Title: Non-invasive measurements of ovarian activity in Beira antelope (*Dorcatragus megalotis*)

Running title: Ovarian cycle in Beira antelopes

Authors: Tanja E Wolf[1], Abdi Arif[2], Nigel C Bennett[1], André Ganswindt[1]

Affiliations: [1] Mammal Research Institute, Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa

[2] Wildlife & Veterinary Service, Al Wabra Wildlife Preservation, Doha, State of Qatar

Contents

As the natural habitat of more and more species becomes depleted, captive breeding programmes have become established to bring species back from the brink of extinction. Monitoring the reproductive status of an individual is essential in order to improve breeding success. Traditional methods have involved stressful blood sampling and thus non-invasive methods have been proven to be reliable alternatives for monitoring reproductive function in both captive and free-ranging animals. Subsequently, non-invasive methods have become an invaluable tool in longitudinal studies and conservation efforts, as animals can be observed without, or minimal human contact. The Beira antelope is a small antelope endemic to the northern part of the Horn of Africa. Population numbers of the Beira have been declining over the last few decades due to habitat fragmentation. We show here that the reproductive cycle of female Beira antelopes can be monitored non-invasively, by using faecal samples to analyse oestrogen (fEM) as well as progestogen (fPM) metabolites. The profiles of fPM and fEM of both females showed regular cyclic patterns in which the

follicular and luteal phases could be distinguished. The overall mean cycle length is 22 days (range 21-25 days), with a mean length of the follicular phase of 6 days (range 4-7 days) and a mean length for the luteal phase being 15 days (range 12-16 days). The suitability of these non-invasive techniques should assist in optimising breeding efforts of this endemic small antelope in captivity. Being non-invasive this method could also be a useful tool for monitoring reproductive function in the dwindling wild populations.

Keywords: Progestagen, Oestrogen, non-invasive, faecal samples, reproduction

1. Introduction

On a global scale, space for free-roaming wildlife is becoming more restricted. Thus breeding programmes of threatened species have been initialised over the last decades in an attempt to conserve and subsequently reintroduce species into similar habitat or small protected areas. Under captive conditions it is possible to extensively examine the reproductive status of individual animals in order to enhance breeding success by monitoring the pattern of ovulation or pregnancy (Hodges et al., 2010). Traditionally reproductive steroid hormones are measured in plasma, however, this involves an unnecessary regular handling of the animals possibly inducing stress, and consequently reducing reproductive success (Heistermann, 2010). In addition, handling and regular blood sampling from free-roaming species is logistically challenging. Thus, non-invasive methods have been proven to be reliable alternatives for monitoring reproductive function in captive and free-ranging animals, and as such, have become an invaluable tool in longitudinal studies and conservation efforts requiring observation of animals with no or minimal human contact

(Heistermann, 2010; Pickard et al., 2001). These non-invasive methods include the monitoring of steroid concentrations in urine, faeces, or saliva as hormone matrices. Although rather minimally invasive, measurements in saliva come with some limitations concerning the collection from wild animals, as it requires the animals to chew for example on a cotton pad, and is therefore mainly used when collecting material from animals in captive settings (Behringer and Deschner, 2017; Heistermann, 2010). Urine has been the matrix of choice to monitor reproductive activity in females for many years (for detailed review see Hodges et al., 2010), however, urine collection in wild animals is often complicated, especially in sandy terrain and if the animals cannot easily be approached closely (Thompson et al., 1998). Thus, faecal sampling is now the preferred method for monitoring female reproductive activity in, for example, a variety of antelope species (e.g. sable antelope (*Hippotragus niger*) (Thompson et al., 1998); Mohor gazelle (*Gazella dama mhorr*) (Pickard et al., 2001)).

The Beira antelope (*Dorcatragus megalotis*) is a small antelope endemic to the northern part of the Horn of Africa (northern Somalia, north-east Ethiopia, and southern Djibouti) (Giotto et al., 2008; Künzel and Künzel, 1998). Population numbers of the Beira have been declining over the last decades due to habitat fragmentation and it is therefore listed as 'vulnerable' by the IUCN (IUCN, 2016). Not much is known about the ecology or the behaviour of this species, mainly due to the rarity and the inaccessibility of its habitat (Giotto and Gerard, 2010). Currently only one *ex-situ* population exists at the Al Wabra Wildlife Preservation. In the wild births have been reported to occur bi-seasonally during the spring and autumn (Giotto et al., 2008), but in captivity this seasonal pattern disappears, like in other antelope species, including idmi gazelles (*gazella gazella ssp.*) or Soemmering's gazelles (*G. soemmerringii berberana*) (Hammer, 2011; Piening Schuler et al., 2009). Soon after

parturition females mate again and the next calf is born approximately 6 months later (Hammer, 2011). The only evidence of cycle length in female Beira comes from observation of mating behaviour in captivity revealing an ovarian cycle length of approximately 25 days (Hammer, 2011). The aim of this study was therefore two-fold: 1) to examine the suitability of two enzyme immunoassays (EIAs), detecting faecal oestrogen and progestagen metabolites, respectively, for monitoring female reproductive function in *Dorcatragus megalotis*; and 2) monitoring luteal activity and defining ovarian cyclicity.

2. Material and Methods

2.1 Animals and housing

The animals were housed in small family groups comprising one male, one to four females and their offspring at the Al Wabra Wildlife Preservation, Qatar. The enclosures offered natural vegetation as sight barriers and for shade. The animals were fed twice a day with *ad libitum* access to fresh water and mineral licks. The study was conducted following the ethical guidelines of the Al Wabra Wildlife Preservation and the European Association of Zoos and Aquaria (EAZA).

2.2 Faecal sample collection:

Faecal samples were collected every two to three days from two individual females between February and April 2015. Faecal samples were collected within 30 minutes of defaecation and stored frozen at -20°C until analysis at the Endocrine Research Laboratory at the University of Pretoria.

2.3 Faecal hormone extraction and analysis:

Hormone extraction followed already established protocols, with faeces being freeze-dried, pulverized, and sieved through a thin metal strainer in order to remove fibrous material (Fieß et al., 1999). Subsequently, 0.10 - 0.11 g of faecal powder was vortexed for 15 minutes with 80% ethanol in water (3 ml). The suspension was centrifuged for 10 min at 1500 g and the supernatant aliquoted and stored at -20˚C until analysis.

Steroid extracts were measured for faecal oestrogen (fEM) and progestagen (fPM) metabolites using two enzyme immunoassays (EIA), which reliably allowed the monitoring of ovarian activity in a variety of mammals (e.g. boars, (Palme and Möstl, 1993); black rhinoceros, (Schwarzenberger et al., 1996); lesser bushbaby, (Scheun et al., 2016). The fEM EIA uses antibodies against 17β-oestradiol-17-HS:BSA (Palme and Möstl, 1993), and the fPM EIA uses antibodies against 5β-pregnane-3α-ol-20-one-3HS:BSA (Schwarzenberger et al., 1996). Both assays have been biologically validated during this study by showing distinguishable hormone profiles linked to luteal activity in the two study animals. The assay procedure followed established protocols (Ganswindt et al., 2002). Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curve of the EIAs (relative variation (%) of the slope of respective trend lines < 5% for both assays. Sensitivity of the assays at 90% binding was 0.24 ng / g faecal dry weight (DW) for the fEM EIA and 9.6 ng /g DW for the fPM EIA. Inter-assay coefficients of variation as determined by repeated measurement of high- and low-value quality controls was 7.3% and 12.3% for the fEM EIA, and 11.0 and 15.3% for the fPM EIA, respectively. The coefficient for intra-assay variance also determined by repeated measurement of high- and low-value quality controls

was 8.9% and 9.7% for the fEM EIA, and 5.6 and 7.1% for the fPM EIA, respectively. All hormone analyses were performed at the Endocrine Research Laboratory, University of Pretoria, South Africa.

2.4 Data analysis

To determine the lengths of individual cycles and the presumed time of ovulation we used the fPM profiles. In this regard, a defined rise of fPM levels above a threshold was used to indicate the onset of the luteal (postovulatory) phase. For this we evaluated individual baseline levels of fPM concentrations using an iterative process (Aujard et al., 1998; de Bruin et al., 2014), where all values greater than the mean + 1.25 standard deviation (SD) of an individual's dataset were removed, the average was then recalculated and the procedure repeated until no values exceeded the baseline level. Oestrus cycle length was calculated as the interval between the two consecutive luteal phases. The follicular phase was defined as the period in which the progestagen levels were below the threshold and ended with the rise in oestrogen concentrations, indicating ovulation.

3. Results

A total of 91 faecal samples were analysed for the two individuals (45 from animal 1 and 46 from animal 2). The profiles of fPM and fEM of both females showed regular cyclic patterns in which the follicular and luteal phases could be distinguished. Both females showed similar absolute fPM concentrations, with maximum fPM concentrations during the luteal phase

(mean 43.67 µg / g DW) being five times higher than respective fPM concentrations during the follicular phase (mean 8.58 µg / g DW).

For female 1, two complete ovarian cycles could be identified, whereas for female 2 three complete cycles have been found (fig. 1). Overall mean cycle length, derived from the cycle lengths of the two females, is 22 days (range 21-25 days), with a mean length of the follicular phase of 6 days (range 4-7 days) and a mean length for the luteal phase being 15 days (range 12-16 days) (table 1).

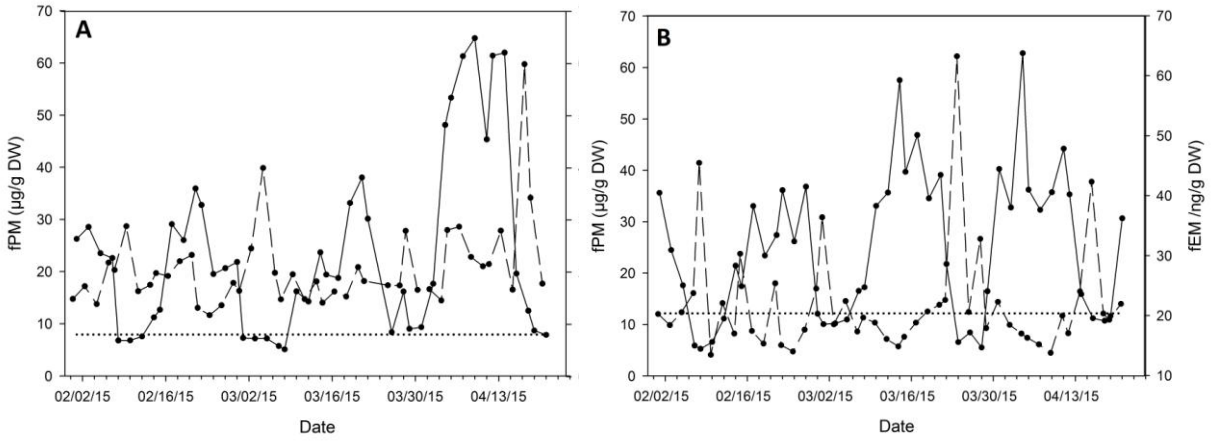

Fig. 1 Faecal oestrogen (dashed line) and progestagen (solid line) profiles of female 1 (A) and female 2 (B). Dotted line indicates progestagen baseline.

Ovulation, observed as an increase in fEM concentrations took place on average 9 days (range 7 – 12 days) before the increase in fPM concentrations. Although female 2 shows an overall higher elevation in fEM concentration (mean 46.84 ng / g DW) compared to female 1 (mean 37.42 ng / g DW), both females similarly show a two-fold increase in fEM concentrations.

Table 1: Ovarian cycle length in two Beira antelopes. All values are given in days.

	Female 1	**Female 2**
Cycle length (mean)	21 – 25 (23)	21 – 23 (22)
Follicular phase (mean)	5 – 7 (6)	4 – 5 (5)
Luteal phase (mean)	14 – 16 (15)	12 – 16 (15)

4. Discussion

Little is known about the reproduction of Beira antelopes. We show here that the reproductive cycle of female Beira antelopes can be monitored non-invasively by using faecal samples to quantify oestrogen as well as progestagen metabolites.

The determined cycle length in the two females is between 21 and 25 days, which is consistent with observational data on oestrus behaviour reporting an approximately 25 day cycle length (Hammer, 2011). This is coherent with the cycle length reported for other similar sized antelopes, for example the Guenther's dik-dik (*Madoqua guentheri*) and the suni (*Neotragus moschatus zuluensis*), for which cycle length of 20 days and 21 days, respectively, have been reported (Loskutoff et al., 1990; Robeck et al., 1997).

Female Beira antelopes have been reported to mate shortly after birth, a pattern that can be seen also in other ungulates (e.g. giraffes (Hall-Martin and Skinner, 1978)). The two females showed regular cycles over the course of the study, but did not conceive during this time, thus a prolonged monitoring period would be needed to evaluate potential differences between fEM and fPM alterations related to cyclicity and pregnancy in hormonal profiles of Beira antelopes. Captive Beira have been reported to breed throughout the year (Hammer, 2011), although a bi-seasonal birthing pattern is apparent in the wild (Giotto et al., 2008). This loss of seasonality when housed under human care has also been reported for other antelopes, such as the idmi and Soemmering’s gazelles (Piening Schuler et al., 2009). In non-

seasonal breeders, birth peaks often arise during times that offer the best conditions for mother and offspring survival (Rutberg, 1987; van Schaik and van Noordwijk, 1985). In this regard, the period of lactation is the energetically most demanding period for mammalian females, which in the majority of species coincides with the weaning period, and therefore should coincide with the annual time of food abundance (van Schaik and van Noordwijk, 1985). Another possibility for birth peaks is the synchronisation of birth in order to reduce the risk of predation, as it is often seen in ungulates (Rutberg, 1987). We do not have supporting data from the wild and thus our explanation for the lack of potential seasonality is speculative, but in the case of the Beira it is more likely that food abundance is the driving effect for birth peaks due to their social system. Beira antelopes live in small groups comprised of one male and several females and in this respect have a social system similar to dik-diks (Giotto et al., 2008).

Faecal hormone analysis has been used in a variety of mammal species to define female cycle length, including primates (Heistermann et al., 2001), elephants (Fieß et al., 1999), or Mhorr gazelles (Pickard et al., 2001), but there is a lack of information especially in small antelope species. Although we only monitored two females over a comparatively short period, resulting in a limited data set, this has been, to our knowledge, the first study monitoring luteal activity and describing ovarian cyclicity in Beira antelope. With this approach, we also managed to confirm the suitability of two EIAs, for monitoring fEM and fPM concentrations in female Beira antelopes, respectively.

The suitability of these non-invasive techniques should assist in optimising breeding efforts of this endemic small antelope in captivity. As regular faecal sampling every two to three days appear adequate to assess ovarian cyclicity in female Beira, this method could also be a useful tool for monitoring reproductive function in the dwindling wild populations.

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Conflict of Interest

None of the authors have any conflict of interest to declare.

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