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# UNIQUE REPRODUCTIVE HORMONE PROFILES IN THE CRITICALLY ENDANGERED IBERIAN LYNX (*LYNX PARDINUS*)

***Katharine M. Pelican, DVM, PhD,[1]* Astrid Vargas, DVM, PhD,[2] Teresa Abaigar, PhD,[3] Jose Manuel Rodríguez,[2] Juana Bergara,[2] David Rodríguez,[2] Antonio Rivas,[2] Janine Brown, PhD,[1] and David E. Wildt, PhD[1]***

***[1]Conservation & Research Center, Smithsonian's National Zoological Park, 1500 Remount Road, Front Royal, VA 22630 USA; [2]Iberian Lynx Ex Situ Conservation Program, Iberian Lynx Captive Breeding Center, El Acebuche, Doñana National Park, 21760-Matalascañas, Huelva, Spain; [3]Estación Experimental de Zonas Áridas, CSIC, General Segura 1, 04001-Almería, Spain***

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## Abstract

The Iberian lynx is the most endangered of the world's felid species. The current population is estimated at fewer than 200 animals, most of which are living in fragmented populations *in situ*. In 2004, an Iberian lynx captive breeding program was initiated in Doñana National Park (Huelva), Spain both as a hedge against extinction and to extend our understanding of species biology. In March 2005, three Iberian lynx cubs were born from a three-year-old dam, the first successful birth in captivity.

The present study evaluated the hormonal control of reproduction in the Iberian lynx. Daily fecal samples were collected from four adult females (2 to 14 yr old at study onset) and three adult males (1.5 to 3 yr old) from April 2004 through May 2005 to characterize seasonal and reproductive endocrine profiles. All of the evaluated females were wild-caught, two from the Sierra Morena Mountains and two from Doñana National Park. Samples were shipped frozen from Spain to the National Zoo's Conservation & Research Center, and metabolites of estrogen and progestin (female) or testosterone (male) were quantified using enzyme immunoassays. Hormonal metabolite concentrations greater than twice baseline were considered elevated.

There were marked seasonal changes in estrogen metabolites in all females. Estrogen concentrations fell to baseline from 11 May through 20 July 2004 and remained at nadir until January 2005. In the presumed early breeding season (January through July), estrogen metabolite levels increased above baseline from 17 January through 30 January 2005. Once elevated, estrogen metabolite profiles remained static with no evidence of estrogenic cycling, rather only an increased and sustained pattern. The mean and peak estrogen metabolite concentrations were lower ($P < 0.05$) in the 2004 non-breeding (mean ± SEM; mean, 1.2 ± 0.2 μg/g feces; peak, 6.0 ± 2.4 μg/g feces) compared to both the 2004 and 2005 breeding seasons (mean, 7.9 ± 2.7 and 10.2 ± 4.3 μg/g feces; peak,

10.2 ± 4.3 and 47.3 ± 22.6 μg/g feces, respectively). Compared to other felids species studied in our laboratories, estrogen metabolite concentration in the female Iberian lynx was ~10-fold greater during the breeding season. In contrast to estrogens, there were no ($P > 0.05$) seasonal variations in progestin metabolite concentrations (overall mean and peak, 32.1 ± 3.6 and 132.0 ± 10.9 μg/g feces, respectively). In 2005, signs of estrus (vocalization and conspecific sexual interest) were observed from 17 January, and breeding was observed in all females from 24 January through 11 February. Initial seasonal elevations in estrogens occurred from 0 to 5 d following initiation of estrous behaviors. One female that copulated multiple times on 24 January gave birth to three cubs on 28 March 2005 (gestation interval = 64 days). Despite multiple copulations in the other intensely monitored females, no other cubs were produced. Mean estrogen and progestin metabolite concentrations during pregnancy in the singleton female were 18.4 and 30.2 μg/g feces respectively, with the progestin concentrations no different than those seen throughout the year or in other non-pregnant females. There were no seasonal changes in testosterone metabolites in any of the adult males (overall mean, 0.7 ± 0.2 μg/g feces).

These results demonstrated that the female Iberian lynx is a seasonal breeder, with reproductive activity reflected in fecal estrogen, but not progestin metabolite profiles. The females of this species excreted much higher quantities of estrogen in the feces compared to other felid species studied to date. Contrary to estrogens, progestin metabolites (at least using standard felid assays) were a poor indicator of reproductive status in the Iberian lynx and even failed to fluctuate in either animals known to have copulated or in the single female that produced living young. The unusual endocrine profiles also extended to the males, which failed to exhibit fluctuations in excreted testosterone metabolites, even though the species seems to have a well defined (January to July) breeding season. Overall, this single milestone birth combined with an emerging database on endocrine profiles and reproductive behaviors provides encouragement that a scholarly and applied approach may be helpful in achieving successful captive breeding in this critically endangered felid.