Biology of Reproduction, 62 (Suppl 1) 2000

289. EXPRESSION OF THE TELOMERASE CATALYTIC SUBUNIT (TERT) IN BOVINE AND HUMAN GRANULOSA AND LUTEAL CELLS. Tina C Lavranos and Raymond J Rodgers. Department of Medicine, Flinders University of South Australia, Bedford Park, S.A., Australia.

During ovarian folliculogenesis granulosa cells divide to produce two lineages: cumulus and mural granulosa cells. Mural granulosa cells can be further divided into antral and basal cells. The pluripotential of granulosa cells suggests they arise from stem cells (1,2). Tissues with actively dividing stem cells express telomerase, a ribonucleoprotein consisting of an RNA component and a catalytic subunit, which is necessary to generate telomeres during successive rounds of cell division. In support of our hypothesis (1) that the mature granulosa and cumulus cells arise from pluripotent stem cells during follicle development, the aim of this study was to investigate the expression of TERT mRNA in both bovine and human granulosa cells at different stages of follicle development. Bovine TERT was first cloned by RT-PCR from bovine fetal lung using sequence homology from the known human and mouse sequences. The expression of TERT was measured by RT-PCR of RNA isolated from bovine follicles (0.5mm-9mm, n = 3 pools of follicles, 5 individual follicles), and corpora lutea (CLs, n = 3), and human granulosa cells from ovulatory follicles from an IVF program (n =3), a human granulosa cell line, HGL5 (n=3) and human corpora lutea (CLs, n=7). Telomerase activity was also measured (2). The levels of TERT mRNA were greatest in smaller bovine follicles, declining in larger follicles and corpora lutea. The activity of bovine large (6-8mm) follicles (telomerase activity, arbitary units 11.8-27.6 /mg protein; n=3) was greatest than in human ovulating follicles (0.01-1.2/mg protein; n=5), or HGL5 cells (0.98-4.02/mg protein; n=4), and neither of these or the human CLs had detectable levels of TERT mRNA. These data support the hypothesis that stem cells exist during folliculogenesis and that their numbers decline as follicle cells mature. 1. Rodgers et al 1999, Mol Cell Endo 151:171-179 2. Lavranos et al 1999, Biol Reprod 61: 358-366

290. ULTRASONOGRAPHIC CHARACTERIZATION OF UNIQUE FOLLICULAR DYNAMICS AND ASSOCIATED ESTROGEN PRODUCTION IN AN INDIAN RHINOCEROS (*Rhinoceros unicornis*). Terri L Roth and Justine K O'Brien. Center for Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH.

Ultrasonography is becoming a valuable tool in reproductive studies of nondomestic species because the information it yields can significantly increase our understanding of reproductive function in these animals. Our goal was to use ultrasonography to correlate follicular dynamics with estrogen production in a 7 yr old Indian rhinoceros over an extended interval. Serial rectal ultrasound examinations were conducted and urine samples collected for 14 consecutive months. Ovarian follicles were measured and day of rupture recorded. Urine samples were analyzed by EIA for estrone conjugates (EC). A total of ten reproductive cycles were monitored. EC concentrations were 12.5 ± 1.1 ng/mg creatinine (CR) (mean ± sem) at baseline and achieved peak levels of 759.7 ± 94.6 ng/mg CR during estrus. EC values > 48.1 ng/mg CR were considered above baseline and marked the start of the follicular phase which lasted 13.0 ± 1.5 days. Based on the number of days from the start of one follicular phase to the next, the reproductive cycle was 50.6 ± 2.5 days. However, based on the number of days between ovulations (confirmed by ultrasound), the reproductive cycle was 42.6 ± 2.2 days. Using either criteria, the cycle length varied considerably (range, 42-62 and 35-48 days, respectively). Of greatest interest was the size of the dominant follicle prior to ovulation. For each cycle, the dominant follicle grew to ≥90 mm in diameter before EC concentrations rose above baseline. These follicles achieved maximum sizes of 100-120 mm and persisted 12.4 ± 1.3 days (range, 9-17 days) before ovulating. These results suggest that the Indian rhinoceros develops larger ovarian follicles than any other mammal studied to-date. Due to the extended and variable interval during which the dominant follicle maintains maximum size prior to ovulation, both serial ultrasound and endocrine monitoring may be required to accurately predict the day of ovulation. (Funded by the International Rhino Foundation)

291. ROLE OF THE FAS ANTIGEN (CD95) PATHWAY IN SERUM WITHDRAWAL-INDUCED APOPTOSIS OF BOVINE GRANULOSA CELLS. Che-Lin Hu, Robert G Cowan, Rebecca M Harman, Dale A Porter and Susan M Quirk. Department of Animal Science, Cornell University, Ithaca NY.

Ovarian follicular atresia occurs by apoptosis of granulosa and theca cells. Apoptosis may be triggered by removal of survival factors critical for follicle development and/or by cytotoxic stimuli. Fas ligand (FasL) is a cell surface protein which induces apoptosis by ligation to its receptor, Fas antigen (Fas). A possible role of the Fas pathway in mediating serum withdrawal-induced apoptosis of granulosa cells was examined. Granulosa cells collected from 5-10 mm bovine follicles were cultured in DMEM-F12 containing serum for 72 h, deprived of serum, and counted at various times after serum withdrawal. Experiments were repeated with 3 separate granulosa cell preparations. Cell death, as determined by cell counts using the trypan blue exclusion method, increased significantly 6 h after serum withdrawal (21±7%; $P<0.05$ vs 0 h) and continued to increase until 24 h (43±6%). Detection of the translocation of phosphatidylserine to the outer surface of the cell membrane by annexin V binding indicated that cells died by apoptosis. Quantitative reverse transcriptase- polymerase chain reaction (RT-PCR) assays showed no changes in Fas mRNA levels but a 4.7-fold increase in Fas-L mRNA