TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Mammary growth during pregnancy and lactation and its relationship with milk yield¹

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ABSTRACT: The number of secretory cells in the mammary gland is often cited as a major determinant of milk production. However, literature data for proxy measures of secretory cell number do not fully support such a claim. In particular, measurements of total mammary DNA in livestock explain only <25% of variation in milk yield, probably because of tissue heterogeneity for DNA concentration. Relative to BW, measurements of udder size in dairy cattle, as total DNA or udder weight, are approximately double those seen in most other livestock classes. Therefore, selection for dairy production, not surprisingly, has resulted in cows with greater secretory capacity. There is limited evidence that genetic selection is still increasing udder size in some cattle populations, but more recent data are needed. It is contended that the most important period of mammary growth for determination of milk yield is that occurring in pregnancy and early lactation. Mammary development is largely complete, at term, in sheep, goats, and cattle, but in pigs, the udder continues to grow during the first 3 wk of lactation, depending, in part, on litter size. Increased litter size in sheep and goats will enhance the extent of mammary

development at the end of gestation (and milk yield) by 20 to 25%. However, twinning in dairy cattle does not affect milk production and, by inference only, is not likely to affect numbers of secretory cells at term. Milking frequency and suckling intensity in very early lactation will increase milk yield in cows and increase mammary development and milk yield in sheep, indicating that even at term, the ruminant gland retains some capacity for further development, if demand requires it. There is limited understanding of the hormonal signals in pregnancy that regulate mammary development relative to the number of young carried. Furthermore, the genetic differences between dairy and beef cattle that lead to substantially greater udder size in the dairy breeds have not been identified. During lactation, the drivers for secretory cell loss in relation to milking frequency and nutritional status are still not known. Measurement of mammary development and using this phenotype in genomewide association studies to identify key genetic variants for mammary development will provide knowledge that is fundamental to understanding the quantitative regulation of milk production.

Key words: lactation, mammary development, mammary DNA, udder capacity, udder volume

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INTRODUCTION

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Most studies addressing the association of mammary size (as a proxy for cell numbers) and milk yield in livestock have used just a few animals, a variety of time points, and measurements of udder volume, weight, or capacity as estimates of udder size, with or without measurement of mammary DNA content. Correlations of milk yield with any

of these parameters were moderate in many reports (summarized in Table 1). In particular, total mammary DNA content explained only <25% of the variance in milk yield. Yet the number of secretory cells in the lactating mammary gland is often cited as being critically important to milk production (e.g., Tucker, 1969; Knight and Peaker, 1984; Boutinaud et al., 2004). Notwithstanding the above, it is important to understand drivers of the extent of mammary development (as the number of secretory cells or their proxy) and their role in the determination of the rate of milk production in both dairy and meat-producing livestock. At the very least, assessing secretory cell numbers enables the calculation of secretory cell productivity.

Dairy cattle appear to have responded to genetic selection by increasing udder size relative to BW compared with beef cattle (Linzell, 1972; Keys et al., 1989). Use of appropriate methods to measure udder size in genomewide association studies with cattle could identify genetic variants that have driven, or are still driving, mammary development, providing a fundamental understanding of key genes and pathways under selection with dairy cattle improvement.

The objective of this review is to evaluate data from various methods that have assessed udder size in pregnancy and lactation, in relation to lactation performance, and to highlight knowledge gaps. Details of the physiological, nutritional, and endocrine regulation of lactation are available elsewhere (Capuco et al., 1997; Knight and Sorensen, 2001; Hovey and Trott, 2004; Rowson et al., 2012; Capuco and Ellis, 2013; Farmer, 2013).

MEASUREMENT OF MAMMARY DEVELOPMENT IN PREGNANCY AND LACTATION

This review focuses on mammary growth in pregnancy and early lactation, a period where the majority of mammary growth occurs. In general, only a weak relationship between mammary development before the first pregnancy and milk yield has ever been reported (Elliott, 1957). However, rapid growth before puberty (Sejrsen et al., 2000) in heifers reduces mammary development and milk yield and enhancing milk intake before weaning can increase milk yield (Soberon and van Amburgh, 2013). Either or both of these mechanisms may also contribute to variation in lactation performance.

Several methods are available for estimation of udder size, including measurement of weight and/or volume, udder capacity, histomorphometric assessment, or quantification of total mammary DNA. Early work was thoroughly reviewed by Munford (1964).

Table 1. Correlation coefficients between milk yield and measures of mammary development in lactating livestock. Note that for pigs, correlations are with litter growth rate

Measurement	Correlation coefficient (<i>r</i>) with milk yield	Reference
Udder weight		
Cattle	0.45	Anderson et al. (1974)
Cattle	0.77	Tucker et al. (1973)
Goats	0.80	Linzell (1966)
Pigs	0.35	Nielsen et al. (2001)
Pigs	0.20 to 0.31	Farmer et al. (2010)
Udder volume		
Cattle	0.70	Knight and Dewhurst (1994)
Cattle	0.75 to 0.80	Davis et al. (1985)
Cattle	0.51	Magaña-Sevilla and Sandoval-Castro (2003)
Cattle	0.78	Knight (2000)
Goats	0.82	Fowler et al. (1990a)
Goats	0.84	Knight (1987)
Sheep	0.69	Davis et al. (1980)
Udder capacity		
Cattle	0.60 to 0.70	Carruthers et al. (1993)
Cattle	0.84	Davis et al. (1998)
Cattle	0.37	Matthews et al. (1949)
Udder DNA		
Cattle	0.22	Anderson et al. (1974)
Cattle	0.34	Swanson et al. (1967)
Cattle	0.34	Tucker et al. (1973)
Cattle	0.00	Knight (2000)
Pigs	0.41	Nielsen et al. (2001)
Pigs	0.49 to 0.57	Farmer et al. (2010)

Many of these methods were developed to quantify experimental induction or manipulation of mammary growth rather than to understand the role of secretory cell numbers in determining milk yield. Some of the advantages and drawbacks of the main approaches to quantifying mammary development are discussed below. Methodology to provide snapshots of rates of mammary cell proliferation and apoptosis has been discussed elsewhere (Capuco et al., 2001).

Udder Weight and DNA Content

Measurement of udder weight, postmortem, has been combined with determination of the DNA content of representative samples of tissue, enabling estimation of total cell numbers in the gland. Udders were trimmed of skin, external connective, and adipose tissue, and nucleic acid contents were determined in dry, fat-free tissue. Capuco et al. (2001) estimated that only 70 to 80% of mammary DNA represented the secretory epithelium, with this value declining with advancing lacta-

Figure 1. Relationship between milk yield and mammary weight and mammary DNA using the New Zealand data set shown in Table 1 (S.R. Davis, unpublished data). Measurements were made in early, peak, and late lactation. Correlation of milk yield with trimmed udder weight was $r = 0.79$ ($n = 19$) and $r = 0.37$ for all cows ($n = 24$). Correlation between milk yield and DNA overall was $r = 0.33$. Removing the cows with a history of mastitis ($\square$) increased the correlation to $r = 0.69$ ($n = 19$). Fitting a model nesting weight or DNA in lactation stage increased the correlation of milk yield ($\blacksquare$) with trimmed mammary weight to $r = 0.84$ ($n = 19$) and with total mammary DNA to $r = 0.78$ ($n = 19$).

tion. The proportion of secretory cell DNA may also vary depending on location within the udder. For example, it might be expected that the concentration of secretory cell DNA would be less in the cisternal regions of ruminant udders, although no published data are available. Furthermore, some secretory cells may be inactive (nonsecretory) at the time of sampling, hence complicating the interpretation of relationships between milk yield and mammary DNA (Molenaar et al., 1992).

In older, and larger, udders of cattle, mastitis may add to the spatial heterogeneity of DNA concentration (see Fig. 1), thereby reducing the accuracy of total mammary DNA measurement. In rodents, measurement of total mammary DNA can be achieved with greater reliability because of less heterogeneity in the tissue. This likely explains the strong relationship ($r = 0.85$ to 0.88) between total mammary DNA and litter weight gain observed in rats (Tucker, 1966).

Knight (2000) reported that although udder volume and peak milk yield in 9 dairy cows were highly correlated ($r = 0.79$), estimating mammary DNA concentration in a single needle biopsy and computing total DNA in the udder showed no relationship between total mammary DNA and milk yield. Potential problems in obtaining a representative sample of mammary tissue in cattle are illustrated in Fig. 1. Five outlier data points from cows with low milk production relative to udder weight and DNA content are shown. These 5 cows had a previous history of mastitis, and the data suggest that the severity of the infection was such that considerable scar tissue had been generated, leading to increased udder weight and inaccurate estimation of total mammary DNA. This highlights the need to examine animal health history prior to such slaughter studies.

Mammary weight and nucleic acid contents in pregnancy, and sometimes early lactation, have also been determined in cattle (Tucker et al., 1973; Anderson et al., 1974; Swanson and Poffenbarger, 1979), sheep (Anderson, 1975), goats (Anderson et al., 1981; Knight and Peaker, 1984), and pigs (Farmer, 2013), generally with the purpose of defining mammary growth profiles.

Udder Volume

Udder volume measurements have proven useful to assess the pattern of mammary development in livestock species suited to such measurements (cows, goats, and sheep), because repeated measurements can be made in the same animals, through time. However, the technique lacks the ability to track changes in mammary tissue composition. Nevertheless, udder volume measurements have been successfully combined with mammary biopsies for nucleic acid determination in dairy goats (Knight and Peaker, 1984) but not cows (Knight, 2000).

Various approaches have been used to assess udder volume in ruminants. Casting and water displacement methods produced estimates of udder volume in goats that were highly correlated with udder weight (Linzell, 1966). In cows, udder volume was assessed by a variety of molding methods including polyurethane (Knight and Dewhurst, 1994) and aluminum foil (Magaña-Sevilla and Sandoval-Castro, 2003). As an alternative method in cows, udder volume can be calculated from measurement of external height, length, and average width of the udder and assuming a wedge shape (Davis et al., 1983b). Udder volume measurements have been used to define mammary growth pro-

Figure 2. (a) Pattern of mammary development, as udder volume, in single- ($n = 23$) and twin-bearing ewes ($n = 19$) through late pregnancy and lactation, measured by water displacement. (b) The response of udder volume (after milk removal with oxytocin) in single-bearing ewes to fostering an additional lamb within 24 h after parturition ($n = 15$; data from Davis et al. 1980).

files during pregnancy and lactation in sheep (Table 2; Fig. 2a) and cattle (Fig. 3) in New Zealand as well as to define the relationship between udder volume and peak milk production (Table 3).

Fowler et al. (1990b) used magnetic resonance imaging to assess mammary parenchymal volume in goats (excluding fluid volume). The correlation between parenchymal volume and milk yield was high at wk 2 of lactation for 6 goats but weakened as lactation progressed (Fowler et al., 1990a).

Functional Udder Capacity

Udder capacity is related, inter alia, to the internal surface of the ruminant udder, including the alveolar and cisternal compartments. Udder capacity has been determined by fluid injection into udders obtained postmortem in cows (Matthews et al., 1949) and by fluid injection in vivo in goats (Peaker, 1980). Allowing the udder to accumulate milk until the secretion rate reached zero was used to measure (functional) udder capacity in cows (Turner, 1955; Davis and Hughson, 1988). This latter approach was particularly useful for repeated determinations of udder capacity in lactating dairy cows (Davis et al., 1998, 2000). In studies on sheep, the research emphasis has been on determining the relationship of cisternal storage capacity in relation to improvement of lactation persistency (Pulina et al., 2007).

Ultrasound Methods

Ultrasound scanning of the udder has been applied in multiple studies to monitor mammary development but mostly for assessment of parenchymal development in younger animals and determination of teat

length and cistern size and development (Olechnowicz and Jaskowski, 2009; Fasulkov, 2012). Esselburn et al. (2015) successfully measured mammary parenchymal development in heifers, before weaning, but commented that defining parenchymal boundaries would be problematic in more-developed udders. Nevertheless, Strzetelski et al. (2004) scanned udders of dairy heifers through pregnancy and showed significant correlations of the proportion of secretory tissue in 1 quarter and milk yield of 3 breeds of primiparous cows. A time series of 2-dimensional mammary scans in heifers conducted through late pregnancy was reported by Nishimura et al. (2011). Ultrasound methods have also been applied to measurement of mammary development in pregnant gilts, but gland dimensions were better assessed by simple measurement of the distance between the teat and the junction of the mammary gland with the abdomen (C. Farmer, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, personal communication).

MAMMARY SIZE AND ITS RELATIONSHIP WITH MILK YIELD

It is desirable to understand how closely, and consistently, milk yield can be predicted from a particular methodology for udder size. Data examining the correlation between milk yield and udder size measurements are shown in Table 1. For udder volume, with 1 exception, the correlation coefficients at peak lactation fall in the 0.69 to 0.84 range. However, in dairy cows, the correlation with milk yield declined as lactation progressed (Davis et al., 1983b), presumably because secretory cell numbers varied independently in relation to external udder dimensions. Furthermore, correlations between milk yield and udder weight were relatively

Table 2. The effect of hemimastectomy of ewes at d 90 of pregnancy on udder volume, milk production, and lamb growth. Data from Davis et al. (1983a). Values in parentheses in the main table are SEM

Group	Ewe BW, kg	Pregnancy udder volume, mL				Lactation udder volume, mL			Peak milk yield, mL/d	Final milk yield, mL/d	Lamb growth rate, g/d
		Day of pregnancy				Week of lactation					
		116	123	130	138	1	3	10	3	10	1–10
Hemimastectomy (n = 8)	60.5 (2.6)	366 (36)	358 (59)	439 (83)	555 (86)	1,124 (97)	1,144 (67)	771 (77)	1,948 (198)	791 (105)	223 (16)
Sham-operated (n = 9)	61.6 (2.5)	354 (22)	464 (56)	804 (80)	931 (79)	1,568 (108)	1,251 (53)	952 (41)	2,269 (153)	1,027 (85)	252 (13)
Control (n = 9)	57.8 (2.3)	337 (28)	440 (29)	588 (62)	710 (44)	1,318 (64)	1,280 (59)	904 (58)	2,270 (121)	941 (135)	247 (9)
Hemimastectomy as % of sham		103	77	55	60	72	91	81	86	84	88

low in some studies (Table 1), possibly because of a lack of mammary tissue uniformity in the test animals.

Milk yield and total mammary DNA were poorly correlated in lactating dairy cattle (Table 1) as were total mammary DNA and growth rate in pigs. The data sets are very limited and likely encompass some, if not all, of the errors previously outlined. The similar decline in milk yield and mammary DNA after peak lactation (Table 4) in cows does point to a much closer relationship between mammary DNA and production than has been described. Regressing milk yield on total mammary DNA for the data from New Zealand dairy cattle shown in Fig. 1 (excluding cows with a history of mastitis) generated a correlation of $r = 0.69$. However, fitting DNA nested in lactation stage improved the correlation ($r = 0.78$, $n = 19$), presumably through accounting for variation associated with a 35% decline in mammary DNA concentration in mammary tissue between early and late lactation (Table 4).

Mammary DNA content in livestock close to peak lactation is summarized in Table 3. United States dairy cattle have the largest udders and most total mammary

DNA at peak lactation, in fact, around 50% more DNA than measured in the smaller, New Zealand dairy cows. Dairy cows had a greater milk yield per kilogram BW and, for mature cows, approximately double the mammary DNA per kilogram BW than pigs, sheep, beef cattle, and goats. In a direct comparison of contemporaries, after 49 d of lactation, total mammary DNA in Holstein heifers was 4-fold greater than that seen in Hereford cattle (Keys et al., 1989).

Early measurements of udder capacity involved fluid filling of udders postmortem. This work with 473 udders from a mix of dairy breeds demonstrated that capacity increased with age but decreased with advancing lactation (Matthews et al., 1949). The data showed a low correlation of udder capacity with milk yield ($r = 0.37$). Udder capacity in cattle and goats measured in vivo was highly correlated with empty udder volume (Peaker, 1980; Davis and Hughson, 1988) and was also moderately correlated ($r = 0.6$ to 0.84) with milk yield (Table 1). Udder capacity declined as lactation progressed (Turner, 1955; Eichler

Table 3. Comparison of milk yield and udder size measures taken close to peak lactation in livestock

Type	BW, kg	Trimmed mammary weight, g	Mammary DNA, g	Peak milk yield, kg/d	Udder weight, g/kg ^{0.75}	Udder DNA, g/kg ^{0.75}	Milk yield/kg udder	Reference
U.S. Holstein	628	24,200	68.9	47.3	194	0.55	1.95	Capuco et al. (2001)
New Zealand Friesian	459	12,700	45.9	22.0	128	0.47	1.73	See Table 4
Dairy heifers	530	16,350	37.8	20.4	149	0.34	1.25	Keys et al. (1989)
Dairy heifers	440	9,310	26.1	16.1 ¹	97	0.27	1.72	Valentine et al. (1987)
Beef	450	4,900	19.0	3.5	50	0.19	0.71	Keys et al. (1989)
Goats	60	1,363 ²	5.78	3.8	64	0.27	2.79	Knight and Peaker (1984)
Goats	50	1,061	3.5	NA ³	56	0.18	NA	Anderson et al. (1981)
Sheep	47	911	2.27	NA	51	0.13	NA	Anderson (1975)
Pig	186	3,327	9.4	NA	66	0.19	NA	Farmer et al. (2010)

¹Average milk yield in the first 10 wk of lactation

²Estimated as 72% of udder volume from trimmed udder weight/total udder weight data published by Anderson et al. (1981).

³NA = not available.

Table 4. Differences in udder size and milk yield between U.S. Holstein and New Zealand Friesian dairy cattle in early, peak, and late lactation. Data for U.S. Holstein are from Capuco et al. (2001). New Zealand data are from S.R. Davis (unpublished data) for multiparous Friesian cows grazing ryegrass/white clover pastures. Values in parentheses in the main table are SEM for New Zealand data and, for U.S. data, the pooled SE, where available

Measurement	Stage of lactation					
	Early		Peak		Late	
	U.S. Holstein (n = 4)	New Zealand Friesian (n = 5)	U.S. Holstein (n = 5)	New Zealand Friesian (n = 6)	U.S. Holstein (n = 5)	New Zealand Friesian (n = 8)
Days in milk	14	17	90	80	240	253
BW, kg	727 (37)	402 (13)	628	459 (13)	706	440 (16)
Udder parenchyma, kg	27.3 (1.7)	10.9 (0.4)	24.2	12.7 (0.3)	23.1	8.7 (0.4)
Total mammary DNA, g	96.4 (6.7)	47.0 (2.3)	68.9	45.9 (2.0)	58.1	24.3 (2.0)
DNA/kg parenchyma	3.53	4.34 (0.25)	2.85	3.62 (0.10)	2.52	2.77 (0.14)
DNA, g/kg BW ^{0.75}	0.70	0.52 (0.02)	0.55	0.47 (0.01)	0.42	0.25 (0.02)
Udder, g/kg BW ^{0.75}	195	121 (2)	194	128 (4)	169	91 (5)
Milk yield, kg/d	39.9 (2.1)	16.4 (1.3)	47.3	22.0 (1.2)	36.5	12.0 (0.8)
Milk yield, kg/d per g mammary DNA	0.42 (0.06)	0.35 (0.03)	0.67	0.48 (0.03)	0.66	0.50 (0.03)

and McFadden, 1996; Davis et al., 2000), reflecting the loss of secretory tissue.

PATTERNS OF MAMMARY DEVELOPMENT IN LIVESTOCK

The pattern of mammary growth is important to understanding how, and when, mammary growth is regulated in relation to meeting the demands or potential demands of the offspring. Anderson and coworkers examined udders, postmortem, in cattle (Anderson et al., 1974), sheep (Anderson, 1975), and goats (Anderson et al. (1981) and ultimately were able to compare the mammary growth trajectories in pregnancy in 8 different species (Sheffield and Anderson, 1985), including laboratory species and livestock (but not pigs). Growth was best defined by exponential equations for both DNA and dry, fat-free tissue. Across the species, DNA doubling time was estimated at 87, 36, and 63 d in cows, goats, and sheep, respectively, and 7.8 and 6.7 d for rats and mice, respectively. These values were broadly in proportion to gestation length. Overall, there was approximately a 9-fold increase in total mammary DNA over gestation (Sheffield and Anderson, 1985).

In sheep (Rattray et al., 1974), goats (Hayden et al., 1979), and possibly pigs (Kensinger et al., 1986), the extent of mammary growth at parturition was increased with increasing numbers of young carried during pregnancy. In cattle, twinning does not appear to be associated with greater milk yield (Syrstad, 1977; Patel et al., 1996), but no data are available to suggest whether twinning in cattle enhances bovine mammary development by the start of lactation. Patterns of mammary growth in individual livestock species are briefly discussed below.

Pigs

In sows, overt development of the 6 to 7 pairs of mammary glands occurs in the latter part of pregnancy, and it is in this period that the most marked accumulation of DNA occurs (Hacker and Hill, 1972; Kensinger et al., 1982; Nielsen et al., 2001). Histological appearance and DNA concentration in mammary tissue indicate maximum differentiation of secretory cells by d 90 of pregnancy. Substantial accumulation of secretion occurs between d 90 and 115 (term) of pregnancy in sows (Kensinger et al., 1982). Kensinger et al. (1986) suggested that mammary growth to the end of pregnancy in pigs was related to the number of young carried. There was a weak curvilinear relationship between mammary development at d 110 of pregnancy and number of young in sows (range 4 to 11), where mammary development was maximal with a litter size of 7 to 8.

Mammary weight and DNA content in sows and gilts almost doubled through to d 28 of lactation (Kim et al., 1999). The increase in weight represented both hypertrophy and hyperplasia of the secretory cells (Kim et al., 1999). Of further interest was the observation that the extent of mammary development during early lactation in sows was associated with parity. The extent of the increase in wet weight was greatest in parity 1 (70%), declining to 20 and 30% in parities 2 and 4, respectively (Beyer et al., 1994).

Genetic differences in mammogenesis among breeds have been noted, with less mammary development in Upton–Meishan gilts than in Large White gilts at d 110 of pregnancy (Farmer and Sorensen, 2001). Furthermore, nutrition during pregnancy will also affect mammary development, at least in gilts. A number of studies reviewed by Farmer (2013) indicated

that excessive energy intake during pregnancy (and resulting excess fat) impaired mammary development whereas varying protein intake was without effects on mammary development. Mammary growth during lactation was positively associated with both energy and protein intake (Farmer, 2013).

Goats

Linzell (1966) profiled the pattern of udder volume growth in goats through pregnancy and lactation. There was an exponential increase in volume through pregnancy and a gradual decline in empty udder volume in lactation. As with the pig, overt mammary development occurred only in the last third of pregnancy. However, in contrast to pigs, no further development (accretion of empty mammary volume) occurred after parturition.

Others have suggested that there is an increase in mammary DNA in goats after kidding. Knight and Peaker (1984) provided some evidence that total mammary DNA continued to accumulate for the first 3 wk of lactation. This finding corroborated an observation made by (Anderson et al., 1981) of a small increase in goat mammary DNA immediately postpartum in a study with approximately 40 mixed-breed dairy goats. The total amount of mammary DNA at peak lactation in Saanen dairy goats was about 50% greater than that observed in sheep (Knight and Peaker, 1984; Table 3), probably reflecting the dairy breed used.

Cattle

Data on mammary growth during pregnancy in dairy and beef cattle are few. Measurement of mammary weight and DNA content was undertaken in heifers at various stages of pregnancy (Anderson et al., 1974; Swanson and Poffenbarger, 1979), although the results were variable. Nevertheless, a large increase in udder weight and udder DNA was demonstrated by the end of pregnancy. Akers et al. (1981) observed a doubling in total mammary DNA in dairy cattle in the period -10 to +10 d postpartum (from approximately 25 to 50 g), indicative of a final wave of cell division through this period.

In New Zealand Jersey cattle of high genetic merit for milk production, udder volume was significantly greater at parturition compared with low-genetic-merit contemporaries. This difference in udder volume was associated with a similar percentage difference in milk production (Fig. 3) relative to low-genetic-merit Jerseys (Davis et al., 1983b, 1985) and persisted through lactation. In contrast, in New Zealand Holstein-Friesian cattle, productivity of the udder (milk yield per unit udder mass) was increased in high-, relative to low-, genetic-merit animals, but there was no difference in ud-

der size (Davis et al., 1985). In Scotland, high-genetic-merit dairy cattle produced 1.3 times more milk from 1.3 times more mammary tissue relative to low-genetic-merit cattle (Knight, 2000). Therefore, it appears that in some dairy populations, ongoing selection for milk yield was achieved by increasing mammary development.

Capuco et al. (2001) showed that mammary DNA in Holstein cattle at the start (14 d) of lactation was at a maximum and declined throughout lactation (Table 4). Similar data were obtained with New Zealand cattle (Table 4). There are no data that directly support any further growth in secretory cell numbers after the start of lactation in dairy cattle, apart from evidence of increased cell proliferation with increased milking frequency in the first 2 wk after calving provided by Hale et al. (2003) and discussed below.

Sheep

The pattern of mammary growth during pregnancy in sheep assessed by serial measurements of udder volume is shown in Fig. 2a. The growth profile was very similar to that observed with goats by Linzell (1966), with growth ceasing at or close to parturition. Furthermore, mammary growth rate and ultimate size was enhanced in twin-bearing ewes (see below). Anderson (1975), from a slaughter study, observed that no more than 2% of mammary growth occurred during lactation in sheep.

MODULATION OF UDDER GROWTH IN PREGNANCY AND LACTATION

The majority of overt mammary growth occurs in the last third of pregnancy. Species differ in the amount of mammary growth that occurs in early lactation (most evident in pigs) and their ability to increase the extent of mammary growth in pregnancy in relation to the number of young carried (sheep and goats, in particular).

There is a quantitative signal for pregnancy mammary development in sheep and goats that increases mammary development in relation to litter size. In twin-bearing ewes, a size differential for the udder was generated in the last 3 wk of pregnancy, with udders being 20 to 30% larger at term relative to single-bearing ewes, thereby providing greater milk yield potential for 2 suckling lambs (Rattray et al., 1974; Davis et al., 1980; Fig. 2a). The extent of mammary growth (and milk yield) was greater through to peak lactation in dairy (machine-milked) goats if multiple young were carried in the previous pregnancy (Hayden et al., 1979) compared with a single young. How the presence of multiple fetuses signals the mammary gland to enhance growth has yet to be established, although speculation that placental lactogen from the concep-

Figure 3. Pattern of change in udder volume in high-genetic-merit (◆—◆; $n = 24$) and low-genetic-merit (■---■; $n = 17$) Jersey cattle through late pregnancy and lactation. Data are from Davis et al. (1983b). The dotted line shows the accelerated loss of udder volume in a group of low-genetic-merit cows that had a high level of residual milk, which caused a low and rapid regression of milk yield, prematurely curtailing lactation.

tus is the primary signal has often been made for both sheep (Mellor, 1987) and goats (Hayden et al., 1979; Anderson et al., 1981). This differential growth signal may be absent in dairy cattle, where twinning does not result in increased milk yield (Syrstad, 1977; Patel et al., 1996), but measurements of mammary size at calving are needed.

Placental lactogen was, and is, a strong candidate as a regulator of mammary development during pregnancy in ruminant species. However, mammary growth regulation must differ in the pig, which does not appear to express placental lactogen (Forsyth, 1986). Prolactin may assume the mammary growth role in pregnant sows (Farmer, 2013). Placental lactogen treatment increased mammary growth in a steroid-primed heifer model and also appeared to stimulate feed intake (Byatt et al., 1994, 1997). Genetic variants in the placental lactogen gene were associated with milk production in a study with Holstein cattle (Zhang et al., 2009), but a definitive role for placental lactogen in mammary development in cattle has not been described (Takahashi, 2006).

In pigs, inhibition of prolactin release by treatment of sows with the dopamine agonist bromocriptine (Farmer et al., 2000; Farmer and Petitclerc, 2003) was effective in reducing mammary growth (by 42.5%) in a defined window from d 90 to 109 of gestation. Bromocriptine treatment earlier in pregnancy was without effect. Relaxin also appears to play a key

role in mammary development in pigs (reviewed in Farmer, 2013), but evidence is lacking in ruminants. In pregnant goats, bromocriptine treatment from 60 to 120 d reduced, but did not abolish, mammary growth (Buttle et al., 1979). This result was used to support a role for placental lactogen in lobuloalveolar development, especially as blood plasma concentrations of placental lactogen were substantially greater in polytocous animals. Overall, the outline of evidence given above indicates that there are significant differences among livestock species in the endocrine regulation of the extent of mammary growth in pregnancy. A comprehensive discussion of the endocrine regulation of mammary development is given in Rowson et al. (2012). Interactions between the conceptus and maternal tissues were reviewed by Thatcher et al. (1980).

Increased growth of the gland with multiple young carried in pregnancy implies that the size of the fat pad is not a primary constraint to mammary development (Hovey et al., 1999) in goats and sheep. The compensatory growth response to hemimastectomy discussed below also supports this contention.

Hemimastectomy

Experiments have been undertaken in sheep and goats to examine the effect of removal of a single

mammary gland on subsequent milk production of the remaining gland.

Goats hemimastectomized in wk 5 or 8 of lactation ($n = 9$) usually showed a compensatory increase in milk yield of the remaining gland, although the response varied from zero in 1 goat, to a 50% increase (Knight, 1987). Gland volume increased, on average, by 25%, after hemimastectomy. Furthermore, mammary DNA was better maintained in hemimastectomized goats through the remainder of lactation, relative to control animals.

In sheep, hemimastectomy was performed before pregnancy as well as on d 45 and 90 of pregnancy (Davis et al., 1983a), and a period of compensatory growth of the udder was observed to occur around parturition. Results were similar irrespective of the timing of the hemimastectomy. Data from animals operated on d 90 are shown in Table 2. Size of the mammary gland in hemimastectomized animals remained close to half the size of sham-operated udders until the time of lambing. From d 137 of pregnancy to 3 d after lambing, there was a period of catch-up growth. Overall, mammary size in hemimastectomized ewes, measured as volume (Table 2) or total mammary DNA (not shown), was >70% of that seen in sham-operated controls by 5 d postpartum and milk yield and lamb growth rates were >80% of control glands (Table 2). Therefore, hemimastectomy resulted in a partial compensation of mammary growth in the peripartum period. The mechanism driving this response is unknown.

Increasing Litter Size or Milking Frequency

In an experiment parallel to the hemimastectomy study, the milk production response to fostering an extra lamb at birth was also tested. When an extra lamb was successfully adopted by a single-bearing ewe, there was a period of mammogenesis through the first 14 d of lactation (Fig. 2b), which was associated with an increase in milk production to a rate similar to that seen in twin-bearing ewes (Davis et al., 1980). Milk yield and lamb growth rates in the foster groups were similar to those seen in twin-bearing ewes, indicating a remarkable ability of the ovine udder to adapt milk output to increased suckling demand.

Many studies have examined the milk yield response to increased milking frequency in early lactation in dairy cattle. A summary was provided by Wall and McFadden (2008). The increased milk yield was often sustained beyond the period of frequent milking (e.g., Bar-Peled et al., 1995), and this carryover response may be indicative of an increase in mammary cell numbers. In support of this, Hale et al. (2003) provided evidence that there was an increase in cell proliferation (measured by enhanced ^3H -thymidine

incorporation into mammary slices in vitro) on d 7 of lactation in response to increased milking frequency.

MAMMARY SECRETORY CELLS AND LACTATION PERSISTENCY

Knight and Peaker (1984) proposed a plausible scheme defining changes in secretory cell productivity and cell number that defined the lactation curve of goats. This scheme was broadly corroborated by Capuco et al. (2003) in dairy cattle. In the goat study, up to peak lactation, there appeared to be an increase in cell number (DNA) as well as an increase in per cell productivity. However, this conclusion was based on 2 independent slaughter groups with small numbers of goats (6 vs. 9) and has yet to be replicated. After peak lactation, milk yield declined in parallel with cell loss (measured as DNA loss) from the udder (Knight and Peaker, 1984).

The main drivers of secretory cell loss are nutrition (e.g., energy balance) and milk removal efficiency. Accelerated loss in udder volume was observed in Jersey cows known to have impaired milk let down (Fig. 3). Accelerated udder regression was also observed with reduced milking frequency (Carruthers et al., 1993).

With regard to nutritional influences on mammary regression, the data shown in Table 4 are relevant. Udder and milk yield data from separate studies with grazing, New Zealand Holstein-Friesian cows and U.S. Holstein cattle on total mixed ration (TMR) diets are compared for a single lactation. A notable difference between the 2 data sets is that the New Zealand cows lost 21.6 g mammary DNA (47%) whereas the U.S. cows lost only 10.8 g DNA (16%) from peak to late lactation (approximately 160 d). The percentage loss of mammary DNA after peak lactation was 0.7%/wk in the TMR-fed cows and 1.9%/wk in the grazing cows, rates of loss that were closely associated with the rates of decline in milk yield. These data corroborate a direct comparison of TMR and pasture diets of Friesian cattle in New Zealand whereby the effects of TMR were to promote retention of udder capacity and increase lactation persistency relative to cows whose diets consisted solely of grazed pasture (Davis et al., 2000). Therefore, reduced dietary energy intake not only effects an immediate reduction in milk production but also increases cell loss from the udder and reduces lactation persistency.

Hormonal changes are possible drivers of milk yield in relation to feed intake. In particular, GH treatment has been associated with mammary cell hypertrophy and improved lactation persistency in both cows (Bauman et al., 1999) and goats (Knight et al., 1990). Nevertheless, the nutrition-related mechanisms that produce an

increased rate of secretory cell loss during lactation and reduced lactation persistency still need to be understood.

MAPPING OF BOVINE GENETIC VARIANTS ASSOCIATED WITH MAMMARY MORPHOLOGY, MILK YIELD, AND BW

Pausch et al. (2016) reported on a meta-analysis of genomewide association studies for udder morphology traits in Fleckvieh cattle. Among the traits were measurements that relate to udder size, such as udder depth and fore udder length. Such udder dimensions in cattle are highly repeatable across lactations, moderately heritable, and correlated with milk yield (Petersen et al., 1985). Several candidate genes for mammary morphology traits across 12 QTL were identified, including *ADAMI2* and *SP5*, both of which have putative roles in pathways associated with mammary development. Furthermore, in a *Bos indicus* × *Bos taurus* pedigree, BTA5 appeared to contain several QTL related to udder conformation (Tolleson et al., 2017).

Genetic improvement was associated with increased udder size in New Zealand Jersey cows (Davis et al., 1983b), Friesian cows (Knight and Sorensen, 2001), and U.S. Holstein cows (Petersen et al., 1985). Whether ongoing genetic improvement of dairy cattle is through increased udder growth needs to be determined. Among causative genes and variants that map to the larger QTL for milk yield in dairy cattle, none are obvious candidates for influencing milk production through effects on mammary growth. These genes include *DGAT1* (Grisart et al., 2004), *AGPAT6* (Littlejohn et al., 2014), *ABCG2* (Cohen-Zinder et al., 2005), and *MGST1* (Littlejohn et al., 2016). Many genes of smaller effect on yield and composition were recently described and summarized (Nayeri and Stothard, 2016). It may be that genetic improvement in dairy cattle in many current populations is being mediated largely through increased productivity of the udder and not mammary growth per se.

Milk yield and mammary weight were closely related to metabolic body size in analyses across multiple species undertaken by Linzell (1972) and Anderson (1976). Genes that can drive an increase in BW might be expected to result in larger udders and increased milk yield. However, genetic selection for milk yield is likely to be only weakly associated with increased body size, at least within breeds. Moderate ($r_g = 0.35$) positive genetic correlations between BW and milk yield at midlactation were reported by Manzanilla-Pech et al. (2014). Nevertheless, Hansen (2000) reported an ongoing increase in live weight of U.S. Holstein cows in association with selection for milk yield.

Gene variants associated with BW in cattle have been identified close to *PLAG1* (BTA14; Karim et al., 2011), *CYP19A1* (BTA10; Mao et al., 2016) and the *NCAPG-LCORL* region on BTA6 through genomewide association tests in Holstein breeds and cross-breeds (Mao et al., 2016) and in some beef breeds (Saatchi et al., 2014). It is notable that there is little reported commonality among these genes for both milk yield and BW traits, although *PLAG1* may be an exception (Fink et al., 2017). Genes that increase milk yield relative to BW are desirable in the context of feed conversion efficiency through increased nutrient partitioning to milk (Bauman et al., 1985). Hence, gene variants that can enhance mammary development relative to BW are relevant and need to be identified.

Divergence of dairy and beef cattle types has occurred in association with enhanced secretory cell numbers in dairy breeds (Table 3), presumably driven by selection of relevant genetic variants driving mammary growth. Determination of udder size in a genotyped dairy and beef pedigree would facilitate identification of the genes and variants associated with udder size, providing understanding of the genetic regulation of a trait that is fundamental for dairy performance.

Milk yield is constrained in early lactation when the cow is in negative energy balance. This raises the question as to whether it is desirable to achieve increase udder size and function in early lactation or will that make cows more susceptible to metabolic disease. Certainly, potential animal health benefits are an argument to place more focus on lactation persistency in selection programs. This will provide lactation capability when the cow is better able to consume sufficient feed to meet the metabolic demands of the udder.

CONCLUSIONS

Existing methods for assessing mammary development in large animals have been applied successfully to understand the relationships among udder size, udder productivity, and milk yield. The poor correlations between milk yield and total mammary DNA content that have been obtained in livestock could probably be improved through more representative sampling of mammary tissue for DNA estimation. Two studies confirm that the mammary gland of dairy cows is far larger in terms of mass and total DNA content, relative to BW, than that seen in other livestock species. More data are needed to answer the question as to whether genetic selection for milk production continues to increase udder size or the mechanism underlying genetic improvement is an increase in mammary productivity. Furthermore, the reviewed data raise multiple questions as to how ultimate udder size is regulated.

For example, we do not know the nature of the quantitative signal in polytocous animals that increases the rate of udder growth in late pregnancy and ultimate size of the udder. Certainly, it does not appear that the size of the mammary fat pad limits the extent of mammary growth, at least in sheep, given the growth response seen in the remaining gland after hemimastectomy. Furthermore, there is a lack of understanding of the mechanisms through which nutrition and milking frequency impact secretory cell survival and proliferation during lactation. Addressing the knowledge gaps outlined above will provide a better understanding of the regulation of milk production and enable improvements in efficiency.

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