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465 PERIOVULATORY LH, FSH, PROGESTERONE AND 17-HYDROXY-PROGESTERONE DURING SPONTANEOUS OVARIAN CYCLES IN NORMAL AND LEVONORGESTREL-TREATED DOGS.

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Dogs cycle every 5-12 mos with a 1-3 wk follicular phase, 2 mo luteal phase, and 3-9 mo anestrus in fertile and non-bred cycles. A rapid rise in progesterone (P) during the 1-2 day preovulatory LH surge, simultaneous with a fall in estradiol (E), decreases the E to P ratio, facilitates surge release of LH and estrus behavior, and is used to time ovulation. The present study evaluated peri-ovulatory and luteal phase changes in 17-OH-P vs P and characterized changes in P, 17-OH-P, LH and FSH in control and levonorgestrel (LN) treated bitches. Dogs are very sensitive to many contraceptive progestins, but LN has not been extensively studied in dogs. Serum was collected from 15 dogs every 24-28h, 5-7 days per week, from proestrus until end of estrus, and assayed for LH, FSH, P, and 17-OH-P content (ng/ml). There were 9 control cycles and 6 which occurred during 2-8 mos of treatment with human-contraceptive doses of levonorgestrel (LN) implants. P and 17-OH-P routinely increased simultaneous with the LH surge. Mean (±SEM) levels of 17-OH-P vs P at days -3, 0, 4 and 8 from the onset of the LH surge were 1.1 ± 0.2 vs 0.4 ± 0.1 ; 3.1 ± 0.4 vs 1.3 ± 0.1; 5.1 ± 0.5 vs 9.6 ± 2.5; and 11.1 ± 1.5 vs 22.6 ± 3.1, respectively. There were no consist nt differences between control and LN treated bitches in P or 17-OH-P, in LH at mid-proestrus (1.1±0.3) or day 0 (15.8±3.3), or in FSH in mid-proestrus (41±8) or at Day 1 peaks (260±36). There were no differences in luteal phase P/17-OH-P ratios between pregnant and nonpregnant cycles. The high ratio of 17-OH-P to P during the LH surge (2.5:1) suggests that 17-OH-P as a progestin could play a role in facilitating preovulatory surge release of LH and estrus behavior, and might be diagnostically useful. The reduced 17-OH-P/P ratio during the early (1:2) and mid/late (1:4) luteal phase was similar to that observed in women. The general lack of efficacy of human doses of LN implants in dogs suggests that it is a weak progestin in dogs and/or is metabolized more rapidly than in other species. Supported by the Morris Animal Foundation.

466 PRELIMINARY RESULTS OF FECAL PROGESTAGEN EVALUATIONS IN THE WHITE RHINOCEROS *(CERATOTHERIUM SIMUM)* INDICATE AN ESTROUS CYCLE LENGTH OF ≈10 WEEKS.

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Captive breeding of the two species of African rhinoceroses (R) is going to be essential for their long term survival, and non-invasive methods to support breeding management are urgently needed. Using fecal steroid analysis we have determined the estrous cycle length of the black R (*Diceros bicornis*) as 25 days [1]. In this study we describe fecal progestagen patterns of non-pregnant white R. Fecal samples of southern (n=2; *Ceratotherium simum simum)*, northern (n=2; *Ceratotherium Simum cottoni)*, and a crossbred (n=1) between the two subspecies, were collected 2-3 times/week for periods of 14-24 months. Feces (0.5g) was extracted with aqueous methanol as described [1], except that 1.0 g aluminium oxide was added prior to extraction. Methanol aliquots were analyzed in two different enzyme-immunoassays (EIA), using antibodies against 5α-pregnane-3ß-ol-20-one 3HS:BSA and 5ß-pregnane-3α-ol-20-one 3HS: BSA, respectively. Results are considered as quantification of total immunoreactive progestagens containing a 20-oxo group. Regular estrous cycles (n=9; duration 68.4±3.4 days) were observed in one southern white R; follicular (FP) and luteal phases (LP) were 12.2±0.8 and 56.5±3.3 days, respectively. Estrus behavior coincided with presumable FP. FPs and LPs concentrations in both EIAs were <250 and >1200 ng/g feces, respectively. Progestagen levels in one northern white R also indicated luteal activity. Low values resembled FP of the southern subspecies, however, luteal progestagen concentrations were considerable lower (<500 ng/g). Intervals between LPs (n=4), and duration of LPs (9,19,20 and 28 days, respectively) were irregular. A similar situation was observed in the crossbred R, were one 15 day LP with <500 ng/g values was observed. Luteal activity was not detectable in the other two R. Our data noticeable contrast a recent study [2], which by means of urinary steroid analysis suggested estrous cycle lengths of 25 and 32 days for the northern and southern subspecies, respectively. However, our long term investigation of white R reveals, (a) missing or erratic cyclicity as a considerable problem, and (b) depicts an estrous cycle length of ≈10 weeks. Resolution of differences between these, and the data of [2] is important for future captive-breeding protocols. [1] Schwarzenberger et al., J. Reprod. Fert. 1993; 98:285-291. [2] Hindle et al., J. Reprod. Fert. 1992; 94:237-249.

467 URINARY ANDROSTANEDIOL GLUCURONIDE IS A MEASURE OF ANDROGENIC STATUS IN ELD'S DEER STAGS *(CERVUS ELDI THAMIN)*.

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Understanding reproductive-endocrine relationships in non-domesticated ungulates often is impossible because of the difficulty of collecting serial blood samples. Although urinary and fecal ovarian steroid metabolites now are commonly assayed as an index of reproductive status in diverse mammalian species, relatively few reports have monitored androgen metabolites in males. To determine the primary excretory by-products of testosterone (T), 85 µCi ^{3}H-T was administered i.v. to 2 adult Eld's deer stags, and blood (10 ml) was collected by jugular venipuncture at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 480 min after isotope infusion; all urine and feces were collected for 96 h post-infusion. Seventy % of labeled circulating steroid was conjugated by 30 min post-infusion. The majority (80.4±3.2%) of T metabolites were excreted into urine, and 95.0±0.9% of these were conjugated, 95.8±0.2% being hydrolyzable with glucuronidase. Androsterone, etiocholanolone, androstanediol, 11-oxo-etiocholanolone, 11-oxo-androsterone and 11β-hydroxytestosterone were identified in glucuronidase-hydrolyzed, ether-extracted urine by GC-mass spectrometry. An ^{125}I, double-antibody radioimmunoassay (RIA) kit for 5α-androstanediol-3α,17β-diol, 17-glucuronide (AdG) was validated for unprocessed urine. Serial dilutions yielded displacement curves parallel to standard preparations, and the mean recovery of added AdG (range, 1.25- 50 ng/ml) was 99.2±9.1% ($y = 1.12x + 0.43$, $r^2 = 0.99$). Assay sensitivity was 0.5 ng/ml; the inter-assay coefficients of variation for 2 separate internal controls were 8.6% and 10.4%, respectively, and intra-assay variation was < 10%. RIA of eluates after HPLC revealed that all immunoreactivity was associated with a single peak that co-eluted with standard AdG. Longitudinal assessments of urine samples collected from 6 stags for 3 yr revealed distinct circannual oscillations in urinary AdG immunoreactivity that corresponded with seasonal changes in serum T, antler growth, body weight, testicular size, ejaculate characteristics and behavior. Overall correlation *(r)* of urinary AdG with T assayed in matched 'same day' serum samples was 0.58, ($P < 0.001$). Thus, non-invasive monitoring of urinary AdG provides useful data for characterizing male endocrine interrelationships in an endangered ungulate species. (Smithsonian Scholarly Studies Program and NIH HD 00903)

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SUPPRESSION OF THE PROLIFERATION OF A MYELO-MONOCYTE CELL LINE, FDC-P2 BY NOVEL INHIBITORS FOR 20α-HYDROXYSTEROID DEHYDROGENASE ACTIVITY

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We have purified 20α-hydroxysteroid dehydrogenase (20α-HSD), an enzyme converting progesterone to biologically inactive 20α-dihydro-progesterone, from rat ovaries and found two distinct isoforms designated as HSD-1 and HSD-2 (1). The expression of these activities differs during the course of pseudopregnancy (2,3), suggesting that they play different roles in regulating luteal function. In this study, to further investigate the physiologycal function of 20α-HSD isoforms, we attempted to find specific inhibitors for the activity of each isoforms. Based on analogy of the progesterone skeleton, 26 steroid derivatives were synthesized. Six of them were found to possess an inhibitory effect on 20α-HSD activity in the rat ovarian cytosol containing both HSD-1 and HSD-2. Among these 6 derivatives, 2 derivatives also suppressed the enzymatic activity in the rat luteal cell culture, which exclusively expressed HSD-1. This suggested that these 2 derivatives could penetrate the cell membrane and inhibit preferentially the activity of HSD-1. Further, these 2 derivatives suppressed the proliferation, as well as 20α-HSD activity, of myelomonocyte FDC-P2 cells, which was known to express 20α-HSD activity in response to IL-3. These results suggest that the steroid derivatives found in this study would be useful for analyzing physiological roles of 20α-HSD isoforms, and that 20α-HSD is involved in the proliferation of FDC-P2 cells.

REFERENCES

1. K. Noda, K. Shiota, M. Takahashi (1991) Biochem Biophphys Acta 1079, 112-118
2. K. Shiota, K. Noda, M. Takahashi (1988) In the 22nd NIBB Conference: The Molecular Basis of Communication within Cells, Okazaki, Japan, February, 1989 (Abstract 14) Ed. Y. Nagahama. National Institute of Basic Biology, Japan
3. H-H. Seong, K. Shiota, K. Noda, A. Ogura, T. Asano, M. Takahashi (1992) J Reprod Fert 96, 573-580