Growth and Development Symposium: Participation of Adult Tissue-Restricted Stem Cells in Livestock Growth and Development

737 Regulation of skeletal muscle satellite cell chemotaxis. R. E. Allen* and X. Liu, *University of Arizona, Tucson.*

The objective of this study was to determine the role of the stromal derived factor 1 and its receptor CXCR4 in chemotaxis of skeletal muscle satellite cells. Skeletal muscle satellite cells in postnatal muscle serve as a pool of muscle precursor cells for muscle growth and muscle repair. Over the past 3 decades, a great deal has been learned about the regulation of satellite cell activation, proliferation and differentiation into muscle fibers; these events are associated with muscle fiber growth and hypertrophy. Repair of damaged muscle, however, requires an additional biological response, migration of satellite cells to the site of fiber damage. The response has not been well characterized. Recent experiments have identified the chemokine receptor CXCR4 and its ligand, stromal-derived factor 1 (SDF-1), as potential regulators of satellite cell chemotaxis. In experiments with rat muscle, CXCR4 was upregulated when satellite cells were activated from quiescence and entered the cell cycle. Furthermore, SDF-1 was not expressed in uninjured muscle, but it was found at the site of muscle injury. In vitro experiments demonstrated satellite cell chemotaxis in response to SDF-1. The ability of satellite cells to migrate toward SDF-1 in living muscle was demonstrated in experiments where isolated satellite cells were injected into the proximal end of rat tibialis anterior muscle, and SDF-1 was deposited in the distal end of the muscle in a collagen gel. In these experiments satellite cells were labeled with Quantum Dots in culture, before injection into muscle. Within 3 d labeled cells were found at the injury site. Treatment of rats with the CXCR4 inhibitor, AMD3100, inhibited cell migration to the SDF-1 injection site, and AMD3100 inhibited migration of injected cells to sites of muscle injury. Skeletal muscle sustains minor and even major injury throughout life, and normal muscle function and subsequent meat quality depend on the ability of satellite cells seek out sites of injury and repair the damage. Results from these and additional experiments suggest that CXCR4 and SDF-1 play a role in this process.

Key Words: muscle, satellite cell, chemotaxis

738 Potentials of male germline stem cells to influence the efficiency of beef cattle production. J. M. Oatley,* *College of Veterinary Medicine, Washington State University, Pullman.*

Genetic gain is the increase in performance characteristics from generation to generation and in cattle populations is primarily made through the male germline. Sperm are the vehicle by which male genetic contributions are passed to the next generation and expanded use of these cells from specific sires has a major impact on production characteristics of cattle populations. Artificial insemination technology has been widely used as a reproductive tool in dairy cattle production to exploit this concept; however, utilization in beef cattle production is limited due to intensive management required for effective implementation which is not conducive with current strategies for managing most the world's beef cattle populations. Thus, there is need for alternative reproductive tools that expand availability of sperm from specific sires. The foundation for spermatogenesis is provided by the actions of testicular germline stem cells which possess the capacity for unlimited self-renewal and generation of committed progenitor cells that terminally differentiate into sperm. Research with rodents has shown that these cells can be isolated from testes of a donor male and transplanted into testes of recipient males in which donor-derived spermatogenesis occurs and offspring with donor haplotype produced after natural breeding. Adapting this methodology for cattle could provide an efficient means to expand the use of germlines from specific sires without the requirement for intensive management. Because stem cells are rare, a period of in vitro expansion following isolation from donor testes is required to provide an abundance of cells for efficacious engraftment following transplant. In recent studies, we have developed a multiparameter selection approach for isolating a culture-viable cell fraction from bovine testes that is enriched for germline stem cells. Moreover, we have devised a method for long-term maintenance of these cells in vitro that is xeno-free, thereby meeting a key requirement for practical application. These advances provide crucial building blocks for developing germline stem cell transplantation methodology into a utilizable reproductive tool for modern beef cattle production that will influence genetic gain.

Key Words: germline stem cells, cattle, reproduction

739 Tenocytic potential of equine umbilical cord derived stem cells. S. A. Reed*[1] and S. E. Johnson[2], *[1]University of Connecticut, Storrs, [2]University of Florida, Gainesville.*

Mesenchymal stem cells offer promise as therapeutic aids in the repair of tendon, ligament, cartilage, and bone damage in the sport horse. Umbilical cord blood (UCB) contains a population of putative stem cells that may be harnessed to aid in the repair of these injuries. Equine UCB derived stem cells express Oct4, Nanog, and Sox2 in a manner similar to embryonic stem cells and can differentiate into a variety of less naïve cell types including those with osteogenic, chondrogenic, hepatogenic and myogenic properties. The objective of this work was to determine if equine UCB derived stem cells (eUCB-MSC) are capable of differentiation into tenocyte-like cells. Equine UCB stem cells (n = 3) were cultured on gelatin or matrigel coated dishes or gelatin coated beads and treated with 10 ng/mL fibroblast growth factor (FGF)-2, - 4, or −5. Expression of *scleraxis* (*scx*), a transcription factor required for tendon development, and *Tenascin-C* (*TnC*), a protein abundant in developing tendons, was measured by real-time PCR (reported as mean ± SEM). Growth kinetics were measured following FGF supplementation. Equine adipose derived stem cells (AdMSC) were used to compare eUCB-MSC to a more adult stem cell population. Both eUCB-MSC and AdMSC express *scx* and *TnC*. When cultured on varying matrices, eUCB-MSC and AdMSC adapted different morphologies. Further, culture in matrigel increased *scx* expression in both cell populations (eUCB-MSC: 6.73 ± 2.1 fold; AdMSC: 39.5 ± 26.6 fold, $P < 0.05$) and increased *TnC* 12.3 ± 1.85 fold in AdMSC ($P < 0.05$). In AdMSC culture in matrigel, supplementation with FGF2 or FGF5 increased *TnC* expression 1.84 ± 0.22 fold and 2.66 ± 0.48 fold, respectively. Fibroblast growth factors affected growth kinetics in AdMSC and eUCB-MSC. In conclusion, both eUCB-MSC and AdMSC express markers of early tenocytes. *Scx* expression is increased when cultured in matrigel, a 3 dimensional matrix. FGF supplementation further increased *TnC* expression in AdMSC. These results demonstrate a potential use for AdMSC and/or eUCB-MSC in future therapies to improve tendon healing.

Key Words: umbilical cord blood, horse, tendon

740 Development, characterization and use of a porcine epiblast-derived liver stem cell line: ARS-PICM-19. T. J. Caperna,* W. M. Garrett, and N. C. Talbot, *USDA/ARS, Beltsville, MD.*

Totipotent embryonic stem cell lines have not been established from ungulates, however, we have developed several somatic cell lines from the in vitro culture of pig epiblast cells. One such cell line, PICM-19, was isolated via colony-cloning and was found to spontaneously differentiate into hepatic parenchymal epithelial cell types, viz., hepatocytes (H) and bile duct cells (BD). Hepatocytes form as monolayers and BD as 3D bile ductules. PICM-19 cells are sensitive to variations in extracellular pH in that low pH induces H differentiation and high pH elicits BD differentiation. Transmission electron microscopy revealed that BD were composed of radially arranged, monociliated cells with their cilia projecting into the ductuleâ€s lumen, whereas H were arranged in monolayers with lateral canalicular structures containing numerous microvilli and connected by tight junctions and desmosomes. Extensive Golgi and rough endoplasmic reticulum networks were also present in H and BD, indicative of active protein synthesis. Analysis of conditioned media by 2D-electrophoresis and mass spectrometry indicated a broad spectrum of serum protein secretion by H. The PICM-19 cell line maintains a range of inducible cytochrome P450 activities, and, most notably, is the only non-transformed cell line that synthesizes urea in response to ammonia challenge. In collaboration with NASA, PICM-19 cells were placed on orbit (Shuttle Mission STS 126) where it was determined that short-term (14 d) microgravity exposure did not affect hepatic cellular differentiation. A PICM-19 subclone which only differentiates into H was isolated. It was evaluated as a platform for toxicity testing and has been utilized in a commercial artificial liver rescue device bioreactor (Hepalife Biosystems). Methods are currently under development to improve the growth of PICM-19 cells without feeder cells. Feeder-cell independent growth will facilitate the study of mesenchymal-parenchymal interactions that influence the divergent differentiation into H or BD, will enhance our ability to genetically modify the cells, and will provide a better model system to investigate porcine hepatic metabolism.

Key Words: liver stem cells, hepatocyte, bile duct

741 Mammary stem cells: Novel markers and novel approaches to increase lactation efficiency. A. V. Capuco*[1], R. K. Choudhary[1,2], C. M. Evock-Clover[1], and K. M. Daniels[3], *[1]Bovine Functional Genomics Lab, USDA-ARS, Beltsville, MD, [2]Department of Animal and Food Sciences, University of Kentucky, Lexington, [3]Department of Animal Sciences, The Ohio State University, Wooster.*

Mammary stem cells (MaSC) provide for net growth, renewal and turnover of mammary epithelial cells, and are therefore potential targets for strategies to increase production efficiency. Appropriate regulation of MaSC can potentially benefit milk yield, persistency, dry period management and tissue repair. Accordingly, we and others have attempted to characterize and alter the function of bovine MaSC. Approaches used have included flow cytometry and in vitro cultivation to enrich for and characterize these cells. Recent data indicate that MaSC retain labeled DNA for extended periods. Relying on this long-term retention of bromodeoxyuridine-labeled DNA, we identified putative bovine MaSC and hypothesized that the label retaining epithelial cells (LREC) present in the basal layer of the mammary epithelium represent MaSC. As in other species, these cells were present in low abundance within mammary epithelium (<1%) and were estrogen receptor-negative. We recently excised LREC and control cells from the mammary epithelium, using laser microdissection, and characterized their transcriptome by microarray analysis. Molecular profiles were consistent with the concept that LREC represent populations of MaSC and progenitor cells, that basal LREC are enriched for MaSC, and that LREC in suprabasal locations are enriched for committed progenitors. Analysis also provided novel candidate biomarkers for MaSC/progenitors. Potential biomarkers currently under investigation include NR5A2, NUP153, FNDC3B and HNF4A. Cells bearing these biomarkers are present in abundance and localization consistent with their utility as MaSC markers. We have attempted to modulate MaSC number in vivo and in vitro. Infusing a solution of xanthosine through the teat canal and into the mammary ductal network of prepubertal heifers and treatment of bovine mammary epithelial cells in vitro, increased the number of putative MaSC/progenitors. This was evidenced in vivo by an increase in the percentage of LREC and increased telomerase activity and in vitro by increased FNDC3B labeling and telomerase activity. The exciting possibility that stem cell expansion can influence milk production is under investigation.

Key Words: stem cells, progenitor cells, mammary