COURSE CODE:	*ANP 501*
COURSE TITLE:	*Physiology of Lactation*
NUMBER OF UNITS:	*3 Units*
COURSE DURATION:	*3 hours per week*

COURSE DETAILS:

Course Coordinator:	**Dr. Ikechukwu Joseph James** *B.Agric., M.Agric., PhD*
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Office Location:	Animal Physiology Staff Office, COLANIM
Other Lecturers:	Dr. Tolulope Julius Williams

COURSE CONTENT:

Mammary gland anatomy- Meaning of mammary gland, Exterior anatomy: udder, teat, supernumerary teats, external meatus. Interior anatomy: Connective tissues such as skin and ligaments, secretory tissues such as alveoli, other interior components such as milk transport system involving duct system, milk storage system such as gland cistern teat cistern. Comparative mammary gland anatomy of Monotremes such as duck-billed platypus and porcupine, Marsupials such as Kangaroo (Tammar wallaby) and eutherian mammals such as cattle, pigs, sheep and goats. Location and comparison of mammary gland of various animal species including man. Mammary tissue histology and cell biology: mammary tissue organization, mammary tissue lobules, the mammary alveolus, the mammary cell, pathways of precursor uptake in mammary cells (protein, lactose, milk fat, vitamins and minerals formation). Mammary gland growth and development: Meaning of mammogenesis, development during the foetal period, development during the pre pubertal period, development during the post pubertal period, development during pregnancy, development during lactation. Induction of lactation hormone treatment, lactaogenesis: meaning and principles of lactogenesis, mammary cytology and hormonal changes associated with lactogenesis, progesterone involvement in lactogenesis. The neonate and colostrums: The neonate, intestinal absorption of immunoglobulin, colostrums formation, immunoglobulin transport in the mammary gland, intestinal protective factors in colostrums and milk, bioactive factors in colostrums and milk. Galactopoeisis: meaning of galactopoeisis, roles of hormones and milk removal in galactapoeisis. Milk ejection: nursing frequency, oxytocin, milk ejection reflex, oxytocin

surge, other roles of oxytocin, other mechanisms of milk ejection, involvement of autonomic nervous system and stress in milk ejection, residual milk.

Practical

Study of udder anatomy of cow and doe: the gross anatomy, sagital section of udder and teats of cow and doe showing alveoli, gland cistern, teat cistern, streak canal, folds of Furstenburg's etc. Lactation curves of West African Dwarf, Red Sokoto and Sahel goats- pre-peak, peak and post peak curvature and equation for estimating lactation persistency in goats. Factors affecting lactation curve such as diseases, nutrition, frequency of milking etc.

COURSE REQUIREMENTS:

This is a compulsory course for all students in the Department of Animal Physiology and as elective Course for other Departments that discovers its relevance in their programme. In view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. Linzell, J.L. and Peaker, M. Mechanism of milk secretion. Physiological Reviews, 51: (3) 564 - 592. 1971.
2. Cowie, A.T. Lactation, in: Austin, C.R., and Short, R.V. (Eds.), Hormonal control of reproduction. Cambridge Univ. Press; New York, Portchester, Melbourne and Sydney, 2nd Edition, pp. 195-231. 1984
3. O.A. Osinowo. Introduction to Animal Reproduction. Sophie Academic, Abeokuta, Nigeria. 2006
4. Hafez, E.S.E., Jainudeen, M.R. and Rosnina, Y. Hormones, growth factors, and reproduction. In: Reproduction in farm animals. Edited by E.S.E. Hafez and B. Hafez, Lippincott Williams and Wilkins Publishers, USA, 7th edition. Pp. 33-54. 2000.
5. Walter L. Hurley. Lactation Biology. http://classes.ansci.illinois.edu/ansc438/
6. Linzell, J.L. and Peaker, M. Mechanism of milk secretion. Physiological Reviews, 51: (3) 564 - 592. 1971.
7. Cowie, A.T. Lactation, in: Austin, C.R., and Short, R.V. (Eds.), Hormonal control of reproduction. Cambridge Univ. Press; New York, Portchester, Melbourne and Sydney, 2nd Edition, pp. 195-231. 1984
8. O.A. Osinowo. Introduction to Animal Reproduction. Sophie Academic, Abeokuta, Nigeria. 2006

9. Hafez, E.S.E., Jainudeen, M.R. and Rosnina, Y. Hormones, growth factors, and reproduction. In: Reproduction in farm animals. Edited by E.S.E. Hafez and B. Hafez, Lippincott Williams and Wilkins Publishers, USA, 7th edition. Pp. 33-54. 2000.
10. Walter L. Hurley. Lactation Biology. http://classes.ansci.illinois.edu/ansc438/

LECTURE NOTES

ANATOMY OF MAMMARY GLANDS OF FARM ANIMALS

Mammary gland is the milk-secreting gland in female animals *post partum*, although some species produce milk *peri partum*. Mammary gland development commences embryonically and continues *post natal* even during lactation. In order to understand mammary gland physiology, anatomical features of the gland should be studied. The anatomical features could be broadly divided into:

1. Exterior Anatomy
2. Interior Anatomy

1. The exterior anatomy

This consists of udder (which is the apposition of mammary glands into a unit structure), teats, supernumerary teats, and external meatus (Figures 1 and 2)

I. Udder

The udder is the apposition of mammary glands into a unit structure. It is located in the inguinal region of the ventral or underside of the cow. It is found in Horse, Cattle, Sheep and Goats but not found in Pigs, Rabbit and Humans. In cow and mare, four mammary glands form an udder but in sheep and goats 2 mammary glands form an udder. The udder is usually covered with hairs except in the teats of cows. Each mammary gland has one teat with one opening. The size of udder determines milk yield.

II. Teat

Teat is otherwise known as P*apilla mammae*. It functions as the only exit for (mammary secretions) and through which the neonate receives milk. Usually one teat drains one gland. No hairs, sweat or sebaceous glands are found on cow's teat. Teat size and shape determine milk flow rate rather than milk yield.

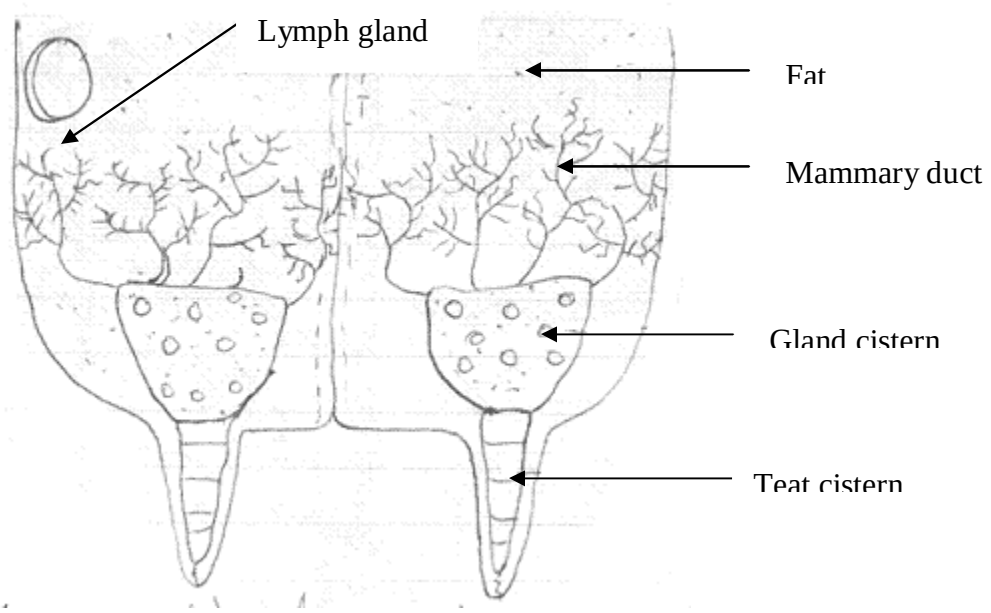


Figure 1. A longitudinal section of the udder

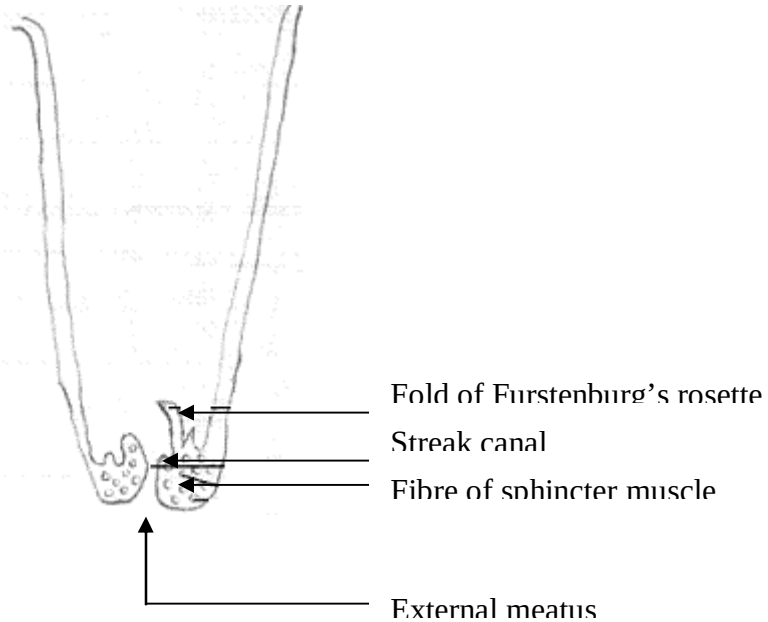


Figure 2. A sagital section of a teat

III. Supernumerary teats

About 50% of cows have extra teats that are called supernumerary teats. Few are usually functional as they open into a normal gland but many do not. They are removed before 1 year of age. A pseudo-teat has streak canal and therefore no connection to the internal structure of the gland.

IV. External meatus

This is the external origin that leads into the streak canal and through which milk comes out to the external environment. The size and shape of both external meatus and streak canal increases and changes respectively with increasing milking frequency and stage of lactation.

2. Interior Anatomy

The interior anatomy is divided into:

I. Connective tissues

II. Secretory tissues

I. Connective tissue

Connective tissue consists of fibrous tissues made of collagen and fatty tissue made of adipose cells. The essence of these tissues is to provide, support to the mammary gland in order to exhibit optimal performance (milk production). Seven tissues provide some degree of support for the udder and they are:

(a) Skin: The skin provides minor support but is numbered among the 7 tissues.

(b) Superficial fascia or Areolar Subcutaneous tissue: This attaches the skin to an underlying tissue.

(c) Coarse areolar or cord-like tissue: This forms a loose bound between the dorsal surface of the front quarters and abdominal wall. Weakening of the bonds makes the udder break away from the abdominal attachment.

(d) Subpelvic tendon: This is not part of the suspensory system but it gives rise to superficial and deep lateral suspensory ligaments which are elastic in nature.

(e) Superficial layers of lateral suspensory ligaments: It is mostly composed of fibrous tissue. This extends downward and forward from the pubic area and spread out. Upon reaching the udder, it attaches itself closely to the alreolar tissues.

(f) Deep lateral suspensory ligaments: This is thicker than the superficial lateral suspensory ligaments. It extends down over the udder and almost enveloping it. Collectively, the lateral suspensory ligaments provide substantial support for the udder. They do not join udder the bottom of the udder. The udder, therefore, pull away from the abdominal region when field with milk.

(g) Median Suspensory ligament: This is the most important part of the suspensory system in cattle. It consists of two adjacent heavy yellow elastic sheets of tissues which arise from the

abdominal wall and which attach to the medial flat surfaces of the two udder halves. It has enormous tensile strength; it is able to stretch some what as the gland fills with milk to allow for the increased weight of the gland.

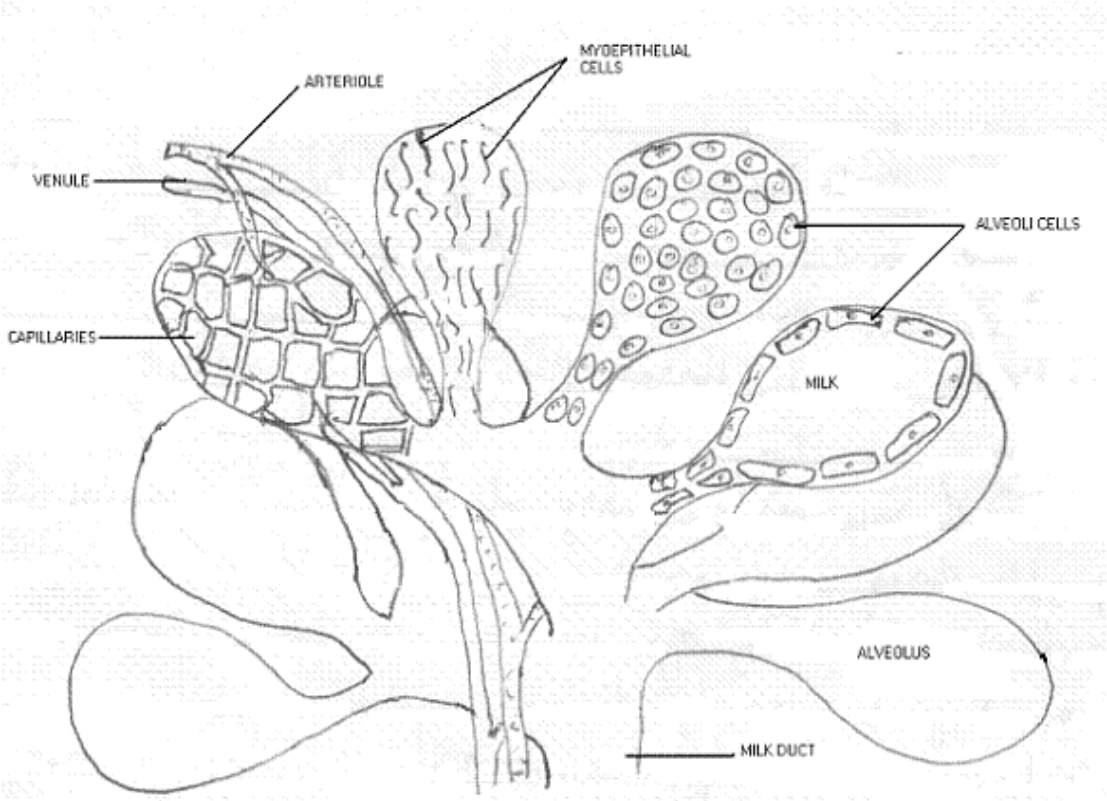
II. Secretory tissues

These are tissues made up of cells, which produce milk. They are organized into lobes, with each lobe made up of many lobules. Each lobule contains 150-220 microscopic alveoli (Fig. 3) as in cow. Alveoli are sac-like structures where milk is synthesized and secreted. An alveolus is the discrete milk-producing unit. The lumen of the alveolus is lined with a single layer of secretory epithelial cells. The epithelial cells contract in response to the hormone oxytocin, resulting in milk been squeezed out of the alveolar lumen and into small ducts. Clusters of 150-220 alveoli are encapsulated by a connective tissue sheath and are organized as a lobule (about 0.7 – 0.8mm diameter in cow). Group of lobules are surrounded by a connective tissue sheaths and comprise a lobe. Each mammary gland is made of numerous lobes.

3. Other interior components: This consists of the milk storage system and milk drainage system.

I. Milk storage system: This includes gland cistern and teat cistern.

i. Gland cistern (Sinus lactiferous): It is also referred to as udder cistern; it opens directly into teat cistern. In cows, sometimes a septum forms between the two cisterns thus, preventing milk removal/drainage (This can be corrected surgically). The major function of the cisterns is for milk storage. The size and shape of the cisterns varies with species, stage of lactation and physiological status of the animal. It is located within the udder and has greater milk storage capacity than the teat cistern.



Source: Cowie, A.T. Lactation, in: Austin, C.R., and Short, R.V. (Eds.), (1984)

Figure 3. Diagram of a cluster of alveoli in the mammary gland of goat (Redrawn)

ii. Teat cistern: This is also a milk storage lumen. It is located within the teat and has smaller capacity for milk storage *vis-a-vis* gland cistern. Usually milk stored in the gland cistern is passed unto the teat cistern for onward removal by milking or suckling.

II. Milk transport system (Duct System): Ducts are the tubules by which milk drains from the alveoli down to the gland cistern. Inter lobar or primary ducts drains multiple lobes. These are generally lines with 2 layers of non-secretory cells and have many myoepithelial cells. Intra lobar ducts are within a lobe and drain several regions of the lobe. Inter lobular or secondary ducts drain multiple lobules. They are lined with one layer of secreted cells and surrounded by myoepithelial cells, and so participate in the oxytocin-induced milk ejection. Intra lobular ducts are small ducts within a lobule. Intercalary or tertiary ducts are the small ducts, which exist from the alveolus. While this organized classification of ducts provides basis for understanding the duct system of the gland, there is no uniformity in the system of duct branching.

COMPARATIVE UDDER ANATOMY OF CATTLE, PIGS, SHEEP AND GOATS

1. Monotremes

They are egg laying mammals. They are considered to have the most primitive mammary gland. Examples are duck-billed platypus (*Ornithorhynchurs*) and Porcupine (Australian) anteater (*Tachyglossus aculeatus*). The former lays eggs and hatches then in an underground tunnel much like a bird. Upon hatching between 10-14 days, they live totally on milk for at least 3 months. The mother has not nipple but milk comes out from 100-150 separate gland tubes that open at the base of a stiff hair. There is no internal storage of milk. Milk is secreted onto the hairs and is lapped off by the young. The latter, upon laying eggs transfer then into a pouch in the ventral abdominal region. Mammary glands are distributed within the pouch, under the surface of the skin. Like the former she has no nipple, milk reservoir but there is milk ejection reflex. Milk oozes from several ducts into indentations in the skin where the young lap it up.

2. Marsupials

The young are born after a short gestation without a true placenta. Example is Tammar Wallaby (a kangaroo) – *Macropus eugenii*. The young is developmentally very immature. They quickly find their way into the pouch where the mammary glands are found. The pouch is located in the ventral region of the abdomen around inguinal region. Mammary glands are located inside the pouch, each with teat. Generally there are four teats each with 10-20 lactiferous ducts. The neonate partially swallows a teat and hangs on it for several months. Development of the mammary gland depends on degree of suckling by the neonate. The teat and gland develop in size as the young grows. Like, monotremes, they have no milk cisterns.

3. Eutherian Mammals (Cattle, Horses, Pigs, Sheep and Goats)

Females relatively have well developed mammary gland unlike in males where rudimentary structures are found. Generally, milk is utilized only by the neonate with an obvious exception of cows, sheep and goats used by humans for their nutrition. As the gland becomes more specialized, the secretion is less like body fluids (serum) in composition. Comparison of udder anatomy and morphology of some farm animals are presented in table 1.

Table 1: Comparative udder anatomy of some farm animals.

Anatomical/ Morphological traits	**Species of Farm Animals**				
	Cow	Mare	Sow	Sheep	Goat
No. of M/gland	4	4	12-14	2	2
No. of teats	4	2	12-14	2	2
No. of streak canal	4	4	24-28	2	2
Location	Inguinal	Inguinal	Thoracic Abdominal Inguinal	Inguinal	Inguinal
Size	>Mare	>Goat	<Sheep	>Sow	>Sheep
*Udder shape	Usually bowl shape	?	?	*Usually bow	*Usually bowl shape
Teat shape	More of cylindrical	More of cylindrical	More of funnel shape	More of cylindrical	More of funnel shape
Distribution of hairs	Only udder	Only udder	Udder + teats	Udder + teats	Udder + teats
Supernumerary teats	Present	?	Present	Present	Present

*Varies with breed.

MAMMARY GLAND HISTOLOGY AND CELL BIOLOGY

Organization of Secretory tissue

The secretory tissue in the udder is organized into lobes, with each lobe made up of 150-220 microscopic alveoli. A lobule can be visualized as a clump of grapes, with the stems acting as the small ducts leading from the alveoli and joining with a larger duct leading out of the lobule. The space between the grapes (alveoli) would be the stomal tissue area. The single layer of epithelial cells lining the alveolar lumen is essential to milk production. The cells and their contents are polar in nature. There are basal and apical membranes of the alveoli cells. The former is on the blood side while the latter is exposed to the alveolar lumen. The nucleus is somewhat located at the basal half of the cells, the golgi apparatus usually just apical to the nucleus and most of the secretory structures (secretory vesicles, fat droplets) generally on the apical half of the cell. The basal membrane is in contact with and attached to the basement membrane, which is a thin layer of connective tissue proteins that provide structural support for the epithelial cells. The epithelial cells are joined together by junctional complexes (tight junctions) which generally are not permeable to molecules. The polarized nature of the cells is at the heart of how and why the mammary epithelial cells secrete milk.

All precursors and substrate of milk components must pass into the epithelial cells before they can be converted into milk lactose, fat or proteins. These precursors leave the blood and enter the extracellular fluid between the capillaries and the epithelial cells. The precursors are then taken up from the extracellular fluid through the basolateral membrane of the epithelial cells: Once inside the cell, the precursors enter the appropriate synthetic pathway.

1. Synthesis of milk protein

Amino acids, which are precursors for protein synthesis, are absorbed through the basal membrane of the cell by several specific amino acid transport systems. Once inside the cell, amino acids are covalently bound together to form proteins at the polysomes (Poly-ribosomes) on the Rough Endoplasmic Reticulum (RER). Proteins synthesized at RER include milk protein casein, β-lactoglobulin, and α-lactalbumin) and membrane bound proteins (Proteins involved in cell-cell contacts) and membrane bound enzymes. Newly synthesized proteins are transferred from the RER to the golgi apparatues (GA) where they are processed for transport out of the cell. Casein is secreted as micelle, which is formed in the GA from Casein molecules, calcium and phosphorus. Caseins and other proteins undergo post-translational processing in G.A. Milk proteins and lactose are transported to the apical membrane of the cell through secretory vesicles that bud off of the Golgi apparatus. At the apical membrane, the membrane of the secretory vesicle fuses with the inner surface of the apical membrane, resulting in an opening through which the vesicle contents are discharged into the alveolar lumen. These vesicle contents are essentially, the skin milk components, water, lactose, casein micelles and whey proteins (Figure 4).

2. Synthesis of Lactose

Glucose enters the cells via the basolateral membrane via specific transport system. Some glucose is converted to galactose in the cell. Both glucose and galactose enter the GA and react resulting in the formation of lactose. The process of lactose formation in the GA results to osmotic drawing of water into the cell, GA and ultimately becoming part of the milk (Figure 4).

3. Synthesis of milk fat

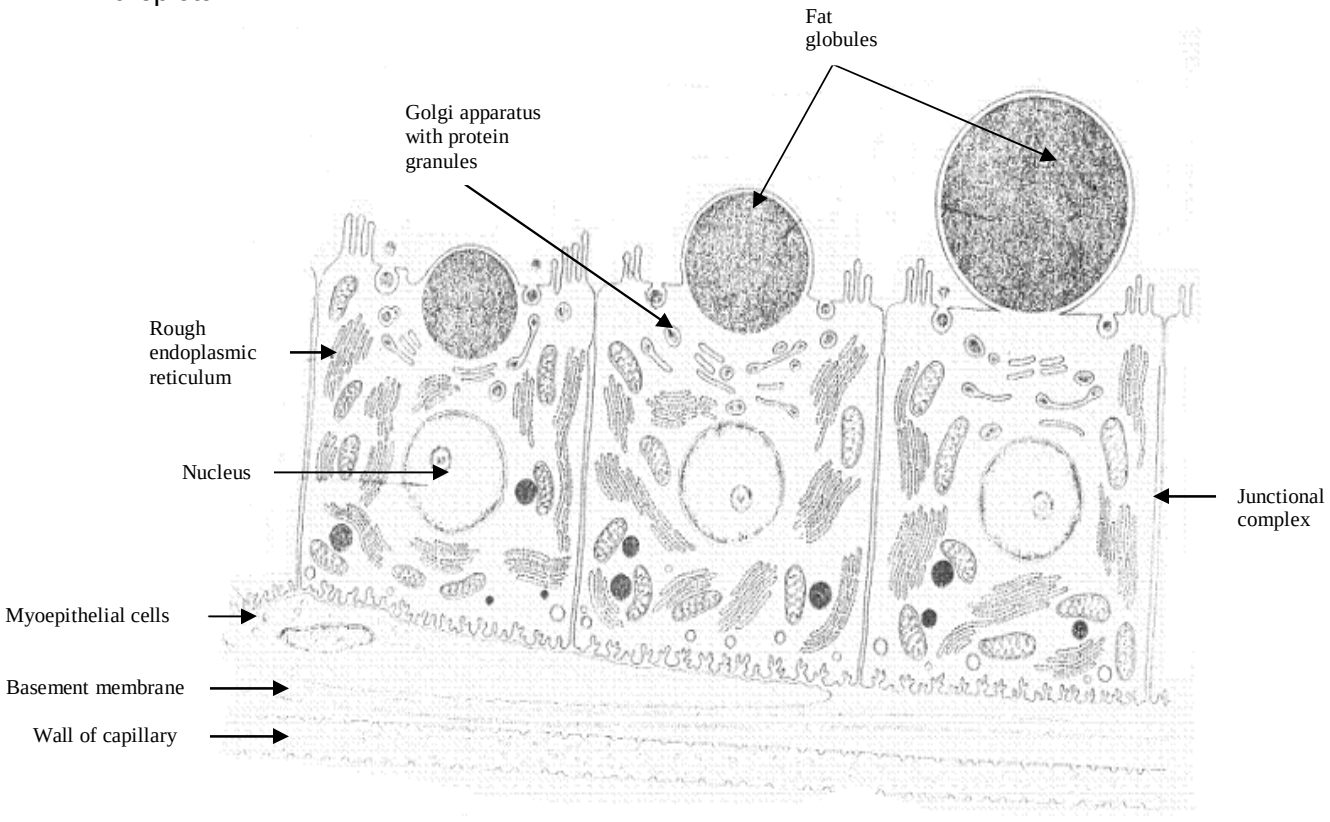
Acetate and β-hydroxybutyrate are important precursors of fatty acids (FA) synthesis in mammary cells, in ruminant especially. These precursors are absorbed through the basolateral membrane. In addition, preformed FA, glycerol and monoacylglyceride are absorbed at the basolateral membrane. All these components enter into the synthesis of triglycerides of milk. Milk fat triglycerides are synthesized on the smooth endoplasmic reticulum (SER) and from small

droplet. Numerous small lipid droplets will fuse together as the growing lipid droplet moves toward the apical membrane. At the apical membrane, the large lipid droplet forces out the apical membranes of the cell, the apical membrane surrounds the lipid droplet until it pinches off and enters the lumen (Figure 4).

TRANSPORT OF MILK COMPONENTS NOT SYNTHESIZED

IN THE EPITHELIAL CELLS

A number of other components pass across the epithelial cell barrier essentially unchanged from their form in blood. These include immunoglobulins and serum albumim. They bind to specific receptors on the basolateral surface of the cell, and are taken into the cell in endocytic vesicle and are transported to the apical side of the cell via the endocytic vesicles (transport vesicles), where the membrane of the transport vesicles fuses with the surface of the apical membrane of the cell and releases them into the lumen of the alveolus. As the transport vesicles transverse the cell, they do not seen to interact with GA, secretory vesicles or lipid droplets.



Source: Linzell and Peaker (1971).

Figure 4. The ultra structure of three alveolar cells and a myoepithelial cell

Paracellular Pathway

This occurs when substances and molecules are allowed to pass through the junctional complexes. When udder is inflamed during involution and infection by mastitis or when oxytocin is injected for total milk removal, the tight junctions opens and become leaky. This condition allows lactose and potassium to move down their concentration gradient from the alveolar lumen into the extracellular matrix and movement of sodium and chlorine from extracellular matrix into the alveolar lumen. Thus resulting in a change in electrical conductivity of milk (used in detection of mastitis), as well as increase in concentration of lactose and other milk components in the blood. Other components that can enter alveolar human without passing through epithelial cells are called leukocytes (somatic cells found in milk).

Dr. T.J. Williams

THE NEONATE:

The newborn mammal, called the **neonate,** is constantly and rapidly changing, both structurally and physiologically. In nature except for air, the only external source of everything to the neonate, is the dam's colostrum and milk. At birth, most neonates -

- have limited fat stores
- the fat stores which are present are not readily available for metabolism
- use up their limited glycogen stores rapidly after birth
- have poor gluconeogenic capacity (synthesis of glucose by the liver)
- are agammaglobulinemic (they have very low concentrations of immunoglobulin in their blood)
- neonate of many species have low iron stores
- have stucturally immature intestines
- have immature digestive capabilities, including:
- low activities of all pancreatic enzymes
- low activities of stomach pepsin
- low activities of many intestinal enzymes
- immature stomach acid generating mechanism (stomach pH is ~3.5)

However they do have -

- high rennin activity - precipitation of casein, curd formation in the stomach
- increasing lactase activity - breakdown of lactose in the intestine
- high salivary lipase activity - breakdown of milk triglycerides.

Source: Walter L. Hurley. Lactation Biology. http://classes.ansci.illinois.edu/ansc438/
Fig.1 Piglets suckling a sow

THE COLOSTRUM

Colostrum is a pale, milky fluid secreted by the mammary glands at the beginning of lactation. In mammals, colostrum is known to contain larger amounts of specific proteins than milk. The most important of these are antibodies, also known as immunoglobulins and antimicrobial peptides (eg,

lactoferrin and lactoperoxidase), and other bioactive molecules, including growth factors. They are formed by the dam's immune system to protect against a variety of infectious agents such as vira and bacteria. During the end of pregnancy and throughout lactation these antibodies are transportated via blood to the mammary glands, where they are actively secreted into the colostrum and subsequent milk. Upon suckling, the neonate absorbs these colostral immunoglobulins (Ig) intact from the gut into the blood during the first few hours to days of life. Because the ingested immunoglobulins originate from the dam, they are also called maternal antibodies. The protection of the neonate against various infectious agents through the maternal antibodies is known as passive immunity. Active immunity is when the animal develops its own protective antibodies. However under certain circumstances, the maternal

antibodies may also attack and destroy the newborns red blood cells, thereby causing fatal incompatability reactions known as **hemolysis** of the newborn or **neonatal isoerythrolysis (NI)**. Thus colostrum may be valuable by protecting against infectious diseases or be detrimental by causing neonatal losses.

INTESTINAL ABSORBTION OF IMMUNOGLOBULIN

After ingestion of colostrum by the neonate, the immunoglobulins are absorbed intact into the neonate's blood stream. This process of immunoglobulin absorption in the intestine stops after a time postpartum depending on the species. This halt in intestinal absorption of immunoglobulins and other macromolecules is called closure.

IMMUNOGLOBULIN TRANSPORT IN THE MAMMARY GLAND

The young of most and perhaps all mammalian species do not develop an effective immune system until after birth. Humeral immune protection (immunoglobulins) is supplied to the neonate by a process of transfer of passive immunity from the dam to the neonate. This generally occurs by transfer of maternal serum IgG from the dam to the offspring either in utero or, after birth, by ingestion of immunoglobulin-rich colostrum by the neonate. These maternal immunoglobulins offer immune protection until development of a competent immune system in the neonate and even may be involved in modulating the neonate's developing immune system. Therefore, lack of colostrum intake shortly after birth can lead to neonate mortality rates approaching 100%.

Immunoglobulin isotype G1 (IgG1) is the major immunoglobulin transported by the cow mammary gland during colostrum formation. The IgG1 and IgG2 make up the majority of immunoglobulin in cow colostrum and primarily come from the blood (that is they are pre-formed). Most of the IgA and IgM that are transported into colostrum are synthesized by the plasma cells (B lymphocytes) that reside in the mammary tissue. Transport of the IgGs and the IgA/IgM occurs through the epithelial cells by a process involving small transport vesicles. However, the receptors for the IgGs and the IgA/IgM are different receptors. The receptor for IgA/IgM is called secretory component (SC) and is proteolytically cleaved off the membrane during transport of the IgA. The SC remains bound to the IgA and the SC-IgA complex is called

secretory IgA. There is also a lot of non-bound SC in milk and colostrum, suggesting that the proteolytic cleavage of SC does not require that it be bound to IgA. The receptor(s) for IgG tr aport has not been completely identified at this time.

Transport of maternal immunoglobulins into colostrum probably occurs in all mammals to varying extents, but the significance of the immunoglobulins in colostrum depends on the species. Humans and other primates transport immunoglobulins to the fetus through the placenta via a receptor-mediated, intra-epithelial mechanism similar to that in the mammary gland. Therefore, when the infant is born it already has a full complement of immunoglobulins in its blood to protect it for disease until its own immune system is fully functional. Transport of immunoglobulins into colostrum in primates does occur (primarily IgA/IgM) but to a more limited extent. However, in most species immunoglobulins are not transported across the plactenta, therefore the colostral immunoglobulins are critically important to neonate survival. In the dairy cow, as much as 2 kilograms of IgG can be secreted into the colostrum during the first five milkings. Another exception is the rat which transports some immunoglobulin across the placental yolk sac and some via the colostrum.

Immunoglobulin concentrations decline rapidly over the first 24 hr after parturition. The total amount of immunoglobulins secreted in the colostrum increases with parity of the mother. For example, the first lactation cows will have about one half the IgG1 concentration that third and fourth lactation cows will have. Concentrations of colostral IgG2 and IgM also are lower in first lactation cows, while the concentration of IgA is only slightly lower.

INTESTINAL PROTECTIVE FACTORS IN COLOSTRUMS AND MILK

The gastrointestinal tract is constantly under attack from acid, proteolytic enzymes, and ingested noxious agents, such as aspirin or alcohol. The presence of multiple defense mechanisms— including the mucus-bicarbonate layer in the stomach, a rapid mucosal turnover, and a good blood supply—ensure that the mucosa remains intact most of the time. If a small area of injury is sustained, the healing process usually proceeds successfully

via standard mechanisms. Surviving cells from the edge of the wound migrate over the denuded area to re-establish epithelial continuity. This process begins within a few minutes after injury and is termed *restitution*. This is followed by increased proliferation and remodeling, which begins <24–48 h after the injury. Many factors, including peptide growth factors, stimulate these various processes.

BIOACTIVE FACTORS IN COLOSTRUMS AND MILK

Colostrum and milk contain many factors that can influence cell growth, differentiation, and function. Several nonpeptide constituents of colostrum, when added to cells in vitro or when infused into animal models, have resulted in increased proliferation. These factors include glutamine, polyamines, and nucleotides. These factors play an important role in maintaining gastrointestinal mucosal mass and modulating the immune system via multiple mechanisms, eg, altering intestinal flora and influencing the actions of growth factors.

Hormones : It is well established that milk and colostrum contain many hormones, which, when infused systemically, influence a wide variety of end-organ systems. These systems include the hypothalamic- hypophyseal system (because milk contains prolactin, somatostatin, oxytocin, and luteinizing hormone-releasing hormone), thyroid gland (because milk contains thyroid-stimulating hormone, thyroxine, and calcitonin), sexual glands (because milk contains estrogen and progesterone), and adrenal and pancreatic glands. It is probable that at least some of these hormones

(eg, luteinizing hormone-releasing hormone) influence plasma concentrations and the development of various end organs of suckling neonates because of the passage of the hormones through the bowel wall into the systemic circulation.

GALACTOPOEISIS

Galactopoeisis is the maintenance of lactation once lactation has been established. Two key interrelated components contribute to the maintenance of lactation, **galactopoietic hormone**s and **removal of accumulated milk**. Because of the importance of galactopoietic hormones in milk production, sometimes the word galactopoiesis also is used to indicate enhancement of lactation, especially in dairy animals. Inhibition of secretion of key galactopoietic hormones will depress milk production to varying degrees depending on the species, stage of lactation, and the

particular hormone being suppressed. Much of the fundamental knowledge that we have on galactopoietic hormones comes from classic studies demonstrating that inhibition of hormone secretion will inhibit milk production. Conversely, administration of additional amounts of galactopoietic hormones during lactation can enhance milk production, again depending on the species, stage of lactation, and particular hormone. The most widely known example of this has led to the common practice of administration of bovine somatotropin (bST, or bovine growth hormone) to lactating dairy cattle resulting in relatively dependable increases in milk yield.

The role of *galactopoietic hormones* such as **prolactin** in maintenance of lactation is well established. Prolactin is considered the major galactopoietic hormone in nonruminants. Prolactin is released at the time of milk removal in ruminants and nonruminants, and it remains a key systemic modulator of milk secretion during lactation. Conversely, **growth hormone** is generally considered to be the predominant galactopoietic hormone in ruminants. Inhibition of prolactin secretion or administration of prolactin to lactating cows has little effect on milk yields. However, these apparently clear-cut roles of prolactin vs. growth hormone in maintenance of lactation in nonruminants vs. ruminants are probably an oversimplification. For example, in lactating sheep both prolactin and growth hormone seem to be important for galactopoiesis. Even in the rat, recent studies have demonstrated an important role for growth hormone, independent of the role of prolactin.

Regardless of the hormones involved, all attempts to evaluate milk secretion must account for continued **removal of milk**. This is a reminder of the critical role of **local mammary factors** in maintenance of milk secretion. One such factor that plays a major role in regulating milk secretion in many species is a **feedback inhibitor of lactation** (FIL) found in milk. FIL is thought to be produced by the mammary cells as they synthesize and secrete milk. Accumulation of FIL in the milk-producing alveoli results in feedback inhibition of milk synthesis and secretion. Frequent removal of milk from the gland minimizes local inhibitory effects of FIL and increases milk secretion. Milk removal involves several mechanisms that impact milk production, including removal of local inhibitory components, regulation of local blood flow, and even physical factors in the alveolus. The effects of frequency of milk removal are tied closely with the local regulation of milk secretion.

MILK EJECTION

The mechanism by which the alveoli physically express milk from the lumen during milk removal is called **milk ejection**. Stimulation of the mammary gland, particularly the teats or nipples, results in secretion of the hormone **oxytocin** from the posterior pituitary. Oxytocin travels via the blood to the mammary gland and causes contraction of the myoepithelial cells surrounding the alveolus. This results in expulsion of the luminal milk from the alveolus into the ducts and out of the gland, resulting in the physical removal of milk from the alveoli.

The role of milk removal complicates interpretation of the hormonal requirements for milk synthesis and secretion. Without frequent emptying of the mammary gland (milk removal), milk synthesis will not persist in spite of adequate hormonal status. Conversely, maintenance of intense suckling or milking stimulus will not maintain lactation indefinitely. Nevertheless, **suckling** or actual removal of milk from the gland is required to maintain lactation.

Milk ejection is important during milking or suckling to obtain the alveolar milk fraction, which can represent more than 80% of the milk stored in the udder of dairy cows. In response to tactile teat stimulation, either manually or by the milking machine, milk ejection is induced by the release of oxytocin and resultant myoepithelial contraction. The time from the start of a tactile stimulation until the occurrence of milk ejection spans between 40 s to >2 min and increases with decreasing degree of udder filling. Therefore, cows need a longer pre-stimulation in late stages of lactation or if the milking is performed shortly after the previous milking, whereas in full udders pre-stimulation is less important. Milk ejection is disturbed under several conditions such as during milking in unfamiliar surroundings or for several weeks immediately after parturition in primiparous cows. Disturbed milk ejection is due to a reduction of or absence of oxytocin release from the pituitary. The process of milk ejection can occur under many conditions. Milk ejection can occur under water - as for whales, porpoises, sea-cows, sea otters, hippopotamus. Milk ejection also can occur while in flight as for some bat species.

MILK EJECTION REFLEX

The milk ejection reflex (let-down) actually is a neuroendocrine reflex. The reflex has an afferent pathway (neural) and an efferent pathway (hormonal, blood-borne).

Afferent Pathway: The greatest amount of innervation in the mammary gland is in the teats, where there are pressure sensitive receptors in the dermis. Mechanical stimulation of the teats activates pressure sensitive receptors in the dermis where the pressure is transformed into nerve impulses that travel via the spinothalamic nerve tract to the brain. These nerves synapse in the paraventricular nucleus and in the supraoptic nucleus in the hypothalamus. When the cell bodies of the oxytocin-containing neurons are stimulated by these impulses originating in the teat, an action potential moves down the oxytocin-containing neurons from the cell body in the hypothalamus down the axon to the neuron ending in the posterior pituitary. This causes release of oxytocin and neurophysin into the blood. The efferent pathway starts at this point.

Efferent Pathway: The efferent pathway begins with the release of oxytocin into the blood. The oxytocin then travels to the mammary gland via the blood, binds to oxytocin receptors on the myoepithelial cells, causing the myoepithelial cells to contract, and resulting in increased intra-lumenal (intramammary) pressure and ejection of milk from the alveolar lumen. Oxytocin receptors are associated with the myoepithelial cells, not the smooth muscle of the mammary gland. In mice these receptors increase through-out gestation, but are fairly constant through lactation.

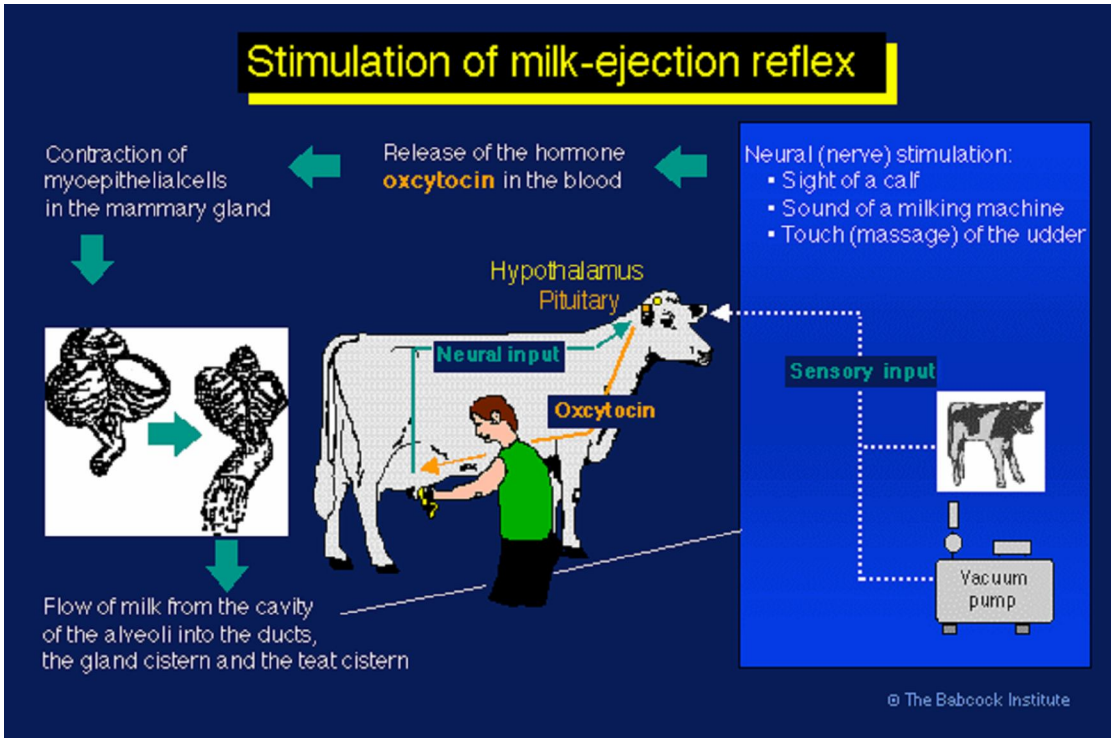


Fig. 2 Milk let-down

Other Mechanisms of Milk Ejection

1. Myoepithelial cells will also contract in response to vasopressin (ADH or antidiuretic hormone). Vasopressin has about 20% the oxytocic activity of oxytocin.
2. Visual or auditory stimuli can cause milk ejection. Milk ejection is a condition response.
3. Stimulation of the genital tract such as vaginal distention causes release of large amounts of oxytocin.
4. The mechanical tap stimulus does not involve oxytocin. It will occur under anesthesia or denervation of the udder. It is not inhibited by epinephrine. Kneading or butting of the udder by the young may elicit this response. This may involve distortion of the alveolar structure or the myoepithelial cell structure, resulting in milk ejection.

Timing is very important : The timing of oxytocin release relative to milk removal is an important factor affecting milk ejection. Oxytocin has a short half-life in the blood = 0.55 to 3.6 min. This means that the removal of milk by machine or by nursing must be closely timed with stimulation of the teats. The oxytocin concentration in the blood normally increases within 1-2 minutes after udder stimulation, but the amount released declines during milking. It is

estimated that the bovine pituitary gland has about 800 mg of oxytocin. This is ~40 times the blood level in resting condition. Only ~1/3 of pituitary oxytocin is released at milking.

The sensitivity of the neuroendocrine reflex seems to decline as lactation progresses. Peak oxytocin occurs 1 minute at 1-2 weeks of lactation, 2 min at 5-6 weeks. Maximum oxytocin concentration during milking also declines as lactation progresses. The minimum amount oxytocin required to cause milk ejection is about 0.02 IU. However, an injection of 10 IU of exogenous oxytocin is used to cause milk letdown.

STRESS IN MILK EJECTION

Inhibition of Milk Ejection: Various stressful stimuli that inhibit milk ejection are associated with increased activity of the sympathetic nervous system. Oxytocin action can be blocked be catecholamines (epinephrine and norepinephrine). The hormones are usually released in response to stressful situations and increase the tone of the smooth muscles of the mammary ducts and blood vessels. This results in the reduction of oxytocin reaching the myoepithelial cells and partial occlusion of the mammary ducts. Moreover, epinepherin directly blocks oxytocin from binding to myoepithelial cells. This is termed ***peripheral inhibition of milk ejection***. Thus, exogenous oxytocin will not cause milk ejection in animals exhibiting peripheral inhibition.

A common cause of failure to milk ejection is associated with stress of milking in the early postpartum period especially for primiparous cows. The stress inhibits the release of oxytocin from the posterior pituitary gland (central inhibition of milk ejection). Exogenous oxytocin is usually administered in these cases causing milk ejection. Based on the above discussion about peripheral and central inhibition of milk ejection, it can be stated that milk
ejection occurs as a result of oxytocin release, which is normally couples with inhibition of the central and peripheral inhibitory controls.

Role of Autonomic Nervous System

The autonomic nervous system is part of the central nervous system. It mainly controls viseral function. The autonomic nervous system is made up of two types of nerves, the ***parasympathetic*** nerves and the ***sympathetic*** nerves. There is no direct innervation of alveoli or myoepithelial cells

1- **Parasympathetic nerves:** The neurotransmitter of parasympathetic nerves is acetylcholine. There is no parasympathetic innervation in the mammary gland.

2- **Sympathetic nerves:** The neuroendocrine components of sympathetic nerves are epinephrine and norepinephrine. Epinephrine (adrenaline) is primarily from adrenal medulla.

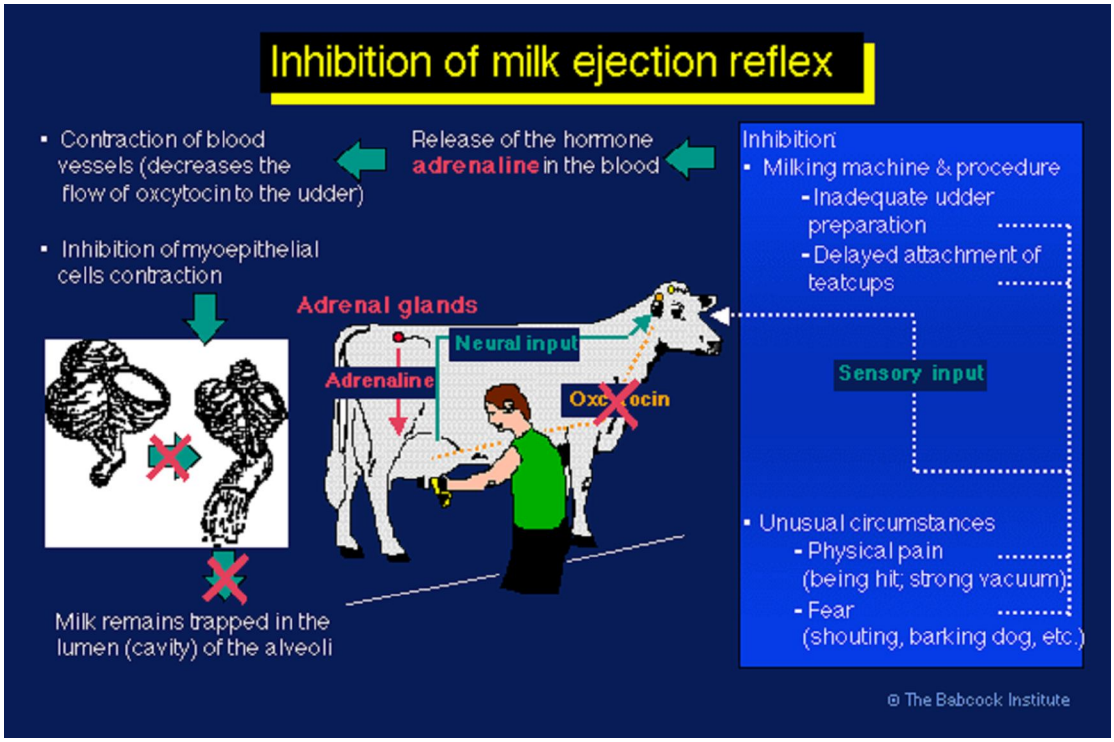
Norepinephrine is a neurotransmitter from peripheral nerves and nerves in the brain. Norepinephrine can also come from the adrenal medulla. It is well known that fright and stress interfere with the milk ejection reflex and may inhibit milk ejection. Fright and stress activate the neuroadrenal system and cause the release of epinephrine. The inhibitory effect of epinephrine on milk ejection occurs both centrally in the brain and in the mammary gland.

Norepinephrine and epinephrine can inhibit oxytocin-induced contraction of myoepithelial cells. Stressful stimuli will inhibit milk ejection. This occurs via epinephrine or norepinephrine derived from the adrenal gland or the sympathetic nerves by the following
mechanisms :

1- Norepinephrine reduces myoepithelial cell contractile response to oxytocin; this is a direct inhibition at the myoepithelial cell level.

Norepinephrine decreases mammary blood flow (amount of oxytocin to the gland); this is an inhibition at the mammary tissue level.

2- Norepinephrine reduces oxytocin release from the pituitary; this is an indirect effect mediated by inhibition of oxytocin release at the hypothalamic level. In the bovine species norepinephrine is the primary catecholamine. Injections of norepinephrine into cows, which increase blood levels to 2 to 5X above normal will cause a decrease of milk yield by 10%. Oxytocin is not altered. Emotional disturbances can cause inhibition of the CNS part of the milk ejection reflex. This may especially occur after calving in the first-calf heifer. Injection of oxytocin may be needed to remove milk because failure to remove the milk will result in reduced yield through lactation.



--Fig. 3 Inhibition of milk let-down