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Title: Reproductive and adrenal endocrinology of the giant panda (*Alluropoda melanoleucs*)

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ABSTRACT

This study sought to utilize non-invasive tactics to evaluate gonadal and adrenal hormones to better understand the reproductive biology and well-being of the endangered giant panda. Specifically, the studies of this dissertation were to: (1) to develop and validate fecal hormone monitoring techniques to quantify excreted gonadal and adrenal steroid metabolites of male and female giant pandas; (2) utilize fecal hormone monitoring to analyze the hormonal milieu of periestrus in the female; (3) investigate relation between gonadal hormone excretion and reproductive physiology during the luteal phase, with particular emphasis on pregnancy/pseudopregnancy, delayed implantation and acyclicity; (4) evaluate male reproductive and adrenal biology by assessing trends in androgen and glucocorticoid excretion in the feces; (5) determine relation between excreted adrenal steroids in the feces of the female giant panda and reproductive phenomena such as, estrus, pregnancy/pseudopregnancy, lactation, seasonality and acyclicity. Strong correlations between concomitant urinary and fecal estrogen conjugate (EC; r = 0.69 - 0.79; $P < 0.05$) and progestagen (r = 0.62 - 0.79; $P < 0.05$) metabolites across known reproductive events (periestrus and luteal phase) demonstrated biological relevance of fecal measures in tracking ovarian function. Longitudinal assessments of 17 females further revealed that, on average, fecal estrogen concentrations began to rise {$P <$ 0.05) above baseline (64.5 ± 5.9 ng/g; range, 20.0 - 103.5 ng/g) 5 d before the pre-ovulatory estrogen peak (Day 0, 468.1 ± 83.9 ng/g; range, 126.9 - 1,546.8 ng/g), which

was followed by a gradual 4-d descent back to baseline All females that experienced estrus exhibited a biphasic luteal fecal progestagen profile during the post-ovulatory interval that included an initial 1.6-fold "primary" increase (368.1 ± 17.7 ng/g; range, 15.9- 1,456.1 ng/g, P > 0.05) above baseline concentrations (101.9 ± 4.5 ng/g; range, 4.1 - 1,245.4 ng/g) during the immediate post-ovulatory interval (88.9 ± 6.5 d; range, 63 - 122 d) which was followed by a "secondary" 8.5-fold increase (3,110.9 ± 283.5 ng/g; range, 101.4 - 16,894.5 ng/g, *P* > 0.05) in progestagen excretion lasted approximately 40 d (39.5 ± 2.8 d; range, 28 - 52 d). Additionally, there were no general differences in the duration or progestagen excretion during the secondary or primary rise of the luteal phase between parturient and nonparturient females. In the male, matched urinary and fecal androgen *(r* =0.61) and OC (r = 0.53) were strongly correlated with one another in single male that was assessed over a 2-year interval. Longitudinal fecal androgen and GC excretory profiles in male giant pandas housed at North American and Chinese facilities revealed similar excretory profiles. In general, fecal androgens increased *(P* > 0.05) 2.3-fold (252.9 ± 15.9 ng/g; range, 205.5 - 294.1 ng/g; *P* < 0.05) above baseline concentrations (112.0 ± 12.6 ng/g; range, 78.8 - 156.3 ng/g) coincident with the onset of the 5-month annual breeding season. In all males *(n* = 5), androgen metabolite concentrations declined and were basal by the end of the breeding season (June). Fecal GC measures generally tracked androgen excretion patterns (r = 0.53 - 0.76), with mean peak GC excretion (362.6 ± 23.1 ng/g; 302.5 - 431.5 ng/g) representing a 2.1 fold increase (P < 0.05) over baseline concentrations (173.8 ± 24.1 ng/g; range, 122.3 - 233.3 ng/g). Fecal androgen and GC in a single male tracked during the transition from subadult (3 years of age) to sexual maturity (6 years of age) were excreted in parallel. In this male basal fecal androgen values were positively correlated with age (r = 0.93; *P* < 0.05) and increased 88% *{P* < 0.05) from age 5 (70.4 ± 23 ng/g) to 6 (132.8 ± 5.0 ng/g) years. Similarly, baseline fecal GC concentrations were positively correlated with age (r = 0.82; *P* < 0.05) and increased 66% *{P* < 0.05) form age 5 (139.0 ± 4.7 ng/g) to 6 (231.3 ± 7.1 ng/g) years. Periestrual EC and GC measures were examined in five females, with four demonstrating a positive *{P* < 0.05) correlation (r = 0.57 - 0.92) between the two

measures, suggesting that GC may play a facilitating role in the hormonal milieu associated with estrus. Among the reproductive states, fecal GC values for both nonparturient (495.9 ± 100.7 ng/g) and parturient (654.1 ± 106.5 ng/g) females highest *(P* < 0.05) during the periestrus interval. Further, nonparturient females excreted lower *{P* < 0.05) GC concentrations during the secondary period of the luteal phase (334.8 ± 24.8 ng/g) than nonparturient females (470.4 ± 54.0 ng/g), suggesting possible physiological differences between the two subsets of females Although fecal GC concentrations in cyclic nonparturient females were not different across all seasons *(P >* 0.05), seasonal differences were found in acyclic, nonlactational females (winter, 302.1 ± 33.4 ng/g; spring, 212.7 ± 18.1 ng/g; summer, 214.3 ± 14.8 ng/g; autumn, 155.1 ± 9.0 ng/g). Overall, fecal GC concentrations in cyclic and acyclic females were similar, which suggests that stress may not be the primary cause of reproductive inactivity in these females. Collectively, these data are a significant contribution to the gonadal and adrenal endocrine databases of the giant panda and provide valuable insight into reproductive biology and well-being that will aid in conserving this unique endangered species.