T263 Risk factors for undergoing lactations >15 months in high-producing Holstein cows in a hot environment. Jessica María Flores-Salas*[1] and Miguel Mellado[2], [1]*Universidad Autonoma Agraria Antonio Narro Unidad Laguna, Torreón, Coahuila, México,* [2]*Universidad Autonoma Agraria Antonio Narro, Saltillo, Coahuila, México.*

An epidemiological study of risk factors for involuntary extended lactations (15 to 40 mo) using a multiple variable logistic regression was carried out on 3278 high-yielding dairy cows in an intensive well-managed Hostein herd, milked 3 times daily in northern Mexico. Additional objectives were to assess the association of lactation length (15 to 40 mo) with milk yield and to assess the effect of multiple services (4 to ≥14) on pregnancy per artificial insemination. Also, a survival analysis was performed using the Cox proportional hazards model to test how the occurrence of pregnancy affects lactation length. Retained placenta (odds ratio (OR) = 1.3), metritis (OR = 1.8), ketosis (OR = 1.4), peak milk yield (<50 vs > 50 kg, OR = 1.4), temperature-humidity index at 60 d postpartum (<82 vs > 82 units, OR = 1.4), and 305-d milk yield (<11,000 vs > 11,000 kg, OR = 1.6) significantly increased the risk for lactations > 15 mo. Primiparous cows had less than half the risk of extended lactations (OR = 0.3) compared with multiparous cows. Once a cow had conceived, her risk of having a prolonged lactation dropped sharply ($P < 0.01$). A strong linear association was found between lactation length and total milk yield for primiparous (450 to 1349 d in milk, maximum milk yield 37,852 kg; r = 0.71) and pluriparous (450 to 1221 d in milk, maximum milk yield 38,021 kg; r = 0.75). Pregnancy per artificial insemination (P/AI) in cows with extended lactation decreased linearly as number of services increased (P/AI = 50.5% for 4 services and 12.8% for ≥14 services). The data showed that well-managed Holstein cows milked 3 times daily were capable of lactating up to 40 mo with remarkable high persistency and with high milk yield at dry-off. Additionally, this study supports previous findings indicating that reproductive and metabolic disorders associated with calving are important risk factors for extended lactations, derived from the link of periparturient diseases withdepressed reproduction in dairy cows.

Key Words: milk production, hot environment, extended lactations

T264 Enhanced pre-weaning nutrition stimulates mammary gland development in dairy heifer calves. Adam J. Geiger*[1], Robert E. James[1], Catherine L. Parsons[1], Anthony V. Capuco[2], and Robert M. Akers[1], [1]*Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*United States Department of Agriculture-Agricultural Research Service, Beltsville, MD.*

It is established that feeding heifers a high plane of nutrition post-weaning but before puberty negatively affects mammary gland (MG) development and milk yield. The mass of the MG parenchyma (PAR) increases up to 60-fold from birth until 3 mo of age. Interestingly, enhanced nutrition during this time (pre-weaning) does not appear to negatively affect MG development and may in fact be beneficial. Therefore, the objectives of this study were to clarify the effects of feeding a higher plane of nutrition pre-weaning to Holstein heifer calves on MG growth, and particularly PAR and fat pad (MFP) development. Thirty-six Holstein heifer calves (<1 wk old) were reared on 1 of 2 dietary treatments to create 2 physiologically diverse groups of animals. Diets included (1) a control milk replacer (MR) fed at 454 g powder per day (CON; 20% crude protein [CP], 20% fat), or (2) an accelerated MR fed at 1,135 g powder per day (ACC; 28% CP, 25% fat). Milk replacer mixed at 15% solids was fed as indicated until the 8th week when feeding was reduced 50% to induce weaning. Calves were housed individually with ad libitum access to water. Starter feeding was initiated at wk 5 and balanced between treatments. Udders were examined visually and by palpation with teats measured weekly. At weaning, a subset of calves were killed (n = 6/diet). Whole MG were removed, dissected, and weighed. ACC calves consumed more MR liquid pre-weaning (4575 vs 3199 mL MR/day; $P < 0.01$). At wk 8, ACC calves had longer front (1.3 vs 0.9 cm; $P < 0.01$) and rear (1.2 vs 0.8 cm; $P < 0.01$) teats. Whole untrimmed udders of ACC-fed calves were heavier (255 vs 66 g; $P < 0.01$). Differences were more pronounced after skin was trimmed (198 vs 38 g; $P < 0.01$). Dissected PAR and MFP were both greater for ACC calves (10.5 vs 1.4 g and 173 vs 29 g, respectively; $P < 0.01$). Overall differences remained if expressed on a body weight basis ($P \leq 0.05$). Results provide compelling evidence that pre-weaning nutrition markedly affects MG development in dairy calves. Efforts in our lab are underway to uncover the cellular and molecular mechanisms responsible for these differences.

Key Words: mammary gland, milk replacer, parenchyma

T265 Bovine, caprine and ovine serotonin receptors expression in the mammary gland during lactating and dry off by immunohistochemistry. Aridany Suarez-Trujillo*[1], Miguel A. Rivero[2], Anastasio Argüello[1], Juan Capote[3], and Noemi Castro[1], [1]*Department of Animal Science, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain,* [2]*Department of Morphology, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain,* [3]*Canarian Agronomic Science Institute, La Laguna, Tenerife, Spain.*

Serotonin (5-HT) functions as a feedback inhibitor of lactation. This action is mediated by 5-HT receptors (5-HTR). In situ hybridization studies showed 5-HTR subtypes are expressed in bovine mammary epithelial and myoepithelial cells as well as blood vessels, and vary by receptor subtype. The aim of this study was to determine the distribution of the 5-HTR subtypes (1B, 1E, 2A, 2B, 4 and 7) in cattle, goat and sheep mammary glands using immunohistochemistry, and to compare distribution among lactating and dry udders. Hypothalamus and mammary gland samples were taken at slaughter from 3 lactating and 3 nonlactating animals of each species. Hypothalamal tissue was used as positive control. Tissues were fixed, paraffin embedded and 5-µm sections were placed on slides. Immunohistochemical staining was performed using rabbit primary antibodies against 5-HTR for the 1B, 1E, 2A, 2B, 4 and 7 subtypes. Anti-rabbit secondary antibody was conjugated with streptavidin peroxidase, and visualization of binding was realized using diaminobenzidine substrate. Tissues were counterstained with hematoxylin. Myoepithelial cells were identified in serial sections by positive immunohistochemical staining for calponin. All 6 receptor subtypes were expressed in mammary epithelial cells in the 3 species studied. Furthermore, 5-HTR 1E was expressed in the myoepithelial cells in all 3 species, and in the blood vessels of cows. Receptor subtype 4 was found in myoepithelial cells of cows and sheep. In all 3 species blood vessels were positively stained for receptors 1B, 2A and 2B. In the lactating animals, receptor distribution in the mammary epithelial cells was cytoplasmatic. However, in mammary tissue from nonlactating animals, distribution changed to the apical membrane in all species. In conclusion, 5-HTR are expressed in epithelial, myoepi-

thelial and blood vessels within mammary tissue of cattle, sheep and goats, with distribution of subtype varying by cell-type and species. Furthermore, distribution of the receptors on mammary epithelial cells changes between lactating and nonlactating states.

Key Words: serotonin receptors, mammary gland, immunohistochemistry

T266 Effect of the supplementation of a functional additive in prepartal cows of two breeds on the level of IgG in colostrum. Angela Moreno and Claudia Ariza-Nieto*, *Corporacion Colombiana de Investigacion Agropecuaria CORPOICA, Bogota, Colombia.*

The purpose of this study was to estimate the effect of supplementation of a functional additive during the last third of gestation in cows on the level of IgG in colostrum. Twenty-eight (28) Holstein and White Orejinegro (WON) cows were assigned to a randomized complete block design with 2 factors (breed and supplementation) with repeated measures (i.e., days of lactation). The supplementation factor included 3 treatments: CTL) Control without supplementation, S0) Supplement without additive, S1) Supplement with additive. Colostrum samples were obtained during the first 3 d of lactation for the determination of crude protein (CP), immunoglobulin G (IgG) and Brix grades. A correlation analysis between Brix grades an IgG was performed. Concentrations of IgG determined by the ELISA technique were strongly correlated with the percentages of Brix grades (n = 82, r = 0.954, $P < 0.0001$), indicating that it can be used as an estimate of the concentration of IgG. The concentration of crude protein in colostrum was affected by the breed × day interaction ($P < 0.05$), it was lower in WON cows (10.3%) than Holstein cows (11.5%) on d 1, whereas on d 2 and 3 WON cows had greater concentrations of protein (5.9% and 6.5%, respectively) compared with Holstein cows (4.8% and 4.1%, respectively). There was a breed × day × supplementation interaction on colostral IgG ($P < 0.05$). On d 1, S1 cows of both Holstein (55.1 mg/mL) and WON breeds (47.3 mg/mL) had greater concentrations of colostral IgG than CTL Holstein (44.4 mg/mL) and WON (32.5 mg/mL) cows. The decrease in colostral IgG from d 1 to d 3 was greater in Holstein (95%) than in WON (86%) cows. It can be concluded that dietary supplementation with functional additives increased the colostral concentration of IgG in both cow breeds.

Key Words: feed additive, cow, colostrum

T267 The effects of cabergoline administration at dry-off of lactating cows on udder engorgement, milk leakages, lying behavior, and udder health at calving. Alex Bach*[1,3], Naomi Isaka[2], Audrey Deflandre[2], and Anna Arís[3], *[1]ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain, [2]CEVA Santé Animale, Libourne, France, [3]Department of Ruminant Production-IRTA, Caldes de Montbui, Spain.*

Cabergoline is an ergot-derivative with high affinity for the D_2 dopamine receptors whose dopaminergic effects cause inhibition of prolactin (PRL) secretion, and thus it could be considered a molecule that acts as a potential dry-off facilitator. One hundred ninety-nine Holstein cows (102 primiparous; 97 multiparous) producing ≥ 18 kg/d at dry-off were split in 2 treatments with the objective of evaluating the effects of cabergoline at dry-off (between 70 and 50 d before the expecting calving date) on PRL secretion, udder engorgement, milk leakages, udder health at calving, and cow wellbeing after dry-off. Treatments consisted of a single i.m. injection of 5 mL of a solution containing 5.6 mg of cabergoline (CAB) or 5 mL of saline solution as a placebo (CTRL). Each animal was evaluated for presence or absence of milk leakages during the 4 d following drying-off and udder engorgement determined using a digital algometer. Lying behavior was recorded during 10 d after drying-off. Twenty-five cows from each treatment were randomly chosen and blood sampled at 3 and 15 d after dry-off and at 5 and 3 d before the expected calving date to determine serum PRL concentrations. Data were analyzed using a mixed-effects model for repeated measures. Cows on CTRL had greater ($P < 0.05$) serum PRL concentrations than cows on CAB at 3 and 15 d after dry-off. Cows on CTRL had a greater ($P < 0.01$) udder engorgement (24.0 ± 0.33 Newtons) than cows on CAB (22.4 ± 33 Newtons) throughout the 4 d following dry-off, and it decreased ($P < 0.05$) as days since dry-off increased. The overall incidence of milk leakage in cows on CAB (3.1 ± 0.88%) was 73.5% of that obtained in cows on CTRL (11.7 ± 1.64%); and cows on CAB had 0.2 lesser odds ($P < 0.001$) to incur in milk leakage than cows on CTRL. The day following dry-off, CTRL cows lied about 1.5 h/d less ($P < 0.05$) than cows on CAB. It is concluded that an i.m. administration of 5.6 mg of carbegoline at dry-off effectively reduces PRL secretion, udder engorgement, milk leakages, and improves lying time the day following dry-off.

Key Words: involution, mammary gland, prolactin

T268 Dry-off facilitator cabergoline hastened the GLUT-1 decrease and lactoferrin increase in the mammary tissue during drying-off in dairy cows. Marion Boutinaud*[1,2], Naomi Isaka[3], Audrey Deflandre[3], Sandra Wiart[1,2], Philippe Lamberton[1], Ana Isabel De Prado Taranilla[3], and Vanessa Lollivier[1,2], *[1]INRA UMR1348, Saint Gilles, France, [2]Agrocampus Ouest UMR1348, Rennes, France, [3]CEVA Santé Animale, Libourne, France.*

In ruminants, the early phase of drying-off is a period of mammary gland involution where lactose secretion is inhibited and lactoferrin is stimulated. GLUT-1 is a glucose transporter that has a key role in supplying substrate for lactose synthesis. The analysis of the changes in lactose and lactoferrin contents in mammary secretions and in GLUT-1 and lactoferrin contents in the udder can provide valuable information about the speed of the mammary involution. To assess the effect of prolactin inhibition by cabergoline on udder involution, 14 Holstein dairy cows were injected with a single i.m. administration of 5.6 mg cabergoline (n = 7) or placebo (n = 7) within 4 h after the last milking the day of drying off (D0). After D0, hay and water ad libitum was supplied to the cows for 10 d. Mammary secretion samples, collected during lactation (D-6) and at D1, D2, D3, D4, D8 and D14 after the drying-off, were used for lactose and lactoferrin analysis. Mammary biopsy samples, collected at D-6, D1 and D8, were used for GLUT-1 mRNA and lactoferrin analyses. Lactose content of mammary secretions progressively decreased during involution, whereas their lactoferrin content increased. The change in lactose content was associated with paralleled change in GLUT-1 mRNA level in the udder. These decreases were faster in cabergoline treated cows compared with controls with lower lactose content in cabergoline treated cows already by D1 than in controls ($P < 0.05$) and significant decrease in GLUT-1 mRNA levels at D1 and D8 respectively for cabergoline and control treatments compared with D-6 ($P \leq 0.05$). The rise of lactoferrin content in mammary secretions was significant starting at D4 in the cabergoline treated cows ($P \leq 0.05$) whereas it only happened at D8 in controls ($P < 0.05$). Overall, cabergoline treatment decreased GLUT-1 mRNA level ($P < 0.05$) and increased lactoferrin content ($P = 0.10$). Similarly, lactoferrin immunostaining intensity in the mammary tissue was higher at D1 than at D-6 in cabergoline treated cows ($P \leq 0.05$), whereas it tended to be higher only at D8 in controls ($P < 0.10$). Our results indicate that cabergoline treatment was efficient to hasten the udder involution and therefore facilitates the dry-off.

Key Words: cow, drying-off, prolactin

T269 Transcriptome analysis of the mammary gland reveals new insights for the role of serotonin in lactation. Jimena Laporta*[1], Francisco Peñagaricano[1], and Laura L. Hernandez[2], *[1]University of Florida, Gainesville, FL, [2]University of Wisconsin-Madison, Madison, WI.*

Serotonin (5-HT) in the mammary gland is known to regulate processes such as calcium homeostasis, tight junction permeability, and milk protein gene expression. The rate-limiting enzyme in the synthesis of non-neuronal 5-HT is tryptophan-hydroxylase (TPH1). Our objective was to further explore the roles of 5-HT in the mammary gland during lactation. We used whole body TPH1 knockout dams (KO; 5-HT deficient, n = 4) and compare them to wild-type (WT; n = 4) and rescue (RC; KO + 100 mg/kg 5-hydroxytryptophan injected daily, n = 4) dams. Mammary tissues were collected on d 10 of lactation. Total RNA extraction, amplification, library preparation, and sequencing were performed following the Illumina mRNA-seq protocol. Sequencing reads were mapped to the mouse reference genome using Tophat. The resulting alignments were used to reconstruct transcript models by Cufflinks. Differential gene expression was analyzed using Cuffdiff. Overall, 97 and 204 genes (false discovery rate, FDR ≤ 0.01) showed at least a 2-fold expression difference between WT and KO and between WT and RC, respectively. Most of the differentially expressed genes (DEG) are related to calcium homeostasis, regulation of apoptosis, cell cycle and cell differentiation, proliferation, and the immune response, among others. Additionally, enrichment analysis using Gene Ontology (GO) and Medical Subject Headings (MeSH) databases revealed the alteration of several biological processes (FRD ≤0.01) including fat cell differentiation and lipid metabolism, regulation of extracellular signal-related kinases and mitogen-activated kinase cascades, insulin resistance, nuclear transport, regulation of membrane potential, release of calcium from the endoplasmic reticulum into the cytosol. The majority of the biological processes and metabolic pathways altered in the KO dams are required for mammary gland homeostasis. Our study reveals the importance of non-neuronal 5-HT for normal mammary gland function and lactation. The potential modes of regulation of the bovine mammary gland during lactation by 5-HT should be further investigated.

Key Words: RNA sequencing, tryptophan-hydroxylase 1, lactation

T270 Lactation performance of dairy cows milked 3 times per day and supplemented with two different formulations of bovine somatotropin. Jozivaldo P. G. Morais*, Andressa P. da S. Cruz, Natalia S. Minami, Luiz P. Veronese, Tiago A. Del Valle, and Simone D. Sartorio, *Universidade Federal de São Carlos, UFSCAR, Araras, SP, Brazil.*

The aim of this randomized field study was to evaluate the lactation performance of dairy cows supplemented with 2 different 500-mg bovine somatotropin (bST) formulations. Sixty Holstein cows were allocated to a control, 90 Holstein cows to a somatotropin fast release formulation (FR-bST, Boostin-S, LG Life Science), and 90 Holstein cows to a somatotropin slow release formulation (SR-bST, Lactotropin, Elanco Animal Health). Treatments (TRT) were delivered every 14 d starting at 57–70 d post calving until the end of lactation, or 17 injections. Before TRT assignment, animals were paired by milk yield, lactation number, and randomly assigned to one of the 3 TRT. Evaluated parameters were milk production, milk protein, milk fat, milk SCC, and BCS. Somatotropin treatment increased milk yield ($P < 0.001$). SR-bST treated cows, yielded 1.6 kg/day more milk than FR-bST treated cows ($P = 0.008$). Somatotropin increased milk protein % ($P = 0.002$), decreased BCS ($P < 0.001$), and had no effect on milk fat % ($P = 0.120$) and SCC ($P = 0.964$). Results are shown in Table 1.

Table 1 (Abstr. T270). Production variables of cows milked 3 times per day without bST and with 2 different bST formulations[1]

	Treatment				*P*-value[2]	
Item	No bST	FR-bST	SR-bST	SEM	C1	C2
Pre-TRT period						
Milk, kg/d	40.04	40.38	39.96	0.464	0.880	0.635
Fat, %	3.36	3.17	3.28	0.063	0.349	0.469
Protein, %	2.68	2.73	2.69	0.014	0.448	0.216
SCC, $\times 10^3$/mL	38.74	39.99	40.92	2.459	0.350	0.885
BCS	2.94	2.94	2.95	0.016	0.851	0.975
TRT period						
Milk, kg/d	34.63	38.03	39.60	0.131	<0.001	0.008
Fat, %	3.18	3.06	3.10	0.001	0.120	0.576
Protein, %	3.01	3.11	3.07	0.001	0.002	0.086
SCC, $\times 10^3$/mL	242.6	181.1	166.8	11.50	0.964	0.865
BCS	3.04	2.95	2.93	0.003	<0.001	0.263

[1]FR-bST = Boostin-S, LG Life Science; SR-bST = Lactotropin, Elanco Animal Health. Starting on d 63 bovine somatotropin was administered at 14-d intervals to cows on the groups to receive bst.
[2]C1 = control vs. bST; C2 = FR-bST vs. SR-bST.

Key Words: milk composition, milk yield, recombinant bST

T271 Effects of milking frequency and prolactin on milk production and expression of prolactin receptors in the mammary gland of dairy cows. Izabella Thompson*[1], Severine Ollier[1], Xin Zhao[2], and Pierre Lacasse[1], *[1]AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada, [2]Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada.*

A better understanding of how biological processes affect milk production in dairy cows is important to improve lactation persistence. The present study investigated the sensitivity of the mammary gland to prolactin. Eleven mid-lactation cows were milked twice daily during the first 2 experimental weeks. During wk 3–10 cows were differentially milked; the right quarters being milked thrice daily (3×), while the left were milked once daily (1×). During wk 11–14, all quarters were milked twice daily. After 4 weeks of differential milking, cows received daily i.m. injections of the dopamine antagonist domperidone (300 mg; DOMP; n = 6) or DMSO (CTL: n = 5) for 8 weeks (wk 7–14). Blood and milk samples were collected weekly for measurement of prolactin (PRL) as well as milk components for the latter. Milk was also collected from front quarters every 2 weeks and RNA was extracted from milk fat fraction. Expression of PRL receptors (long; PRLRL and short; PRLRS) were measured by real-time RT-PCR. Mammary gland biopsies were taken from rear quarters on wk 2, 6, 10 and 14 for subsequent gene and protein analyses. Differential milking improved milk production in the 3× quarters compared with the 1× quarters ($P < 0.01$). Increases in fat ($P = 0.04$), protein ($P = 0.05$) urea ($P = 0.01$) and lactose ($P < 0.01$) were observed in the 1× quarters, while greater SSC ($P < 0.01$) was observed in the 3× quarters. Blood PRL concentrations were greater for DOMP than CTL cows (24.8 ± 2.3 vs 13.9 ± 2.5 ng/mL; $P < 0.01$). There was no effect of injections on milk production, but milk from DOMP cows had greater concentrations of lactose ($P = 0.02$) than CTL. Interestingly, DOMP cows had greater expression of both forms of PRLR ($P < 0.01$) in milk fat compared with CTL. Additionally, greater PRLR expression was observed in the 3× than 1× quarters ($P < 0.01$). Results show that milk frequency improves milk production and alters composition and expression of PRLR in the mammary gland. Moreover, administration

of domperiodone increased PRL concentration and is related to greater expression of both forms of PRLR in the mammary gland.

Key Words: milk production, mammary gland, prolactin

T272 Effects of inhibiting the lactogenic signal at calving on milk production and metabolic perturbations in cows. Noémie Vanacker*[1], Ollier Séverine[1], Blouin Richard[2], and Lacasse Pierre[1], *[1]AAFC-Dairy and Swine Research and Development Centre, Sherbrook, Québec, Canada, [2]Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, Canada.*

During the periparturient period, the abrupt increase in energy demand for milk production induces metabolic disorders. Our previous work has shown that reducing milk output by milking once a day or partially in the first days of lactation reduces these disorders. The aim of this study was to reduce metabolic disorders by limiting milk production during the first week of lactation through inhibition of the lactogenic signal driven by prolactin. Twenty-two late gestation cows were assigned into 2 groups based on parity and milk production of the first 100 d of the previous lactation. Both groups received 8 i.m. injections of either quinagolide (Quin; 2mg of an inhibitor of prolactin release) or water (CTL) just after calving, and the subsequent 7 injections were given every 12h. Milk production was measured until d 28 post-calving. Blood samples were taken from d 1 (calving) to 5 and then on d 7, 10, 14, 21 and 28 to measure concentrations of urea, phosphorus, calcium, glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate and prolactin (PRL). Prolactin concentrations were lower ($P < 0.01$) in Quin cows from d 2 to d 5. Interestingly, between d 10 and d 28, PRL concentrations were greater ($P < 0.05$) in Quin than CTL cows. Milk production was lower in Quin cows from d 2 to d 6 than in control (24.3 ± 6.4 kg/d vs 34.8 ± 4.1 kg/d; $P < 0.05$). There was no residual effect of quinagolide on milk production after d 6. The blood glucose and calcium concentrations were greater ($P < 0.05$) in Quin cows whereas the concentration of β-hydroxybutyrate was greater ($P < 0.05$) in CTL cows during the first week of lactation. Blood NEFA, urea and phosphorus were not affected by treatment. In conclusion, reducing the prolactin peak at calving is effective to reduce milk production during the first week of lactation without compromising the global productivity of the dairy cow. This reduction in milk production allows a reduction of the metabolic stress during this period.

Key Words: prolactin, quinagolide, energy balance

T273 Effect of increased milking frequency in early lactation on milk production, proliferation and apoptosis of mammary cells in dairy cow. Juliana Mergh Leão*[1], Juliana Aparecida Mello Lima[1], Sandra Gesteira Coelho[1], José Reinaldo Mendes Ruas[2], Anilton César Vasconcelos[1], Ângela Maria Quintão Lana[1], Ronaldo Braga Reis[1], and Helton Mattana Saturnino[1], *[1]Universidade Federal de Minas Gerais-UFMG, Belo Horizonte, Minas Gerais, Brazil, [2]Empresa de Pesquisa Agropecuária de Minas Gerais-EPAMIG, Felixlândia, Minas Gerais, Brazil.*

The objectives of the present study were to evaluate the effect of increased milking frequency (IMF) during early lactation on milk yield, proliferation and apoptosis in mammary epithelial cells of F_1 Holstein × Zebu cows. Fourteen cows F_1 Holstein × Zebu cows were randomly distributed into 2 groups. The control group (2×) was milked twice a day up to 210 d in milking (DIM), and the group subjected to IMF (4×) was milked 4 times a day from 2 to 21 DIM, and twice a day from 22 to 210 DIM. Milk production was measured daily from 4 to 30 DIM and then each 15 d until 210 d. Mammary biopsies were performed on d 2, 7, 14, 21, and 28 postpartum. Biopsies were obtained using a biopsy needle (12 g × 12). Mammary tissue was used to measure rates of cell proliferation and apoptosis using Ki-67 nuclear proliferation antigen localization and terminal deoxynucleotidyl transferase nick-end labeling (TUNEL), respectively. Data were analyzed with a repeated measures design and means were compared by Tukey›s test ($P < 0.05$). During IMF, the 4× produced 2.6 ± 0.2 kg/d more milk than the 2× ($P < 0.05$). After IMF phase, milk production from the 4× decreased and was similar to 2× up to 210 DIM ($P > 0.05$). Mammary cell proliferation and apoptosis were not affected by milking frequency ($P > 0.05$). Rates of proliferation and apoptosis were influenced by days of lactation ($P < 0.05$). On the second day of lactation, the highest apoptosis rate (0.3%) was observed. Days 7 (0.16%), 14 (0.13%), 21 (0.11%) and 28 (0.06%) had similar and lower rates in comparison with the second day of lactation. There was a higher percentage of proliferation in mammary epithelial cells on d 2 (2.12%). On d 7 (0.99%) and 14 (0.87%), the proliferation rate dropped to about half the value observed on d 2 and in the following week (0.47%) it was observed another reduction which remained until the 28th of lactation (0.43%). Changes in milking frequency during early lactation did not alter milk yield after IMF and mammary cell population dynamics suggesting that maybe the mammary biopsy procedure used in the current study influenced these responses negatively.

Key Words: biopsy, Ki-67, terminal deoxynucleotidyl transferase nick-end labeling (TUNEL)

T274 Effect of cortisol on mammary epithelial cell turnover: milk synthesis, proliferation and apoptosis. J. A. Negrao*, G. M. Krempel, S. A. Oliveira, G. F. Bomfim, H. Z. Polato, and F. C. Lahr, *Basic Science Department, FZEA/USP, Pirassununga, SP, Brazil.*

Cortisol is essential for copious synthesis of milk components, and cortisol administration delayed the decline of milk yield in late lactation. However, the effect of cortisol in proliferation, activity, and survival of the epithelial cells remains controversial. The objective of this study was to evaluate the effect of cortisol on the survival and proliferation of epithelial cells. Twenty-four Saanen goats were submitted to administration of ACTH (Cortisol group) or placebo (Control group). Milk yield and milk quality (fat, protein, lactose, and CCS) were measured daily and weekly, respectively. Cortisol concentrations in plasma were measured before and after ACTH or Placebo administration at 60, 90, and 120 d of lactation. All mammary biopsies were conducted 1 h after ACTH or placebo administration. Biopsies were taken of 4 goats submitted to ACTH and 4 goats submitted to placebo at 60, 90 and 120 d of lactation. Apoptosis was determined using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) kit, and proliferating cell was determined using a proliferating cell nuclear antigen (PCNA) kit. The mRNA levels of Bax and Bcl-2 were analyzed by RT-PCR. At the same time, Bax and Bcl-2 synthesis were measured by ELISA kit. Statistical analysis was performed by ANOVA using mixed model, and the level of significance was set at $P < 0.05$ for main effects and interactions. Although, cortisol increased significantly after ACTH administration milk yield, milk quality, casein and lactose in milk were similar for both groups. Furthermore, percentage of apoptotic cells measured by TUNEL, percentage of proliferation measured by PCNA, and the expression and synthesis of Bax (promoter of apoptosis) were similar in ACTH and placebo groups (control). However, the expression and synthesis of Bcl-2 (inhibitor of apoptosis) were significantly higher after ACTH administration when compared with placebo administration. Furthermore, the relationship between Bax/Bcl-2 was significantly lower after ACTH administration when compared with placebo administration.

These results support the hypothesis that cortisol can delay apoptosis in the mammary gland.

Key Words: mammary gland, Bax, Bcl-2

T275 Effects of growth hormone and insulin-like growth factor on synthesis and secretion of β-casein, β-lactalbumin and lactoferrin in mammary epithelial cells. J. A. Negrao*, G. M. Krempel, S. A. Oliveira, G. F. Bomfim, F. C. Lahr, and H. Z. Polato, *Basic Science Department, FZEA/USP, Pirassununga, SP, Brazil.*

Growth hormone (GH) and insulin-like growth factor (IGF-I) are associated to milk production traits, however the mechanism how GH and IGF-I increase the milk synthesis remains unclear. The objective of this study was to evaluate the effect of GH and IGF-I on the synthesis and secretion of β-casein, β-lactalbumin, and lactoferrin by mammary epithelial cells. Twenty-four Saanen goats were used, and 5 goats were biopsied. Milk yield and milk quality (fat, protein, lactose and CCS) were measured daily and weekly, respectively. Growth hormone and insulin-like growth factor-I concentrations in plasma were measured once a day, at 30 and 60 d of lactation. All mammary biopsies were conducted just after blood samples. Biopsies were taken of 5 goats at 30 and 60 d of lactation. Epithelial cells were isolated and cell culture was used to study expression and synthesis of β-casein, β-lactalbumin, and lactoferrin. Four treatments were imposed to epithelial cell culture: (1) control (basal medium); (2) GH (10 ng/mL); (3) IGF-I (10 ng/mL); (4) GH + IGF-I (10 ng/mL + 10 ng/mL). The mRNA levels of β-casein, β-lactalbumin, and lactoferrin were analyzed by RT-PCR. At the same time, the synthesis of β-casein, β-lactalbumin, lactoferrin, and lactose in were measured by ELISA kit in culture medium. Statistical analysis was performed by ANOVA using mixed model, and the level of significance was set at $P < 0.05$ for main effects and interactions. Growth hormone (GH) and insulin-like growth factor (IGF-I) were associated with milk yield, and goats classified as higher producing (4 from 24 experimental goats) presented high GH and IGF-I levels. β-Casein expression and synthesis were significantly higher for IGF-I and GH + IGF-I treatments when compared with control. Lactoferrin expression and synthesis were significantly higher for GH and GH + IGF-I treatments when compared with control. However, β-lactalbumin expression and synthesis were not change by hormone treatments when compared with control. These results support the hypothesis that GH and IGF-I can modulate directly the synthesis of milk.

Key Words: growth hormone (GH), insulin-like growth factor (IGF-I), milk synthesis

T276 The barrier integrity of bovine mammary epithelial cells in vitro in response to lipopolysaccharide (LPS) and lipoteichoic acid (LTA) treatment. Christina Zbinden*[1,2], Rupert M. Bruckmaier[1], and Olga Wellnitz[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.*

The blood-milk barrier of the bovine mammary gland prevents an intermixture of blood and milk. During mastitis, the permeability of the blood milk barrier is increased, which is reflected by an exchange of blood constituents into milk, and vice versa. The aim of this study was to investigate the role of mammary epithelial cells in the regulation of blood-milk barrier alteration during mastitis induced by cell wall components of *Escherichia coli* (LPS) and *Staphylococcus aureus* (LTA). Low passage primary bovine mammary epithelial cells (bMEC) from 3 different cows were grown separately on Transwell inserts. The level of integrity of the epithelial barrier was measured by means of transepithelial electrical resistance (TEER), as well as by diffusion of the fluorophore Lucifer Yellow (LY) across the cell layer. The formation of tight junctions between adjacent epithelial cells was examined by immunofluorescence staining of zona occludens-1 and by transmission electron microscopy. The cultured cells formed tight cell layers sealed by tight junctions. The barrier integrity was reduced after 3 h ($P < 0.05$; paired *t*-test relative to unchallenged cells) in response to 500 µg/mL LPS from *E. coli*, and after 7 h in response to 20 mg/mL LTA from *S. aureus*. At these dosages, the fluorescence values of LY in the apical compartment of the Transwell insert dropped within 24 h from 5386 to 560 ± 235 RFU in response to LPS, and from 5386 to 1416 ± 144 RFU in response to LTA. No significant changes in barrier permeability were observed in response to 200 µg/mL LPS, or to 2 mg/mL LTA Although LPS and LTA affected the barrier permeability most likely due to an opening of the tight junctions, LPS additionally caused considerable cell damage reflected by increased LDH concentrations in cell culture medium ($P < 0.05$). These results confirm a pathogen-specific impairment of the blood-milk barrier during mastitis, which involves differences in cell degradation.

Key Words: bovine mastitis, blood-milk barrier, tight junction

T277 Intravenous challenge with lipopolysaccharide does not induce a mammary immune response in dairy cows and does not affect the blood-milk barrier. Olga Wellnitz*[1,2], Emmanouil Kalaitzakis[1,2], Heinrich Bollwein[2], and Rupert M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Clinic of Reproductive Medicine, Vetsuisse Faculty University of Zurich, Zurich Switzerland.*

An intramammary challenge with lipopolysaccharide (LPS) from *Escherichia coli* is known to induce a considerable immune response of the mammary gland and an impairment of the blood-milk barrier. The aim of the current study was to investigate a potential immune reaction in the mammary gland and potential changes of the integrity of the blood-milk barrier in response to an intravenous, i.e., systemic challenge, with LPS. Ten lactating dairy cows in wk 3 of lactation were challenged intravenously (V. jugularis) with 0.5 µg/kg BW *E. coli* LPS (O26:B6). Rectal temperature was measured hourly. Milk samples of one udder quarter were taken immediately before and then every 30 min until 5 h after challenge. Mammary gland biopsies of one quarter were taken immediately before and 8 h after LPS challenge for RT-qPCR of immunorelevant factors. Rectal temperature increased ($P < 0.001$) within 1 h of LPS administration from 38.3 ± 0.1 to 39.2 ± 0.1°C and stayed elevated throughout the 5 h of experiment. The milk somatic cell count was 83.3 ± 19.1 × 10^3/mL immediately before challenge and did not change throughout the experiment. Lactate-dehydrogenase concentrations in milk as a marker for blood-milk barrier impairment was 48.1 ± 6.0 U/L immediately before challenge and did not change throughout the experiment. Relative mRNA expression of immunorelevant factors; that is, the cytokines TNF-α and interleukin-1β in mammary gland tissue did not change in response to intravenous LPS injection. In conclusion, in contrast to intramammary injections, an intravenous injection of 0.5 µg/kg BW LPS induces a systemic immune response shown by an increase in rectal temperature, but has obviously no effects on factors of mammary gland immune response which typically change during LPS-induced mastitis. Also the blood-milk barrier integrity does not appear to be influenced by systemic LPS.

Key Words: intravenous lipopolysaccharide, mammary gland, blood-milk barrier

T278 Characterization of mammary glucose metabolism during milk fat depression. Natalie L. Urrutia*[1], Kevin J. Harvatine[1], and Dale E. Bauman[2], [1]*The Pennsylvania State University, University Park, PA,* [2]*Cornell University, Ithaca, NY.*

Milk fat synthesis requires butyrate, acetate, and reducing equivalents (NADPH) as substrates. NADPH is provided from glucose through the pentose phosphate pathway and isocitrate dehydrogenase (IDH1). During milk fat depression (MFD), reduced milk fat synthesis may result in decreased uptake of nutrients by the mammary gland, including glucose. The objective of this study was to characterize expression of glucose metabolism and NADPH synthesis enzymes in mammary tissue during MFD using tissue from a previously published experiment. Cows were arranged in a 3 × 3 Latin square design with 14-d experimental periods. Treatments were control (CON), CLA-induced MFD (CLA; 3 d i.v infusion of 10 g/d of *trans*-10,*cis*-12 CLA in an intralipid emulsion), and a low forage, high oil diet (LF/HO; 45.9% forage, 3.0% soybean oil, and 1.5% fish oil). Milk fat yield was decreased 24% by CLA and 38% by LF/HO. Mammary biopsies were performed 1 to 3 h after milking at the end of each treatment. Gene expression of enzymes involved in glucose metabolism and NADPH synthesis were determined by Real-Time PCR and analyzed relative to the geometric mean of housekeeping genes (18S ribosomal subunit, ribosomal protein S9 and β2-microglobulin). Data were analyzed using the fit model procedure of JMP Pro and the model included fixed effects of treatment and geometric mean of housekeeping genes and random effects of cow and period. Glucose transporter 1 (GLUT1) and IDH1 were significantly reduced by the LF/HO treatment when compared with CON (28 and 36%, respectively; $P < 0.05$). Phosphogluconate dehydrogenase expression was lower in the LF/HO treatment when compared with CLA ($P < 0.05$), but not different compared with CON. Other genes involved in glucose metabolism such as HK2, ACLY, G6PDH and GLUT8 were not affected by treatments.These results suggest that during diet-induced MFD the reduction in glucose uptake coincides with decreased expression of GLUT1 and that the decrease in use of NADPH corresponds to a downregulation of the isocitrate pathway. The smaller decrease in milk fat in the CLA treatment may have limited the ability to observe a treatment effect during CLA-induced MFD.

Key Words: glucose, NAPDH, isocitrate

T279 Transcriptome adaptation of the bovine mammary gland to a diet supplemented with linseed oil. Eveline M. Ibeagha-Awemu[1], Ran Li[1], Adolf A. Ammah[1], Nathalie Bissonnette*[1], Chaouki Benchaar[1], and Xin Zhao[2], [1]*Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Sherbrooke, Quebec, Canada,* [2]*Department of Animal Science, McGill University, Ste-Anne-De-Bellevue, Quebec, Canada.*

Diets rich in unsaturated fatty acids (UFA) have been shown to increase the contents of some milk beneficial fatty acids (FA) including conjugated linoleic acid (CLA). The mechanism by which UFAs modulate cow's genetics resulting in increased milk CLA content is not clear. This study examined the transcriptome of the bovine mammary gland following dietary supplementation with linseed oil. Twelve Holstein cows (35±10 kg milk; 150±50 DIM) were used in a complete randomized block design and fed a total mixed ration (control diet) for 28 d (d 1-28; control period, CP) followed by a treatment period (TP; d 29-56) consisting of control diet + 5% linseed oil (57% α-linolenic acid). Milk samples were collected weekly for fat, protein and individual FA determination. Mammary gland biopsies were performed on 6 cows on d 14 (CP) and d 35 and 56 (TP). Global transcriptome was analyzed using RNA-sequencing. Milk fat percent decreased ($P < 0.0001$) from 3.62% (CP) to 2.52% (TP) while protein percent was unchanged by treatment. The proportions of C4:0, C8:0, C14:0, C16:0 and C14:1 decreased ($P \leq 0.0003$) while C18:1n-11t, C20:3n-3, C20:5n-3, C22:5n-3, CLA10t12c and CLA9c11t increased ($P \leq 0.035$) during the TP. RNA-sequencing generated 459 million reads out of which 89% mapped to unique positions on the bovine genome. Eight genes (*CSN2*, *CSN1S1*, *CSN1S2*, *LGB*, *CSN3*, *LALBA*, *COX1*, and *GLYCAM1*) out of 11121 expressed constituted 73.70% of mapped reads. One hundred seventy-nine genes were significantly regulated (79 up- and 100 down-regulated) after FDR correction. Most affected genes were *FBP2* (11-fold up-regulated) and *MROH2B* (4-fold down-regulated). Further regulated genes with roles in FA synthesis/uptake included *LPIN1*, *SREBF1*, *INSIG1*, *FASN*, *BDH1*, *ACSS1*, *ACADVL*, *SLC25A34*, *SLC39A11*, *TIEG2*, and *CYP2B6*. Differentially expressed genes were significantly enriched in several KEGG pathways including PPAR and insulin signaling pathways. This study has provided a broader picture of the transcriptomics events that are involved in mammary gland adaptation to a diet rich in UFA. Our study provides further knowledge on mammary lipogenesis and data that can be used to develop new nutritional strategies for a better management of milk increased beneficial FAs.

Key Words: bovine mammary gland transcriptome, linseed oil, lipogenesis