Lactation failure in crossbred Sahiwal Friesian cattle

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Summary. Milk producers in Malaysia make extensive use of crossbred Sahiwal Friesian dairy cattle. These animals have, however, been found susceptible to lactation failure. A survey of cows in an experimental herd of F1 Sahiwal Friesian animals indicated that, in 30% of animals, milk yield decreased to negligible levels within the first 8 weeks *post partum*. Lactation failure was associated with a progressive increase in the amount of residual milk left in the udder after normal milking. By week 3 of lactation, residual milk volume was significantly greater than that in animals that, based on previous lactation history, were not susceptible to lactation failure, and accounted for up to 30% of milk available at the morning milking. The cellular consequences of residual milk accumulation were evident in the activities of acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase, key enzyme markers of cellular differentiation, which decreased in glands undergoing lactation failure and were lower than values measured in tissue of control cows. Mammary cell number, estimated by tissue DNA content, was also reduced in animals undergoing lactation failure. These indices of mammary development indicate that lactation failure is the result of premature involution in susceptible animals. Premature involution is a predictable consequence of progressive milk stasis in failing lactation, and attributable to an increase in autocrine feedback by inhibitory milk constituents. The progressive increase in residual milk is, on the other hand, unlikely to be attributable to impaired mammary development. Measurements of milk storage during milk accumulation showed no differences between control and lactation failure cows in the distribution of milk between alveolar and cisternal storage compartments. We conclude that lactation failure in Sahiwal Friesian cows is due to a failure of milk removal, and probably the result of an impaired milk ejection reflex rather than to the glands' milk storage characteristics.

Keywords Lactation, milking, residual milk, mammary development, milk yield.

Milk producers in South Eastern Asia make extensive use of crossbred Sahiwal Friesian cattle. These cattle are relatively heat-tolerant (Lemerle & Goddard, 1986) and, in general, sufficiently productive to make smallholder dairy units in Malaysia financially viable (Wan Hassan *et al.* 1989). It has, however, been recognized for some

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time that a proportion of Sahiwal Friesian are prone to lactation failure in the first third of lactation, a phenomenon observed in both imported and locally-bred animals (Edwards, 1985). Consistent loss of > 60% of a lactation's potential production in up to 30% of a dairy herd is economically unattractive, but the cause of lactation failure has received little attention.

In this study, lactation failure was examined in an experimental herd, using standard physiological and biochemical analyses of mammary development and function. Attention focused on the milking process, since differences in lactation failure rates between animals milked by machine and those milked by hand (Edwards, 1985) suggested that lactation failure could be the result of ineffective milk removal. Ineffective milk removal could be the result of impaired milk ejection, and due to oxytocin deficiency (Bruckmaier & Blum, 1998). Alternatively, or in addition to this, cows undergoing lactation failure may have udder anatomy that favours milk storage in alveolar lumina rather than cistern. If so, they would be more adversely affected by residual milk (Knight *et al.* 1994) through the actions of a feedback inhibitor of milk secretion present in milk (Wilde *et al.* 1995).

The developmental consequences of inefficient milking are well documented (Wilde *et al.* 1989; Wilde & Knight, 1990; Li *et al.* 1999) and could, in severe conditions, precipitate the premature termination of lactation (Quarrie *et al.* 1994; Li *et al.* 1999). If lactation failure is, indeed, a consequence of inefficient milk removal, assessment of mammary development should identify characteristic changes in mammary cell number and differentiation. Since the relationship between milk secretion rate and milk removal arises through autocrine feedback by a milk constituent (Peaker & Wilde, 1996), this would, in turn, suggest a cellular mechanism for lactation failure.

METHODS

Animals

Sahiwal Friesian cows were from the Unit Tensu of the Department of Veterinary Services, Malaysian Agriculture Research and Development Institute (MARDI). F1 generation animals were routinely allowed free access to grazing and fed 3 kg concentrates at each milking. Animals were milked twice daily at 05.30 and 15.30 unless stated otherwise. Fat, protein and lactose composition of milk from the morning milking was analysed at weekly intervals (Milk-O-Scan analyser 133B; N. Foss Electric-Denmark). Cows were identified as normal or susceptible to lactation failure (LF), based on their previous lactation history, the average lactation number of animals used in the studies being $4{\cdot}2 \pm 0{\cdot}27$. Lactation failure was defined as a loss of milk production within 80 d of calving under normal milking conditions. Normal cows were usually in milk for > 300 d.

Measurement of residual milk and milk storage characteristics

Residual milk was measured after the morning milking in a random sample of Shaiwal Friesian cows in the herd, and in two groups of 20 animals identified as normal or subject to lactation failure based on their previous lactation history. Cows were milked by machine until milk flow ceased, and yield was recorded to an accuracy of 100 g. Residual milk was recovered by machine milking following an intravenous injection of 5 IU oxytocin (Syntocin; Troy Laboratories, Pty. Ltd, Australia); the injection was repeated after 3 min to ensure complete emptying of the udder. Residual milk percentage was calculated as a percentage of the total volume obtained by machine and oxytocin milking.

Cisternal and alveolar milk storage was measured in groups of six normal and LF cows as described previously (Knight *et al.* 1994) at 2 h intervals after recovery of residual milk. Measurements were made at 1 h, and then at 2 h intervals up to 12 h, after recovery of residual milk, and were performed in weeks 4–5 of lactation. Cisternal milk was obtained by catheter drainage of each gland, milk ejection being prevented by performing the operation in unfamiliar surroundings away from the milking parlour (Bruckmaier & Blum, 1998). Animals were transferred to the milking parlour, and alveolar milk was recovered by machine milking aided by two injections of oxytocin (5 IU i.v.), with a 3 min interval between injections. Each cow was studied only once at each time point, with at least 2 d between consecutive measurements. The order of time point measurements was randomized, and results were corrected for within-cow variation in milk yield with stage of lactation as described previously (Knight *et al.* 1994). Udder volume was measured after emptying of the gland by the method of Davis & Hughson (1988).

Mammary biopsy

Mammary tissue was sampled on days 20, 40 and 60 of lactation by biopsy under local anaesthesia of three control and three LF cows not used for other measurements (Knight *et al.* 1992). Briefly, cows were sedated with xylazine (5,6 dihydro-4H-1,3-thiazine-hydrochloride (Rompun solution, 0·25 mg/50kg body weight i.m.; Bayar Leverkusen, Germany), and an L-block was performed using xylocaine (10 ml 2% solution (v/v); Jurox PTY Ltd, Australia) to provide regional anaesthesia at the site of incision. Mammary secretory tissue was exposed by an incision made in the upper back quadrant of one quarter, and a 5–10 g portion was removed with a scalpel. Bleeding was controlled by ligation, and the incision was closed using simple and interrupted blanket sutures for deep and superficial tissues respectively. Animals were treated post-operatively with oxytetracycline. Milk yield returned to pre-treatment values within 2 d of biopsy. Depression of milk yield was seen in both biopsied and non-biopsied quarters, suggesting it was the result of starvation prior to surgery rather than to tissue removal. Biopsy samples were trimmed to remove connective and fatty tissue, and immediately frozen in liquid nitrogen for subsequent analysis.

Tissue analysis

Biopsy tissue was homogenized and assayed for the activities of acetyl-CoA carboxylase, fatty acid synthetase, galactosyltransferase and aryl esterase as described previously (Wilde *et al.* 1986), under conditions where enzyme activity was linearly related to the amount of sample and incubation time, and expressed per mg DNA. DNA concentration was measured in tissue homogenates by a fluorimetric method (Labarca & Paigen, 1980), and total DNA was calculated as the product of DNA concentration and udder mass (Knight & Peaker, 1984).

Statistics

Results were compared by ANOVA using SAS program release 6.08 (SAS Institute Inc. Cary, NC, USA).

RESULTS

Milk yield

Sahiwal Friesian cows varied considerably in lactation performance, with falling milk production evident in ~ 30% of animals by 6 weeks *post partum*. In these cattle, lactation effectively failed after 8 weeks, and milking was discontinued. This

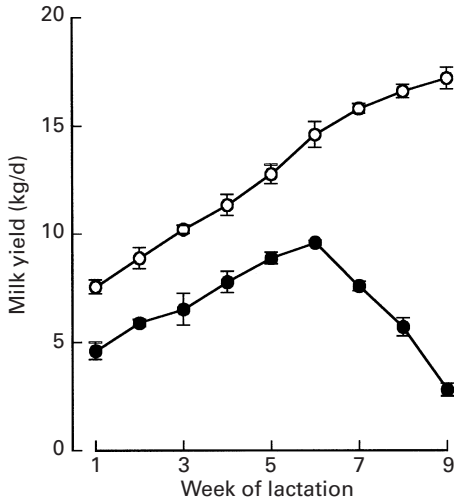

Fig. 1. Mean daily milk yield in Sahiwal Friesian cows exhibiting lactation failure. Cows were in their third lactation, and identified as normal or exhibiting lactation failure (LF) based on previous lactation history. ○, normal cows; ●, LF cows. Values are the mean ± SEM for 20 animals. All LF values: * $P < 0{\cdot}05$ compared with normal cows.

Table 1. *Mammary enzyme activities and DNA content in normal and lactation failure (LF) cows during early lactation*

(Values are the mean ± SEM for groups of three normal or LF cows)

	Normal cows			LF cows		
	Day 20	Day 40	Day 60	Day 20	Day 40	Day 60
Acetyl CoA carboxylase, nmol/min per mg DNA	72·4 ± 14·5	74·7 ± 24·6	76·0 ± 18·5	68·2 ± 13·5	70·8 ± 16·7	48·2 ± 15·5[ab]
Fatty acid synthetase, μmol/min per mg DNA	0·26 ± 0·05	0·28 ± 0·07	0·29 ± 0·05	0·22 ± 0·08	0·25 ± 0·09	0·13 ± 0·18[ab]
Galactosyltransferase, μmol/min per mg DNA	0·28 ± 0·07	0·30 ± 0·03	0·32 ± 0·07	0·25 ± 0·06	0·27 ± 0·07	0·17 ± 0·08[ab]
Aryl esterase, μmol/min per mg DNA	0·18 ± 0·06	0·21 ± 0·05	0·22 ± 0·06	0·14 ± 0·02	0·17 ± 0·03	0·10 ± 0·02[b]
DNA, mg/g tissue	4·77 ± 0·15	4·81 ± 0·17	4·83 ± 0·14	4·67 ± 0·15	4·59 ± 0·13	3·79 ± 0·17[ab]
Total DNA, g	37·23 ± 0·38	36·61 ± 0·45	36·51 ± 0·67	33·81 ± 0·67	32·68 ± 0·70	25·51 ± 0·30[ab]

[a] Significantly lower than previous values for that group: $P < 0{\cdot}05$.
[b] Significantly lower than values for normal cows at the same stage of lactation: $P < 0{\cdot}05$.

performance was repeated in successive lactations, such that groups of normal and LF cows were easily identified and their milk yields compared (Fig. 1).

Residual milk volumes and mammary DNA and enzyme activities were compared by random design ANOVA using SAS program release 6·08 (SAS Institute Inc. Cary, NC, USA). Differences in alveolar and cisternal volumes between groups of cows and within each cow across time were analysed using Student's test for unpaired and paired observations respectively (Knight *et al.* 1994).

Cell number and differentiation

Lactation failure occurred without any gross change in milk composition (results not shown), but was accompanied by marked changes in mammary development indicative of premature involution. At day 60 of lactation, the activities of acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase, three enzyme

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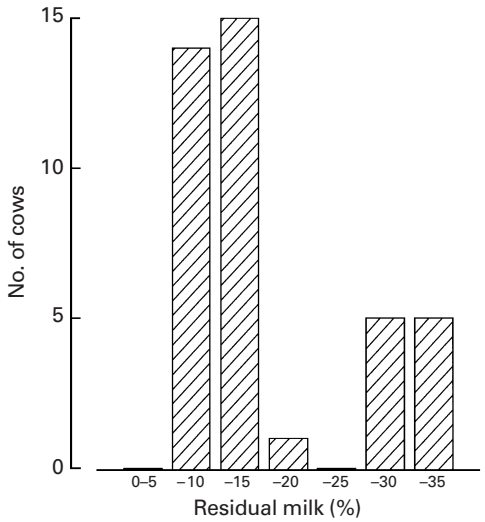

Fig. 2. Residual milk percentage in a random sample of Sahiwal Friesian cows at 50–70 d in milk. Residual milk was measured in a random sample of 300 cows in the unit herd, and calculated as a percentage of the total milk available at the morning milking. The distribution of values for those cows that were 50–70 d in milk is shown.

markers of epithelial differentiation, had fallen in LF mammary tissue ($P < 0{\cdot}05$) and were respectively 37 %, 55 % and 47 % lower than in mammary tissue of normal cows (Table 1). The activity of aryl esterase did not change significantly with stage of lactation in LF cows, and was significantly lower than in normal cows on day 60 of lactation (Table 1). Mammary DNA concentration in LF cows, and total DNA content calculated as the product of concentration and udder volume (Knight *et al.* 1994), decreased between days 40 and 60 of lactation, and both were significantly lower than normal cows on day 60 (Table 1). A fall in mammary DNA content indicates that 22 % of the cell population was lost during lactation failure, such that the cell number was 30 % lower than in normal cows.

Residual milk

A survey of 300 Sahiwal Friesian cows selected at random from the experimental herd showed that the proportion of stored milk retained after milking increased with stage of lactation, this increase being particularly evident in the first 90 d *post partum* (results not shown) . The range of residual milk values measured in early lactation (7–32 % at 60–80 d in milk) was, in addition, indicative of the presence of two groups of cows in this random population (Fig 2). One group, comprising the majority of animals, retained relatively little residual milk. Examination of these animals' production history showed that they were normally in milk for > 300 d. The other group, comprising 30 % of the population, had higher residual milk and was subsequently found to comprise those animals prone to lactation failure, based on previous lactation history. This suggested that milking was relatively ineffective in those cows that consistently exhibited lactation failure.

Comparison of selected normal and LF cows of the same parity confirmed the results of the random survey, showing that, as milk yield increased in the early weeks of lactation, residual milk volume remained relatively constant in normal cows but increased significantly in the LF group (Fig. 3). In week 7 of lactation, residual milk accounted for 5·5 % and 26·5 % of total milk recovered at the morning milking from

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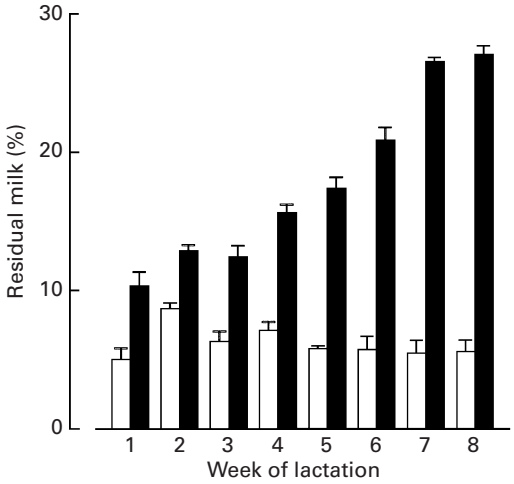

Fig. 3. Change in residual milk with stage of lactation in normal and lactation-failure (LF) cows. Residual milk is expressed as a percentage of total milk available at the morning milking. □, Normal cows; ■, LF cows. Values are the mean ± SEM for 20 animals. All LF values were significantly greater ($P < 0{\cdot}05$) than those for normal cows.

Table 2. *Least mean square of residual milk percentage in normal and lactation-failure Sahiwal Friesian cows of different parities*

(Values are the least mean square ± SEM for six animals)

	Residual milk (%)†	
Lactation number	Normal	Lactation failure
2	8·2 ± 0·5	32·3 ± 0·8[a]
3	9·5 ± 0·8	33·1 ± 0·3[a]
4	9·4 ± 0·6	33·7 ± 0·4[a]
5	10·7 ± 0·7	34·8 ± 0·5[a]

† Residual milk is expressed as a percentage of the total available at the morning milking.
[a] Significantly higher than values for normal cows: $P < 0{\cdot}05$

normal and LF cows respectively. High residual milk in cows destined to lactation failure was a recurrent phenomenon in successive lactations. Residual milk volume (results not shown) and residual milk percentage (Table 2) were consistently greater in LF cows irrespective of parity ($P < 0{\cdot}05$). Together, the results demonstrated that animals susceptible to lactation failure had higher residual milk volumes after milking, that residual milk increased in early lactation coincident with the onset of lactation failure, and that high residual milk was a recurrent phenomenon in multiparous cows.

Milk storage characteristics

Within the bovine mammary gland, milk is stored in the cisternal space (including the duct system) and within the lumen of the secretory alveoli. Distribution of milk between the two compartments changes during milk accumulation, and those animals which store relatively more of their secretion in the alveolar space have a lower rate of milk secretion per unit mass of secretory tissue (Knight, 1995). The extent to which the cistern fills with milk soon after milking is not related to residual milk volume, but increases with stage of lactation, making efficient milking particularly important in early lactation (Knight *et al.* 1994). To determine if gland anatomy, and therefore milk storage, may also contribute to

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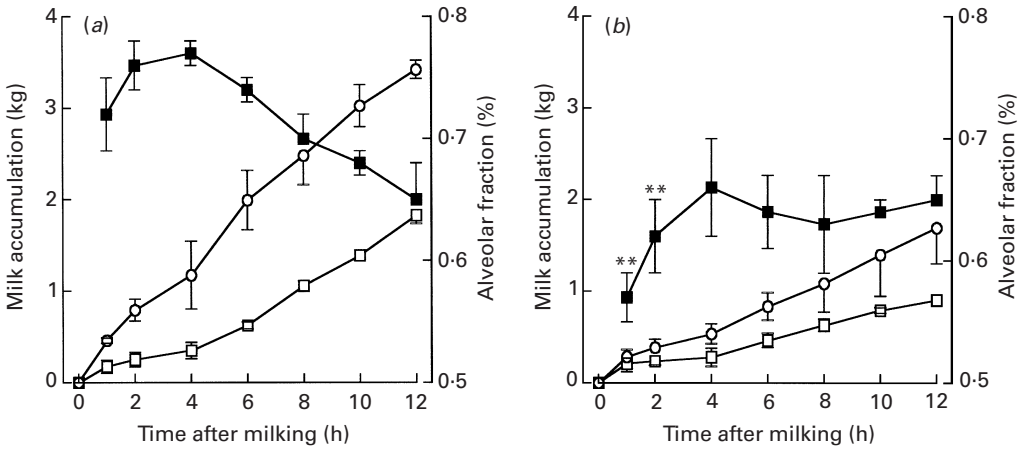

Fig. 4. Alveolar and cisternal milk accumulation in glands of Sahiwal Friesian cows exhibiting lactation failure. Alveolar milk (○), cisternal milk (□) and alveolar fraction (■) were measured in (*a*) normal and (*b*) lactation failure cows at intervals after complete evacuation of the udder. Values are the mean ± SEM for six animals. ** $P < 0{\cdot}01$ compared with normal cows.

lactation failure, alveolar and cisternal milk accumulation was compared in normal and LF cows. Milk stored in alveolar and cisternal spaces was measured at intervals after complete evacuation of the udder. In normal cows, alveolar milk storage increased in a linear manner with time during milk accumulation, although a small volume of milk was transferred to the cisternal space soon after milking (Fig. 4*a*). There was then little further accumulation of cisternal milk until 4 h after milking, after which constant rate of cisternal accumulation was observed (Fig. 4*a*). A similar pattern of milk accumulation was observed in an earlier study of Friesian cattle (Knight *et al.* 1994). In animals susceptible to lactation failure, alveolar storage also increased in a linear manner, whereas cisternal volume accelerated after an initial lag period (Fig. 4*b*). The fraction of milk stored in alveoli, expressed relative to total milk recovered, was initially higher in normal cows, but decreased thereafter, such that after 12 h of milk accumulation there was no difference in the alveolar fraction between normal and LF cows (Fig. 4*b*). That the fraction of alveolar milk in normal cows decreased with time after 4 h of milk accumulation but remained relatively constant in LF cows suggests that, at a lower rate of milk secretion, the elasticity of ductal tissue reduced transfer of milk between alveolar and cisternal spaces.

DISCUSSION

Farm records and veterinary surveys of Sahiwal Friesian cattle in Malaysia identified a proportion of cows with very short lactations. This was confirmed systematically by the present study, lactation failure, despite adequate nutrition and husbandry, being observed in 30 % of cows, with milk production remaining low and failing only 8–9 weeks after calving. A possible explanation for lactation failure was suggested by the beneficial effect of exogenous oxytocin injections which, in preliminary trials, increased milk yield in LF cows and postponed the onset of lactation failure (M. Murugaiyah, unpublished results). This observation suggested that lactation failure in Sahiwal Friesian cows may be due to ineffective milking, such that milk synthesis and secretion are increasingly subject to the inhibitory effects of accumulated milk (Peaker & Wilde, 1996).

Screening of cows under normal milking conditions confirmed the existence of a population of animals with high residual milk, and comparison with farm records identified these animals to be susceptible to lactation failure. Conversely, cows identified as susceptible to lactation failure, based on previous lactation history, were found to have higher residual milk volumes, a trait that increased as lactation advanced. By week 7, when lactation was failing, residual milk accounted for $> 25\%$ of the total available, a value higher than that for cows with normal lactations, and considerably in excess of residual milk values determined for Friesians elsewhere (Knight *et al.* 1994). Further, the recurrent failure of this population of cows in successive lactations was reflected in the persistence of residual milk accretion in cows of increasing parity. The coincidence of high residual milk and lactation failure, both in terms of animals affected and their development with stage of lactation, suggests that the two are causally related.

The effects of milking frequency, milking efficiency and milk stasis on mammary development and function are well established, and support a causal relationship between high residual milk and lactation failure. Infrequent milking of goats (once instead of twice daily) decreased both the rate of milk secretion and the degree of mammary epithelial differentiation (Wilde & Knight, 1990). When sustained, infrequent milking caused a decrease in alveolar cell number, a consequence of a stimulation of cell death by apoptosis (Li *et al.* 1999). Inefficient milking, in which the proportion of residual milk was deliberately increased, also inhibited milk secretion and, with time, decreased mammary differentiation (Wilde *et al.* 1989). Loss of cellular differentiation during lactation failure was indicated by the fall in mammary acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase activities, with no significant change in the house-keeping enzyme aryl esterase (Wilde *et al.* 1986). Tissue DNA content indicated that cell number was also falling, possibly due to induction of apoptosis. Thus, the developmental adaptations seen in lactation failure were those to be expected if milk removal were impaired. The progressive increase in residual milk, which in practice culminated in milk stasis around the ninth week of lactation, would thereafter be expected to induce widespread mammary involution. Complete cessation of milking precipitated bovine mammary involution, with elimination of milk protein gene expression and the induction of cell death by apoptosis (Wilde *et al.* 1997). In those circumstances, involution could be attributable to falling concentrations of galactopoietic hormones. Mammary involution is, on the other hand, induced in individual goat mammary glands by local milk stasis (Quarrie *et al.* 1994; Li *et al.* 1999), showing that milk accumulation could by itself be the trigger of lactation failure.

Ineffective milking, and the accumulation of residual milk, down-regulates milk yield and mammary development probably through feedback inhibition by milk constituents. Screening of goats' milk constituents has identified a small milk protein that inhibits milk secretion acutely (Wilde *et al.* 1995) and decreases cellular differentiation, both in cell culture (Wilde *et al.* 1991; L. M. B. Finch, J. M. Bryson and C. J. Wilde, unpublished results) and in lactating animals (Wilde *et al.* 1988). The same factor may also induce mammary apoptosis (A. Streets, S. A. Baldwin, D. J. Flint and C. J. Wilde, unpublished results). Feedback inhibition is autocrine in nature, the inhibitory protein (termed FIL, feedback inhibitor of lactation) being secreted by the mammary epithelial cells which it subsequently inhibits (Wilde *et al.* 1995), and degree of inhibition being related to FIL concentration in alveolar milk. Milk movement and storage dictate that autocrine feedback cannot be simply a matter of secretion of the inhibitor and its removal during milking. Incomplete

milking necessitates some form of inhibitor processing, either inactivation of secreted inhibitor or activation of a precursor form; otherwise FIL in residual milk would prevent relief of feedback after milking. Whatever its details, the mechanism would become increasingly inefficient with increasing residual milk volume. Accordingly, glands milked by catheter, such that all alveolar milk was retained, showed no relief of autocrine feedback and therefore no response to frequent milking (Henderson & Peaker, 1987). Similarly, natural dysfunction of milk ejection in a proportion of Sahiwal Friesian cows would increase autocrine feedback from residual milk, with adverse effect on subsequent milk secretion, cellular differentiation and cell number.

The degree of autocrine feedback also depends on the site of inhibitor storage, and the movement of milk between alveolar and cisternal storage spaces, because it is only FIL in alveolar milk that is in contact with the secretory cells and therefore active in feedback inhibition (Knight, 1995). Thus, milk production for a given mass of secretory tissue is greater in goats that store relatively little milk in the alveolar space (Peaker & Blatchford, 1988). For the same reason, the extent to which autocrine feedback is overcome between milkings is likely to be influenced by an initial efflux of milk from alveoli to cistern that occurs within 1 h after milking (Knight *et al*. 1994). This efflux occurred in Sahiwal Friesian cows, but was greater in normal cows, perhaps because of greater compliance of the cisternal compartment. Alveolar fraction measured after 8 h of milk accumulation was, however, found to be a better indicator of sensitivity to autocrine feedback in Friesian cattle (Knight *et al*. 1994). Inasmuch as alveolar fraction did not differ between normal and LF cows at 12 h post-milking, it appeared that the effect of high residual milk in cows was not exacerbated by, and probably not a consequence of, mammary anatomy. Conversely, inefficient milking of LF cows was not compensated by more rapid efflux of milk from alveoli to cistern. Overall, therefore, mammary anatomy, and the resultant distribution of milk between alveolar and cisternal spaces, was unlikely to have contributed to lactation failure. Indeed, the higher alveolar fraction in normal cows soon after milking suggests that, other factors being equal, those cows should be at greater risk of feedback inhibition on milk secretion.

High residual milk, and by implication lactation failure, is likely to be due to impaired milk ejection. This could be due to pituitary dysfunction, to impaired oxytocin release, or to mammary insensitivity to the hormone. Animal temperament prevented reproducible measurement of systemic oxytocin by venepuncture during milking. Meaningful measurements will require catheterization of blood vessels during milking, a technique not practicable at the time of the study. Temperament itself may contribute to the poor milk ejection and lactation failure, since stress imposed by, for example, unfamiliar surroundings has been found to inhibit oxytocin release mediated by the central nervous system (Bruckmaier & Blum, 1998). If lactation failure resulting in high residual milk is the result of oxytocin deficit, its occurrence may be preventable by administration of oxytocin in early lactation (as suggested by preliminary experiments; M. Murugaiyah, unpublished results) or by alteration in husbandry. Familiarization of susceptible animals with milking routine prior to calving may alleviate subsequent stress and improve milk ejection (Bruckmaier *et al*. 1992). Prestimulation before milking may also improve both milk ejection and milking characteristics (Bruckmaier & Blum, 1996). Alternatively, hand milking, whilst impractical in production situations, is reported to improve udder evacuation and reduce residual milk accumulation (Hamann & Tolle, 1980), a benefit which probably reflects a greater induction of oxytocin release than that obtained by machine milking (Gorewit & Aromandom, 1985). Accordingly, hand milking was

found to reduce the incidence of lactation failure in Friesian crossbreeds (Edwards, 1985) and could, if applied for a short period in early lactation or intermittently through lactation, serve to clear residual milk and prolong lactation.

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