

greatest personnel availability. Creating planned kidding periods has been previously recognized as advantageous to resource utilization within dairy operations. However, there have been concerns raised that this induction of parturition may possibly lead to increased complications in does such as abnormal cervical/vaginal dilation, insufficient ligament relaxation or general dystocias. Additionally, there were questions surrounding the potential production of underdeveloped or compromised kids. Therefore, a retrospective analysis was conducted to determine whether the induction of parturition in dairy goats was associated with increased complications in the doe or increased kid mortality at kidding. From 2004 to 2005, 362 dairy goats of varying breeds (predominantly Saanen/Alpine crosses) were treated intramuscularly with dexamethasone (10 mg) and Lutalyse® (5 mg) to induce parturition between days 145 and 149 of gestation. The outcomes of these kiddings were compared to 1595 natural kidding events that occurred from 2000 to 2005. Pregnancies in both groups were derived from multiple assisted reproductive technologies (ART) including standard pen breeding, artificial insemination, in vitro fertilization, microinjection, and somatic cell nuclear transfer. The complications identified at birth ranged from dystocias (including deliveries by caesarian section) to uterine tears, vaginal tears, vulvar tears, and vaginal/vulvar bruising. In some cases, these complications led ultimately to doe mortality. Kid mortalities included kids that presented either dead at birth with no discernible cause for death or kids that failed to thrive in the first 5 days after birth that were subsequently euthanized. Data was analyzed using Chi-square analysis. There was no difference in dam complications between natural kiddings and induced kiddings (13.1% versus 14.4%, respectively). However, there was a significant difference in mortality rates between 2700 kids born from natural kiddings compared with 652 kids born from induced kiddings with a higher loss of kids born through the natural birthing process (7.0% versus 2.6%, respectively). It was apparent from this retrospective analysis that management practices associated with induced kidding events can lead to increased kid survival which is beneficial to herd efficiencies without causing increased complications to the does. Additionally, induction of parturition creates defined kidding windows that allow for careful attendance of the kidding process while maximizing utilization of resources, and this practice leads to a beneficial earlier intervention in the event of any kidding complications. In summary, induction of parturition using dexamethasone and Lutalyse® is a beneficial tool to be considered when mana-

ging kidding events to maximize resource utilization while at the same time increasing kid survival.

Keywords: Caprine; Induced parturition; Birth complications

EFFECTS OF FSH AND PTU ON SERTOLI CELL PROLIFERATION IN PORCINE TESTICULAR TISSUE

S.G. Clark¹, J.A. Ford Jr.², J. Baldrighi¹, M. Mello³, L. Franca⁴, K.I. Carnes², R.A. Hess²

¹*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA;* ²*Department of Veterinary Biosciences, University of Illinois, Urbana, IL, USA;* ³*Department of Animal Sciences, University of Illinois, Urbana, IL, USA;* ⁴*Federal University of Minas Gerais, Belo Horizonte, Brazil*

Regulation of the cell cycle involves a complex and orderly sequence of events that are critical in determining the fate of eukaryotic cells toward proliferation or differentiation. Very little is known regarding the factors involved in Sertoli cell cycle in mammals in general and pigs in particular. Follicle-stimulating hormone (FSH) is the major factor responsible for postnatal Sertoli cell proliferation in laboratory rodents and primates, although studies in the pig suggest that FSH is not as tightly correlated with the increase in number of Sertoli cells. Additionally, the goitrogen 6-*n*-propyl-2-thiouracil (PTU), when given to induce transient neonatal hypothyroidism, leads to a tremendous increase in testis size, number of Sertoli cells and sperm production in rodents. In pigs, however, Sertoli cell proliferation is diminished by the postnatal depletion of thyroid hormone. The objective of this study was to determine the effects of PTU and FSH hormones on Sertoli cell proliferation in the pig testes. Testes were collected from 14-day old piglets, and the tissue was dissected into 5 mm³ pieces for transplantation under the skin of castrated nude mice. Two days post-surgery mice were randomly assigned to one of four treatment groups: control, PTU (0.015%), FSH (5 IU rFSH), and PTU + FSH. Following 14 days of treatment testes pieces were harvested, fixed by immersion in neutral buffered formalin, embedded in paraffin, and sectioned. For immunohistochemistry paraffin was removed, sections rehydrated, endogenous peptidases blocked, and target antigens were unmasked by boiling in 0.01 M citrate buffer. The following antibodies were used to examine Sertoli cell proliferation: GATA-4 (1:200 dilution; transcription factor specific for developing and adult pig Sertoli cells) and Ki-67 (1:500 dilution; a

nuclear protein present in all phases of the cell cycle except G0). The sections were incubated overnight at 4 °C with the appropriate antibody. The slides were then incubated with an appropriate secondary antibody, visualized with DAB chromagen, and counterstained with Mayer's hematoxylin. GATA-4 stained intensely in all treatments. Statistical differences between treatment groups were detected using paired Student's *t*-tests. Positive staining for Ki-67 was found in $19.9 \pm 0.7\%$ and $20.0 \pm 1.8\%$ of the Sertoli cells from testes in control and FSH treated nude mice, respectively. The percentage of Sertoli cells positive for Ki-67 was increased in PTU ($27.9 \pm 3.4\%$, $p = 0.06$) and slightly increased in the PTU + FSH ($22.1 \pm 5.6\%$, $p = 0.36$) treated nude mice when compared with control treated mice. The results indicate that PTU is a key regulator of Sertoli cell proliferation in pigs, although the interactions between PTU and FSH remain unclear. Further studies of the interaction of these and other regulatory factors are necessary to completely understand the porcine Sertoli cell cycle.

Keywords: Testis; Porcine; Follicle stimulating hormone; Propylthiouracil

CLONED HOLSTEIN BULLS WITH DIFFERING RESPONSES TO HEPARIN-INDUCED IN VITRO SPERM CAPACITATION

H. Galantino-Homer¹, S. Kurosaka², C.E. Marshall³, M. Modelski¹, Y. Miller-Lux¹, K.J. McLaughlin², I. Dobrinski¹, G. Althouse¹

¹Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA; ²Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA; ³Select Sires, Inc., Plain City, OH, USA

Recent reports of semen analysis, in vitro fertilization (IVF), and breeding trials to compare genetically identical (cloned) bulls generated by either blastomere splitting or somatic cell nuclear transfer (SCNT) have revealed no significant differences in these parameters. As more details of the many molecular changes in sperm required for fertilization are determined, more sensitive and specific assays of sperm function can be added to the standard semen analysis, including assays for sperm capacitation, the complex maturational event required for fertilization. The objective of this study was to determine the similarity of semen parameters for two cloned 3-year old Holstein bulls (A and B) derived via SCNT from a single donor bull. Assays utilized semen

collected by artificial vagina and included semen analysis, in vitro fertilization and in vitro sperm capacitation. Data were analyzed by ANOVA and Tukey test using SigmaStat 3.0 (SPSS inc., Chicago, IL). Using Society for Theriogenology guidelines, both bulls were categorized as satisfactory potential breeders at the start of the 6 month study, with no abnormalities of external genitalia or accessory sex glands detected, normal scrotal circumference (A: 38.5 cm, B: 37.5 cm), sperm morphology (A and B: 85% normal), and sperm motility (A and B: 90% motile). Sperm viability (carboxyfluorescein diacetate/propidium iodide fluorescence assay) following three 5 min washes via centrifugation for 8 min at $380 \times g$ and resuspension in capacitation medium (TALP) did not differ between bulls (A: $89.6 \pm 1.7\%$, B: $89.8 \pm 0.8\%$, mean $\pm$ S.E.M., $n = 5$). IVF was performed utilizing in vitro matured bovine oocytes and sperm from four separate ejaculates per bull that was incubated for 4 h in TALP with 10 μ g/ml heparin at 39 °C in 5% CO₂/95% air in a humidified environment. No significant differences in fertilization, as assessed by cleavage rate (A: 68.4% of 155 oocytes; B: 62.6% of 195 oocytes), or embryo development, as assessed by the percentage of >4 cell embryos at day 2 of culture (A: 45.8%; B: 41.0%) and the percentage of blastocysts at day 7 of culture (A: 26.5%; B: 28.2%), were detected. Sperm capacitation was assessed via the ability to undergo the lysophosphatidylcholine-induced acrosome reaction (LC AR) and protein tyrosine phosphorylation as detected by antiphosphotyrosine protein immunoblotting before and after incubation for 4 h at 39 °C in 5%CO₂/95% air in TALP with and without 10 μ g/ml heparin. In contrast to the lack of detected differences between the two bulls by all other assays, a significant difference in heparin-induced sperm capacitation was noted with Bull A displaying a greater increase than bull B in heparin-induced, capacitation-associated protein tyrosine phosphorylation ($n = 5$). In addition, preliminary LC AR data ($n = 3$) indicates that bull A more consistently (3/3 assays) displays a positive response to heparin than bull B (1/3 assays).

Keywords: Sperm capacitation; Bovine; Cloning; Fertilization; Semen analysis

Acknowledgements: Bulls graciously provided by Select Sires, Inc. Supported by the University of Pennsylvania Reference Andrology Laboratory and a grant from NIH/NICHD to HGH (5-K08-HD041430).