

## **6. SUMMARY**

The experimental work of this study was carried out at Sakha Experimental Station, belonged to Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Chemical analysis of blood samples was executed at laboratory of Ruminant Unit, Sheep and Goat Research Division, Animal Production Research Institute. While, chemical analysis of taken milk samples was conducted at The International Livestock Management Training Center (ILMTC), Sakha, Animal Production Research Institute. Histological study was executed at laboratory of Pathology Division, Faculty of Veterinary Medicine, Cairo University. However, cytological study was executed at Central Laboratory, Faculty of Science, Ain Shams University (ASU-CL). Both DNA and RNA of mammary tissue analysis were executed at Molecular Genetic Laboratory, The International Livestock Management Training Center (ILMTC). The field work of the experiment lasted for eight months starting from November, 2004.

The objective of this study is to get more information concerning:

1. The changes that occur in the mammary gland and its secretion during physiological transitions of the bovine udder from lactation to involution and from involution to colostrogenesis stages.
2. Modulation of mammary cell growth, differentiation, and milk biosynthesis near parturition

3. Effect of dry period length on the subsequent lactation in Friesian cows.

A total of 16 pregnant Friesian cows aged between 3.5 and 6.5 years, and had 2 to 4 parities. These cows were divided into two groups (8 cows in each) the first one included cows with dry period more than 60 days (above maximum). While the second included cows with dry period less than 45 days (lower minimum).

**The results obtained in this study could be summarized as follows:**

Dry period length for cows having long dry period length was 72.38 days and its different phases (active involution, steady state involution, and redevelopment and colostrogenesis) were 28.00, 16.38, and 28.00 days, respectively. While means dry period length for cows having short dry period length was 33.25 days and both active involution, and redevelopment and colostrogenesis phases were 16.63 days.

**1. Mammary gland function during the dry period:**

There were gradual changes occur in biochemical composition of mammary secretions during dry period, including:

- A. Concentration of fat in secretions decreased gradually during dry period attaining its lowest value was 0.6 % during steady state involution phase. Then beginning to increase gradually reached its heights value during 1<sup>st</sup> day after parturition (5.51 and 5.90 % for cows having long and short dry periods, respectively). Then beginning to decrease gradually again

accompanied with milk yield increased. Also, concentrations of fat in secretions during all studied stages were greater for cows having short dry period compared with those having long dry period.

- B. Concentrations of total protein in secretions increased significantly ( $P < 0.05$ ) during dry period. During redevelopment and colostrogenesis phase protein % was the highest (18.64 and 17.69 %) for cows having long and short dry period lengths, respectively. The lowest values of protein concentrations (3.19 and 3.09 % for cows having long and short dry period lengths, respectively) were recorded on 7<sup>th</sup> day postpartum (whole milk).
- C. The increase in total protein concentrations was observed during dry period occurred despite of the decline in the major milk proteins (caseins,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin). This dramatic increase thought to arise from increasing antibacterial proteins (immunoglobulins, lactoferrin, and serum albumin), which play a role in protects the gland against bacterial infection.
- D. Cows having short dry period showed a significant increase ( $P < 0.05$ ) in protein % during the end of lactation and active involution periods. While during redevelopment and colostrogenesis phase and postpartum it was greater for cows having long dry period. This may have occurred because immunoglobulin production was probably lower in cows having short dry period.
- E. Lactose concentrations in secretions declined rapidly after the last milking whereas were 1.43 and 1.30 % for cows

having long and short dry period lengths, respectively during active involution phase, then declined to 0.81 for cows having long dry period length during steady state involution phase. Then increased gradually, although at parturition it was usually still lower than at drying-off, but it increased slightly just before parturition. This is explaining decreased lactose synthetase during dry period. Overall, concentrations of lactose were lower for cows having short dry period length in comparison with cows having long dry period length during all stages.

- F. The highest values of solids not fat (SNF) concentrations (20.84 and 20.69 % for cows having long and short dry period lengths, respectively) were recorded during redevelopment and colostrogenesis phase. This suggests that in the days immediately before parturition there was a rapid increase in the secretory activity of the alveolar cells coupled with increase concentrations of total protein. While on 7<sup>th</sup> day postpartum (whole milk) SNF % had the lowest values.
- G. The highest values of total solids (TS) concentrations were recorded during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum. This raise coupled with increase in concentrations of total protein and fat. This resulted from active milk synthesis which characterized by intense mammary growth, rapid differentiation of secretory epithelium, and increased synthesis and secretion of fat, protein, and carbohydrates as procures of colostrum.
- H. There was a significant rise ( $P < 0.05$ ) in somatic cell count (SCC) level at active involution phase for both cows having

long and short dry period lengths ( $1.798$  and  $1.997 \times 10^6/\text{ml}$ , respectively), this increase is probably due to cessation of milk removal as well as resorption of milk components. While at the next phase of the studied dry period (steady state involution) the level of SCC had a significant rise ( $P < 0.05$ ) for cows having long dry period length ( $2.542 \times 10^6/\text{ml}$ ). During the last phase of the dry period (redevelopment and colostrogenesis) the level of SCC had a significant decrease ( $P < 0.05$ ) for cows having long dry period length ( $1.774 \times 10^6/\text{ml}$ ), while for cows having short dry period length the level of SCC rise was ( $2.107 \times 10^6/\text{ml}$ ) but not significant ( $P < 0.05$ ). In general, the levels of SCC decrease markedly during postpartum period, reaching the lowest values in whole milk at 7<sup>th</sup> day postpartum.

- I. The values of pH of mammary secretions increases significantly ( $P < 0.05$ ) from (6.80 and 6.69) during end of lactation to (7.39 and 7.22) during active involution, then declines significantly ( $P < 0.05$ ) to (6.35 and 6.52) on 1<sup>st</sup> day postpartum, before its increasing again to (6.83 and 6.82) in whole milk at 7<sup>th</sup> day postpartum for both cows having long and short dry period lengths, respectively.

## **2. Histological and ultrastructural changes of the mammary tissue:**

- A. *Histological changes during involution indicated marked shifts in the secretory activity of the gland during the active involution phase of the nonlactating period. As evidenced by a reduction in alveolar luminal area with a concomitant increase in connective tissue area.*

- B. *Cytoplasmic organelles declined in the involuting cells, particularly those involved in the extensive milk protein synthesis and secretion function of the lactating cell, such as the RER, Ribosomes, Golgi, and secretory vesicles.*
- C. *Despite the apparent breakdown of the protein synthesis and secretion pathway associated with milk proteins, the involuting epithelial cells maintained intact cellular organelles involved in metabolic and secretory function. Ribosomes and segments of RER were present at all stages, as were mitochondria and microvilli.*
- D. *Invading macrophages have been implicated in removal of luminal milk fat globules in involuting mammary tissue and also may be involved in removal of cellular debris.*
- E. *The structure of alveoli at steady state involution phase (light microscope level) resembled the solid mass of cells. The morphology of bovine mammary tissue and the composition of mammary secretions remain relatively constant.*
- F. *The involution of bovine mammary tissue does not involve extensive detachment of mammary epithelial cells from the basement membrane as occurs in rodents.*
- G. *During the redevelopment and colostrogenesis phase, increased synthetic and secretory activity were apparent by increase areas of epithelium and lumen*

*and a decrease area of connective tissue. Expansion of alveolar lumina area resulted in a concomitant compression of surrounding connective tissue area. As parturition approached, fully active cells also became more numerous. The cell cytoplasm contained abundant RER, well-developed Golgi apparatus, and numerous mitochondria.*

- H. Although the progression of ultrastructural changes in alveolar epithelial cells was consistent between the cows receiving the long dry period and the cows receiving the short dry period, obtained results suggest that the rate at which this progression occurred was faster in the cows receiving the short dry period.*
- I. Ultrastructural changes of cow mammary tissue suggest that a mammary involution as it relates to the epithelia, occurs as an organized and controlled process that apparently leads to minor loss of epithelial cells. As well as a dry period is important for replacing senescent mammary epithelial cells and increasing the epithelial component of the gland prior to the next lactation.*

### 3. The DNA and RNA changes in mammary gland tissue:

A. Mammary cell number and its secretory activity were estimated by using multiple biopsies to quantitatively evaluate changes in DNA and RNA content, respectively. During end of lactation an average concentrations of DNA were 3.25 and 3.15 mg/g decreased to reach 3.00 and 2.86 mg/g during active involution for cows will having long and

short dry period lengths, respectively. This would suggest that milk stasis during active involution has been demonstrated to stimulate DNA laddering in mammary tissue. These indicate that mammary epithelial cells were lost during involution phase in the mammary gland, but this process of cell loss does not seem to be as dramatic. Concentration of DNA decreased (7.69 and 9.21 %) from the end of lactation during active involution phase for cows having long and short dry periods, respectively this indicate that mammary epithelial cells were greater lost in mammary tissue for cows having short dry period during this phase.

B. During steady state involution phase for cows having long dry period length, the concentration of DNA decreased to 2.87 mg/g (11.69 % from the end of lactation). This indicate that the lost of mammary epithelial cells during active involution and steady state involution phases were greater in mammary tissue for cows having long dry period.

C. The average concentrations of DNA during redevelopment and colostrogenesis phase increased to 3.91 and 3.62 mg/g (20.31 and 14.92 % from the end of lactation) for cows having long and short dry periods, respectively. This demonstrates that mammary epithelial cells increased during this phase for cows having long dry period greater.

D. During the end of lactation period an average concentrations of RNA were 5.71 and 5.91 mg/g decreased to attained 4.22 and 4.80 mg/g during active involution phase for cows will having long and short dry period lengths, respectively. Then it decreased to reach 3.01 mg/g during steady state involution

phase for cows having long dry period length. This demonstrates that secretory activity for mammary tissue decreased gradually during active involution and steady state involution phases. While during redevelopment and colostrogenesis phase concentrations of RNA increased to 6.01 and 6.26 mg/g for cows having long and short dry periods, respectively.

E. The ratio of RNA: DNA was greater for cows having short dry period throughout the experimental periods. This show that secretory activity for mammary epithelial cells was greater for cows having short dry period compared with those having long dry period.

F. These obtained data suggest that the value of the dry period might be to repair or replace damaged or senescent mammary epithelial cells prior to the next lactation.

#### 4. The blood components changes in cows during dry period:

- A. Plasma insulin-like growth factor 1 (IGF-1) concentration decreased significantly ( $P < 0.05$ ) as parturition approached. After that, it gradually decreased to reach its lowest values at 7<sup>th</sup> day postpartum. Also, it significantly ( $P < 0.05$ ) greater for cows having long dry period lengths compared with those having short dry period lengths.
- B. Plasma glucose concentrations decreased gradually during dry period. At 1<sup>st</sup> day postpartum plasma glucose concentration fell sharply its lowest value, then increased significantly ( $P < 0.05$ ) after that. And generally it was greater for cows having long dry period during the end of lactation period and active

involution phase, while during redevelopment and colostrogenesis phase and postpartum period it was greater for cows having short dry period.

- C. Total plasma protein concentrations decreased gradually during dry period. While at 1<sup>st</sup> day postpartum it decline significantly ( $P < 0.05$ ) this may be due to the production of colostrum, which is rich in proteins especially immunoglobulin, then it began to increase gradually after that. And it was also, greater for cows having long dry period compared with cows having short dry period length during all study stages.
- D. Total plasma lipids concentration decreased gradually during dry period. While at 1<sup>st</sup> day postpartum it decline significantly ( $P < 0.05$ ), then beginning to increase gradually after that. And it was greater for cows having long dry period compared with cows having short dry period length during all study stages.

### **5. Changes in cow body weight and mammary gland dimensions during the dry period:**

- A. Cows gained averages 10.62 and 6.01 % of body weight during the dry period, while they lost 8.50 and 7.14 % at calving, also during 1<sup>st</sup> week postpartum they lost 4.83 and 4.47 % for cows having long (72.38 days) and short (33.25 days) dry period lengths, respectively.
- B. Means of distance of fore and rear quarters from the floor increased gradually during active and steady state involution where they reach the highest values (71.21 and 68.63 cm) for cows having long dry period, respectively. Then they

decreased gradually until they reach the lowest values (52.13 and 49.50 cm, respectively) for cows having long dry period at the 7<sup>th</sup> day postpartum.

- C. Udder fore and rear depths decreased gradually during active and steady state involution phases, and reached their lowest values (19.26 and 17.21 cm) for cows having long dry period respectively. At 1<sup>st</sup> day postpartum they increased significantly ( $P < 0.05$ ). But differences between cows having long and short dry period lengths during all study stages for means of udder fore and rear depths were not significant.
- D. Udder fore and rear widths decreased during active and steady state involution phases and reached the lowest values (18.74 and 13.53 cm) for cows having long dry period, respectively. While at 1<sup>st</sup> day postpartum they increased significantly ( $P < 0.05$ ). Generally difference between cows having long and short dry period lengths during end of lactation, dry periods and at 7<sup>th</sup> day postpartum were not significant except, during colostrum period they were significant ( $P < 0.05$ ).
- E. Udder periphery decreased during active and steady state involution phases for cows having long and short dry periods, and reached the lowest values (84.53 cm) for cows having long dry period. While during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum it increased significantly ( $P < 0.05$ ). Difference between cows having long and short dry period lengths during dry period were not significantly ( $P < 0.05$ ). But it was significantly ( $P < 0.05$ )

for cows having long dry period length during both end of lactation and postpartum periods.

- F. Udder volume decreased during active involution (27.49 and 17.38 % for cows having long and short dry periods, respectively) and steady state involution (46.18 % for cows having long dry period). While during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum it increased significantly ( $P < 0.05$ ) for cows having long dry period (167.97 and 90.48 %, respectively) and were (47.62 % and 44.97 %, respectively) for cows having short dry period. After that the udder volume decreased but not significantly ( $P < 0.05$ ).
- G. Length of fore and rear teats decreased during active and steady state involution phases and reached the lowest values (4.42 and 4.18 cm, respectively). While during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum they increased significantly ( $P < 0.05$ ) for cows having long dry period, and insignificantly for cows having short dry period.
- H. Diameter of fore and rear teats decreased during active and steady state involution phases and reached the lowest values (1.75 and 1.62 cm, respectively). While during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum they increased significantly ( $P < 0.05$ ) for cows having long dry period, and insignificantly for cows having short dry period.
- I. Distance between fore teats, distance between rear teats and distance between side teats decreased during active and

steady state involution phases, and reached the lowest values (9.58, 5.50 and 5.54 cm, respectively). While during redevelopment and colostrogenesis phase and at 1<sup>st</sup> day postpartum they increased significantly ( $P < 0.05$ ).

**6. Effect of dry period length on milk production in subsequent lactation:**

- A. Total milk yield increased by 18.82 % for cows having long dry period length (4697.25 kg) compared with cows having short dry period length (3953.13 kg).
- B. 100-Day milk yield was higher by 35.71 % for cows having long dry period length (2335.88 kg) compared with those having short dry period length (1760.13 kg).
- C. Lactation period was longer by 12.55 % for cows having long dry period length (325.00 days) when compared with cows having short dry period length (288.75 days).
- D. Average daily milk production was higher by 5.41 % for cows having long dry period length (14.43 kg/day) when compared with cows having short dry period length (13.69 kg/day).

**Summarization:**

The dramatic changes occur in mammary tissue and mammary secretion composition during the redevelopment and colostrogenesis phase are essentially the opposite of events that occur during active involution. Progression to a state of active milk synthesis is characterized by intense mammary growth, rapid differentiation of secretory epithelium, and increased synthesis and secretion of fat, protein, and carbohydrates that results in the accumulation of colostrum.

A dry period, typically 45 to 60 days, between lactations is believed to be required to maximize milk yield in the subsequent lactation. Several hypotheses have been proposed to explain the requirement for the dry period, including (1) replenishment of body reserves, (2) regeneration of mammary tissue, and (3) optimization of benefits from endocrine events near the time of parturition.

For the dairy cow, a 45 to 60 days dry period is required for optimal milk yield in the subsequent lactation. This period probably represents an active involution phase of about 3 to 4 weeks until the involution process is completed, followed immediately by redevelopment of the gland beginning 3 to 4 weeks prior to parturition. Such an optimal dry period would have no “steady state” phase of involution. If the dry period is shortened to less than 45 days, then the process of involution would overlap the prepartum redevelopment phase, presumably resulting in less mammary development for the next lactation. Therefore hypotheses have been proposed to explain reduced milk yields in cows having short dry period (less than 45 days): 1) inadequate body reserves, 2) endocrine differences, 3) reduced mammary epithelial cell numbers, and 4) reduced mammary functionality and mitotic capacity.

Dry period of greater than 72 days increases feed and management costs with no associated return and may result in lowered production in the current lactation, however decrease the cow’s lifetime production.