Lactation Biology II

TH278 Changes in the mechanical microenvironment of the bovine mammary gland and their effect on mammary function. J. Biet*[1], K. Stelwagen[2], J. Margerison[3], CA Poole[4], A. Cullum[1], and K. Singh[1], *[1]AgResearch Ltd., Hamilton, New Zealand, [2]SciLactis Ltd., Hamilton, New Zealand, [3]Massey University, Palmerston North, New Zealand, [4]University of Otago, Dunedin, New Zealand.*

Mammary gland engorgement leads to changes in cell morphology and has been recognized as a potential key initiator for mammary remodeling processes culminating in a decline in milk production. These changes in the mechanical forces (mechanotransduction) may initiate a cascade of signaling networks involved in mammary involution. The goal of this study is to determine the effects of physical distension of bovine mammary epithelial cells on changes in cell sensing and signaling, and the initiation of the involution process. Multiparous, non-pregnant Friesian cows (n = 8) at peak lactation (78 ± 20 DIM) were milked with prior administration of oxytocin (20 IU per cow; IM) to ensure complete milk removal. Subsequently, one rear quarter was aseptically infused with a sterile iso-osmotic (300 mOsm) saline solution equivalent in volume to 5 h worth of individual milk secretion. Fifteen hours later, mammary gland biopsies were collected from both hind quarters, with the infused and the non-infused glands respectively providing treatment and control samples. Alveolar tissue was fixed, wax embedded, sectioned at 7µm. Histological analysis (haematoxylin and eosin staining) and grading of tissue sections for degree of milk stasis and signs of involution showed morphological differences between the control and treatment tissues. While the control tissues had the appearance of lactating mammary glands, the treatment tissues showed more signs of involution ($P <$ 0.002). Moreover, qRT-PCR results showed a significant decrease in α-lactalbumin expression (1.44 fold, $P < 0.05$) indicating a decline in milk production and volume due to changes in the mechanical microenvironment. Thus, increased udder filling may play a fundamental role in the change of mammary epithelial cell shape which in turn may initiate the involution process

Key Words: mechanotransduction, mammary gland, dairy cow

TH279 Determining the effect of chronic light:dark shifts on dairy cow milk production. J. Crodian*, T. Casey, and K. Plaut, *Purdue University, West Lafayette, IN.*

Photoperiod affects milk production and growth of dairy cattle. However, mechanisms underlying responses to photoperiod are not well understood. Virtually all aspects of physiology are controlled by the circadian system, which coordinates behaviors and physiological processes with the environment. Chronic shifts in light (L), dark (D) cycle disrupt circadian system and are associated with development of metabolic disease in rodents and humans. The objective of this study was to develop a model system to study circadian disruption in dairy cows by determining the effects of exposing mid-lactation dairy cows to chronically shifting LD cycles on feed intake, milk yield, milk composition and mammary gene expression. Six first lactation Holsteins, 90 ± 7.3 d in milk were maintained on a 16 h L: 8 h D cycle and milked at 0500 h and 1600 h for 7d (control). Immediately following control period, cows were exposed to continuous cycles of 8 h L: 8 h D, but were maintained on the same milking and feeding schedule for 7d. Cows were allowed 5d of acclimation; milk yield, composition and mammary gene expression were measured during the last 48 h of each period, and differences were analyzed with paired *t*-test. Exposure to chronic 8 h LD cycles significantly depressed milk yield ($P < 0.05$; 31.5 ± 1.5 versus 30.0 ± 1.3 kg) but did not affect daily feed intake. Percent milk fat, protein and lactose were not different, but milk urea nitrogen (MUN) significantly increased (12.5 ± 0.8 versus 14.8 ± 0.6 mg/dl). On the last day of each period, mammary gene expression was measured using Q-PCR of total RNA isolated from the cytosolic components of milk fat globules. Expression of core clock gene Bmal1 was 38%, beta-casein 68%, alpha-lactalbumin 48%, fatty acid synthase 11% and acetyl CoA-carboxylase 37% of control period level ($P < 0.05$). Disruption of circadian rhythms by alternating LD cycle decreased milk production, decreased expression of genes involved in milk synthesis and increased MUN content of milk. Further, decreased BMAL1 expression supports that alterations of mammary clock genes may be part of the mechanism responsible for photoperiod induced alterations in milk production.

Key Words: photoperiod, dairy cow, milk

TH280 Lipoprotein lipase (LPL), molecular cloning, tissue expression, and regulation of milk fat synthesis in goat mammary epithelial cells. W. S. Zhao, J. Luo*, and S. L. Hu, *Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

Lipoprotein lipase (LPL) served as a central factor in hydrolysis of triacylglycerol and uptake of free fatty acid. However, very little data are accessible about the action of LPL on the regulation of milk fat synthesis in goat mammary gland. In this investigation, we described the cloning and sequencing of LPL gene from Xinong Saanen dairy goat mammary gland, along with a study of its phylogenetic relationships. Sequence analysis showed that LPL in goat contributes many similarities with other mammals, including sheep, bovine, human and rat. The presence of mRNA levels for LPL in goat various tissues, using RT-qPCR, revealed the highest mRNA level in the white adipose tissue, less in heart, lung, spleen, rumen, small, intestine, mammary gland, and kidney, following almost undetectable in liver and muscle, respectively. The expression profiles of LPL gene in mammary gland at early, peak, mid, late lactation stages and dry period of Xinong Saanen dairy goat was also accessed. There was a dramatic increase at early lactation, following a rapid decrease in LPL mRNA appearing at peak lactation and mid lactation compared with early lactation and then a slight increase from mid lactation to late lactation, finally arrive at lowest levels at dry period. The expression patterns of LPL were very similar by using Orlistat (an inhibitor of LPL gene) and RNAi of LPL (69% and 31%). RNAi of LPL reduced mRNA abundance of SREBP, FASN, HSL and PPARG by 16%, 17%, 32% and 82%, respectively, and accelerated the expression of PRLR and GPR41 by 266% and and 3390%, without significant effect on ACC gene. Nonetheless, by using Orlistat, the expression of HSL (58%) was dramatically downregulated and the levels of GPR41 (578%) and SREBP (243%) were upregulated sharply. Meanwhile, the level of PPARG (125%) was upregulated, together with the downregulation of ACC (18%) and PRLR (57%).

Key Words: LPL gene, goat mammary epithelial cells, Orlistat

TH281 Use of quantitative real-time PCR for diagnosis of culture negative mastitis cases. K. E. Merriman, J. Laporta, T. L. Peters, M. J. Fuenzalida, P. L. Ruegg, and L. L. Hernandez*, *University of Wisconsin, Madison.*

Mastitis is the most common health problem and greatest source of economic loss in the dairy industry. The current gold standard method for identifying mastitis-causing pathogens is through the use of microbiological testing. To treat mastitis, culturing of milk should be done as efficiently as possible to determine the bacteria causing the infection. However, culturing is not the most reliable method to find the pathogens because 25–45% of milk samples yield no bacterial growth (NBG), even after 48 h of incubation. Quantitative real-time PCR (qPCR) is one option of detecting bacterial DNA in milk samples that do not contain sufficient number of live bacteria to The objective of this study is to assess use of qPCR to detect mastitis-causing pathogens in milk samples that were determined as NBG using standard microbiological testing. Quarter milk samples were aseptically collected from cows that were diagnosed with clinical mastitis at 2 dairy farms. Initial microbiological testing was performed according to NMC specifications. Only samples that were determined to be NBG were further subjected to qPCR testing (n = 51). DNA was subsequently extracted from all milk samples. The DNA from each sample was assessed by qPCR for 9 different bacteria using primer sequences for *E. coli* (EC), *Streptococcus* spp. (SC), *Strep agalactiae* (SAg), *Staphylococcus* spp. (ST), *Staphylococcus* aureus (SAu), *Enterococcus* spp. EG), *Enterococcus faecalis* (EF), *Streptococcus uberis* (SU) and *Klebsiella pneumoniae* (KP). When probed for ST, all milk samples demonstrated a 32-fold increase in gene expression. Only 2 of the milk samples tested for EC demonstrated a 5-fold increase in EC. When tested for EF and KP only one of the samples had increases in gene expression of 2- and 5-fold, respectively. When samples were tested for SU, SAg, EG, and SAu there were no changes in gene expression detected. Despite milk samples being identified as NBG using microbiological techniques, qPCR analysis was able to detect DNA of specific bacteria in milk.

Key Words: mastitis, no bacterial growth, qPCR

TH282 Circulating serotonin (5-HT) concentrations on day 1 of lactation as a potential predictor of transition-related disorders. J. Laporta, SAE Moore*, MW Peters, and LL Hernandez, *University of Wisconsin, Madison.*

Serotonin (5-HT) has been described as a homeostatic regulator of lactation. Recently, it has been determined that 5-HT is involved in regulating calcium and glucose homeostasis during the transition period in rats. It has been shown that 5-HT is important for initiating calcium mobilization from bone during the transition period. Additionally, 5-HT has been demonstrated to affect hepatic and mammary gland energy metabolism during the transition period. Therefore, we investigated the correlation of circulating 5-HT concentrations in relation to circulating calcium and parathyroid hormone related-protein (PTHrP) concentrations on d 1 of lactation, as well as the correlation of circulating 5-HT with the incidence and severity of ketosis in Holstein cows in relation to circulating 5-HT concentrations. Blood samples were collected from 50 multiparous cows on d 1 of lactation at the Emmons Blaine Dairy Cattle Research Center. Serum and plasma were harvested from samples and analyzed for 5-HT, ionized calcium, and PTHrP. Incidence and extent of ketotic events were recorded for all animals during the first 10 d postpartum and ketosis severity was defined as follows: 0 = no signs of ketosis, 1 = mild ketosis with no treatment, 2 = mild ketosis with treatment, 3 = moderate ketosis, and 4 = severe ketosis. Serum 5-HT and ionized calcium were positively correlated ($P = 0.0182$, r = 0.3672), as were serum 5-HT and plasma PTHrP ($P = 0.0419$; r = 0.3562). Additionally, serum 5-HT was negatively correlated with ketosis incidence and severity ($P = 0.0481$; r = 0.3137). These data suggest that 5-HT potentially plays a role in regulating calcium and glucose homeostasis during the transition period in cattle, which has been previously demonstrated in rodents.

Key Words: serotonin, lactation, transition period

TH283 Hormonal regulation of α-tocopherol transfer related molecules expression in bovine mammary epithelial cells. S. Haga*[1,2], Y. Kobayashi[1], M. Nakano[1], H. Ishizaki[1], SG Roh[2], and K. Katoh[2], *[1]NARO Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan, [2]Lab of Animal Physiology, Graduate School of Agriculture Science, Tohoku University, Sendai, Miyagi, Japan.*

α-Tocopherol (α-Toc) is the most biologically active form of vitamin E in animals. α-Toc is the major lipid-soluble vitamin and an important integrant in milk for newborn animals. However, little is known on the secretory mechanism of α-Toc in milk and the regulation of α-Toc transfer protein (αTTP), scavenger receptor class B type 1 (SR-B1) and ATP-binding cassette transporter A1 (ABCA1) in the mammary gland. In this study, therefore, we investigated the mRNA expression levels of αTTP, SR-B1 and ABCA1 in mammary glands among different lactation stages, and the effects of lactogenic hormones (dexamethasone, insulin and prolactin), E2 and GH on these genes expression in cultured bovine mammary epithelial cells (bMEC). The mammary tissues were sampled from 9 dairy cows (peak lactation (n = 3, 2 mo after parturition), late lactation (n = 3, 8 mo after parturition) and the dry-off stage (n = 3, 3 years after parturition). The cloned bMEC were grown until confluence in a collagen type IV coated transwell and treated with lactogenic hormones, E2 and GH at various concentrations for 24 h. Total RNA was extracted from the mammary tissues and the cultured bMEC, and then αTTP, SR-B1 and ABCA1 mRNA were analyzed by Q-RT-PCR. Values were considered to be statistically significant if their P-value was < 0.05 (SAS). In mammary tissues, the expression levels of αTTP and SR-B1 mRNA were significantly higher at lactation stages than at dry off stage. In bMEC, lactogenic hormones increased the expression levels of αTTP, SR-B1 and ABCA1 mRNA. Dexamethasone or E2 treatment significantly upregulated the expression levels of SRB1 mRNA in a dose-dependent manner. The expression level of ABCA1 mRNA was increased by E2, but decreased by GH in dose-dependent manner. These results indicate that hormonal changes in different lactogenic stage differentially regulate the expression of αTTP, SR-B1 and ABCA1 genes related with α-Toc transfer for vitamin E secretion in milk.

Key Words: α-tocopherol, bovine mammary epithelial cell, milk secretion

TH284 Cellular composition and expression of potential stem cell markers in mammary tissue of cows consuming endophyte-infected fescue seed during the dry period and early lactation. R. K. Choudhary*[1], R. L. Baldwin VI[2], C. M. Evock-Clover[2], P. Grossi[3], T. H. Elsasser[2], G. Bertoni[3], E. Trevisi[3], K. R. McLeod[1], and A. V. Capuco[2], *[1]Department of Animal Sciences, University of Kentucky, Lexington, [2]Bovine Functional Genomics Lab, USDA-ARS, Beltsville, MD, [3]Istituto di Zootecnica, Universitá Cattolica del Sacro Cuore, Piacenza, Italy.*

We evaluated the effect of consuming endophyte-infected fescue during late pregnancy on parameters of mammary development in Holstein cows. Cows (n = 16) were fed 10% of their ration as tall fescue seed that was free from (CON) or infected with endophyte (INF) from 90 d before expected calving until 10 d of lactation. Mammary tissue was biopsied during dry period (−32 d) and early lactation (+10 d). The percentage of tissue area that was occupied by epithelium, stroma and lumina was

quantified. Epithelial proliferation was assessed by nuclear expression of the Ki67 antigen, detected by immunohistochemistry. Staining for putative mammary stem cell markers, nuclear receptor subfamily 5 group A member 2 (NR5A2), fibronectin type III domain containing 3B (FNDC3B) and musashi1 (MSI1), was evaluated and expressed as a percentage (% DAB pixels out of DAB plus hematoxylin pixels). Epithelial content of mammary tissue did not differ between CON and INF cows, nor did stromal and luminal areas differ between treatments in dry cows ($P > 0.05$). However, in lactating cows, tissue areas reflected greater milk yield in CON than INF cows (luminal area in CON > INF; stromal area in INF > CON; $P < 0.05$). Proliferation indices did not differ between mammary epithelia of CON and INF cows ($P > 0.05$). Similarly, nuclear staining of NR5A2, FNDC3B and MSI1 did not differ in INF vs. CON. However, there were differences ($P < 0.05$) in staining of all 3 markers between dry period and lactation (−32 d vs. +10 d). FNDC3B staining was greater during early lactation than the dry period ($P < 0.001$) and cytoplasmic staining of myoepithelial cells was observed during lactation. During early lactation, FNDC3B (r = 0.86; $P = 0.13$) staining tended to correlate with milk yield. Data indicate that fescue toxicity did not alter cellular composition of mammary tissue, epithelial proliferation rate, or expression of mammary stem cell markers. Immediate effects of fescue toxicosis on milk yield are likely mediated by influences on mammary differentiation and secretory activity.

Key Words: fescue toxicosis, mammary stem cell, lactation

TH285 Influence of intramammary lipopolysaccharide challenge on milk and plasma adiponectin in dairy cows. S. P. Singh*[1], S. Häussler[1], O. Wellnitz[2], R. M. Bruckmaier[2], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, Bonn, Germany, [2]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland.*

Intramammary LPS infusion induces local and systemic inflammatory responses. Circulating concentrations of the adipokine adiponectin (Aq) decrease during inflammation and Aq was therefore suggested as a negative acute phase protein. Our objective was thus to investigate the changes in milk and blood Aq concentrations after inducing an experimental mastitis by intramammary LPS infusion in dairy cows. Eight lactating, non-pregnant Holstein cows (1st - 6th parity) in wk 18 to 37 of lactation were used. One front and one rear quarter was intramammarily injected with 200 µg LPS from *E. coli* (O26:B6) in 10 mL NaCl (0.9%). Cisternal milk samples were collected before and 8 h after LPS challenge. Skim milk was prepared by centrifugation (3,000 × *g*, 20 min, 4°C). Blood plasma was prepared (3,000 × g, 20 min) from blood samples taken hourly. Skim milk and plasma Aq concentrations were measured by ELISA. The intra- and interassay variation were 7.0% and 11.0%; 4.5% and 11.9% for plasma and skim milk, respectively. The limit of detection was 0.03 ng/mL. Assay accuracy for skim milk was indirectly determined by linearity of serial samples dilutions. Differences in plasma Aq between time points were tested for significance ($P < 0.05$) by repeated measure ANOVA with Bonferroni correction. Wilcoxon signed rank test was used for analysis of skim milk Aq concentrations. The results are presented as means ± SEM. The plasma Aq concentrations (µg/mL) were not different across all times of LPS infusion (average concentrations: 29.8 ± 0.6), whereas milk Aq was significantly increased after 8 h of LPS challenge (0.5 ± 0.7 vs. 1.1 ± 0.5; $P = 0.028$). In view of the fairly constant Aq concentrations in plasma, the Aq secretion from adipose tissue was apparently not affected by intramammary LPS treatment. The increase in milk Aq after LPS challenge probably resulted from transfer of blood Aq through the disturbed blood milk barrier.

Key Words: lipopolysaccharide, milk, adiponectin

TH286 Transcriptomics differences between liver and mammary tissue in mid-lactation dairy cows. D. P. Bu[1], M. Bionaz[2], X. M. Nan[1], and J. Q. Wang*[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Animal and Rangeland Sciences, Oregon State University, Corvallis.*

Liver and mammary gland are among the most prominent organs during lactation in dairy cows. With the purpose to understand both the different and the complementary roles of those 2 organs during lactation, a transcriptome analysis was performed on liver and mammary tissues of 10 dairy cows in mid-lactation (136 ± 3.7 d). The analysis was performed using a 4 × 44K Bovine Agilent microarray chip. The data were normalized by Lowess and uploaded into ArrayStar. A paired *t*-test with false discovery rate correction (FDR) uncovered 7,813 genes differentially expressed (DEG) between the 2 tissues with an FDR <0.001. The functional analysis was performed using the Database for Annotation, Visualization and Integrated Discovery and the Dynamic Impact Approach (DIA). The most enriched biological terms in DEG more expressed in mammary vs. liver were related to translation, extracellular matrix, cell adhesion, cytoskeleton, and vesicle transport. The mitochondria and related respiration, immune reaction (i.e., complement and coagulation cascades, acute inflammatory response) and metabolism of drugs, fatty acid, and steroid were the most enriched terms in DEG more expressed in liver vs. mammary tissue. The DIA analysis revealed a larger induction of almost all metabolic pathways in liver compared to mammary, with exception of fatty acid biosynthesis. The liver had also overall more induced pathways related to immune, endocrine, and digestive system, peroxisome, and PPAR signaling. The DIA uncovered an overall larger protein synthesis capacity and related signaling pathways (e.g., mTOR), cell communication (including cell adhesions), cell cycle, cellular innate immune system (e.g., toll-like receptor), protein export, and secretion in mammary vs. liver. In summary the transcriptome analysis revealed that the liver is more metabolically active compared to the mammary gland with a larger production of energy through mitochondria and peroxisomes while the mammary gland synthesizes more protein with greater export, cell-to-cell interactions, and proliferative capacity compared with the liver.

Key Words: transcriptomics, mammary gland, liver

TH287 Differential proteome analysis of lactating and nonlactating bovine mammary gland. H. Y. Liu*[1], J. X. Yang[1], X. D. Zhang[2], and J. X. Liu[1], *[1]Institute of Dairy Science, Zhejiang University, Hangzhou, China, [2]Department of Animal Science and Technology, Zhejiang Agriculture and Forestry University, Hangzhou-Lin'an, China.*

The initiation and maintenance of lactation are complex phenomena involving general biochemical and endocrinological processes in the mammary glands. It has been extensively studied over the years at the genetic, physiological and morphological levels. However, only limited data are available concerning proteomic changes of lactating and nonlactating bovine mammary gland. To determine the differentially expressed protein profiles between periods of lactation and nonlactation, the comparative proteomes were analyzed using 2-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF/TOF protein identification. Bovine mammary gland tissues were obtained from 3 healthy multiparous dairy cows in each period, respectively. The analyses of multiple 2-DE gels indicated that a total of 80 protein spots

(corresponding to 59 altered proteins) differentially expressed in both periods, including 56 upregulated proteins and 3 downregulated proteins. Database search and identification of functional protein analysis showed that upregulated proteins were mainly related to transportation, macromolecular biosynthesis, metabolism, protein-folding, apoptotic, secretion and pentose-phosphate shunt. Downregulated proteins were involved in cytoskeleton, transport and lipid degradation. These proteins, through various pathways and actions, may regulate either lactogenesis, galactopoiesis, or involution within the mammary gland. Moreover, 30 corresponding genes of the differentially expressed proteins were quantified by real-time RT-PCR to examine the transcriptional profiles and validate the proteins identified by MS/MS between lactating and nonlactating bovine mammary gland. Thus, this study provides useful dynamic protein-related information to facilitate further investigation of the underlying lactation mechanism of dairy cows.

Key Words: lactation, bovine mammary gland, differential proteomics

TH288 AKT/mTOR and JAK2/STAT5 pathway act synchronously on the synthesis of β-casein in bovine mammary epithelial cells. L. L. Shi, F. Zhao, X. J. Gao, Q. Z. LI*, and N. Zhang, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China.*

Both AKT and STAT5 are important to cell apoptosis, survival, proliferation and differentiation, and the latter contributes to milk protein gene transcription in bovine mammary epithelial cells (BMECs), but a role for AKT in mammary epithelial cell differentiation has not been established. A lactogenic phenotype is generally induced in BMECs by the differentiation medium including dexamethasone, insulin and prolactin, referred to as DIP and has typically been assessed by milk protein production. The previous studies have shown that these hormones are necessary in vitro for differentiation and milk protein synthesis of BMECs by activating JAK2/STAT5 and PI3K/AKT1/mTOR pathway. To address the interaction between the 2 key pathways, we inhibited AKT1 or STAT5 expression by RNA interference with DIP+IGF-1, DIP+GH, or only DIP, and detected their effects on the synthesis of β-casein in BMECs. In the presence of the differentiation medium, AKT1 inhibition by RNAi compared with control cells, which led to lower mRNA and protein level of AKT1 and mTOR, but no change in their phosphorylation. Interestingly, reduced STAT5 and β-casein expression were also observed. While the similar downregulation of STAT5 expression occurred when STAT5 was inhibited, the above 3 expression levels of AKT1 and mTOR were decreased synchronously. AKT1, mTOR and STAT5 expression were greater for DIP +IGF-1 or DIP+GH than for just DIP, followed by increasing β-casein mRNA. Overall, PI3K/AKT1/mTOR and JAK2/STAT5 pathway both act on the synthesis of β-casein in BMECs. Although they had been negatively regulated separately, the abundance of β-casein mRNA decreased resulting from the 2 inhibited pathways synergically. IGF-1 or GH can enhance β-casein mRNA expression by upregulated JAK2/STAT5 and AKT/mTOR activity, whereas IGF-1 can't replace GH due to its little effect on AKT1 and mTOR phosphorylation.

Key Words: AKT1, STAT5, β-casein

TH289 The effects of laminin on the proliferation of dairy cow mammary epithelial cells are mediated by α6 and β4 integrin. F. Zhao, C. Liu, X. J. Gao, and Q. Z. LI*, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China.*

Laminin is a key extracellular matrix (basement membrane) protein and exerts function by its cell membrane surface receptor integrins to affect cell behavior including cell adhesion, apoptosis, survival and cell differentiation. In bovine mammary gland epithelial cells (BMECs), 2 important integrin heterodimers α6β1 and α6β4 are close related to laminin function. In this study, 14 primiparous Holstein dairy cows were chosen from 7 development stages during lactation cycle. Mammary gland biopsies were taken in virgin (postnatal 12 mo), pregnant (6 mo into gestation), lactating (7, 50, 140, 280 d postpartum), and dry period (30 d after weaning). By immunohistochemistry we found that α6 and β4 subunits collocated in basal side cells toward basement membrane in mammary tissue slices. Both mRNA and protein level of 2 subunits were higher during pregnancy and lactation than during virgin and dry period. The proliferation of mammary epithelial cells which originated from a lactating cow was enhanced with the presence of laminin substrate in comparison with plastic culture surface. In vitro, PRL, GH, IGF-1, IGF-2 or 10% serum affect cell growth differently, and IGF-1 and GH were more effective mitogen. We confirmed that BMECs proliferation with laminin substrate induced by GH, IGF-1 or 10% serum could be dramatically inhibited to the same degree to that without laminin by blocking integrin α6 function, whereas it only had a weak inhibitory effect by blocking integrin β4 function. It could result from the different number of heterodimers, and it has been well known that α6 subunit is involved in 2 heterodimers α6β1 and α6β4, but β4 subunit had been detected only to form the later at present. In conclusion, once cell-matrix and cell-cell junctions have established, α6 integrins will play an important role in mammary epithelial cell proliferation by α6β1 and α6β4 heterodimers mediated signaling from laminin or other mitogen such as IGF-1 and GH. These results allow us understand better the regulation of mammary epithelial cells proliferation associated with integrins.

Key Words: integrin α6, integrin β4, bovine mammary epithelial cell