Lactation Biology I

W289 Changes in parathyroid hormone-related protein concentrations in bovine milk from the early stage of lactation. K. Onda*, R. Sato, K. Kazama, H. Ochiai, and Y. Wada, *Azabu University School of Veterinary Medicine, Sagamihara, Japan.*

The concentration of parathyroid hormone-related protein (PTHrP) in the blood of healthy animals is extremely low. However, milk contains a relatively large amount of PTHrP, and the changes in its levels in the early stages of lactation and the biological implication of these changes in cows remain unclear. To understand the characteristics of parturient changes in milk PTHrP content, we first measured the changes in milk PTHrP concentrations as a function of time after parturition in 8 primiparous and 8 multiparous Holstein cows at 7 intervals until 21 d postpartum. Second, based on these results, we collected milk samples from 47 primiparous and 66 multiparous Holstein cows at 3 d postpartum and investigated the relationship between the milk PTHrP concentration and the age of the cow or milk yield. Consequently, the concentration of PTHrP in milk of both age groups of cows was lowest on the day of parturition (3.1 ± 1.5 n*M* in primiparous and 1.6 ± 0.8 n*M* in multiparous cows) and significantly increased on d 7 of lactation and stayed on the same level until d 21 of lactation (5.9 ± 2 n*M* in primiparous and 6 ± 1.3 n*M* in multiparous cows on d 21). Comparing the 2 groups, milk PTHrP concentrations in primiparous cows were higher than those of multiparous cows in the first 3 d of lactation ($P < 0.05$). The milk PTHrP concentration at 3 d postpartum exhibited a negative correlation with increasing age of cows ($r = -0.57$, $P < 0.0001$), and a positive correlation with milk yield was observed only in the multiparous cows ($r = 0.29$, $P < 0.02$). This experiment identified the unique characteristics of milk PTHrP in the early stage of lactation; milk PTHrP concentrations are higher in primiparous cows than in multiparous cows and lower in colostrum than in later milk. The difference in milk PTHrP concentration observed between primiparous and multiparous cows at 3 d postpartum was more strongly influenced by age than by milk yield.

Key Words: age, parity, PTHrP

W290 SND1 regulates milk protein synthesis of dairy cow mammary epithelial cells in vitro. W. W. Bi, C. C. Luo, Y. Lin, X. J. Gao*, and Q. Z. Li, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China.*

Tudor-SN (SND1, p100) has been shown to function as a transcriptional coactivator, which is highly conserved through evolution from yeast to mammals. The conservation of SND1 along evolution suggests it may have important functions. However, the physiological function of SND1 in mammary epithelial cells of dairy cow has not been characterized. In this study we constructed transfected dairy cow mammary epithelial cells (DCMECs) with SND1 to analyze the expression and localization of SND1 and the function in transfected DCMECs by CASY, fluorescent immunostaining, Western blot and RT-qPCR. Analysis indicated that SND1 was widely expressed in nucleus of DCMECs. Overexpression and siRNA inhibition of SND1 experiments showed that SND1 promoted the cells proliferation and lactation relative genes expression, regulated milk protein synthesis through Stat5 and mTOR pathway. These findings indicated that SND1 involves in regulation of milk synthesis, and sheds new insights for understanding the mechanisms of milk protein synthesis.

Key Words: SND1, dairy cow mammary epithelial cell, milk protein synthesis

W291 Heat-induced stress and response of bovine mammary epithelial cells. H. Hu, D. P. Bu, J. Q. Wang*, L. Y. zhou, and X. M. Nan, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Study objectives were to determine the cell-stress response to heat stress (HS) in Chinese bovine mammary epithelial cells (CMECs). CMECs were exposed to either 38 or 42°C for 0.5, 1, 3, 5, 8, and 12 h, and cell apoptosis rate and expression of apoptosis regulation genes were quantified by flow cytometry and qRT-PCR, separately. Calculation data of mean, SD, and *P*-values were performed on triplicate experiments using SARS (9.0). The Student's *t*-test was used to calculate *P*-values for comparison, and the significance was set at a *P*-value <0.05. Thermal temperature significantly induced cell apoptosis and the peak of apoptosis appeared at 3 h with the apoptosis rate up to 14% ($P < 0.05$). The expression level of heat shock proteins (Hsps), Bcl-2, caspases and tumor necrosis factor receptor (TNFR) family genes were detected to discuss the cell response to heat stress. The genes, which were in development of apoptosis, death receptor gene (TNF-R1), cysteine proteases genes (caspases-3, -7 and -8) and Apaf-1 were markedly upregulated by HS ($P < 0.05$). However, Fas, caspase-6 and -9 were downregulated ($P < 0.05$) with different exhibition. For Bcl-2 protein family, which plays a critical role in pro- or anti-apoptotic processes, the transcription levels of anti-apoptotic protein genes (Bcl-2, Bcl2Al and Mcl-l) were significantly increased ($P < 0.05$) and pro-apoptotic protein genes (Bax, Bak and Bid) were significantly decreased ($P < 0.05$) during thermal temperature treat. The gene ratio of Bcl/Bax shown positive suggested its blocking activity to apoptosis and determined the survival of cells following an apoptotic stimulus. Simultaneously, Hsps genes (hsp27, 70 and 90) were generally upregulated at 42°C and this was especially apparent for hsp70 transcription as it was increased 14-fold at 1 h ($P < 0.05$). The sudden increasing of Hsps promoted cells effective recovery and survival under HS conditions. The changes of cell apoptosis rate and gene transcription induce by HS began to recover to normal after 8 h. These results suggested that HS induced the apoptosis of CMECs, and aroused its intracellular thermotolerance machinery.

Key Words: bovine mammary epithelial cell, heat stress, apoptosis

W292 Study on differential intake free amino acids of mammary gland in dairy cows. X. Y. Wang, N. Zhang, Q. Z. Li*, and X. J. Gao, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China.*

Free amino acids in the blood constitute the major precursors of milk proteins, and the availability of these amino acids to the mammary system is critical to optimizing milk production. The study was to investigate the difference of amino acid intake between dairy cows producing high-quality milk and cows producing low-quality milk. Chinese Holstein dairy cows (n = 18) were allotted in 2 groups according to results of milk composition analyzer: one is high milk quality group (n = 9, high protein and fat ratio) and the other is low milk quality group (n = 9, Low protein and fat). We collected the blood from the external pudic artery and abdominal mammary vein of the 2 groups of cows, and used the amino acids autoanalyzer to determine 17 free amino acids concentration. The results showed that the concentration of 5 essential amino acids (Met, Leu, Ile, Thr, Val) of the high milk quality group were significantly higher than the low milk quality group ($P < 0.05$), and the concentration of 2 nonessential amino acids (Glu, Tyr) were

significantly higher than the low milk quality group ($P < 0.05$). The uptake rate of 6 essential amino acids (Thr, Val, Met, Ile, His, Arg) in the high milk quality group were significant higher than the low milk quality group ($P < 0.05$), and nonessential amino acids detected were also significantly higher than the low milk quality group ($P < 0.05$). In conclusion, differential utilization of Met, Leu, Ile in the High and Low milk quality groups maybe the main reason to cause the considerable difference of milk protein rate.

Key Words: dairy cow, milk quality, free amino acids

W293 Prolactin-inhibitor cabergoline hastened the mammary involution during drying-off in dairy cows. M. Boutinaud*[1,2], N. Isaka[4], A. Deflandre[4], E. Gandemer[1,2], P.-G. Marnet[1,2], F. Dessauge[1,2], and V. Lollivier[1,2], *[1]INRA UMR 1348 PEGASE, Saint Gilles, France, [2]Agrocampus UMR 1348 PEGASE, Rennes, France, [3]Université Européenne de Bretagne, Rennes, France, [4]CEVA Santé Animale, Libourne, France.*

In ruminants, the early phase of drying-off is a period of mammary gland involution that is marked by the cessation of prolactin (PRL) release. The analysis of the changes of mammary secretion composition can provide valuable information about the speed of the mammary involution. To assess the effect of PRL inhibition by cabergoline on mammary gland involution, 14 Holstein dairy cows were injected with a single i.m. administration of 5.6 mg cabergoline (n = 7) or placebo (n = 7) at the first day of drying-off (D0). Mammary biopsy samples were collected one week before drying-off (D-6), at D1 and at D8 and used for RNA extraction and RT-PCR analyses. Mammary secretion samples were collected using a teat-cannula once during lactation (D-6) and at D1, D2, D3, D4, D8 and D14 after the drying-off. The mammary secretion samples were used for milk fat, lactose, true protein, a-lactalbumin, lactoferrin and citrate analysis. The decrease in lactose content of mammary secretions seemed to be faster in cabergoline treated cows compared to controls, demonstrated by lower levels of lactose in cabergoline treated cows already by D1 than in control cows ($P < 0.05$). Cabergoline treatment tended to increase fat content at D3 after drying-off ($P < 0.10$), whereas an increase in fat content was only observed at D4 in the control group. The rise of lactoferrin was significant starting at D4 in the cabergoline treated cows whereas it only happened at D8 in controls, and overall there was a tendency towards greater lactoferrin content in cabergoline treated cows ($P = 0.10$). Cabergoline did not seem to alter citrate content. However, the decrease in lactoferrin/citrate ratio happened faster in cabergoline treated cows compared to controls on D1 ($P = 0.01$). No significant effects of cabergoline treatment were observed both in true protein and in a-lactalbumin contents in mammary secretions or in a-lactalbumin and k-casein mRNA levels in mammary tissues. These changes in lactose, lactoferrin, lactoferrin/citrate ratio and fat, indicate that cabergoline treatment was efficient to hasten the mammary gland involution without affecting milk synthesis in the mammary tissue

Key Words: cow, drying-off, prolactin

W294 Effects of omitting the dry period on plasma progesterone and prolactin during lactogenesis and on colostrum IgG content in dairy cows. R. S. Zbinden[1], H. A. van Dorland[1], G. Remmelink[3], B. Kemp[2], A. T. M. van Knegsel[2], and R. M. Bruckmaier*[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Adaptation Physiology Group, Wageningen University, Wageningen, the Netherlands, [3]Livestock Research, Wageningen University and Research Centre, Lelystad, the Netherlands.*

Omitting the dry period represents a strategy that may reduce metabolic stress in early lactating cows. A drawback of continuously milking is discussed to be a compromised colostrum quality, although insufficient studies have been done to confirm this. The objective of this study was to evaluate the effects of omitting the dry period on key hormone patterns during lactogenesis and the IgG content of colostrum in periparturient dairy cows. Twenty Holstein-Friesian dairy cows were randomly assigned to 2 experimental groups (0 or 60 d dry period; DRY0 or DRY60, respectively). Milk yields were recorded daily and plasma concentrations of progesterone and prolactin were determined from d 5 pre- until d 3 postpartum. Milk samples were collected for analysis of IgG concentration from d 7 pre- until d 3 postpartum for DRY0, and from d 0 until d 3 postpartum for DRY60 cows. Data were analyzed with a mixed model procedure including dry period length, day and their interaction as fixed effects. Plasma prolactin did not differ between DRY0 and DRY60 and started to increase significantly from one day before calving in both groups ($P < 0.05$). Progesterone dropped prepartum and followed a similar pattern in both groups, but was significantly lower on one day before calving in DRY60 compared with DRY0. This may point to a stronger decrease in progesterone concentration for DRY0 and could imply a faster calving process after the progesterone drop. IgG concentration started to increase from d 6 prepartum in DRY0 cows. From calving up to d 3 postpartum, IgG level decreased, but no difference was observed between the groups. However, calculated IgG mass was significantly higher for DRY60 compared with DRY0 ($P < 0.05$) across the study time, due to the higher milk yield in DRY60 (21.8 versus 13.5 kg/d on d 0; $P < 0.05$). In conclusion, the endocrine profiles supporting lactogenesis remained unaffected, and the colostrum quality was not compromised by omitting the dry period in dairy cows. It can be speculated that milking related oxytocin releases during the peripartal period induced an increased labor activity and faster birth.

Key Words: colostrum, immunoglobulin, dry period

W295 Characterization of mammary circadian rhythms of wild-type C57BL/6J mice and the role of thyroid hormone responsive spot 14 (S14) in circadian regulation of milk fat synthesis. L. Ma*, Y. Ying, A. Clarke, P. Bartell, and K. J. Harvatine, *Penn State University, University Park.*

Peripheral circadian rhythms are well described in liver and adipose tissue and S14 expression follows a circadian rhythm. The objectives of the current study were to characterize the mammary circadian rhythm of wild-type (WT) mice and investigate the role of S14 in the rhythm of milk fat synthesis. Wild-type and S14 null mice were euthanized on d 14 of lactation (0600, 1200, 1800, or 2400 h; n = 6 per time point per genotype) and dam mammary tissue and pup stomach milk clots were collected. Dam intake and body weight and litter gain were recorded twice per day. Data were analyzed by ANOVA with genotype, time, and the interaction of genotype and time as fixed effects and second fit to a cosine function with a 24 h period for rhythm analysis. Wild-type mice consumed more feed during the dark than the light phase (66% vs. 34%; $P < 0.01$). Total intake and eating patterns were maintained in S14 null. Litters gained 22% more during daytime compared with nighttime in both WT and S14 null ($P < 0.05$). Pup milk clot fat concentration followed a circadian rhythm and the amplitude and phase were decreased and delayed by 18.7% and 15.6 h in S14 null mice ($P < 0.05$). In S14 null mice, the amplitude and phase of 16-carbon FA were decreased by 66% and advanced by 2.9 h, compared with WT ($P < 0.05$). In the mammary gland, expression of the core clock genes (CLOCK, BMAL1, CRY1&2, and PER1&2), lipogenic regulators (SREBP1c and S14), and lipogenic enzymes (FASN and SCD1) followed a circadian rhythm in

WT. A 15 to 23% decrease in the amplitude of BMAL1 and PER1&2 and a 58% increase in the amplitude of CRY1 rhythm were observed in S14 null mice, although the phases were similar ($P < 0.05$). Moreover, the amplitudes of SREBP1c and SCD1 were diminished by 52 and 19%, respectively ($P < 0.05$). In conclusion, there is a circadian rhythm in the mammary gland of wild-type C57BL/6J mice. Spot14 is important for maintaining the circadian rhythm of milk fat synthesis by affecting mammary core clock genes and expression of lipogenic enzymes.

Key Words: spot 14, circadian rhythm, milk fat synthesis

W296 Suitability of refractometer and densimeter for on-farm determination of colostrum quality in dairy cows and heifers. J. J. Gross*, E. C. Kessler, and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland.*

Instruments for on-farm colostrum quality determination are increasingly used in dairy practice. The objective of this study was to elucidate the relationships between colostrum quality as assessed by 2 common on-farm instruments and composition in colostrum in cows and heifers. Twelve multiparous cows and 14 heifers were milked for the first time exactly 4 h post-calving. Colostrum was analyzed for total IgG by ELISA and for fat, protein and lactose by Foss Milkoscan (previously validated for use with colostrum). A Brix sugar refractometer (BRIX) and a Kruuse colostrum densimeter (DENS) were used to assess colostrum quality at 20°C. In cows, BRIX was closely correlated with DENS (r = 0.72, $P < 0.05$), protein (r = 0.88, $P < 0.01$) and lactose concentration (r = −0.85, $P < 0.01$). Also DENS showed a clear relationship with milk protein (r = 0.74, $P < 0.01$) and lactose (r = −0.73, $P < 0.05$) in cows. Both BRIX and DENS did not correlate with IgG concentration in cows. In colostrum of heifers, BRIX was less correlated with DENS (r = 0.43) and lactose (r = −0.50) than in cows, but still high with protein concentration (r = 0.75, $P < 0.01$). Also DENS was less correlated with protein (r = 0.44) in heifers than in cows. For heifers, BRIX and DENS did not correlate with IgG concentration. In conclusion, the refractometer and the densimeter closest correlated with protein content in colostrum but not necessarily with total IgG concentration. The easier handling of the refractometer and the higher correlations of its results with colostrum constituents make it a more reliable instrument than DENS for on-farm assessment of colostrum quality.

Key Words: colostrum, densimeter, refractometer

W297 PPARgamma agonists and antagonists fail to overcome *trans*-10, *cis*-12 conjugated linoleic acid (CLA) inhibition of lipogenesis and lipogenic gene expression in bovine mammary epithelial cell culture. D. E. Oliveira*[3,1], K. J. Harvatine[1], Y. R. Boisclair[2], and D. E. Bauman[2], *[1]Penn State University, University Park, [2]Cornell University, Ithaca, NY, [3]Santa Catarina State University, Lages, Santa Catarina, Brazil.*

Trans-10, *cis*-12 CLA decreases fat synthesis in mammary tissue and cell culture. We have previously observed that expressions of none of the 3 peroxisome proliferator-activated receptor (PPAR) isoforms were changed in mammary tissue during diet- and CLA-induced milk fat depression. However, PPARs are regulated at the level of ligand activation and specifically by some fatty acids (FA). The effect of PPARgamma (PPARg) on lipid synthesis and its role in the response to *trans*-10, *cis*-12 CLA was investigated using PPARg specific chemical agonist (Troglitazone, TG) and antagonist (T0070907). Bovine mammary epithelial cells (Mac-T) were treated with *trans*-10, *cis*-12 CLA (75 m*M*) complexed to BSA in serum free or growth media in the presence or absence of 9-*cis* retinoic acid (9cRA; retinoid X receptor agonist). Data was analyzed by ANOVA and protected LSD. Lipogenesis, measured as ^{14}C acetate incorporation into FA, was decreased 69.5 and 70.9% by CLA in serum free and growth media, respectively ($P < 0.05$). CLA also decreased expression of fatty acid synthase (FASN), sterol response element binding protein 1 (SREBP1), and thyroid hormone responsive spot 14 (S14), but not PPARg. PPARg agonist and antagonist did not change rates of lipogenesis. Additionally, neither PPAR agonist nor antagonist was able to inhibit CLA effects on lipogenesis (80 and 70% lower, respectively) or inhibit the reduction in expression of FASN, SREBP1, and S14. No interaction was observed between PPARg agonist or antagonist and 9cRA or growth media. Overall, there is no evidence for a role of PPARg in CLA inhibition of lipogenesis or downregulation of FASN, SREBP1 and S14.

Key Words: PPARg, conjugated linoleic acid, milk fat depression

W298 The inflammatory response of primary bovine mammary epithelial cells to *Staphylococcus aureus* strains reflects the molecular background of the bacteria. C. Zbinden[1,3], R. Stephan[2], S. Johler[2], RM Bruckmaier*[1], and O. Wellnitz[1], *[1]Veterinary Physiology Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [3]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.*

Using a latex agglutination-based diagnostic tool (Staphaurex test) we grouped bovine *S. aureus* strains into: Staphaurex-negative SLAT(−) and -positive SLAT(+) isolates. SLAT(−) strains belong to clonal complex (CC) 151, SLAT(+) strains can be assigned to various CC's. Genetic changes in spa, clfA and fnbpA genes and loss of the fnbpB gene (important virulence factors) were detected in SLAT(−) isolates. Recently we showed that microarray profiles of SLAT(−) isolates were highly similar but differed largely from those of SLAT(+) isolates. Based on these molecular data we postulate that SLAT(+) strains are more virulent than SLAT(−) strains. This study aimed to investigate if the immune response of the mammary gland to SLAT(−) and SLAT(+) strains differs, which could play a role in mastitis development. Primary bovine mammary epithelial cells in 4th passage were stimulated in sixtuplicates with 10 and 25 multiplicity of infection of inactivated suspensions of 3 SLAT(+) and 1 SLAT(−) strain isolated from bovine mastitis of different clinical severities. After 1, 6, and 24 h cells were harvested. At several time points the relative mRNA abundance (ΔΔCT) of selected immune factors was higher ($P < 0.05$) after stimulation with SLAT(+) compared to SLAT(−). Differences were highest after 6 h of incubation (1.3 to 2.5 threshold cycles [CT] for tumor necrosis factor alpha, 0.9 to 3.0 CT for interleukin-8, 1.5 to 3.6 CT for RANTES [Regulated And Normal T cell Expressed and Secreted] and 0.8 to 5.5 CT for serum amyloid A, respectively). The overall immune response was more pronounced with higher MOI. The mRNA expression of tight junction proteins zonula occludens-1 and occludin was not affected in both groups. These data are supported by an adhesion assay where the adherence of SLAT(−) to epithelial cells (Hep2) was lower than of SLAT(+) strains. The results indicate that *S. aureus* strains with varying virulence and different latex agglutination cause differences in the immune response of bMEC in vitro, which may reflect the severity of mastitis.

Key Words: *Staphylococcus aureus*, cow, mastitis

W299 Identification and characterization of microRNAs in a dairy cattle mammary gland epithelial cell line. X. M. Nan[1], D. P. Bu*[1], J. Q. Wang[1], J. J. Loor[2], and H. Hu[1], *[1]State Key Laboratory*

of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana.

Our objective was to identify and characterize the microRNAs (miRNAs) expressed in bovine mammary gland epithelial cells (MEC) isolated by our laboratory. Cells were cultured and passaged in DMEM/F12 basic medium with 10% fetal bovine serum. For experimental assays, cells at 80% confluence were cultured in lactation medium (containing insulin, epidermal growth factor, transferring, hydrocortisone and progesterone) for 24 h. MicroRNAs of MEC were isolated using TRIzol reagent and PAGE. Subsequently, small RNAs were amplified and sequenced by PCR and Solexa sequencing technology. Novel miRNAs were identified by stem-loop RT-PCR and sequencing of PCR products. To confirm the tissue specificity, expression of novel miRNAs was measured in mammary gland, liver, adipose, ileum, spleen and kidney tissues from 3 lactating Holstein cows (L350 ± 40 d) by stem-loop RT-PCR and sequencing of PCR products. After bioinformatics analysis, a total of 12,323,451 reads were obtained by solexa sequencing and 11,979,706 were clean reads. Among clean reads, 9,428,122 reads belonged to miRNAs. Further analysis revealed that bta-mir-184 had the most abundant expression and 388 loci possessed the typical stem-loop structures matching the known miRNA hairpins, whereas 38 loci with novel hairpins were identified as novel miRNAs. One of novel miRNAs named as bta-U21 was tissue specific in lactating mammary gland. Seven novel miRNAs, including bta-U21, had tissue-restricted distribution. In conclusion, the study identified 388 known miRNAs and 38 novel miRNAs expressed in dairy cattle MEC and some of them had tissue specificity and/or tissue-restricted distribution.

Key Words: microRNA, mammary gland epithelial cell line

W300 Evaluation of udder development shortly before parturition. V. Bjerre-Harpøth*[1], E. C. Kessler[2], J. J. Gross[2], and R. M. Bruckmaier[2], *[1]Department of Animal Science, Aarhus University, Foulum, Tjele, Denmark, [2]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Udder development shortly before parturition involves a final stage of mammogenesis and increasingly early stages of lactogenesis including colostrogenesis. Both, mammogenesis and lactogenesis contribute to an increased size of the udder in the period around parturition. The aim of the study was to investigate the udder development, and thereby the possibility of predicting the time of parturition. The observation technique for udder development should be a simple, on farm technique. Starting a week before expected parturition, 10 multiparous dairy cows were daily measured. Blood was sampled every 8 h until parturition and analyzed for progesterone. For determination of udder growth, the distance between 2 marks on the upper part and 2 marks on the lower part of the hindquarters was measured. Additionally, udder tension (scored: 1 = soft to 4 = hard) and the tension of the tail head ligaments (scored: 1 = hard to 3 = soft) was determined by manual palpation. A first index, Total Difference (TotDif) was calculated as the total difference in the upper and lower udder measurements per day. A second index, Tension in Udder and Ligament (TenUdLig) was the udder tension plus the tail head ligament scores. An exponential regression line depending on the time relative to parturition was used to describe prepartum udder growth expressed by TotDif and TenUdLig. Exponential growth of the udder already started before the drop of blood progesterone concentration. The equations were TotDif = $0.5838+9.8460 \times \exp(0.0002 \times x)$ and TenUdLig = $2.8446+3.1764 \times \exp(0.0002 \times x)$, x = the time in minutes before parturition. TenUdLig was the single best predictor of time to parturition. Alone, a TenUdLig score at minimum 5 could predict 20% chance of parturition within 24 h, 70% chance within 48 h, and 80% chance within 72 h. Cows with a combination of a TenUdLig score at minimum 5 and an increase in TotDif score on minimum 4 between 2 daily measurements calved within 24 h (100%). In conclusion, the prepartum udder development followed an exponential pattern, and in regards to prediction of parturition, the tension in the udder (combined with tension in the tail head ligament), was a better parameter than the growth of the udder.

Key Words: udder development, ligament

W301 Adiponectin receptor gene expression and adiponectin regulation of glucose uptake and cell growth in mammary epithelial cells. I. S. Yuh*[1,2] and L. G. Sheffield[3], *[1]Department of Animal Biotechnology, College of Animal Life Sciences, Kangwon National University, Chunchon, Republic of Korea, [2]Institute of Animal Resources, Kangwon National University, Chunchon, Republic of Korea, [3]Department of Dairy Science, University of Wisconsin, Madison.*

The objective of the research is to identify the presence of adiponectin receptors and to study adiponectin action on glucose uptake and growth in mouse mammary epithelial cells as an experimental model. In the real time RT-PCR analysis, these cells expressed both types of adiponectin receptors, AdipoR1 and AdipoR2, and the expression levels of these receptors were similar in the presence of 10% FBS. Some of lactogenic or growth factors, insulin (10 ng/mL) or IGF-I (10 ng/mL) alone or each of these in the presence of 1% FBS, did not alter AdipoR1 and AdipoR2 gene expression pattern for 0, 0.5, 1, 2, 4, 12 or 24 h incubation ($P > 0.05$). Prolactin (10 ng/mL) or EGF (10 ng/mL) alone or each of these in the presence of 1% FBS, did not affect the AdipoR1 and AdipoR2 mRNA induction ($P > 0.05$). In glucose uptake experiment, adiponectin plus pre-incubation of insulin for 2 h before adiponectin stimulation (1 mg/mL, 30 min) significantly increased 2-deoxy-D-glucose,[1,2-^{3}H] uptake compared with no treatment ($P < 0.01$)), but adiponectin alone or insulin alone (pre-incubation) did not. In a similar way, insulin alone for 30 min incubation or adiponectin for 2-h pre-incubation did not increase glucose uptake ($P > 0.01$) but insulin plus pre-incubation of adiponectin significantly increased glucose uptake compared with no treatment ($P < 0.01$). The interaction effects of these hormones were not significant when these hormones were pre-activated by the other side ($P > 0.05$) suggesting additive effect between these hormones in glucose uptake. In cell growth experiments, addition of 1 m*M* adiponectin in the presence of 1% FBS, significantly decreased DNA synthesis of mammary epithelial cells compared with control ($P < 0.01$). In addition, AICAR(100 or 200 μ*M*), AMPK activator and adiponectin signaling intermediate, significantly decreased mammary epithelial cell growth for 2 day and 4 day incubation periods in the presence of 2% FBS ($P < 0.05$). These results indicate that adiponectin has inhibitory effect on mammary epithelial cell growth.

Key Words: adiponectin receptor, glucose uptake, mammary growth inhibition