(Sample Invited Minisymposium Abstract)

Disruption of the Sperm-specific *Ldhc* Gene Is Detrimental to Sperm Function and Male Fertility.

Erwin Goldberg, Fanny Odet, Chongwen Duan, William Willis, Eugenia Goulding, Aisha Kung, Mitch Eddy. Northwestern University, Evanston, IL; National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC

The lactate dehydrogenase isozymes are key catalysts in the glycolytic pathway of energy metabolism and it is well known that the distribution of the LDH isozymes vary in accordance with the metabolic requirements of different tissues. There are three subunit types (A, B and C) and exquisite tissue specificity is exemplified by the *Ldhc* gene. It is abundantly expressed in male germ cells during spermatogenesis and encodes the only LDH isozyme for which activity can be detected in spermatozoa. Spermatozoa rely almost exclusively on aerobic glycolysis to produce the ATP necessary for capacitation, motility and fertilization. Why *Ldhc* gene expression has been conserved in mammalian testes where *Ldha* and *Ldhb* genes are also transcribed remains an enigma. We have chosen targeted disruption of the *Ldhc* gene as the method to address the question of why testes and sperm need this unique form of LDH. Preparation of a targeting construct proved challenging since the intronic sequences are composed of 43% repetitive elements. The final construct contained Neo sequences bounded by loxP elements for conditional expression with TK for selection. Redundancy in this metabolic pathway would predict a wild-type phenotype in the mutant animal. Reliance on LDHC for glycolysis in germinal epithelial cells would predict impaired spermatogenesis most likely during pachytene of the first meiotic division or in early spermatids which prefer lactate over glucose as energy substrate. We hypothesized that disruption of ATP production via this metabolic pathway would impair motility and fertilizing capacity of spermatozoa similar to that observed with GAPDHS null mice. However, here we demonstrate that targeted disruption of *Ldhc* affects only male fertility. LDHC has been studied as an immunocontraceptive in females. The present results not only satisfy proof of principle but also suggest that this protein may be a useful target in developing a male contraceptive. This research was supported by NIH HD05863 (EG) and in part by the Intramural Research Program of the NIH, National Institutes of Environmental Health Sciences (EME).

(Sample Poster/Platform Abstract)

Variation In Number of Follicles Growing During Follicular Waves Reflects Size of the Ovarian Reserve in Cattle.

Janet Ireland, Fermin Jimenez-Krassel, George Smith, Pat Lonergan, Alex Evans, James Ireland. Michigan State University, East Lansing, MI; University College Dublin, Dublin, Ireland

Whether variation in the ovarian reserve (total number of healthy follicles and oocytes in ovaries) impacts fertility in humans and other species is unknown. We have shown (using extensive ultrasonography) that peak number of antral follicles ≥ 3 mm in diameter growing during different follicular waves of bovine estrous cycles varies 7-fold among animals, but is very highly repeatable (0.95) within animal. This observation indicates that cattle can be phenotyped reliably based on peak follicle number during waves. We have also shown that cattle of similar ages with consistently low (≤15) vs high (>25) follicle numbers during follicular waves have characteristics associated with ovarian aging and infertility, including higher circulating FSH concentrations, lower serum inhibin-A and progesterone concentrations, and poorer responsiveness to superovulation with reduced number of high quality oocytes and embryos. Based on these findings, we hypothesized that variation in peak number of antral follicles (≥ 3mm) during follicular waves is positively associated with the variation in number of healthy follicles in the ovarian reserve. Two studies were conducted to test this hypothesis: In the first study, beef heifers (n = 13) were subjected to daily ultrasonography to determine alterations in peak number of follicles during 2 to 4 different follicular waves in animals before and 6 weeks after unilateral ovariectomy. The results showed that peak follicle number per wave was reduced 50% after surgery in the unilaterally ovariectomized animals relative to peak numbers for the same animals before surgery. This observation illustrates that a 50% reduction in the ovarian reserve following unilateral ovariectomy results in a correspondingly large decrease in peak number of follicles growing during follicular waves. In the second study, a single ovary was removed surgically 1 d after ovulation from animals of similar ages and weights with low vs high follicle numbers during waves (n = 5 per group). Each ovary was weighed, cut into 8 longitudinal strips, immersed in fixative, sectioned at 8 lm intervals, stained, and total number of healthy primordial, primary, secondary and antral follicles counted in every 40th section in two ovarian strips per animal. The results showed that ovarian weight and total number of healthy follicles in the ovarian reserve were 58% and 83% lower in animals with low vs high follicle numbers during waves, indicating that cattle with low vs high follicle numbers during follicular waves have smaller gonads and a diminished ovarian reserve. In summary, our novel results indicate that variation in peak number of antral follicles during follicular waves is positively associated with total number of healthy follicles in the ovarian reserve, thus peak follicle number during waves is a reliable phenotypic marker to predict relative size of the ovarian reserve in cattle. These observations are important because they imply that the bovine is a unique model to study not only the role of the ovarian reserve in fertility, but also to elucidate the mechanisms involved in infertility in healthy single ovulating species like cattle and humans. Research supported by USDA 2004-35203-14781 to JJI.