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UNIVERSIDAD AUTÓNOMA DE BARCELONA
DEPARTMENT DE CIENCIA ANIMAL I DELS ALIMENTS

Modifying the Lactation Curve in Dairy Goats: Effects of Milking Frequency, Dry Period, and Kidding Interval

Modificación de la Curva de Lactación en Cabras Lecheras: Efectos de la Frecuencia de Ordeño, el Periodo de Secado y el Intervalo entre Partos

TESIS DOCTORAL

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Tesis Doctoral presentada por Ahmed A. K. Salama, dirigida por los Drs. Gerardo Caja y Xavier Such del Departament de Ciència Animal i dels Aliments de la Universitat Autonoma de Barcelona, para obtener el grado de Doctor.

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LIST OF ABBREVIATIONS

1X, 2X, ..., 6X	1-, 2-, ..., 6-times daily milking
AIF	apoptosis-inducing factor
BrdU	Bromodeoxyuridine
bST	bovine somatotropin
CI	calving interval
CLA	conjugated linoleic acid
CN	casein
D0, D27, D56	0-, 27-, 56-d dry off period
ECM	extracellular matrix
EGF	epidermal growth factor
FA	fatty acids
FIL	feedback inhibitor of lactation
FGF	fibroblast growth factor
GH	growth hormone
Ig	immunoglobulin
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IMI	intramammary infection
K12 and K24	12- and 24-mo kidding intervals
KGF	Keratinocyte growth factor
LIF	leukemia inhibitory factor
MBF	mammary blood flow
MDGI	mammary-derived growth inhibitor
MEC	mammary epithelial cells
MF	milking frequency
OT	oxytocin
PBS	phosphate-buffered saline
PCD	Programmed cell death
PCNA	proliferating cell nuclear antigen
PRL	prolactin
SCC	Somatic cell count
SG	specific gravity

TGF	transforming growth factor
TJ	tight junction
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling

ABSTRACT

Seventy-nine Murciano-Granadina dairy goats were used in 4 experiments to study the effects of milking frequency, omitting dry period, and kidding interval on lactational performance and udder compartments. In Exp. 1, 32 goats were milked once- (1X, n=17) or twice-daily (2X, n=15). Once-daily milking reduced milk yield by 18% compared to 2X, with the reduction being greater in early lactation. Goats of < 4 parities lost more milk during 1X than older goats. Milk of 1X goats contained higher percentages of total solids, fat and casein than milk of 2X goats, but milk protein percentage and SCC did not differ. In Exp. 2, udder compartments (cisternal and alveolar) and cisternal recoil at different milking intervals (8, 16, and 24h) were studied in 14 of the goats used in experiment 1 and milked 1X (n=7) or 2X (n=7). Alveolar milk increased from 8 to 16 h after milking, but did not change thereafter. Cisternal area measured by ultrasonography and cisternal milk increased linearly ($R^2 = 0.96$ to 0.99) up to 24 h, indicating continuous milk storage in the cistern at any alveoli filling degree. Despite extended milking intervals, cisterns of 1X goats did not become larger than 2X goats. Primiparous goats had smaller cisternal areas and less cisternal milk than multiparous goats. No changes in cisternal area were observed after oxytocin injection, indicating the absence of cisternal recoil in goats. In Exp. 3, 17 pregnant goats were assigned to 2 groups: goats that were dried off 56 d before expected kidding (D56; n=9), and goats without dry off (D0; n=8). Five goats in the D0 group dried off spontaneously at d 27 before kidding (D27). The D0 kids had lower birth weight than D27 and D56. Colostrum of D0 goats contained less IgG than D27 and D56 goats. In the subsequent lactation, D0 goats produced less milk than D27 and D56 goats, with no differences between D27 and D56. Apoptosis and proliferation indices increased during involution in D56 goats. At wk 7 of the subsequent lactation, mammary cell turnover did not vary between groups. In Exp. 4, 30 goats were divided to kid annually (K12; n=16) or biennially (K24; n=14). Over 92 wk, K12 goats had 2 lactations from wk 1 to 42 and from wk 51 to 92, whereas K24 goats had 1 extended lactation from wk 1 to 92. Pregnancy reduced milk yield in K12 goats from wk 10 of pregnancy. From wk 51 to 79, K12 goats produced 32% more milk than K24 goats, but their milk contained lower fat and protein than K24 goats. No changes were detected for milk lactose and SCC from wk 51 to 79. From wk 80 to 92, milk yield and milk composition did not vary between groups. Over the 92 wk, K12 (1192L) and K24 (1093L) produced similar total milk yield. At wk 39, milk fat of K12 goats contained greater conjugated $C_{18:2}$ fatty acid and desaturase index than K24 goats. At wk 55, milk of K12 goats contained higher $C_{18:2}$ and $C_{18:3}$, and lower $C_{16:0}$ fatty

acids, resulting in a lower atherogenicity index compared to K24. Cisternal milk at wk 39 was lower for K12 than K24 goats, whereas alveolar milk did not differ. Fat content was greater for alveolar milk than cisternal milk for K12 goats at wk 55 and for K24 goats at wk 39 and 55. No differences in milk protein or lactose were detected between cisternal and alveolar milk. In conclusion, Murciano-Granadina dairy goats used in this work are adapted to once daily milking, need the dry period and can do extended lactations.

RESUMEN

Se utilizaron un total de 79 cabras Murciano-Granadinas en 4 experimentos destinados a estudiar los efectos de la frecuencia de ordeño, la omisión del secado y el intervalo entre partos sobre la producción de leche y su reparto en los compartimentos de la ubre. En el Exp.1, 32 cabras se ordeñaron una (1X, n = 17) o dos veces diarias (2X). Un ordeño diario redujo la producción de leche un 18% respecto a 2X, siendo la reducción mayor al principio de lactación. Las cabras de < 4 partos perdieron mas leche que las > 4 partos en 1X. La leche de las cabras 1X contuvo mas sólidos totales, grasa y caseína que la leche de las cabras 2X, aunque el porcentaje de proteína y el RCS no varió. En el Exp. 2, se estudiaron los compartimentos de ubre (cisternal y alveolar) y el retorno de leche hacia los alvéolos a diferentes intervalos entre ordeños (8, 16 y 24 h) en 14 cabras utilizadas en el Exp. 1 (1X, n = 7; 2X, n = 7). La leche alveolar aumentó hasta las16 h, sin observarse cambios entre las 16 y las 24 h. La área cisternal (medida por ecografía) y la leche cisternal aumentaron linealmente (R^2 = 0.96 a 0.99) hasta las 24 h, indicando que el almacenamiento de leche en la cisterna es de tipo continuo, independientemente del grado de llenado del los alvéolos. A pesar del intervalo extendido entre ordeños, las cisternas de las cabras 1X no fueron mas grandes que las cisternas de las 2X. Las cabras primíparas tuvieron menor área cisternal y menor leche cisternal que las cabras multíparas. No se observaron cambios en el área cisternal después de una inyección de oxitocina, indicando la ausencia del fenómeno de retorno elástico de leche de la cisterna a los alvéolos. En el Exp. 3, 17 cabras gestantes se dividieron en 2 grupos: cabras que se secaron 56 d antes de la fecha esperada del parto (D56; n = 9), y cabras sin secado (D0; n = 8). Cinco cabras del grupo D0 se secaron espontáneamente 27 d antes del parto (D27). Los cabritos de D0 nacieron con menos peso que los cabritos de D27 y D56. El calostro de las cabras D0 contuvo menos IgG que el de las cabras D27 y D56. En la lactación siguiente, las cabras D0 produjeron menos leche que las cabras D27 y D56, sin diferencias entre estas últimas. Los índices de apoptosis y proliferación del tejido mamario aumentaron en las cabras D56 durante la involución de la ubre. A la semana 7 de la siguiente lactación, no se observaron diferencias entre grupos en la dinámica celular mamaria. En el Exp. 4, 30 cabras se sometieron a distinto ritmo reproductivo para parir anualmente (K12; n = 16) o bianualmente (K24; n = 16). Durante las 92 semanas experimentales, las cabras K12 tuvieron 2 lactaciones (semanas 1 a 42 y 51 a 92), mientras las cabras K24 siguieron una sola lactación extendida de la semana 1 a 92. La gestación redujo la producción de leche en las cabras K12 a partir de la semana 10 de gestación. Entre las semanas 51 y 79, las cabras K12 produjeron 32% mas leche que las

cabras K24, aunque su leche contuvo menos grasa y proteína que las cabras K24. No se detectaron diferencias entre grupos en la lactosa y RCS de leche desde la semana 51 a la 79. Entre las semanas 80 y 92, la cantidad y composición de leche no varió entre grupos. Durante las 92 semanas experimentales, las cabras K12 (1.192 L) y K24 (1.093 L) produjeron cantidades similares de leche. En la semana 39, la grasa de la leche de K12 presentó un mayor contenido en ácido linoleico conjugado (CLA) con un mayor índice de desaturación respecto que las cabras K24. En la semana 55, la leche de K12 contuvo mas $C_{18:2}$ y $C_{18:3}$, y menos $C_{16:0}$, resultando en un menor índice de aterogenicidad que la de K24. La leche cisternal de las cabras K12 fue menor que la de las cabras K24 a la semana 39, mientras la leche alveolar no varió. La leche alveolar de las cabras K12 presentó un mayor contenido en grasa que en la leche cisternal en la semana 55, lo que también se observó en las K24 en las semana 39 y 55. El contenido en proteína y lactosa no varió entre leche cisternal y alveolar. En conclusión, las cabras Murciano-Granadinas utilizadas en esta Tesis demostraron una buena adaptación a 1X, la necesidad del secado y la posibilidad de realizar lactaciones extendidas.

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CHAPTER 1: INTRODUCTION

CHAPTER 1

INTRODUCTION

Economically, there are two unprofitable periods in the life of a dairy animal: the period from birth to first parturition, and the dry period. Milk yield and the shape of the lactation curve are determined by the number of mammary secretory cells and the secretory activity per cell. During lactation, the mammary gland undergoes different phases of secretory cell proliferation (hyperplasia) and differentiation (hypertrophy). Traditionally, research has concentrated on factors that maximize milk yield at the peak of lactation in the belief that this would increase economic efficiency. However, factors that affect maintenance of milk secretion during the declining phase of lactation (persistency) have received relatively little attention despite their impact on milk yield. Persistency is a heritable trait, is greater in primiparous than multiparous animals, is improved by increasing milking frequency and growth hormone injection, and is reduced by poor nutrition and concurrent pregnancy. Preventing or even reversing factors that decrease lactation persistency should be an important goal of the modern dairy industry. If persistency of lactation could be increased, the dry period would be eliminated or at least shortened and lactation would be extended. With extended lactation, the preparturient and early lactation periods with their increased health risks and associated costs would be shorter. The elimination of the dry period increases profitability when milk yield in the subsequent lactation does not suffer significant losses. Little is known about the effects of dry period length and extended kidding intervals on milk yield and quality in dairy goats.

Machine milking is an efficient process when animals with high milkability are used, because this means that the maximum amount of milk can be obtained in short time with the minimum of manual intervention. Milkability is affected by many factors such as: genetic potential, udder and teat morphology, and milk storage characteristics within the udder. From the economic point of view, machine milking is more costly and therefore it is desirable to reduce milking frequency without negative effects on milk secretion. The magnitude of these negative effect again depend on the internal structure of the udder and therefore on the milk storage sites. Therefore, the study of the internal udder structure and milk distribution within the udder is a key point in determining milkability and the adequate daily milking frequency.

The objectives of this thesis were therefore to study the effects of daily milking frequency on milk yield, composition and lactation persistency in dairy goats. The partitioning of milk within the udder during different intervals between milking was also evaluated. Milk yield, colostrum quality, and mammary cell turnover (proliferation and apoptosis) were studied in dairy goats that did not have a dry period between lactations. Finally, the impact of pregnancy on lactation persistency and milk quality was investigated in goats that kidded annually compared with goats that kidded biannually.

CHAPTER 2: LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

1. Udder Anatomy

The udder is a gland originated from the ectoderm and mesoderm layers, located in the inguinal region and attached to the ventral body wall. The goat udder consists of two separate halves with a single gland in each half which is drained by a single teat. Blood vessels, the lymph and nerves enter and leave the udder through the inguinal canal.

1.1. Suspensory System

The goat udder is supported by a suspensory apparatus made up of medial and lateral laminae which form a saclike support for the right and left halves of the udder. The medial suspensory ligament is compromised of strong sheets of elastic tissue which attach to the pelvic arch. Strong support from this ligament is required during peak lactation to prevent the formation of a pendulous udder type (Haenlein and Caccese, 1992). The lateral laminae are immediately lateral to the external pudic vessels near the superficial inguinal ring (Figure 1), they support the lateral part of the udder by sending collagenous septa deep into the gland, and they are suspended caudally from the symphysial tendon and cranially from the tunica flava abdominis (Figure 1).
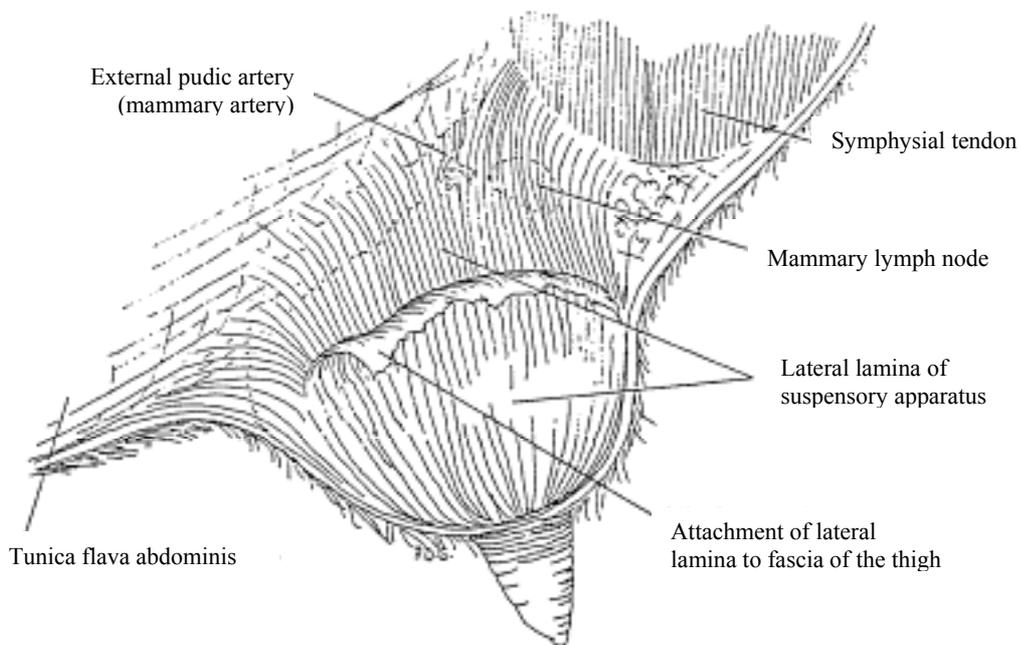
Figure 1. Left lateral view of the suspensory apparatus of the goat udder (Garrett, 1988).

1.2. Blood Supply

Blood circulation in the goat udder is shown in Figures 2 and 3. The udder is supplied with blood by the external iliac artery. The external pudic artery, originated from the external iliac artery, is the only blood supply to the goat udder (Garrett, 1988). In cows, the ventral perineal artery joins the pudic artery to help supply the udder. This anastomosis seldom, if ever, develops in goats (Garrett, 1988). Haenlein and Caccese (1992) reported a relationship of 400 volumes of blood for each volume of milk in dairy goats.
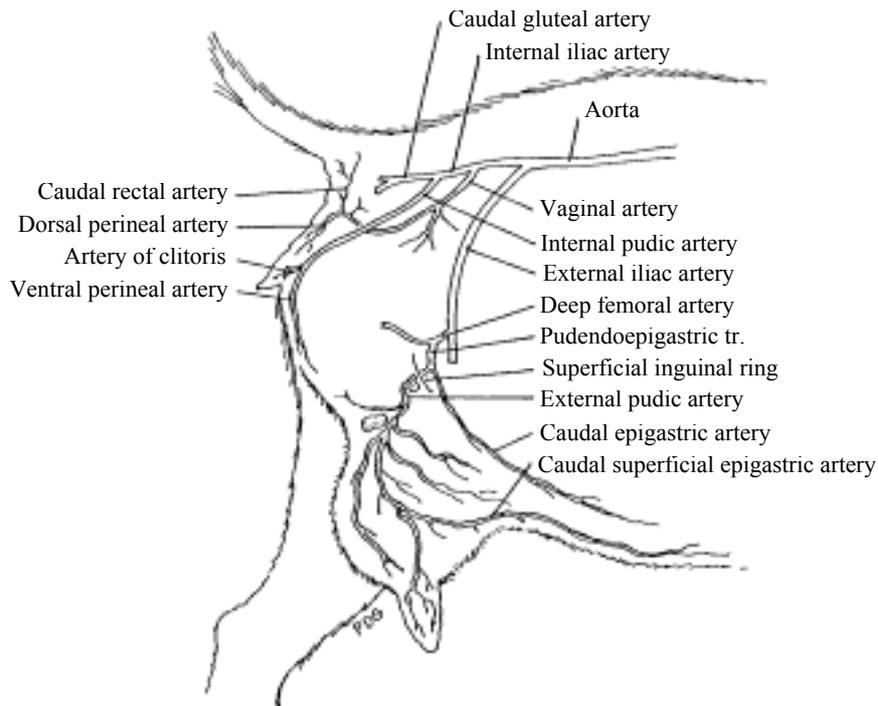
Figure 2. Right-side view of the blood supply to the udder of the goat (Garrett, 1988).
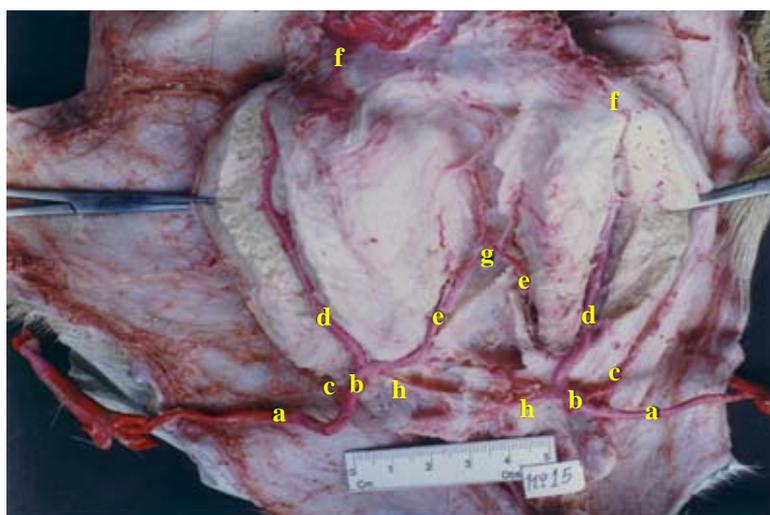
Figure 3. Dorsal view of the goat udder. (a) External pudic artery (b) Mammary artery (c) Superficial mammary branch. (d) Cranial mammary artery (e) Medial mammary artery (f) Caudal superficial epigastric artery (g) Anastomosis of medial mammary arteries (h) Ventral labial artery (Luiz and Miglino, 2000).

The venous system is more pronounced and evident than the arterial system, and blood vessels that can be seen on the udder or abdomen are veins, not arteries (Haenlein and Caccese, 1992). The ventral perineal artery drains blood from the perineal region toward the udder, where it anastomoses with the external pudic vein. Blood can leave the udder by way of the external pudic vein or by the subcutaneous abdominal vein (milk vein) cranial to the udder. The milk vein is formed during the first pregnancy by the anastomosis of the cranial and caudal superficial epigastric veins.

1.3. Nerve Supply

The genitofemoral nerve (3rd – 4th lumbar pair) supplies all of the interior of the udder and most of the skin except for a small area in the cranial and caudal parts of the udder (Figure 4). These small areas are supplied cranially by the lumbar cutaneous nerve branches and caudally by the mammary branch of the pudic nerve (Figure 4).

The udder is innervated by 2 types of nerves: sensory fibers (afferent) in teats and skin, and sympathetic fibers (efferent). The milk ejection reflex during milking and suckling is a neuro-endocrine reflex (Crowley and Armstrong, 1992). This reflex has an afferent pathway (conducted from the teats to the brain via neurons) and an efferent pathway (conducted from the pituitary to the mammary gland via blood-borne hormones). Mechanical stimulation of the teats activates pressure sensitive receptors where the pressure is transformed into nerve impulses that travel to the hypothalamus resulting in the release of oxytocin (**OT**) from the pituitary to the blood. The OT then travels to the mammary gland, binds to oxytocin receptors on the myoepithelial cells, causing the myoepithelial cells to contract, resulting in the ejection of milk from the alveolar lumen.
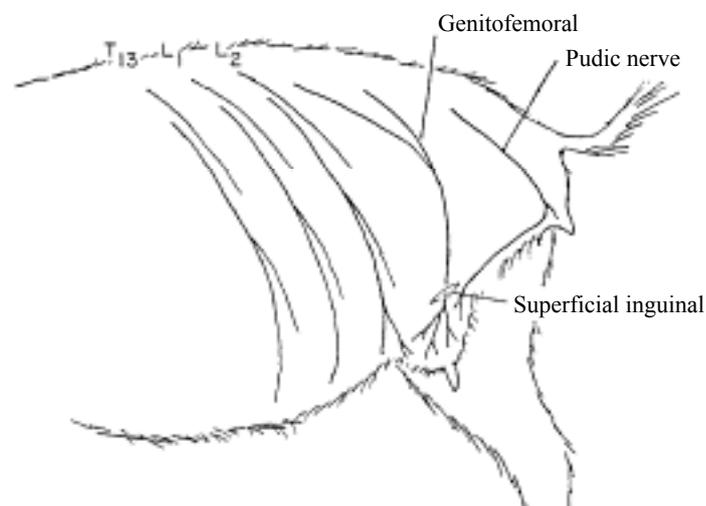
Figure 4. Diagram of a left-side view of the nerves supplying the goat udder (Garrett, 1988).

2. Udder Compartments

In an adult animal, the mammary gland grows during pregnancy (mammogenesis), then cells differentiate to start milk secretion before parturition (lactogenesis), milk secretion is maintained during lactation (galactopoiesis), and finally the mammary gland regresses after drying off (involution). During lactation, milk is synthesized within the alveolar compartment and its amount depends on the activity and number of the mammary epithelial cells (**MEC**). A system of intra-lobular and inter-lobar ducts allows for milk transport from the alveoli lumen to the gland cistern. Cisternal compartment serves as a storage space for milk during the period between sucklings and/or milkings.

2.1. Activity of the Alveolar Cells

Activity of MEC can be studied at the transcriptional level by determining the concentration of RNA (Boutinaud et al., 2003) or mRNA for some mammary key enzymes such as Acetyl-coA carboxylase, Fatty acid synthetase and Galactosyl transferase in biopsy samples using complementary DNA probes (Wilde et al., 1990; Travers and Barber, 1993; Bryson et al., 1993). Also, MEC activity has often been measured directly at the post-transcriptional level by determining the synthesis rates of lactose, casein (**CN**) and total protein in short-term mammary explant culture (Wilde et al., 1986).

Mammary cell differentiation in cows, goats and rats (Figure 5: a, b and c) increased markedly between parturition and lactation peak and continued after peaking during later lactation in non pregnant animals (Wilde et al., 1986; Wilde and Knight, 1989; Capuco et al., 2001). Wilde et al. (1986) studied the activities of the mammary key enzymes in dairy goats at different stages of lactation and indicated that the degree of cellular differentiation at wk 33 of lactation (when milk yield was on average 52% of its peak value) was greater than at wk 7 of lactation (when milk yield was at its peak). Since the activity of mammary cells did not decrease as lactation advanced in non pregnant animals, the main reason for milk decline during late lactation appears to be reduced MEC number. However, during late lactation, when animals were concomitantly lactating and pregnant, the secretory activity per cell also declined due to conflicting metabolic demands of gestation and lactation (Capuco et al., 2003).

Compensatory mammary growth in response to milk stasis in the opposite udder half was accompanied by an increase in the cell activity in beef cattle (Capuco and Akers, 1990) and dairy goats (Henderson and Peaker, 1983).

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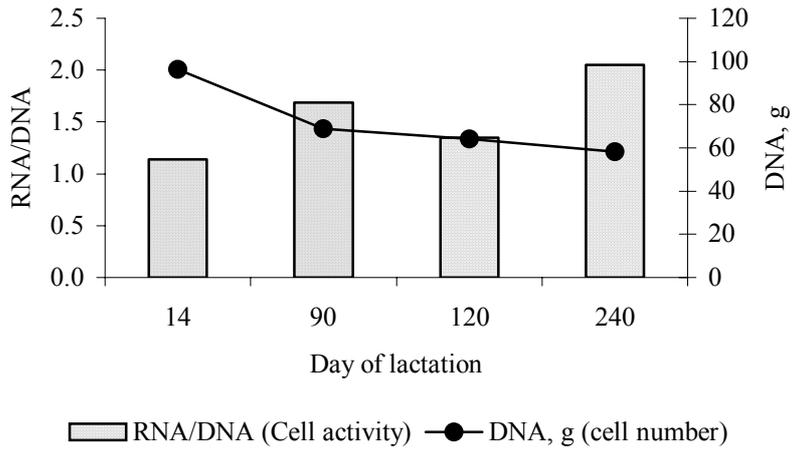
Figure 5a. Changes in mammary cell number and activity in the udder of multiparous non pregnant dairy cows (Capuco et al., 2001).
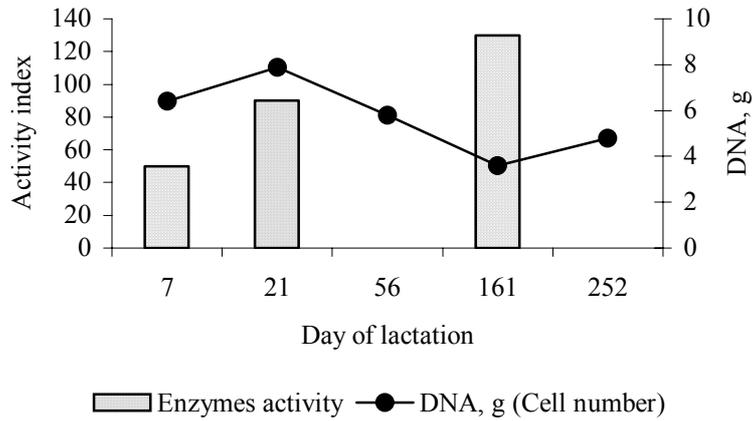
Figure 5b. Mammary cell growth and differentiation in goats udder (Knight and Peaker, 1984; Knight and Wilde, 1987,1993).
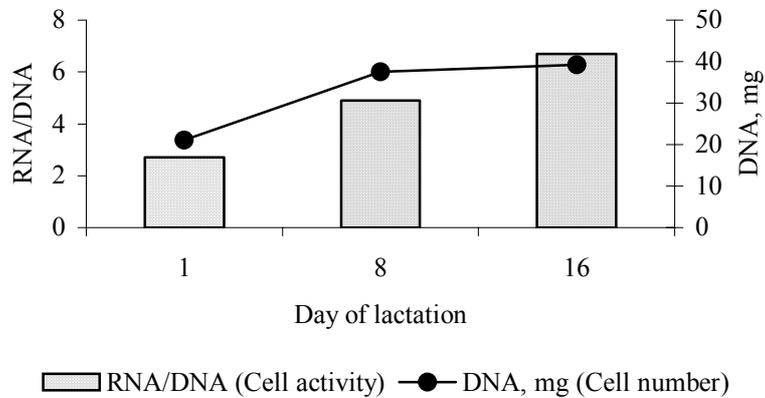
Figure 5c. Mammary cell number and activity during lactation in rats (Paape and Tucker, 1969).

2.2. Number of the Alveolar Cells

Cell number in the mammary gland is the function of the rates of cell proliferation and cell death. The mammary gland grows when the rate of proliferation exceeds the rate of death, and it regresses when the rate of death exceeds the rate of cell proliferation (Capuco et al., 2001).

Udder size, as an index of mammary growth, correlated positively with milk yield in goats (Linzell, 1966) and dairy cows (Dewhurst et al., 1993). Magaña-Sevilla and Sandoval-Castro (2003) developed a simple, quick, and precise technique to measure udder volume in dairy cows using aluminum foil. Computed tomography (Soresen et al., 1987) and magnetic resonance imaging (Fowler et al., 1990) were used to estimate proportions of secretory and non-secretory tissue within the udder. The DNA content per mammary cell nucleus is constant during pregnancy and lactation (Tucker, 1987). Thus, total DNA content was also used to indicate MEC population in many studies and a close correlation between milk yield and the amount of secretory tissue has been reported in ruminants (Linzell, 1966; Tucker et al., 1973; Capuco and Akers, 1990; Baldi et al., 2002; Boutinaud et al., 2003).

2.2.1. Mammary Cell Proliferation

Cells proliferate in response to specific extracellular factors, which include growth stimulatory and growth inhibitory cytokines, the extracellular matrix (ECM) with which the cell makes contact, and the type and degree of proximity of neighboring cells (Oshima and Campisi, 1991).

Cells exist in a viable, non-proliferating (quiescent) growth state or in a proliferating growth state (Figure 6). Quiescent cells may remain metabolically active for long periods of time and may be in a reversible growth arrested state or irreversible differentiated state. Proliferating cells may continuously divide (i.e. the epithelial stem cells of the intestinal crypts) or may undergo cell division only in response to a specific stimulus (i.e. mammary cell proliferation in response to specific hormonal signals).

Whether proliferation is continuous or induced in a quiescent cell, it proceeds via 4 sequential temporally and biochemically separate phases:

- Mitosis (M).
- Pre-synthetic gap phase (G_1).
- Synthetic DNA phase (S).
- Second gap phase (G_2) that allows cells to prepare for M.
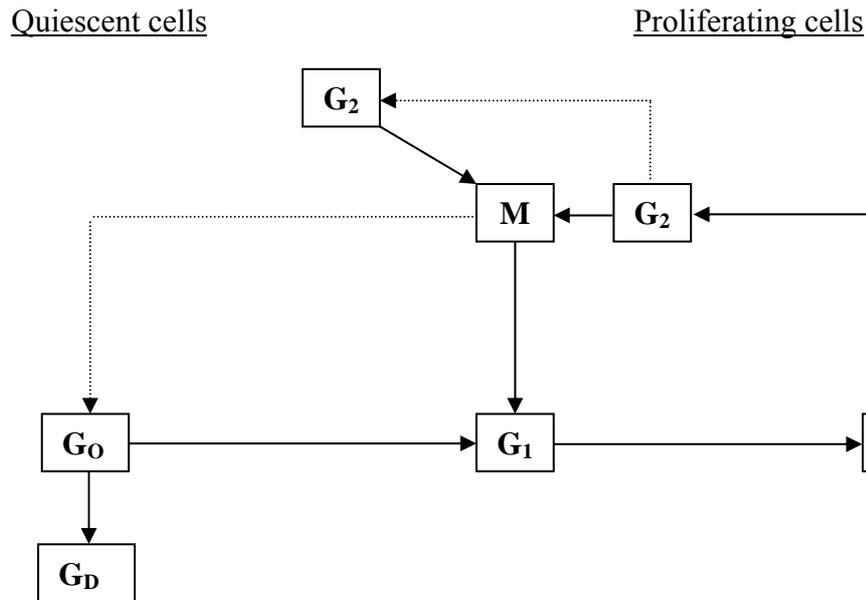
Figure 6. Organization of the cell cycle (Oshima and Campisi, 1991). G = Gap phase; M = Mitosis; S = DNA synthetic phase; D = Terminally differentiated cell.

At the M phase, the replicated genome is segregated into two nuclei, the nuclei and cytoplasmic contents of the cell are physically partitioned, and the cell splits into 2 cells. The newly divided cells may prepare for another cell cycle (G_1 phase) or enter a non-proliferating quiescent state (G_O). Cells in G_O state may re-enter the cycle or may, as terminally differentiated cells, have growth arrested irreversibly (G_D). However, Franke and Keenan (1979) reported that fully differentiated MEC (i.e. after lactation has started) can divide, which may explain the increasing cell number during early lactation in goats (Knight and Peaker, 1984) and when daily milking frequency (**MF**) is increased or growth hormone (**GH**) is injected (Boutinaud et al., 2003). The length of the cell cycle in bovine MEC was determined as 19 to 21 h, with G_1, S, and G_2 + M phases of 6 to 7, 7 to 9, and 5 to 6 h, respectively (Zavizion et al., 1998).

Proliferation rate differed according to cell type in the mammary gland. Different types of cells present in the mammary gland are shown in Figure 7. Capuco and Akers (1990) reported that 81% of proliferating cells are epithelial and 19% are fibroblast in beef cattle. Epithelial and fibroblast cells presented 85 and 12%, respectively of proliferating cells in the mammary gland of dairy cows in late lactation (Capuco et al., 1997).

Mammary cell number in goats increased by 23% between wk 1 and 3 (Knight and Peaker, 1984; Figure 5b) and by 51% between d1 and d15 of lactation (Anderson and Wahab, 1990). Moreover, Carretero et al. (1999) studied the alveolar development in dairy

ewes using epoxy resin casts and detected an alveolar growth between wk 1 and 5 of lactation. Nevertheless, no net mammary growth was observed during early lactation in cows (Figure 5a) or in meat sheep (Anderson, 1975) because cell death was greater than proliferation (Capuco et al., 2001; Colitti et al., 2004a).
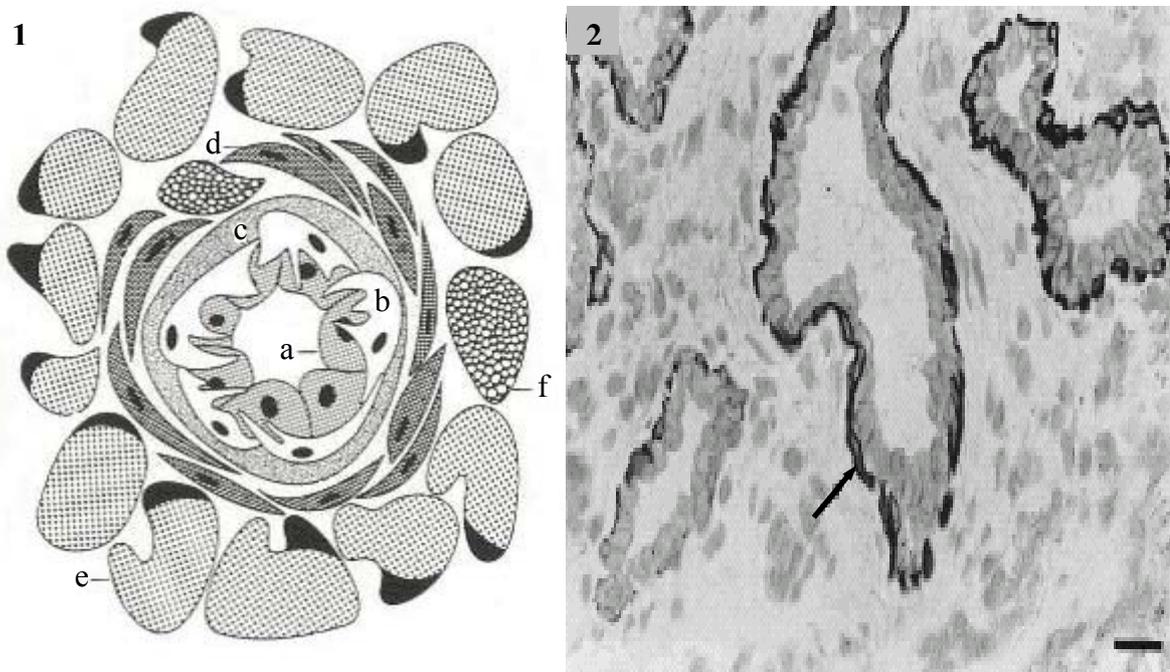
Figure 7. [1] Representation of mammary cell types. The epithelial compartment contains epithelial cells (a) surrounded by myoepithelial cells (b) and separated from the stromal compartment by a basement membrane (c). The stromal compartment consists of the fibroblasts (d) and adipocytes (e). Mast cells (f) are also present (Haslam, 1988). [2] Immuno-histochemical labelling of myoepithelial cells. Arrow indicates myoepithelial cell surrounding the epithelial cells (Hellmen and Isaksson, 1997).

The increase in milk yield until lactation peak (from wk 3 to wk 8 in goats, and from wk 2 to wk 13 in cows) appeared to be due to increased synthetic capacity of the MEC (hypertrophy), rather than to an increase in number of secretory cells (Capuco et al., 2001). The progressive decline in milk yield after peaking was associated with a decrease in the total DNA content of the mammary parenchyma, representing a net fall in cell number (Knight and Peaker, 1984; Anderson and Wahab, 1990; Fowler et al., 1990; Knight and Wilde, 1993; Capuco et al., 2001).

The apparent lack of mammary growth after wk 3 of lactation in goats and throughout lactation in cows does not preclude the mammary gland's ability to grow in response to different management conditions such as increasing MF or GH injection. The existence of undifferentiated potential stem cells capable of generating other differentiated cells in the mammary gland has been reported in mice (Smith and Medina, 1988), dairy goats (Li et al.,

1999b) and cows (Capuco et al., 2003). It has been shown by transplanting portions of developing or lactating mouse mammary gland that the gland is capable of fully regenerating itself (Smith and Medina, 1988). Similarly, a mammary gland can be entirely regenerated from a single cell transplanted in adipose mammary gland tissue (Kordon and Smith, 1998). Capuco and Akers (1990) reported that compensatory proliferation in mammary parenchyma occurred in response to cessation of milk removal from the opposite udder half in beef cattle. Moreover, Knight (1987) showed that MEC number increased during compensatory growth in hemimastectomized dairy goats. After hemimastectomy, milk yield and total mammary DNA of the single remaining gland increased in a compensatory fashion.

Mammary gland in goats, but not cows, can grow during early lactation. In rodents, mammary proliferation occurs during early lactation (Figure 5c). This growth during early lactation is important; for instance, gestational mammary development can be reduced experimentally in mice with no deleterious effects on subsequent milk production (Knight and Wilde, 1987). In the same manner, this may occur in goats. So, if mammary development is affected during pregnancy in goats (i.e. omitting drying off or very short dry period), would mammary growth be compensated during the subsequent lactation?.

2.2.2. Mammary Cell Death

Any cell can be killed by a wide range of pathological stimuli, such as certain toxins or viruses. These murdered cells die by a process known as necrosis. In contrast, programmed cell death (**PCD**) involves the stereotypic loss of individual cells at specific times during development (Schwartz et al., 1993). Unlike necrosis, which is a passive process, PCD is induced by a physiological stimuli (Figure 8), like hormones and growth factors. Programmed cell death requires a gene or set of genes that would code for protein (or proteins) having a destructive effect in the same cells harboring the gene(s).

2.2.2.1. Mammary Cell Death by Apoptosis

The terminology "apoptosis" (from the Greek: the dropping of leaves from a tree) describes a type of PCD that plays an important role in early development and growth of normal adult tissues. Out of 10^{14} cells in the human body, about 10^{7} are known to undergo apoptosis daily (Melino et al., 2001). According to the later authors, apoptosis has been among the fastest growing fields in the last decade as reflected by the number of publications that has increased from fewer than 1000 in 1990 to over 13,000 in 2000.

Moreover, there are now specialized journals on apoptosis such as Apoptosis and Cell Death and Differentiation.
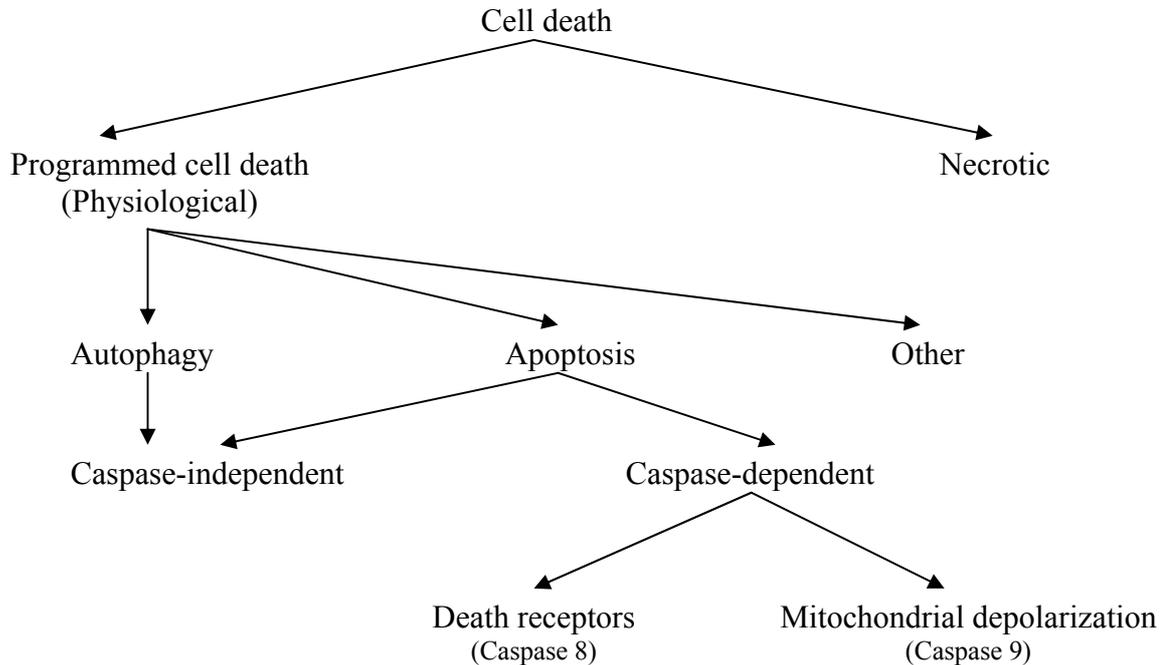
Figure 8. Categories of the cell death. Deaths are programmed (physiological) or not (necrosis). See below for the explanation of some types of programmed cell death. Adapted from Lockshin and Zakeri (2004).

Cell death by apoptosis differs fundamentally from necrotic cell death (Figure 9). Firstly, apoptosis is a response to physiological stimuli, whereas necrotic cell death is mainly the result of tissue injury (Kerr, 1971). Secondly, the cellular mechanism of apoptosis involves an orderly sequence of events that involves the compaction of chromatin into crescent-shaped areas abutting the nuclear envelope, condensation of the cytoplasm and subsequently, the vesiculation of genomic DNA in so-called apoptotic bodies (Kerr et al., 1972).

One of the principal criteria by which apoptosis is identified is the internucleosomal cleavage of DNA to generate fragments of 180 base pairs and multiples thereof (DNA laddering), which can be detected as DNA ladders when chromatin of apoptotic cells is subjected to agarose gel electrophoresis (Wyllie et al., 1980). In addition, the detection of apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (**TUNEL**) is based on the tagging of theses DNA fragments.

In contrast to necrosis, cell death by apoptosis takes place in the absence of an immune response. Apoptotic bodies are rapidly taken up and degraded by adjacent cells or

are engulfed by neighboring macrophages (Figure 9). Moreover, anti-inflammatory cytokines including transforming growth factor-β (**TGF-β**) are released preventing an influx of neutrophils and the consequent tissue damage and inflammation. Lastly, dying by apoptosis requires energy in the form of ATP, whereas necrosis results from ATP depletion to a level incompatible with cell survival (Edinger and Thompson, 2004).
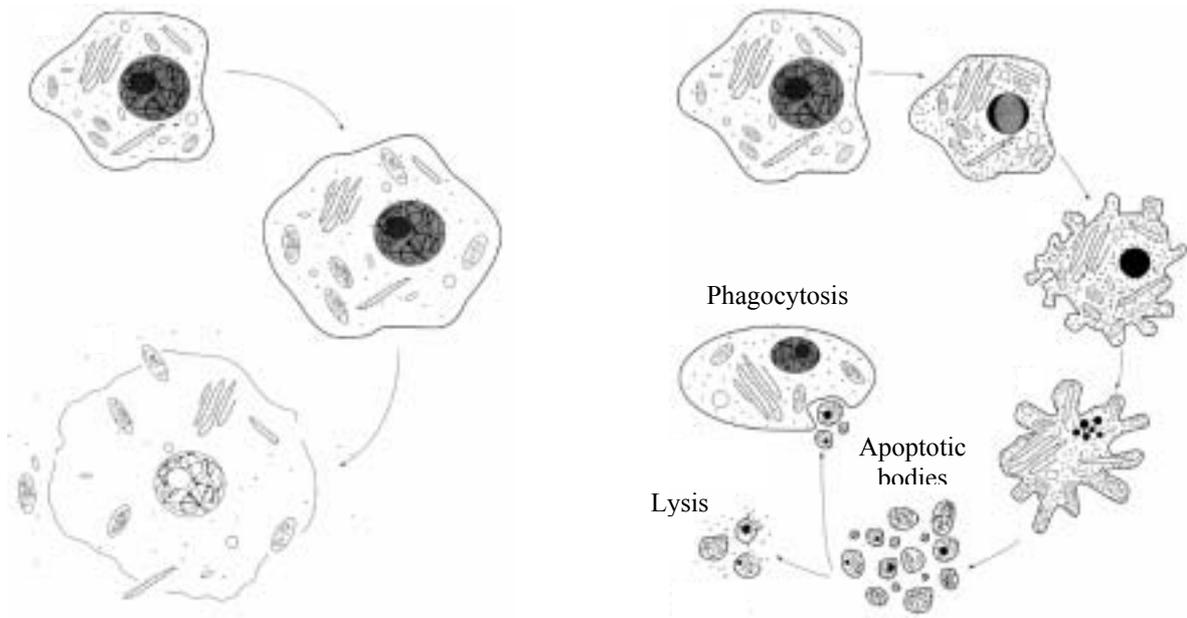
Figure 9. Cell death by necrosis (left) and apoptosis (right). In necrosis a normal cell swells before losing its membrane integrity and spills its contents into the surrounding area. In apoptosis normal cell shrinks and the condensed chromatin collapses into crescents around the nuclear envelope. The membrane then begins to bulge and bleb, while the nucleus ultimately collapses. The blebbing increases and the cell finally breaks apart into a number of apoptotic bodies, which lyse in vitro and are phagocytosed in vivo (Mastrangelo and Betenbaugh, 1998).

2.2.2.1.1. Mechanism of Apoptosis

In apoptosis triggered by many types of stimuli, mitochondria (the source of cell energy) represent the central control point of apoptosis (Figure 10). The release of "cytochrome *c*" from mitochondria leads to the activation of caspases that execute apoptosis (Desagher and Martinou, 2000). Cytochrome *c* is a small heme protein found loosely associated with the inner membrane of the mitochondrion. It is a soluble protein and is an essential component of the electron transfer chain. Caspases are a family of aspartate specific cysteine proteases that are believed to be major effectors of apoptosis. Twelve mammalian caspases have been identified, and each one may play a more- or less-significant role in apoptosis depending on the cell type and the stimuli that induced the cascade of apoptosis (Mastrangelo and Betenbaugh, 1998; Roy, 2000).

Once cytochrome *c* is released towards the cytosol, it binds to the apoptosis-activating factor-1 (Apaf-1). In the presence of ATP or dATP, this complex recruits and activates procaspase 9 to generate a functional apoptosome (Figure 10). Activated caspase 9 can, in turn, activate other caspases that are in charge of the apoptosis execution (Desagher and Martinou, 2000).
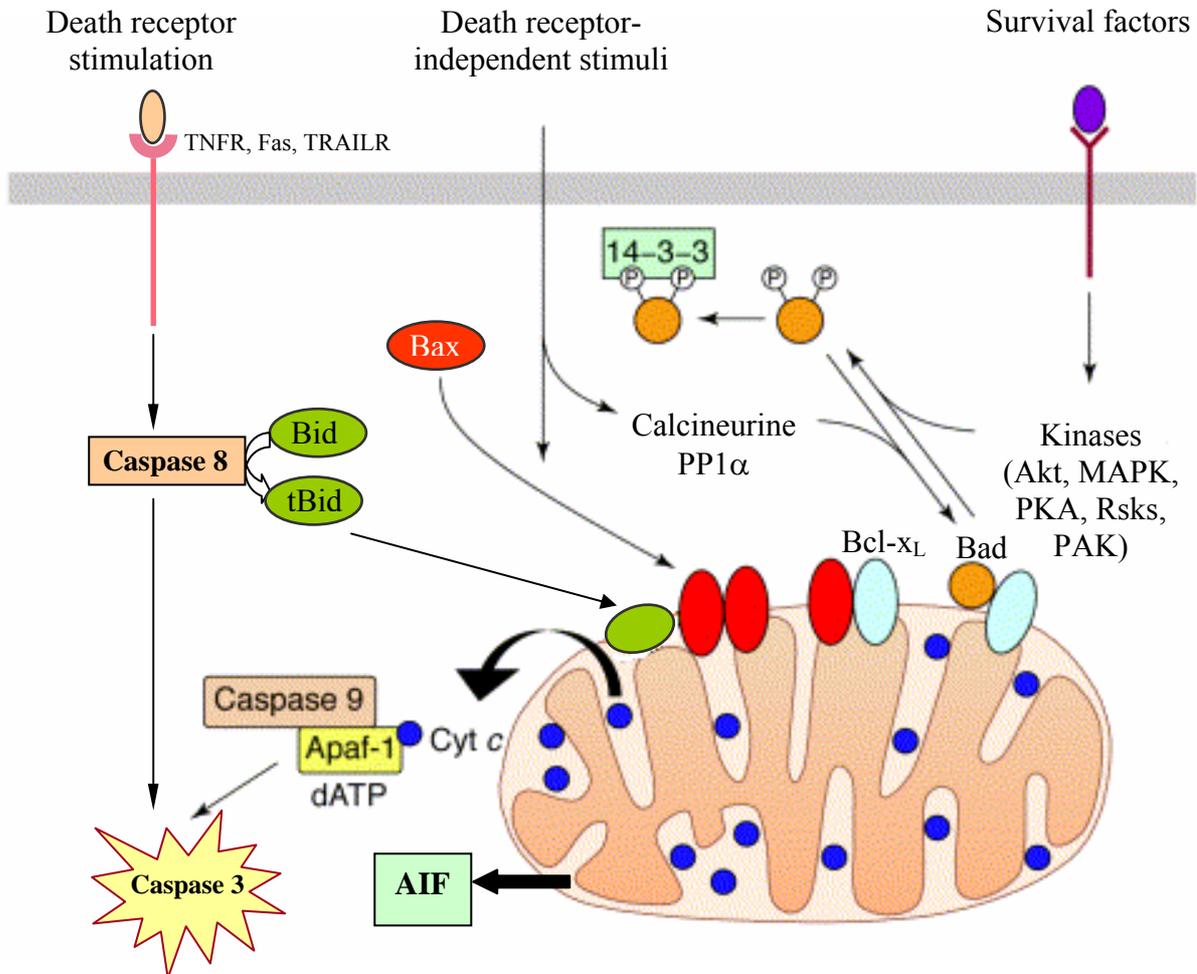
Figure 10. There are 2 major pathways for caspase-dependent apoptosis. 1) Binding of death ligands to their receptors lead to a signaling cascade that activate caspase 8 and the translocation of Bid to mitochondria where it activates Bax protein resulting in cytochrome *c* (cyt *c*) releasing. 2) Death receptor-independent stimuli and growth factor deprivation trigger apoptosis by inducing translocation of Bax or Bad to mitochondria resulting in cyt *c* releasing. Once in the cytosol, cytochrome *c* activates caspase 9 by binding to Apaf-1 and dATP (Desagher and Martinou, 2000; Porter, 1999).

In addition to cytochrome *c*, another protein normally located in the mitochondrial intermembrane space, known as apoptosis-inducing factor (**AIF**), is transported to the cytosol and the nucleus during apoptosis causing DNA condensation and fragmentation (Susin et al., 2000). The release of AIF occurs before that of cytochrome *c* and before

caspase activation; therefore, death induced by AIF is caspase-independent (Daugas et al., 2000).

The release of cytochrome c is controlled by the Bcl-2 proteins family. More than 15 Bcl-2 proteins have been identified in mammals (Adams and Cory, 1998). Some of these genes, including Bcl-2, Bcl-x_L, Bcl-w, Mcl-1 and A1, act to prevent cell death (antiapoptotic), while others, such as Bcl-x_s, Bax, Bid, Bad, Bak, Bik, Bim and BNip promote apoptosis (Porter, 1999; Zhang et al., 2003).

Among the various Bcl-2 proteins identified to date, Bcl-2, Bcl-x_L and Bax represent the most well characterized members (Hou et al., 2003). Upon the induction of apoptosis, Bax is believed to undergo a conformational change, leading to the exposure of its hydrophobic segment to enable its translocation from the cytosol to mitochondria. Bax subsequently oligomerizes and forms pores on mitochondrial outer membrane surface to cause the release of cytochrome c (Figure 10). Bcl-2 and Bcl-x_L antagonize the pro-apoptotic activities of Bax through the inhibition of Bax translocation from the cytosol to mitochondria. In addition, Bcl-x_L may inhibit the association of Apaf-1 with procaspase-9 and thereby prevent caspase-9 activation (Adams and Cory, 1998).

Mammary apoptosis in mice is associated with increasing Bax levels, down-regulation of Bcl-w, cytochrome c release and processing of numerous caspases, including caspase-1, -3, -7, -8, and -9 (Heermeier et al., 1996; Metcalfe et al., 1999; Marti et al., 2000, 2001). The overexpression of Bcl-2 inhibited mammary apoptosis in mice (Marti et al., 2001). Moreover, the ratio of Bcl-x_s (apoptotic) versus Bcl-x_L (antiapoptotic) increased during the first 2 d of involution (Heermeier et al., 1996), coinciding with the highest activity of caspases (Marti et al., 2000).

Levels of Bcl-2 were greater than levels of Bax in early lactating dairy heifers favoring the cell survival (Colitti et al., 2004a). As lactation advanced (from d 45 to 60), ratio of apoptotic to proliferating cells increased, Bax to Bcl-2 ratio increased, and AIF was expressed on d 60 of lactation (Colitti et al., 2004a). After 10 d of drying off in dairy cows, AIF is transported from the intermembrane space of mitochondria to the cytosol and the nucleus causing DNA fragmentation in those cells undergoing apoptosis (Colitti et al., 2004b). In goats the expression of Bax and caspase 3 was lowest in early lactation, increased in late lactation and was elevated during drying off (Wareski et al., 2001). Bax protein was detected for 8 d after weaning in sheep and was highest on the 8th d (Colitti et al., 1999).

2.2.2.1.2. Clearance of Apoptotic Cells in the Mammary Gland

Apoptotic cells have been detected in milk during normal lactation and may represent a normal mechanism for reducing MEC during gradual involution (Monks et al., 2002). The apoptotic cells are shed into the alveolar lumen by a process that does not compromise the integrity of the epithelium. Additionally, unknown signals from a dying cell to its neighbors cause them to extrude it from the monolayer by an actin- and myosin-dependent mechanism that maintains the integrity of the tight junctions (**TJ**). If these cells are not removed by milking, they age into the late apoptotic cells seen in the alveolar lumen during early involution. The removal of these cells occurs in a phagocytic process by macrophages. Thus, apoptotic cells during mammary involution of non pregnant ewes were phagocytozed by intraepithelial macrophages and alveolar epithelial cells, and occasional apoptotic epithelial cells were observed in the alveolar and duct lumina (Tatarczuch et al., 1997). Also, highly vacuolated cells, neutrophils and lymphocytes were found in the intralveolar areas as well as in the alveolar lumen. These highly vacuolated cells seen in the alveolar lumina were believed to be desquamated alveolar epithelial cells, but recently they were confirmed as macrophages containing phagocytozed liquid droplets (Tatarczuch et al., 1997).

2.2.2.1.3. Apoptosis and Lactation

The DNA laddering was not observed in early lactating mouse mammary tissue (Marti et al., 2000), but was detectable at 16 to 18 d of lactation and increased by d 22 (the first day of weaning) to a level similar to that observed during induced involution by litter removal (Wilde et al., 1999).

In dairy animals, gradual regression begins after the peak of lactation, as milk yield declines progressively. Colitti et al. (2004a) studied mammary apoptosis and proliferation in primiparous cows in early lactation and found that the apoptosis : proliferation ratio increased from d 45 to 60 of lactation (Figure 11). In late lactation (217 to 252 d), the apoptotic index averaged 2.4% in Friesian cows (Wilde et al., 1997b). The index reported by the later authors was approximately 4-fold greater than the average value (0.56%) determined by Capuco et al. (2001) in non pregnant lactating cows from the same breed at d 90 to 240 of lactation (Figure 11). Wilde et al. (1997b) did not indicate the reproductive status of cows used in their study. As pregnancy increased the rates of mammary cell apoptosis and proliferation in lactating cows (Capuco et al., 2001), a difference in pregnancy status could account for the discrepancy between the two studies. In the study of

Capuco et al. (2001) average daily apoptotic rate after lactation peak (0.56%) exceeded the average rate of cell proliferation (0.3%), explaining the gradual decrease in milk yield after peak.
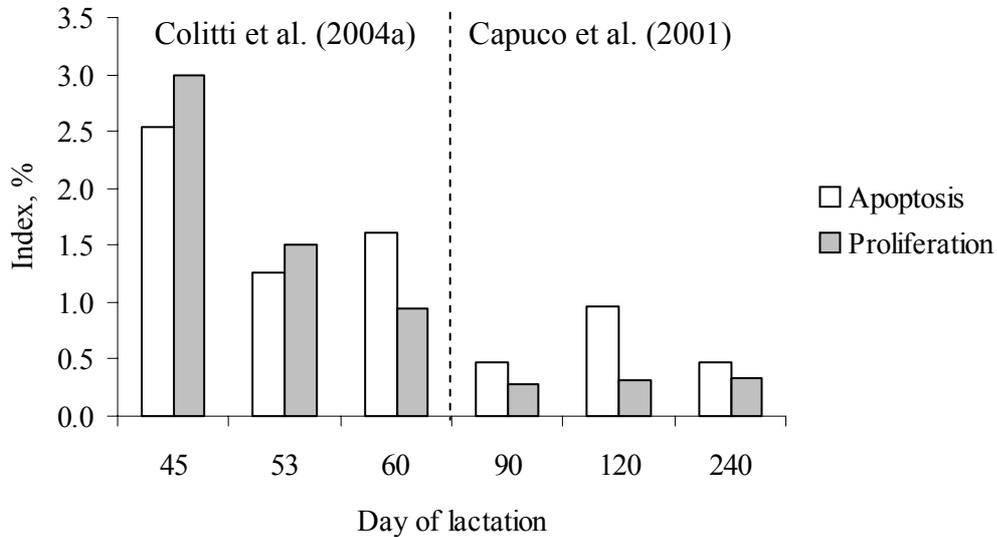
Figure 11. Indices of apoptosis and proliferation in the mammary tissue of dairy cows at different stages of lactation.

The duration of apoptosis has been estimated to be approximately 3 h (Bursch et al., 1990) and TUNEL only marks fragmented DNA that is present during this short period. Therefore, Capuco et al. (2001) proposed that the labeling index measured by TUNEL should be multiplied by 8 to compute the daily rate of cell death. Nevertheless, Colitti et al. (2004a) considered that TUNEL data reflect the daily apoptosis rate in the mammary gland because the observation of Bursch et al. (1990) was obtained in rat liver using haematoxylin and eosin rather than TUNEL. Considering species, tissue and methodology differences, the data of Bursch et al. (1990) can not be extrapolated to the apoptosis in the mammary gland measured by TUNEL (Colitti et al., 2004a). The lifespan of MEC is unknown.

Less than 1% of cells in the lactating tissue of non pregnant goats were dying (Li et al., 1999a). The cell loss after peak lactation in dairy goats appears to account for the decline in milk production, since surviving cells retain metabolic activity (Wilde and Knight, 1989), and detection of DNA laddering indicates that it occurs by apoptosis (Li et al., 1999a).

In addition to the apoptotic death, another potential mechanism for declining cell numbers during lactation is the continuous loss of MEC in milk. High somatic cell count (**SCC**) has been reported in goat milk (Hinckley, 1983; Contreras et al., 1996; Salama et al., 2003b), and epithelial cells represent 26% of milk SCC (Boutinaud et al., 2002). Assuming

that milk yield during lactation in goats is 600 L and a range of SCC of 250 to 1000×10^3 cells/mL with a DNA content of 6 pg/cell, cumulative loss of mammary DNA will be 0.23 to 0.94 g throughout lactation. The average mammary DNA loss between d 7 and 252 of lactation was 3.1 g in dairy goats (Wilde and Knight, 1989). Thus, DNA losses via milk can represent 7 to 30% of the total DNA loss during lactation in goats. DNA losses in milk of dairy cows does not significantly contribute to the declining number of MEC. Capuco et al. (2001) estimated that losses of DNA in milk was 0.62 g during 240 d, representing only 1.6% of the net loss of mammary DNA (38 g).

2.2.2.1.4. Apoptosis and Mammary Involution

Involution in the mouse mammary gland has been characterized as a two-stage process (Walker et al., 1989; Lund et al., 1996). During the first stage, mammary involution is triggered by local stimuli (milk stasis) that initiate apoptosis, but the process can be reversed by reinitiating milk removal (Walker et al., 1989). However, the second phase of involution (3 to 5 d) is irreversible because of the activation of matrix metalloproteinases which destroy the lobular-alveolar structure by degrading the ECM and basement membrane, and also causes a massive loss of alveolar cells (Lund et al., 1996). Heermeier et al. (1996) reported that the rate of apoptosis in mice reached a maximum (4.8%) on d 3 after weaning, coinciding with the second stage of mammary involution. Apoptosis during the second phase may also include the "anoikis" (a term for detachment-induced apoptosis) as cell-ECM interaction becomes perturbed (Green and Streuli, 2004).

Percentages of apoptosis after drying off in ruminants varied according to stage of involution, specie and reproductive state (Table 1). Concurrent pregnancy and advanced lactation at the time of milk stasis in dairy cows results in a rapid, but small increase in mammary apoptosis during involution and maintenance of alveolar structure and integrity throughout the dry period (Annen et al., 2003). The highest incidence of apoptosis was detected at 2 d after drying off in pregnant cows and was 4- to 8-fold greater than that in lactating tissue (Annen et al., 2003). By d 8, the number of apoptotic MEC did not differ from pre-stasis values (Annen et al., 2003). Apoptosis in the alveolar epithelial cells of non pregnant ewes (Tatarczuch et al., 1997; Colitti et al., 1999) begins at d 2 after weaning with a peak at d 4 to 8 (Table 1). Three days after cessation of milking in non pregnant dairy goats, mammary tissue was found to retain a lactating morphology, and apoptotic index was less than 1% (Li et al., 1999a). Apoptosis reached a maximum level at wk 2 of involution

(Table 1) and then decreased to a level similar to that found in involuting mouse mammary tissue (Li et al., 1999a).

Table 1. Percentages of apoptosis after drying off in cows, sheep and goats.

Specie	Reproductive state	Days after dry off	Apoptosis, %	Reference
Cow	Unknown	7	4.8	Wilde et al., 1997b
"	"	10	4.4	Colitti et al., 2004b
Sheep	Non pregnant	2	2.9	Tatarczuch et al., 1997
		4	3.6	
		7	1.0	
		15	0.8	
"	"	1	0.2	Colitti et al., 1999
		3	0.7	
		5	1.0	
		8	4.1	
Goats	"	3	0.9	Li et al., 1999a
		7	3.0	
		14	5.0	
"	Pregnant	7	1.8	Salama et al., 2005b

Apoptosis was never observed in the myoepithelial cells during mammary involution in sheep (Tatarczuch et al., 1997), goats (Li et al., 1999a) or rats (Walker et al., 1989), while elimination of myoepithelial cells was by apoptosis in mice (Walker et al., 1989). The preservation of myoepithelial cells during involution in sheep, goats and rats is vital, providing a supportive framework for the alveoli and/or synthesizing some components necessary for the ECM remodeling during involution (Tatarczuch et al., 1997).

Factors that can affect the rate of mammary gland involution include: 1) systemic hormones and local growth factors, 2) pregnancy status, and 3) stage of lactation when milk removal ceases (Capuco and Akers, 1999).

Mammary involution in mice can be reduced by systemic progesterone and glucocorticoids (Lund et al., 1996) or by pregnancy (Capuco et al., 2002). In dairy cows, milking is usually stopped when the cow is in the last 2 mo of pregnancy and milk yield is low. Therefore, it is not surprising that involution should proceed at a slower rate than would be observed if milking was terminated during a high production period (Capuco and

Akers, 1999). In addition, concurrent pregnancy inhibits involution in cows and may account for the maintenance of the alveolar structure during the dry period (Capuco et al., 1997), although the alveolar structure is partially maintained for several weeks after milking cessation in non pregnant dairy cows (Holst et al., 1987), ewes (Tatarczuch et al.,1997) and beef cows (Akers et al., 1990).

Maintaining the alveolar structure in the involuted mammary glands of non pregnant animals may be due to the concurrent cell proliferation. Clearly, proliferation occurs in pregnant animals during the dry period between successive lactations (Capuco et al., 1997), but may also occur during involution in non pregnant dairy animals. There are high rates of proliferation during the first 5 to 7 d of involution in non pregnant beef cows (Capuco and Akers, 1990). In rats, when pups were separated from their mothers for 2 d, mammary proliferation was doubled, but apoptosis rates was multiplied by 20 during the separation period (Knight, 2000). When pups were returned to their mothers, proliferation and apoptosis were 3 fold their values before separation. This proliferation may immediately precede apoptosis of the cell (Capuco and Akers, 1999) or it is possible that the same cue which starts apoptosis also starts a totally different series of events leading to cell proliferation (Figure 12). Alternatively, it may signal proliferation of the mammary epithelium which inhibits mammary involution and permits restoration of milk synthesis during the early stages of milk stasis. Thus, partial reinitiation of lactation is possible in non pregnant dairy cows after 11 d of milk stasis (Noble and Hurley, 1999) and in non pregnant beef cows after 4 wk of weaning (Lamb et al., 1999).
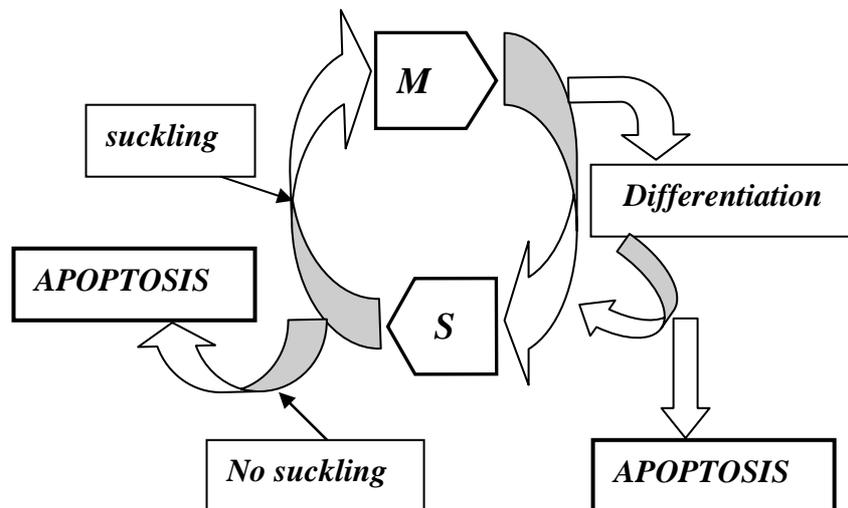
Figure 12. Schematic representation of the cell cycle to show the possible routes to apoptosis. M: mitosis. S: DNA synthesis. In the absence of continued suckling, differentiated mammary secretory cells may enter directly into apoptosis or, more likely, re-enter the cell cycle, synthesize DNA and then either undergo apoptosis or, if suckling is resumed, proliferate (Knight, 2000).

It has been reported that the early period of mammary gland involution coincides with a period of acutely increased susceptibility to mammary infections, despite the extensive leucocytic infiltration into the mammary gland during this stage (Olvier and Sordillo, 1989). These authors explained that this could be due to the loss of the physical barrier by sloughing of the alveolar epithelium. However, alveolar cell loss during involution is performed by apoptosis with very limited shedding of alveolar epithelial cells into the alveolar lumina, maintaining the integrity of the epithelial lining of the gland. This excludes the hypothesis of Olvier and Sordillo (1989). The most probable explanations for this are: 1) it is possible that the leukocyte types (especially the lymphocyte phenotypes) infiltrating into the gland at this stage are not sufficient (in terms of number of cells, types of cells or both) to protect the gland, and 2) the phagocytotic leucocytes residing in the alveolar and ductal lumina are less active since they are working to remove the lipid droplets and milk proteins and are replete with engulfed material (Tatarczuch et al., 1997).

2.2.2.2. Mammary Programmed Cell Death by Autophagy

In numerous biological systems the PCD has been found to involve the autophagic/lysosomal compartment. Autophagy means to eat oneself (Edinger and Thompson, 2004). Cell death by autophagy is caspase-independent (Lockshin and Zakeri, 2004). Autophagic cell death, or type II PCD, exhibits extensive autophagic degradation of Golgi apparatus, polyribosomes and endoplasmatic reticulum, which precedes nuclear fragmentation (Bursch et al., 2000). Autophagy has been described in yeast (Figure 13; Klionsky and Emr, 2000). A signal transduction event leads to the following: 1) The induction of autophagy. 2) Membrane from an unknown source sequesters cytosol and/or organelles (a mitochondrion is depicted) resulting in the formation of a double-membrane vesicle termed an autophagosome. 3) On completion, the autophagosome docks with the lysosome or vacuole. Fusion of the autophagosome outer membrane with the vacuole releases the inner vesicle into the vacuole lumen. The inner vesicle is termed an autophagic body. 4) Breakdown within the vacuole allows recycling of the degraded autophagic body and its hydrolyzed cargo [amino acids, fatty acids (**FA**), sugars, and nucleotides]. The morphology of macroautophagy in mammalian cells is similar to that shown in Figure 13; however, in mammalian cells autophagy can be induced by environmental cues other than starvation.
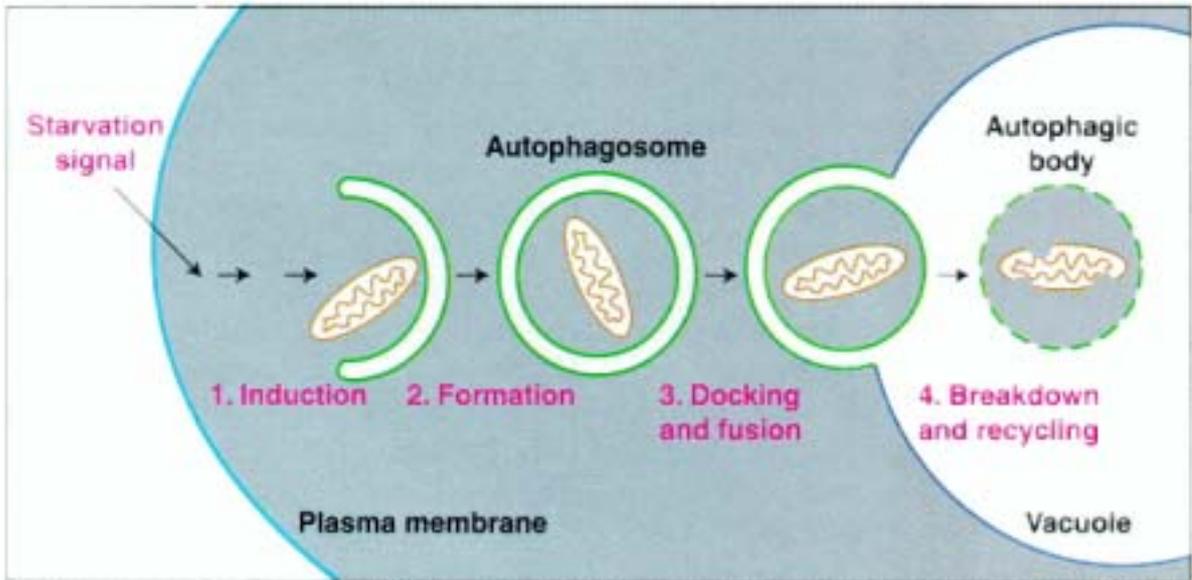
Figure 13. Schematic model of macroautophagy in yeast (Klionsky and Emr, 2000).

At the level of the mammary gland, autophagic cell death has mainly been observed during involution accompanied by apoptosis (Monks et al., 2002; Lockshin and Zakeri, 2004). Hurley (1989) reported that autophagocytosis occurred during bovine mammary involution by the appearance of autophagocytic structures called cytosegresomes and cytosomes. Both are found in the cytoplasm of epithelial cells. The presence of these autophagocytic structures coincides with the period of greatest decline in the rough endoplasmic reticulum and Golgi apparatus, which seem to occur at about 48 h after cessation of milk removal. Moreover, at d 4 of mammary involution in ewes, autophagosomes were present in the cytoplasm of some alveolar epithelial cells (Tatarczuch et al., 1997). Detailed mechanisms of autophagy in the mammary gland have not yet been reported.

2.3. Milk Stasis within the Alveoli

As time after milking increased, there is:

    1) An increase in cisternal filling and intramammary pressure (Peaker, 1980).

    2) A decrease in blood flow (Stelwagen et al., 1994b).

    3) An increase in TJ permeability (Stelwagen et al., 1997).

    4) An accumulation of putative feedback inhibitor of lactation (Wilde et al., 1995).

2.3.1. Alveolar Distension

Physical distension of the mammary gland results in the cessation of milk secretion. Milk removal with immediate replacement of milk volume by isotonic sucrose progressively stopped milk secretion within 1 to 2 d (Peaker, 1980). Alveolar lumen fills to capacity at approximately 17 to 18 h after milking (Stelwagen, 2001), causing significant physiological changes. Alveolar distension rather than chemical feedback may be a more important, or the sole stimulant, of apoptosis during milk stasis (Wilde et al., 1997a). Sealing one teat during lactation in the mouse induces an accumulation of milk and involution in that gland, while the other glands in the same animal continue to lactate (Marti et al., 1997).

The effect of alveolar distension to induce apoptosis is mediated by focal adhesion kinase signal transduction pathway (Davis et al., 1999). Changes in focal adhesion kinase activity are indicative of the cell perceiving a new set of environmental conditions such as those generated during milk accumulation. As milk accumulates, the intra-alveolar pressure will increase and flatten the MEC (Richardson, 1947; Stelwagen, 2001), establishing a strain affecting focal adhesion sites. Consequently, there is a change in genes expression and ultimately this leads to apoptosis (Li et al., 1997). In addition, mammary cells appear to respond to extended milking interval by producing an insulin-like growth factor binding protein-5 (**IGFBP-5**), which inhibits insulin-like growth factor (**IGF**) action by restricting the availability of IGF-I at the epithelial cell (Flint et al., 2000).

Cell number loss due to extended milking intervals is a long-term response. Resumption of 2 times daily milking (**2X**) after short periods of 1 time daily milking (**1X**) resulted in complete recovery of milk yield, indicating that there is no measurable permanent effect of short-term 1X in dairy cows (Davis et al., 1999; Ayadi et al., 2003b). Similarly, after 3 wk of 1X in dairy cows, empty udder volume was not decreased, suggesting that no cell loss occurred (Stelwagen and Knight, 1997). However, after extended periods of 1X yield recovery may only be partial on resumption of 2X reflecting an enhancement of cell loss via apoptosis (Davis et al., 1999).

2.3.2. Mammary Blood Flow

There is a close relationship between mammary blood flow (**MBF**) and milk production because blood supplies mammary gland with nutrients and hormonal stimuli necessary to sustain milk synthesis (Prosser et al., 1996). The close arterial infusion of adrenaline (a mammary vasoconstrictor), at a rate adjusted to maintain a 50% reduction in

MBF in goats, reduced milk yield by 30 to 50% (Prosser et al., 1996). Nevertheless, the close relationship between MBF and milk yield does not always hold. Nielsen et al. (1990) reported a decline in the ratio of MBF to milk yield as yield increased in goats. Further, during acute exposure of goats to cold, milk yield fell, but MBF was unaffected (Thompson and Thomson, 1977).

A reduction in MBF is an early consequence of milk accumulation in goats (Stelwagen et al., 1994b) and cows (Guinard-Flament and Rulquin, 2001), although Fleet and Peaker (1978) could not detect any change in MBF until after 24 h of milk accumulation in goats. Moreover, 1X reduced milk yield by 24% ($P < 0.01$), MBF by 16% ($P < 0.01$), and udder O_2 consumption by 18% ($P = 0.16$) in dairy cows, suggesting that the energy expenditure of the udder is diminished in response to the milk yield reduction caused by 1X milking (Delamaire and Guinard-Flament, 2004).

Lacasse and Prosser (2003) reported that increasing MBF (by infusion of a vasodilator to the external pudic artery) did not enhance milk production in dairy goats. Thus, it appears much more likely that rates of mammary metabolism control MBF than vice-versa. In other words, optimal milk yield is dependent on at least a minimal blood supply, but any increase above this minimum may not necessarily result in greater milk yield. So, if milk secretion rate is reduced during 1X, then MBF is also expected to decrease.

2.3.3. Tight Junction Leakiness

One of the most important consequences of the extended milking interval (i.e. 1X) is the increase in TJ permeability. The TJ are extracellular structures that are in close proximity to the apical domain and form semi-permeable barriers between adjacent epithelial and endothelial cells (Stelwagen et al., 1994b). Intact TJ prevent paracellular leakage of blood serum components into milk and prevent milk components from passing into the blood. The TJ also maintain a small transepithelial potential difference between blood and milk (Peaker, 1977).

The TJ disruption began after 21 h of milk accumulation, while milk secretion began to decline after 19 h in goats in mid-lactation, suggesting that impairment of mammary TJ integrity is associated with decreased milk secretion during extended milking interval in goats (Stelwagen et al., 1994b). Stelwagen et al. (1997) reported that concentration of α-lactalbumin and lactose in plasma and Na:K ratio in milk are suitable indicators of TJ status in vivo. Plasma lactose concentration increased in cows milked 1X in late lactation,

indicating an increase in TJ permeability (Lacy-Hulbert et al., 1999). In addition, Stelwagen et al. (1997) concluded that milk stasis during early lactation in dairy cows induces TJ leakiness after 18 h and to revert to the closed state shortly after milking. Leakiness of TJ does not seem to be responsible of all milk yield losses during 1X. Lactose leakage calculated from clearance data was only 3% of the lactose synthesized by the mammary gland, while the loss in milk yield was 15% (Stelwagen et al., 1997). Therefore, almost all of the decline in milk yield was due to a decrease in milk secretion. These results suggest a relationship between TJ permeability and milk secretion. Similarly, milk secretion rate in goats declined when calcium chelators, known to open TJ such as EDTA (Stelwagen et al., 1995) or citrate (Neville and Peaker, 1981), were injected into the lumen of the mammary gland to induce TJ to open.

Stelwagen et al. (1994c) and Kelly et al. (1998) reported elevated levels of plasmin and plasminogen during 1X milking in cows, which was explained by an increase in their paracellular transport from blood into the milk through the leaky TJ (mammary tissue does not produce plasmin or plasminogen). Another possible entry route for plasmin, plasminogen, or both from blood into milk is via damaged alveolar cells during mastitis (Saeman et al., 1988). Plasminogen is converted to plasmin by plasminogen activators synthesized by the MEC (Politis, 1996). Plasmin degrades α_s-CN and β-CN to boiling-resistant peptides (proteose-peptones), which disturb TJ integrity, reduce milk secretion, and induce mammary involution when injected into udders of dairy goats (Shamay et al., 2002) and dairy cows (Shamay et al., 2003).

The trigger for the change in TJ permeability is not clear. There is no evidence that an increase in the physiological pressure within the mammary gland during milk accumulation could increase mammary damage and hence mammary permeability (Stelwagen and Lacy-Hulbert, 1996). Colditz (1988) found that milk from the distended udder in sheep contains more inflammatory activity than newly secreted milk, which suggests a regulatory mechanism involving the production of pro-inflammatory factors in accumulated milk affecting TJ permeability. The TJ formation is dependent on glucocorticoids, and treatment with glucocorticoids reduces TJ permeability in dairy cows (Stelwagen et al., 1998b) and prevents mammary involution in the mouse (Feng et al., 1995).

2.3.4. Feedback Inhibitor of Lactation

Beside the mechanical compromises (alveoli distension and TJ leakiness) in the mammary gland during extended periods of milk accumulation, chemical feedback inhibitor of lactation (**FIL**) is present in milk and can further reduce milk secretion during long milking intervals.

Henderson et al. (1983) showed that 3 times daily milking (**3X**) of one gland of lactating goats increased milk yield from that gland, but not from the contralateral gland milked 2X, by increasing the rate of secretion (local effect). This can only be a consequence of something other than the release of galactopoietic hormones (which would affect both glands) and must, therefore, be a result of the removal of milk or a bioactive factor contained in this milk (Henderson et al., 1983). Henderson and Peaker (1984) reported that this local response was not due to the pressure of stored milk. They infused into the lumen of one gland a volume of inert, isotonic solution, equal in volume to the milk removed at milking and they noticed that the rate of milk secretion increased even with the degree of distension caused by milk accumulation. Moreover, when milk in the mammary gland was diluted with isotonic lactose or sucrose solutions, the rate of secretion increased (Henderson and Peaker, 1987).

Further in vitro studies demonstrated that a whey fraction in goat milk with a molecular weight of 10 to 30 kDa inhibited synthesis of lactose and CN in rabbit mammary explants in a dose-dependent manner (Wilde et al., 1987a). Inhibition by FIL was rapid, consistent with the acute response elicited by changes in MF, and was readily reversible. When explants exposed to the inhibitory fraction for 6 h were washed and cultured again, rates of lactose and CN synthesis returned to control levels (Wilde et al., 1987a). In addition, FIL inhibited milk secretion when injected into the glands of lactating goats via the teat duct (Wilde et al., 1995; 1996). The effect of FIL injection on milk yield in goats was also dose-dependent (milk yield reduced by 0.9 and 17.4% with doses of 100 and 750 μg FIL, respectively) and persisted for up to 3 d at the higher doses tested (Wilde et al., 1995). The dose-dependence of the effect exerted by the FIL on milk secretory rate in goats, and the demonstration that FIL was active when introduced via the teat canal, together suggest that the degree of feedback inhibition during normal lactation is determined by the concentration of FIL in the alveolar lumen (Wilde et al., 1996). The epithelial secretory cells are both the source and the target of FIL, hence the process is an autocrine mechanism (Wilde et al., 1995).

The dose-dependent effects of FIL in vivo and in vitro suggests a mechanism in which the concentration of FIL in milk increases with time, hence increasing MF stimulates milk secretion by reducing FIL concentration during milk accumulation (Wilde et al., 1995). The question now is how does FIL concentration increase during milk accumulation?. Two mechanisms may explain the increase of FIL with time. First, a pro-inhibitor is secreted in milk and with time the active inhibitor is formed, giving an increase in FIL concentration with time (Peaker and Wilde, 1996). Second, Wilde et al (1996) suggested the existence of FIL specific receptors on the luminal membrane of MEC. Thus, it is possible that as the luminal membrane of the cell increases in area during the accumulation of milk and expansion of the alveoli, the specific receptors might be more exposed to FIL (Wilde et al., 1996). Whatever the mechanism by which inhibition is relieved, increasing MF decreases the time of milk storage preventing the transformation of the inactive inhibitor to active form and the alveoli will not expand, hence the specific receptors on the apical membrane will be less exposed to FIL.

Both autocrine and endocrine controls can interact to regulate milk production. One consequence of milking at different frequencies/efficiencies is that the mammary cell prolactin (**PRL**) receptor number is altered. McKinnon et al. (1988) reported that mammary cell PRL receptor number in goats was increased by 3X milking and reduced by incomplete milking. This is most probably a direct effect of the FIL, since a reduction in cell surface PRL receptor number occurred when mouse MEC incubated with FIL in vitro (Bennett et al., 1990). Knight et al. (1990a) reported that the increase in milk yield due to more frequent milking in goats was greater during bromocriptine treatment, suggesting that MF alters MEC sensitivity to circulating galactopoietic hormones and that PRL sensitivity is one factor influencing milk yield, at least when PRL concentration is low.

If FIL could be neutralized by immunization, the disadvantages of extended milking intervals would be avoided. Wilde et al. (1996) studied the effect of immunization against FIL during the declining stage of lactation in goats and reported that the rate of decline in milk secretion was significantly reduced in immunized goats compared with sham-immunized controls. In the same experiment, when one gland of immunized goats was switched from 2X to 1X, the ipsilateral decrease in the rate of milk secretion was reduced significantly compared with sham-immunized goats.

2.4. Factors that Affect the Number and Activity of Mammary Cells During Lactation

2.4.1. Frequency and Efficiency of Milking

Milking more than 2X results in increasing milk yield and milk secretion rate in dairy cows (Hillerton et al., 1994), goats (Knight et al., 1990b), ewes (McKusick et al., 2002a) and camels (Alshaikh and Salah, 1994).

The increase in cell differentiation appears to be an early response to increasing MF. An increase from 2X to 3X in goats (Wilde et al., 1987b) and to 3X (Norgaard et al., 2005) or 4 times daily milking (**4X**) in cows (Hillerton et al., 1990) stimulated mammary cell differentiation, as measured by key enzyme activities (acetyl-CoA carboxylase, fatty acid synthetase, and galactosyl transferase), within 2 to 4 wk. These increases in maximum enzyme activity by greater MF suggest that there is a greater accumulation of the enzymes, either through increased enzyme synthesis or decreased enzyme degradation (Wilde et al., 1987b). Travers and Barber (1993) reported that an increase in MF from 2X to 3X increased significantly the expression of Acetyl-coA carboxylase and fatty acid synthetase genes in the goat mammary gland. This increase in gene expression was accompanied by an increase in enzyme activities/mg DNA. In contrast, unilateral 1X of goats for 4 wk decreased secretory cell differentiation compared with contralateral glands milked 2X (Wilde and Knight, 1990). In cows, mammary tissue from glands milked 1X had significantly lower enzyme activities after 23 h of milk accumulation than tissue from glands milked 2X after 8 h of milk accumulation (Farr et al., 1995). Nevertheless, changing MF unilaterally in goats from 2X to 1X or 3X did not alter β-CN or α-lactoalbumin gene expression (Bryson et al., 1993).

Increasing MF incremented the number of secretory cells. Henderson et al. (1985) found that the goat mammary gland milked 3X was larger than that milked 2X, suggesting either growth or reduced regression (involution) of the 3X gland. Similarly, Wilde et al. (1987b) studied the effect of unilateral 3X vs. 2X for 37 wk and found that the weights of both the whole mammary gland and the mammary parenchyma, and the amount of parenchymal DNA were, significantly greater for the 3X glands, indicating an increased number of cells compared with that of the glands milked 2X. A greater number of epithelial cells per secretory alveolus was detected in dairy cows milked 4X (Hillerton et al., 1990) and goats milked 3X (Li et al., 1999a). Similarly, 3X or 4X milking of dairy cows in early lactation increased mammary cell proliferation (Hale et al., 2003; Norgaard et al., 2005) and

reduced apoptosis (Norgaard et al., 2005), indicating relatively more MEC. On the other hand, DNA laddering was induced and the proportion of apoptotic cells was increased in goat glands milked 1X compared to glands milked 3X (Li et al., 1999a). As previously mentioned, apoptosis is regulated by a local mechanism sensitive to milk stasis. Frequent milking may have inhibited MEC death by decreasing the alveolar distension. FIL inhibited goat mammary cell proliferation in vitro, at least in part by stimulating apoptosis (Wilde et al., 1999). If FIL exerts the same effect in vivo, then an increase in FIL concentration with infrequent milking will contribute to the induction of apoptosis.

Another explanation for the positive effect of increasing MF on milk yield is the release of PRL and OT. In addition to the role of OT in milk ejection, OT released during milking has a galactopoietic effect in dairy cows and goats (Lollivier and Marnet, 2005). Thus, increasing MF will lead to increasing OT surges, producing a positive effect on MEC. On the other hand, PRL promotes differentiation of MEC, and an increase in the number of differentiated MEC would be expected to be associated with greater milk yield (Capuco et al., 2001). Circulating PRL increases in response to milking in cows (Johansson et al., 1999) and goats (Hart and Linzell, 1977); thus, animals milked 2, 4 or 6X would have double the number of daily PRL release events that 1, 2 or 3X animals experience, respectively. Crawford et al. (2004) reported that cows in early lactation milked 2X and injected twice daily with PRL were as productive as cows milked 4X, indicating that PRL produces the effects of increasing MF in early lactation. Moreover, increasing MF is associated with an increment in the expression of PRL receptors in goats (McKinnon et al., 1988) and cows (Dahl et al., 2002). This increase in PRL receptors suggests that animals milked more frequently are more sensitive to PRL and might have a greater number of MEC that differentiate and produce milk (Figure 14).

The positive effect of increasing MF on MEC number would suggest that increased milk yield when animals are more frequently milked might persist after treatment stopped (Hillerton et al., 1990; Knight et al., 1992; Bar-Peled et al., 1995; Hale et al.,. 2003; Dahl et al., 2004b). When MF increased from 2X to 4X (Hale et al., 2003; Fernandez et al., 2004) or from 3X to 6X (Dahl et al., 2004b) during the first 21 d after calving, greater milk yields persisted for 4X and 6X at least up to the 6th mo of lactation. These results suggest that a "window" in early lactation (first 21 d) exists during which increasing MF causes milk yield responses that persist for the entire lactation, even after MF was reduced to 2X or 3X. Nevertheless, Norgaard et al. (2005) tested the effects of 2X versus 3X milking in cows in early lactation (d 7 to 56 postpartum) and found that the positive effect of increasing MF on

milk yield did not persist after d 56 when 3X cows were switched to 2X. In addition, increasing MF from 3X to 6X during the first 7, 14, or 21d postpartum in multiparous dairy cows resulted in similar milk yield during the first 9 wk of lactation (VanBaale et al., 2004).
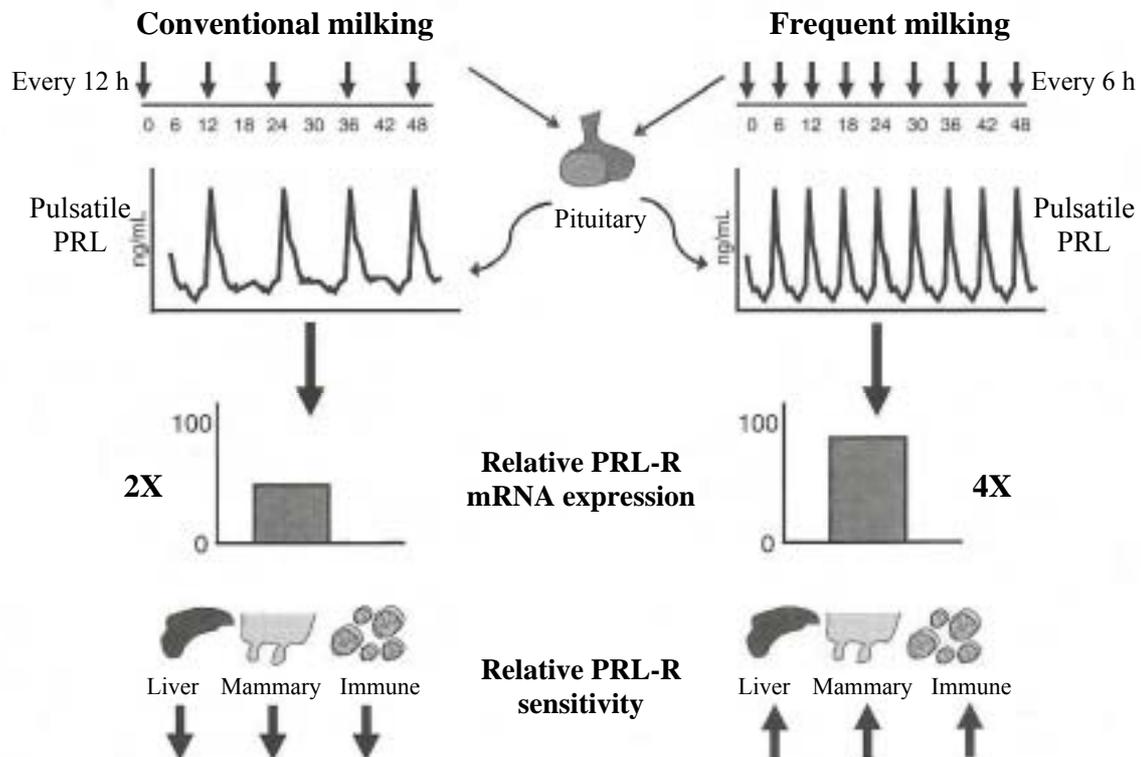
Figure 14. Effect of milking frequency on PRL sensitivity. Each milking induces a surge of PRL. Therefore, cows milked four times daily (4X) would have twice the number of PRL surges relative to cows milked twice daily (2X). Basal secretion of PRL is not affected, as the milking induced surge is transient. The pattern of PRL release causes upregulation of PRL-receptor (PRL-R) in mammary, hepatic and lymphocytic tissue, thereby increasing the sensitivity of these tissues to PRL (Dahl et al. 2004a).

If residual milk volume is too large due to incomplete milking or udder morphology, the effect of FIL is relieved only slightly or not at all (Wilde et al., 1996). Therefore, frequent milking by catheter (which removes only cisternal milk) did not increase milk yield in goats (Henderson and Peaker, 1987). Peaker and Blatchford (1988) reported that the rate of milk secretion in individual glands of lactating goats was inversely related to the fraction of milk left after milking, irrespective of the actual volume of milk in the gland at the time of milk removal.

Milking efficiency depends on the technique and technology employed in the milking. Singh and Dave (1985) found that glands of cows milked by machine alone produced less milk than those which were subsequently hand-milked to minimize the volume of residual milk. The proportion of residual milk in the gland after milking has been found to increase in the second and subsequent lactations (Ebendorff et al., 1987; Barnes et al., 1990).

Therefore, although yield tends to increase in successive lactations, milk secretion may become less efficient, and autocrine inhibition may be an increasingly important factor (Wilde et al., 1996). It is clear that omission of hand milking after machine milking had a greater effect on milk yield in the second lactation than in the first (Ebendorff et al., 1987). Davis et al. (1999) suggested that paying attention to the efficiency of milk removal will minimize yield loss during 1X. Stripping and use of OT have both been shown to enhance milk yield as a result of high efficient milk removal and hence reducing FIL concentration, analogous to more frequent milking (Carruthers et al., 1991; 1993a). Omitting the hand stripping in Manchega ewes decreased milk yield and milk lactose by 17 and 7%, respectively, but increased milk protein by 3% (Molina et al., 1989ab). However, the omission of machine stripping in East Friesian dairy ewes reduced milk yield by only 14% without affecting lactation length, milk composition, or SCC (McKusick et al., 2003). The milking machine in the later work was set to provide 180 pulses/min. This elevated pulsation frequency was reported to reduce the amount of residual milk left behind in the udder, and it causes less change in teat end thickness (better massage), therefore benefiting the teat end in permitting it to return to its normal thickness much sooner after milking (Marnet and McKusick, 2001).

2.4.2. Hormones and Growth Factors

The hormones primarily responsible for maintaining milk production are GH and PRL. The importance of PRL in lactogenesis is well known (Tucker, 2000). However, PRL has been considered to be relatively unimportant for galactopoiesis in ruminants. This consideration is questionable because minimal PRL secretion is sufficient to maintain milk production (Buys et al., 1995). Moreover, ruminant mammary tissue seems to be able to sequester significant amounts of PRL even when little is present in the circulation and there is evidence of local production of PRL in the mammary gland (Knight, 2001). Also, by the inhibition of GH and PRL in rats, it has become clear that the individual effects of GH and PRL could only be observed in the absence of the other. So, it might be possible that effects of PRL on lactation in cows can only be observed in the absence of GH (Tucker, 2000). For these reasons, injection of PRL or its depletion by bromocryptine (which never totally removes PRL from circulation) is expected to have less or no effect on milk production, which may explain why many studies, specially in cows (Tucker, 2000), concluded that PRL is unimportant in ruminants. However, PRL depletion by bromocryptine reduced milk yield by 15 to 20% in goats (Knight, 2001) and by 50% in ewes (Buys et al., 1995).

Similarly, administration of exogenous PRL increased milk yield by 10% in dairy goats (Knight, 2001). There is increasing evidence that PRL plays an important role in the galactopoiesis and is probably a minor, but significant, player in ruminant lactation persistency (Knight, 2001).

While the importance of PRL in ruminants is controversial, in rodents PRL is a key hormonal regulator of mammary cell survival. Prolactin has been demonstrated to inhibit apoptotic cell death in the mouse mammary gland after litter removal (Sheffield and Kotolski, 1992). In PRL-deficient rats, TJ opened, total DNA content decreased and apoptosis increased (Flint and Gardener, 1994). Barber et al. (1992) demonstrated that PRL regulates Acetyl-coA carboxylase gene (the major enzyme regulating de novo lipid synthesis) expression in lactating rat mammary gland. Depletion of both circulating PRL and GH for 48 h in rats by treatment with a combination of bromocriptine and anti-GH serum stimulated mammary apoptosis (Travers et al., 1996). In the same study, apoptosis was reduced by PRL treatment after 24 h and was abolished by dual hormone replacement.

The role of PRL in favoring mammary growth may by related to its influence on components of the IGF axis. PRL induces mammary gland expression of IGF-II mRNA, a known mammary mitogen and survival factor, in pregnant mice (Hovey et al., 2003) and pregnant dry cows (Wall et al., 2005). In addition, PRL suppresses the expression of IGFBP-5 mRNA in the mammary gland of pregnant rats (Tonner et al., 1997). This binding protein has been reported to sequester both IGF-I and IGF-II, thereby inducing apoptosis (Tonner et al., 1997). In addition, the antiapoptotic effect of PRL is associated with the up-regulation of Bcl-2, the down-regulation of Bax and suppression the transcription of TGF-β_1 that has an apoptotic action (Motyl et al., 2000).

Administration of bovine somatotropin (**bST**) increased milk yield and improved lactation persistency in dairy cows (Bauman et al., 1999), ewes (Fernandez et al., 2001) and goats (Baldi et al., 2002). Total milk yield correlated positively with plasma concentration of GH and negatively with insulin in dairy cows (Sorensen and Knight, 2002). This can be explained by the role of both hormones in energy partitioning; GH favors the mammary gland by directing more available substrates for milk secretion (Figure 15), whereas insulin has the opposite effect. As stated before, the decrease in milk yield with advancing lactation is due to a decline in MEC number and not cellular activity. The effect of bST on increasing the lactation persistency seems to be also due to maintenance of the MEC population (more proliferation or less apoptosis) rather than maintenance of cellular secretory rate. In dairy

cows (Capuco et al., 2001) and dairy ewes (Kann, 1997), GH increased cell proliferation without affecting cell apoptosis. Growth hormone increased cell hypertrophy (Boutinaud et al., 2003) and total mammary DNA (Knight et al., 1990b; Baldi et al., 2002; Boutinaud et al., 2003) without affecting mammary apoptosis (Baldi et al., 2002) in goats in late lactation.
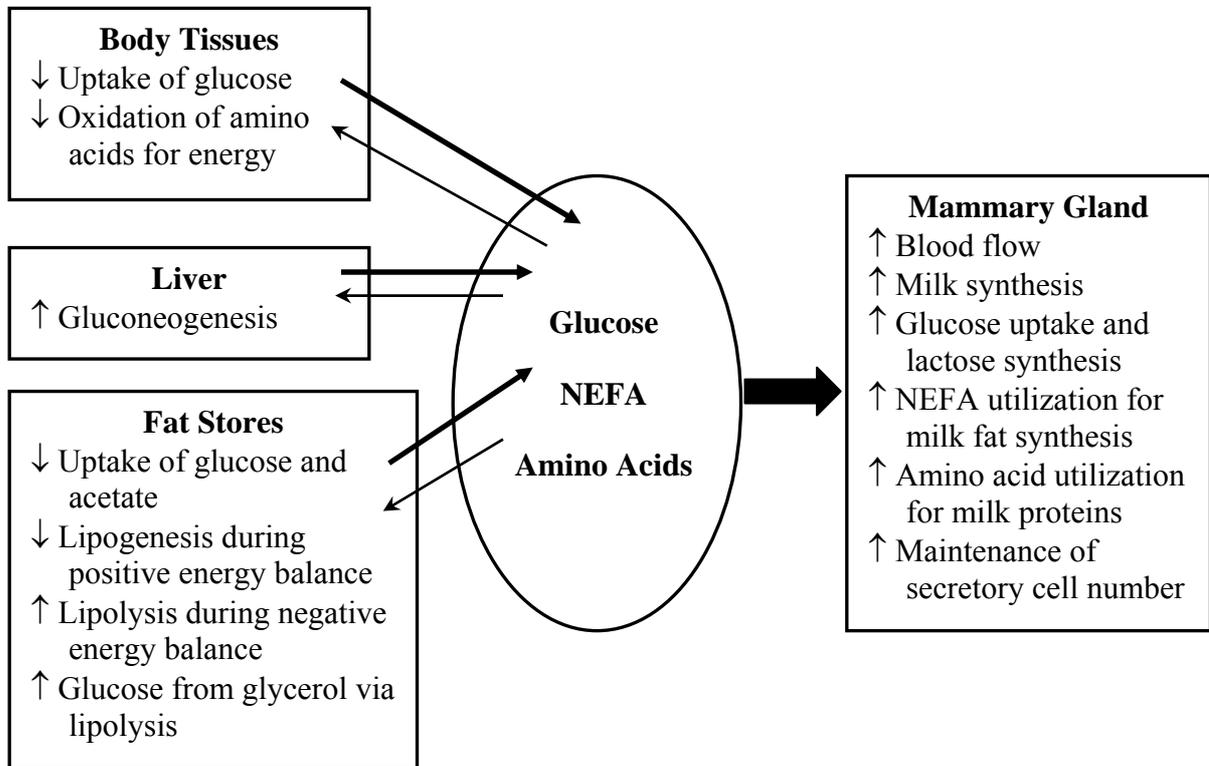
Figure 15. Metabolic adaptations induced by bST. Administration of bST induces metabolic adaptations of tissues in a fashion that provides nutrient partitioning and metabolic support for increased milk production. Glucose, nonesterified fatty acids (NEFA), and amino acids are partitioned toward the mammary gland (Akers, 2002).

While PRL seems to act directly on mammary tissue through its receptors, the effects of GH are considered to be caused by IGF-I, mainly synthesized by the liver, but also produced locally by the stromal mammary cells (Tucker, 2000). However, GH may directly influence mammary function through the GH receptor detected in vivo (Sinowatz et al., 2000) and in vitro (Sakamoto et al., 2005). Moreover, mRNA of the GH receptor was detected in vivo during mammogenesis, lactogenesis, galactopoiesis, and involution in the bovine mammary gland (Plath-Gabler et al., 2001). The GH affects directly MEC in vitro to increase the expression and synthesis of $\propto$-CN (Sakamoto et al., 2005) and β-CN (Yang et al., 2005), suggesting a role of GH in stimulating cell differentiation.

IGF-I is a mammary mitogen and antiapoptotic factor, but its action is controlled by a family of IGFBP, that bind to IGF with different affinities inhibiting or enhancing the

biological effects of IGF-I (Cohick, 1998). Milk from lactating animals contains very low levels of all the IGFBP. However, during mammary involution in rats there is an increase in IGFBP-5, inhibiting IGF-I mediated cell survival (Flint et al., 2000). There is an evidence that GH and PRL interact to prevent mammary apoptosis in dairy cows; GH by stimulating local production of IGF-I, and PRL by inhibiting local production of IGFBP-5 (Accorsi et al., 2002). IGFBP-5 induces mammary apoptosis by: (a) sequestration of IGF-I, preventing the interaction of IGF-I with its receptor, and (b) binding plasminogen activator inhibitor, allowing activation of plasminogen activator and consequent ECM remodeling as a result of the generation of plasmin (Flint et al., 2000). Despite the mitogenic effect of IGF-I, no correlation was found between lactation persistency and plasma concentration of IGF-I in cows (Sorensen and Knight, 2002). These authors proposed that IGF-I concentration in the mammary tissue would be more highly correlated with persistency than the systemic level.

Once lactation became established, progesterone administration had no effect on milk yield, probably because progesterone receptors are not present in the mammary gland during this physiological state, and progesterone has a higher affinity for milk fat than for its own intracellular receptor (Tucker, 2000). Therapeutic doses of synthetic glucocorticoids suppressed milk yield in dairy cows, but glucocorticoids were strongly galactopoietic in rats (Tucker, 2000). Administration of progesterone and glucocorticoids reduced apoptosis in the involuting mammary gland (Berg et al., 2002). Also, apoptosis in the mammary was inhibited by endogenous progesterone and glucocorticoids during normal lactation in rats (Berg et al., 2002). The presence of either steroid alone was sufficient to prevent apoptosis, suggesting that their antiapoptotic effects in the lactating mammary gland may be mediated via similar signaling pathways.

Growth factors are involved in the development and function of the mammary gland, and they can act as mediators of the endocrine hormones in an autocrine or paracrine manner. Beside IGF-I, IGF-II, and TGF, there are several growth factors that participate in the regulation of mammary growth and differentiation (reviewed by Oka et al., 1991). These growth factors include epidermal growth factor (**EGF**), fibroblast growth factor (**FGF**), hepatocyte growth factor, mammary-derived growth inhibitor (**MDGI**), leukemia inhibitory factor (**LIF**), and keratinocyte growth factor (**KGF**).

While TGF-α has a proliferative effect in the mammary gland, TGF-β_1 inhibits mammary growth (Oka et al., 1991) and the concentration of its mRNA increased during involution in cows (Plath et al., 1997) and goats (Wareski et al., 2001) when PRL was at its

lowest level. Moreover, TGF-β_1 was reported to exert both antiproliferative and apoptotic action in bovine MEC (Kolek et al., 2003). TGF-β_1 induces cell death by decreasing the expression of Bcl-2 (Rosfjord and Dickson, 1999) and enhancing the expression of Bax, caspase-3, and IGFBP-3 (Kolek et al., 2003; Gajewska and Motyl, 2004). In addition, the mRNA and protein levels of TGF-β_3 increase rapidly at the onset of involution, suggesting an involvement with apoptosis regulation in mice (Green and Streuli, 2004).

Expression of FGF-2 was also high during involution in bovine mammary gland, and since FGF-2 was found to activate the latent TGF-β form in bovine endothelial cells, FGF-2 may have a role during mammary gland involution (Plath et al., 1998). The EGF inhibited apoptosis in mouse MEC through the increase in the expression of Bcl-x_L (Rosfjord and Dickson, 1999). MDGI inhibited MEC growth in vitro (Spitsberg et al., 1994). MDGI may play an important role during established lactation by inhibiting cell proliferation and thereby favoring functional differentiation of mammary cells (Erdmann and Breter, 1993). However, concentration of MDGI in milk fat globule membranes is unaffected by both MF and bST administration, suggesting that MDGI does not play an important role in acute regulation of mammary cell synthetic activity during established lactation (Stelwagen et al., 1994d). Schere-Levy et al. (2003) found that LIF is expressed in postpubertal, adult virgin and pregnant mouse mammary gland. LIF expression was undetectable during lactation, but sharply increased after weaning by milk stasis, suggesting that LIF may play a relevant role during mammary involution. The mammary fat pad can differentially transcribe mRNA of KGF, which is a paracrine mitogen for ruminant MEC (Hovey et al., 2001).

Hormonal treatments can interact with MF affecting milk yield and lactation persistency. When GH administration and increasing MF were tested in goats, milk yield increased in both treatments, but the greatest effect was produced by the combined treatment (Knight et al., 1990b; Boutinaud et al., 2003). The persistence of lactation increased in glands of goats treated with GH and milked 3X as a result of more secretory cells retention (Knight and Wilde, 1993). Boutinaud et al. (2003) proved that MF and GH affect different mechanisms involved in milk production in dairy goats: MF increases cell number and alveolar diameter, while GH induces cell hypertrophy and cell number maintenance. Similarly, Hale et al. (2003) concluded that the increase in milk yield with more frequent milking may be the result of increased mammary cell proliferation. A compensatory effect of GH in association with 1X has been reported in dairy cows but not in dairy goats. Thus, GH was able to reverse the decrease in milk yield by more than 10% (Carruthers et al., 1991) or to exceed the loss of milk yield (Stelwagen et al., 1994a) in dairy

cows, but GH failed to counteract the decrease in milk yield induced by 1X in goats (Boutinaud et al., 2003).

2.4.3. Nutrition

Nutrition affects mammary development during pregnancy and lactation in both rodents and ruminants. During early lactation, mammary proliferation is proportionally greater in species that exhibit less negative energy balance (Stefanon et al., 2002). This may explain why rat mammary proliferates during early lactation, while in dairy cows, that suffer negative energy balance during early lactation, mammary cells do not proliferate after parturition (Figure 5: a and c). Similarly, the mitogenic effects of GH that have been observed in pre-partum goats (Knight et al., 1994c) and cows (Collier et al., 1993), disappeared during early lactation, and returned in mid lactation when the animal's energy balance is restored (Capuco et al., 2003). Moreover, when the animal is in an undernutrition situation (similar to negative energy balance in early lactation) administration of bST resulted in increased circulating levels of bST, but levels of IGF-I did not increase, resulting in no positive effects of bST on milk yield (Figure 16).

Knight and Sorensen (2000) reported that additional concentrate improved lactation persistency in dairy cows. Epithelial cell proliferation at wk 8 postpartum was greater in cows fed high-energy density diet compared with cows fed low-energy density diet, whereas epithelial cell apoptosis did not differ (Norgaard et al., 2005). These results indicate that the cell number of the mammary gland accommodates to nutrient availability, i.e., a decrease in nutrient availability will lead to a decrease in the number of mammary cells.

In addition to energy levels, energy fuels can also modulate mammary cell turnover. Colitti et al. (2005) found that mammary apoptosis in lactating ewes fed high starch or high fat diets was lower than in ewes fed control diets; the 3 diets were iso-energetic. Inversely, Bcl-2 to Bax ratio in high starch and high fat diets was greater than in the control diet, but the apoptosis to proliferation ratio did not differ between diets.

Deficiencies in the dietary supply of specific amino acids (histidine, methionine and lysine) in early and late lactation, markedly reduced milk yield with no clear evidence of corresponding changes in measurements of mammary cell number, activity or proliferation rate (Yeo et al., 2003). Esteve et al. (1999) have emphasized the role of oxidative stress as an exogenous modulator of nuclear DNA damage and apoptosis and showed that dietary antioxidants, like vitamin E, protect against apoptosis. While vitamin E was reported to

protect against apoptosis in normal mammary cells, Kline et al. (2001) stressed its effect in inducing apoptosis in human breast cancer cells by the cell-cycle arrest in the G1 phase.
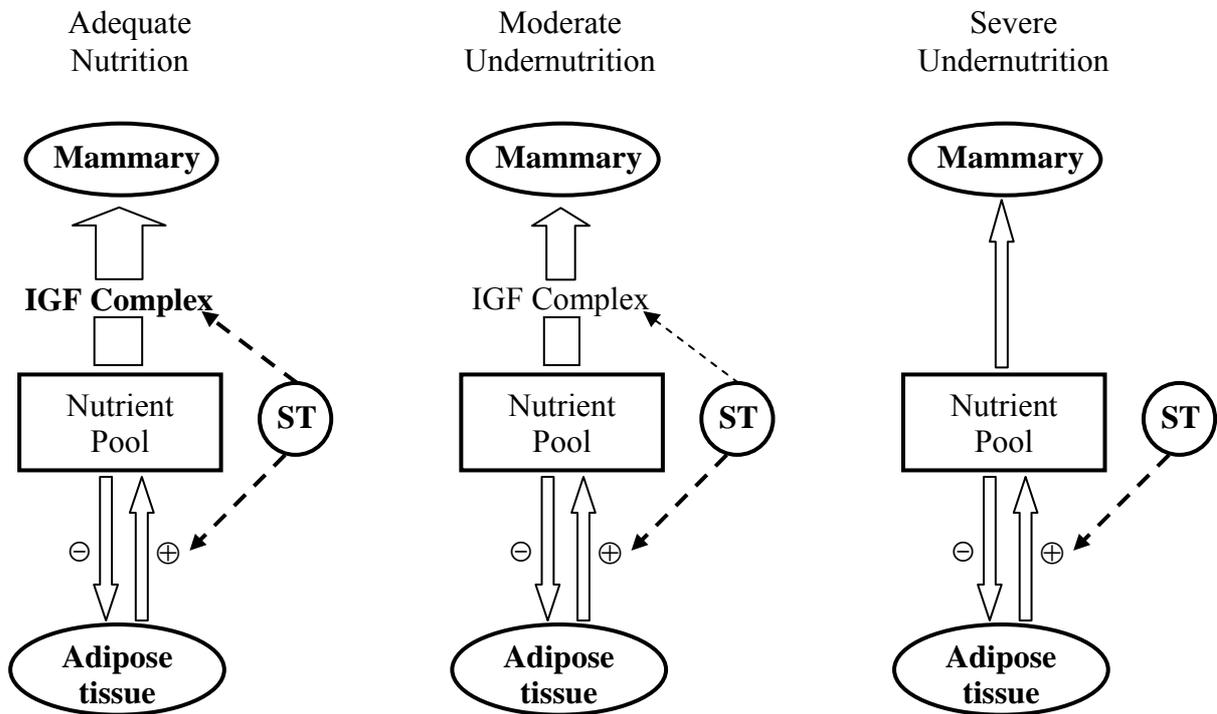
Figure 16. A model of the effects of somatotropin (ST). Direct effects include alterations in activities of key enzymes and tissue response to homeostatic signals as indicated by + and – symbols on adipose tissue rates of lipolysis and lipogenesis, respectively. Indirect effects involve the IGF complex (IGFs and IGFBPs), and these are modulated by nutritional status (Bauman and Vernon, 1993).

2.4.4. Intramammary Infection

Bovine intramammary infection (**IMI**) in vivo (Sheffield, 1997; Long et al., 2001) and in vitro (Wesson et al., 2000) increased the number of apoptotic cells. Infection elicited increases in expression of proapoptotic (Bax, caspase-3, caspase-7, and FAS) and pro-inflammatory (TNF-α and IL-1β) genes, whereas expression of the antiapoptotic gene (Bcl-2) was decreased (Long et al., 2001; Wesson et al., 2001; Didier and Bruckmaier, 2003). Induction of matrix metalloproteinases-9, stromelysine-1, and plasminogen activator were also increased, consistent with degradation of the extracelluar matrix and cell loss during mastitis (Long et al., 2001). Mastitis also increased mammary cell proliferation and levels of mRNA for IGF-I, FGF, EGF, and TGF-α, perhaps as a tissue repair mechanism after mastitis (Sheffield, 1997; Long et al., 2001). Taken together, these changes could be important in a variety of processes that occur during infection, such as protection against injury or tissue repair and recovery processes.

2.5. Cisternal Compartment

Cistern size is an important factor in determining the milkability (Labussière, 1988; Caja et al., 1999a) and the adequate interval between milkings (Knight and Dewhurst, 1994; Ayadi et al. 2003a; Salama et al., 2003b) since animals with large cisterns are milked faster with simplified routines and tolerate extended milking intervals. Moreover, cistern size plays an important role in controlling milk secretion because when the cistern reaches its full capacity, physical pressure and FIL concentration within alveoli are increased.

Since FIL acts directly on the secretory cells, it is only effective when present within the secretory alveolar lumen. Thus, animals with large cisterns should be less subject to FIL than those with small cisterns and are forced to store a high proportion of their milk within the alveolar space (Wilde et al., 1996).

Goats (Knight et al., 1989; Salama et al., 2003b) and cows (Dewhurst and Knight, 1994) with a relatively high cisternal : alveolar ratio had a lower response to increasing MF than those with a low ratio. In dairy cows, the decrease in the amount of milk yield as a result of 1X was significantly and inversely correlated (r = 0.07 to 0.81) with the proportion of cisternal milk (Knight and Dewhurst, 1994; Stelwagen and Knight, 1997; Ayadi et al., 2003b). Those authors concluded that the ability of individual cows to tolerate 1X is related to their cisternal storage characteristics; yield is reduced less in cows that store a greater proportion of their milk within the cistern. Results in dairy goats support this conclusion where losses during 1X in Canarian goats (7%), that are characterized by larger udders (Capote et al., 1999), were lower than in Murciano-Granadina goats (22%), that are characterized by medium size udders (Salama et al., 2003b). Moreover, Manchega ewes with small cisterns lost more milk during 1X than Lacaune ewes with large cisterns (Castillo et al., 2005). In contrast, Nudda et al. (2002) reported that sheep breeds with large cisterns (Sarda and Awassi) suffered more milk losses during 1X than Merino sheep.

The possibility that storage characteristics may be change in the long term by 1X can not be disregarded. Cisternal volume in goats increased after relatively short periods of 1X (Knight and Dewhurst, 1994). In addition, lactating goats become more tolerant of 1X in successive treatment periods, apparently because a greater proportion of stored milk was accommodated in the cistern of the gland rather than in the alveolar lumen (Wilde et al., 1996). Long term 1X was reported to increase udder volume (Lopez et al., 1999) and residual milk volume (Caja et al., 1999b) compared to goats milked 2X. However, Salama

et al. (2004) showed that neither cisternal milk volume nor cisternal size became larger after 5 wk of 1X milking, with 1X goats having more alveolar milk volume than 2X goats.

Cistern size and correlation coefficients between volume of cisternal milk and cistern area measured by B-mode (B = brightness) ultrasonography in different species and milking intervals are shown in Table 2.

Table 2. Cisternal area and correlation between cisternal area and cisternal milk.

Specie	Stage of lactation	Milking interval (h)	Probe freq. (MHz)	Cistern area (cm^2)	r	Reference
Cow	Mid	8	5	25.6	-	Bruckmaier & Blum (1992)
	Late	4	5	9.8 & 19.5	0.44	Ayadi et al. (2003a)[1]
	"	8	5	12.2 & 24.4	0.88	" " "
	"	12	5	14.6 & 30.3	0.84	" " "
	"	16	5	24.4 & 41.1	0.63	" " "
	"	20	5	21.5 & 41.0	0.51	" " "
	"	24	5	22.0 & 41.0	0.46	" " "
	Early	12	5	12.1	0.82	Caja et al. (2004)[2]
	Mid	"	5	8.0	0.74	" " "
	Late	"	5	4.2	0.80	" " "
Buffalo	Early	12	6	15.4 & 21.2	-	Thomas et al. (2004)[1]
	Mid	"	6	14.1	-	" " "
	Late	"	6	7.1 to 7.2	-	" " "
Sheep	Mid	8	5	19.0	-	Bruckmaier & Blum (1992)
	Late	24	3.5	19.0	0.82	Nudda et al. (2000)
	Whole	8	5	14.0 & 24.0	0.76	Rovai et al. (2002)[3]
Goat	Mid	8	5	16.5	-	Bruckmaier & Blum (1992)
	Early	8	5	11.1	0.74	Salama et al. (2004)
	"	16	5	21.2	0.63	" " "
	"	24	5	27.3	0.36	" " "

[1] Data of cistern area for front and rear quarters, respectively.
[2] Data of cistern area are for front quarters.
[3] Data of cistern area are for Manchega and Lacaune breeds, respectively.

Ultrasonography creates cross sections of body tissues based on the intensity of high frequency sound waves reflection. Different reflection intensities are reproduced in different degrees of brightness, i.e. in different tones of gray on the screen. The intensity of sound reflection depends on the degree of variation in acoustic impedance of adjacent structures within a certain tissue. The terms hyperechoic (high intensity), hypoechoic (low intensity), and anechoic (no reflection) are used to describe these reflection intensities (Cartee et al., 1986). Homogenous structures with relatively uniform density or stiffness tend to transmit rather than reflect the ultrasound and tend to create large acoustic impedance differences between them and adjacent non-homogeneous material. Thus, fluids (which are homogenous) appear anechoic on ultrasound images (black color) and allow good visualization of the boundaries between them and more non-homogenous tissues (Figure 17). Therefore, ultrasonography is a very suitable method for measuring cisternal size in a non-invasive way and used frequently in udder measurements in dairy cows (Bruckmaier and Blum, 1992; Bruckmaier et al., 1994b; Ayadi et al. 2003a; Caja et al., 2004), meat sheep (Ruberte et al., 1994; Caja et al., 1999a), dairy sheep (Nudda et al., 2000; Rovai et al., 2002), dairy goats (Bruckmaier and Blum, 1992; Salama et al., 2004) and buffaloes (Thomas et al., 2004).
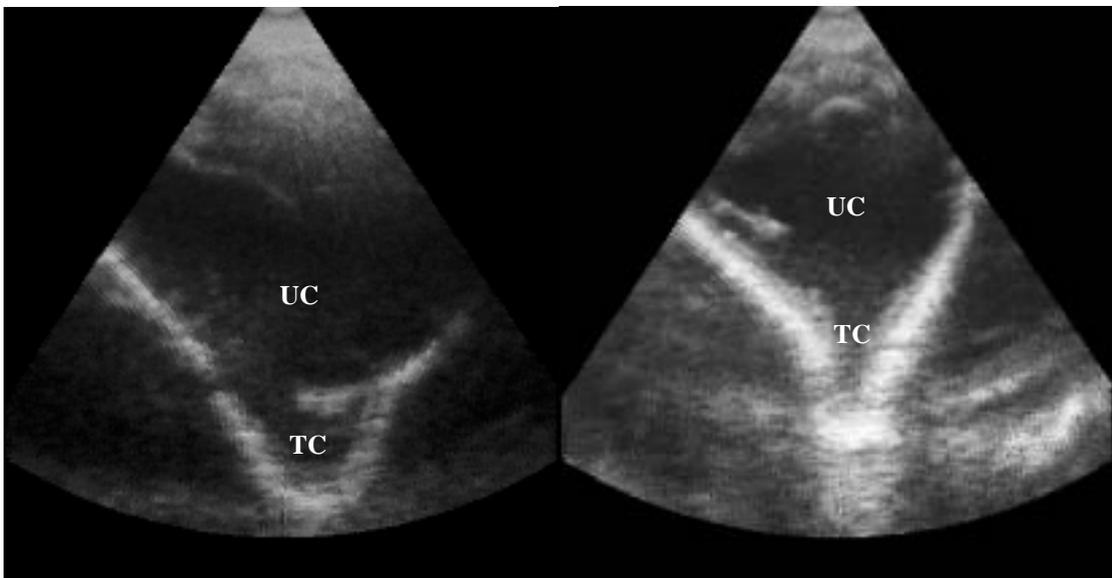
Figure 17. Udder ultrasound scans in dairy goats (Salama et al., unpublished). UC = udder cistern, TC = teat cistern.

2.6. Milk Distribution between Alveolar and Cisternal Compartments

2.6.1. Fractional Milk Yield

As previously shown, milk in the udder is distributed between alveolar and cisternal compartments. Cisternal milk is obtained passively by overcoming the teat sphincter barrier, whereas alveolar milk is actively obtained by inducing the process of milk ejection. Milk ejection during suckling and machine milking occurs via the binding of OT with its receptors on the mammary myoepithelial cells (Linzell, 1955). The number of OT receptors does not change during lactation (Soloff, 1982). If the binding between endogenous OT and its receptors is blocked by an OT receptor antagonist, cisternal milk can be separated reliably during machine milking (Knight et al., 1994b; Wellnitz et al., 1999; McKusick et al., 2002ab; Rovai et al., 2002; Ayadi et al., 2003ab; Salama et al., 2004, 2005a; Thomas et al., 2004; Castillo et al., 2005). Separation of both milk fractions can also be performed using catheter drainage or adrenalin injection (Knight et al., 1994ab; Davis et al., 1998). Direct teat cannulation may induce a transient OT release despite careful catheterization (Mayer et al., 1991), which can result in an overestimation of the quantity of cisternal milk. In accordance, Caja et al. (2000) reported significantly lower values of cisternal milk when an OT-blocking agent was used in dairy ewes compared with catheter drainage. Knight et al. (1994b) observed lower percentages of cisternal milk using direct teat cannulation or milking under the effects of an OT receptor blocking agent (57 to 59% of total milk) than when using adrenalin (85%). However, cisternal proportion obtained by catheter drainage was similar to that obtained by adrenalin injection in dairy cows (Davis et al., 1998).

Milk partitioning between alveolar and cisternal compartments varies widely according to specie, breed, lactation stage, parity and milking interval (Figure 18). Percentages of cisternal milk for normal milking intervals (8 to 16 h) are low (10 to 30%) in dairy cows (Pfeilsticker et al., 1996; Davis et al., 1998; Ayadi et al., 2003a). Range of variation is greater in dairy ewes (40 to 80 %) according to sheep breed (Nudda et al., 2000; McKusick et al., 2002a; Rovai et al., 2002). In goats, Peaker and Blatchford (1988) reported greater cisternal milk percentages, ranging according to time elapsed after milking from 65% (1 h) to 88% (16 h) in Saanen goats. Using goats of the same breed 5 h after milking, Knight et al. (1994b) recorded lower values than the previous study (58%). Moreover, Salama et al. (2004) reported that cisternal to alveolar milk ratio in goats increased from 57:43 to 75:25 at 8 and 24 h after milking, respectively. Cisternal milk represented only 5%

of the total milk in buffaloes (Thomas et al., 2004). This low cisternal fraction in buffaloes means that cisternal milk will be milked out rapidly before the occurrence of milk ejection, resulting in limped collapsed teats and incomplete milking (Thomas et al., 2004). Therefore, it is crucial to elicit milk ejection before machine milking begins in buffaloes (Thomas et al., 2004).
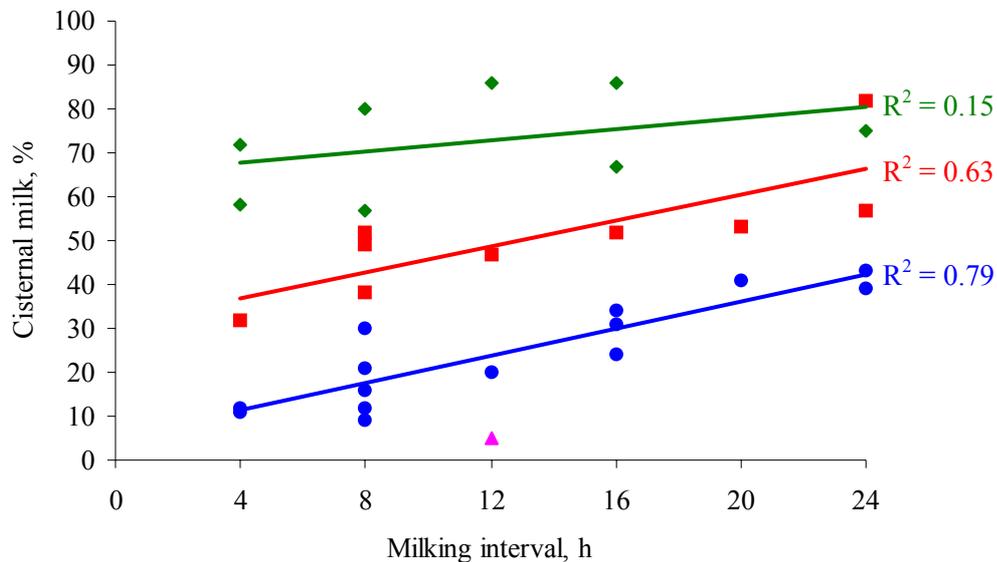
Figure 18. Cisternal milk fraction in dairy cows (●; Knight and Dewhurst, 1994; Knight et al., 1994a; Stelwagen and Knight, 1997; Davis et al., 1998; Ayadi et al., 2003a), dairy sheep (■; Nudda et al., 2000; McKusick et al., 2002a; Rovai et al., 2002), dairy goats (♦; Peaker and Blatchford, 1988; Knight et al., 1994b; Salama et al., 2004), and buffaloes (▲;Thomas et al., 2004).

The quantity of cisternal milk decreased, while its proportion increased as stage of lactation advanced due to the decrease in total milk yield over time (Dewhurst and Knight, 1993; Pfeilsticker et al., 1996; Caja et al., 2004). Nevertheless, Stelwagen and Knight (1997) reported no effect of lactation stage on cisternal milk amount or proportion. Salama et al. (2005a) reported that amounts of both cisternal and alveolar milk in goats in early lactation were greater than in late lactation. However, cisternal milk proportion in early lactation (76%) was greater than in late lactation (58%), which contradicts the results obtained in dairy cows. Moreover, cisternal milk volume was greater for multiparous compared to primiparous dairy cows (Pfeilsticker et al., 1996) and goats (Salama et al., 2004), which agrees with the greater cisternal size in multiparous cows (Bruckmaier et al., 1994b) and goats (Salama et al., 2004).

2.6.2. Composition of Milk Fractions

Little information exists in literature on the fractional composition of milk in dairy goats. Cisternal milk contains lower fat than alveolar milk in dairy cows (Davis et al., 1998; Waldmann et al., 1999; Ontsouka et al., 2003; Ayadi et al., 2004), dairy ewes (McKusick et al., 2002ab) and dairy goats (Salama et al., 2005a). Milk fat globules are large and do not pass freely from alveoli to cistern, and therefore more fat is retained in the alveolar compartment (McKusick et al., 2002a; Ayadi et al., 2004). As time after milking increased, concentration of fat decreased in cisternal milk and increased in alveolar milk (McKusick et al., 2002a; Ayadi et al., 2004). McKusick et al. (2002a) and Ayadi et al. (2004) indicated a transfer of milk fat from alveoli to cistern during early udder filling; however, this transfer was no longer taking place during the longer intervals, resulting in an accumulation of milk fat in the alveolar compartment. This underlines the importance of milk ejection and complete milking for the recovery of milk that is rich in fat (Labussière, 1969; McKusick et al., 2002ab; Ontsouka et al., 2003; Ayadi et al., 2004).

Differences between alveolar and cisternal contents of milk protein and lactose are minimal in dairy cows (Davis et al., 1998; Ayadi et al., 2004), dairy ewes (McKusick et al., 2002ab) and dairy goats (Salama et al., 2005a). Synthesis of protein and lactose is regulated by a final shared metabolic pathway (Fitzgerald et al., 1970), thus it is expected that both components change similarly. Milk protein is found in the form of small CN micelles (Cowie and Tindal, 1971) and therefore could pass easily between alveolar and cisternal compartments between milkings, resulting in minimal differences in protein concentration of milk fractions. Protein concentration increased in the cisternal and alveolar milk with extended milking intervals in cows (Ayadi et al., 2004) and ewes (McKusick et al., 2002a).

The SCC in cisternal milk collected before milking was similar to that of later milk fractions during milking in dairy cows (Ontsouka et al., 2003). However, SCC in foremilk was lower than in stripped milk in dairy ewes (Peris et al., 1991) and dairy cows (Bruckmaier et al., 2004). In dairy ewes, cisternal and alveolar SCC was similar for milking intervals of 4, 8 and 12h, however, at longer milking intervals cisternal SCC was lower than alveolar SCC (McKusick et al., 2002a). In goats milked 1X, alveolar SCC was greater than cisternal SCC in early lactation, but not in late lactation (Salama et al., 2005a).

3. Effect of Once Versus Twice Daily Milking on Milk Production

3.1. Milk Yield

Reducing MF from 2X to 1X resulted in milk yield losses in different dairy species (Table 3). From the information previously discussed on milk stasis within the alveoli and effect of MF on MEC number and activity, the response to a change in MF can be considered at 3 levels:

- Acute response, takes place within hours according to alveolar distension and FIL concentration.

- Short term response, takes place within days involving changes in MEC activity.

- Long term response, takes place over a period of weeks and involves changes in MEC number through the balance between proliferation and apoptosis.

In addition, the ability of the alveoli to drain between milkings affects the response to MF. Milk drainage from alveoli is determined by the tone of the milk ducts which is regulated through α-adrenergic receptors (Bruckmaier et al., 1997). Moreover, valve-like structures have been identified at the exist sites of the milk ducts and these may have a role in generating resistance to alveolar drainage (Caruolo, 1980). Cows with a greater cisternal filling at 8-h (Knight and Dewhurst, 1994; Stelwagen and Knight, 1997) and at 24-h (Davis et al. 1998) milking intervals were more tolerant to 1X because of elevated levels of milk drainage from the alveoli to the cistern.

Parity number also affects the response to MF. Yield losses during 1X were greater in heifers than mature cows (Carruthers et al., 1993a) and in primiparous goats than multiparous goats (Salama et al., 2003b), which could be linked to the relative immaturity of cisternal development in primiparous animals. Nevertheless, Bach and Busto (2005) reported that primiparous dairy cows milked with robot were less sensitive to irregular milking (unequal milking intervals and teat cup attachment failure) than multiparous dairy cows.

Table 3. Milk yield loss during 1X compared to 2X in dairy animals.

Species	Stage of lactation	1X duration	% loss	Reference
Cow	Early	3 wk	38	Stelwagen and Knight (1997)
	"	2 d	20	Auldist and Prosser (1998)
	"	3 wk	32	Rémond et al. (2002)
	Mid	5 d	15	Stelwagen and Lacy-Hulbert (1996)
	"	2 wk	18	Carruthers et al. (1993b)
	Late	2 wk	7	Stelwagen et al. (1994a)
	"	2 wk	11	Davis et al. (1998)
	"	5-13 wk	11-15	Lynch et al. (1991)
	"	2 wk	13	Carruthers et al. (1993b)
	"	4 wk	13	Lacy-Hulbert et al. (1999)
	" (low SCC)	4 wk	14	Kamote et al. (1994)
	" (high SCC)	"	26	" " "
	"	3 wk	28	Stelwagen and Knight (1997)
	"	1 wk	35	Kelly et al. (1998)
	"	10 wk	30	O'Brien et al. (2002)
	Whole	Whole	35	Holmes et al. (1992)
	"	"	30	Rémond et al. (2004)
Overall mean			**22**	
Sheep	Early	8 d	19	Morag (1968)
	Early-Mid	8 wk	8-34	Castillo et al. (2005)
	Mid	6 d	15	Negrao et al. (2001)
	Mid-Late	12 wk	20	Knight and Gosling (1994)
	Mid-Late	26 wk	28	Papachristoforou et al. (1982)
	Late	4 d	18-24	Nudda et al. (2002)
	Whole	Whole	35	Labussière et al. (1974)
	"	Whole	48	Knight et al. (1993)
Overall mean			**25**	
Goat	Early	4.5 wk	26	Wilde and Knight (1990)
	"	Whole	21	Salama et al. (2003b)
	Mid	6 wk	7	Papachristoforou et al. (1982)
	Late	8 wk	6	" " "
	"	"	17	Salama et al. (2003b)
	Whole	Whole	35	Mocquot (1978)
	"	"	6	Capote et al. (1999)
	"	"	19	Salama et al. (2003b)
Overall mean			**17**	

3.2. Milk Composition

Production of more concentrated milk may be a feature of adaptation to extended milking intervals as in the case of 1X. Carruthers et al. (1993b) explored the possibility that dairy cows which produce relatively more concentrated milk (i.e., produce less lactose, relative to fat and protein) may be more tolerant to 1X. Many of the changes in milk composition during 1X can be related to changes in the permeability of the TJ between the MEC leading to increased exchange of milk and interstitial fluid (Davis et al., 1999).

Milking 1X caused significant increases in fat and total protein percentages in dairy cows (Carruthers et al., 1991; 1993b; Stelwagen et al., 1994a; Lacy-Hulbert et al., 1999; O'Brien et al., 2002; Rémond et al., 2004). Nevertheless, Holmes et al. (1992) and Rémond et al. (2002) reported no changes in fat and protein during 1X.

Milk of 1X ewes had a greater percentage of protein and a lower percentage of lactose than milk of 2X ewes; there was no difference in fat percentage (Knight and Gosling, 1994). Goats milked 1X had slightly lower fat and protein in milk than 2X (Capote et al., 1999). Nevertheless, Salama et al. (2003b) reported that milk of 1X dairy goats contained greater fat and similar protein compared to milk of 2X goats. Variation in results between studies may be due to differences in breed, cisternal size, 1X duration and lactation stage when 1X was applied.

Because of the large size of the CN micelles, CN does not leak out of the milk pool via leaky TJ (Stelwagen et al., 1998b). Thus, while milk volume is lower with 1X, CN becomes more concentrated in the milk of dairy cows (Lacy-Hulbert et al., 1999) and dairy goats (Salama et al., 2003b). Nevertheless, there was no difference in milk CN between cows milked 1X and 2X during early (Rémond et al., 2002), late (O'Brien et al., 2002) and whole lactation (Rémond et al., 2004). A decrease in milk lactose content is the most consistent change induced by 1X due to leaky TJ (Davis et al., 1999). Nevertheless, 1X did not affect milk lactose in dairy cows (Rémond et al., 2002; 2004) and goats (Capote et al., 1999).

Once daily milking for 14 d (Stelwagen et al., 1994a), 26 d (Lacy-Hulbert et al., 1999), 3 wk (Rémond et al., 2002), 10 wk (O'Brien et al., 2002), and whole lactation (Rémond et al., 2004) did not affect milk SCC compared with 2X in dairy cows. Similarly, 1X throughout lactation in dairy goats did not affect milk SCC (Salama et al., 2003b). Nevertheless, 1X for 8 mo (Holmes et al., 1992) increased milk SCC in cows. A short

period of 1X (6 d) increased yield and concentration of SCC, percentage of neutrophils and bovine serum albumin (Stelwagen and Lacy-Hulbert, 1996). During a subsequent 4-d 2X period, SCC decreased, but bovine serum albumin content and percentage of neutrophils remained high.

The increase in SCC during 1X was not associated with damage to MEC. The cause of SCC increase during 1X is not entirely clear, but impairment of the TJ barrier may have facilitated a paracellular influx of somatic cells into milk (Stelwagen and Lacy-Hulbert, 1996). The 1X-related increase in the proportion of Polymorphonuclear neutrophil may affect milk quality because Polymorphonuclear neutrophil can ingest milk fat globules and CN (Frost et al., 1984). Once daily milking in late lactation did not affect yield of somatic cells, but increased SCC, with the effect being greater in the cows with high initial SCC suggesting that cows with low SCC are more resistant to the adverse effects of 1X in late lactation (Kamote et al., 1994; Holmes et al., 1992). These last two studies suggest that if the initial SCC level is low, 1X leads to a small increase in SCC, but if the initial level of SCC is high (as the case of animals with subclinical mastitis), 1X will result in SCC rising to very high levels. This also implies that using 1X as a management practice needs to be coupled to improved mastitis management.

4. Effect of Dry Period Length on Milk Production

Throughout the last four decades, a dry period length of 50 to 60 d has been widely recommended for dairy cows. It is anticipated that the milk yield that is lost during the non-lactating period will be recovered during the subsequent lactation. However, the profitability will increase if milk yield during the subsequent lactation is sustained after dry periods that are shorter than this classical standard. Thus, the objective is to identify the shortest dry period length that does not result in a decrease in milk yield during the subsequent lactation.

Studies conducted to determine the effect of dry period length on milk yield in the subsequent lactation can be classified to two categories (Bachman and Schairer, 2003):

- Retrospective analysis of observational milk production data: in this type of studies, the number of records for animals with the conventional 60-d dry period is greater than the number of records for animals with shorter dry period length. Moreover, animals are not assigned randomly to each dry period length and therefore, results obtained from these studies (Schaeffer and Henderson, 1972; Funk et al., 1987; Makuza and McDaniel, 1996) are biased. Additionally, many of the retrospective studies, upon which the present standard of the 60-d dry period is largely based, did not consider factors that can affect milk yield subsequent to a dry period of any given length. Those factors are: herd, cow within herd, year, season, breed, parity, diet, body condition, health, bST use protocol, MF, previous milk yield, previous days open, and current days open (Bachman and Schairer, 2003). However, Kuhn and Hutchison (2005) showed that correction for the previous lactation milk yield and cow effects from an animal model reduces the bias in observational data. In addition, observational studies with a larger sample size have the potential to provide much more information than designed trials (the effects of dry period length on herd life, lifetime production, and interactions with other factors such as parity, calving season and previous lactation milk yield, days open, or SCC).

- Planned animal trials: in these studies each animal is assigned to a planned dry period length at random, irrespective of the milk production level and the amount of milk at the moment of drying off. Thus, results are reliable because a similar number of animals, with varied genetic ability to produce milk, are assigned to each planned dry period length (Swanson, 1965; Smith et al., 1966; Coppock et al., 1974; Sorensen and Enevoldsen, 1991; Rémond et al., 1992; 1997; Bachman, 2002; Gulay et al., 2003a, b; Annen et al., 2004d; Rastani et al., 2005).

4.1. Why the Dry Period is Important?

At the time of drying off, dairy cows are most often in the last trimester of pregnancy, while ewes and goats may be nopregnant or in the second trimester of pregnancy. When milk stasis occurs in pregnant animals, the mammogenic and lactogenic stimulation of pregnancy opposes stimuli for mammary involution. Traditionally, the sequential events occurring within bovine mammary tissue during a 60-d dry period were classified as: 1) active involution (transition from lactating to non-lactating state), 2) steady state involution (non-lactating state), and 3) differentiation (colostrum formation); with respective durations of approximately 21, 18, and 21 d (Hurley, 1989). Thus, a dry period of < 40 d would be considered insufficient time for the sequential processes of involution to occur (Hurley, 1989). However, the importance of steady state involution has never been established (Grummer and Rastani, 2004).

Swanson et al. (1967), based on comparisons of mammary weight, total mammary DNA content and histology, concluded that little cellular involution occurred during dry periods that ranged from 15 to 75 d in dairy cows. Similarly, Capuco et al. (1997), using a combined evaluation of tissue morphology and total udder DNA, reported that throughout a 60-d dry period no net loss of MEC occurred, tissue area occupied by epithelium did not decrease, and alveolar structures remained intact when compared with 0-d dry period (**D0**).

However, a non-lactating dry period between successive lactations is necessary to permit replacement of damaged or senescent epithelial cells through the processes of cell turnover (Capuco et al. 1997; Fitzgerald et al., 2004), which, in turn, allows maximum milk production to occur during the ensuing lactation. The process of cell turnover during dry off in pregnant animals includes apoptosis (see above) and proliferation. Senescent cells die by apoptosis and are then replaced by new cells (proliferation). Figure 19 shows apoptosis and proliferation indices in MEC during dry off in dairy cows (Wall et al., 2005).

Proliferation of MEC in glands dried off for 60 d was greater than in D0 glands of primiparous dairy cows, indicating that milk yield losses of D0 glands in the subsequent lactation are due to reduced cell proliferation during the dry period (Fitzgerald et al., 2004; Annen et al., 2004b). The fact that total cell number remains constant despite reductions in MEC proliferation, suggests an alteration in mammary cell turnover that would result in less replacement of senescent MEC during late pregnancy. If old MEC have reduced secretory and miotic capacity (Capuco and Akers, 1999), this change in MEC turnover which increases carryover of old MEC into the ensuing lactation may reduce mammary

functionality in D0 udders (Capuco et al., 1997; Annen et al., 2004a). Senescent cells can not be stimulated to proliferate by any known physiological signals, despite apparently normal growth factor receptor number and affinity (Oshima and Campisi, 1991).
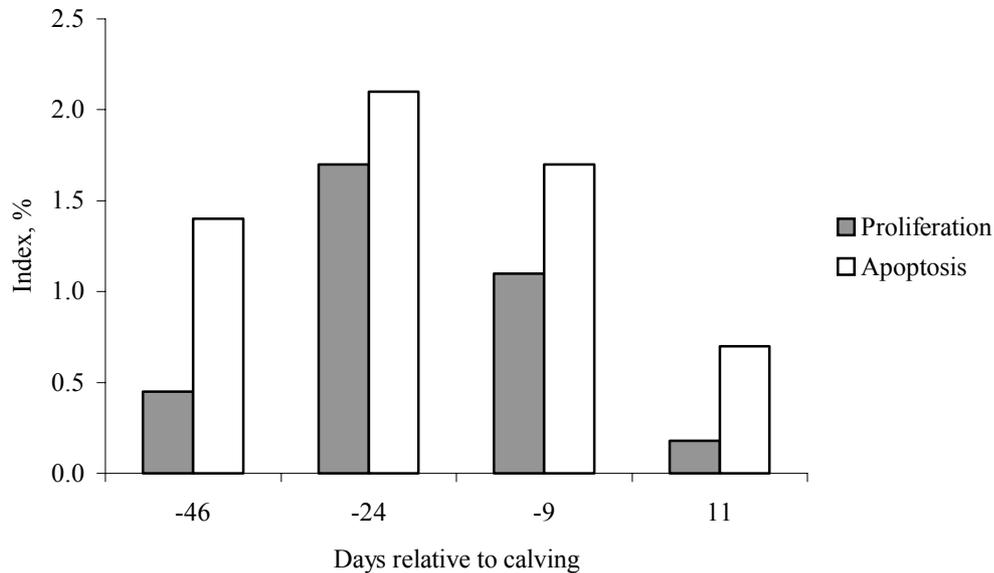
Figure 19. Indices of apoptosis and proliferation at -46, -24, -9, and 11 d relative de parturition in dairy cows (Wall et al., 2005).

4.2. Effect of Dry Period Length on Subsequent Milk Yield

As shown in Table 4, omitting the dry period in dairy cows reduced milk production in the subsequent lactation by 6 to 52%, suggesting that a dry period between lactations is necessary in dairy cows. These milk losses are due to reduced functionality of MEC rather than genetic, endocrine or nutritional factors. Moe (1981) showed that lactating cows convert metabolizable energy into fat with about 75% efficiency; but when dry, efficiency decreases to 60%. So, for maximum energetic efficiency, reserves should be restored during late lactation making the dry period unnecessary to replenish energy reserves depleted during lactation. Due to their positive effect on MEC number and activity, administration of bST and increasing MF may alleviate milk yield losses due to omitting the dry period (Annen et al., 2004d). Nevertheless, negative effects of continuous milking in dairy cows were not overcome by bST supplementation or increasing MF (Annen et al., 2004b; Collier et al., 2004). No carryover effect of omitting the dry period was detected after animals were given a dry period (Swanson, 1965; Coppock et al., 1974).

No significant differences were reported in the subsequent milk yield values for cows dry for 60 d vs. those dry for 30 to 40 d (Table 4). That 30 d is a sufficient period for drying

off is supported by the observation that mammary growth was initiated within the first 25 d of a 60-d dry period (Capuco et al., 1997). Nevertheless, cows which had a 30-d dry period produced 10.2% less daily milk yield during 84 d of lactation compared with cows that were dry for 50 d (Sorensen and Enevoldsen, 1991). Moreover, using the within-cow experimental design, half udders that had been dry for 30 d produced less milk yield (-22%), during the first 30 d of the subsequent lactation, than those half udders that had been dry for 70 d (Gulay et al., 2003b).

Table 4. Effect of dry period length on milk yield in the subsequent lactation in dairy cows.

Days dry	Change (%)	Reference
0 vs. 60 (2nd lactation)	-25	Swanson (1965)
0 vs. 60 (3rd lactation)	-38	" "
0 vs. 60	-38 to -44	Smith et al. (1966)
0 vs. 60	-17	Rémond et al. (1992)
0 vs. 60	-23	Rémond et al. (1997)
0 vs. 49	-24	Madsen et al. (2004)
0 vs. 60	-20	Rastani et al. (2005)
0 vs. 60 (primiparous)[1]	-18	Annen et al. (2004d)
0 vs. 60 (multiparous)[1]	-6	" " " "
0 vs. 60 (primiparous)	-40	Fitzgerald et al. (2004)
0 vs. 60 (primiparous)	-52	Annen et al. (2004b)
20 and 30 vs. 50	-7 to -10	Coppock et al. (1974)
30 vs. 50	-10	Sorensen & Enevoldsen (1991)
30 vs. 60	-2	Bachman (2002)
30 vs. 60	-3	Gulay et al. (2003a)
30 vs. 60	-11	Rastani et al. (2005)
30 vs. 60 (primiparous)	-13	Annen et al. (2004d)
30 vs. 60 (multiparous)	-3	" " " "
30 vs. 70	-22	Gulay et al. (2003b)

[1] Cows treated with bST.

Parity number seems to affect the response to dry period length (Table 4). In primiparous and multiparous cows treated with bST, D0 reduced milk yield during the first 17 wk of the subsequent lactation in the primiparous cows but not in the multiparous cows (Annen et al., 2004d). This implies that profitability in multiparous cows was improved

through increased net milk income generated by omitting the dry period using bST. Similarly, Schaeffer and Henderson (1972) and Funk et al. (1987) noted that cows completing their first lactation benefit more from longer dry periods than older cows. Nevertheless, Sorensen and Enevoldsen (1991) found no evidence that first lactation cows respond differently to dry period length than older cows.

A dry period between successive lactations is also necessary for optimal lactation in the rat (Paape and Tucker, 1969). Litters weight and mammary DNA were highest when primiparous mothers had a 4- or 8-d dry period as compared with 0-, 12-, or 16-d dry periods, indicating that subsequent lactation yield increases with increasing length of the dry period up to a certain point, but a further increase has no beneficial effect on the yield of milk in the following lactation. Pitkow et al. (1972) also demonstrated reduced milk secretion in D0 rats, but provided evidence that omitting the dry period resulted in an increase in mammary cell carryover from one lactation to the next.

In humans, nursing throughout the last trimester of pregnancy caused a 15% reduction in weight gain of infants during the first mo postpartum (Marquis et al., 2002). Moreover, the infants from D0 women breastfed longer per feeding in a 24-h time period than infants from women that had not breastfed during pregnancy. Despite the longer feeding times, infants from D0 mothers had a tendency for lower intakes (Marquis et al., 2002). These results suggest a reduced milk secretion in D0 mothers, possibly due to reduced MEC activity or number.

It is almost clear that a dry period between lactations is necessary in dairy cows, rats and human. The situation seems to be different in dairy goats because one study with Saanen goats (Fowler et al., 1991) concluded that D0 did not reduce subsequent milk production. Moreover, half udders that started the subsequent lactation with no dry period had greater parenchyma weight and secretory cell number at wk 16 of lactation than half udders that were dried off for 23 wk (Figure 20). Those authors suggested that involution occurred in the continuously milked glands but was delayed. Therefore, growth of mammary glands that were dried off was completed pre-partum, while glands with no dry period continued to proliferate post-partum (Figure 20). Similarly, when the dry period was omitted in mice by re-mating at the post partum estrus, alveolar integrity was lost and DNA laddering was maximal at d 20 of the first lactation (Wilde et al., 1999). Alveolar integrity was partially restored by d 22 (the 1st day of the following lactation), but DNA laddering remained intense, indicating that apoptosis did not cease with parturition. Not until d 5 of

the 2nd lactation tissue morphology and laddering intensity was comparable to that observed in established lactation. Thus, it appears that a period of mammary involution is an obligatory requirement for the subsequent lactation, and concurrently-pregnant, lactating mice accommodate this by the induction of tissue remodeling and apoptosis at the start of the subsequent lactation compared with their non pregnant counterparts.
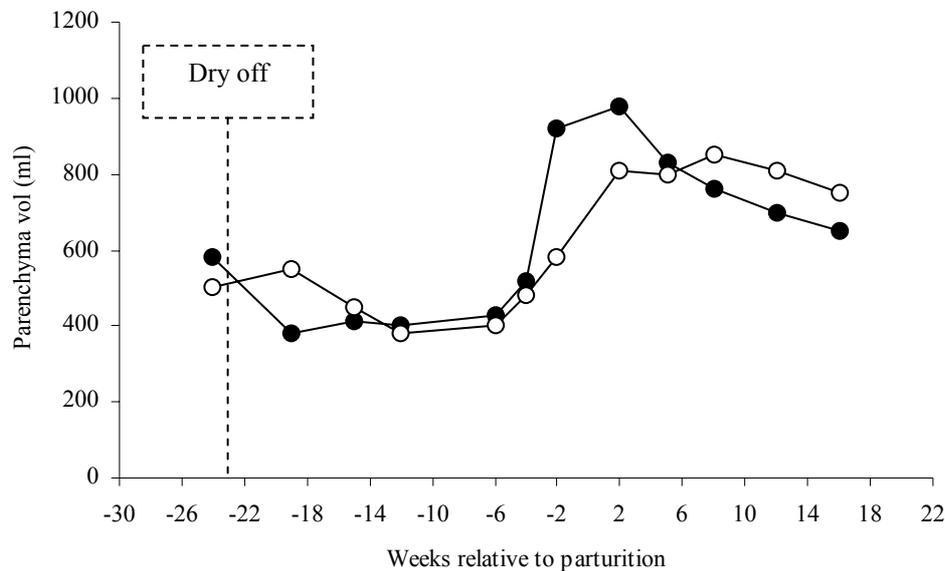
Figure 20. Mammary parenchyma volume for individual glands with (●) or without (○) dry off (Fowler et al., 1991).

In the study of Fowler et al. (1991), half udders were dried off 2 wk before mating (dry period length was 23 wk), which is three times the optimal length for cows. This long period may have obviated the benefit of a dry period and results might differ if goats had shorter dry periods. Additionally, the effect of the half udder experimental design should be considered. When one gland is no longer milked, milk yield (Hamann and Reichmuth, 1990) and mammary growth (Capuco and Akers, 1990) increased in a compensatory manner in the lactating glands within the same udder, and involution was partially inhibited in the nonlactating gland (Akers and Keys, 1985). Since significant mammary growth occurs during early lactation in goats (as discussed earlier; Figure 5b), and milking induces the release of somatotropin in goats (Hart and Linzell, 1977), but not in cows (Lefcourt et al., 1995), the mammary gland of goats may have a greater capacity to continue cell-renewal processes, such as those detected during a bovine dry period, into early stages of lactation, thus largely decreasing the importance of a dry period in goats. However, omitting the dry off period in both udder halves reduced milk yield by 28% compared with 56-d dry off period (**D56**) in dairy goats mated at wk 29 of lactation (Salama et al., 2005b).

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4.3. Effect of Dry Period Length on Milk Composition and Colostrum Production

Milk fat, protein and SCC in D0 cows increased during the last 2 mo of pregnancy with a more substantial increase occurring during the last 4 wk of pregnancy (Wheelock et al., 1965; Rémond et al., 1992; Annen et al., 2004a). Postpartum milk fat content was not affected by dry period length (Wheelock et al., 1965; Rémond et al., 1992; Annen et al., 2004c; Madsen et al., 2004), but FA profile in milk fat may be altered. As D0 cows have less negative energy balance at the start of the subsequent lactation due to lower milk yield and/or improved DMI (Rémond et al., 1992, 1997; Rastani et al., 2005; Madsen et al., 2004), a decrease in long chain FA in milk of D0 animals is expected due to lower body fat mobilization (Rémond et al., 1992). Postpartum milk protein content was greater (Rémond et al., 1992; Annen et al., 2004a; Madsen et al., 2004) or unchanged (Smith et al., 1967; Annen et al., 2004c) in D0 cows compared with cows that dried off. The increase in milk protein content may be the result of improved energy balance or protein balance in D0 cows; thereby sparing AA and energy for protein synthesis (Rémond et al., 1992).

Milk SCC after parturition tended to be greater (Annen et al., 2004a) or unchanged (Annen et al., 2004c) in D0 cows compared with cows that dried off for 60 d. The elevated SCC in D0 cows may be due to the fact that cows with 60-d dry period were treated with antibiotics before drying, while udders of D0 cows were not treated (Rémond et al., 1997).

Mammary TJ are leaky during late pregnancy and close around parturition (5 d before kidding) due to progesterone withdrawal and elevated levels of PRL (Linzell and Peaker, 1974; Nguyen et al., 2001). The increase in TJ permeability has been reported to be associated with decreased milk secretion rate (Stelwagen et al., 1995) and an increase in milk contents of plasma constituents (Rémond and Bonnefoy, 1997). Therefore, very low milk yields in some D0 animals before parturition are more similar to dry secretions than milk, affecting negatively the physical properties and composition of that milk.

Colostrogenesis results in enhanced protein and immunoglobulin (**Ig**) concentrations in the colostrum (Wheelock et al., 1965). The absence of this accumulation period in D0 animals may result in reduced colostrum quality. Little is known about the effect of dry period length on colostrum quality. Cows with D0 may produce colostrum with low Ig concentrations (Brandon and Lascelles, 1975; Rémond et al., 1997). In humans, an overlap of breast-feeding and late pregnancy also resulted in reduced Ig concentrations in colostrum (Marquis et al., 2003). Omitting the dry period in dairy goats reduced the colostrum content of IgG compared with D56 goats (Salama et al., 2005b).

5. Effect of Pregnancy on Milk Production

Pregnancy causes a significant decline in the milk yield of dairy cows. The mechanism by which pregnancy influences milk yield is not fully understood, but it is believed to be caused by the estradiol and progesterone hormones that maintain the pregnancy (Stefanon et al., 2002). Circulating estradiol is low until d 60 of pregnancy in goats, increases gradually until the last wk of pregnancy, and then rises rapidly to a peak value before kidding (Dhindsa et al., 1981). Estradiol concentration in blood was greater in goats carrying multiple fetuses than in those carrying only one, suggesting that the feto-placental unit is the source of estrogen during pregnancy (Dhindsa et al., 1981; Manalu et al., 1996). Plasma progesterone concentrations throughout pregnancy in dairy goats (5 to 15 ng/mL) were similar to levels observed during the luteal phase (Kornalijnslijper et al., 1997) being greater in twin- than in single-bearing does (Manalu et al., 1996).

With regard to the estrogen impact on lactation persistency, Peaker and Linzell (1974) found that administration of estrogen at doses designed to match the secretion rate in late pregnancy caused a significant decline in milk yield in goats. Moreover, estradiol injection for several days before drying off accelerated mammary tissue remodeling and involution in dairy cows (Athie et al., 1996) and goats (Mellado et al., 1998) by promoting plasminogen activation (Athie et al., 1997). Placental lactogen has also been proposed to mediate the effect of pregnancy on mammary development in declining lactation and thereby on lactation persistency (Capuco et al., 2002; Stefanon et al., 2002).

The effect of progesterone on mammary development in pregnant animals in late lactation, could be the promotion of MEC survival. Administration of progesterone or glucocorticoids inhibited apoptosis in mammary gland during lactation (Berg et al., 2002) and involution (Feng et al., 1995) in non pregnant mouse. In addition, pregnancy delays involution of the mouse mammary gland after forced weaning on d 10 of lactation by a mechanism that involves the combined stimulation of cell proliferation and inhibition of apoptosis (Capuco et al., 2002). This result is consistent with the concept that mammogenic effects of pregnancy partially offset the local effects of milk stasis on mammary involution.

In addition to the hormonal effect, yield losses may be due to the energy demand of the fetus, specially after d 190 of pregnancy in dairy cows (Bell et al., 1995). The later authors reported a quadratic increase in fetal energy content, with energy deposition increasing by 10% from d 190 to 210 of gestation and in each subsequent 20-d period. However, Prior and Laster (1979) reported an increase in the energy demand of the fetus

after d 90 of pregnancy. Moreover, Roche (2003) calculated that the energy demand of the fetus in pregnant cows of USA is 18% greater than in New Zealand because calf size at birth in New Zealand is smaller than in USA. Similarly, Coulon et al. (1995) noted that the effect of pregnancy was greater in the cows that had given birth to heavier or twin calves.

Few studies have examined the effect of pregnancy on milk yield and milk composition. In dairy cows, some authors (Erb et al., 1952; Coulon et al., 1995; Olori et al., 1997; Bertilsson et al., 1997; Sorensen and Knight, 2002; Roche 2003; Brotherstone et al., 2004) reported milk yield losses from the 5 to 6th mo of pregnancy onwards, whereas other authors (Bormann et al., 2002) reported a decline in milk yield from the 3rd mo of pregnancy. Milk yield of goats mated when they were at peak lactation decreased at the same rate as that of non pregnant goats during the first 8 wk of pregnancy (Knight and Wilde, 1988). Thereafter, yield decreased more quickly in the pregnant goats and in the last wk before parturition was 57% of the value of non pregnant goats (1.3 vs. 2.3 kg/d). In goats mated when they were in wk 29 of lactation, pregnancy reduced milk yield by 21, 33, 61 and 79 % at wk 11, 12, 13, and 14 of pregnancy, respectively (Salama et al., 2005a).

The effect of pregnancy depends on the stage of lactation when pregnancy occurs (Brotherstone et al., 2004). Cows that conceive in the 1st, 2nd, or 3rd mo of lactation suffered more milk losses during 300-d lactation than non pregnant cows or cows becoming pregnant in mid- or late-lactation. Similarly, Tekerli et al. (2000) demonstrated that cows that conceived shortly after calving had lower lactation persistency and that the decline in the slope of the lactation curve after peak yield decreased with increased days open.

Milk fat, protein and lactose contents in pregnant cows were greater than in non pregnant cows after d 126 of pregnancy and differences increased as pregnancy advanced until drying off at d 266 of lactation (Roche, 2003). With the traditional 305-d lactation, Olori et al. (1997) reported little effect of pregnancy on milk protein and lactose content but milk fat content increased significantly from the 6th mo of pregnancy.

6. Effect of Extended Lactation on Milk Production

In the normal lactation of mice, mammary cell numbers increased up to d 5 of lactation, cellular differentiation continued until d 15, and thereafter there was a gradual decline in mammary cell number and activity (Shipman et al., 1987). Substitution of younger pups for the natural litter at d 15 prevented the normal fall in mammary cell number and differentiation, and lactation was extended.

Cows are traditionally bred at the peak of lactation with the aim of establishing a calving interval (**CI**) of 12 to 13 mo. As the milk production per cow has increased dramatically during recent decades, the traditional 12-mo CI could be extended to avoid udder health problems resulting from the drying off at high production levels (25 to 30 kg/d). In addition to udder health risks at drying off, insemination at lactation peak (d 50 to 60), to reach a 12-mo CI, may be related to fertility problems such as silent estrus and ovarian cysts that increase with increasing milk yield (Grohn et al., 1994). Finally, it seems wasteful not to utilize the genetic potential of modern cows in the case of the traditional 12-mo CI (Osterman and Bertilsson, 2003).

Several studies indicated that 12-mo CI is optimal and increased profitability compared with extended CI (Louca and Legates, 1968; Oltenacu et al., 1981; Holmann et al., 1984; Weller and Folman, 1990). On the other hand, some studies demonstrated the benefit of an extended CI (Weller et al., 1985; Arbel et al., 2001). Cumulative yield of the current and subsequent annualized lactations was greatest at 117 and 98 d open for primiparous and multiparous cows, respectively (Weller et al., 1985).

Van Amburgh et al. (1997) compared the advantages of 13.2- vs. 16.5-mo CI in bST-treated cows and found that the extended CI resulted in fewer calvings, lower incidence of postpartum metabolic diseases, lower veterinary costs, less culling with fewer replacements needed, and an overall improvement in herd life, animal well-being and dairy farm profitability. However, CI had no effect on milk production in other studies. Average milk yield per day of CI was similar between dairy cows conceived at 88 or 121 d postpartum (Schneider et al., 1981). Osterman and Bertilsson (2003) studied the effect of CI (12 vs. 18 mo) combined with MF (2X vs. 3X) on energy corrected milk yield in dairy cows and found no differences in the yield per day of CI between 18- and 12-mo CI irrespective of MF. However, 18-mo CI combined with 3X milking resulted in greater energy corrected milk yield per day of CI compared with cows calved every 18 mo and milked 2X.

Cows in New Zealand bred to 24-mo CI and fed only pasture or supplemented with concentrates were producing 55 and 80% of their peak production, respectively, at d 440 of lactation with a second peak in spring (Klover, 2004).

The advantages of extended CI are greater for primiparous than multiparous cows because of their greater lactation persistency and flatter lactation curve (Arbel et al., 2001; Osterman and Bertilsson, 2003). Moreover, extended CI resulted in greater weight gains between calvings and greater body weights at the subsequent calving (Schneider et al., 1981). Since body weight gain is especially important for heifers, extended CI could well be beneficial for primiparous cows (Louca and Legates, 1968). Arbel et al. (2001) found that insemination 61 d later than normal increased the average value-corrected milk yield (a calculated value of milk yield that includes fat and protein) by 0.8 kg per day of CI in primiparous cows, whereas a delay of 53 d in multiparous cows resulted in an increase of only 0.2 kg per day of CI. Similarly, 18-mo CI resulted in an increased average energy corrected milk yield of 1.3 kg per day of CI in primiparous cows, while the multiparous cows decreased their energy corrected milk yield by 1.0 kg per day of CI (Osterman and Bertilsson, 2003).

Cows with extended CI reached drying off with greater milk fat and protein contents than cows with 12-mo CI. Osterman and Bertilsson (2003) reported that fat and protein percentages increased from 4.60 and 3.55% at mo 1 of lactation to 4.80 and 3.75%, respectively at mo 10 of lactation in cows with 12-mo CI. The corresponding values for cows with 18-mo CI increased from 4.70 and 3.60% at mo 1 of lactation to 5.80 and 4.20% at mo 16 of lactation. Milk SCC was not affected by extending the CI from 12 to 18 mo in dairy cows (Osterman et al., 2005).

Lactation in high yielding dairy goats usually lasts for nearly 10 mo and ceases in late pregnancy for 2 mo, to reach a 12-mo kidding interval. However, some dairy goat producers in France (Chastin et al., 2001) and the USA (Gipson and Wiggans, 2001) milk their does for an extended period before rebreeding and drying off. Dairy goats that were not remated continued to lactate for 2 to 4 yr and showed new peaks of milk yield during spring (Linzell, 1973). Salama et al. (2005a) compared milk production in dairy goats with 12- and 24-mo kidding intervals and found that both groups produced similar total milk yield throughout 2 yr (1192 vs. 1093 L for 12- and 24-mo kidding intervals, respectively). In the second yr, goats with the 24-mo kidding interval produced milk with greater contents of fat and protein than goats with the 12-mo kidding interval with no differences in milk SCC.

CHAPTER 3: OBJECTIVES

CHAPTER 3

OBJECTIVES

The main objective of this thesis was to study the effect of some management and physiological factors on milk yield, milk quality and lactation persistency in Murciano-Granadina dairy goats.

The specific objectives were:

1. The effects of once- versus twice-daily milking throughout lactation in lactating goats.

    Effects on milk yield, milk composition, milk SCC and udder health (Chapter 4).

    Effects on the secretion of milk and its components at different milking intervals (Chapter 4).

    Changes in the cisternal compartment at different milking intervals (Chapter 5).

    Evaluation the phenomenon of cisternal recoil in the udder (Chapter 5).

2. The effects of omitting the dry period on colostrum quality, milk yield, and mammary cell turnover (Chapter 6).

3. The effects of the modification of lactation cycle in dairy goats.

    Lactational effects of a typical 12-mo lactation cycle compared to an extended lactation cycle of 24 mo in dairy goats milked once-daily (Chapter 7).

    The impact of pregnancy on the lactation curve (Chapter 7).

CHAPTER 4: ONCE DAILY MILKING

CHAPTER 4

EFFECTS OF ONCE VERSUS TWICE DAILY MILKING THROUGHOUT LACTATION ON MILK YIELD AND MILK COMPOSITION IN DAIRY GOATS

ABSTRACT

The effects of once (1X) vs. twice (2X) daily milking throughout lactation on milk yield, milk composition, somatic cell count (SCC) and udder health were studied in 32 Murciano-Granadina dairy goats. Goats were assigned at wk 2 of lactation to 2 treatment groups; once daily milking at 0900 (1X, n = 17), or twice daily milking at 0900 and 1700 (2X, n = 15). Milk yield was recorded weekly until wk 28, and milk composition and SCC were evaluated for each individual udder half at each milking at wk 2 and 4 of lactation and then, monthly until the end of the experiment. Once daily milking resulted in an 18% reduction in the yield of 4% fat-corrected milk compared to twice daily milking (1.61 vs. 1.95 L/d, respectively). This reduction was more marked from wk 2 to 12 than in mid and late lactation. Response to milking frequency also varied according to parity number where goats of less than 4 parities suffered more milk yield losses during 1X than older goats. Milk of 1X goats contained higher percentages of total solids (13.6 vs. 12.9%), fat (5.10 vs. 4.62%) and casein (2.57 vs. 2.35%) than milk of 2X goats, but milk protein percentage did not differ between treatments (3.28 vs. 3.20%). Yields of total solids, fat, protein and casein tended to be higher for 2X than 1X. Milk SCC did not differ between treatments. We conclude that application of once daily milking in Murciano-Granadina dairy goats moderately reduced milk yield without negative effects on milk composition and udder health. Losses in milk yield would be reduced if 1X is practiced during mid or late lactation and in older goats. An increase in labor productivity and a higher farmer's standard of living is also expected.

INTRODUCTION

Number of daily milkings is of great importance in determining milk yield in dairy animals. The 1X of dairy cows is practiced in some countries either in early lactation to reduce metabolic stress or in late lactation to improve quality of farming life (Davis et al., 1999). Compared with 2X milking, 1X milking reduced milk yield by 7 to 38% in dairy cows (Stelwagen et al., 1994a; Stelwagen and Knight, 1997), 15 to 48% in ewes (Knight et

al., 1993; Negrao et al., 2001) and 6 to 35% in dairy goats (Mocquot, 1978; Capote et al., 1999). The wide variation in yield losses during 1X reported by other authors may be due to differences in breed, lactation stage, level of production, duration of 1X milking and individual characteristics.

Bewly et al. (2001) reported that more frequent milking requires more variable costs (labor, utilities, milking supplies, and additional feed costs). In Spain, and other countries where the goat production systems are extensive or semi-extensive, high MF is a major cost for dairy goat farms. Under these conditions, lower MF increases labor productivity and reduces milk storage risks. However, for infrequent milking to be a practical strategy, it should have no long-term deleterious effects on milk yield or milk quality.

Somatic cell count in goat milk has become an important quality index since goat milk was officially defined in the grade A Pasteurized Milk Ordinance in 1989 in the USA (Zeng and Escobar, 1995), and a European regulation (92/46 EEC) to control SCC in goat milk was issued in 1992. Moreover, the relatively low cost and rapidity of SCC determination have resulted in it being widely used as an indicator of milk quality and as a management tool to determine the prevalence of IMI in dairy animals. It is well established that SCC in dairy goats is affected by IMI (Zeng and Escobar, 1995), parity number (Sánchez et al., 1999), stage of lactation (Wilson et al., 1995), breed (Sung et al., 1999), level of milk production (Hinckley, 1983), nutritional status and milking method (Salama et al., 2003a), and estrus (McDougall and Voermans, 2002). However, there is no information on the effect of milking frequency on SCC in dairy goats.

The objectives of this study were to investigate the effects in lactating Murciano-Granadina goats of 1X vs. 2X throughout lactation on: 1) milk yield and chemical composition, 2) milk SCC and udder health, and 3) secretion of milk and its components at different milking intervals.

MATERIALS AND METHODS

Animals and Management Conditions

Two milking frequencies were studied over 2 consecutive years in a total of 32 Murciano-Granadina dairy goats (17 and 15 goats for the first and second yr, respectively) from the herd of the experimental farm of the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autonoma of Barcelona. Goats were divided into 2 groups

and milked from parturition to wk 42 of lactation, when they were dried-off. Kids were separated from their dams within 8 h of birth and reared on milk substitutes and all goats were milked twice daily until wk 2. In both years, groups were balanced with respect to parity, milk yield and SCC; baseline measurements were recorded on 2 consecutive days in wk 2 of lactation. Goat parities were: first, 5; second, 9; third, 7; and, fourth or more, 11. Treatments were randomly applied to the groups from wk 2 to dry-off, and were: goats milked 1X at 0900 (n = 9 and 8, for the first and second yr, respectively), and goats milked 2X at 0900 and 1700 (n = 8 and 7, for the first and second yr, respectively). New goats were used for both treatments in the 2nd year. Both groups grazed on natural pastures for 6 h daily and were supplemented with concentrate in the milking parlor (1.53 Mcal NE_L/kg, 16% CP, as fed) at a flat rate of 0.5 to 1 kg/doe per day according to lactation stage, and with 0.5 kg/doe alfalfa and 0.5 kg/doe alfalfa pellets in the shelter.

Goats were milked in a double-12 stall parallel milking parlor (Westfalia Landtechnik, Granollers, Spain) equipped with recording jars (2 L ± 5%) and low-line milk pipeline. Milking was performed at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 66% according to the milking parameters used in the breed (Peris et al., 1996). Milking routine included machine milking, machine stripping before cluster removal, and teat dipping in a iodine solution (P3-cide plus, Henkel Hygiene, Barcelona, Spain).

Sample Collection, Analysis, and Measurements

Milk recording and sampling were done from wk 2 to 28 of lactation. Milk yield of individual goats was recorded weekly at every milking by using the recording jars in the milking parlor. Milk samples were taken from each udder half after each milking for analysis of composition, SCC and bacteriology at wk 2 and 4 of lactation and then monthly until wk 28. Milk yield was also recorded by udder half on the sampling days. Yield of FCM in 300 DIM was estimated according to Thomas et al. (2000).

Milk yield and milk composition recording at each milking (a.m. and p.m.) allowed the study of the secretion of milk and milk components during the different periods of milk accumulation (8 and 16 h for 2X goats, and 24 h for 1X goats).

For analysis of milk composition, a sample of approximately 100 mL was collected and preserved with $K_2Cr_2O_7$ (0.3 g/L) at 4 °C. Unhomogenized milk samples were analyzed with a near infrared spectrometer (Technicon InfraAlyzer- 450, Bran+Luebbe SL,

Nordersted, Germany), using the method of Albanell et al. (1999), for content of TS, fat, CP (N × 6.38), and CN.

For SCC, a sample of approximately 50 mL was placed in a plastic vial, preserved with an anti-microbial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems Inc., San Ramon, CA) and kept at 4 °C until analysis. The SCC was determined in the Dairy Herd Improvement laboratory of Catalonia (Allic, Cabrils, Barcelona, Spain) using an automatic cell counter (Fossomatic 250, Foss-Electric, Hillerød, Denmark). Routine bacteriological culture was performed on aseptic milk samples obtained from each udder half before milking. An infection was assumed to have occurred if 5 or more similar cfu were present in the incubated sample of milk.

Statistical Analysis

Data from 1 goat in each treatment were excluded from the statistical analysis because of an IMI. Data were analyzed by the PROC MIXED for repeated measurements of SAS (SAS 8.1; SAS Inst. Inc., Cary, NC). The statistical mixed model included the fixed effects of milking frequency, year, parity, prolificacy, and wk of lactation; the random effects of the animal and half udder nested within animal; and the interactions of milking frequency to the factors of year, parity, and wk of lactation; and the residual effect. Random effect of udder half nested within animal was excluded from the model in the analysis of milk yield. For data analysis of milk secretion during different milking intervals, the fixed effect of milking frequency was replaced by the fixed effect of milking interval (8 and 16 h corresponded to 2X goats and 24h corresponded to 1X goats). Parity number was grouped into 3 categories corresponding to first and second parities (n = 14), third parity (n = 7), and fourth or more parities (n = 9). Goats of first and second parities were grouped together because they had in many cases the same age. The prolificacy effect corresponded to 2 levels defined as single or multiple kids. Logarithmic transformations ($\log_{10}$) of SCC values were used in statistical analysis. Data from wk 2 were used as a covariate to correct for differences in initial values when necessary. Significance was declared as $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Milk Yield

Year significantly affected FCM yield throughout lactation and milk yield during mid lactation (wk 13 to 28), but interaction between yr and treatment was not significant.
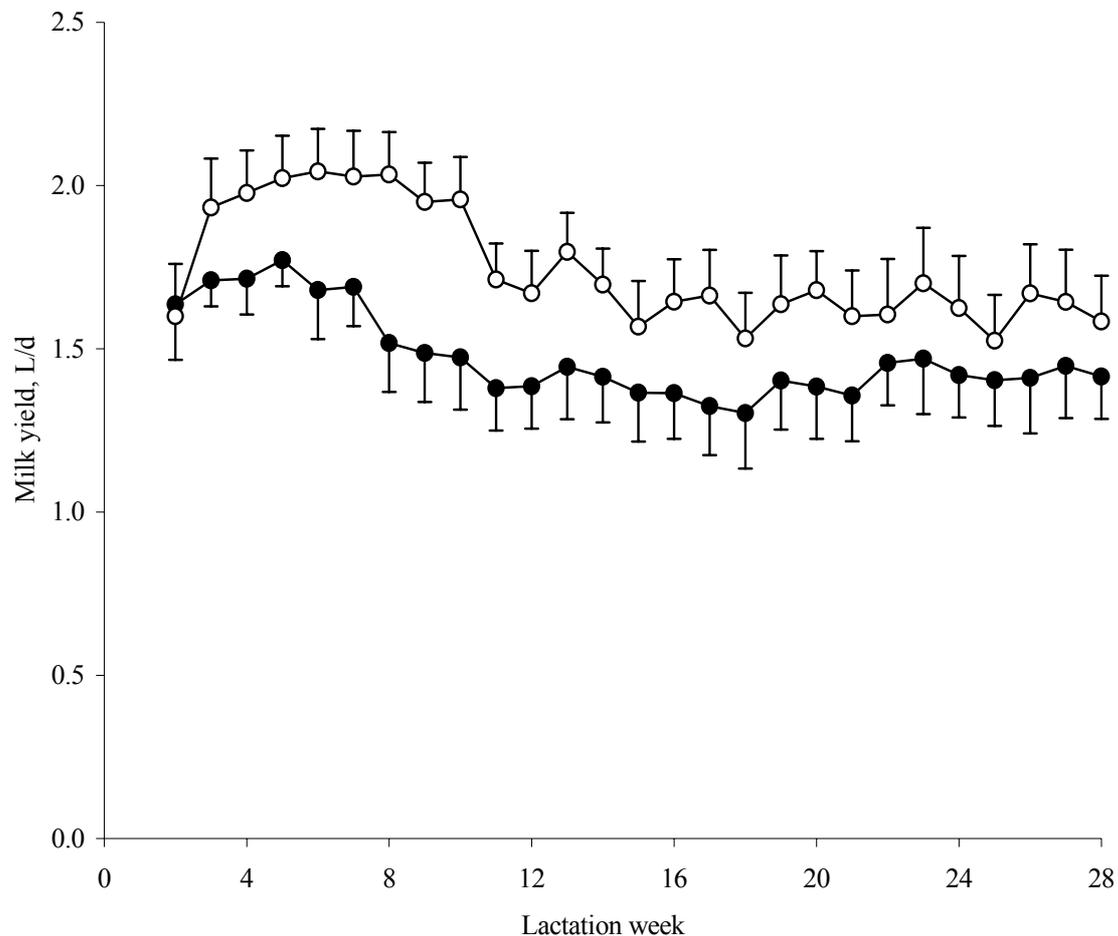
Figure 1. Milk production of dairy goats milked once (•; n=16) or twice (○; n=14) daily. Values are means with SEM indicated by vertical bars.

As shown in Table 1 and Figure 1, 1X resulted in a 18% significant reduction in FCM daily yield compared to 2X (–0.34 L/d) during the experimental period. This reduction was greater than the values previously reported in Canarian goats (6%) by Capote et al. (1999) but smaller than in Alpine goats (36%) by Mocquot (1978) for overall lactation. Moreover, Wilde and Knight (1990) reported a loss of 26% in Saanen goats in a short-term experiment in early lactation, while losses ranged between 6 and 7% in Damascus goats during middle and late lactation, respectively (Papachristoforou et al., 1982). The increase in the concentration of the FIL, synthesized by the mammary gland (Wilde et al., 1995) and the

intramammary pressure (Peaker, 1980) may cause the decrease in milk synthesis in 1X goats. Also, the loss of TJ integrity after about 20 h of milk accumulation, may play a role in the milk yield losses in 1X (Stelwagen et al., 1994b). When less frequent milking is prolonged, the decrease in milk yield is sustained by sequential developmental adaptations, initially as a down-regulation of cellular differentiation (Wilde et al., 1987b) and later as a net loss in mammary cell number via apoptosis (Li et al., 1999a).

Table 1. Effect of milking frequency on milk yield, chemical composition, and somatic cell count in dairy goats.[1]

Dependent variable	Milking frequency[2]		SED
	1X (n = 16)	2X (n = 14)	
Milk yield			
wk 2 to 12, L/d	1.50[b]	1.89[a]	0.16
wk 13 to 28, L/d	1.34[b]	1.61[a]	0.20
wk 2 to 28, L/d	1.43[b]	1.76[a]	0.19
Estimated 300 DIM, L	460[b]	531[a]	31
FCM[3]			
wk 2 to 12, L/d	1.70[b]	2.09[a]	0.17
wk 13 to 28, L/d	1.55	1.81	0.17
wk 2 to 28, L/d	1.61[b]	1.95[a]	0.15
Estimated 300 DIM, L	504[b]	590[a]	28
Milk components			
Total solids, %	13.6[a]	12.9[b]	0.2
Total solids, g/d	198.0[b]	225.8[a]	20.5
Fat, %	5.10[a]	4.62[b]	0.21
Fat, g/d	69.1	81.0	7.1
Protein, %	3.28	3.20	0.08
Protein, g/d	45.2	54.4	5.3
Casein, %	2.57[a]	2.35[b]	0.07
Casein, g/d	33.8	40.6	4.1
SCC, $\log_{10}$/mL	5.95	5.86	0.07
SCC, $\log_{10}$/d	8.77	8.76	0.07

[1] Data are least square means and standard errors of the difference (n = 30).
[2] Once (1X) or twice (2X) daily milking.
[3] 4% FCM = 0.4 (L of milk) + 15 (kg of fat).
[a, b] Means with different superscripts within the same row are different ($P < 0.05$).

The reduction in 4% FCM yield due to 1X was more marked during the first 3 mo of lactation (19%) than in late lactation (14%; $P < 0.08$). In accordance, Stelwagen and Knight (1997) reported that milk yield losses in dairy cow during 1X in late lactation were lower than in early lactation. McKusick et al. (2002a) reported no differences in milk yield between milking every 16 h compared with every 12 h during mid- to late-lactation in dairy ewes, but milk yield was reduced after more than 16 h of milk accumulation in a short term experiment conducted in the same flock.

Mammary gland cisternal capacity appears to be a constraint in cows milked 1X (Knight and Dewhurst, 1994) and cisternal milk proportion increases during the course of a normal lactation in cows (Dewhurst and Knight, 1993) and in dairy ewes (Rovai et al., 2002). Our data also suggest that the cisternal capacity is critical in dairy goats during early lactation when higher levels of milk are produced, while in late lactation (descending phase), the cisterns may have been able to better accommodate the level of milk production, resulting in lesser effect of FIL, and thus lower milk yield losses as previously indicated in Canarian goats that have large cisterns (Capote et al., 1999).

The reduction in FCM yield during 1X varied also according to parity number: 38% in first and second parities, 22% in third parity, and 11% in more than third parity (Table 2). Primiparous cows are reported to have a proportionately greater response to increased MF than multiparous cows (Amos et al., 1985). As parity number increased, cistern capacity increased in cows (Dewhurst and Knight, 1993) and dairy ewes (Rovai et al., 2002). Increased cistern capacity might reduce the negative effect of FIL on milk secretion when milk accumulates in the alveoli, thereby decreasing the positive response to 2X in older animals. Positive correlations between milk yield and proportion or volume of cisternal milk in dairy cows milked 1X were reported (Stelwagen and Knight, 1997).

There is no documentation on the effects of 1X on lactation persistency in dairy goats. Both 1X and 2X groups had a maximum yield of 1.75 ± 0.13 and 2.00 ± 0.10 L ($P < 0.01$) during wk 4 and 5 of lactation, respectively (Figure 1). Persistency of lactation as defined recently by Grossman et al. (1999) is the number of days during which the level of peak yield is maintained. Applying this definition, 1X goats persisted for 21 ± 3 d which was lower ($P < 0.01$) than in 2X goats (40 ± 5 d). By the end of the study (196 DIM), goats were producing approximately 83 and 79% of their peak production for 1X and 2X, respectively, which was higher than the 30% recorded by dairy cows at a similar stage of lactation (Lacy-Hulbert et al., 1999). Milking frequency did not affect lactation length in any of the studied

treatments and all goats in this experiment were dried-off routinely for 300 DIM on average. Total 4% FCM for 300 DIM was 14.6% lower for 1X than for 2X (Table 1). Dairy ewes milked every 12 h had similar lactation length and milk yield than did ewes milked every 16 h (McKusick et al., 2002a).

Table 2. Effect of milking frequency according to parity number on fat corrected milk yield, chemical composition, and somatic cell count in dairy goats.[1]

| Dependent variable | Parity category[3] | Milking frequency[2] | | SED |
		1X	2X	
FCM[4], L/d	1	1.52[b]	2.43[a]	0.220
	2	1.38[b]	1.79[a]	0.210
	3	1.68	1.87	0.172
Milk total solids, %	1	13.46[a]	12.36[b]	0.301
	2	14.08[a]	13.45[b]	0.311
	3	13.52	13.01	0.264
Milk fat, %	1	5.33[a]	3.95[b]	0.286
	2	5.72	5.20	0.282
	3	5.15	4.81	0.243
Milk protein, %	1	3.22	3.14	0.090
	2	3.36	3.27	0.094
	3	3.40[a]	3.15[b]	0.078
Milk casein, %	1	2.39	2.40	0.086
	2	2.59[a]	2.25[b]	0.084
	3	2.75[a]	2.38[b]	0.072
Milk SCC, $\log_{10}$/mL	1	5.47	5.31	0.175
	2	5.87	5.86	0.077
	3	6.51	6.38	0.112

[1] Data are least square means and standard errors of the difference (n=30).
[2] Once (1X) or twice (2X) daily milking.
[3] Parity number was grouped into 3 categories: 1 (1st and 2nd, n = 14), 2 (3rd, n = 7), and 3 (4th or more, n = 9).
[4] 4%-FCM = 0.4 (L of milk) + 15 (kg of fat).
[a, b] Means with different superscripts within the same row are different ($P < 0.05$).

We have to take into account that goats used in this study came from a herd initially bred under 2X milking conditions. The same herd is at present managed under 1X milking and the goats yielded more than 2.0 L of 4% FCM daily (Salama et al., 2002; 2003a), which

is similar to the production of 2X goats in this experiment. Indeed, the possibility exists that during breeding under 1X milking conditions goats may become more tolerant and adapted to less frequent milking and, consequently, losses in milk yield for 1X would be reduced.

Milk Composition

Year significantly affected yield and percentage of milk components with the exception of protein percentage (P = 0.619) and CN percentage (P = 0.195). A tendency was also observed for CN yield (P = 0.096), but interaction between yr and treatment was not significant.

Milk of 1X goats was more concentrated than milk of 2X goats and had higher concentrations of TS (+6%), fat (+10%) and CN (+9%), as indicated in Table 1. This could be expected as a consequence of the concentration of milk components when milk yield decreased as well as a result of changes occurred in the synthesis of milk components. In dairy cows, concentration of fat, protein and CN increased as a result of 1X for short periods at late lactation (Lacy-Hulbert et al., 1999) or during an entire lactation (Holmes et al., 1992). In contrast, as milking interval increased, milk fat content decreased while milk protein content increased in a short term experiment in dairy ewes in which OT was used to remove alveolar milk (McKusick et al., 2002a). The same authors observed that ewes milked every 12 h had similar milk production, milk fat and protein percentages and yields than did ewes milked every 16 h, in a trial conducted during mid-lactation (McKusick et al., 2002a). Changes in fat concentration in milk may be related to differing regulatory mechanisms for secretion of milk fat globules relative to the components in the aqueous phase of milk and to the transfer between alveolar and cisternal compartments (Davis et al., 1999; McKusick et al., 2002a).

Percentage of milk protein did not vary significantly between treatment groups (P = 0.260) in our results, as reported in dairy ewes milked every 12 h vs. 16 h (McKusick et al., 2002a). Total protein in milk is the result of the proteins synthesized in the mammary gland and the serum proteins entering the milk when mammary TJ are disrupted. Casein does not move through leaky mammary TJ, presumably because of the large size of their micelles (Stelwagen et al., 1998a). Thus, while milk volume was lower with 1X, CN synthesized remained and became more concentrated in the milk. Nevertheless, 1X milking is often associated with increased plasmin and plasminogen activities (Stelwagen et al., 1994c) which may lead to the breakdown of β-CN to γ-CN without changes in total CN. Moreover,

mammals have the ability to provide milk that is consistent in protein concentration regardless of the most environmental stresses (Cowie and Tindal, 1971).

Daily yield of TS significantly decreased by 12% in 1X goats, and daily yields of fat ($P =$ 0.073), protein ($P =$ 0.086) and CN ($P =$ 0.098) tended to be reduced by 15, 17 and 16%, respectively, in agreement with the significant reduction in the daily milk volume (19%).

Milk SCC and Udder Health

SCC is an important index for milk quality and in many countries it is used as a criterion for milk payment to producers, penalizing goat milk that contains more than 1×10^6 cells/mL. However, there is no data on the effect of 1X on milk SCC in dairy goats. No significant effects were detected for yr or interaction between yr and treatment on milk SCC. Moreover, SCC did not differ ($P =$ 0.190) between treatments (Table 1) and the geometric means of SCC throughout lactation were 979 and 917×10^3 cells/mL for 1X and 2X, respectively. However, these values were greater than previously reported by Salama et al. (2003a) in the same conditions.

Available information on SCC in cows during 1X is contradictory. In agreement with our results, both Stelwagen et al. (1994a) and Lacy-Hulbert et al. (1999) indicated no significant effect of 1X on milk SCC at late lactation. In contrast, milking dairy cows 1X throughout lactation (Holmes et al., 1992) or for short periods at early (Stelwagen and Lacy-Hulbert, 1996) or late (Kelly et al., 1998) lactation increased SCC. This increase may be due in part to a concentration effect as milk yield decreased during 1X (Kamote et al., 1994) and in part to the impairment of the TJ barrier facilitating a paracellular influx of somatic cells into the milk without damage to the mammary secretory cells (Stelwagen and Lacy-Hulbert, 1996). Kamote et al. (1994) suggested that if the initial SCC level is low, 1X results in a small increase in SCC. Goats in this experiment started lactation with relatively high SCC but SCC did not increase significantly during1X. Dairy ewes milked every 12 h had similar SCC in milk than did ewes milked every 16 h (McKusick et al., 2002a).

Bacteriological culture revealed that 1 half udder of 1 goat in each treatment had an IMI. Clinical mastitis was not observed in any of the studied goats suggesting that 1X had no deleterious effect on udder health. Similarly, udders of dairy cows milked 1X throughout lactation did not suffer any mastitis problems, although milk with higher SCC was produced, when compared to 2X cows (Holmes et al., 1992).

Overall means of milk SCC increased as lactation stage advanced ($P < 0.01$) and milk from the 4th mo of lactation or later exceed the limit of 1×10^6 cells/mL. These results agree with the increase in SCC in goats as lactation advances (Wilson et al., 1995; Salama et al., 2003a). The reason for this rise in SCC as lactation progresses may be due to both a concentration effect as less milk is produced and to the presence of chemostatic cytokines that draw polymorphonuclear leukocytes into milk in higher concentrations during late lactation (Manlongat et al., 1998). Also, SCC significantly increased ($P < 0.001$) as parity number increased (Table 2) as previously reported by Sánchez et al. (1999) in dairy goats. This increase could be attributed to the increased prevalence of bacteria in the mammary gland of older animals, or to the cumulative stress of the mammary tissue from several pregnancies and lactations (Boscos et al., 1996).

Secretion of Milk and its Components

Hourly milk secretion rate had the greatest value for the 8-h milking interval and significantly decreased as time after milking increased (Table 3). The reduction was more marked for the 16 to 24-h interval (–18%) than for the 8 to 16-h interval (–11%) indicating a secretion rate saturation effect with time, as reported in goats (Peaker and Blatchford, 1988), dairy ewes (McKusick et al., 2002a) and cows (Knight et al., 1994a; Davis et al., 1998; Ayadi et al., 2003a). Hourly milk secretion rates in our results were greater than those calculated from data reported (52 to 54 g/h) by McKusick et al. (2002a) in dairy ewes milked every 12 h or every 16 h.

Fat secretion rate was greater for the 8-h milking interval, but the values did not differ for the 16 and 24-h milking intervals (Table 3). Alveolar milk is richer in fat content than cisternal milk, which may explain the higher fat content during the 8-h period after milking as compared with 16 and 24 h after milking and confirms the importance of milk ejection during milking for recuperation of alveolar milk that is rich in TS (McKusick et al., 2002a). However, fat percentage did not differ between 8 and 24 h after milking. Despite the milk yield and specie differences, hourly fat secretion rates in our results for the 16 and 24-h milking intervals were also similar to those calculated (2.7 to 2.9 g/h) from McKusick et al. (2002a) in dairy ewes milked every 12 or 16 h. This may be a result of the plateau observed in the transfer of fat from alveolar milk to cisternal milk during the longer milking intervals, resulting in an accumulation of fat in the alveolar compartment (McKusick et al., 2002a).

Table 3. Secretion rate of milk and its components, and milk composition according to milking interval in dairy goats milked at different milking frequencies.[1]

| Dependent variable | Milking frequency[2] | | | |
| | 2X | | 1X | |
	8 h	16 h	24 h	SEM
Milk, mL/h	80[a]	71[b]	58[c]	3
Milk fat, g/h	4.34[a]	2.96[b]	2.94[b]	0.14
Milk fat, %	5.43[a]	4.14[b]	5.11[a]	0.21
Milk protein, g/h	2.41[a]	2.30[a]	1.89[b]	0.09
Milk protein, %	3.04[b]	3.23[a]	3.29[a]	0.07
Milk casein, g/h	1.81[a]	1.64[b]	1.48[b]	0.06
Milk casein, %	2.26[c]	2.33[b]	2.56[a]	0.05
Milk SCC, $\log_{10}$/mL	6.06[a]	5.69[b]	5.98[a]	0.06

[1] Data are least square means and standard error of the mean (n = 30).
[2] Once (1X) or twice (2X) daily milking.
[a, b, c] Means with different superscripts within the same row are different ($P < 0.05$).

Protein secretion rate was significantly greater for the 8 h after milking, but the value did not differ for the 16-h milking interval (Table 3). The lowest secretion rate of milk protein was observed at the 24-h milking interval. Hourly milk protein secretion rates in our results were smaller than the value calculated from McKusick et al. (2002a) in dairy ewes milked every 12 or 16 h (2.5 g/h). Protein percentage increased as time after milking increased (Table 3), indicating a significant concentration effect in the milk accumulated in the udder after a 16- or 24-h milking interval, as discussed above. Regarding milk CN, secretion rate was decreased as milking interval increased and the concentration effect was more clear when CN percentage increased significantly according to time after milking (Table 3).

Milk at 8 h had the highest SCC (Table 3), which is consistent with reports of lower SCC in fore-stripped milk compared to stripped milk (Gonzalo et al., 1993) and milk SCC according to milking interval (McKusick et al., 2002a) in dairy ewes. At 16 h, as cisternal milk percentage increased, milk SCC significantly decreased. However, milk SCC increased at 24 h, which may be associated with leaky TJ between MEC occurred after 20 h in dairy

goats (Stelwagen et al., 1994b) facilitating the paracellular influx of somatic cells into milk (Stelwagen and Lacy-Hulbert, 1996). Therefore, if milk quality in dairy goats was based on SCC, the 16-h interval would appear to be the most appropriate interval to produce milk with high quality. Milking 3 times every 48 h (16-h interval between milking) may also be a better alternative to 1X, and McKusick et al. (2002a) showed that milking East Friesian ewes every 16 h did not affect milk yield, milk components, or SCC as compared with twice daily milking at an interval of 12 h.

CONCLUSIONS

Once daily milking in Murciano-Granadina dairy goats moderately reduced milk yield without negative effects on milk composition and udder health. Since milk of 1X goats contained more fat without significant increases in SCC, reduced revenue due to lower milk yield could be partially offset if payment for milk was based on milk quality. Once daily milking for dairy goats in early lactation and for dairy goats of less than 4 parities may not be a suitable management decision because of higher losses. However, the reduction in total labor when once daily milking is adopted permits farmers more time to devote to other farming practices and/or to other activities off the farm, improving their productivity and standard of life.

CHAPTER 5: CISTERNAL COMPARTMENT

CHAPTER 5

CHANGES IN CISTERNAL UDDER COMPARTMENT INDUCED BY MILKING INTERVAL IN DAIRY GOATS MILKED ONCE- OR TWICE-DAILY

ABSTRACT

Fourteen Murciano-Granadina dairy goats were used to evaluate udder compartments (cisternal and alveolar) and cisternal recoil after an oxytocin (OT) challenge at different milking intervals (8, 16, and 24 h) during wk 7 of lactation. Goats were milked once- (1X; n = 7) or twice- (2X; n = 7) daily from wk 2 of lactation. Averages of milk yield for wk 4 and 8 were 1.76 and 2.24 L/d, for 1X and 2X, respectively. For each half udder, cisternal area by ultrasonography and cisternal milk by machine milking were measured after i.v. injection of an OT receptor blocking agent. Alveolar milk was then obtained after i.v. injection of OT. Regardless of milking frequency, alveolar milk increased from 8 to 16 h after milking, but did not change thereafter. Cisternal area and cisternal milk increased linearly (R^2 = 0.96 to 0.99) up to 24 h, indicating continuous milk storage in the cistern at any alveoli filling degree. Cisternal to alveolar ratio increased with milking interval (from 57:43 to 75:25), but difference between milking intervals was significant at 8 h only, at which time 2X goats showed a greater ratio (1X, 51:49; and, 2X, 62:38). Despite extended milking intervals, cisterns of 1X goats did not become larger than 2X goats after 5 wk of treatment. The highest correlation between cisternal area and cisternal milk was detected at 8 h after milking (r = 0.74). Primiparous goats had smaller cisternal areas and less cisternal milk than multiparous goats at all milking intervals. Cisternal recoil was studied in a sample of 1X (n = 4) and 2X (n = 4) multiparous goats by scanning cisterns by ultrasonography at 0, 5, 15 and 30 min after an OT challenge for each milking interval. Cisternal area increased after OT injection for the 8- and 16-h milking intervals, but no differences were observed for the 24-h interval. Unlike cows, no changes in cisternal area were observed after OT injection, indicating the absence of cisternal recoil in goats. We conclude that goats show a large cisternal compartment that increases linearly after milking. Nevertheless, cisternal size did not increased after once daily milking, probably due to less milk yield. Multiparous goats had larger cisterns than primiparous goats and were able to store more milk in their cisterns at all milking intervals. Due to the high capacity of goat cisterns, no milk return from cistern to alveoli is expected if milking is delayed after milk letdown.

INTRODUCTION

Milk in the udder is stored in 2 interconnected compartments (cisternal and alveolar) that determine milkability. Animals with large cisterns are milked faster with simplified routines and tolerate extended milking intervals better (Knight and Dewhurst, 1994; Ayadi et al., 2003a; Salama et al., 2003b). Milk partitioning between the 2 compartments varies according to species, breed, milking interval, and stage of lactation.

In dairy goats, Peaker and Blatchford (1988) reported elevated cisternal milk percentages, ranging according to time elapsed after milking from 65% (1 h) to 88% (16 h). Cistern size may become larger in goats after a short period of 1X to accommodate greater milk accumulation in the udder (Knight and Dewhurst, 1994). Measurement technique also may affect milk partitioning as a consequence of the possible OT release during udder manipulation. Use of OT analogues has been proposed to block the spontaneous milk ejection (Wellnitz et al., 1999), allowing a reliable separation between cisternal and alveolar milk. This is especially critical when milking intervals are long or when machine milking is used instead of teat cannulation to harvest milk fractions separately. Although the methodology of OT blocking agents was validated in Saanen goats (Knight et al., 1994b), it has not been used previously in goats for separating cisternal and alveolar milk during different milking intervals.

Cisternal size also has been measured efficiently by ultrasonography in ruminants (Bruckmaier and Blum, 1992; Ruberte et al., 1994; Ayadi et al., 2003a). This technique allows noninvasive investigation of the cistern, and could be useful as a new approach to study udder changes to accommodate milk accumulation during different milking intervals and after milk letdown.

Linzell (1955) demonstrated in mice a back-flux of milk from ducts into alveoli when milk removal was delayed after milk letdown as a consequence of the elastic properties of the ducts. This effect was confirmed recently by using real-time ultrasonography in dairy cows and termed cisternal recoil (Caja et al., 2004). No references to its occurrence exist in sheep and goats, but Peaker and Blatchford (1988) claimed that due to cisternal recoil the final distribution of milk in the udder of dairy goats was not affected by repeated OT injections without milking. The aim of the present study was to investigate the effect of milking interval on cisternal size and milk partitioning in the udder of goats adapted to 1X or 2X. Dairy goats in both MF treatments also were used to test the phenomenon of cisternal recoil.

MATERIALS AND METHODS

Management of Goats

Four primiparous and 10 multiparous (second parity [n = 4] and third or greater parity [n = 6]) Murciano-Granadina dairy goats with symmetrical and healthy udders located on the experimental farm of the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autonoma of Barcelona in Bellaterra were used. Goats were allocated at wk 2 of lactation into 2 balanced groups on the basis of parity and milk yield and assigned randomly to either of 2 MF treatments: 1X (0900; n = 7) or 2X (0900 and 1700; n = 7). During wk 7 of lactation, an experimental period of 5 consecutive days was allowed in which milk partitioning in the udder, cisternal size (from a.m. milking of d 1 to a.m. milking of d 3), and cisternal recoil (from p.m. milking of d 3 to a.m. milking of d 5) were evaluated. After wk 7, each goat was returned to her previous MF treatment until drying off (300 DIM).

Goats grazed natural pastures (6 h/d) and were supplemented in the shelter with concentrates in 2 separate pens according to stage of lactation. During the 5-d experimental period, goats remained in pens and their individual daily ration was a dehydrated mixture of whole-plant corn and alfalfa hay fed ad libitum, plus 0.2 kg of barley grain, 0.3 kg of alfalfa pellets, and 0.8 kg of concentrate mixture pellets (1.53 Mcal NE_L/kg; 16% CP, as fed). Goats were milked in a double-12 stall parallel milking parlor (Westfalia Surge Ibérica, Granollers, Spain) equipped with recording jars. Typical milking settings were used (vacuum, 42 kPa; pulsation rate, 90 pulses/min; and pulsation ratio, 66%) for goats of this breed (Peris et al., 1996). Milking routine included machine milking without udder preparation or teat cleaning, machine stripping, and teat dipping in an iodine solution (P3-cide plus, Henkel Hygiene, Barcelona, Spain). Average milk yield of each goat was calculated by using daily milk records of half udders before (wk 4) and after (wk 8) the experimental period.

Experimental Procedures

Cisternal size and milk partitioning in the udder. At the end of the a.m. milking of d 1, all goats were injected i.v. with 2 IU of OT (Veterin Lobulor, Laboratorios Andreu, Barcelona, Spain) to remove the residual milk in the udder. The following milkings, until the a.m. milking of d 3, were used to measure cisternal size and milk partitioning in the half udders (cisternal and alveolar milk) at the following milking intervals: 24-, 8- and 16-h and, 8-, 16-, and 24-h, for 1X and 2X goats, respectively, to minimize changes in the regular

milking schedule. Thus, on d 2, 1X goats were milked again at 1700, corresponding to the 8-h milking interval, whereas 2X goats missed this milking to achieve the 24-h milking interval.

To prevent undesired milk letdown during scanning and evaluation of milk partitioning in the udder, each goat was injected (i.v.) with 0.8 mg of an OT receptor blocking agent (Atosiban, Ferring Lab., Mallmö, Sweden) while in their pens before being taken to the milking parlor. Cisternal size was evaluated for each half udder by measuring cisternal area by ultrasonography. Ultrasonography was conducted using a real time B-mode ultrasonograph (Ultra Scan 900, Ami Medical Alliance Inc., Montreal, Canada) equipped with a 5 MHz sectorial probe (2 dB power, 80° scanning angle, 0.5 mm axial and 1.5 mm lateral resolution). The probe was placed directly against the upper part of the medium suspensory ligament, caudally to the udder, and between the inguinal lymph nodes (Ruberte et al., 1994) using the teat as scan axis. Contact gel was applied to udder skin to exclude air between probe and udder (Geleco, Laboratorios Carreras, Barcelona, Spain). Two scans were done for each udder half and transferred to a personal computer for image analysis. Cisternal area was calculated in triplicate for each scan using image treatment software (MIP4 Advanced System, Microm España, Barcelona, Spain). A conversion rate of 1,024 pixels/cm^2 was used.

As the calculated half life of the OT receptor blocking agent is 18 min (Wellnitz et al., 1999), a single dose was sufficient to prevent milk letdown while goats were moved individually to the milking parlor (approximately 4 min) to perform duplicated scans for both half udders (approximately 8 min) and to evacuate cisternal milk by machine milking (less than 3 min). Approximately 20 min after the Atosiban injection, goats were injected (i.v.) with 2 IU of OT and machine-milked to obtain letdown alveolar milk.

Cisternal recoil. Cisternal recoil phenomenon was studied in 8 (4 per MF) randomly selected multiparous goats, using repeated udder scanning after an OT challenge as proposed by Caja et al. (2004). Cisternal recoil was evaluated at 3 milking intervals (8, 16, and 24 h) for which milk partitioning in the udder was previously measured in both treatments. The experimental period lasted from the p.m. milking of d 3 to the a.m. milking of d 5.

For the initial udder scanning (0 min), each goat was injected (i.v.) with 0.8 mg of Atosiban and the cistern of each half udder was scanned as previously described. Afterwards, 2 IU of OT were injected (i.v.) to induce milk letdown and cisterns were

scanned at 5, 15, and 30 min after injection to measure changes in cisternal area of the unmilked half udders. Two scans were done for each udder half and cisternal area was calculated in triplicate by using the image treatment software previously indicated. After the last scan, goats were moved to the milking parlor and machine milked.

Statistical Analyses

Data from half udders were analyzed by ANOVA using mixed model procedure for repeated measurements (PROC MIXED; SAS 8.1, SAS Inst., Inc., Cary, NC). The mixed model used included the fixed effects of MF (treatment), milking interval, and parity; the random effects of the animal and the half udder nested within the animal; and the interactions between milking interval and MF, and between milking interval and parity; plus the residual error. For analyses of the cisternal recoil results, the fixed effect of scanning time after OT injection and the interaction between scanning time and milking interval were added to the model. Pearson's correlation coefficients between measurements were also calculated. Significance was declared as $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Cisternal Area

Values and changes in cisternal area were not different for each milking interval in 1X and 2X goats (Table 1). Cisternal area increased linearly ($P < 0.001$) as milking interval increased in 1X ($R^2 = 0.96$) and 2X ($R^2 = 0.99$) goats. Average cisternal size observed 8 h after milking (11.1 ± 1.3 cm^2) was slightly smaller than that measured in Saanen goats (Bruckmaier and Blum, 1992) for the same milking interval and probe frequency (5 MHz). At 24 h after milking, average cisternal area (27.3 ± 1.4 cm^2) increased proportionally to elapsed time (246%) indicating that cisterns became larger to accommodate more efficiently milk accumulation in the udder. Larger cisternal area may explain the small negative effects of long milking intervals on milk yield previously reported in Murciano-Granadina goats (-17%; Salama et al., 2003b). No references are available describing cisternal size at long milking intervals in different breeds of dairy goats, so the ability of Murciano-Granadina goat udders to adapt to 1X cannot be corroborated.

Although udder halves of goats are much smaller than udder quarters of cows, cisternal area values in our results were similar or greater than that reported by Ayadi et al. (2003a) for front quarters of dairy cows (12 to 21 cm^2 for 8- to 24-h milking interval), confirming the importance of cisternal size in dairy goats. Similarly, cisternal area reported

at 8-h in Manchega and Lacaune dairy ewes (14 to 24 cm^2; Rovai et al., 2002) and at 24-h milking intervals in Sarda ewes (19 cm^2; Nudda et al., 2000) were both greater than those reported in cows.

Table 1. Cisternal size, milk partitioning, and milk accumulation rates at different milking intervals in dairy goats milked once (1X) or twice (2X) daily.[1]

Item	1X			2X			SEM
	8 h	16 h	24 h	8 h	16 h	24 h	
Milk yield, mL/d[2]	——— 881[b] ———			——— 1118[a] ———			105
Cisternal area, cm^2	8.7[c]	21.9[b]	28.3[a]	13.4[c]	20.4[b]	26.2[a]	2.5
Cisternal milk, mL	181[c]	469[b]	670[a]	249[c]	453[b]	743[a]	51
Alveolar milk, mL	173[bc]	234[a]	238[a]	134[c]	209[ab]	230[a]	23
Total milk, mL	357[c]	706[b]	906[a]	382[c]	661[b]	971[a]	68
Cisternal fraction, %	51[d]	66[bc]	73[a]	62[c]	68[b]	76[a]	2.7
Milk accumulation, mL/h							
Cisternal	21.4[b]	28.3[a]	26.8[a]	30.1[a]	28.1[a]	31.0[a]	1.8
Alveolar	22.0[a]	15.0[bc]	10.2[df]	16.6[b]	12.7[cd]	9.3[f]	1.5
Total	42.9[ab]	42.8[ab]	36.6[c]	46.8[a]	41.0[bc]	40.4[bc]	1.8

[1] Data are least square means for half udders.
[2] Average half-udder milk yield before (wk 4) and after (wk 8) the experimental period.
[a, b, ... f] Means with different superscripts within row differ ($P < 0.05$).

No significant differences ($P = 0.26$) were detected between left and right half udder cisternal areas in our machine milked goats, in contrast to the results of Nudda et al. (2000), who found that left udders had greater cisterns than right udders in hand milked Sarda dairy ewes. This asymmetry in sheep was attributed to the different stress applied to udder halves during hand milking that was not produced by machine milking in our case.

Milk Partitioning in the Udder

Average milk yield during the experimental period was normal for the breed and losses in milk yield due to 1X (−21%) agree with those reported in Murciano-Granadina goats in early lactation (Salama et al., 2003b). Volumes of total milk yield and cisternal milk increased linearly ($R^2 = 0.97$ to 0.99; $P < 0.001$) with milking interval up to 24 h in both 1X and 2X goats (Table 1). Total milk stored in the udder at 8, 16, and 24 h after milking represented 41, 80 and 103%, and 34, 59 and 87% of daily milk yield recorded in

1X and 2X goats, respectively. Daily milk yield calculated by applying the appropriate individual milking interval did not differ from actual daily milk yield at each MF (1X, +3%; $P = 0.72$; and, 2X, −7%; $P = 0.28$).

Although greater cisternal size was expected in 1X goats to accommodate greater milk accumulation in the udder, as indicated by Knight and Dewhurst (1994) in Saanen goats after a short period of 1X, volumes of cisternal milk and cisternal areas did not differ between 1X and 2X goats at either milking interval. Lack of difference indicates that the 5-wk period of 1X treatment was not sufficient in Murciano-Granadina goats to produce appreciable changes in cisternal size, or that its effect was on the alveolar compartment to become larger due to the elevated pressure of stored milk (goats milked 1X stored numerically more milk in the alveoli than 2X goats).

Volume of alveolar milk increased from 8- to 16-h milking interval in a similar manner for both 1X and 2X goats and remained unchanged thereafter (Table 1). These results agree with the pattern of milk accumulation reported in dairy ewes (McKusick et al., 2002a) and in dairy cows (Davis et al., 1998; Ayadi et al., 2003a), but disagree with those of Peaker and Blatchford (1988) in Saanen dairy goats in which alveolar milk reached a plateau 6 h after milking. This apparent contradiction could be due to differences in the experimental techniques used. In the present study we used an OT receptor blocking agent, whereas Peaker and Blatchford (1988) used direct cannulation without the blocking of OT receptors, which may have induced a transient OT release (Mayer et al., 1991).

Percentage of cisternal milk increased with time since milking for as much as 24 h, but the value at 8-h milking interval was smaller ($P < 0.05$) in 1X goats than in 2X goats (Table 1). As discussed above, the decrease in percentage of cisternal milk in 1X goats at 8-h may be a consequence of their numerically greater alveolar milk fraction at all milking intervals. Percentages of cisternal milk continued to increase at 16- and 24-h milking intervals, but differences between 1X and 2X groups disappeared. Cisternal milk previously reported in 2X milked Saanen goats (Peaker and Blatchford, 1988; Knight et al., 1994b) ranged from 55% (1 h) to 85% (16 h), which is greater than the average values reported in Table 1 (8 h, 51%; and, 16 h, 68%) in Murciano-Granadina goats. These differences are less likely to be due to breed differences and more likely a consequence of different techniques used to separate milk fractions.

On average 75% of total milk stored in the udder 24 h after milking was cisternal milk in our study. Although no data exist to compare with our results at this 24-h milking interval

in goats, this maximum value was greater than that reported in East Friesian dairy ewes (57%; McKusick et al., 2002a) and in Holstein cows (40%; Ayadi et al., 2003a). A greater cisternal milk percentage was observed in Sarda dairy ewes (82%; Nudda et al., 2000) at 24-h milking interval, but this value may be overestimated because the ewes were in late lactation and adrenalin was used as an inhibitor of milk ejection. Knight et al. (1994b) reported smaller percentages of cisternal milk using direct teat cannulation or milking after applying an OT receptor blocking agent (57 to 59% of total milk) than when using adrenalin (85%).

Larger cisternal storage capacity observed in the present study in goats allows inhibitory factors of lactation to be diverted away from secretory cells in the alveolar compartment (Wilde et al., 1995). Moreover, large-cisterned udders also reduce alveolar pressure, thus avoiding possible damage to MEC (Peaker, 1980) and impairment of their TJ (Stelwagen et al., 1994b). This high cisternal capacity may explain why loss in milk yield during 1X in medium-cisterned goats, such as Murciano-Granadina dairy goats (−17%; Salama et al., 2003b) is smaller than in small-cisterned goats, such as Saanen (−26%; Wilde and Knight, 1990) and Alpine (−35%; Mocquot, 1978), but greater than in large-cisterned goats such as Canarian dairy goats (−6%; Capote et al., 1999). Similarly, cows with a greater cisternal filling at 8-h (Knight and Dewhurst, 1994) and 24-h (Davis et al. 1998) milking intervals, were more tolerant to 1X.

Correlations among Cisternal Area and Milk Fractions

The correlation between cisternal milk volume and cisternal area has not been reported previously in dairy goats. The highest correlation between cisternal milk volume and cisternal area was detected 8 h after milking (Table 2). But as milking interval increased, correlation between cisternal milk volume and cisternal area decreased in both 1X and 2X goats. Correlations were not significant 24 h after milking. Similarly, Ayadi et al. (2003a) reported that correlations between cisternal milk volume and cisternal area in dairy cows varied quadratically with milking interval, peaking between 8- and 12-h, and lowest for the 24-h milking interval. This quadratic pattern was explained by the fact that at longer milking intervals enlarged cisterns could not be completely visualized by ultrasonography as also was true for goats. In our case, milk stored in the large ducts was not included in the estimation of the cisternal area of the udder. Because of the large cisterns observed in goats after 8-h milking interval (>50% cisternal milk), correlation between volume of cisternal milk and cisternal area should improve when using a lower frequency probe, which gives a

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deeper and wider exploration field. Nudda et al. (2000) reported a greater correlation (r = 0.82; 24 h after milking) in large-cisterned Sarda dairy ewes using a 3.5 MHz probe.

Correlation values in our study were similar to those reported by Rovai et al. (2002) in dairy ewes, but lower than those reported in meat sheep (Caja et al., 1999a) or dairy cows (Bruckmaier et al., 1994b; Ayadi et al., 2003a), which corresponds with their relatively smaller size of cistern. Cisternal area also correlated positively with total milk yield, but only values at 8 h were significant at both milking frequencies (Table 2). Correlation of cisternal area with alveolar milk also was positive, but only significant for 2X goats at the 8-h milking interval.

Table 2. Correlations among cisternal area and different milk fractions in Murciano-Granadina dairy goats milked once (above diagonal) or twice (below diagonal) daily.

	Milking interval	Cisternal area	Cisternal milk	Alveolar milk	Total milk
Cisternal area	8		0.76**	0.19	0.70*
	16		0.68*	0.48	0.84***
	24		0.29	0.20	0.30
Cisternal milk	8	0.72**		-0.29	0.59*
	16	0.57*		-0.05	0.69*
	24	0.42		0.38	0.95***
Alveolar milk	8	0.69**	0.61*		0.76**
	16	0.20	0.70**		0.69*
	24	0.39	0.61*		0.65*
Total milk	8	0.77**	0.96***	0.80***	
	16	0.46	0.95***	0.88***	
	24	0.46	0.98***	0.77**	

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

The correlation between milk fractions in the udder supported the role played by the cisterns in milk accumulation. A very high positive correlation between cisternal milk and total milk was observed for 2X goats at all milking intervals, but 1X goats only reached similar values at 24 h (Table 2). This difference indicates that the role of the cistern in storing milk is important at all milking intervals in 2X goats. For 1X goats, the cistern is

more important at longer milking intervals (e.g., 24 h), after the alveoli have become full, which agrees with the values reported in Table 1. Cisternal milk correlated positively with alveolar milk in 2X, but not in 1X (Table 2). Correlation coefficients between alveolar milk and total milk were smaller and less affected by milking interval and MF than the correlation between cisternal milk and total milk (Table 2). This difference indicates that the milk yield potential of the alveolar compartment may be not expressed if the size of the cisternal compartment or the MF prevents milk drainage from the alveoli.

Effect of Parity Number on Cistern Size and Milk Partitioning in the Udder

Values of cisternal area and cisternal milk volume according to time after milking in primiparous and multiparous goats are shown in Figure 1. Primiparous goats had smaller cisternal area and lesser cisternal milk than multiparous goats (15.0 vs. 22.3 cm^2; and, 330 vs. 512 mL, respectively; $P < 0.01$). Although no data exist for comparison with goats, our results are in accordance with the trend reported in dairy cows (Bruckmaier et al., 1994b; Pfeilsticker et al., 1996) and in dairy ewes (Rovai et al., 2002), suggesting an enlargement of the mammary cistern with increasing age.

Differences in storage capacity of the cisterns between primiparous and multiparous goats were more evident after 24 h of milk accumulation, in which multiparous goats had larger cisternal area (29.9 vs. 22.6 cm^2; $P < 0.05$) and were able to store more volume of milk in the cistern (791 vs. 481 mL; $P < 0.01$) than primiparous goats. Moreover, after 24 h of milk accumulation, parity number correlated positively with cisternal area (r = 0.44; $P < 0.05$), cisternal milk (r = 0.56; $P < 0.01$), alveolar milk (r = 0.35; $P < 0.08$), and total milk (r = 0.57; $P < 0.01$). These results suggest that smaller cisterns would be full sooner to cause greater intra-alveolar pressure and more autocrine factors in milk (putative FIL), which is only effective in the alveolar compartment (Wilde et al., 1995) to inhibit milk secretion. Our data agree with the previous results in which primiparous goats milked 1X suffered greater losses (−30%) than older goats (−11%; Salama et al., 2003b).
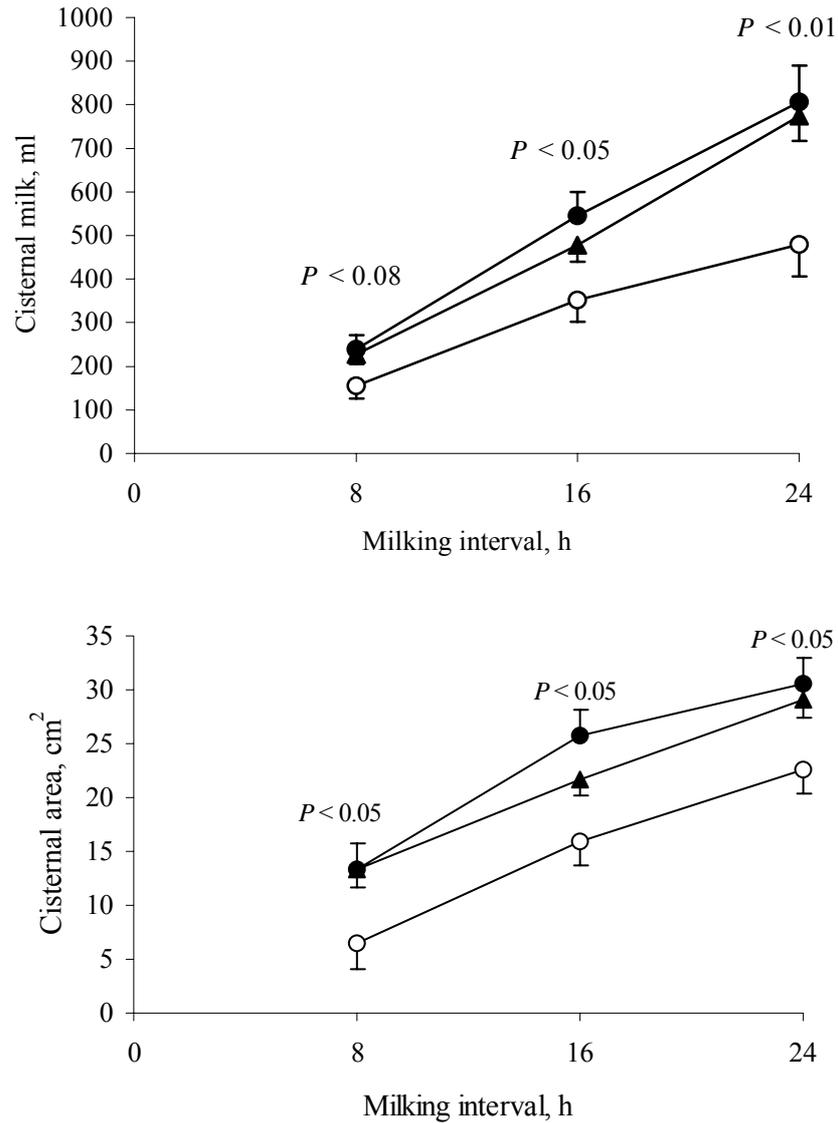
Figure 1. Effect of milking interval on cisternal milk (upper panel) and cisternal area (lower panel) by half udder based on parity number: $\bigcirc$, 1st parity (n = 4); $\bullet$, 2nd parity (n = 4); $\blacktriangle$, $\geq$ 3rd parity (n = 6). Vertical bars represent SE.

Cisternal Recoil

Cisternal area changes produced according to time since the OT challenge are shown in Table 3. The MF did not affect cisternal area and interaction between treatment and milking interval also was not significant. Therefore, data from both treatments were analyzed jointly (n = 8 goats).

Initial cisternal area (before OT injection) was smaller ($P < 0.05$) for the 8-h than 16- and 24-h milking intervals, but no differences were detected between 16- and 24-h. Cisternal areas reached their greatest values (29.5 cm^2 on average) for the 24-h milking

interval and did not vary at any time point, suggesting that the maximum cisternal storage capacity was reached after 24 h milk accumulation regardless the MF. However, cisternal area increased 5 min after the OT injection for the 8-h (79%, $P < 0.001$) and 16-h (19%, $P < 0.05$) milking intervals, but remained constant thereafter, indicating that the cisternal recoil phenomenon reported by Caja et al. (2004) in dairy cows was not produced in goats. Lack of cisternal recoil in goats agrees with another report (Pfeilsticker et al., 1996), in which no change in the amount of cisternal milk occurred for as much as 120 min after teat stimulation in dairy cows. Moreover, the greater cisternal milk percentages detected in our goats (ranging from 51 to 76% of total milk) and the small contact surface between the alveolar and cisternal compartments made the return of a significant amount of cisternal milk to the alveoli difficult. These results confirm the general statement of good milkability of dairy goats and their weak dependency on OT release for milk removal (Bruckmaier et al., 1994a).

Table 3. Change in cisternal area measured by ultrasonography after an oxytocin challenge on the basis of milking interval in dairy goats.[1]

Milking interval, h	Time after i.v. oxytocin (min)				SEM
	0	5	15	30	
8	14.4[bd]	25.4[ad]	25.8[a]	24.2[ad]	2.1
16	24.9[bc]	29.6[ac]	29.5[a]	29.5[ac]	2.0
24	28.4[c]	30.2[c]	29.2	30.0[c]	2.1

[1] Data are least square means for half udders in cm^2.
[a, b] Means with different superscripts within row differ ($P < 0.05$).
[c, d] Means with different superscripts within column differ ($P < 0.05$).

CONCLUSIONS

Cisternal changes according to time after milking were easily monitored by udder scanning (after 8 h of milk storage) in dairy goats. No apparent changes in the storage characteristics of the cisternal compartment of the udder of dairy goats were observed as a consequence of once daily milking during early lactation. Multiparous goats had greater cisternal area and are able to store more milk in the cisternal compartment than primiparous goats. No cisternal recoil occurs in goats indicating that, once milk is ejected, it is unable to return to the alveoli regardless whether milking is performed.

CHAPTER 6: OMITTING THE DRY PERIOD

CHAPTER 6

EFFECTS OF OMITTING THE DRY OFF PERIOD ON MILK YIELD, COLOSTRUM COMPOSITION AND MAMMARY CELL TURNOVER IN DAIRY GOATS

ABSTRACT

Seventeen pregnant multiparous Murciano-Granadina dairy goats (577 L in 300 DIM) were used to study the effects of dry off period length on lactational performance in the subsequent lactation. Goats were kept in a semi-intensive exploitation system with once daily milking throughout lactation and 1 kidding per year. Goats were mated after estrus induction by the buck effect at wk 29 of lactation and were assigned to 2 experimental groups according to dry off treatment: goats that were dried off 56 d before expected kidding (D56; n = 9) and goats without dry off (D0; n = 8). After parturition, kids were weighed and removed from their mothers before sucking. Goats were hand milked to obtain colostrum and machine milked thereafter. Colostrum was sampled for composition and IgG analysis. Milk yield was recorded weekly during the preceding and subsequent lactations. Udders were biopsied in a sample of goats at −70 (late lactation), −49 (during dry off) and 48 d (early lactation) to kidding. Apoptotic and proliferating cells in mammary tissues were detected immunohistochemically. Five goats (63%) in the D0 group dried off spontaneously at d 27 ± 4 before kidding and were considered as a separate group (D27). The rest of the D0 goats yielded 0.86 L/d from d −56 to kidding. Goats kidded 2.25 kids/goat, but D0 kids had lower birth weight (1.7 kg) than D27 (2.2 kg) and D56 (2.1 kg). Colostrum of D0 goats contained less IgG (5.6 mg/mL) than D27 (32.9 mg/mL) and D56 (42.4 mg/mL). In the following lactation (210 DIM), D0 goats produced less milk (1.78 L/d) than D27 (2.51 L/d) and D56 (2.24 L/d), with no differences between D27 and D56. Apoptosis and proliferation indices increased from 0.51 and 2.09%, at d −70, to 1.75 and 7.12% at d −49 (d 7 of dry off) in D56 goats. Despite differences in milk yield at early lactation (d 48) between D0, D27 and D56 groups (1.85, 2.74, and 2.43 L/d; respectively), no differences in apoptosis or proliferation indices were detected (D0, 0.65 and 2.48%; D27, 0.68 and 1.37%; and, D56, 0.71 and 2.95%), indicating that dry period length did not affect mammary cell turnover in the subsequent lactation. Omitting the dry period between lactations reduced the quality of the colostrum and had negative effects on milk yield in dairy goats. Goats dried off for 27 d

were as productive as goats dried for 56 d, indicating that 1 mo of drying off may be enough in practice.

INTRODUCTION

A non lactating dry period before parturition is necessary to permit replacement of damaged or senescent epithelial cells (Capuco et al., 1997), and hence to maximize milk production during the ensuing lactation. Omitting the dry period in dairy cows reduced milk yield by 15 to 25% in the subsequent lactation (Swanson, 1965; Remond et al., 1992; Rastani et al., 2005), indicating that a dry period between lactations is indispensable for dairy cows. However, omitting the dry period accompanied with bST injection resulted in no loss of production in multiparous cows, but in primiparous cows there was a loss (Annen et al., 2004a).

Available information indicates that the dry period seems to be unnecessary in dairy goats. The only study carried out on multiparous goats with the half-udder design (Fowler et al., 1991) showed that continuously milked half-udders were as productive as half-udders with the dry period.

Acquisition of passive immunity by the neonate depends on consumption of a sufficient amount of colostral IgG prior to cessation of macromolecular transport by the intestine (Stott and Fellah, 1983). Continuous milking of 1 half-udder throughout the dry period in dairy cows reduced the massive selective transfer of blood IgG into the colostrum of these glands (Brandon and Lascelles, 1975). In contrast, colostrum formation was normal in the contralateral, unmilked glands. These results show that there is a local regulation of colostrum formation and indicate that local signals, such as continuous milking throughout the dry period, can impede colostrogenesis, even during late gestation when hormonal influences favor its establishment. If colostrum contains a low concentration of IgG, the neonate has to be fed a large amount of colostrum to obtain a sufficient amount of IgG. However, Stott and Fellah (1983) suggested that large amounts of colostrum containing a low concentration of IgG would not be absorbed adequately; instead limited amounts of colostrum with a high concentration of IgG may be more beneficial. The effect of omitting the dry period on colostrum quality has not been studied before in dairy goats.

Milk yield depends on the activity and number of mammary epithelial cells (Knight, 2000). The number of mammary cells is determined by the rates of cell apoptosis and proliferation. Apoptosis and proliferation occur in the mammary gland throughout lactation,

resulting in a considerable turnover of mammary cells (Capuco et al., 2001; Colitti et al., 2004a). Capuco et al. (2001) reported daily rates of proliferation and apoptosis in the bovine mammary gland of 0.3 and 0.56%, respectively. Assuming that these indices are constant throughout lactation and that cells which die are not those which have proliferated, Capuco et al. (2001) calculated that 90% of mammary cells are renewed during lactation. Moreover, they estimated that if mammary cells die after proliferating, then more than half of the cells could renew themselves during a 240-d lactation. Besides the stage of lactation, some management factors such as GH injection (Capuco et al., 2001), milking frequency (Hale et al., 2003), and diet energy density (Norgaard et al., 2005) affect the levels of mammary apoptosis and proliferation. It is not known whether omitting the dry period can affect mammary cell turnover in the subsequent lactation in dairy goats.

The objective of this study was to investigate the effects of omitting the dry period on colostrum quality, milk yield, and mammary cell turnover in the subsequent lactation in Murciano-Granadina goats.

MATERIALS AND METHODS

The experimental procedures and animal care conditions were approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona.

Animals and Management Conditions

Seventeen pregnant multiparous Murciano-Granadina dairy goats from the herd of the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autonoma de Barcelona were used. Goats were mated naturally at wk 29 of lactation (April) after estrus induction by the buck effect. Eight wk before the expected kidding date (293 ± 1 DIM), goats were assigned to 2 experimental groups: goats that were dried off for 56 d (D56; n = 9) and goats without dry off (D0; n = 8). Five of the 8 goats assigned to the D0 group dried off spontaneously at d 27 ± 4 before kidding and were considered as a separate group (D27). Goats grazed for 6 h daily and were supplemented indoors with a commercial concentrate (6.4 MJ NE/kg and 160 g CP/kg; as fed) at a flat rate of 0.5 to 1.0 kg/d according to lactation stage, and with 0.5 kg alfalfa hay and 0.5 kg alfalfa pellets.

Goats were milked 1X at 9 a.m. in a double-12 stall parallel milking parlor (Westfalia-Separator Ibérica, Granollers, Spain) with recording jars (2 L ± 5%) and a low milk pipeline. Milking was at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and

a pulsation ratio of 66%. Milking routine included machine milking, stripping before cluster removal, and teat dipping in an iodine solution (P3-cide plus, Henkel Hygiene, Barcelona, Spain).

Sample Collection, Analysis and Measurements

Milk yield was recorded weekly using the recording jars in the milking parlor until wk 30 of the subsequent lactation (wk 80 of the experiment) at which goats were mated. After parturition, kids were weighed and separated from their mothers before sucking. Goats were hand milked, and fresh colostrum samples were collected to determine specific gravity (SG) and colostrum composition. For the IgG concentration, colostrum samples were frozen at -20 °C until analysis. The SG of colostrum was measured using a density flask (Afora S.A., Madrid, Spain) according to Lewis (1987). Colostrum TS were calculated by drying in an oven at 103 ºC overnight. Fat was analyzed by the Gerber method. Non CN protein was measured by Kjeldahl analysis of the filtrate after precipitation with 10% acetic acid and 1M sodium acetate, and NPN was measured by Kjeldahl analysis of filtrate after precipitation with 20% TCA (International Dairy Federation, 1993). Total protein was expressed as N × 6.38, and CN was calculated as the difference between total protein and non CN protein.

Concentration of IgG in the colostrum was determined by the radial immunodiffusion technique using VET-RID kit plates specific for goat IgG (Bethyl Laboratories, Inc., Montgomery, TX). Colostrum samples were diluted (1:10) using PBS (pH = 7.4) and then the wells on the plates were filled with 5 μL of diluted colostrum. Plates were incubated at room temperature for 24 h, after which the diameters of the ring-shaped precipitates were measured to the nearest tenth of a millimeter using a scaled ocular of a microscope (Olympus SZH, Olympus Corporation, Tokyo, Japan). A reference curve was constructed by plotting the diameter of the precipitated rings of 3 standard concentrations (250, 1000, and 2000 mg/dL) on two-cycle semilogarithmic graph paper. The concentration of IgG in the samples was determined by interpolation from the reference curve.

Mammary Biopsies and Immunohistochemistry

The first biopsy was obtained from 7 animals (3 D56 and 4 D0) at d 284 ± 2 of the preceding lactation. Three of the 4 biopsied D0 animals became D27 thereafter. The second biopsy was obtained from the same 3 goats in the D56 group at 7 d after drying off. The last biopsy was obtained from 9 goats (3 D56 biopsied twice previously, 3 D27 biopsied once previously, 1 D0 biopsied once previously, and 2 D0 biopsied for the first time) at d 48 ± 1

of the subsequent lactation. Mammary biopsies were taken after milking using a 14 gauge needle (Bard Max·Core, Covington, GA) after subcutaneous local anesthesia (2 ml Lidocaine 2%, B. Braun Medical SA, Barcelona, Spain) and skin incision in 1 half udder. The biopsy needle was introduced approximately 5 cm in the parenchyma 2 or 3 times to obtain sufficient tissue. Mammary samples were fixed in 10% neutral buffered formalin overnight at 4°C before being transferred to 70% ethanol until further processing. Samples were embedded in paraffin, cut at 3 μm, and were stained with hematoxylin-eosin, or processed for detection of cell proliferation by localizing proliferating cell nuclear antigen (PCNA) and cell apoptosis using the TUNEL.

PCNA localization. Deparaffinized rehydrated tissue slides were incubated in 3% H_2O_2 for 30 min to inhibit endogenous peroxidase. To unmask the antigens, tissue slides were placed in a covered glass staining dish containing 10 m*M* citrate buffer (pH 6.0) and microwave heated for 10 min. The slides were then incubated with bovine albumine in PBS (60 min) to block nonspecific binding of antibodies. To stain PCNA, slides were then incubated with 100 μL of diluted mouse monoclonal antibody to PCNA (clone PC10, BioGenex, San Ramon, CA) overnight at 4 °C. In the morning, slides were incubated for 60 min at room temperature with 1:200 goat anti-mouse Ig biotin (DakoCytomation, Glostrup, Denmark) as a secondary antibody. Slides were then incubated with 1:100 avidin-biotin peroxidase complex (Immuno Pure ABC Peroxidase Staining kit Standard, Pierce Biotechnology, Rockford, IL) for another 60 min at room temperature. Slides were incubated for 5 to 10 min with the chromagen, 3,3`-diaminobenzidine tetrahydrochloride (Sigma, Madrid, Spain) in PBS containing 0.04% H_2O_2. Finally, slides were counterstained with hematoxylin.

Detection of cell death by TUNEL. A commercial kit (Apop Tag[®], Serologicals Corporation, Norcross, GA) was used to visualize apoptotic cells. After deparaffinization and rehydration, slides were incubated with proteinase K (20 μg/mL of PBS). Slides were quenched with 2% H_2O_2 in PBS for 5 min, incubated in equilibration buffer for 20 min and then incubated with terminal deoxynucleotidyl transferase for 60 min in a humidified chamber at 37 °C. Slides were washed with stop / wash buffer for 20 min at room temperature and then incubated with anti-digoxigenin-peroxidase for 30 min at room temperature in a humidified chamber. Tissue sections were incubated for 5 to 10 min with 3,3`-diaminobenzidine tetrahydrochloride (Sigma, Madrid, Spain) in PBS containing 0.04%

hydrogen peroxide. Sections were washed with distilled water and then counterstained with hematoxyline.

Cell counting. Tissue sections were viewed by light microscopy (Olympus BH2, Olympus Corporation, Tokyo, Japan) to quantify PCNA antigen expressing cells and apoptotic cells. For each tissue section, 5 microscopic fields were quantified. A field was selected under low power and slightly out of focus, then the objective was switched to higher power, a digital image of the microscope field was taken at 100× magnification and cells were counted on a computer monitor. On photographs of mammary tissue, number of cells per alveolus was counted.

Statistical Analysis

Data were analyzed by the PROC MIXED for repeated measurements of SAS (SAS 8.2; SAS Inst. Inc., Cary, NC). The statistical mixed model contained the random effect of the animal within the dry period length (treatment); the fixed effects of treatment and week of lactation; the interaction between treatment and week of lactation; and the residual error. Milk yield during 300 DIM of the previous lactation was used as a covariate. Data of milk persistency, colostrum composition and mammary tissue parameters were analyzed by one way ANOVA using PROC GLM with a model containing the effect of the treatment and the residual error. Logarithmic transformations ($\log_{10}$) of IgG concentrations values were used in statistical analysis because this variable was not normally distributed. Pearson's correlation coefficients between colostrum components were also calculated.

RESULTS AND DISCUSSION

Of the 8 goats assigned to D0 treatment, only 3 goats (37%) were able to maintain lactation until kidding. The other 5 goats decreased milk yield dramatically and dried off spontaneously 27 d before kidding. According to the usual management conditions of the breed (Salama et al., 2005a), goats during this experiment were milked 1X and kept in pens in late lactation and advanced pregnancy (summer), which probably led to the low number of goats that can be milked throughout pregnancy. With higher daily milking frequency and mating at earlier lactation stage, more goats are expected to start the subsequent lactation without drying off. Actual days dry were 56 ± 1 and 27 ± 4 d for D56 and D27, respectively. Kidding period was 8 d long in the present study.

Birth Weight of Kids and Colostrum Composition

Goats kidded 2.44, 1.80, and 2.33 kids/goat for D56, D27 and D0, respectively. Kids of D0 goats had lower ($P < 0.05$) birth weight than kids of D56 and D27 (Table 1). This decrease in birth weight of D0 kids may be the consequence of the nutritive stress due to the competition between lactation and pregnancy specially in the last third of gestation when the fetus growth is highest. Prepartum energy or protein restriction reduced birth weight by 6 to 10% in goats (Sahlu et al., 1995). Alternatively, D0 goats that tolerated the omission of the dry period might have been able to resist the negative effect of pregnancy because they were bearing fetuses with lighter weight. In dairy cows, shortening or eliminating the dry period did not affect calf birth weight (Grummer and Rastani, 2004). The impact of pregnancy on milk yield is partially thought to be due to the secretion of estrogen by the fetal-placental unit (Bachman et al., 1988). Estradiol concentration during pregnancy is proportional to the calf's birth weight (Guilbault et al., 1985).

Table 1. Effect of dry period length on the birth weight of kids, colostrums specific gravity and colostrum composition in dairy goats (values are least square means $\pm$ SE).

Item	Dry period length[1]		
	D56	D27	D0
Kids birth weight, kg	$2.11^a \pm 0.07$	$2.24^a \pm 0.13$	$1.71^b \pm 0.07$
Colostrum characteristics			
Specific gravity	$1.053^a \pm 0.002$	$1.048^a \pm 0.003$	$1.032^b \pm 0.004$
Log IgG, mg/mL	$1.61^a \pm 0.04$	$1.51^a \pm 0.06$	$0.74^b \pm 0.07$
Total solids, %	$23.0^a \pm 1.0$	$20.1^{ab} \pm 1.5$	$15.7^b \pm 1.9$
Fat, %	6.37 ± 0.39	5.78 ± 0.61	6.28 ± 0.78
Protein, %	$13.19^a \pm 0.75$	$10.52^a \pm 1.15$	$4.34^b \pm 1.49$
Casein, %	3.64 ± 0.20	2.92 ± 0.31	2.77 ± 0.39
Casein, % of CP	$28.2^b \pm 1.9$	$28.1^b \pm 2.9$	$63.1^a \pm 3.8$
Whey protein, %	$9.05^a \pm 0.66$	$7.18^a \pm 1.02$	$1.28^b \pm 1.31$
NPN, %	$0.51^a \pm 0.02$	$0.42^b \pm 0.04$	$0.29^c \pm 0.04$

[1] Dried off for 56 d (D56), dried off for 27 d (D27), or without dry off (D0).
[a,b,c] Means with different superscripts within row differ ($P < 0.05$).

Specific gravity of D0 colostrum was lower ($P < 0.01$) than in D56 and D27, indicting the lower quality of colostrum from goats milked throughout pregnancy (Table 1). No values of SG in the colostrum of goats are available to compare with our results. Colostral

SG of D0 (1.032) was slightly higher than values for normal goat milk which ranged from 1.022 to 1.026 (Guo et al., 2001). Measurement of colostral SG provides an inexpensive and practical method for estimating colostral Ig concentration in cows (Fleenor and Stott, 1980). Since kids of D0 had lower birth weight and their mothers produced colostrum with low SG, colostrum of D27 and D56 was used to feed those kids.

Colostrum of D0 goats contained a lower ($P < 0.01$) concentration of IgG (5.6 mg/mL) than D56 (42.4 mg/mL) and D27 (32.9 mg/mL) goats. The IgG concentration in colostrum of D0 was greater than the concentration reported by Levieux et al. (2002) in the normal milk (0.20 to 2.21 mg/mL). Nevertheless, this concentration seems to be insufficient to achieve the recommended serum level of IgG in newborn kids (>12 mg/mL; O'Brien and Sherman, 1993). In addition, a positive correlation (r = 0.86) was found between the concentration of IgG in the colostrum and in the serum of newborn kids (Argüello et al., 2004). In the case of omitting the dry period, a very large amount of colostrum has to be fed to supply kids with sufficient mass of IgG. However, large amounts of colostrum containing a low concentration of IgG would not be absorbed adequately (Stott and Fellah, 1983). IgG concentrations in D27 and D56 goats ranged from 23.5 to 64.0 mg/mL (overall mean, 39.6 mg/mL). These values are consistent with those reported in Alpine and Saanen goats (Levieux et al., 2002) and Canarian goats (Argüello et al., 2003).

Continuous milking adversely affected colostrogenesis and resulted in colostrum with a low concentration of IgG in D0 goats in our experiment. Approaching parturition, massive selective transfer of blood IgG into the colostrum in dry mammary gland causes a high concentration of IgG in the colostrum (Brandon and Lascelles, 1975). This selective transfer of IgG is locally controlled by mammary epithelial cells and when milk secretion is maintained, this selective transfer decreased (Guy et al., 1994). Therefore, continuous milking during pregnancy in D0 goats in our study maintained the activity of mammary epithelial cells, preventing the accumulation of IgG in D0 colostrum.

Casein constituted 28.2, 28.1 and 63.1% of total protein in the colostrum of D56, D27 and D0, respectively. Colostrum of D0 is close to normal goat milk in the same breed, where CN represents 73 to 76% of total milk protein (Salama et al., 2003a). Whey protein, most of which is Ig, constitutes over two-thirds of total protein in the colostrum of D56 and D27, and was greater ($P < 0.01$) than in D0 (Table 1).

As far as we know, correlations among IgG concentration, SG and other components of the colostrum have not been reported before in dairy goats (Table 2). Excluding values of

D0, concentrations of IgG correlated positively with SG (r = 0.88; *P* < 0.001), which was similar to the 0.84 reported by Fleenor and Stott (1980), but higher than the 0.53 and 0.63 reported by Morin et al. (2001) and Quigley et al. (1994), respectively in dairy cows. Colostral SG was more correlated with concentrations of protein (r = 0.91) and whey protein (r = 0.92) than with IgG concentration. The same observation was reported by Morin et al. (2001) who showed the limitations of using colostral SG as an indicator of IgG concentration in bovine colostrum.

Table 2. Correlation coefficients between colostral IgG and other colostrum components in dairy goats dried off for 56 or 27 d before kidding (n = 14).

	Total solids	Fat	Total protein	True protein	Casein	Whey protein	NPN	Specific gravity
IgG	0.85[***]	0.44	0.91[***]	0.91[***]	0.51[*]	0.92[***]	0.38	0.88[***]
Total solids		0.76[***]	0.93[***]	0.93[***]	0.74[***]	0.86[***]	0.45	0.85[***]
Fat			0.48	0.47	0.62[**]	0.37	0.39	0.32
Total protein				0.99[***]	0.64[**]	0.97[***]	0.43	0.96[***]
True protein					0.63[**]	0.97[***]	0.40	0.95[***]
Casein						0.45	0.55[*]	0.57[*]
Whey protein							0.30	0.95[***]
NPN								0.31

[*] *P* < 0.05
[**] *P* < 0.01
[***] *P* < 0.001

Milk Yield

Data of milk yield for lactation that preceded the dry period and lactation that followed the dry period are shown in Figure 1 and Table 3.

Preceding lactation. Goats that tolerated continuous milking throughout pregnancy were not the best milk producers during the first 29 wk of the preceding lactation (2.25, 2.12, and 1.98 L/d for D56, D27 and D0, respectively). However, D0 goats produced more (*P* < 0.01) milk than D27 during wk 43 and 46 of lactation. Milk persistency was similar among groups before pregnancy, but decreased as pregnancy advanced (Table 3).

In the last 3 wk before parturition, milk yield increased for D0 goats (Figure 1). Circulating prolactin in dairy goats remained low up to d 120 of pregnancy and then increased sharply during the last 20 d of pregnancy (Kornalijnslijper et al., 1997). Similarly,

an increase in milk yield during the days preceding calving was observed in dairy cows (Wheelock et al., 1965), although Remond et al. (1992) did not detect such increase.
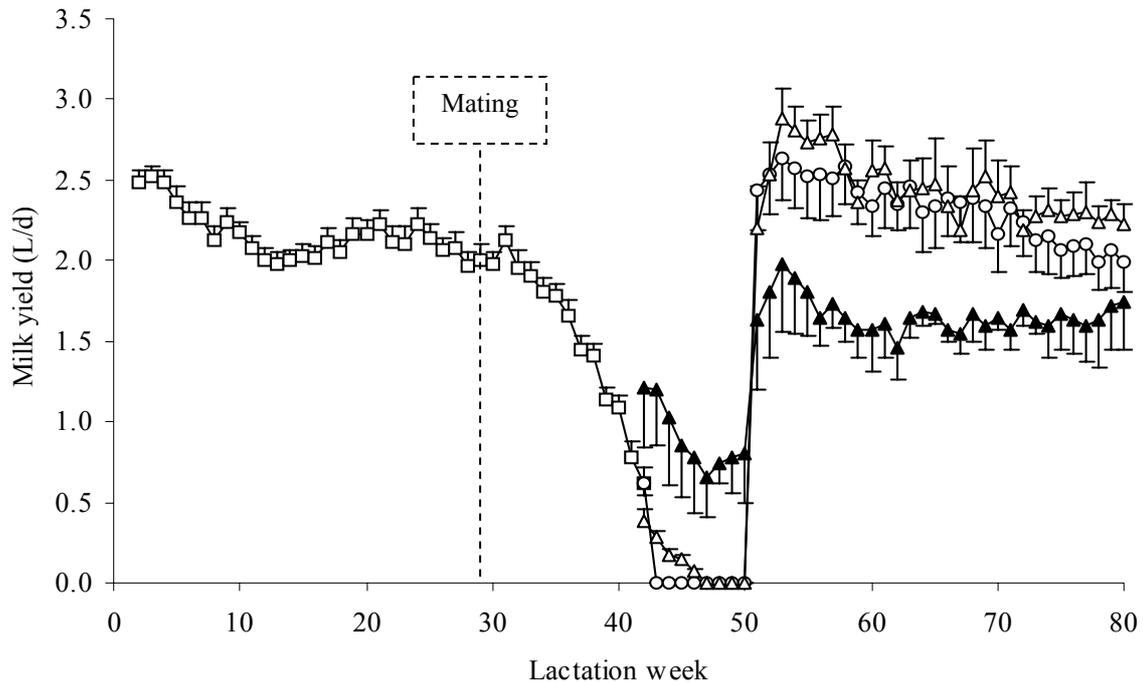
Figure 1. Milk yield of dairy goats during the pre-experimental period (□; n = 17). At wk 42 of lactation, goats were divided into 2 groups: goats that were dried off for 56 d (○; n = 9) and goats without dry off (n = 8). At wk 47 of lactation, 5 of the 8 goats assigned to no dry off produced lees than 0.25 L/d and therefore, they were dried off for 27 d (Δ; n = 5), and the rest were continuously milked (▲; n = 3).

Subsequent lactation. Due to omitting the dry period, D0 goats produced 16% less ($P < 0.01$) milk in the subsequent lactation than they did in the previous lactation. Moreover, milk yield of D0 goats in the subsequent lactation was reduced ($P < 0.05$) by 21 and 29% compared with the yield of D56 and D27 goats, respectively (Figure 1 and Table 3). On the other hand, D27 and D56 goats in the subsequent lactation produced 15% ($P < 0.01$) and 4% ($P = 0.122$), respectively more milk than they did in the equivalent period in the previous lactation. Omitting the dry period in dairy cows also reduced milk production in the subsequent lactation by 15 to 40% (Swanson, 1965; Remond et al., 1992; Rastani et al., 2005), although bST administration alleviated theses losses in multiparous cows (Annen et al., 2004d).

Table 3. Effect of dry period length on milk yield and milk persistency in dairy goats (values are least square means ± SE).

Item	Dry period length[1]		
	D56 (n = 9)	D27 (n = 5)	D0 (n = 3)
Preceding lactation			
Milk yield, L/d			
wk 2 to wk 29	2.25 ± 0.10	2.12 ± 0.13	1.98 ± 0.16
wk 30 to wk 42	1.50 ± 0.11	1.46 ± 0.13	1.65 ± 0.16
wk 43 to wk 46	...	0.15[b] ± 0.10	0.96[a] ± 0.14
wk 47 to wk 50	...	...	0.75 ± 0.10
Milk persistency, %			
Before pregnancy[2]	67.6 ± 2.3	64.2 ± 2.9	69.7 ± 3.8
Third mo of pregnancy[3]	26.0[b] ± 2.7	27.6[b] ± 3.5	45.3[a] ± 4.5
Fourth mo of pregnancy[4]	...	4.8[b] ± 3.6	34.0[a] ± 6.6
Subsequent lactation			
Milk yield, L/d			
wk 2 to wk 20	2.35[a] ± 0.12	2.60[a] ± 0.16	1.79[b] ± 0.20
wk 21 to wk 30	2.05[ab] ± 0.08	2.32[a] ± 0.10	1.76[b] ± 0.12
wk 2 to wk 30	2.24[a] ± 0.10	2.51[a] ± 0.13	1.78[b] ± 0.16
Milk persistency[5], %	65.1 ± 4.6	66.5 ± 5.8	75.8 ± 7.5

[1] Dried off for 56 d (D56), dried off for 27 d (D27), or without dry off (D0).
[2] Milk yield during wk 26 to 29 / milk yield during wk 2 to 6.
[3] Milk yield during wk 39 to 42 / milk yield during wk 2 to 6.
[4] Milk yield during wk 43 to 46 / milk yield during wk 2 to 6.
[5] Milk yield during wk 27 to 30 / milk yield during wk 2 to 6.
[a,b] Means with different superscripts within row differ ($P < 0.05$).

Fowler et al. (1991) with the half-udder design, reported that the dry period between lactations is not necessary in Saanen dairy goats. In the latter study, half udders were dried off 2 wk before mating (dry period lasted 23 wk), which is unusual and 3 times the length of the dry period in our study. Additionally, when 1 half udder was dried off, activity and number of mammary cells increased in the lactating half within the same udder (Capuco and Akers, 1990), and involution was partially inhibited in the non-lactating half (Akers and Keys, 1985). In our study both udder halves were dried off for a shorter period when goats were in late lactation and advanced pregnancy, which might explain the discrepancy with the results of Fowler et al. (1991).

Milk persistency was numerically higher ($P = 0.25$) in D0 than in D56 and D27 (Table 3). Consequently, milk losses decreased from 27% in the first 5 mo of lactation to 18% during the sixth and seventh mo of lactation and difference between D56 and D0 became no significant (Figure 1 and Table 3). Similarly, milk of cows with no dry period (Remond et al., 1997) or with a 30-d dry period (Bachman, 2002) persisted more than in cows with the conventional 60-d dry period.

No differences in milk yield were detected between D56 and D27 groups indicating that the shorter dry periods (i.e. 27 d) are sufficient for mammary involution in goats. Capuco et al. (1997) showed that mammary growth in cows was initiated within the first 25 d of the 60-d dry period. Moreover, cows with a 30-d dry period produced similar milk yield as cows with the 60-d dry period (Bachman, 2002; Gulay et al. 2003a).

Mammary Cell Turnover

Indices of apoptosis and proliferation in D56 goats at 284 DIM of the preceding lactation, 7 d after drying off, and 48 DIM of the subsequent lactation are shown in Figure 2. Indices of apoptosis (0.61%) and proliferation (2.52%) did not vary between d 284 and d 48 of the preceding and subsequent lactations, respectively. Our values of apoptosis are in the range (0.2 to 3.0%) reported in lactating cows (Capuco et al., 2001; Colitti et al., 2004a; Norgaard et al., 2005) and lactating goats (Li et al., 1999a; Wareski et al., 2001). Similar to our values of proliferation, cells expressing PCNA accounted for 0.17 to 2.99% of mammary cells during lactation in dairy cows (Colitti et al., 2004a; Norgaard et al., 2005). In our results, cell proliferation exceeds cell death which gives the impression that the mammary gland grows throughout lactation rather than regresses. The TUNEL test detects the proportion of cells dying at a given moment before being phagocytosized by macrophages and adjacent alveolar cells, whereas PCNA indicates proliferating cells for approximately 24 h (Knight, 2000). In addition, milk may be a vehicle for the elimination of mammary apoptotic cells in addition to phagocytosis. We observed some apoptotic cells in the alveolar lumen and most of these cells were localized in the alveolar epithelium (Figure 3) as previously reported in ewes (Tatarczuch et al., 1997).

Comparing d 284 (late lactation) with d 7 after dry off (involution) in D56 goats, an increase in mammary apoptosis (0.51 to 1.75%; $P < 0.06$) and proliferation (2.09 to 7.12%; $P < 0.05$) was observed (Figure 2). Similarly, Li et al. (1999a) indicated that the apoptosis index was less than 1% of cells in lactating tissues and increased at wk 1 (2 to 3%) and 2 (5%) after drying off in nonpregnant goats
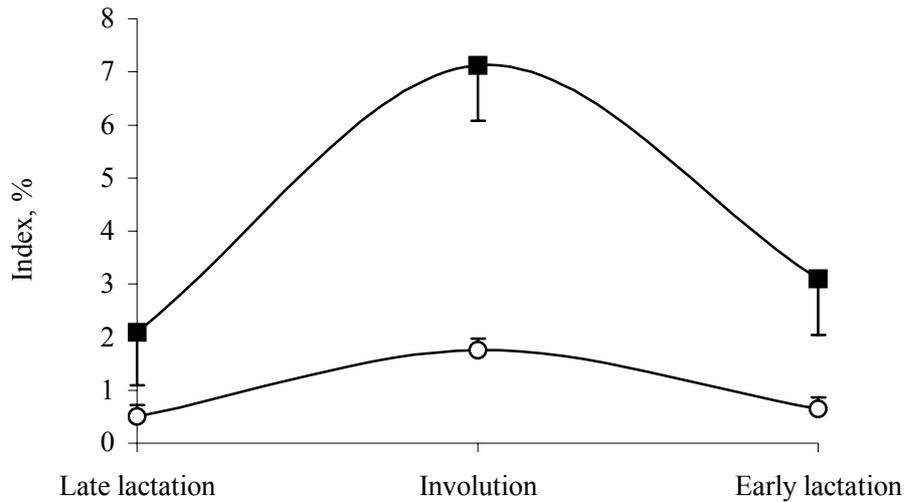
Figure 2. Apoptosis (○) and proliferation (■) indices in D56 goats (n = 3). Biopsies were taken at late lactation (284 DIM), during involution (7 d after drying off) and early lactation (48 DIM in the subsequent lactation).
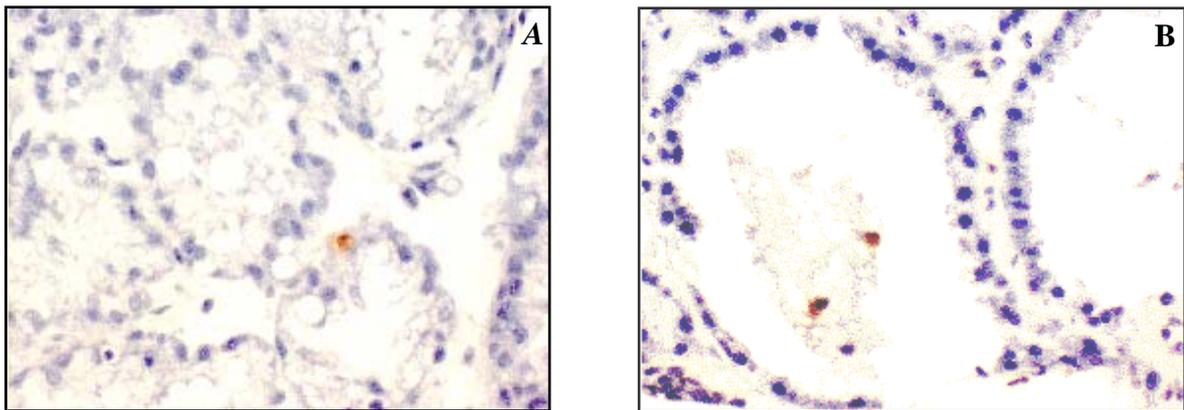
Figure 3. Identification of apoptotic cells in the mammary gland of dairy goats at wk 7 of lactation. Apoptotic cells were mainly localized in the alveolar epithelial cells (A) and some were in the alveolar lumen (B).

The peak of apoptosis probably occurred before d 7 of involution in our goats because they were in late pregnancy at drying off. Annen et al. (2003) detected the greatest incidence of apoptosis in pregnant cows at d 2 after drying off, and by d 8 the number of apoptotic cells did not differ from lactating values. High apoptosis and proliferation indices during dry off in our results indicate significant mammary cell turnover as previously observed by Capuco et al. (1997) in dairy cows, where the proliferation of mammary epithelial cells in dry cows was higher than in cows without dry off to replace senescent and damaged cells.

In the subsequent lactation (48 DIM), number of cells per alveolus in D56 ($P = 0.14$) and D27 ($P = 0.19$) goats was greater than in D0 goats (Table 4) in accordance with milk

yield levels. In our results, milk yield correlated positively with cell number per alveolus (r = 0.81; $P < 0.05$). No differences were detected between groups in apoptosis or proliferation indices for D56, D27 and D0, respectively (Table 4). These results indicate that the length of the dry off period did not affect mammary cell turnover in the following lactation.

Table 4. Effect of preceding dry period length on number of epithelial cells per alveolus and indices of apoptosis and proliferation in the mammary gland of dairy goats at 48 DIM.

Item	Dry period length[1]		
	D56 (n = 3)	D27 (n = 3)	D0 (n = 3)
Milk yield, L/d	2.43[a] ± 0.23	2.74[a] ± 0.19	1.85[b] ± 0.25
Cell number per alveolus	38.6 ± 7.1	36.3 ± 7.0	24.1 ± 5.0
Apoptotic cells, %	0.71 ± 0.28	0.68 ± 0.28	0.65 ± 0.23
Proliferating cells, %	2.95 ± 1.04	1.37 ± 1.04	2.48 ± 0.85
A : P ratio	0.24 ± 0.34	0.93 ± 0.33	0.34 ± 0.28

[1] Dried off for 56 d (D56), dried off for 27 d (D27), or without dry off (D0).
[a,b] Means with different superscripts within row differ ($P < 0.05$).

However, the absence of the dry period might have negatively affected cell renewal before kidding in D0 goats, since these goats tended to have a lower ($P < 0.20$) number of cells per alveolus than D56 and D27 goats in the subsequent lactation (Table 4). Fitzgerald et al. (2004) observed that proliferation of mammary epithelial cells in glands of primiparous dairy cows dried off for 60 d was greater than in glands without dry off at 7 d before calving. These authors reported no differences in cell proliferation between glands with or without dry off after parturition, indicating that reduced cell proliferation before parturition caused milk yield losses in the subsequent lactation in glands without dry off.

CONCLUSIONS

Omitting the dry period between lactations in Murciano-Granadina dairy goats had negative effects on milk production, probably due to impaired cellular replacement during dry off. Milking during late pregnancy reduced the immunologic quality of the colostrum. Goats dried off for 27 d before kidding were as productive as those goats dried off for 56 d and the colostrum quality was similar.

CHAPTER 7: PREGNANCY & EXTENDED LACTATION

CHAPTER 7

EFFECT OF PREGNANCY AND EXTENDED LACTATION ON MILK PRODUCTION AND UDDER COMPARTMENTS IN DAIRY GOATS MILKED ONCE DAILY

ABSTRACT

Thirty multiparous Murciano-Granadina dairy goats milked once daily were used to study the lactational effects of an extended 24-mo kidding interval (K24; n = 14) compared to the traditional 12-mo kidding interval (K12; n = 16). Over a period of 92 wk, K12 goats were mated twice at wk 29 during the first lactation and 79 during the second lactation, whereas K24 goats were mated once at wk 79 of extended first lactation. The K12 goats were dried off from wk 14 to 21 of pregnancy (wk 43 to 50 of lactation). Milk yield was recorded from wk 2 to 92, whereas milk composition were studied from wk 29 to 92. Milk fatty acids were analyzed in milk samples taken at wk 39 (wk 10 of pregnancy) and 55 (wk 5 of subsequent lactation), when milk in udder compartments (cisternal and alveolar) was also evaluated. Average milk yield during the first 29 wk was 2.23 L/d. Pregnancy reduced milk yield in K12 goats from wk 39 to 42 of lactation compared to K24 goats. During the dry period for K12 goats, milk yield of K24 goats averaged 1.53 L/d. From wk 51 to 79, K12 goats produced 32% more milk than K24 goats, but their milk contained lower fat and protein than K24 goats. No changes were detected for milk lactose and SCC from wk 51 to 79. From wk 80 to 92, differences in milk yield and milk composition between groups were not significant. Milk of pregnant K12 goats contained higher $C_{16:1}$ and conjugated $C_{18:2}$ fatty acids, and had a higher desaturase index than milk of open K24 goats at wk 39. In the following lactation (wk 55), milk of K12 goats contained higher $C_{18:2}$ and $C_{18:3}$, and lower $C_{16:0}$ fatty acids, resulting in a lower atherogenicity index compared to K24 goats. Cisternal milk at wk 39 was lower for K12 than K24 goats, whereas alveolar milk did not differ. In K12 goats, values of cisternal milk tripled, but alveolar milk only doubled at wk 55 (wk 5 of subsequent lactation) compared to wk 39, indicating the importance of the cistern in accommodating high milk yield at early lactation. Values of cisternal and alveolar milk did not differ between wk 39 and 55 for K24 goats. Fat content was higher for alveolar milk than cisternal milk for K12 goats at wk 55 and for K24 goats at wk 39 and 55. No differences in milk protein or lactose were detected between cisternal and alveolar milk. In conclusion, pregnancy reduced milk yield from wk 10 after conceiving onwards. Extended

lactation did not significantly decrease milk yield (-8.2%), but increased milk components that may contribute to cheese yield, and may be a useful strategy for reducing metabolic stress in early lactation and for simplifying herd management in dairy goats.

INTRODUCTION

Pregnancy has been shown to cause a significant decline in milk yield of dairy cows due to hormonal changes (Bachman et al., 1988; Akers, 2002) and the nutritive requirements of the fetus, especially during the third part of gestation (Bell et al., 1995). Some authors (Olori et al., 1997; Brotherstone et al., 2004) reported milk yield losses in dairy cows from 5 mo of pregnancy onwards, whereas other authors (Bormann et al., 2002) reported an earlier decline. In the only available report on lactational effects of pregnancy in dairy goats mated at peak of lactation (Knight and Wilde, 1988), milk yield decreased at the same rate in pregnant goats during the first 8 wk of pregnancy as in non-pregnant goats. Nevertheless, milk yield decreased more quickly in the pregnant goats thereafter, reaching 57% of the milk yield of non-pregnant goats in the last wk before parturition. No information on the effects of pregnancy on milk composition and milk partitioning in the udder was reported in the study of Knight and Wilde (1988). Moreover, the impact of pregnancy on milk production in late lactation (as in the case of dairy cows) has not been reported before in goats.

Extended lactation in dairy cows is an alternative to typical 300-DIM lactation. It reduces the number of dry days within the animal's lifetime and the metabolic stress related to negative energy balance during early lactation (Knight, 1997), and may be profitable for dairy farmers (Arbel et al., 2001). Linzell (1973) observed that well fed non-pregnant goats, milked twice daily, can lactate continuously for 2 to 4 yr. There is no information on the quality of milk produced from goats under extended lactation.

The objective of this study was to investigate the lactational effects of 2 different kidding intervals, producing a typical 12-mo lactation cycle in contrast to an extended lactation cycle of 24 mo in Murciano-Granadina dairy goats milked once daily. Effects of pregnancy on lactation were also evaluated.

MATERIALS AND METHODS

The experimental procedures and animal care conditions were approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona (reference CEEAH 02/400).

Animals and Management Conditions

Thirty multiparous Murciano-Granadina dairy goats located on the experimental farm of the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autonoma de Barcelona in Bellaterra were used after fall kidding (second wk of October) with a litter size of 2.12 ± 0.15 kids/goat. Murciano-Granadina goats have a wide reproduction season in Spain (Falagan, 1988), with a shallow seasonal anoestrus from February to June, and frequent out of season kidding for producing Christmas milk kid ('cabrito lechal').

Goats were divided into 2 balanced groups at wk 29 of lactation with respect to parity, milk yield (recorded from wk 2 to 29), and SCC (recorded on 2 consecutive d in wk 29). The groups were: traditional 12-mo kidding interval (K12; n = 16), where goats were impregnated twice at wk 29 and 79 and dried off twice from wk 43 to 50 (first lactation) and from wk 93 to 100 (following lactation); and extended 24-mo kidding interval (K24; n = 14), where goats were impregnated once at wk 79 and dried off once from wk 93 to 100. The average litter size in the previous kidding was 2.12 ± 0.15 and 2.23 ± 0.20 kids/goat for the goats in K12 and K24 groups, respectively. Mating was carried out in April at wk 29 and 79 after natural estrus induction by the buck effect without light treatment. A teaser buck was introduced for 4 d and then replaced by a fertile one that impregnated 82% of goats between d 5 and 9 of introduction.

During a 2-yr period, K12 goats had 2 lactations (from wk 1 to 42 and from wk 51 to 92), whereas K24 goats had one extended lactation (from wk 1 to wk 92). The period from wk 30 to wk 42 allowed us to study the effect of pregnancy on milk production in K12 pregnant goats compared to K24 open goats.

Goats grazed for 6 h/d and were supplemented in the shelter with 0.5 to 1.0 kg/d of a commercial concentrate (1.53 Mcal NE_L/kg; 16% CP, as fed) according to lactation stage, and with 0.5 kg/d alfalfa pellets. During the dry period (wk 43 to 50), goats were kept in pens and their individual daily ration consisted of a dehydrated mixture of whole-plant corn and alfalfa hay (1:1) fed ad libitum, 0.2 kg whole barley grain, 0.3 kg alfalfa pellets, and 0.5 kg concentrate pellets.

Goats were milked once daily at 0900 in a double-12 stall Casse system parallel milking parlor (Westfalia-Surge Ibérica, Granollers, Spain) with recording jars (2 L ± 5%) and low milk pipeline. Typical milking settings were used (vacuum, 42 kPa; pulsation rate, 90 pulses/min; and pulsation ratio, 66%) for goats of this breed (Salama et al., 2004). Milking routine included machine milking without udder preparation or teat cleaning, machine stripping, and teat dipping in an iodine solution (P3-cide plus, Henkel Hygiene, Barcelona, Spain).

Procedures, Sample Collection and Analysis

Milk yields of individual goats were recorded weekly by using the recording jars in the milking parlor throughout the experiment. Milk composition and SCC were recorded biweekly from wk 29 to 41 and monthly thereafter. A sample of approximately 50 mL was placed in a plastic vial, preserved with an anti-microbial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems Inc., San Ramon, CA) and kept at 4°C until analysis. Milk fat, protein, lactose and SCC were determined in the Dairy Herd Improvement laboratory of Catalonia (Allic, Cabrils, Barcelona, Spain) using infrared spectroscopy and an automated somatic cell counter (MilkoScan 4000 + Fossomatic 5000, Foss-Electric, Hillerød, Denmark).

During wk 39 and 55 (wk 10 of pregnancy and wk 5 of the subsequent lactation, respectively for K12 goats), a 50 mL milk sample from K12 and K24 goats was collected, immediately cooled, and the fat fraction was separated by centrifugation for 30 min at 6000 × g and 4°C and then stored at −80°C. Methylation of FA was performed according to Sukhija and Palmquist (1988) and the modifications of Kramer et al. (1997). Fatty acid methyl esters were analyzed by gas chromatography (HP 6890, Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector and HP-23 (cis/trans FAME) capillary column (60 m × 0.25 mm i.d. with 0.25-μm film thickness; Agilent Technologies, Palo Alto, CA). Initial temperature was 40°C (for 6 min) increased to 230°C (for 12 min) at a rate of 5°C/min. Individual FA were identified by comparison of retention times to those of pure standards (Sigma-Aldrich Quimica, Madrid, Spain) and expressed as percentages of the total FA detected as fatty acid methyl esters.

Milk partitioning in the udder (cisternal and alveolar) of K12 and K24 goats was determined at wk 39 and 55. To prevent undesired milk letdown during evaluation of milk partitioning in the udder, each goat was i.v. injected with 0.8 mg of Atosiban (Tractocile, Ferring, Spain), an OT receptor blocking agent, while in their pens according to Salama et

al. (2004). Thereafter, goats were taken to the milking parlor and machine milked to evacuate cisternal milk. Approximately 20 min after the Atosiban injection, goats were i.v. injected with 2 IU of OT (Laboratorios Ovejero, León, Spain) and machine-milked to obtain the letdown alveolar milk. Samples of cisternal and alveolar milk were taken for the analysis of fat, protein, lactose and SCC as previously described.

Statistical Analysis

Data were analyzed by ANOVA using the PROC MIXED for repeated measurements (SAS Inst., Inc., Cary, NC, Version 8.2). The statistical mixed model contained the fixed effects of treatment (K12 or K24), wk of lactation and parity, the random effect of animal within treatment, the interactions between treatment and wk of lactation and between treatment and parity, and the residual error. For data analyses of milk fractions composition, the fixed effect of fraction (cisternal or alveolar) and the triple interaction between treatment, wk of lactation and fraction were added to the model. Logarithmic transformations ($\log_{10}$) of SCC values were used in the statistical analysis.

RESULTS AND DISCUSSION

Milk Yield

Average data of milk yield and milk composition, and milk yield changes throughout the experiment (92 wk) are shown in Table 1 and Figure 1. Milk yield before conception (wk 2 to 29 of lactation) and during the first 7 wk of pregnancy (wk 30 to 36 of lactation) was similar for K12 and K24 goats and averaged 2.15 ± 0.14 L/d. Milk yield of K12 goats tended to be lower than K24 goats at wk 8 (1.52 vs. 1.80 L/d; $P < 0.07$) and wk 9 (1.38 vs. 1.65 L/d; $P < 0.12$) of pregnancy. However, the impact of pregnancy started to be significant ($P < 0.05$) in K12 goats at wk 10 of pregnancy (wk 39 of lactation). Milk yield losses were 0.32 ($P < 0.05$), 0.51 ($P < 0.001$), 0.85 ($P < 0.001$) and 1.11 ($P < 0.001$) L/d at wk 10, 11, 12 and 13 of pregnancy, respectively, representing approximately a 1.5-fold increase in the rate of decline every wk. Knight and Wilde (1988) reported that pregnancy had no effect on milk yield during the first 8 wk of pregnancy in Saanen goats, but milk yield decreased rapidly thereafter and was 57% of the value of non-pregnant goats in the last wk of pregnancy. Milk yield in K12 goats at wk 13 of pregnancy (wk 42 of lactation) was 21% of the value of K24 goats (0.3 vs. 1.41 L/d; $P < 0.001$). In our experiment, goats were milked once daily and mated in late lactation (wk 29), which might explain the greater milk yield losses compared to the experiment of Knight and Wilde (1988) where goats were

milked twice daily and mated much earlier than in our study (wk 8). The greater effect of pregnancy on late lactation may be due to the decreasing power of galactopoietic hormones as lactation advanced. Sorensen and Knight (2002) reported that blood concentration of GH decreased, whereas insulin concentration increased, as lactation advanced in dairy cows. Pregnancy also caused a significant decline in milk yield of dairy cows in late lactation from mo 5 of gestation onwards (Olori et al., 1997; Brotherstone et al., 2004) or from as early as mo 3 of pregnancy (Bormann et al., 2002).
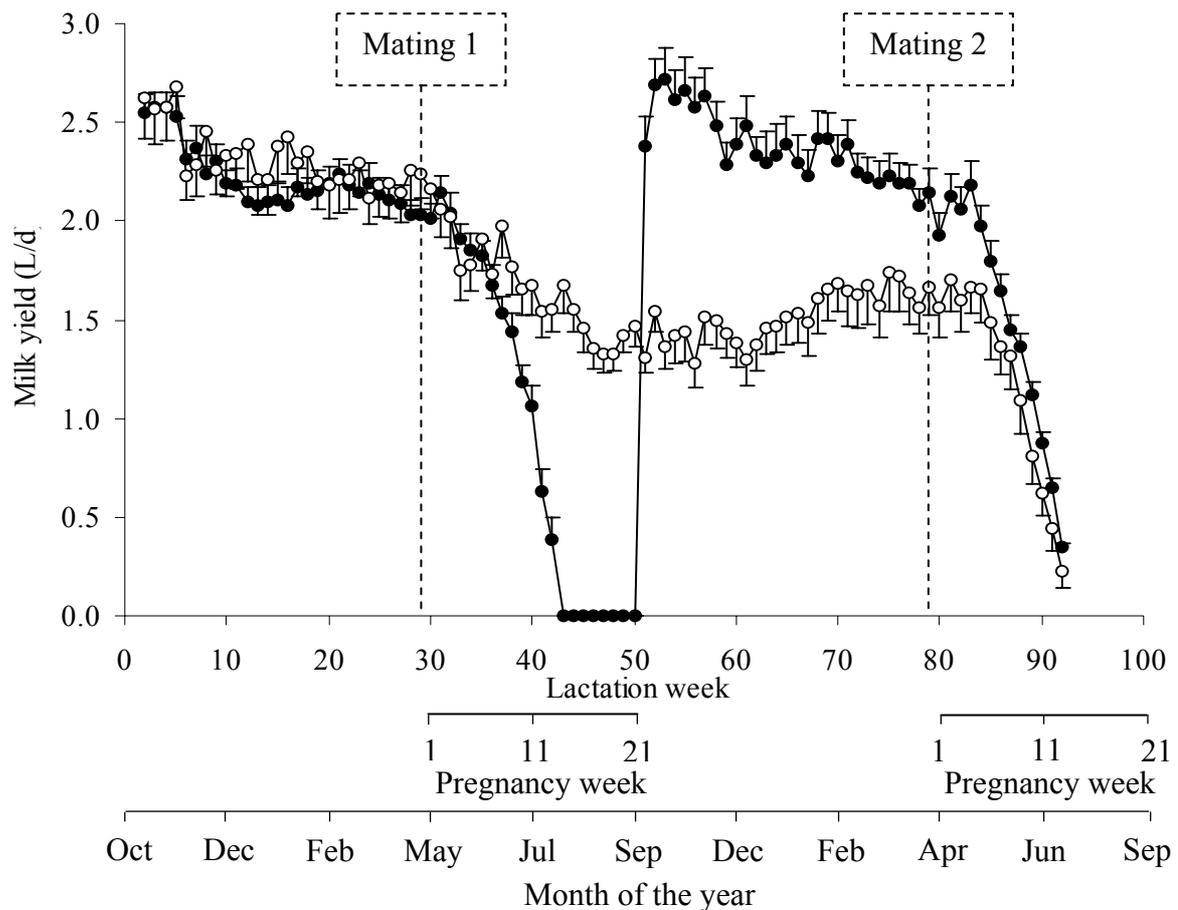
Figure 1. Daily milk yield in dairy goats kidded annually (●) or biennially (○). Values are means with SE indicated by vertical bars.

The mechanism by which pregnancy influences milk yield is not fully understood, but it is believed to be caused by the hormones released during pregnancy, most probably estrogen (Bachman et al., 1988; Akers, 2002). Administration of estrogen caused mammary gland regression with a significant decline in milk yield in lactating goats (Peaker and Linzell, 1974). Moreover, estradiol injection before drying off accelerated mammary tissue involution in dairy cows (Athie et al., 1997) and goats (Mellado et al., 1998) by promoting plasminogen activation (Athie et al., 1997). Circulating estradiol is low until wk 9 of pregnancy in goats, increases gradually until 1 wk before kidding, and then peaks before

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kidding (Dhindsa et al., 1981). Estradiol concentration during pregnancy is proportional to the number of fetuses (Dhindsa et al., 1981; Manalu et al., 1996). Litter size in K12 goats averaged 2.2 ± 0.1 kids/goat, which is relatively high for goats (Amoah et al., 1996), indicating that a marked effect of estrogen on milk secretion must have occurred. Beside the estrogen, placental lactogen peaks during the last third of pregnancy and may influence mammogenesis, lactogenesis and alter the maternal metabolism in order to accommodate the growth and development of the fetus (Akers, 2002).

In addition to the hormonal effect, yield losses might be due to the nutritive requirements of the gravid uterus. Energy requirements for pregnancy do not only include the energy deposited in the conceptus, but also the energy used for the conceptus metabolism and the energy used by maternal tissues to support the conceptus (Freetly and Ferrell, 1998). In the current study, we found a negative correlation ($R^2 = 0.35$; $P < 0.05$) between litter birth weight and milk yield at wk 13 of pregnancy (wk 42 of lactation) before drying off (data not shown), which may indicate the competition for energy between milk production and conceptus. Glucose is the main source of energy for the gravid uterus, and an increase in the net hepatic plasma glucose release in pregnant ewes has been reported from d 40 of pregnancy onwards (Freetly and Ferrell, 1998). Despite this hepatic release of glucose, pregnant goats had lower concentrations of blood glucose than the non-pregnant goats after d 84 of pregnancy (Khan and Lurdi, 2002). This suggests that there may be a competition for glucose between the mammary gland (for lactose synthesis) and the gravid uterus (especially with twins or triplets), which would result in milk yield losses during pregnancy.

From wk 43 to 50, K12 goats were dried off whereas K24 goats produced 1.53 L/d (Table 1 and Figure 1). From wk 51 to 92, milk yield of K12 goats peaked (2.67 L/d) between wk 53 and 55 (wk 3 and 5 of subsequent lactation) and then descended gradually ($P < 0.05$), while milk yield in K24 goats tended ($P < 0.10$) to increase at wk 68 of lactation from 1.43 to 1.65 L/d (Figure 1). This increase in milk yield of K24 goats was maintained throughout spring when grass quality and photoperiod are increasing. A longer photoperiod is related to increases in circulating PRL in goats (Kornalijnslijper et al., 1997), and PRL and IGF-I in dairy cows (Dahl and Petitclerc, 2003). In accordance with our results, non-pregnant goats continued to lactate for 2 to 4 yr and showed a rise in milk yield in the spring either when they were grazing (Mackenzie, 1967) or when they were kept housed under constant values of light and temperature and given dry feeds (Linzell, 1973). It is not clear why the positive effect of longer daylight was more clear in K24 goats than in K12 goats.

Table 1. Lactational performances of dairy goats according to kidding interval and physiological stage during the 92-wk experimental period (data are least squares means ± SE).

Item	Kidding interval, mo	wk 2 to 29	wk 30 to 38	wk 39 to 42	wk 43 to 50	wk 51 to 79	wk 80 to 92
Physiological stage	12	Open & lactating …	—— Pregnant & lactating —— (wk 1 to 9)[1]	(wk 10 to 13)[1]	Dried off (wk 14 to 21)[1]	Open & lactating …	Pregnant & lactating (wk 1 to 13)[1]
	24		———————————— Open & lactating ————————————				Pregnant & lactating (wk 1 to 13)[1]
		…	…	…	…	…	
Goats, n	12	16	16	16	16	16	16
	24	14	14	14	14	14	14
Milk yield, L/d	12	2.24 ± 0.12	1.79 ± 0.14	$0.81^b \pm 0.11$	…	$2.42^a \pm 0.12$	1.55 ± 0.10
	24	2.21 ± 0.15	1.88 ± 0.16	$1.63^a \pm 0.14$	1.53 ± 0.10	$1.65^b \pm 0.17$	1.33 ± 0.13
FCM[2], L/d	12	…	1.90 ± 0.13	$0.84^b \pm 0.10$	…	$2.73^a \pm 0.14$	1.66 ± 0.15
	24	…	1.96 ± 0.16	$1.57^a \pm 0.12$	1.59 ± 0.10	$1.96^b \pm 0.18$	1.45 ± 0.16
Milk fat, %	12	…	4.23 ± 0.16	4.17 ± 0.14	…	$4.72^b \pm 0.14$	4.68 ± 0.24
	24	…	4.17 ± 0.21	3.85 ± 0.18	4.43 ± 0.15	$5.37^a \pm 0.19$	5.02 ± 0.25
Milk protein, %	12	…	3.30 ± 0.10	$4.22^a \pm 0.20$	…	$3.45^b \pm 0.10$	3.97 ± 0.13
	24	…	3.21 ± 0.13	$3.30^b \pm 0.24$	3.52 ± 0.10	$3.81^a \pm 0.13$	3.96 ± 0.14
Milk lactose, %	12	…	4.45 ± 0.05	$3.81^b \pm 0.08$	…	4.51 ± 0.04	4.10 ± 0.06
	24	…	4.34 ± 0.07	$4.15^a \pm 0.09$	4.37 ± 0.08	4.42 ± 0.06	4.15 ± 0.06
Milk SCC, log	12	…	6.08 ± 0.11	6.51 ± 0.14	…	6.10 ± 0.10	6.34 ± 0.10
	24	…	6.04 ± 0.14	6.29 ± 0.17	6.46 ± 0.12	6.13 ± 0.13	6.31 ± 0.09

[1] wk of pregnancy
[2] FCM = 0.4 (L of milk) + 15 (kg fat).
[a, b] Means with different superscripts within the same column for each parameter are different ($P < 0.05$).

From wk 51 to 79 (wk 1 to 29 of subsequent lactation), K12 goats produced 32% more milk ($P < 0.001$) than K24 goats; this difference decreased to 28% when milk yield was corrected for fat content, but the difference was still significant ($P < 0.01$) as shown in Table 1. From wk 80 to 92 (wk 30 to 42 of subsequent lactation), no differences were detected between K12 and K24 goats for milk yield ($P = 0.151$) and FCM (Table 1). Differences in milk yield between groups were maintained until wk 85 and were insignificant thereafter (Figure 1).

In the 92-wk experimental period, K12 and K24 goats produced a similar total milk yield (1,192 ± 58 and 1,093 ± 73 L; for K12 and K24, respectively; $P = 0.319$). No significant losses in milk yield were reported either in dairy cows when the CI was extended from 12 to 14 (Arbel et al., 2001), or 16 mo (Osterman and Bertilsson, 2003). The K24 goats lost the kid crop of one year. The reduced income in K24 goats was partially compensated by the greater price of the milk produced between wk 51 and 79 which was richer in fat and protein than in K12 goats (Table 1). Moreover, the biennial kidding system with extended lactation may be a good alternative for goats that remain open after mating in the first year and those could be remated with their counterparts in the second year.

Goats were mated at wk 79 (wk 29 of subsequent lactation) and all became pregnant and kidded at d 158 ± 1.9 d after the start of mating, indicating that both goat groups were in adequate conditions for reproduction. Similarly, Ratnayake et al. (1998) found no difference in fertility between dairy cows managed for CI of 12, 15 or 18 mo.

Milk Composition and SCC

Values of milk fat, protein, lactose and $\log_{10}$ SCC did not differ between K12 and K24 goats from wk 30 to 38 of lactation (Table 1). However, differences were detected at wk 41 (wk 12 of pregnancy), when milk yield of K12 goats was low and tended to contain more fat (4.63 vs. 3.86%; $P < 0.10$), and more ($P < 0.001$) protein (4.89 vs. 3.43%) and less ($P < 0.001$) lactose (3.58 vs. 4.23%) than milk from K24 goats (Figure 2). Leakiness of TJ at advanced pregnancy may account for milk composition differences between K12 and K24 goats. Elevated levels of progesterone induce TJ leakiness and the injection of a progesterone antagonist in mice in late pregnancy resulted in a rapid closure of TJ (Nguyen et al., 2001). The highest level of circulating progesterone was reported to be between wk 8 (when placenta is functional) and 17 of pregnancy in goats (Kornalijnslijper et al., 1997),

and is proportional to the number of fetuses (Manalu et al., 1996). Thus, elevated progesterone concentrations during advanced pregnancy may favor TJ leakiness.

Comparing wk 2 and 12 of pregnancy (wk 31 and 41 of lactation), increases ($P < 0.001$) in milk fat (4.32 to 4.63%), milk protein (3.21 to 4.89%) and $\log_{10}$ SCC (5.98 to 6.55) were detected in K12 goats, whereas milk lactose decreased ($P < 0.001$) from 4.51 to 3.58% (Figure 2). Similarly, increases in milk fat and protein contents, and a decrease in lactose content were observed in late pregnant dairy cows at the end of lactation (Remond et al., 1992; Olori et al., 1997). Changes in milk composition before drying off in K12 goats may be the result of TJ leakiness, thus reducing milk secretion and allowing the flow of plasma proteins into the alveolar lumen (Nguyen et al., 2001). Leaky TJ may also result in lactose loss from milk to plasma (Stelwagen et al., 1995), which may explain the low content of milk lactose at wk 12 of pregnancy.
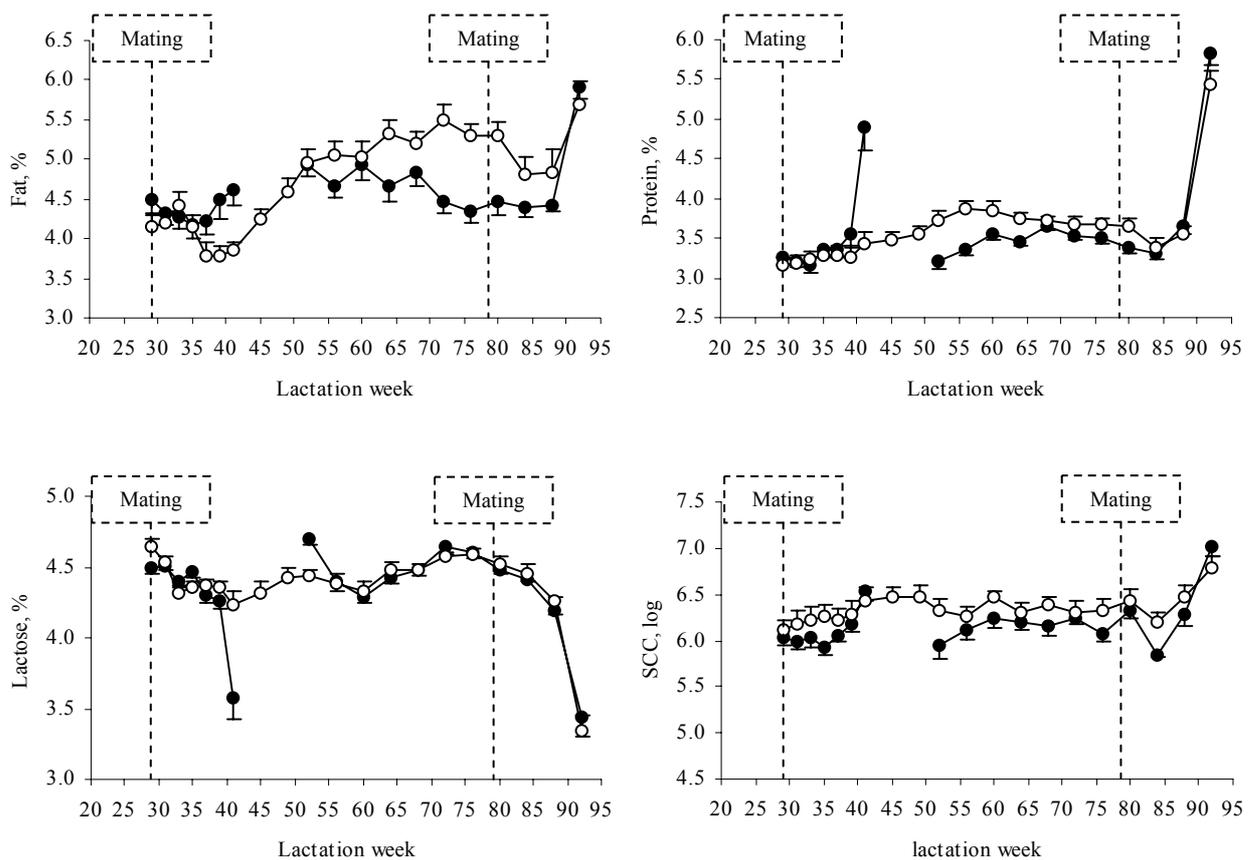

Figure 2. Milk fat, protein, lactose and SCC in dairy goats kidded annually (●) or biennially (○). Values are means with SE indicated by vertical bars.

During the first 29 wk of the subsequent lactation (wk 51 to 79), milk from K12 goats contained less ($P < 0.05$) fat (-14%) and protein (-10%) than milk from K24 goats (Table 1

and Figure 2). The lower fat and protein contents in milk of K12 goats is probably due to a dilution effect as their milk yield was greater than K24 goats. Moreover, the negative energy balance at early lactation in K12 goats (see below) could lead to a lower percentage of protein as previously reported in dairy cows (Remond et al., 1992). From wk 51 to 79 (wk 1 to 29 of subsequent lactation), no differences ($P > 0.10$) between groups were detected for milk lactose and SCC (Table 1 and Figure 2). Lactose is the major osmotic regulator of milk, and a strong positive correlation exists between lactose synthesis and milk yield. Knight and Wilde (1993) found that the number of secretory cells in the udder of goats decreased as lactation advanced, but the cell activity remained unchanged. Reduced milk yield without reduced lactose content in K24 goats suggests a lower number of secretory cells in the udder of K24 goats.

During pregnancy in the subsequent lactation (wk 80 to 92), milk composition and SCC did not differ between groups (fat, 4.85%; protein, 3.97%; lactose, 4.13%; and $\log_{10}$ SCC, 6.32; on average).

Throughout the study, milk SCC exceeded the limit of 1×10^6 cells/ml except for K12 at wk 52 (wk 2 of subsequent lactation). These values were slightly higher than values previously reported by Salama et al. (2003b) in the same breed milked 1X. This greater content of SCC seems to be due to a parity effect rather than an effect of 1X. All goats in the current study were multiparous, and Salama et al. (2003b) reported an increase in milk SCC as parity number increased, with no difference in SCC between 1X and 2X goats.

Fatty Acid Profile of Milk Fat

Only minor differences in FA profile were observed between K12 and K24 goats at wk 39 (wk 10 of pregnancy), when milk fat of K12 goats contained greater ($P < 0.05$) palmitoleic ($C_{16:1}$) and conjugated linoleic acid (CLA) than K24 goats (Table 2). The CLA ($C_{18:2}$ *cis*-9, *trans*-11) is synthesized in the rumen and is also produced in the mammary gland by Δ^9-desaturase enzyme, and its increase in dairy products is considered to be desirable (McGuire and McGuire, 1999). An increase ($P < 0.05$) in the $C_{16:1}/C_{16:0}$ and $C_{18:1}/C_{18:0}$ desaturase indices, related to the activity of the Δ^9-desaturase enzyme (Perfield et al., 2002; Kelsey et al., 2003), was observed in the milk of K12 goats compared to K24 goats (Table 2). Consequently, the CLA content in milk of K12 goat was greater than K24 goats (18.2%; $P < 0.05$).

Table 2. Effect of kidding interval and lactation stage on fatty acid composition of milk fat in dairy goats (data are least squares means of percentages of total methyl esters).

| Fatty acid | Kidding interval, mo | | | | |
| | 12 | | 24 | | |
	wk 39[1]	wk 55[2]	wk 39	wk 55	SEM
Goats, n	16	16	14	14	
Fatty acid					
$C_{4:0}$	0.94	1.01	1.00	0.94	0.04
$C_{6:0}$	1.12^b	1.45^a	1.23^b	1.25^b	0.07
$C_{8:0}$	2.12^b	2.77^a	2.26^b	2.24^b	0.11
$C_{10:0}$	9.34^b	10.86^a	9.74^b	9.68^b	0.37
$C_{10:1}$	0.20	0.18	0.14	0.21	0.05
$C_{12:0}$	5.09	5.30	4.81	5.00	0.36
$C_{14:0}$	11.31^a	10.33^b	10.67^{ab}	10.76^{ab}	0.46
$C_{15:0}$	1.65^a	1.48^b	1.71^a	1.41^b	0.09
$C_{16:0}$	31.00^a	26.58^b	31.40^a	31.23^a	0.94
$C_{16:1}$	1.68^a	1.26^b	1.29^b	1.41^b	0.11
$C_{17:0}$	1.45^b	1.62^a	1.54^{ab}	1.40^b	0.07
$C_{17:1}$	0.30	0.33	0.30	0.31	0.02
$C_{18:0}$	7.37^b	9.47^a	8.42^{ab}	8.34^{ab}	0.63
$C_{18:1}$	20.05	20.33	19.27	19.52	0.87
$C_{18:2}$	2.45^b	2.95^a	2.49^b	2.49^b	0.11
$C_{18:3}$	1.14^b	1.43^a	1.30^{ab}	1.13^b	0.09
$C_{18:2}$ *cis*-9, *trans* 11 (CLA)	0.78^a	0.62^b	0.66^b	0.60^b	0.04
Desaturase index[3]					
$C_{16:1}/ C_{16:0}$	0.054^a	0.048^b	0.041^b	0.045^b	0.003
$C_{18:1}/ C_{18:0}$	2.80^a	2.20^b	2.36^b	2.39^b	0.18
Atherogenicity index[4]	3.12^a	2.76^b	3.16^a	3.13^a	0.16

[1] wk 10 of pregnancy for 12-mo kidding interval.
[2] wk 5 of the subsequent lactation.
[3] According to Perfield et al. (2002).
[4] (C12 + 4C14 + C16)/sum of unsaturated fatty acids (from Ulbricht and Southgate, 1991).
[a, b] Means with different superscripts within row differ ($P < 0.05$).

At wk 55 (wk 5 of subsequent lactation) more FA differences between groups were observed ($P < 0.05$). Milk fat from K12 goats contained greater caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), margaric ($C_{17:0}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$), and lower palmitic ($C_{16:0}$) and $C_{16:1}$ than K24 goats (Table 2). The effect was more marked in $C_{16:0}$ (17.5%) that correlates negatively with C4 to C8 (Chilliard et al., 2003), agreeing with the greater percentages of these short chain FA in the milk of K12 goats at wk 55. Human diets rich in $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ FA are potentially hypercholesterolmic, whereas, $C_{18:0}$ and unsaturated long chain FA have cholesterol lowering and anti-atherogenic properties (Ney, 1991). In our results, the atherogenicity index in K12 was lower ($P < 0.05$) than K24 at wk 55 (wk 5 of subsequent lactation) when $C_{16:0}$ percentages were low and polyunsaturated FA ($C_{18:2}$ and $C_{18:3}$) were high (Table 2).

Stage of lactation markedly affected the FA profile of milk fat in K12 goats as previously reported by Schmidely et al. (2002). Milk fat of K12 goats at wk 39 (wk 10 of pregnancy) or in late lactation contained greater ($P < 0.05$) percentages of myristic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), $C_{16:0}$ and $C_{16:1}$, and lower ($P < 0.05$) $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{17:0}$, stearic ($C_{18:0}$), $C_{18:2}$, and $C_{18:3}$ acids than milk fat at wk 55 (wk 5 of subsequent lactation) or in early lactation. The greater long chain FA (C18) percentages at wk 55 (wk 5 of subsequent lactation) could be a consequence of the mobilization of body reserves during the negative energy balance in early lactation, as indicated previously during early lactation in dairy goats (Schmidely et al., 2002). Negative correlation exists between C18 and medium-chain FA in milk fat of dairy goats (Chilliard et al., 2003) as found in our results for the same FA in the milk of K12 goats at wk 5 of the subsequent lactation (Table 2). The CLA concentration in milk fat of K12 was greater ($P < 0.05$) at wk 39 than at wk 55 in accordance with the greater desaturase activity observed at wk 39. Moreover, the secretion of polyunsaturated FA, known as inhibitors of desaturase activity (Chilliard et al., 2003), was lowest ($P < 0.05$) at wk 39 in K12 goats. We are not aware of any previous research on the effect of lactation stage on CLA or desaturase index in goat milk. Similar to our results, a small, but significant increase in milk fat content of CLA and desaturase index as lactation advanced was reported in dairy cows (Kelsey et al., 2003), but Stanton et al. (1997) found no effect of lactation stage on milk fat CLA levels in dairy cows.

On the contrary, the FA profile of milk fat in K24 goats was unchanged between wk 39 and 55 except for pentadecanoic ($C_{15:0}$) acid, which was greater ($P < 0.05$) at wk 39. This may °be a consequence of the unchanged physiological stage during extended lactation, and of the steady state of nutrition despite the seasonal changes in the quality of pasture.

Milk Fractions in the Udder

Yield of milk fractions. Figure 3 shows milk partitioning and milk accumulation rates in the alveolar and cisternal udder compartments in K12 and K24 goats. Percentages of cisternal milk varied according to lactation wk (58 to 82%) and agreed with values previously reported in Murciano-Granadina dairy goats (Salama et al., 2004).

At wk 39 (wk 10 of pregnancy), K12 goats had lower ($P < 0.05$) total milk (975 vs. 1912 mL), cisternal milk (638 vs. 1560 mL) and percentage of cisternal milk (57.7 vs. 82.1%) than K24 open goats. Nevertheless, volume of alveolar milk was similar for K12 and K24 goats and averaged 345 mL.
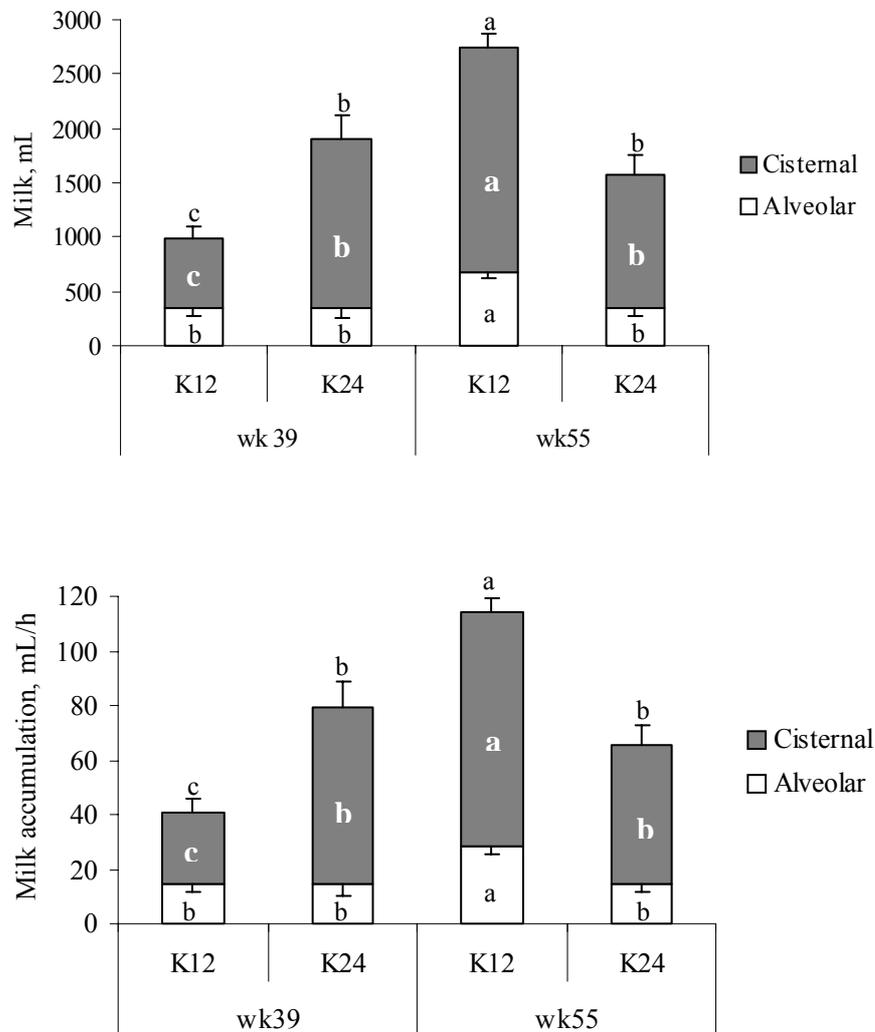
**Figure 3.** Milk partitioning (upper panel) and milk accumulation rates (lower panel) in the udder of dairy goats kidded annually (K12) or biennially (K24). Measurements were carried out at wk 39 (wk 10 of pregnancy) and 55 (wk 5 of subsequent lactation) of lactation. Values are least squares means with SE indicated by vertical bars. Different means ($P < 0.05$) are indicated by different letters.

At wk 55 (wk 5 of subsequent lactation), milk yield of K12 goats peaked after kidding and the volume of cisternal milk tripled ($P < 0.001$) its value at wk 39, while volume of alveolar milk only doubled ($P < 0.001$) as shown in Figure 3. Volumes of cisternal and alveolar milk at wk 55 in K12 goats were greater ($P < 0.01$) than in K24 goats, without differences in cisternal milk percentages. On the contrary to K12 goats, K24 goats were in a similar physiological stage between wk 39 and 55 and their milk yield did not differ, and thereby volumes of cisternal and alveolar milk, as well as cisternal milk percentages, remained unchanged. These results indicate the main role of the cistern for accommodating the udder to changes in milk yield (i.e. K12 goats at early lactation). This conclusion is also supported by the fact that milk accumulation rates were less marked in the alveolar than in the cisternal compartment for both goat groups (Figure 3). Expected differences in cisternal milk between groups should be lower with 2X, according to the results obtained by Salama et al. (2004) in the same breed.

With regard to the effects of stage of lactation on udder compartments, lower ($P < 0.001$) volume and percentage of cisternal milk were observed in late lactating K12 goats when compared to their own values in early lactation (Figure 3). Although no data was available on volume and percentage of cisternal milk in K24 goats in early lactation, a reduction in this udder compartment was expected as milk yield decreased. Caja et al. (2004) reported in dairy cows that volume of cisternal milk decreased, while its proportion increased, as lactation advanced. The difference between goats and cows could be due to the larger cisternal compartment in goats compared to cows, as indicated by Salama et al. (2004). Effect of pregnancy on cisternal milk volume was attributed to a decrease in the nutrients available for milk synthesis, as discussed above, resulting in a decrease in milk yield.

Composition of milk fractions. No references are available on the composition of alveolar and cisternal milk fractions in dairy goats. Alveolar milk contained greater ($P < 0.001$) fat than cisternal milk, except for K12 goats at wk 39 (wk 10 of pregnancy) when milk yield was very low (Table 3). Similar results were reported in dairy cows (Ayadi et al., 2004) and ewes (McKusick et al., 2002a). Milk fat globules are large and do not pass freely from alveoli to cistern, and therefore more fat is retained in the alveolar compartment (McKusick et al., 2002a; Ayadi et al., 2004). This underlines the importance of milk ejection and complete milking for recovery of milk that is rich in fat.

Table 3. Effect of kidding interval and lactation stage on composition of milk fractions in dairy goats (data are least squares means).

| | Kidding interval, mo | | | | |
| | 12 | | 24 | | |
Item	wk 39[1]	wk 55[2]	wk 39	wk 55	SEM
Goats, n	16	16	14	14	
Fat, %					
Cisternal	4.97[a]	3.83[bc,e]	3.02[c,e]	4.38[ab,e]	0.66
Alveolar	5.58[bc]	7.36[a,d]	5.01[c,d]	6.18[b,d]	0.65
SEM	0.48	0.45	0.54	0.55	…
Protein, %					
Cisternal	4.70[a]	3.26[b]	3.02[b]	3.64[b]	0.42
Alveolar	4.99[a]	3.13[b]	3.00[b]	3.69[b]	0.44
SEM	0.31	0.29	0.35	0.35	…
Lactose, %					
Cisternal	4.22[b]	4.47[a]	4.35[ab]	4.28[ab]	0.11
Alveolar	4.20[b]	4.42[a]	4.34[ab]	4.33[ab]	0.09
SEM	0.08	0.08	0.09	0.10	…
SCC, $\log_{10}$/mL					
Cisternal	6.38[a]	5.84[b,d]	6.29[a]	6.41[a]	0.19
Alveolar	6.41[a]	6.09[b,e]	6.34[ab]	6.47[a]	0.19
SEM	0.13	0.12	0.15	0.15	…

[1] wk 10 of pregnancy for 12-mo kidding interval.
[2] wk 5 of the subsequent lactation.
[a, b, c] Means with different superscripts within row differ ($P < 0.05$).
[d, e] Means of cisternal and alveolar fractions with different superscripts within variables differ ($P < 0.05$).

No differences in protein and lactose content between cisternal and alveolar milk were observed (Table 3). Differences between alveolar and cisternal concentrations of milk protein and lactose are minimal in dairy cows (Ayadi et al., 2004) and dairy ewes (McKusick et al., 2002a). Milk protein is found primarily in the form of small CN micelles (Cowie and Tindal, 1971) and therefore could pass freely between alveolar and cisternal compartments between milkings, resulting in minimal differences in protein concentrations of milk fractions. Synthesis of protein and lactose is synergistic and regulated by a final shared metabolic pathway (Fitzgerald et al., 1970), thus it is expected that both components change similarly.

Cisternal and alveolar SCC were similar for open and pregnant goats at wk 39 (Table 3). However, cisternal SCC was lower ($P < 0.05$) than alveolar SCC at wk 55 (wk 5 of subsequent lactation) for K12 goats. This result is consistent with lower SCC in foremilk compared to stripped milk in dairy ewes (Peris et al., 1991) and dairy cows (Bruckmaier et al., 2004). Nevertheless, Ontsouka et al. (2003) found no differences in SCC between milk fractions in dairy cows. The difference between cisternal and alveolar SCC observed in early lactating goats in our study reflects the importance of the methodology of milk sampling for SCC determination. Usually, foremilk samples are taken for SCC determination and udder health monitoring in dairy goats. Thus, these samples may not reflect the actual SCC in the total milk at the end of milking.

CONCLUSIONS

Pregnancy caused a dramatic milk yield drop in dairy goats, which was observable from the second month after mating, and facilitates drying off before the next kidding. When mating was not conducted, goats machine milked once daily under semi-extensive conditions, lactated for 2 consecutive years without significant losses in milk yield. Since milk of K24 goats contained more fat and protein with no significant increase in SCC, reduced revenue due to kid crop loss could be partially offset if payment for milk was based on milk quality. Extended lactation may be a useful strategy for reducing goat stress throughout their lifespan in compromised conditions due to high milk yield or low nutritive resources.

CHAPTER 8: CONCLUSIONS

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CONCLUSIONS

Once Versus Twice Daily Milking

- The 1X in Murciano-Granadina dairy goats moderately reduced milk yield, but increased milk fat.

- No significant increases in SCC were detected and udder health was not negatively affected by 1X.

- The 1X for dairy goats in early lactation resulted in greater milk yield losses than in late lactation.

- Primiparous goats have a smaller cisternal compartment, store less milk in the cisternal compartment and suffer greater milk yield losses 1X than multiparous goats.

- No changes in the storage characteristics of the cisternal compartment of the udder of dairy goats were observed as a consequence of 1X during early lactation.

- No cisternal recoil occurs in goats indicating that once milk is ejected it is unable to return to the alveoli regardless of whether milking is performed.

- The reduction in total labor when 1X is adopted will permit farmers more time to devote to other farming practices and/or to other activities off the farm, improving their productivity and standard of life.

Effects of Dry Period Length

- Omitting the dry off period between lactations in Murciano-Granadina dairy goats reduced milk yield in the subsequent lactation, probably due to impaired mammary cell turnover during dry off.

- Omitting the dry off period reduced the immunologic quality of the colostrum.

- Milk yield and colostrum quality did not vary between goats dried off for 27 or 56 d before kidding.

Effects of Pregnancy

- Pregnancy reduced milk yield significantly from wk 10 after mating in dairy goats in late lactation, which facilitates drying off before the next kidding.

- Milk yield of pregnant goats before drying off was low and contained greater fat, protein and CLA, and less lactose than milk from nonpregnant goats.

- Pregnant goats stored a lower amount of milk in the cistern, in accordance with their lower level of milk yield compared with nonpregnant goats.

Effects of Kidding Interval

- Nonpregnant goats milked 1X under semi-extensive conditions, lactated for 2 consecutive years without significant losses in milk yield.

- Milk of goats kidded biennially contained more fat and protein without a significant increase in SCC in the second year of lactation compared with milk of goats kidded annually.

- Reduced revenue due to kid crop loss in goats kidded biennially can be partially offset by the increase in milk fat and protein.

- Extended lactation may be a useful strategy for reducing goat stress for their lifespan in compromised conditions due to high milk yield or low nutritive resources.

CHAPTER 9: REFERENCES

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REFERENCES

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