ABSTRACTS

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SEASONAL PROFILES OF OVARIAN ACTIVITY IN IBERIAN LYNX (LYNX PARDINUS) BASED ON URINARY HORMONE METABOLITE ANALYSES

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Introduction - The Iberian lynx is the most endangered felid species. An important tool for the ex-situ conservation program for Iberian lynx is the development of non-invasive methods to monitor reproduction. Especially, pregnancy diagnosis is of particular importance as a management tool for the Iberian lynx Conservation Breeding Program. In lynx, however, fecal progestins do not follow the typical pregnancy pattern of felids. Therefore, the aim of the present study was to test whether urine can be used as an alternative for monitoring seasonal ovarian activity.

Materials and methods - During a two year study period (2006 and 2007) urine samples from six Iberian lynx females were collected. Overall five pregnant, three non-pregnant and one abortive cycle were available for urine steroid analysis. Urine samples were collected on a nearly daily basis and immediately frozen. Urinary progestins (P4) and estrogens (E2) were determined by ELISA and related to creatinine (Crea) content. To identify relevant urinary estrogen metabolites, high-performance liquid chromatography (HPLC) analysis of steroid extracts from urine samples collected during first and third trimester of pregnancy and during lactation was performed. Additionally, the HPLC pattern of ³H (tritiated) labelled steroid metabolites in urine was determined based on a radiometabolism study, which was performed in female Eurasian lynx. The Eurasian lynx is considered to be a model species for the highly endangered Iberian lynx.

Results - The urinary progestin was characterized by an increase of mean P4 values within pregnancy (2.8 ± 1.75 ng per mg Crea), compared to 2.11 ± 0.98 ng and 1.96 ± 0.65 ng per mg Crea before mating and after birth, respectively. No difference, however, was found in the P4 level between a pregnant and pseudo-pregnant lynx cycle (time interval of 1 to 65 days after mating = the pregnancy duration in Iberian lynx). Although the P4 level of the pseudo-pregnant animal was slightly lower (2.07 ± 1.23 ng per mg Crea), the broad variance of urinary progestins made a reliable pregnancy diagnosis unattainable.

We were unable to detect a mating related urinary estrogen peak, because the collection method did not allow discriminating between male and female urines. Urine samples collected in the presence of males were characterized by 10x higher estrogens (66.5 ± 6.1 ng E2 per mg Crea; n = 55) in comparison to pure female urines irrespective their reproductive cycle (9.7 ± 0.7 ng E2 per mg Crea, n = 154). Female urinary estrogens showed a significant elevation from 3.3 ± 0.5 ng E2 per mg creatinine after breeding season compared to 10.9 ± 0.8 ng E2 per Crea during pregnancy (mean of all animals). Direct comparisons of a pseudo-pregnant (9.2 ± 1.0 ng E2 per mg Crea) with a pregnant cycle (16.0 ± 2.2 ng E2 per mg Crea) revealed a further elevation of urinary estrogens caused by implantation.

The Eurasian lynx urine is composed of four major polar radiolabelled estrogen metabolites (conjugated steroids) and reasonable amounts of free E2 and estrone, but our E2 ELISA recognized only two of the polar peaks. The HPLC profile of Iberian lynx urines showed the same elution pattern suggesting similar estrogen metabolism in both lynx species. In all three urine extracts of Iberian lynx, estrone was detectable in comparable amounts (8-12% of immunoreactive metabolites), whereas during pregnancy more E2 was measurable (18% of immunoreactive metabolites) in comparison to the sample from lactation period (3%).
Conclusions - the seasonal ovarian activity in Iberian lynx can be monitored by urinary estrogen secretion, which reflects, in contrast to urinary progesterone, the luteal activity during pregnancy. The increased E2 in urine collected during pregnancy of Iberian lynxes in contrast to early and pseudo-pregnant urine samples might indicate a possible E2 secretion of the lynx placenta.