

than S (392 vs 381 ±3.4 kg and 2.6 vs 3.3 ±0.12 mm; P<0.05), but the proportion of heifers that had two estruses was greater for A than W (48.6% vs 30.9%; P<0.05). Prolactin, initially 16.3±1.6 ng/ml, was higher for A than W from 4 to 12 wk, and lower for A than W from 16 to 24 wk (12 wk; 10.1 vs 1.1 and 24 wk; 6.7 vs 20.0 ±1.8 ng/ml, P<0.05). Extended photoperiod in autumn advanced puberty independently of the effects of diet on growth, and acute change in photoperiod influenced prolactin, in heifers housed outdoors.

Key Words: Photoperiod, Puberty, Prolactin

M42 Heat shock increases glutathione in bovine oocytes. R. R. Payton^{*1}, P. Coy², R. Romar², J. L. Lawrence¹, and J. L. Edwards¹, ¹The University of Tennessee, Knoxville, USA, ²The University of Murcia, Murcia, Spain.

Heat shock increases glutathione (GSH) content in a variety of cell types including embryos. Objective of this study was to examine GSH content in bovine oocytes cultured at an elevated temperature during maturation. Cumulus-oocyte complexes were randomly allocated to one of three treatments and then cultured in the following manner: 38.5°C for 24 h (Control), 41°C for 6 h followed by 38.5°C for 18 h (HS 0-6), or 41°C for

12 h followed by 38.5°C for 12 h (HS 0-12). After 24 hours, oocytes presumed mature were denuded of cumulus by vortexing. Pools of oocytes (25-32/treatment group) were solubilized in 0.63 M phosphoric acid and frozen at -20°C until further analysis. Glutathione content was determined using a 5,5'-dithiobis(2-nitrobenzoic acid)-glutathione disulfide reductase recycling assay and was expressed as per oocyte. Intra-assay coefficient of variation was 7.7%. Data were analyzed using an incomplete block design using mixed models of SAS after testing for normality. The experiment was replicated on 5 occasions and included a total of 8 to 11 pools of oocytes per treatment (236-330 total oocytes/treatment). Culture of oocytes at 41°C during the first 12 h of maturation increased GSH content (4.4 versus 2.7 and 1.6 pmol/oocyte for HS 0-12, Control and HS 0-6, respectively; SEM=0.57; P=0.02). Increases in an antioxidant such as GSH, suggest heat-induced increases in free radicals. Cytoplasmic perturbations involving increased free radical production may be one of several mechanisms contributing to reduced developmental competence of heat-stressed oocytes. Supported in part by USDA Initiative for Future Agricultural and Food Systems Program, "Improving Fertility of Heat-Stressed Dairy Cattle"; Grant #2001-52101-11318.

Key Words: Heat shock, Oocyte, Glutathione

Lactation Biology

M43 Intramammary infusion of prostaglandin E₂ (PGE) increases mammary development and milk yield of cows induced to lactate. J. M. Lukas^{*1}, W. J. Weber¹, R. J. Collier², J. L. Vicini³, M. F. McGrath³, and B. A. Crooker¹, ¹University of Minnesota, St. Paul, ²University of Arizona, ³Monsanto Agricultural Group, St. Louis, MO.

Effects of intramammary infusions of PGE on mammary development and milk yield of cows induced to lactate were evaluated using a half-udder model. Multiparous, nonpregnant, healthy, reproductive cull Holstein cows (N = 11) were dry for 50 d prior to treatment initiation. Cows were induced to lactate by twice daily subcutaneous (SQ) injections of 17β-estradiol (0.05 mg/kg BW/injection) and progesterone (0.125 mg/kg BW/injection) on d 1 through 7 and once daily SQ injections of POSILAC[®] (500 mg bST) on d 1, 11 and 21. On d 13, 16, 18 and 20, right and left quarters of each cow were infused with 10 ml of PGE (0.85 mg) or excipient and the quarters massaged to disperse infusate. Intramuscular injections of dexamethasone (0.05 mg/kg BW/d) were administered on d 21, 22 and 23 and 2X milking initiated on d 22. Cows received bST on d 31. Change in mammary gland development was assessed by photo documentation. Milk yield per quarter was determined for 14 d and half udder samples collected at 5 and 12 d in milk (DIM) for composition analyses by DHIA. Data from udder halves were analyzed as repeated measures using PROC MIXED of SAS with P < 0.05. Nine cows had visibly larger PGE treated half-udders by d 18. One cow was milked for only 2 d due to temperament. All cows were induced successfully as the untreated udder halves produced at least 4.5 kg/d by 14 DIM. Milk yields were greater in PGE half-udders in all cows on all days (5.1 vs. 9.4 ± 0.7 kg milk; 6.5 vs. 12.3 ± 0.8 kg FCM; 6.1 vs. 11.4 0.8 kg SCM). Milk composition did not differ between halves at 5 or 12 DIM. Milk fat content was unchanged (5.5 ± 0.2%) but protein (4.3 vs. 3.7 ± 0.1%) and log SCC (5.5 vs. 5.0 ± 0.1) decreased and lactose (4.7 vs. 5.0 ± 0.05%) increased from 5 to 12 DIM. Results indicate PGE either enhanced mammary development or differentiation resulting in increased milk yield from cows induced to lactate.

Key Words: Induced lactation, Mammary development, Milk yield

M44 Effects of induced lactation on milk fatty acid profiles in multiparous Holstein cows. H. C. Hafliker, III^{*1}, L. H. Baumgard¹, W. J. Weber², M. Chahine², G. C. Lamb², T. H. Klusmeyer³, M. F. McGrath³, J. L. Vicini³, and B. A. Crooker², ¹University of Arizona, ²University of Minnesota, ³Monsanto Animal Agriculture Group, St. Louis, MO.

Fatty acid profiles of milk from cows (previous 305 ME > 8,400 kg) that calved or were induced to lactate were compared to determine effects of induced lactation. Nonpregnant, reproductive culls were induced to lactate after a 50-d dry period by 2X/d subcutaneous (SQ) injection of 17β-estradiol (0.05 mg/kg BW/injection) and progesterone (0.125

mg/kg BW/injection) for 7 d (-13 to -7 DIM) and 0 or 1 SQ injection of POSILAC[®] (500 mg bST) at -13 and -3 DIM. An intramuscular injection of dexamethasone (0.05 mg/kg BW/d) was administered at 0 DIM and 3X milking initiated at 1 DIM. Induced cows received bST at 7 and 17 DIM and at 14-d intervals thereafter. Calved cows received bST at 14-d intervals after 63 DIM. Milk samples collected at 2 and 12 weeks of lactation (WOL) from a subset of successfully induced (10 of 34) and calved (10 of 19) cows were analyzed for fatty acid content. Method (calved, induced) and WOL effects were assessed by PROC MIXED of SAS with P < 0.05. Milk yield and composition of the subsets did not differ from their respective groups. During 2 and 12 WOL, milk yield of calved and induced cows averaged 40.6 and 22.4 kg/d. Milk from calved cows contained more protein (3.5 vs. 3.0%) and other solids (5.9 vs. 5.6%) but fat (4.3%) and log SCC (5.6) did not differ from induced cows. On a weight (mg/g) and molar (% of total moles) basis, *de novo* fatty acid synthesis and incorporation of preformed fatty acids into milk fat were similar for calved and induced cows. Substrate to product ratios (a proxy of Δ⁹-desaturase capability) of C_{14:0}/C_{14:1}, C_{16:0}/C_{16:1}, C_{18:0}/C_{18:1} and *trans*-11 C_{18:1}/*cis*-9, *trans*-11 CLA were 16 to 28% less for induced cows. Total percentage of *cis*-9, *trans*-11 CLA did not differ but contents of *trans*-C_{18:1} isomers were 17 to 20% less in induced cows. Although induced cows produced less milk and had an enhanced Δ⁹-desaturase system, overall milk fatty acid profiles were similar.

Key Words: Milk fat, Induced lactation

M45 Effects of different milking intervals on composition of cisternal and alveolar milk in dairy cows. M.A. Ayadi, G. Caja^{*}, X. Such, and E. Albanell, *Universitat Autònoma de Barcelona, Spain.*

Milk composition change in cisternal and alveolar compartments at different milking intervals has been rarely studied. Interest is higher as a consequence of robotic milking and milking omission routines. Four Holstein cows (20.5 kg/d, 215 DIM) regularly milked daily at 0800 and 1800 were used to study the effects of different milking intervals on cisternal milk (CIS) and alveolar milk (ALV) in a 5 wk experiment. Experimental milkings were made at random and in duplicate at 4, 8, 12, 16, 20 and 24 h after a regular milking. A wash-out period of 2 d with regular milkings was allowed between experimental milkings. A teat cannula was used to drain CIS after an i.v. injection of an oxytocin receptor blocking agent (Atosiban; 10 μg/kg). Oxytocin was then injected to remove ALV. Samples of each milk fraction per quarter were analyzed for composition. Ratio of CIS:ALV varied according to milking interval and averaged 30:70. Milk fat content decreased in CIS and increased in ALV as milking interval increased (P<0.001). Minimum fat percentage in front and rear CIS (0.93%) was reached at the same milking interval (16 h). Milk fat content in ALV was constant during the first 16 h, increasing rapidly thereafter. Final fat content in ALV (6.95%) was higher than CIS initial (5.62%; P<0.05) and final (0.96%; P<0.001) values. Total

fat yield tended to increase for CIS with longer milking intervals, but increased markedly for ALV ($P < 0.001$), indicating that fat globules do not pass freely from the alveoli to the cistern between milkings. Milk protein content increased in CIS ($P < 0.001$) and tended to increase in ALV with longer milking intervals. Initial and final milk protein content did not differ between CIS and ALV. Total protein yield increased with milking interval in both fractions ($P < 0.05$) and was greater in the rear quarters than in the front quarters ($P < 0.01$) for both milk fractions. We concluded that effects of milking interval on milk composition can be explained by changes in alveolar and cisternal milk ratio.

Key Words: Milk composition, Alveolar milk, Cisternal milk

M46 Description of glucose transport in isolated bovine mammary epithelial cells by a 3-compartment model. C. T. Xiao*, V. M. Quinton, and J. P. Cant, *University of Guelph, Ontario, Canada.*

The carrier-mediated glucose transport in isolated bovine mammary epithelial cells displays moderate degrees of asymmetry and cooperative interactions between export and import sites when described by a fixed-site carrier model. The present study examines the hypothesis that these model features are due to compartmentalization of intracellular glucose. Net uptake of 3-O-methyl-D-[1-³H]glucose (3OMG) by isolated bovine mammary epithelial cells was measured at 37 °C. The time course curve of 3OMG net uptake could be better fitted by a double exponential equation than a single or triple exponential equation. Compartmental analysis of the time course curve suggested that translocated 3OMG is distributed into two compartments with fractional glucose spaces of $32.6 \pm 5.7\%$ and $67.4 \pm 5.7\%$, respectively. The results support the view that glucose transport in bovine mammary epithelial cells is a multi-step process consisting of two serial steps: fast, carrier-mediated, symmetric translocation of sugar across the cell plasma membrane into a small compartment, and subsequent slow exchange of post-translocated sugar between two intracellular compartments. A 3-compartment model of this system successfully simulated the observed time course of 3OMG net uptake ($R^2 = 0.98$) and the observed dependence of unidirectional entry rates on intra- and extracellular 3OMG concentrations ($R^2 = 0.99$). Parameters of the fixed-site carrier model derived from the simulation results represented a significant degree of asymmetry and a moderate degree of negative cooperativity. The results indicate that compartmentalization of intracellular glucose exerts significant effects on glucose transport behavior and should be considered when modeling this process.

Key Words: Glucose transport, Bovine mammary epithelial cell, Model

M47 Over-expression of IGF-I in lactating porcine mammary tissue has a differential effect on amino acid transport systems. D. E. Gronlund, W. L. Hurley*, M. H. Monaco, M. B. Wheeler, and S. M. Donovan, *University of Illinois.*

Uptake of amino acids (AA) by the lactating mammary gland is critical for milk protein synthesis. Several transport systems are responsible for AA uptake by mammary epithelial cells. Understanding of the regulation of AA transport is limited, but may include regulation by mammary growth factors. We compared the kinetic properties of a sodium-independent AA transport system (for lysine) and a sodium-dependent AA transport system (for taurine) in mammary tissue from nontransgenic sows (control; $n = 5$) and transgenic sows ($n = 5$) that over-express insulin-like growth factor-I (IGF-I) in their mammary gland during lactation, under the direction of the α -lactalbumin promoter. Mammary tissue was collected on day 20 ± 1.8 and 21 ± 2.1 of lactation for control and transgenic sows. Tissue explants were incubated in isosmotic physiological buffer with the respective radiolabeled AA tracer and either lysine from 50 μM to 12.8 mM, or taurine from 1.25 μM to 320 μM . Extracellular volume of explants was determined using radiolabeled sucrose as a nonpermeable tracer. Kinetic parameters for lysine transport were not different between control and transgenic sows ($K_m = 2.1 \pm 0.4$ vs 1.5 ± 0.2 mM; $V_{max} = 6.1 \pm 0.7$ vs 7.5 ± 1.6 mmol/kg cell water/30 min). The K_m of taurine transport was not different between control and transgenic sows (20 ± 3.1 vs 30 ± 5.0 μM). However, the V_{max} of taurine transport was significantly lower for control vs transgenic sows (70 ± 16 vs 118 ± 29 $\mu\text{mol/kg cell water/30 min}$; $P < 0.05$). Mammary over-expression of IGF-I by sow mammary tissue did not affect lysine uptake, a sodium-independent transport system, nor did it affect the affinity of the taurine transport system. However, the maximal rate of

taurine transport was increased in sow mammary tissue of IGF-I transgenic sows, suggesting that the effect of IGF-I over-expression may occur through a regulation of cellular sodium transport systems. Funded in part by the USDA CSREES under project NRICGP 00-35206.

Key Words: Mammary gland, Amino acid transport, Growth factors

M48 The acyclic period postpartum in automatic and conventional milking. D. Weiss*¹, M. Reist², and R. M. Bruckmaier¹, ¹*Inst. of Physiology, Technical Univ. Munich, Germany,* ²*Novartis Centre de Recherche Sante Animal St-Aubin, Switzerland.*

The postpartum acyclic period is prolonged in cows due to frequent suckling as compared to twice daily milking. In conventional milking systems twice daily (2X) milking is typical, while in automatic milking systems (AMS), three and more daily milkings are common in early lactation. This study tested whether increased milking frequency in AMS delays postpartum cyclic activity and pregnancy in dairy cows. Simmental cows ($n=124$) were studied during the postpartum period until the onset of the ovarian cycle and pregnancy. Calvings were evenly distributed throughout the one-year experimental period. Cows were housed in one barn, and 62 cows were milked voluntarily in the AMS (AC), while 62 cows were milked in the conventional milking parlor (PC) 2X. Other management was identical for both groups. Milk yields and number of milkings were recorded in both systems. Milk samples were analyzed for progesterone twice weekly to determine ovarian activity until cows were pregnant. First and second ovulation was determined by progesterone profiles of each individual cow. The mean milking frequency during the first 3 weeks after parturition was higher in AC (3.030.02) than the 2X milking in PC. Individual milking frequency within AC for the first 3 weeks varied between 2.080.11 and 4.280.02 milkings. Milk yield in the first 6 weeks was higher ($p < 0.05$) in AC compared to PC (30.00.1 vs. 22.70.2 kg/d). The day of the first and second ovulation did not differ between groups (27.61.5 and 36.41.4 vs. 27.51.1 and 36.11.3 in AC and PC, respectively). Within AC no relation was observed between milking frequency and duration of acyclic period. The first and second ovulation was earlier ($p < 0.05$) in primiparous cows (24.00.1 and 34.11.3 d) as compared to multiparous cows (30.50.1 and 38.31.4 d). Days open was similar in AC (73.47.1 d) and PC (74.75.7 d). Increased milking frequency in AMS did not delay postpartum cyclic activity and pregnancy in dairy cows.

Key Words: Acyclicity postpartum, Milking frequency, Automatic milking

M49 Change from conventional to automatic milking in cows with and without previous experience. D. Weiss* and R. M. Bruckmaier, *Institute of Physiology, Technical University Munich, Germany.*

Effects of a change from milking in a conventional parlor to an automatic milking system (AMS) on milk yield was investigated. Cows had either no experience in AMS milking ($n=17$) or were previously milked for at least one lactation in the AMS ($n=9$). Experienced cows (EC) were older (3.6 ± 0.5 lactations) than unexperienced cows (UC; 1.8 ± 0.3 lactations). EC were milked for 36.4 ± 5.1 d after parturition in the parlor before changing to the AMS. EC obtained no training before changeover. Milk yields were recorded in the parlor for 10 d prior to changeover. Milk yield in the parlor was 26.3 ± 2.3 kg/d in UC and 37.6 ± 1.7 kg/d in EC. UC were trained intensively in the AMS area for 3 d before changeover, while still being milked in the parlor, and the first AMS milking was performed on the fourth day, i.e., after at least 8 visits to the AMS. Although offered concentrate, UC had to be pushed into the AMS milking stall for the first one to two visits. EC entered the AMS milking stall voluntarily. In UC the rate of voluntary visits were 0, 32, 48, 56, 81, 86, 91, 94, 93 and 97% during the first 10 d of AMS milking. In UC, milk letdown was disturbed during the first visits and mean yield at the first milking in the AMS was lower than in the parlor ($67.5 \pm 4.8\%$, $p < 0.05$). In EC, milk letdown was not disturbed and yield at the first AMS milking was $101 \pm 3\%$ of the yield in the parlor. Rate of voluntary visits and the degree of disturbance of milk letdown in UC was independent of lactation stage and age of the animals. During the first 10 d of milking in the AMS the mean frequency was 2.67 and 2.88 milkings/d in UC and EC. Milk yield at the first 15 AMS milkings was lower in UC ($86.8 \pm 4.7\%$) and higher in EC ($108.5 \pm 1.8\%$) compared to the yield obtained in the parlor ($p < 0.05$). In conclusion, cows with previous experience to AMS milking do not need an adaptation period after transient

parlor milking. Furthermore, milk yield is increased in these cows from the increased milking frequency. In contrast, unexperienced cows need intensive adaptation to the AMS.

Key Words: Automatic milking, Milk yield, Dairy cow

M50 Use of digital pictures to study udder morphology in dairy sheep. M. Rovai¹*, D. L. Thomas¹, Y. M. Berger¹, and G. Caja², ¹University of Wisconsin-Madison, ²Universitat Autònoma de Barcelona, Bellaterra, Spain.

Ewe udder shape and size are related to milk yield and milking time, and culling for undesirable udder traits can improve the efficiency of machine milking. Measurements from digital pictures of ewe udders may provide an easy and accurate method for measuring ewe udders. Udder traits were measured on 120 dairy ewes and from digital pictures of their udders taken at the time of the in vivo measurements. Measurements were taken at wk 5, 11, and 17 of lactation 4 hr before the pm milking. Ewes were milked 2X/d. Udder height, udder width, teat length, teat angle, and cistern height were measured in vivo using a ruler and protractor. Udders also were assigned scores from the 9-point scoring system developed by De la Fuente et al. (1995) for teat size, teat angle, udder height, and udder shape. Following in vivo scores and measurements, digital pictures of the rear udder of each ewe were taken. While taking each picture, a ruler was held parallel to the ground in the same vertical plane as the back of the udder and a few cm below the bottom of the udder to serve as a calibration device for measurements on the digital pictures. Likewise a plumb bob was suspended vertically in back and in the middle of the udder while taking each picture to give a true vertical line as a reference for measuring teat angle. Measurements from the digital pictures were obtained using the public domain software, Image Tool from Texas University, available on the Internet. All digital measurements were significantly ($P < 0.0001$) correlated with those measured in vivo. Correlations were 0.73 for udder height, 0.67 for udder width, 0.47 for teat length, 0.88 for teat angle, 0.68 for teat size score, 0.79 for teat angle score, 0.88 for udder height score, and 0.89 for udder shape score. Advantages of the digital picture method over in vivo measurements are that pictures can be taken faster than the in vivo measurements at the farm, they can be analyzed at your convenience, and they provide a permanent record for future use.

Key Words: Digital pictures, Dairy sheep, Udder traits

M51 Udder traits of dairy ewes on U.S. commercial farms and their effects on milk yield. M. Rovai¹*, D. L. Thomas¹, Y. M. Berger¹, and G. Caja², ¹Univ. of Wisconsin-Madison, ²Univ. Autònoma de Barcelona, Spain.

Rapid scoring systems have been developed in Europe to categorize udder shapes of dairy ewes, and these scores are related to milk yield and milking time. These scoring systems were evaluated in U.S. ewes of dairy-meat crosses. Ewes were scored by one classifier 3 hr before the pm milking using a linear udder scoring system (De la Fuente et al., 1995) based on a 9-point scale per trait: udder depth, teat angle, and udder shape. Udders also were scored for typology (Gallego et al., 1983): 1 = horizontal teats, 2 = teats at 45 degrees, 3 = vertical teats, and 4 = misshaped udder. Ewes were from two commercial dairy sheep farms (A, n = 177; B, n = 166) and one university farm (C, n = 120). Ewes were milked 2X/d. Flocks differed in breed composition: A: East

Friesian (EF) crosses (10 to 50% EF); B: EF crosses (10 to 75% EF); and C: EF (50 or 75% EF), 50% Lacaune (LC), and 25% EF-50% LC (EF-LC) crosses. Remainder of breeding was of non-dairy domestic breeds. Effects of parity and stage of lactation also were considered. Percentage of EF breeding had no effect on milk production or udder trait scores in farms A and B. In farm C, LC ewes had the most ($P < 0.01$) horizontal teats, and EF-LC ewes had the highest ($P < 0.0001$) milk yield. Milk production ($P < 0.05$) and udder depth score ($P < 0.001$) increased significantly as parity number increased in all farms. Udder depth score and milk production decreased ($P < 0.05$) through lactation in farms A and B. Udder depth scores did not change with stage of lactation in farm C. Ewes in later lactation tended to have more horizontal teats and faulty (type 4) udders than ewes in earlier lactation. Udders of type 2 were more frequent than other types in all flocks. Farm C also had a high percentage of ewes with udders of type 1 which may be related to LC ewes. Within all genotypes and farms, positive correlations were observed between udder depth scores and milk yield (0.20 to 0.46; $P < 0.05$). High correlations were observed between udder shape and teat angle scores (0.80 to 0.93; $P < 0.0001$).

Key Words: Dairy sheep, Udder traits, Linear scores

M52 Udder traits of U.S. dairy ewes and their effects on milking time and milk yield. M. Rovai¹*, D. L. Thomas¹, Y. M. Berger¹, and G. Caja², ¹Univ. of Wisconsin-Madison, ²Univ. Autònoma de Barcelona, Spain.

Udder shape and size is related to milk yield and milking time in specialized dairy sheep breeds in Europe. This study determined if similar relationships exist among U.S. dairy-meat cross ewes. Ewes (n=120) of 4 breed groups (50% East Friesian, EF; 75% East Friesian, EE; 50% Lacaune; LC; and 25% East Friesian-50% Lacaune crosses, EF-LC; remainder breeding of each group was domestic non-dairy breeds) were utilized. Ewes were milked 2X/d. Measurement of udder size (depth, height, width, and circumference), teat size (length and width), teat angle, and cistern height was done 6 hr after the am milking by one technician at wk 5, 11, and 17 of lactation. Cistern area by ultrasonography and kinetics of milk emission (lag time, volume the 1st minute, total volume, and milking time) also were measured. Cisternal scans were obtained by a portable ultrasound scanner with 3.5 MHz sectorial transducer. Milk yield was highest ($P < 0.0001$) in EF-LC ewes, increased ($P < 0.001$) with age, and decreased ($P < 0.0001$) through lactation. LC ewes had the shortest ($P < 0.01$) teats (3cm) and highest ($P < 0.0001$) teat insertion (59). Cistern height and udder size were larger ($P < 0.05$) in LC and EF-LC ewes than in the other two breed groups. EE and EF-LC dairy ewes had greater ($P < 0.001$) cistern area (30 and 32 cm²) than ewes of the other two breeds. Udder and teat size increased ($P < 0.001$) with parity. Udder size decreased ($P < 0.0001$) through lactation while teat angle and cistern height increased ($P < 0.0001$). Cistern area decreased through lactation ($P < 0.0001$) and increased ($P < 0.0001$) with parity. Total milking time was greatest ($P < 0.05$) in EF-LC ewes, increased ($P < 0.05$) with parity, and decreased ($P < 0.05$) during lactation. Udder traits correlated with daily milk yield ($r = 0.21$ to 0.50 ; $P < 0.01$) and milking kinetics ($r = 0.15$ to 0.38 ; $P < 0.05$). Cisternal area correlated with daily milk yield ($r = 0.63$; $P < 0.0001$), milk volume during the 1st minute ($r = 0.34$; $P < 0.0001$), measures of teat size ($r = 0.18$ to 0.25 ; $P < 0.01$), and udder height ($r = 0.20$; $P < 0.01$).

Key Words: Dairy ewes, Udder traits, Milking kinetics

Animal Health

M53 Binding of IgM to non-apoptotic bovine blood neutrophils. S. N. Knight^{*}, M. Worku, and P. L. Matterson, NC Agricultural & Technical State University, Greensboro, NC.

Receptors for IgM have been identified on bovine neutrophils. The objective of this study was to evaluate the association of IgM binding with apoptosis of bovine blood neutrophils. A modified assay to detect apoptosis by comparing the effect of actinomycin-D (160 μ M), sodium butyrate (160 μ M), E.Coli lipopolysaccharide (LPS) (10ng/ml) treatments versus untreated isolated neutrophils in the presence or absence of purified bovine IgM was used. Whole blood was collected from healthy, lactating Holstein cows (N=4) in 15 ml vacutainer blood collection tubes pretreated with 250 IU of heparin sodium. The blood was pooled, diluted with 1X PBS, separated by gentle centrifugation and RBC were

lysed with 0.83% ammonium chloride several times until a white pellet and clear supernatant was obtained. Viable, isolated PMN were verified by microscopic observation and counting, using Trypan Blue exclusion for viability (98.0%) and Wright stain differentials. Treated and control PMN were spotted onto poly-L-Lysine, subbed slides. After drying, slides were then assayed for the apoptosis using Promega's Apoptosis Detection Kit which is based on the TUNEL method of labeling fragmented DNA of apoptotic cells with Fluorescein. The percentage of cells incorporating green fluorescence was evaluated microscopically. Neutrophil isolation, Actinomycin D and Dexamethasone induced apoptosis. Bacterial endotoxin, Sodium butyrate and IgM binding showed the least amount of apoptosis. Treatment with IgM had no effect on apoptosis