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Using PGFM (13,14-dihydro-15-keto-prostaglandin F2α) as a non-invasive pregnancy marker for felids

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Abstract

Understanding the complex endocrine interactions that control reproduction in felids is essential for captive breeding management. The most important demand is a quick and reliable pregnancy diagnosis. However, the occurrence of pseudopregnancies in felids complicates matters. We investigated whether the fecal prostaglandin metabolite (PGFM) recently suggested for pregnancy diagnosis in the lynx is suitable for all felid species. We found that increased levels of PGFM during the last trimester indicate pregnancy in seven of the eight main lineages of the carnivore family Felidae. PGFM levels in a sand cat (domestic cat lineage) were basal at mating and remained so until Day 40 post-mating. Day 41 marked the beginning of a distinct increase culminating in peak levels of 6.5 μg/g before parturition and decreasing again to baseline thereafter. Similar pregnancy profiles were obtained from the domestic cat, the leopard cat, the lynx, the ocelot and the caracal lineage, whereas in pseudopregnant individuals (sand cat, Iberian and Eurasian lynx) fecal PGFM remained at basal levels. In pregnant cheetahs (puma lineage) PGFM increased above basal following day ~48 peaking before pregnancy but remained at baseline in pseudopregnant females. Discrepancies existed in the Panthera lineage. While Chinese leopard, Sumatran tiger, and the black panther showed marked increases of PGFM during the last weeks of pregnancy, only moderate increases in PGFM levels were found in the Indochinese tiger and the Persian leopard. Altogether, PGFM as tool for pregnancy diagnosis has been proven to be useful in breeding management of felids.

Keywords: Prostaglandin F2α; Metabolite; PGFM; Felids; Pregnancy; Pseudopregnancy

1. Introduction

All 36 species of wild felids are included in Appendices I and II of CITES and tend to be one of the most endangered and vulnerable groups of mammals in the world. Twenty-three of those cat species are threatened or endangered with extirpation in at least some part of their natural range (http://www.iucnredlist.org). For example, survival of the 10 non-domestic felid species endemic to Latin America is particularly jeopardized by habitat loss, human-animal conflicts, and poaching [1] whereas tiger conservation in Asia is mainly by harvest of animals for traditional medicines used by at least a quarter of the world's human population [2]. One felid species in particular—the Iberian Lynx—is critically endangered (IUCN R. List 2009) mainly due to decimation of European rabbit (*Oryctolagus cuniculus*) populations, the lynx's main prey [3].

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Because of increasing extinction risks there is a growing demand for zoos to sustain genetically healthy felids populations in case of catastrophic extinctions. Most felid species reproduce poorly in captivity, a problem attributed to behavioral incompatibilities, captivity stress, or inappropriate husbandry [1]. Causes of female reproductive failure are challenging to diagnose because of difficulties analyzing the complex endocrine milieu associated with estrous activity, ovarian function, conception, and pregnancy [4]. Therefore, understanding the endocrine principles of reproduction in felids is essential for their captive breeding management and applied conservation efforts. One of the most important demands in captive breeding programs is a quick and reliable pregnancy diagnosis, because females in captivity tend to abort or kill their offspring if management conditions are not appropriate. Thereby primiparous females have a higher rate of failure to raise their young than multiparous ones [5]. A timely installed video-surveillance system permits early detection of problems which may result in the rescue of cubs that were abandoned by their mothers for hand-raising. In this regard, non-invasive endocrine monitoring utilizing urine and fecal samples is preferred. Such techniques avoid repeated blood sampling for reproductive hormone analysis and do not represent a source of additional stress that may increase the risk of abortion in pregnant lynxes.

In several felid species, pregnancy diagnosis utilizing non-invasive fecal hormone metabolite monitoring has become a routine procedure [6]. After successful mating, progesterone level increases in blood plasma due the activity of corpora lutea. Towards the end of pregnancy, progesterone levels decrease and drop to baseline levels before parturition [6]. This plasma profile of progesterone secretion is mirrored by progesterone metabolites in feces. In many felid species, fecal progesterone (P4) metabolite concentrations increase significantly during pregnancy [7]. Pseudopregnancies (non-pregnant luteal phase) are characterized by a shorter duration of fecal progestin elevation, usually approximately one-half to two-thirds of the gestation length. For example, in the cheetah the average pseudopregnancy length is 53 d, whereas a normal full-term gestation length is 94 d [7]. In the clouded leopard average pseudopregnancy lasts 48 d, compared to a full-term gestation of 90 d [7]. The main disadvantages of fecal progestin measurements for reliable pregnancy diagnosis are the necessity of repeated (frequent) sampling as well as highly variable intra- and interspecies baseline concentrations. Furthermore, in a few felid species fecal P4 metabolite analysis failed to demonstrate pregnancy [8–10].

As an alternative to fecal steroid analysis, urine has been utilized for tracking pregnancy specific hormones. In particular, several peptide hormones, such as luteinizing hormone (LH) and human choriogonadotropin (hCG) can be detected in urine and may be related to sexual activity or pregnancy status [11,12]. Recently, it was shown that relaxin is detectable in urine of pregnant domestic cats, leopards and lynxes [10,13]. In our previous study [14] we investigated the urinary prostaglandin F2α metabolite (PGFM), which also seems to be a pregnancy related placental signal in the Iberian lynx [14]. $PGF_{2\alpha}$ is a prostaglandin, that has been portrayed as a locally bioactive hormone detectable in virtually all tissues [15]. It is now widely accepted that uterine and placental prostaglandins play a key role in regulating the function and life span of corpora lutea [16] and exogenous PGFα is luteolytic in both pregnant and pseudo-pregnant bitches [17]. Serum PGFM analyses in the dog revealed different patterns between pregnant and non-pregnant (diestrus) bitches [18].

The same was obvious in the Iberian lynx. Based on the analysis of the urinary $PGF_{2\alpha}$ metabolite PGFM, a clear differentiation between pregnant and pseudopregnant female lynxes was possible [14]. The PGFM patterns revealed a constant hormone increase over the last trimester (21 d) of gestation in pregnant females with peak concentrations around the time of parturition followed by a post-partum drop to baseline. In comparison, in pseudopregnant females baseline profiles were obtained during the entire period of supposed pregnancy [14]. The finding that PGFM is detectable in feces of Iberian lynxes as well and follows similar courses as shown for urine [14] encourages us to extend our investigations to other felids.

We hypothesize that the fecal PGFM detected in the lynx might be representative for other felid species and that our PGFM enzyme linked immunoassay (EIA) can be used as a simple and reliable pregnancy test. To prove this hypothesis, we collected fecal samples from pregnant and pseudopregnant females of different felid species and determined PGFM by an EIA based on a new and more sensitive PGFM-specific antibody and a PGFM peroxidase conjugate [14].

2. Materials and methods

2.1. Animals and sampling

The housing locations of study animals are shown in Table 1. Ten different zoos contributed to the