

Lactation Biology 2

W142 Hormonal regulation of suspected components of bovine IgG1 transcytosis mechanism in primary bovine mammary cells in vitro. A. Stark¹, E. Vaschkova², O. Wellnitz^{*1}, R. M. Bruckmaier¹, and C. R. Baumrucker³, ¹*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland*, ²*Trakia University, Stara Zagora, Bulgaria*, ³*Penn State University, State College*.

Colostrum is distinguished by the specific transfer of IgG1 from the blood to mammary secretions. It occurs during the last 2–3 weeks of pregnancy when steroid concentrations of estradiol (E2) and progesterone (P4) are high. Rodent intestinal uptake of immunoglobulin G has indicated this transcytosis process is mediated by a receptor termed Fc fragment of IgG, Receptor, Transporter, α (FcGRT) and supported by light chain β 2 Microglobulin (β 2M). The bFcGRT has been cloned by others and mRNA exists in the mammary tissue and intestines of ruminant species. We hypothesized that steroid hormone treatments E2 and P4 of bovine mammary cells in vitro would induce changes in mRNA expression indicating which components are involved in IgG1 transcytosis. Two different cultures (passage 6 to 14) of primary bovine mammary cells were evaluated by qPCR. Cells on plastic and rat tail collagen were treated with hormonal combinations (steroids/lactogenic). Evaluated components were bFcGRT, β 2M, and various bRab GTPases; the latter components direct endosomal transcytosis movements in other eukaryotic cells. All tested components showed strong expression of mRNA in the cells. Plastic experiments showed that FcGRT, Rab11b, Rab25, were significantly regulated ($P < 0.05$) by steroid hormones while Rab11A and β 2M were not changed. Experiments on collagen gels showed that lactogenic hormones increased expression of bLf mRNA (8X; $P < 0.0001$) in both mammary cells. Less increased expression of FcGRT was principally stimulated by steroids (E2), while the Rab25 was increased by lactogenic and steroid treatments. These results indicate that some suspected components of IgG1 transcytosis have their mRNA altered by hormones in vitro. However the 2 different primary cultures of bovine mammary cells show different expression patterns, perhaps reflecting animal to animal variation that is experienced by in vivo experiments. Mammary cell bFcGRT, and bRab25 are candidates that are hormonally altered for the colostrum period.

Key words: colostrum, IgG, cattle

W143 Reducing metabolic stress of dairy cows during the transition period by partial milking or nursing. É. Carbonneau^{*1}, A.-M. De Passillé², J. Rushen², B. G. Talbot¹, and P. Lacasse³, ¹*Université de Sherbrooke, Sherbrooke, QC, Canada*, ²*AAFC-Pacific Agri-Food Research Centre, Agassiz, BC, Canada*, ³*AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada*.

During the transition from pregnancy to lactation, the sudden increase in nutrient demand for milk production causes metabolic perturbations and high incidences of metabolic diseases in high yielding cows. We previously showed that limiting milk yield by milking once a day during the first wk of lactation improved metabolic status but reduced milk production during the following weeks. In this study, we examined if limiting milk harvest postpartum while maintaining milking-induced endocrine stimulus could improve the metabolic status of cows without reducing overall milk production. Forty-seven Holsteins cows were allocated to 3 treatments, balanced for parity and milk production: 1) cows were milked completely twice a day from calving (control); 2) cows were partially milked twice a day until d5 after calving

(partial); 3) cows were left with the calf to suckle from the dam until d5 and were milked once a day from d3 to d5 (nursing). All cows were milked twice a day from d6 to the end of the experiment (d63). During the treatment period (d1 to d5), milk production averaged 27.3 and 9.7 kg/d for control and partial, respectively. There was no residual effect ($P = 0.7$) of treatments on milk production which averaged 47.5, 45.9 and 46.4 kg/d for the control, partial and, nursing, respectively, between wk2 and 9. The DMI of the cows were similar during and after treatment ($P > 0.2$). From wk2 to 9, milk protein and lactose content were not affected by treatments, but milk fat content tended ($P = 0.06$) to be higher in control cows than in cows where milk harvest was limited (partial + nursing). Blood concentrations of glucose ($P < 0.001$) and phosphorus ($P < 0.05$) were lower and the concentrations of NEFA ($P < 0.05$) and BHBA ($P < 0.0001$) were higher in control cows than in the other cows during the treatment period. The positive effects on glucose and BHBA remained significant ($P < 0.05$) up to d28. There was no effect of treatments on blood urea, calcium and haptoglobin. These results suggest that reducing milk harvest postpartum while maintaining milking stimuli reduces metabolic stress without compromising productivity of high yielding dairy cows.

Key words: milking management

W144 Analysis of the bovine milk transcriptome by RNA sequencing. S. Wickramasinghe, G. Rincon, A. Islas-Trejo, and J. F. Medrano^{*}, *Dept. of Animal Science, University of California-Davis, Davis*.

Even though the chemical and physical properties of cow milk are well characterized, very limited research has been done on characterizing the milk transcriptome. The objective of this project was to perform a comprehensive expression profiling of genes expressed in milk somatic cells in Holstein cows. Milk samples were collected from 6 Holstein cows at d 15 (transition milk) and 250 (late) of lactation and RNA was extracted from the pelleted milk cells. Expression analysis was performed by RNA sequencing (RNaseq) using the Illumina GAII analyzer. Reads were assembled, annotated and analyzed in CLC Genomics workbench 3.7. Data was normalized by calculating the “reads per kilo base per million mapped reads” (RPKM) for each gene and annotated with Ensemble bovine annotation (24,580 unique genes). *t*-test was performed to identify the genes with significant changes in expression between the 2 stages of lactation. GenMAPP and MAPPFinder applications were used to determine the most significant gene ontology (GO) classifications (permutation $P \leq 0.05$) among these genes. The RPKM value of 0.3 was set as the threshold for detectable gene expression. The transition milk had 11,876 genes and late milk had 12,553 genes above the threshold. Genes encoding milk proteins had the most abundant transcripts in transition milk, and genes involved in immune regulation and cell defense had the most abundant transcripts in late lactation. Transition milk was enriched with gene ontology (GO) terms for Golgi vesicle transport while late milk was enriched with GO terms for DNA replication and signal transduction. ~8,000 genes had ubiquitous expression in milk and most of these genes were localized to intracellular organelles and intrinsic membranes. 4359 genes had significant change in expression ($P \leq 0.05$ and FDR $q \leq 0.2$) between the 2 stages, and these genes were mostly localized in extracellular matrix or vesicles. This is the first study to describe the comprehensive bovine milk transcriptome. Our results revealed that 48–51% of anno-

tated genes are expressed in the bovine milk and provided a valuable insight into the bovine lactome.

Key words: cow milk, gene expression, RNAseq

W146 Residual effects of incomplete udder emptying during milking in dairy cows. J. Guinard-Flament*, A. Albaaj, P.-G. Marnet, and C. Hurtaud, *UMR Production du Lait, INRA/Agrocampus OUEST, Saint-Gilles, France.*

Extended milking intervals reduce milk yield with residual effects on following milking intervals, which could depend on the quantity of milk stored in the udder. The aim of this trial was to simulate milk accumulation by decreasing udder emptying (100, 70, 40, and 0%) at one milking to describe the short-term effects on milk yield in relation with mammary morphology and epithelium permeability. Sixteen dairy cows (55 DIM) were assigned to treatments 100, 70, 40, and 0% according to a Latin square design with 4 7-d periods. Cows were milked twice daily at 0700 and 1730. Treatments were applied at the morning milking called M0 and milk yield was recorded on following milking (from M0 to M7). Changes in the udder morphology were assessed 1 and 10 h after M0 milking by estimating the distance between the 4 teat tips and the cisternal surface area using ultrasonography. The permeability of mammary epithelium was estimated using lactose concentrations in blood plasma measured 1 h before M0 and 4, 7, and 10 h after M0. The quantity of milk collected at M1 linearly increased as udder emptying decreased at M0. Nevertheless, because of milk accumulation in the udder, milk yield of M0+M1 curvilinearly decreased with treatments (42.9, 41.1, 36.4, 26.9 kg for 100, 70, 40 and 0%, respectively; SEM = 1.14). Residual effects on milk yield were observed only for 40 and 0% on M2 and M3 milking, and did not differ between 40 and 0% (e.g., 20.8 and 20.4 vs 21.9 kg for 100% at M2; SEM = 0.745). The udder cistern area was maximal just after M0 for 70, 40 and 0% treatments. However, udder continued to distend as shown by measurement of the distance between teats. Before M1, this distance was higher than after M0 and linearly increased as udder emptying decreased at M0. Increase in concentration of lactose in blood plasma occurred only for 40 and 0% and were observed respectively 10 and 4h after M0. In conclusion, dairy cows are poorly sensitive to low amounts of milk forgotten in the udder at one milking. When observed, residual effects on milk yield were associated with loss of the mammary epithelium integrity.

Key words: milk yield, incomplete milking, dairy cow

W147 Effect of prolactin-release inhibition on milk production and mammary gland involution at drying-off. S. Ollier*¹, X. Zhao², and P. Lacasse¹, 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.*

Drying-off is a challenging period for high-yielding cows as they are often dried off while still producing significant quantities of milk and, consequently, are highly susceptible to new intra-mammary infections. Once active involution is completed, the mammary gland becomes much more resistant to infection. Therefore, it is of critical importance to develop strategies to reduce milk production before the drying-off and to accelerate mammary gland involution. In this study, we assessed the effect of an inhibition of the lactogenic signal driven by prolactin (PRL) on milk production and on evolution of involution markers. Sixteen Holstein cows in late lactation were assigned to 2 treatments based on milk yield, somatic cell count and parity. Eight

cows received twice daily i.m. injection of quinagolide (2 mg per injection), a specific inhibitor of PRL-release, from 4 d before drying-off to 3 d after (Quin). The 8 others received injections of the solvent (water, Control). Blood and milk (mammary secretion) samples were collected on the last 5 d before and 1, 3, 5, 7, 10, and 14 d after the last milking. On the day preceding the first injection and the following day, several blood samples were also collected around milking time. Quinagolide reduced ($P < 0.01$) basal serum PRL concentrations on all the injection days and PRL released in blood during milking. The PRL inhibitor induced a decrease ($P < 0.05$) in milk production before drying-off, which averaged, over the last 3d of lactation, 19.3 and 15.5 kg/d for the control and the Quin groups, respectively. Quinagolide had no significant effect on milk citrate:lactoferrin and Na:K ratios, which decreased and increased respectively ($P < 0.001$) during the first 2 wk of the dry period. Nevertheless, the increases ($P < 0.001$) in somatic cells and bovine serum albumin in milk during early involution were greater ($P < 0.01$) in the Quin than the control cows. This experiment shows that inhibition of PRL-release induces a decrease in milk production of cows in late lactation. Changes in mammary secretion composition suggest that this approach is also hastening mammary gland involution.

Key words: quinagolide, dry period

W149 Putative stem/progenitor cell markers in lactating and re-developing bovine mammary glands. E. Brijs*, K. Singh, and A. Molenaar, *AgResearch Ltd., Ruakura Research Centre, Hamilton, New Zealand.*

The study of stem and progenitor cells in the bovine mammary gland is still developing, especially in the area of stem cell regenerative capacity during lactation. The objective of this study was to investigate whether the putative stem/progenitor cell markers integrin β 3 (CD61), keratin 5 (K5) and integrin β 1 (CD29) used in murine and human studies are expressed in the bovine mammary gland. Mammary gland biopsies were collected from 16 multiparous cows at near-peak (66 ± 3 DIM) and late lactation (226 ± 6 DIM), and at 30 (29 ± 10) and 10 (11 ± 6) days prepartum to the next lactation season. Qualitative immunohistochemistry (IHC) analysis demonstrated the presence of CD61 positively labeled cells in the basal epithelium, intra-alveolar cells and stromal cells at all 4 time points; however the tissue sections from late lactation and 30 d prepartum appeared to have a greater number of positively stained cells. The putative bipotent marker K5 had intense cytoplasmic labeling in the majority of the basal epithelium during the prepartum period in comparison to near-peak lactating tissue. Positive cells were also found in a luminal position. IHC analysis indicated that as alveolar become more differentiated in prepartum tissue there was a decrease in K5 expression. Real-time RT-PCR confirmed this expression pattern showing a 4.4- and 3.3-fold increase at 30 ($P < 0.001$) and 10 ($P < 0.001$) days prepartum, respectively, in comparison to near-peak lactation. Preliminary IHC analysis of CD29 indicated diffuse staining in the majority of basal epithelium at all 4 time points. However, RT-PCR showed a similar expression pattern to K5, with a 1.8-fold increase at 30 d prepartum ($P < 0.001$) and 1.5-fold increase at 10 d prepartum ($P < 0.01$) in comparison to post-peak lactation. Collectively these preliminary data show that these cell markers can be used to study stem/progenitor cells in bovine mammary tissue and suggest that stem cell activity is upregulated during mammary gland re-development.

Key words: stem cell markers, mammary gland, dairy cows

W150 Responses to steroidal doses and growth hormone treatment of nulliparous dairy ewes induced to lactate. M. Ben Khedim, G. Caja, A. K. K. Salama, A. Schalageter, E. Albanell, and M. Rovai*, *G2R, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

With the aim of improving the response to lactation induction protocols in dairy ewes, 2 treatments based on s.c. injections of estradiol and progesterone (d 1 to 7) at reduced doses (half dose: HD, 0.25 and 0.63 mg/kg BW, respectively; one third dose: TD, 0.17 and 0.42 mg/kg BW, respectively) and hydrocortisone (d 18 to 20; 50 mg/d), compared with a previously-tested protocol (Ramírez et al., 2008, *J. Dairy Sci.* 91), were applied in 47 ewes of 9 mo of age (Manchega, n = 24; Lacaune, n = 23). Ewes were penned in 8 groups and fed ad libitum a TMR. Machine milking (twice daily) started on d 21 and lactation success (Manchega, > 0.2 L/d; Lacaune > 0.4 L/d) was evaluated on d 35. Ewes under these thresholds were dried-off. Lactating ewes were treated with growth hormone (bST, 250 mg) on d 48 and 62 of lactation. Group intake was recorded daily during the experiment (4 wk before and 10 wk after milking started) with individual

estimations during lactation by using polyethylene glycol 6000 (50 g/d for 14 d). Treatments decreased DM intake according to the steroidal dose used (HD, -28%; TD, -18%; $P < 0.05$) but recovered thereafter. Lactation success was in the range of the standard protocol (55% on average) and did not vary by steroidal dose or breed. Onset of lactation increased DM intake 16% in both breeds ($P < 0.01$). Lacaune ewes produced nearly 2 times more milk than Manchega on d 14 of lactation and this varied with treatment (HD, 817 mL/d; TD, 458 mL/d; $P < 0.01$), whereas milk yield in Manchega did not vary with treatment (351 mL/d). No differences in milk composition were detected according to breed or treatment during the first 14 d of lactation. Milk yield increased with exogenous bST (Manchega, 114%; Lacaune, 90%; $P < 0.01$), but only a decrease in milk protein content ($P < 0.01$) and a numerically greater DM intake ($P = 0.16$) were detected when bST and control lactating ewe-lambs were compared. In conclusion, lactation induction success did not vary with treatment, but a breed \times treatment effect was observed in ewes induced to milk, and this was related to the hormonal environment and milk potential of each breed.

Key words: lactation induction, bST, sheep