary stroma.

The interactions of many factors have an influence on normal mammary cell development and function, including the animal's genetic background, endocrine and metabolic state. This review of literature will, however, focus primarily on those factors that act to produce and sustain the mammary gland in its fully functional state during lactation.

Mammary Development *pre partum*

A satisfactory lactation can be anticipated when the mammary glands have reached a proper state of development, both in terms of number of cells and in their ability to synthesize and to secrete milk (Knight, 1982b). There are five stages of mammary development that can be distinguished: embryonic and fetal, prepubertal, steady-state cyclic adult growth, and growth during pregnancy and lactation (Laurence *et.al.* 1991). The main development of the mammary gland commences at puberty; pre-pubertal cows have few mammary ducts, but these increase in size, number and complexity after puberty (Linzel, 1966; Delouis *et al.* 1980). At the onset of puberty, budded structures at ductal apices elongate and ramify into the surrounding fat pad to form the mature ductal structure of the ruminant mammary gland. During pregnancy, there is a high degree of cell proliferation and the mammary ductal system grows

further and infiltrates the fat pad (Rillema, 1994). This phase also involve the appearance of terminal alveoli, and the lobuloalveolar system progressively takes over most of the space occupied by stroma and fatty tissue, so that the gland becomes a compact mass of lobules of alveoli separated from each other by septa of connective tissue. The mammary gland in the ruminant develops at first pregnancy and alveolar lobules are formed at the fifth month. The lobular-alveolar system grow extensively by the sixth month and secretory activity develops during the last several months (for further reviews see Erb, 1977).

Development and subsequent differentiation of the gland during pregnancy are dependent on complex interactions between a number of systemic hormones and locally produced growth factors (reviewed by Topper and Freeman, 1980, Tucker, 1987). It was believed that mammary development ceased at the end of pregnancy (Convey, 1974) but now recent findings have indicated that it continues beyond this point. Goat mammary tissue proliferates exponentially during pregnancy and growth continues *post partum*, albeit at a reduced rate (Knight and Peaker, 1984). After lactation the mammary gland reverts to a non-secretory state for a cyclic renewal.

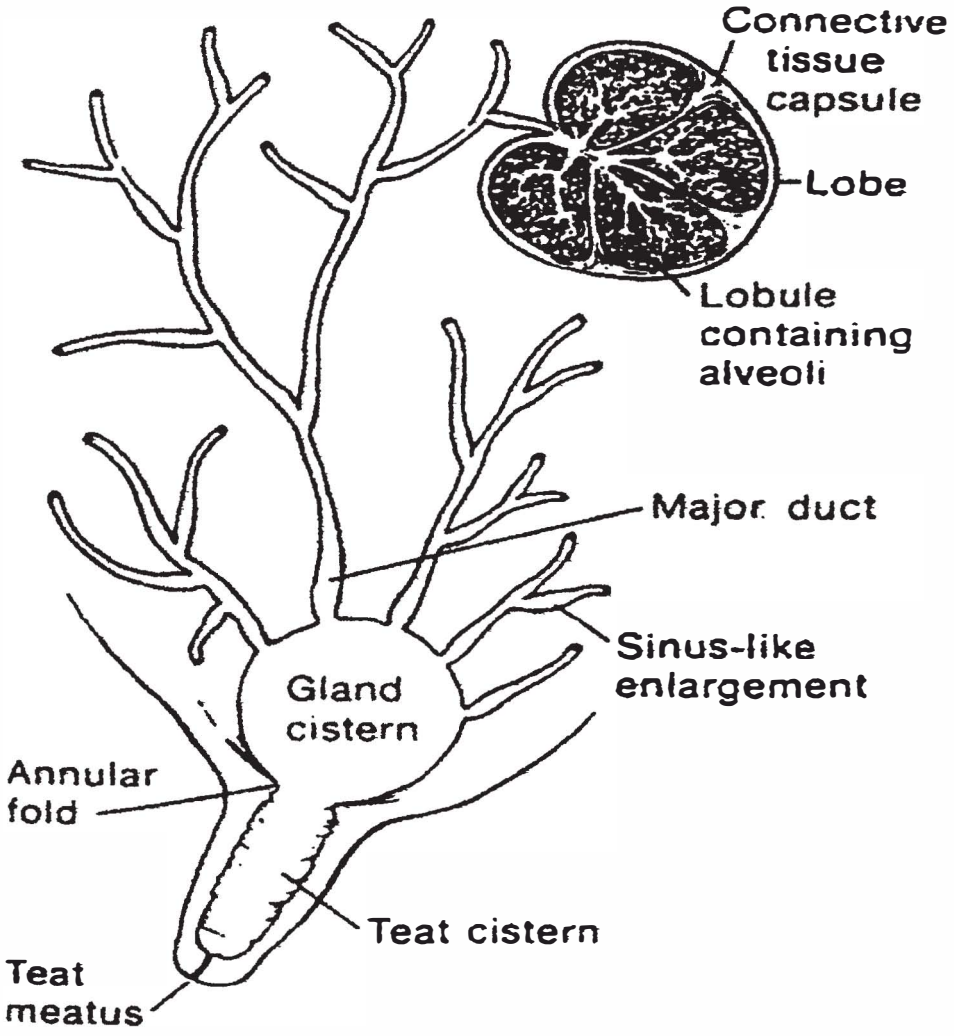

Figure 1.1 : Diagrammatic sketch of the ductal system of one quarter of the bovine mammary gland. Taken from Schmidt (1971)

Mammary Development in Lactation

The regulation of cell number is of critical importance to the lactating mammary gland. The rate of milk synthesis is influenced by many factors but ultimately it is the number and activity of the secretory cells that determine milk yield. Thus the maintenance of milk production depends upon the degree to which growth is completed at parturition and the relative contributions of cell number and secretory cell activity to milk yield throughout lactation, which varies from species to species (Knight and Peaker, 1982a). In ruminants, milk yield rises characteristically to a peak early in lactation and declines gradually thereafter for as long as milking is continued. Maintenance of milk production during the later stages of lactation will depend upon the number of secretory cells lost, the extend of cell replacement (if any) and the retention of synthetic capacity by each cell. If these parameters could be altered during pregnancy or early lactation, then it could potentially alter the magnitude, and/or the profile, of subsequent milk production (Knight, 1987).

Cell number in the mammary gland has been measured in terms of total DNA content (DNA_t) (Knight,1984). Measurement of total mammary DNA in mice suggest that about 50% of total proliferation of cells seem to occur during early pregnancy, with 40% during lactation; but in ruminants a greater proportion of mammary growth during pregnancy is achieved (e.g. 78% in sheep, Anderson, 1975). In goats, increasing milk yield is associated by increasing cell number over the first 3 weeks of lactation (Knight and Peaker, 1984, Fowler *et al. 1990*), but enhanced cellular activity alone accounts for the further increase in milk yield to peak lactation (Wilde *et al.* 1986). In goats, the decline in milk yield after peak lactation is primarily due to a

decrease in secretory cell number (Wilde *et al.* 1986), with individual cells not losing their metabolic capacity for milk synthesis to any significant degree. Thus it is not a decrease in cell differentiation but the loss of secretory cells which is responsible for the decrease in milk yield after peak lactation (Knight and Peaker, 1984; Wilde *et al.* 1986). Further evidence of the dynamic nature of the mammary cell population during lactation comes from developmental responses to frequency of milking.

Milking frequency has an influence on the mammary secretory cell number. A prolonged increase in the frequency of milking results in a great number of secretory cells in the thrice–daily milked gland compared to the contralateral twice-daily milked gland at 37 weeks of thrice-daily milking (Wilde *et al.* 1987). Measurement of thymidine incorporation and DNA, indicated that the difference in cell number between thrice and twice-daily milked glands was due, at least in part, to proliferation of new cells in the more frequently milked gland (Wilde *et al.* 1987). Thus changes in milking frequency leads to physiological adaptations in the secretory cell population of the lactating mammary gland and these changes act to sustain the increase or decrease in milk yield.

Mammary Involution

Mammary tissue starts to undergo the process of involution when milk secretion stops (Sordillo and Nickerson, 1988; Oliver *et al.* 1989) This process is characterized by de-differentiation and apoptosis of mammary epithelial cells, the extent and time course of the latter varying between species (Wilde *et al.* 1999). Cessation of milk removal causes loss of alveolar structure and basement membrane

degeneration (Quarrie *et al.* 1996), induced by extracellular protease activities (Strange *et al.* 1992; Talhouk *et al.* 1992).

There is now evidence that goat mammary cell loss after peak lactation occurs by apoptosis. DNA laddering, indicative of apoptosis, has been observed in lactating ruminant mammary tissue (Quarrie *et al.* 1994), as well as in rodent tissue after peak lactation (Quarrie *et al.* 1995). Apoptosis can be stimulated by a mechanism sensitive to milk stasis in both rodent and ruminants (Quarrie *et al.* 1995; Quarrie *et al.* 1996) and recent work shows that during lactation, apoptosis is subjected to local control, within each mammary gland, by frequency of milking (Li *et al.* 1999). The circumstances of cell death during lactation and involution differ in significant respects (Meites and Hopkins, 1961) .

Mammary Cell Differentiation

Differentiation is defined as the process whereby the mammary epithelial cell acquires the complement of intracellular enzymes and proteins necessary to meet the demands of milk synthesis and secretion. The relationship between cell number, cell activity and milk yield can be assessed by serial biopsy of the mammary gland (see Chapter 5). Cell differentiation commences sometime prior to 7 weeks *pre partum*, proceeds at a modest rate during late gestation and accelerates markedly between parturition and peak lactation (Wilde *et al.* 1986). Cellular activity is largely determined by the degree of differentiation. The activities of a number of key enzymes involved in the synthesis of milk constituents, such as acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS) and galactosyltransferase (GT), and assay of rates of lactose, casein

and total protein synthesis, can act as markers of the degree of cellular differentiation, i.e. metabolic capacity (Wilde, *et al.* 1987a).

Epithelial cell differentiation is characterised by accumulation of milk protein mRNAs and an increase in lipogenic and other enzyme activity involved in milk synthesis (reviewed by Burgoyne and Wilde, 1994). Differentiation of the mammary gland is a sequential process. Milk protein mRNAs are present by mid to late pregnancy but copious milk production does not start until parturition (Harris *et al.* 1991). For example, in mouse, β-casein mRNA is present at mid-pregency and increases progressively up to after parturition (Harris *et al.* 1991). Conversely WAP gene expression increases predominately after the young

1991), while ACC and FAS activities rise in the final days of pregnancy and continue to rise until peak lactation (Shipman *et al.* 1987). This sequential induction of epithelial cell differentiation suggests that milk protein genes are regulated differentially within the secretory cell either by systemic hormones or by local intramammary factors.

Regulation of casein gene expression has been studied primarily in mammary cell culture. The synergistic action of the lactogenic hormones, principally glucocortiod, insulin and prolactin and the extracellular matrix on β-casein gene expression is well documented (Schmidhauser *et al.* 1990; Schmitt-Ney *et al.* 1991).

Alpha-lactalbumin is essential for the production of lactose and thus milk (Stacey *et al.* 1995). Expression of the α-lactalbumin gene requires insulin and prolactin and is maximal in the presence of glucocorticoid (Ono and Oka, 1980),

although high levels of glucocorticoid may inhibit α-lactalbumin expression (Funder, 1989). Progesterone also inhibits α-lactalbumin gene expression and it is the loss of progesterone at parturition, along with the increase in prolactin that allows increased α-lactalbumin protein synthesis (Funder, 1989).

Whey acidic protein (WAP) is expressed in high levels in the lactating mammary glands of mice, rats and rabbits (Hennighausen *et al.* 1982). WAP mRNA accumulates in late pregnancy and by mid-lactation is present at levels 1000 times higher than that seen in early pregency. WAP gene expression is dependent on synergy between prolactin, glucocorticoid and insulin, cell-cell and cell –matrix interactions.

β-lactoglobulin is the major whey protein in ruminant milk. It is expressed by mid-pregenancy, increases slowly until parturition and then increases rapidly, again reaching a peak at mid-lactation (Gaye *at al.* 1986). In cultures of ovine mammary cells induction of milk protein genes appears less dependent on lactogenic hormones than caseins. Glucocorticoid and insulin in synergy with prolactin are only slightly more effective than prolactin alone in inducing β-lactoglobulin gene expression (Puissant *et al.* 1990).

Milk Synthesis and its Components

Milk provides the primary source of nutrition for young mammals until they are able to digest more solid food. While the composition of milk varies widely between species, the main components are water, protein (providing a source of