Minnesota Dairy Health Conference

University of Minnesota

College of Veterinary Medicine

Veterinary Continuing Education

May 19-20, 2010
St. Paul, Minnesota

Boehringer Ingelheim

Elanco

Pfizer Animal Health

Virtus Nutrition

Lactation Biology

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As the name implies, mammary glands are a distinctive feature of the class Mammalia and are found in all members of it's subclasses: the monotremes, marsupials and eutherians. Almost without exception, successful reproduction in the mammals requires lactation from functional mammary glands for its completion. Their development has allowed intense maternal investment in offspring to continue beyond the time of parturition, permitting birth of young at a stage of development where they would otherwise not survive.

Monotremes (prototherians: platypus and echidna), lay eggs and have very altricial young. They do not have nipples, rather the mammary glands discharge directly onto a specialized area of skin, the areola, from where the young suck or lick it up.

Marsupials (metatheria) have a short gestation period (usually just the length of the luteal phase of the estrous cycle) and the young are born at an immature, almost embryonic, stage. Following birth they climb unaided to the pouch and attach to a nipple (located in the pouch). This subsequently swells and they are fixed to it, unable to detach until they have achieved sufficient growth. It has been said that marsupials "have exchanged the placenta for the teat".

Within the eutherians (true placental mammals) neonatal development varies from altricial to precocious but all are dependent on milk for the early part of post-uterine life.

Comparative Mammary Gland Anatomy

External Anatomy

Number, distribution, shape and size of the mammary glands vary by species. In most cases they are paired, and exception being marsupials where occasionally a pair may fuse into a single gland during fetal development, resulting in an odd number.
The *number* varies from 2 (1 pair) in humans, sheep and goats, up to 18 (9 pairs) in the sow.
Positioning ranges from the thorax (primates, elephant, bats), abdominal (whales), inguinal (cow, goat, sheep), all along ventral thorax, abdomen and inguinal region (sow, rat and rabbit) to almost dorsal (coypu) and is determined during embryonic development (see later).
Shape ranges from flattened sheets in the rat, flat but circular in the rabbit, prominent in human, dependent in ruminants.
In the females of all species except the monotremes each mammary gland has a nipple or teat. In some species the nipple is absent in males (mouse and rat).

Internal Anatomy
Internally, the mammary gland consists of 2 basic types of tissue, parenchyma (secretory) and supporting tissue (stroma).

The structure of the milk secreting tissue itself is very similar across species. The basic milk secreting structure is the alveolus. It is a sack lined by a single layer of secretory epithelial cells (these are continuous with those lining the duct system). Outside this is the myoepithelial layer (a.k.a. the basket cells) which has contractile properties important for milk let-down. Beyond this is a basement membrane. Outside the basement membrane is a network of capillaries that brings in the raw materials for milk synthesis. Each alveolus discharges its secretion through a capillary milk duct.

Alveoli are arranged in groups or clusters termed lobules (like grapes in a bunch). The individual capillary milk ducts empty into the intralobular ducts. Which enter into progressively larger ducts to provide the route for milk removal.

While the secretory tissue is similar across species, differences are manifested in the anatomy and arrangement of the duct system.
In the monotremes the major ducts discharge directly onto the skin of the alveolus or mammary patch.
In dogs and humans, 12-20 major ducts have openings on the nipple.
In the cow, goat and sheep, major ducts empty into a large gland cistern, which is continuous with the teat cistern. This is then drained via the single streak canal.

In the mare and sow, 2 gland systems, with their relatively small (compared to ruminants) gland and teat cisterns) are drained by a single teat - the teat has 2 openings or streak canals, one for each gland system.

Ducts discharging at the nipple (or skin in monotremes) are also called *galactophores*.

Outside the glandular tissue is the stroma, a mixture of connective tissue and fat cells, collectively termed the mammary fat pad. It functions as a supporting tissue and is essential for normal development of the parenchyma. The mammary epithelial cells (which create the duct and alveolar systems) cells will not grow unless placed in an environment of adipose tissue. Each lobule is surrounded by connective tissue and groups of lobules are further arranged into lobes.

Supporting structures for the mammary gland are best described in the cow. Here the udder is divided into distinct left and right halves by the major supporting structure, the median suspensory ligament. This is composed of elastin and collagen. In mature lactating cows the elastin predominates, acting as a shock absorber for the gland during movement.
Other supporting structures are the lateral suspensory ligaments and their extensions, the lamellar plates. These project into the parenchyma of the gland and are interspersed with it.

The skin of the udder provides little in the way of support but is a vital barrier to infection.

Mammary Development in the Embryo and Fetus

Embryonic development

Mammary glands are derived from the embryonic ectoderm.
Development of the glands begins as a thickening of the ectoderm on the ventrolateral aspect of the developing embryo. This bilateral linear thickening then develops through a defined series of changes to finish as the *mammary buds*. These are spheres of ectodermal cells that have grown into the underlying mesoderm in locations of the future mammary glands (i.e. they are in species specific numbers and locations). While the mammary gland is derived from ectoderm (=epithelium = embryonic skin), its development is controlled by factors from the local mesenchyme. Mesenchyme, which arises from the mesoderm or middle layer of the embryo (ectoderm-mesoderm-endoderm) forms connective tissues, fat (adipose tissue) and blood among other things. Removal of mammary mesenchyme prevents formation of the mammary gland.

To this end, early in development of the mammary gland, a layer of adipose tissue cells surround the mammary bud. Subsequently other epithelial structures that form during mammary differentiation are also surrounded by adipose tissue. An adequately formed mammary fat pad is a prerequisite for successful progression of mammary growth. From early fetal life the female develops a more extensive fat pad than her male counterpart.

There is now evidence from mice that a gene cascade involving Gli3 →Fgf10 in mesenchyme activates the Fgf2b receptor in overlying ectoderm which then induces a Wnt genetic cascade that determines location and development of at least some glands [Veltmaat et al., Development 133:2325-2335, 2006].
Mammary mesenchyme can also induce mammary formation from other types of epithelium, either from elsewhere on the body or even from different animals. Mammary mesenchyme can even induce formation of rudimentary mammary buds from the epidermis of early chick embryos! The converse is also true, chick mesenchyme can induce primitive feather follicle formation from mouse mammary epithelium.

Fetal development

Primary Cord

As soon as the bud attains its spherical shape the next stage commences. Groups of cells proliferate out of the sphere and form cords of cells that elongate deeper into the dermal tissue to form the primary mammary cord (a.k.a. primary sprout), the base of which (it is growing down into deeper tissues so its base is at the surface) remains attached to the epidermis. The number of these primary cords that grow out of each bud will determine the number of ducts that will open onto the nipple.

Secondary Cords

Once the primary cord attains a certain size, its distal end branches to form 2 or more secondary buds. These in turn elongate into cords which will eventually form the large milk ducts, in the ruminants they will discharge into the gland cistern.

Canalization of the Cords

While they are elongating, the cords also increase in diameter. The result of this is that the cells in the center of the cord get further away from their nutrient source, supplied by small capillaries, and they die. Thus the cords become hollow (canalization) and form the mammary ducts.

Supporting Tissue

Connective tissue supporting structures for the mammary gland also form during fetal life. In the

heifer, with further growth of the 4 glands and their fat pads during fetal life, the median suspensory ligament becomes more prominent. Four distinct quarters are palpable by the time of birth.

There is no requirement for ovarian factors (hormones) in the development of the mammary glands during fetal life.
In species such as mice and rats, the influence of testosterone in the male fetus causes the primary mammary cord to lose its attachment to the surface epithelium during later fetal life. This results in male mice and rats having no nipples. This is also the case in species as diverse as horses and beaver.

Mammary Development: From Birth to Puberty
At birth the mammary gland consists of a rudimentary duct system that opens at a small nipple. The gland shows general growth at an isometric rate (i.e. same rate as rest of the body, it just keeps pace). There is also some maturation of parts not clearly defined at birth such as the teat sphincter.

Several weeks prior to puberty, depending on species, the growth of the mammary gland becomes allometric - faster than general body growth. This is due to the increased secretion of ovarian hormones from developing follicles during this period - mammary gland growth in males and ovariectomized females continues at an isometric rate. There is accumulating evidence that local production of IGF-1 and IGF binding proteins mediate the effects of growth hormone and possibly estrogen on mammary growth at this stage [Akers et al., Dom. Anim Endocrinol 29:259-267, 2005].

Mammary Development Following Puberty
Which component of the mammary tissue grows, how quickly and to what extent during this phase depends on the species examined and appears to depend on the type of estrous cycle they have.

- Animals with ultra short cycles and virtually non-existent luteal phases (mouse, rat) show mostly duct growth and alveoli are rarely formed.

- Primates have a longer cycle and full luteal phase, and here duct development is almost full, to the extent of formation of the fine ductules that indicate the future lobules, which also contain a few alveoli.

- In the bitch, with a long luteal phase that can be considered a pseudopregnancy, this duct growth is accompanied by considerable lobulo-alveolar development. This degree of development is only seen during pregnancy in other species. We mentioned previously that all bitches could be considered to go through a pseudopregnancy every cycle due to the similarity of pregnant and non-pregnant cycles. Indeed many of these non-pregnant bitches will mother small toys around the time of parturition or demise of the CL. They may also show mammary development and lactation. Behavior and endocrinology you may find yourself treating in practice with various prolactin inhibitors.

These observations have lead to the concept that, directly or indirectly: estrogen is largely

responsible for duct growth, while progesterone is responsible for lobulo-alveolar growth.

Attainment of Full Alveolar Development
The time when mammary development reaches the anatomic stage that it would be competent to secrete milk for offspring (full alveolar development) if appropriately stimulated depends on species:

Full alveolar development in *monotremes* occurs in response to egg incubation.

In *marsupials* the development in pregnant and non-pregnant females is identical. Pregnancy generally being the same length or even slightly shorter than an estrous cycle. Full development is dependent on the suckling stimulus of the permanently attached pouch young. Because of this it is possible to foster a neonatal marsupial onto a virgin female as long as her cycle is synchronous with that of the true mother.

In the *eutherian* mammals, full development of the mammary gland is only completed during pregnancy or even early lactation. The growth of the gland during pregnancy in nearly all eutherian species fits an exponential curve, where the overall rate of growth is inversely proportional to gestation length for that species. Usually it is not until the second half of gestation that lobulo-alveolar development really takes off.

During pregnancy, the mammary fat pad is slowly infiltrated and the adipose cells replaced by duct tissue, alveoli, blood and lymphatic vessels, and connective tissue supporting structures. Developing alveoli arranged in lobules take over much of the gland volume previously occupied by stroma. By the last third of pregnancy the stroma is represented by thin bands of connective tissue that divide the lobules and, at a higher level, regions of lobules into lobes.

In some species, such as ruminants, the alveolar cells will begin to secrete in the last third of pregnancy, and the alveolar lumen becomes distended. In other species secretion doesn't commence until just before parturition. In all species there is a burst of secretory activity just prior to, or just after, parturition. Prior to this, full secretory activity is held in check by the high circulating progesterone levels.

Hormonal Control of Mammary Growth in the Post-Pubertal Period
The mammary gland is probably the body organ that has its growth and function most regulated by hormones.
Experiments in endocrinectomized rats (removed ovaries, adrenals and anterior pituitary) suggest that minimum requirements are:

Duct growth depends on estrogen, adrenal steroids and growth hormone.

Lobulo-alveolar growth requires these 3 plus progesterone and prolactin.

Placental lactogens (prolactin-like hormones produced in the placenta) are found in many species (ruminants and rodents are examples), and appear to have important roles in mammary growth

during pregnancy in these species. (There are often many of these hormones arising from the original prolactin gene and they form a prolactin family; in mice there are 26 members including placental lactogens, prolactin-related protein, prolactin-like protein and proliferin. Most seem to be involved in altering metabolism and immune function to ensure successful pregnancy, with variable mammotrophic activity as a side-effect).

To the above mix can be added insulin and thyroid hormone. Local growth factors known to play a role in stimulating mammary growth are insulin-like growth factors I and II (IGF-I, IGF-II), and epidermal (EGF) and fibroblast growth factors (FGF). Transforming growth factor-β has an inhibitory role that influences the pattern of the ductal tree, while matrix metalloproteinases are involved in local breakdown and remodeling of the mammary fat pad to allow epithelial outgrowth.

Given the differences in the physiology of estrous cycles and pregnancy between species it is not surprising that some species differences would exist in the above model.
Thus rats, mice, rabbits and cats exhibit only duct growth when estrogen is administered and lobulo-alveolar growth requires progesterone (as in above model).
Guinea pigs, goats and cows require both estrogen and progesterone for normal duct development and show some lobulo-alveolar development in the presence of estrogen alone.
Bitches show little or no development of either system under estrogen alone.
Note that it was not the steroids alone that gave these changes, all these animals had intact adrenal and anterior pituitary glands that the ovarian steroids could act on to cause release of other hormones and growth factors, which they can then interact with at the mammary gland.

Terminology of final mammary differentiation and lactation
In much of the literature there has been a change in the terms used to describe the final differentiation of the mammary gland that prepare it for lactation, the onset of lactation and its maintenance.

Lactogenesis I was the term used to describe the a limited degree of structural and functional differentiation of the secretory epithelium during pregnancy. This is now termed secretory differentiation.

Lactogenesis II was the term used to describe the completion of this differentiation in the peripartum period and the onset of copious milk production post-partum. This is now termed secretory activation.

Note that in ruminants it appears that differentiation is essentially completed several weeks prior to parturition. In these species all the mechanisms for lactation are in place early but the onset of large scale synthesis and copious secretion (lactation itself) is suppressed. The changes at parturition act as a trigger for full milk synthesis.

Lactation

Differentiation of Alveolar Epithelial (Secretory) Cells

As parturition approaches the alveolar cells are transformed. The nucleus moves to the basal area and becomes rounded. The base and lateral areas of the cell are filled with RER and small lipid droplets. In the apical area (closest to the alveolar lumen) becomes filled with swollen Golgi membrane arrays, developing secretory vesicles, and small lipid droplets. Due to these changes the microscopic appearance becomes characteristic, the apical areas appear lacy while the basolateral areas are darkly stained. The cell becomes polarized:

- The basal area concerned with precursor uptake and synthesis of protein and lipid
- The apical area performing posttranslational modification of proteins, and the packaging of these proteins and lactose ready for secretion.

Control of Lactogenesis/secretory activation

The general model for lactation induction at the end of parturition is as follows:

- *Increase of positive stimulators for lactation*:
  prolactin + glucocorticoids + estradiol + growth hormone (IGFs) ± insulin and an increase in mammary sensitivity to them.
- *Decline in lactogenic enzyme inhibitor*:
  Progesterone

Prolactin

Proliferation and differentiation of secretory cells in late pregnancy. Induces enzyme activity required for synthesis of milk components.

Based on recent evidence, placental lactogen also appears to promote lactogenesis in cattle, but it is not as potent as prolactin.

Glucocorticoids

Assist in regulation of several enzymes needed for milk synthesis. Essential for development of extensive rough endoplasmic reticulum (RER) within the secretory cells.

Progesterone

The main factor thought to be important in preventing induction of milk biosynthetic enzymes prepartum is progesterone, declining progesterone around parturition removes inhibition.

(For example, progesterone has been shown to inhibit transcription of the casein gene in mammary gland tissue of mice - Lee and Oka 1992 *Endocrinology* **131**: 2257-2262).

In some species the stimulus of suckling from the newborn appears important in giving full expression of synthetic and secretory activity (in addition to milk let-down).

Milk Synthesis

Milk is a mixture of proteins, carbohydrates, fats and ions in water.

Milk Precursors

The composition of milk depends on the amount and type of precursors taken up by the gland, and the transformation these undergo during milk synthesis. The major substrates extracted from the blood by mammary cells are glucose, amino acids, fatty acids and minerals. Ruminants also use acetate and β-hydroxybutyrate.

Species may differ considerably in which precursors they use and how they use them, thus both biosynthetic pathways and the final product may vary across species.
For example: monogastrics use glucose as the main precursor for fatty acid production within the mammary gland. Ruminants have lower levels of glucose than other species and what they have is largely made from propionate rather than absorbed as glucose from the intestine; but they do have circulating acetate so they use this as the main precursor for fatty acid production and preserve the glucose for production of lactose.

Carbohydrate

The major sugar in milk is lactose.
Lactose is a disaccharide (2 sugars) composed of one molecule of glucose and one molecule of galactose joined together. Galactose is just modified (epimerized) glucose.

Lactose is synthesized in the mammary gland from glucose supplied by the bloodstream. In the cow this glucose is made in the liver by gluconeogenesis. In a high producing cow 60-85% of the glucose produced may be used by the mammary gland.

Lactose is synthesized from glucose by the enzyme complex lactose synthetase, which consists of 2 proteins: galactosyltransferase and α-lactalbumin.
The lactose synthetase complex is attached to the internal wall of the Golgi membrane and final synthesis of lactose occurs here. The complex undergoes dramatic up-regulation at lactation onset.

Glucose and UDP-galactose (uridine diphosphate-galactose) enter the golgi and, under the influence of the lactose synthetase complex, form lactose and UDP.

UTP + glucose-1-P □ UDP-glucose + P-P

UDP-glucose □ UDP-galactose

UDP-galactose + glucose □ lactose + UDP

Whereas the Golgi membrane was permeable to glucose and galactose (monosaccharides), it is impermeable to lactose (disaccharide). This sugar is the major osmotically active component of milk and draws water into the Golgi vesicle. This osmotic action of lactose accounts for milk volume (since in domestic species milk is mostly water).

Other carbohydrates in milk vary widely by species. They include small amounts of free glucose and galactose, oligosaccharides, glycopeptides and glycoproteins.

Protein

Milk crude protein consists of the caseins (there are several), α-lactalbumin, β-lactoglobin, serum albumin, lactoferrin, lysozyme, immunoglobulins and nonprotein nitrogen compounds (mainly urea). Casein, α-lactalbumin and β-lactoglobin represent >90% of protein in most species and are synthesized in the mammary alveolar epithelium. An exception is human where they represent <50%, human milk containing high quantities of lactoferrin and nonprotein nitrogens.

Amino acids and nonprotein nitrogen compounds from blood provide the building blocks for milk protein synthesis. These are actively transported into the alveolar cells and proteins are assembled on surface ribosomes of the well developed RER.

These newly assembled proteins have a leader sequence of 15-30 amino acids that causes them to be inserted into the lumen of the ER. From here they are transported to the Golgi apparatus.

The Golgi apparatus is the site where modification of the milk proteins occurs and is also where synthesis and addition of other non-lipid components of milk into the secretory vesicle occurs. Protein modifications include phosphorylation and glycosylation of certain proteins, formation of disulfide bonds.

In the Golgi, the casein is combined with calcium and phosphate to form colloidal casein micelles. These are essential for cheese production.
The micelles and other proteins are co-secreted with lactose from the Golgi vesicles.

Lipid

Triglycerides (a.k.a. triacylglycerols) comprise 97-98% of the lipids present in milk. The remainder are mostly phospholipids.

Milk fatty acids range from C_4 to C_{18}. Those under C_{16} are synthesized in the mammary alveolar epithelial cells, those over C_{16} are derived from the blood borne lipids.

Precursors for lipid formation in <u>ruminants</u> are acetate, β-hydroxybutyrate, and triacylglycerides contained in very-low-density lipoproteins and chylomicrons. In dairy cows 70% of milk fat is derived from body stores during the first 2 weeks of gestation. The average for the first 12 weeks of lactation is ~ 40%.

In <u>non ruminants</u> the major blood borne precursors are glucose and triacylglycerides.

Cellular Secretion of Milk Constituents

There are 5 pathways for secretion of components into milk.

1. Proteins, lactose and salts and other non-fat components of milk are packaged into secretory vesicles in the Golgi. The presence of the lactose also draws water into these vesicles. These vesicles bud off from the Golgi and move to the apex of the cell. At the apical surface (i.e. adjacent the lumen of the alveolus) the membrane surrounding the secretory vesicle fuses with the plasma membrane of the cell and the contents are released into the alveolar lumen. This is a process of *exocytosis*.

2. Lipid droplets form near the ER and are transported to the apical membrane for secretion. Here there is lots of spare membrane left over after secretion of the lactose/protein/mineral/water by exocytosis from the Golgi secretory vesicle. This membrane forms the milk fat globule membrane. The membrane beneath the fat globule "pinches off" and the globule is secreted into the alveolar lumen (*reverse pinocytosis*). The membrane around each droplet of fat prevents them coalescing and the membrane itself provides a useful source of phospholipids to the offspring.
Butter results when beating removes the membrane around the fat globule allowing fat droplets to coalesce.

After lactose, fat and secretory proteins the next major component of milk to be secreted are the minerals that contribute to the ash content. Calcium and phosphate are secreted in the Golgi vesicles with proteins and lactose ⇨ by pathway 1 (exocytosis) as part of the casein micelle.

3. Other salts may be pumped into the cell at its base and some may then diffuse passively into the alveolus. The apical plasma membrane is permeable to monovalent ions (sodium, chloride and potassium) and glucose. It is impermeable to divalent cations and disaccharides. The cell membranes are also permeable to many pharmacological agents - concentrations can occur in milk (e.g. systemically administered antibiotics). This diffusion and pumping across basal and apical membranes is termed *transmembrane transport*.

4. *Transcytosis* is a mechanism for proteins that are not synthesized in the alveolar epithelial cell to enter the milk. This includes secretory immunoglobulin (IgA), hormones such as insulin and prolactin, growth factors such as IGF-1 and serum albumin. In this process the protein interacts with a specific receptor at the basal membrane of the cell. The protein-receptor complex is internalized and transported across the cell to the apical membrane where both protein and receptor are released into the alveolar lumen.

5. The final pathway is *paracellular transport*. During lactation tight junctions form between adjacent mammary epithelial cells which serve to separate the interstitial spaces and the alveolar lumen, effectively closing the paracellular transport pathway. During a normal lactation white blood cells appear to be able to pass into the alveolar lumen via this route without opening it to other substances.
During pregnancy, mammary involution and mastitis, the tight junctions open and large proteins and other constituents can be transported via this route.

Maintenance of Lactation

Non-Ruminants

In lab animals the minimum requirements for continued lactation are prolactin, corticosteroids and oxytocin. Additional production is obtained by adding estrogens, insulin and thyroid hormone, but some effects may be indirect - via effects on general metabolic function.

Glucocorticoids regulate the activity of several enzymes in the milk synthesis pathway by controlling their transcription rates. Adrenalectomy causes a 40-50% decrease in milk production, but this is not simply due to reduction in the enzyme levels as none become rate limiting. There are complex interactions between the glucocorticoids and prolactin.

Prolactin acts in a general fashion to maintain enzyme levels and protein synthesis. It increases gene transcription rates and the half-lives of the resulting mRNAs. The effect of prolactin on some enzymes is enhanced by glucocorticoids (here the two hormones are synergistic) while in other areas their effects appear to be only additive.
Prolactin also functions to prevent cell death (apoptosis) of mammary epithelium and maintains tight junctions between the cells.

Oxytocin is required for removal of milk from the alveolus. In its absence the mammary glands degenerate due to milk accumulation.

Ruminants

Ruminants are the most widely studied species outside of lab rodents and there are several differences in their physiology.

Once lactation is established, prolactin is **not** required for its maintenance*.

There is no effect of adrenalectomy on maintenance of lactation. It does not reduce milk production or enzyme levels, thus there is **no** requirement for glucocorticoids.

There **IS** a requirement for somatotropin (growth hormone) in the maintenance of ruminant lactation. Somatotropin has been shown to maintain mammary secretory cell numbers - the major reason that bST treatment of dairy cows gives increased persistency of lactation (it doesn't decline as fast). It also causes greater rates of milk synthesis.

*Despite the fact that prolactin is not required for maintenance of lactation in ruminants, there is some evidence for a role in supporting <u>maximal</u> production.

Local Control of Milk Synthesis

Increasing the frequency of milk removal increases milk production while decreasing milking frequency reduces production. This is seen in several species, including women.

In dairy cows, increasing milking frequency from 2x to 3x daily increases production ~ 10%. Increasing to 6x daily gave a 21% increase. Conversely, reducing frequency from 2x to 1x daily reduced yield by up to 20%.

This effect is mediated by "Feedback inhibitor of lactation" (FIL), a glycoprotein secreted by the secretory epithelial cells of the mammary alveoli. It is an autocrine regulator: it acts to inhibit the same cell that secretes it.

Appears to bind to a receptor on the apical plasma membrane. It has immediate effects to inhibit protein and lactose synthesis and ecretion.

It appears likely that there are actually several factors that act in concert to exert local control of milk production, including casein fragments.

Milk Ejection (Let-down)

This is the process whereby milk that has been synthesized and stored in the mammary alveoli is released by suckling young or the milker.

Milk is secreted into the alveoli relatively continuously from the epithelium. It is stored within the lumen of the alveolus or in expansions of the duct system in species where these exist. The duct storage system is large in ruminants (gland cistern) but still only stores a minority of milk in the udder. Species such as the rat and rabbit have no such storage structures, whereas it exists but is much smaller in women (sinuses). While milk within the storage ducts may be available passively to the suckling offspring, or, in the case of cows, to the milking machine; the majority of milk is stored within the alveoli and smaller ducts. Here the effect of surface tension means the milk cannot be removed solely by suckling, access to this larger store requires that it is actively ejected. In a cow immediately after milking there is essentially no milk stored in the cistern. The fraction stored here gradually increases and when the udder is full (~12 hours from last milking), about 20% of milk is passively available (stored in the cisterns and larger ducts) but the remaining 80% is in the alveoli and requires active ejection. The cisternal fraction is also larger during peak lactation than it is during late lactation. The size of the cistern gradually increases with lactation number so there is a greater fraction of milk passively available in older cows, but still well under 30%.

Milk ejection is controlled by a neuroendocrine reflex (others include the LH surge in induced ovulators, and Ferguson's reflex at parturition).

The nipple is densely innervated with intradermal sensory afferent nerves.

These synapse with nerves in the spinal cord which pass up the cord and after going through

several synaptic relays the stimulus arrives at the hypothalamus.

Here the information passes to the paraventricular and supraoptic nuclei, which contain oxytocin producing neurones. These are synchronously activated and oxytocin pulses are released from nerve endings located in the posterior pituitary gland.

Oxytocin circulates in the blood and causes contraction of the myoepithelial cells, which ejects milk from the alveoli into the duct system from where it is available for removal.
The contraction of these myoepithelial cells causes an increase in pressure within the udder cistern and larger ducts but due to capacity limitations not all milk from the alveoli can be accommodated in these areas. To ensure residual milk is minimized it is important that milk is removed from the udder in a timely manner following letdown, that removal is continued and that there are repeated episodes of milk ejection during the milking process.

Lag time from the start of teat stimulation till commencement of milk ejection ranges from 40 seconds to 2 minutes. Lag time is largely related to udder fill, so is shortest at peak lactation and longer in late lactation. There is no difference in time required for oxytocin release or circulation to the mammary gland – the extra time is that required for the myoepithelial cells to contract sufficiently to force out the lower volume of milk stored in the (incompletely filled) alveolus.

The milk ejection reflex may be conditioned to other stimuli, thus many dairy cows will let-down in response to the sight and sounds of the milking parlor. A similar phenomenon occurs in other species, including women, when they are preparing to nurse.
Likewise milk ejection can be inhibited by stressful situations (first-time heifer, unfamiliar dairy, rough handling) and this is due to central abolition of oxytocin release in response to tactile stimulation of the teat.

Under natural conditions the frequency of nursing (and thus letdown) varies from continuous attachment in the marsupials to once/week in the seals. The pattern of letdown during nursing also varies. In pigs and rabbits there is a single episode of let-down in response to suckling. In rats the young may stay attached for hours and during this time there are regular episodes of oxytocin secretion and milk letdown.