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Non-invasive reproductive monitoring in round-eared elephant shrews (*Macroscelides proboscideus*)

Nicht-invasives Monitoring von Sexualhormonmetaboliten bei Kurzohrrüsselspringern (*Macroscelides proboscideus*)

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Abstract

Breeding elephant shrews is difficult and few is known about their sexual cycle and their reproduction biology. Therefore, fecal estrogen and progesterone metabolite excretion was monitored in round-eared elephant shrews (*Macroscelides proboscideus*) (n=6) for up to four months. Daily fecal samples were collected and analyzed using enzyme immunoassays (EIA) for estrogen and progesterone metabolites. The female elephant shrews monitored in this study showed a variable cycle length of 6-31 days with an average cycle length of 15.5 days. Females with visual and olfactory contact with males had a shorter cycle length (average of 11.5 and 13 days) and higher progesterone metabolite values than females without contact with males. Due to this observation, we hypothesize that female round-eared elephant shrews have induced ovulation. Because of missing periodic variations in the progesterone concentrations, only estrogen can be used for non-invasive estrous cycle diagnostic in round-eared elephant shrews. Due to the small sample size in this study (n=6), further research is necessary.

Keywords: round-eared elephant shrew, *Macroscelides proboscideus*, reproduction biology, sexual cycle

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Introduction

The insectivorous elephant shrews or sengis belong to the mammalian clade *Afrotheria* (Rathbun, 2009). Within this clade, the order of the *Macroscelidea* contains one family (*Macroscelididae*) with four genera, which, depending on the author, comprise 15-17 species (Rathbun, 2009; Rudloff, 2009; Westheide & Rieger, 2015). Except for *Elephantulus rozeti*, the only species in northern Africa, the elephant shrews‘ distribution is restricted to the southern half of Africa (Douady, 2003).

The most common sengi in captivity is the round-eared elephant shrew (*Macroscelides proboscideus*). Its cute appearance and high activity rate make this species one of the most popular small mammals in zoological collections (Olbricht & Sliwa, 2010; Rudloff, 2009; Zootierliste, 2019). According to the literature, the estrus cycle of this monogamous species varies from 14 days to 2.5 months (Rudloff, 2009; Schubert, 2009). This study aimed to specify the female sexual cycle via non-invasive fecal hormone monitoring, a standard method used for endocrine analysis in many wildlife species (Schwarzenberger & Brown, 2013). Due to numerous confusions in sexing elephant shrews (Sicks, personal communication), we further aimed to develop a non-invasive hormonal sexing method.

Material and methods

The feces of three males and three females were collected daily for up to four months (Table 1). Because female “T“ has two sampling periods (T1 and T2), seven different symbols are listed in Table 1 (Table 1). All sand in the terrariums was sieved for daily fecal sample collection, and food debris, such as seeds, was removed. The fecal pellets were frozen at -18°C until the extraction started.

In order to allocate the feces individually, the animals were kept in solitary confinement during the sampling period. No fecal samples were collected on a few days, so the sample size in a those cases differs from the number of days in the sampling period (Table 1). All elephant shrews were of a suitable age for breeding (Olbricht, 2008; Olbricht & Sliwa, 2010).

Tab. 1: Overview of round-eared elephant shrews and sampling period for this study. The female “T“ has two sampling periods (T1 and T2).

symbol	year of birth	sex	keeping conditions	age at the 1st sampling day [months]	sampling period	sample size
A	2014	female	visual and olfactory contact to a male	36	2017-2018	127
T1	2013	female	visual and olfactory contact to a male	39	2016-2017	61
T2	2013	female	no contact to a male	49	2017	118
N	2016	female	no contact to a male	3	2016-2017	61
H	2013	male	visual and olfactory contact to a female	49	2017-2018	124
R	2014	male	visual and olfactory contact to a female	36	2017	12
J	2014	male	no contact to a female	42	2017	9
		total sample size				512

For sample extraction, 0.1 g feces were mixed with 2.0 ml methanol and vortexed for 30 minutes. After centrifugation for 15 minutes, the supernatant was diluted at 1:10, and samples were analyzed using established enzyme immunoassays (EIA) for fecal estrogen, progesterone, and androgen metabolites. The antibodies for the EIAs were raised in rabbits (Schwarzenberger et al., 2000). The following immunogens were used to generate the antibodies: 5α-pregnane-

3ß-ol-20-one 3HS:BSA for the 20-oxo pregnane EIA (Schwarzenberger et al., 1996), estradiol-17β-OH 17-HS:BSA for the estrogen EIA (Patzl et al., 1998) and 5α-androstane-3,17-dione 3-CMO:BSA for the epiandrosterone (=17-oxo-androstanes) EIA (Möstl & Brunner, 1997).

Results

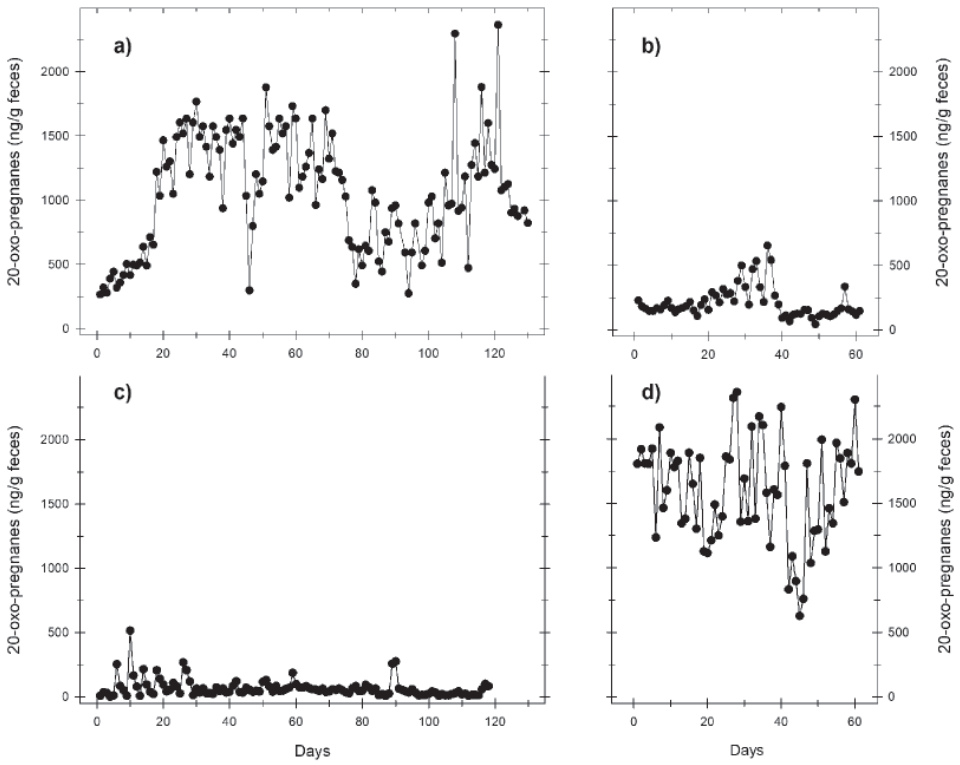

Fig. 1: Fecal 20-oxo-pregnane concentration of female "A" (a), female "N" (b), female "T2" (c) and female "T1" (d). Females "A" and "T1" had visual and olfactory contact to a male whereas Females "N" and "T2" where kept without contact to a male.

The husbandry system influenced the concentration of 20-oxo-pregnanes. Although 20-oxo-pregnanes did not reveal a cyclic pattern, females with visual and olfactory contact with a male had higher concentrations (Fig. 1). In contrast, estrogens were a good indicator of the ovarian cycle, as peak values of these hormones indicated follicular phases (Fig. 2). Females with sight and smell contact with a male had a shorter average cycle length ("A": 8 days; "T1": 14 days) than females without male contact ("T2": 28 days; "N": no cycle detectable) (Figs 1 and 2).

Epiandrosterone concentrations in females varied widely, ranging from 9.3 to 2,400 ng/g feces. The correlation coefficient with 20-oxo-pregnane concentrations was 0.93. In males, epiandrosterone concentrations ranged from 65.7 to 289.4 ng/g feces. However, there was no

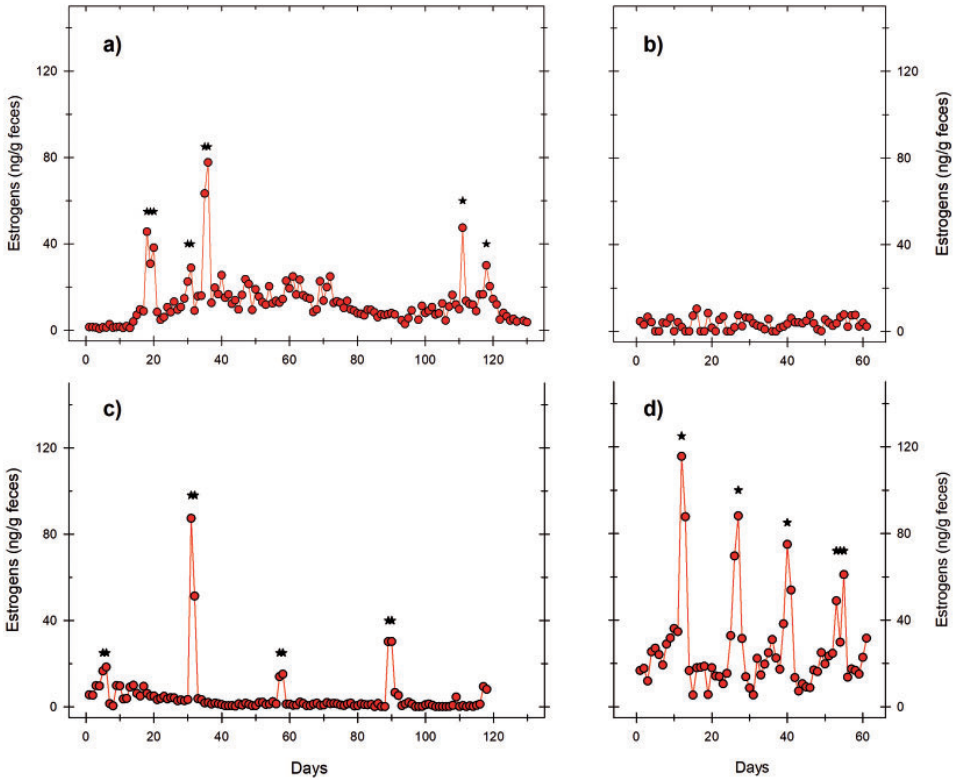

Fig. 2: Fecal estrogen concentration of female "A" (a), female "N" (b), female "T2" (c) and female "T1" (d). Females "A" and "T1" had visual and olfactory contact to a male whereas Females "N" and "T2" where kept without contact to a male. Estrogen values indicating a follicular phase are marked with a *.

significant difference between the epiandrosterone concentration in females and males, and thus it is not possible to identify the sex of the animals by measuring fecal androgen metabolite concentrations.

Discussion

The objective of this study was to obtain data on the female sexual cycle in elephant shrews; for this purpose, methods for non-invasive monitoring of fecal steroid hormone metabolites were established. Female elephant shrews in this study exhibited variable cycle lengths from 6-31 days, with an average cycle length of 15.5 days. The individuals with olfactory and visual contact with males exhibited a significantly shorter cycle and had significantly higher progesterone levels and pronounced estrogen peaks. In contrast, females without male contact exhibited low progestagen concentrations and occasional estrogen peaks. However, in terms of cycle diagnostics, progestagens were not informative. The results of hormone analyses suggest induced

ovulation in elephant shrews. Like in the elephant shrews we studied, higher progesterone concentrations were found in females in opposite-sex groups in species with induced ovulation; for example, in female lions (*Panthera leo*; Schramm et al. 1994) or cheetahs (*Acinonyx jubatus*; Brown et al. 1996). These results suggest that ovulation is triggered by olfactory and visual contact with males (McDermott, 2019; Jorge-Neto et al., 2020). The young female "N" had no contact with a male and showed no ovarian activity during our study, although it could be shown by Olbricht (2008) that other round-eared elephant shrews are reproductively mature in this age and mate successfully for the first time. Our results are supported by similar observations in brush-tailed bettongs (*Bettongia penicillata*; Hinds & Smith, 1992), Pallas' Cat -(*Otocolobus manul*; Brown et al., 2002) and grey short-tailed opossums (*Monodelphis domestica*; Hinds et al., 1992). However, the small sample size of our study must be considered, and further studies with samples from a higher number of individuals are needed to verify these results.

Breeding elephant shrews in captivity is difficult, and one possible reason is the problem of differentiating sexes and the associated pairing of same-sex individuals (Sicks, personal communication). Therefore, we attempted to develop a noninvasive method for sexing this species using androgen metabolite analysis. However, we were unable to detect significant differences in fecal epiandrosterone metabolite concentrations between female and male elephant shrews, leading us to conclude that this method is unsuitable for sex determination.

Another possible explanation for the difficulty in breeding round-eared elephant shrews is the territorial aggressive behavior of many round-eared elephant shrews in captivity, so the animals must be kept individually. It is possible that females prefer males that are least related to them when choosing mates, as seems to be the case with cheetahs (Kirkpatrick et al., 2006; McDermott, 2019). Due to the high inbreeding rate of elephant shrews in captivity in Europe (Olbricht & Sliwa, 2010), establishing breeding pairs is difficult. However, as no studbook records exist and zoos exchange animals with private keepers, genetic studies would be necessary to determine the degree of inbreeding.

Further studies with larger sample sizes collected from at least eight females are necessary to verify our hypothesis of induced ovulation in round-eared elephant shrews. Because hormonal sexing in round-eared elephant shrews was unsuccessful, a DNA-based methodology using fecal samples should be established in the future.

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Zusammenfassung

Die Zucht von Rüsselspringern ist kompliziert und bislang nur sporadisch erforscht. Daher wurde die tägliche Ausscheidung von fäkalen Östrogen- und Progesteronmetaboliten bei Kurzohrrüsselspringern (*Macroscelides proboscideus*) (n = 6) über einen Zeitraum von bis zu vier Monaten mittels Enzymimmunoessay (EIA) analysiert. Die weiblichen Rüsselspringer dieser Studie zeigten eine variable Zykluslänge von sechs bis 31 Tagen und eine durchschnittliche Zykluslänge von 15,5 Tagen. Weibliche Tiere mit Sicht- und Riechkontakt zu einem Männchen

wiesen dabei eine kürzere Zykluslänge (durchschnittlich 11,5 und 13 Tage) sowie eine höhere Progesteronmetabolitenkonzentration im Vergleich zu Weibchen ohne männlichen Kontakt auf. Aufgrund dieser Beobachtung stellen wir die Hypothese auf, dass weibliche Kurzohrrüsselspringer eine induzierte Ovulation haben. Aufgrund fehlender zyklischer Schwankungen der Progesteronkonzentration eignet sich lediglich Östrogen für die nicht-invasive Zyklusdiagnostik in Kurzohrrüsselspringern. Angesichts der geringen Stichprobengrößer dieser Studie (n = 6) ist weitere Forschung notwendig.

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