



A Review on Non-Invasive Pregnancy Diagnosis in Wild Cats



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Submission: February 02, 2017; **Published:** February 28, 2017

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Abstract

Many wild cats around the world are facing the danger of extinction due mainly to habitat destruction, poaching, illegal trade, inbreeding, diseases and conflict with humans. As a result, conservationists moved towards the application of ex-situ conservation programs through captive breeding to save endangered species for future introduction to their natural homes. However, breeding success of these cats in captivity relies largely to the availability of non-invasive techniques using urine and fecal samples to detect pregnancy hormones in the early stages of reproductive management. Wild cats are usually prone to stress when physically restrained for blood collection to carry out pregnancy tests. This stress jeopardizes the success of captive breeding of endangered cat species. Monitoring reproductive hormones in urine and feces provided an alternative and successful tool for reproductive management of these species without exposing the animals to stress or workers to the danger of handling big cats. The detection of prostaglandin F2 alpha and its metabolites PGFM (13, 14-dihydro-15-Keto-PGF2 alpha) in urine and feces using radioimmunoassay (RIA) or enzyme immunoassay (EIA) has been extensively used for this purpose and is a breakthrough in pregnancy diagnosis in wild cats. Further studies are needed to validate the technique in other non-felids species.

Keywords: Captive breeding; Extinction; Stress; Pregnancy; Endangered; Metabolites

Introduction

About 36 species of felines have been recognized around the world where many of them are red listed by IUCN as vulnerable or endangered due to poaching, habitat loss and habitat fragmentation. Human activities are driving many species of wildlife towards extinction [1]. The number of species regarded as threatened more than doubled between 2000-2015 [2]. Many efforts have been exerted to counteract the driving forces that threaten wild felines in their natural habitat by applying ex-situ conservation programs. In the early 90s, captive breeding of threatened species became one of the more popular methods for saving endangered species [3]. However, the success of these efforts relies on better understanding of the reproductive cycles governed by the endocrine profiles of these species to facilitate ex-situ conservation efforts [4].

To launch captive breeding programs for captive felines some non-invasive techniques have been used to diagnose pregnancy in these species without stressing the animals. Stress-induced activities by veterinarians and their assistants to collect blood samples can have a negative impact on the animal welfare and lead to failure in conception [5]. Therefore, monitoring of reproductive hormones using urine and fecal samples provided

an important and alternative approach for pregnancy diagnosis in felines compared with traditional methods [6].

In mammals, uterine and placental prostaglandin F2 alpha is involved in the regulation of reproductive related processes such as embryonic development, parturition and resumption of ovarian activities. The hormone is rapidly metabolized in the liver to its plasma metabolite (PGFM, 13, 14-dihydro-15-keto-PGF2 alpha) which is excreted in urine and feces [7]. The detection of prostaglandin metabolite in the excreta of these species proved to be quick and non-stressful tool for pregnancy diagnosis for a wide collection of captive and free ranging animal species [8].

The present mini-review attempts to demonstrate the applicability of this technique from the point of views of their inventors and theoretically assess its success in feline's reproduction. Wild cats and their conservation status. Wild cats are wild species of the family Felidae that live naturally in different parts of the world within their normal range of distribution except in Australia and Antarctica. There are 36 species of felines that are recognized including lions (Pantheraleo), leopards (Pantherapardus), jaguars (Pantheraonca), tigers

(Pantheratigris), lynx (*Lynx lynx*), bobcats (*Lynx rufus*), cheetahs (*Acinonyx jubatus*), serval (*Leptailurus serval*), caracal (*Caracal caracal*) and other small cats. According to IUCN Red list (2007), one species is critically endangered, four species are endangered, 13 are vulnerable, eight are near threatened, and 11 are least concern. The animal status within the IUCN categories is based on the current population size and/or area of distribution. In the Arabian region including the Arabian Peninsula, Jordan, Syria, Iraq, and North African countries six species have been recognized where two species were assessed as regionally extinct, one species is critically endangered and three species are of least concern [9].

Living cats belong to two subfamilies, Pantherinae and Felinae. Pantherinae includes lions, leopards, and tigers while Felinae includes the rest of non-panther cats. Cats are obligate carnivores and they are sometimes called as hyper-carnivores because the very large proportion of animal protein they require to consume in diets compared to other carnivores [10]. Some of the wild cats, such as tigers are persecuted in their habitats for their medicinal values in China [11]. Similarly, other African cats such as lions, leopards, serval and cheetahs are severely poached. Cheetahs, for example is illegally shipped from Somalia to the Gulf region and to south East Asian countries [12].

Sample collection and storage

Hodges et al. [13] reviewed the methods of urine and feces sampling for steroid hormones detection in captive animals. They mentioned that urine samples can be collected midstream from a container placed under the drainage in the floor of the animal cage or by aspiration of the sample from the floor using a pipette or syringe. As small as 0.2ml of the sample is sufficient to quantify the amount of steroids in the urine. Samples preferably centrifuged before analysis to remove any debris contained and frozen at -20°C. Fecal samples, on the other hand, are collected directly from the floor, homogenized and frozen at -20°C until time of analysis. Long-term storage in ethanol or collection of old samples may alter the concentration of fecal steroid hormones [14-16].

Pregnancy diagnosis techniques in cats

Captive breeding of endangered species in zoos and other animal facilities has taken a considerable attention as a tool for maintaining bio-diversity [17]. In the beginning of 1960s, many studies, focusing on the concentration of relaxin hormone in companion dogs and cats, indicated the potential use of immunoreactive relaxin for pregnancy diagnosis in wild animals but with challenges in some species. Bergfelt et al. [18] stated that relaxin detection may have limited value for pregnancy diagnosis in wild bottlenose dolphins when used alone, but it could be of a confirmatory tool if used in combination with an elevation of progesterone in a single sample analysis. Some of the early techniques of detecting reproductive activities and pregnancy in wild cats measured the urine relaxin hormone using a bench-top serum assay (Witness relaxin kit, synbiotics corp.,

San Diego, California 92127, USA) [19]. Van Drossen et al. [20] stated that detection of urine relaxin for pregnancy diagnosis is a reliable method in domestic cats and other wild felines and canines. However, Dehnhard et al. [6] mentioned that the main disadvantage of this technique as a pregnancy diagnostic tool is its pregnancy related time course. The hormone level is highest at mid-pregnancy and fall to base line during the last trimester and hence prediction of parturition appears unachievable with this approach.

Other modified non-invasive methods for pregnancy diagnosis in wild cats were described by measuring urine and fecal prostaglandin (PGFM) metabolites by either enzyme immunoassay (EIA) or Radioimmunoassay (RIA). In mammals, uterine and placental prostaglandin (PGF2 alpha) is involved in the regulation of reproductive activity including embryonic development, initiation of parturition and resumption of ovarian activity. The hormone is rapidly metabolized to its plasma metabolite PGFM (13, 14-dihydro-15-keto-PGFM) that has been detected in urine and feces [21]. The levels of this hormone increased during the last trimester of pregnancy in seven of the eight main lineages of felidae and therefore, represented a suitable indicator for pregnancy diagnosis in these species [22,23]. Denhard et al. [6] suggested that monitoring PGF2 alpha metabolites in fecal samples is a reliable method for pregnancy diagnosis in cats and a threshold of over 5mg/g of dry feces can serve as indication for near parturition in a week time. Despite its wide applicability in many species the technique was of limited use in some other wild species including white rhinos (*Ceratotherium simum*), red fronted lemur (*Eulemur rufifrons*) and hares (*Lepus europaeus*) [21]. Despite success with assisted reproduction in felids and the humane non-invasive methods for pregnancy diagnosis, the results remain inconsistent [24]. Much of the inconsistency of the results seen in cats can be related to the two ovulation mechanisms observed in felid species: induced versus spontaneous. While success in assisted reproduction has been achieved in some species such as cheetahs and Ocelots (*Leopardus pardalis*) other species such as clouded leopards (*Neofelis nebulosa*), tigers and fishing cats (*Prionailurus viverrinus*) presented very little success [25]. Other research findings [26,27] concluded that prostaglandin F2 alpha is further metabolized to other metabolites that can be successfully used to diagnose pregnancy in Eurasian lynx and leopard cats (*Prionailurus bengalensis*).

Conclusion

To conclude I can say that in almost all-felid species urine and fecal PGFM can be used with an acceptable level to differentiate between pregnant, pseudo-pregnant and non-pregnant individuals. A good non-invasive pregnancy diagnosis can eliminate any source of stress to the species when other invasive methods (blood samples) are used. The technique may be less sensitive in other felid species and not applicable for other non-felid species. Further investigations are required to validate its application in other wild animal species.

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 DOI: 10.19080/JDVS.2017.01.555560

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