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332. MAPPING REPRODUCTIVE QTL IN REPRODUCTIVE CONGENIC STRAINS OF MICE. Spearow JL, Nguyen B*, Low C*, Barker E*, Banova I*, Sariano S*, Le B*, Hartouni S*, DuongT*, Ng D*, Estanol R*, and Barkley M. Section of Neurobiology, Physiology & Behavior, Univ. of California at Davis, CA.

Molecular genetic markers for reproductive quantitative trait loci (QTL) would enhance selection for improved reproductive performance in several mammalian species and improve optimization of hormonal treatments for diverse hormone-response genotypes. The present study examined major genetic differences in the regulation of ovarian function and mapped QTLs controlling ovulation rate and aromatase activity. Quantitative trait loci (QTL) controlling 6-fold genetic differences in hormone-induced ovulation rate (ORI) and 21-fold genetic differences in hormone-induced ovarian aromatase activity (AAI) were mapped between C57BL/6J (B6) and A/J mice. QTL linkage analysis in (B6A)XA backcrosses mapped significant ORI QTL to Chr 6 near D6Mit316 (*Oriq3*) with an effect of 8.1 eggs (LOD= 3.45) and to proximal Chr 10 (*Oriq5*) with an effect of 4.4 eggs (LOD=3.94). To overcome the problem of genetic noise caused by the simultaneous segregation of several reproductive QTL, reproductive congenic strains were developed for regions of mouse Chr 2, 4, 6, 9, and X by repeatedly backcrossing mice with B6 microsatellite markers flanking each reproductive QTL onto the A/J genetic background. Reproductive congenic strains of mice were used to confirm the presence of significant ORI QTL on Chr 2, 6 and X, and AAI QTL on Chr 2 and 4. Higher resolution mapping in congenic testcrosses mapped: Oriq3 near D6Mit210 with an effect of 4.7 eggs ($P<0.0004$); Oriq4 to mid Chr X with an effect of 3.46 eggs ($P<0.00001$); Oriq6 near D2Mit6 with an effect of 6.1 eggs ($P<0.0006$); Aaiq2 near D2Mit66 with an effect of 133%; and Aaiq3 to Chr 2 proximal to D2Mit6 with an effect of 60%. A/J and B6 mice differed dramatically in the induction of aromatase mRNA, and in the apparent Km & Vmax of aromatase enzyme. These reproductive congenic strains allow positional cloning of genes with major effects on reproduction. The present data show that genetic differences in the hormonal induction of ovarian steroidogenesis and ovulation are a major source of variation in the regulation of reproduction. Consideration of these and syntenic genetic differences in hormone response genotypes will allow optimization of hormonal treatments for the desired level of reproduction and enhance selection for prolificacy. Supported by USDA 89-37240-4909; PHS R01-HD28253; NSF IBN 95-07872.

333. THE REPRODUCTIVE PHYSIOLOGY OF A LIVING FOSSIL - THE SUMATRAN RHINOCEROS (*Dicerorhinus sumatrensis*). Roth TL,[1] McRae MA,[1] Brown JL,[2] Kroll JL,[1*] Bellem AC,[2*] O'Brien JK,[1*] and Romo JS.[1*] [1]Center for Research of Endangered Wildlife, Cincinnati Zoo and Bot Garden, Cincinnati, OH; [2]Conserv & Res Ctr, Smithsonian Inst, Front Royal, VA

A descendant of the wooly rhinoceros that lived during the last ice age, the Sumatran rhino is the most primitive of existing rhino species. It also is the most endangered, with fewer than 400 worldwide. Captive propagation has failed, in part, because little is known about reproduction, and males are dangerously aggressive towards non-estrual females. Study objectives were to: 1) characterize the reproductive physiology of the female Sumatran rhino; and 2) use this knowledge to develop a successful captive breeding program. Rectal ultrasound examinations and blood sample collections were conducted 2 to 7 times per week on the only young (7 year old) female Sumatran rhino in the U.S. Follicular dynamics, ovulation and corpus luteum development were recorded. Serum was analyzed for progesterone by ELISA and for LH by RIA. Based on ultrasound data, a breeding program was implemented. Compatible breeding activity occurred on only one day of each cycle (n = 5), culminating in a 30-60 min copulation 1-3 h after introducing the pair. Progesterone patterns, ovarian activity and breeding behavior indicated a 21-day reproductive cycle. Serum progesterone levels were lowest (<200 pg/ml) for 2 to 4 days of the cycle during which breeding and ovulation occurred. Progesterone was elevated (≥ 500 pg/ml) by 5 to 6 days and peaked (1249 ± 184 pg/ml, mean ± SEM) at 10.0 ± 0.8 days post-ovulation. Follicular activity was documented on both ovaries, but only the left produced large (25-29 mm), ovulatory follicles. Serum LH increased from baseline (0.54 ± .03 ng/ml) to peak (14.0 ± 4.0 ng/ml) concentrations on the day of breeding, and the female ovulated by 45 h post-copulation. On one occasion, a 4 mm embryonic vesicle was detected by ultrasound 14 days post-breeding. At 26 days, a fetal heartbeat was observed. Early embryogenesis appeared similar to that in horses, and serum progesterone remained elevated until the pregnancy was lost between 40 and 44 days. The reproductive physiology of the Sumatran rhino is no longer such a mystery. These results demonstrate the successful impact that basic scientific research can have on managing and promoting natural propagation of an endangered species. (Funded by the International Rhino Foundation)

334. COMPARATIVE SPERM FUNCTION AND GAMETE INTERACTION AMONG SMALL FELID SPECIES FROM DIVERGENT EVOLUTIONARY LINEAGES. Swanson WF, McRae MA, Davidson JL[1]*, Levens GP* and Campbell MK*. Cntr Res Endangered Wildlife, Cincinnati Zoo & Bot Garden, Cincinnati, OH; [1]Northwood Felid Res and Educ Found, Canton, OH.

The world's 37 felid species have been classified into three distinct evolutionary groups: the ocelot, domestic cat and pantherine lineages. The relationship between lineage classification and reproductive function may be important for extrapolating assisted reproductive technology for the conservation of endangered cat species. In this study, our objectives were to: 1) evaluate motility longevity and capacitation of spermatozoa from small cats in each lineage; and 2) investigate the ability of these spermatozoa to penetrate salt-stored domestic cat oocytes (DCO) and fertilize viable DCO. Semen was collected via electroejaculation from eight males (one cat/species) representing the three lineages (ocelot: Geoffroy's cat, ocelot; domestic cat: sand cat, jungle cat, domestic cat; pantherine: golden cat, caracal, serval). Spermatozoa were incubated (Ham's F10 medium; 38°C, 5% CO_2) in microdrops under oil. At 0, 3 and 6 h, aliquots were assessed for sperm motility (SMI=[% motile sperm + (rate of progressive motility × 20)]/2), treated 30 min with calcium ionophore A23187 (CI) and stained with FITC-PNA to assess acrosomal status. Spermatozoa (2×10^5 motile sperm/ml) from each species were co-incubated with both salt-stored and viable DCO (8-15 oocytes/sample) for 18-20 h. Salt-stored oocytes were examined for penetration (sperm within the perivitelline space). Viable oocytes were evaluated for fertilization, and embryos cultured for 5 d and stained with Hoescht 33342 to determine nuclei number. Across species, SMI (69.4 ± 1.8, 0 h; 39.1 ± 7.0, 6 h; mean ± SEM) declined ($P < 0.01$) during culture whereas the percentage of acrosome-reacted sperm (31.6 ± 4.2%, 0 h; 71.6 ± 7.0%, 6 h) increased ($P < 0.01$) after CI treatment. Spermatozoa from each species were capable of fertilizing viable oocytes (59.1 ± 9.6%, mean; 13-82%, range), with most (63%) fertilized oocytes cleaving. Of these, 70% developed to the early-late morula stage (29.4 ± 4.0 cells/embryo). Although spermatozoa from most (6 of 8) species penetrated salt-stored oocytes (42.5 ± 14.6 %), there was no correlation (r =.016, $P > 0.05$) between salt-stored oocyte penetration and fertilization success. In conclusion, these findings suggest that sperm function and gamete interaction have been highly conserved among small cat species during evolutionary divergence of the Felidae. (Supported by NIH grant RR0009801)