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ORIGINAL RESEARCH

Endocrine correlates of female reproductive activity in the roan antelope (*Hippotragus equinus*)

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Abstract

Although ovarian endocrine activity has been described in most members of the tribe Hippotragini, little is known about the reproductive endocrine correlates of the roan antelope (*Hippotragus equinus*), an endangered antelope species of southern Africa. This study characterised the endocrine pattern of the reproductive activity (oestrous cycle, pregnancy and post-partum period) in the female roan antelope by monitoring faecal progestogen metabolite (fPM) concentrations and oestrogen metabolite (fEM) concentrations for cyclicity and observing reproductive behaviours. The study was carried out over the course of 12 months. At the beginning of this study, all 18 focal females (nulliparous, primiparous and multiparous) were monitored for cyclicity (for between 3 and 4 months). Following observed copulation, pregnancy in multiparous females (n = 10) was subsequently monitored until parturition and 21 days post-partum. Individual faecal samples were collected three times per week during the presumed period of cyclicity, twice per week during pregnancy and daily for 21 days post-partum. Mating births, courtship events, and copulations were recorded when observed. In eight of the 18 females monitored, the patterns of fPM and fEM concentrations indicated oestrous cycle lengths (n = 10) of approximately 30.8 ± 2.1 days. The determined lengths of luteal and follicular phases were approximately 15.8 ± 1.5 days and 14.6 ± 2.1 respectively. Pregnancy was marked by a pronounced increase in fPM concentrations until parturition, and lasted approximately 280.4 ± 4.8 days; whereas the intercalving period ranged between 306 and 380 days (n = 9; mean: 333.2 ± 7.4 days). Twenty-one day after parturition, 78% of the focal females (n = 5) did not show a resumption of the ovarian activity. This study demonstrated that monitoring faecal reproductive hormone metabolite patterns is a valuable approach for estimating ovarian activity in roan antelope and may be used to assist respective conservation breeding programmes by improving management practices.

Introduction

The global impact of anthropogenic activity has threatened the existence of many species and caused the extinction of others (Sayer & Whitmore, 1991; De Vos et al., 2014). As a consequence, over the past few decades, captive breeding has been applied to the conservation of threatened ungulate species such as the Mhorr gazelle (*Nanger dama mhorr*) and the Arabian oryx (*Oryx leucoryx*; Abáigar et al. 2019; Spalton et al., 1999), thereby promoting an increase in the global populations of these endangered species (Ralls & Ballou, 2013). However, developing adequate reproductive management practices to improve the breeding outcome remains a major challenge for captive breeding facilities as it fundamentally relies on understanding the reproductive physiology of the species in question, which is often not known in detail (Sontakke, 2018). Monitoring the pattern of female reproductive hormones, such as oestrogens and progestogens, can provide valuable information about the length of the oestrous cycle and the pregnancy period, the two main processes of female reproductive success. Besides this, information on the resumption of ovarian activity post-partum may be acquired. Oestrogens and progestogens are not only involved in the onset and regulation of the oestrous cycle and pregnancy, but also in the display of sexual behaviours (Christensen et al., 2012; Kauffman & Micevych, 2019; Rathbone et al., 2001). Hence, a deep

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understanding of these key processes is fundamental to successfully apply advanced techniques in conservation breeding programmes, including oestrus synchronisation and artificial insemination. When correctly applied, these tools can greatly enhance the breeding success of respective species in such programmes (Andrabi & Maxwell, 2007; Loskutoff et al., 1995; Sontakke, 2018).

Traditionally, the steroid hormones oestrogens and progestogens were quantified in plasma (Schwarzenberger & Brown, 2013), but since the late 1970s, there has been an increasing interest in the application of non-invasive techniques to assess the reproductive activity and fertility status of females (Graham, 2004; Loskutoff et al., 1995; Schwarzenberger, 2007). Measuring hormones in blood is ideal when the respective focal females are easily and frequently accessible. However, when studying free-roaming individuals, this approach may become challenging as blood collection often requires anaesthesia and restraint which may be stressful and comes with health risks for the animal (Hodges et al., 2010; Pukazhenthi & Wildt, 2003). An alternative non-invasive method is the quantification of related hormone metabolites in faeces. Faecal sample collection is comparatively easy, logistically less challenging, and safe for the collector and the animal (Schwarzenberger & Brown, 2013; Sontakke, 2018). Furthermore, this approach facilitates a frequent longitudinal sample collection from individuals, which is often required for an accurate evaluation of cyclicity (Graham, 2004; Hodges et al., 2010). However, when quantifying hormone metabolites non-invasively for the first time in a species, there is a need to validate methods, such as enzyme immunoassays (EIAs), to ensure a reliable quantification of respective hormone metabolites (Kersey & Dehnhard, 2014).

Non-invasive monitoring of faecal oestrogen (fEM) and progestogen (fPM) metabolite concentrations has proven to be a valuable tool in understanding the reproductive physiology of various antelope species (Mohammed et al., 2011; Pickard et al., 2001; Thompson et al., 1998; Wolf et al., 2019). Evidence from previous studies on sable antelope (*Hippotragus equinus*), scimitar-horned oryx (*Oryx dammah*) and Arabian oryx (*Oryx leucoryx*) has shown that the differences in length of oestrous cycle and pregnancy within the tribe Hippotragini does not differ distinctively. Generally, hippotragine species exhibit an oestrous cycle length of approximately 24 days (Morrow et al., 1999; Sempéré et al., 1996; Thompson et al., 1998) and the lengths of pregnancy are around 9 months (Dittrich, 1974; Sempéré et al., 1996). For hippotragines and bovine species that have been investigated thus far, progestogen concentrations increase throughout pregnancy and suddenly decline with parturition (Ostrowski et al., 2005; Robertson, 1972). Ovarian activity appears to resume within 18–25 days after parturition in Hippotragini such as the Arabian oryx (Asa et al., 1996; Ostrowski et al., 2005), whereas, in other ungulate species such as cattle (*Bos taurus*), this has been reported to vary between 16 and 69 days (Webb et al., 1980).

The roan antelope (*Hippotragus equinus*) is a large antelope widely distributed across the western, central, eastern and southern parts of Africa. This antelope once occurred in large numbers in savannas and grasslands, but numbers have drastically declined over the last few decades (Havemann et al., 2016). Although this species is classified as 'least concern' by the International Union for the Conservation of Nature (IUCN, 2008), roan antelopes are currently classified as vulnerable in southern Africa and have been reported to be critically endangered in areas such as the Kruger National Park (South Africa; Dörgeloh, 2001; Harrington et al., 1999; Kruger et al., 2016). This situation has led to intensive breeding programmes in southern Africa, where the numbers of captive individuals have increased considerably over the last few decades (Havemann et al., 2016; Taylor et al., 2016). Despite this situation, there is still a scarcity of endocrine-related information on the oestrous cycle and pregnancy period for this species. According to previous reports, roan antelopes are non-seasonal breeders with an inter-calving period of around 320 days and a pregnancy length ranging from 268 to 286 days (Dittrich, 1974; Skinner & Chimimba, 2005). However, these reports were based on observations only and information on ovarian endocrine activity is lacking. The current study set out to determine the oestrous cycle, pregnancy period and post-partum period in captive roan antelopes by assessing faecal steroid metabolite concentrations. The information gleaned can be used to improve management practices of captive breeding programmes. More specifically, the objectives of this study were: (1) to establish two EIAs for measuring fEM and fPM concentrations in female roan antelopes; (2) to characterise the ovarian activity and its endocrine correlates for this species, and (3) to evaluate the applicability of quantifying fPMs to diagnose pregnancy in roan females.

Materials and methods

Study area and animals

This study was conducted at Lapalala Wilderness Nature Reserve, Limpopo, South Africa (23°53′3.59″S; 28°18′0.61″ E). This reserve is a privately owned, 48 000 ha conservation area located within the UNESCO Waterberg Biosphere, where a founder group of roan antelope arrived in 2010 as part of a reintroduction programme. The vegetation comprises open grassland, open shrubland, as well as open to closed woodland (Low & Rebelo, 1996; Mucina et al., 2006). Eighteen females aged between 1.8 years to 6.0 years (~150–250 kg) were monitored for 3–4 months to determine cyclicity, whereas 10 out of those 18 individuals were monitored for a period of 12 months (from August 2017 to July 2018) to evaluate endocrine correlates of pregnancy, and 5 out of those 10 females were monitored for an additional 3 weeks to look at post-partum period. The 18 females were selected from three breeding herds each consisting of one reproductive male, adult females (Herd A: n = 9; Herd B: n = 10; Herd C: n = 4), heifers (Herd B: n = 2; Herd C: n = 9), juveniles (Herd A: n = 2; Herd B: =2; Herd C: n = 2) and calves (Herd A: n = 6; Herd B: n = 5; Herd C: n = 4). In this study, adult females are those that have calved at least once, heifers are females from 2 years old that have never calved; juveniles are animals (male or female) between 1 and 1.5 years, and calves

are animals (male or female) up to 1 year. The focal females were classified as being either multiparous, primiparous or nulliparous, and the age of each animal was determined from their recorded date of birth and assigned at the beginning of the study (Table 1). Information about previous births of all the multiparous females monitored were also provided by the reserve management. The breeding herds were housed in 10- to 20-ha camps that were not adjacent to each other. This study was carried out without interfering with the actions of reserve management. All the reproductive males were introduced in the breeding camps approximately at the same period of the year to synchronise births. The females were exposed to the breeding males throughout the study period, through visual (VC) and olfactory (OC) contact when the males were housed in adjacent camps. The herds grazed on pasture and were supplemented daily with A-grade lucerne (*Medicago sativa*) and protein feed (160 g/kg; 0.9 kg per animal per day) during the study period. Water was available *ad libitum*. All females included in this study were identified by a unique tag number on the ear as well as physical features such as horn shape, ear shape, colour of the fur and natural markings.

Ovarian activity and pregnancy monitoring

All 18 females selected for this study were monitored to assess oestrous cycle length (Table 1). For oestrous cyclicity, females selected from Herd A and B were monitored from August through to early October 2017, whereas those from Herd C were monitored from May 2018 through to July 2018. At the beginning of the study, two multiparous females were at the late phase of pregnancy, three multiparous females calved on the first day and the remaining primiparous and multiparous females were lactating. Thus, sample collection began between day 0 and day 60 post-parturition. The reproductive males were introduced into the breeding camps in September (from August: Herd A and B) and June (from May: Herd C) onwards.

For pregnancy, females (n = 10) were monitored from observed copulation until parturition. Following birthing, some females (n = 5) not showing frequent horn threat (indicating aggressive behaviour due to maternal instinct) were monitored daily for 21 days to determine the resumption of ovarian activity.

Behavioural observations and faecal sample collection

All focal females (n = 18) were observed daily for a minimum of 2 h. *Ad libitum* and scan sampling techniques (Altmann, 1974) were used to assess reproductive behaviours such as flehmen, courtship and copulation (Kamgang et al., 2021). Successful copulatory and parturition events were also recorded. After birthing, the calves were observed to determine the length of their concealment period. In parallel with observations, faecal sample collection was carried out three times per week for each female from the beginning of the oestrous cycle until 4 weeks after observed copulation (for females monitored for cyclicity only), and/or until the male showed reduced interest in a female (for females monitored for cyclicity and pregnancy). For pregnancy monitoring, faecal samples were collected from every multiparous female presumed pregnant (i.e. from 4 weeks following the last observed copulation) twice a week until delivery; whereas for post-partum, collection was carried out daily for 21 consecutive days for some females (n = 5). Each faecal sample (15–20 g) was collected within 1 h following defecation as soon as

Table 1 Characteristics of the females monitored in the study

Herd	Female ID	Age (years)	Reproductive history	Lactating	RS	RE	NSC
A	F2	6	M	✔	P	OC, P	48 (OC)
A	F3	6	M	✔	C	OC, P, PP	24 (OC), 63 (P), PP (21)
A	F5a	6	M	✔	PP	OC, P, PP	24 (OC), 63 (P), (21) PP
A	F4	6	M	✔	PP	OC, P, PP	23 (OC), 71 (P), 21(PP)
A	F5b	6	M	✔	PP	OC, P	23 (OC), 64 (P)
B	F11	3	M	✔	PP	OC, P, PP	24 (OC), 74 (P), 21 (PP)
B	F13	3	M	✔	C	OC, P, PP	23 (OC), 76 (P), 21 (PP)
B	F16	3	M	✔	PP	OC, P	22 (OC), 71 (P)
B	F17	3	M	✔	P	OC, P	22 (OC), 71 (P)
B	F18	3	M	✔	pp	OC, P	24 (OC), 64 (P)
B	F20	6	M	✔	PP	OC, P	24 (OC), 66 (P)
C	F23	2	N		C	OC	37
C	F22	2	P	✔	PP	OC	33
C	F24	2	P	✔	PP	OC	41
C	F25	2	P	✔	PP	OC	34
C	F26	2	P	✔	PP	OC	36
C	F30	2	N		C	OC	31
C	F33	1.8	N		C	OC	35

The females from Herds A and B were monitored from August 2017 to early October 2017, whereas females from Herd C were monitored from May to July 2018. RH (reproductive history): Multiparous (M), primiparous (P), nulliparous (N). RS (reproductive status at the beginning of the experiment): pregnant (P), cycling (C), post-partum (PP). RE: reproductive event monitored: oestrous cycle (OC), pregnancy (P), post-partum (PP); NSC, number of sample collected per reproductive state.

the animal had moved away. Faeces were collected with gloves and placed in a 30-ml plastic container. The container was immediately stored in a cooler box and was placed in a freezer within 1-h post-collection (−20°C), and stayed frozen until further processing.

Steroid extraction and analysis

Frozen samples were lyophilised, pulverised and sieved using a thin mesh to remove fibrous material as described by Fieß et al. (1999). Following this, 0.100–0.110 g of faecal powder was extracted by adding 3 ml of 80% ethanol. Subsequently, the suspension was mixed, vortexed for 15 min and centrifuged for 10 min at 1500×***g***. The supernatant was aliquoted into 1.5 ml microcentrifuge tubes and stored at −20°C until analyses. Concentrations of fEM and fPM were determined by EIAs utilising antibodies against 17β-oestradiol-17-HS and 5β-pregnane-3α-one-3HS: BSA respectively. Further assay characteristics including antibody cross-reactivities are provided for the fEM by Palme & Möstl (1993) and for the fPM EIA by Schwarzenberger et al. (1996). Both assays were biologically validated by demonstrating significant increases in fEM (d.f. = 4; t = 3.7; P = 0.021) and fPM (d.f. = 4; t = −7.8; P = 0.002) concentrations in females (n = 5) when pregnant (late gestation prior to parturition) compared to non-pregnant. These females were pregnant or calved at the beginning of the study (on the first day of sample collection). Serial dilutions of faecal extracts (n = 5) gave displacement curves that were parallel to the respective standard curve of the utilised EIAs (relative variation (%) of the slope of respective trend lines <3% for the fEM EIA and <5% for the fPM EIA). The sensitivity of the assays was 0.24 ng/g dry weight (DW) for the fEM EIA and 9.6 ng/g DW for the fPM EIAs respectively. Intra-assay coefficients of variance (CV) of high- and low-concentration controls were 5.33 and 6.35% for the fEM and 7.49 and 7.56% for the fPM assay respectively. Inter-assay CV of high and low-concentrations were 11.69 and 14.48% for the fEM and 13.55 and 14.89% for the fPM assay respectively. All analyses were conducted at the Endocrine Research Laboratory, University of Pretoria, South Africa.

Data analyses

To determine the length of individual oestrous cycles and presumed time of ovulation, individual baseline fPM concentrations were determined for each female using an iterative process (slightly modified) based on Morrow et al. (1999), whereby all values exceeding the mean + 1.25 SD were removed and the mean and SD calculated again. This process was repeated until no values exceeded the criterion value (mean + 1.25 SD), thus representing the baseline fPM concentration for this individual. For determining individual baseline fEM concentrations, the same process was applied with the mean + 2 SD (Morrow et al., 1999). All fPM values exceeding the baseline for two or more consecutive data points were considered indicative of a luteal phase. The length of a luteal phase was determined from the first day that fPM concentrations rose above baseline until the day the fPM concentrations returned to baseline. The follicular phase was defined as the period from which two consecutive fPM values did not exceed the baseline and/or might be marked with a rise in fEM concentrations above baseline. The oestrous cycle length was defined as the interval of time between the start of two consecutive luteal phases. For each female the luteal and follicular phase length were determined by calculating the average length for the luteal/follicular phases identified.

Conception was determined from the first-day fPM concentrations rose after observed copulation and stayed elevated 6–8 weeks following the onset of this increase (Polegato et al., 2018; Sempéré et al., 1996). Pregnancy length was defined as the day of conception until the day of parturition. The length of pregnancy was divided into two stages: early (from conception until 6 months after conception) and late pregnancy (from 6 months following conception until parturition). The inter calving period was defined as the period between the day of the previous calving (available in the records of the reserve) and the day calving had been observed.

For all females, individual means of fEM and fPM concentrations were calculated for each phase of the oestrous cycle. Log-transformation failed to normalise mean fEM concentrations during the phases of the oestrous cycle. Subsequently, we calculated median fEM concentrations for each female. This data set was also not normally distributed when log-transformed. Thus, a Wilcoxon rank test was used to compare respective median fEM concentrations between the follicular and luteal phase of the oestrous cycle. A paired-sample t-test with Bonferroni correction was used to test for differences in fPM concentrations between: Baseline concentrations and respective concentrations during the luteal phase of oestrous cycle; during the different phases of pregnancy (early pregnancy: from conception up until 6 months; late pregnancy: from 6 months until calving); baseline concentrations, concentrations at birthing and after parturition (10 and 21 days post-partum). Paired sample T-test were performed to test difference in fPM concentrations between the pregnant state and the non-pregnant state in individuals, between the luteal phase and early phase of pregnancy for females with a regular oestrous cycle.

All statistical analyses were carried out using the IBM package Statistical Package for Social sciences (SPSS) version 26.0 (2019) Armonk, NY, USA:IBM Corp. The results are presented as mean ± standard error (SE; fPM) or median ± interquartile range (fEM) and were found to be significant at $P < 0.05$.

Results

fPM and fEM baseline concentrations

Combined data from all the females monitored herein indicate that overall, the mean female fPM baseline concentration was approximately 9.1 ± 1.2 μg/g DW (n = 18). In nulliparous females, overall, mean fPM baseline concentration was approximately 18.4 ± 0.1 μg/g DW; whereas respective overall mean fPM baseline concentrations in multiparous and the primiparous female were approximately 8.7 ± 0.4 μg/g DW and 3.4 ± 0.3 μg/g DW respectively. Overall median fEM baseline concentration in all females was 59.6 ± 3.5 ng/g DW; with

multiparous females showing an overall median baseline fEM concentration of 62.8 ± 5.4 ng/g DW; the primiparous female showing a median baseline of 55.7 ± 2.3 μg/g DW, and nulliparous females an overall median baseline of 53.12 ± 1.2 ng/g DW.

Endocrine correlates of the oestrous cycle

For eight of the 18 females monitored (multiparous: n = 5; primiparous: n = 1; nulliparous: n = 2) at least one ovarian cycle with a distinct variation in fPM concentrations could be identified (Table 2). However, not all identified cycles showed a variation in fEM levels. Among these females, one multiparous female (F2) showed three consecutive oestrous cycles of similar length (Fig. 1).

Overall, the average lengths of the luteal and the follicular phases were approximately 15.8 ± 1.5 days and 14.6 ± 2.1 days respectively. The length of the luteal and the follicular phase was 16.3 ± 1.7 and 16.8 ± 2.2 days, respectively, in multiparous females, and 17.1 ± 1.1 and 16.8 ± 2.2 days in the primiparous and nulliparous females. The length for the ovarian cycles identified was approximately 30.8 ± 2.1 days ranging between 16 and 39 days (Table 2). Faecal PM concentrations during the luteal phase (17.4 ± 2.1 μg/g DW, range: 10.5–28.7 μg/g DW) were significantly higher compared to baseline concentrations (10.3 ± 2.1 μg/g DW; P = 0.048, t = −5.84). Similarly in all females with an identified oestrous cycle, fEM concentrations were significantly elevated (Z = 2.4; Fig. 3; Table S1) during the follicular phase (median: 70.7 ± 25.2 ng/g DW) compared to the luteal phase (median: 54.6 ± 12.1 ng/g DW; P = 0.017) and baseline concentrations (median: 52.26 ± 1.9 ng/g DW; P = 0.012). The follicular phase was also marked by the display of receptive behaviours such as copulation and courtship circling between the male and the female (Kamgang et al., 2021) that occurred at the beginning of the follicular phase (Fig. 1; Fig. S1).

The remaining 10 females monitored for oestrous cycle length lacked an apparent cycle. These females were immediately impregnated by the reproductive male after his introduction to the herd. The multiparous females that did not show a cycle (n = 6) exhibited an anoestrus period of between 14 and 42 days. With the resumption of luteal activity, copulation occurred and fPM levels increased up until parturition (Fig. 2a). The same pattern was observed for primiparous females (n = 3), which showed an anoestrous period of between 48 and 62 days followed by sustained elevated levels of fPM concentrations after observed copulation (Fig. 2b). In this study, both primiparous and multiparous females that were monitored showed high fEM concentrations at the beginning of the cycling period (period immediately following postpartum, when the study began). Results showed that these high levels decreased between 8 and 20 days (Fig. 1 and 2).

Endocrine correlates of pregnancy

Among the 10 multiparous females monitored throughout pregnancy, one female (F20) had a miscarriage (indicated by vaginal bleeding). The length of pregnancy determined by monitoring the elevation of fPM concentrations post-copulation (calculated conception date) until parturition was approximately 280.4 ± 4.8 days and ranged between 264.0 and 307.0 days. The intercalving period varied between 306 and 380 days (333.2 ± 7.4 days). Females showed a significant increase in fPM concentrations during pregnancy compared to the non-pregnant state (33.3 ± 2.0 μg/g; DW vs. 10.7 ± 0.7 μg/g DW; d.f. = 9; P = 0.001).

For the nine multiparous pregnant females monitored until parturition, fPM concentrations increased significantly throughout the different phases of pregnancy (P = 0.001; n = 9; Fig. 3; Fig. 4; Table S3; Fig. 5). For multiparous females (n = 5) that showed ovarian cyclicity, fPM concentrations at early pregnancy (mean: 21.7 ± 1.1 μg/g DW) were higher compared to respective concentrations during the luteal phase of oestrous cycle (mean: 14.9 ± 2 μg/g DW; t = −2.7; P = 0.05). For all females monitored throughout pregnancy, during early pregnancy, individual fPM concentrations (mean: 22.56 ± 0.9 μg/g DW) were significantly higher (P = 0.001) than respective baseline fPM concentrations (mean: 8.7 ± 0.5 μg/g DW). Respective fPM concentrations during late pregnancy (56.8 ± 2.7 μg/g DW) were significantly higher compared to individual fPM concentrations during early pregnancy (P = 0.001; Fig. 4). Mean individual fPM levels during early and late pregnancy for all females with successful pregnancies were 40.6%, and 38.5% higher than the respective concentrations measured for the miscarrying female F20. In females sampled on parturition day (n=6) fPM levels were 63% lower compared to levels 72h prior to parturition.

Table 2 Faecal progestogen and oestrogen metabolite baseline levels and concentrations; oestrous cycle lengths, follicular and luteal phase lengths during oestrous cycle in 8 female roan antelope

Individuals	F2	F3	F4	F13	F16	F22	F30	F33	Mean ± SEM
Number of cycles monitored	3	1	1	1	1	1	1	1	1.3 ± 0.3
Oestrous cycle length (days)	37, 33, 32	32	29	26	29	16	35	39	30.8 ± 2.1
Follicular phase length (days)	11, 12, 13	24	14	12	14	7	16	23	14.6 ± 2.1
Luteal phase length (days)	24, 19, 15	8.5	11	14	15	9	19	16	15.8 ± 1.5
fEM baseline (ng/g DW)	43.5	56.8	50.28	52.0	64.8	52.41	52.16	56.7	53.2 ± 2.2
fEM follicular phase conc. (ng/g. DW)	42.5	63.0	89.3	84.3	72.3	64.3	65.7	69.0	68.4 ± 5.1
fEM luteal phase conc. (ng/g. DW)	37.5	54.0	52.3	56.8	56.8	52.4	62.7	60.4	54.5 ± 2.9
fPM baseline (μg/g DW)	8.4	10.4	7.9	6.5	7.9	2.6	18.5	18.4	10.3 ± 2.1
fPM follicular phase conc. (μg/g DW)	7.4	7.5	7.8	7.9	8.8	4.2	16.7	19.4	10.0 ± 1.9
fPM luteal phase conc. (μg/g. DW)	21.6	14.6	16.9	11.0	10.5	15.8	25.3	25.6	17.4 ± 2.1

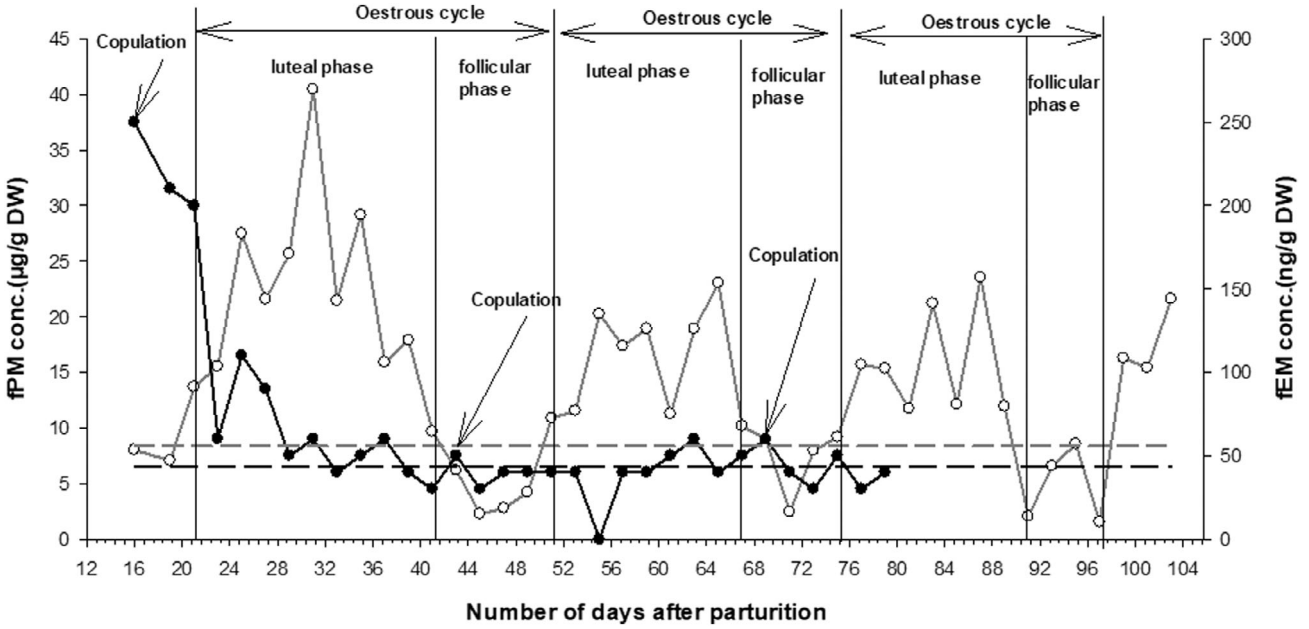

Figure 1 Longitudinal profile of faecal oestrogen metabolite (fEM) and faecal progestogen metabolite (fPM) concentrations profiles during three consecutive regular oestrous cycles in a multiparous female (F2). The solid black line with black dots represents the fEM profile and the solid dark grey line with white dots represents the fPM profile. The dashed dark grey and black lines represent fPM and fEM baseline levels respectively. The *x*-axis represents the number of days post-partum.

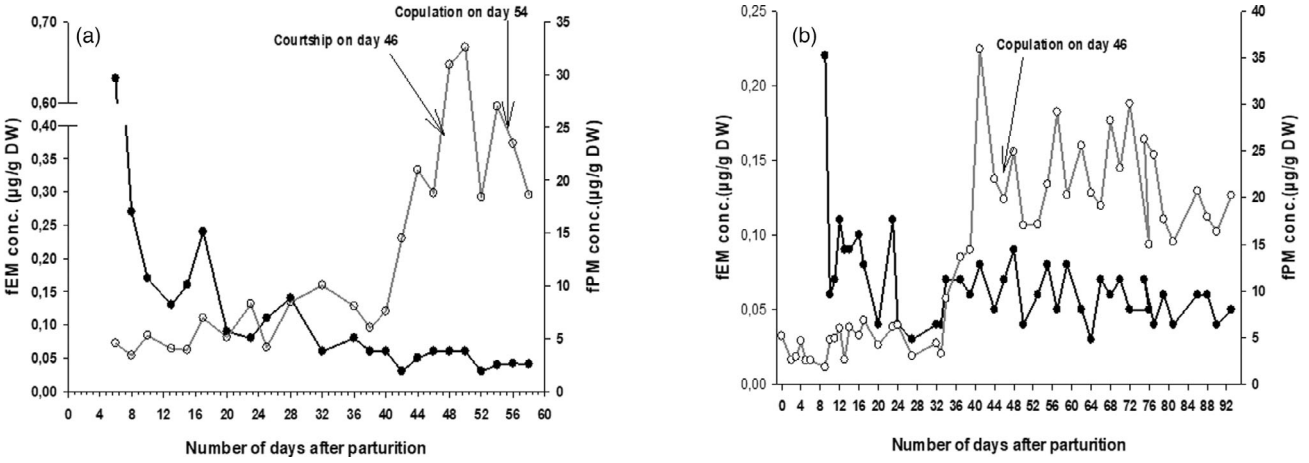

Figure 2 Longitudinal profiles of faecal oestrogen metabolite (fEM) and faecal progestogen metabolite (fPM) concentrations during the period 0–92 days after-parturition for a multiparous female (a) and a primiparous female (b) with irregular luteal activity and no clearly identified cycle. The black line with black dots represents the fEM profile and the dark grey line with white dots represents the fPM profile. The *x*-axis represents the number of days after-parturition.

Endocrine correlates of the ovarian activity following parturition

After calving, females concealed the calves within thickets inside the camps for a period of between 8 and 12 days (mean: 8.1 ± 0.6 days). After parturition, fPM levels gradually and significantly decreased ($P < 0.01$; mean 10 days post-partum 10.3 ± 3.47 µg/g DW and mean 21 days post-partum: 6.3 ± 1.3 µg/g DW; Table S4). Although females showed variation in fPM concentrations post-partum, no cyclical pattern could be identified within the 21 days post-partum period in four of the five females monitored.

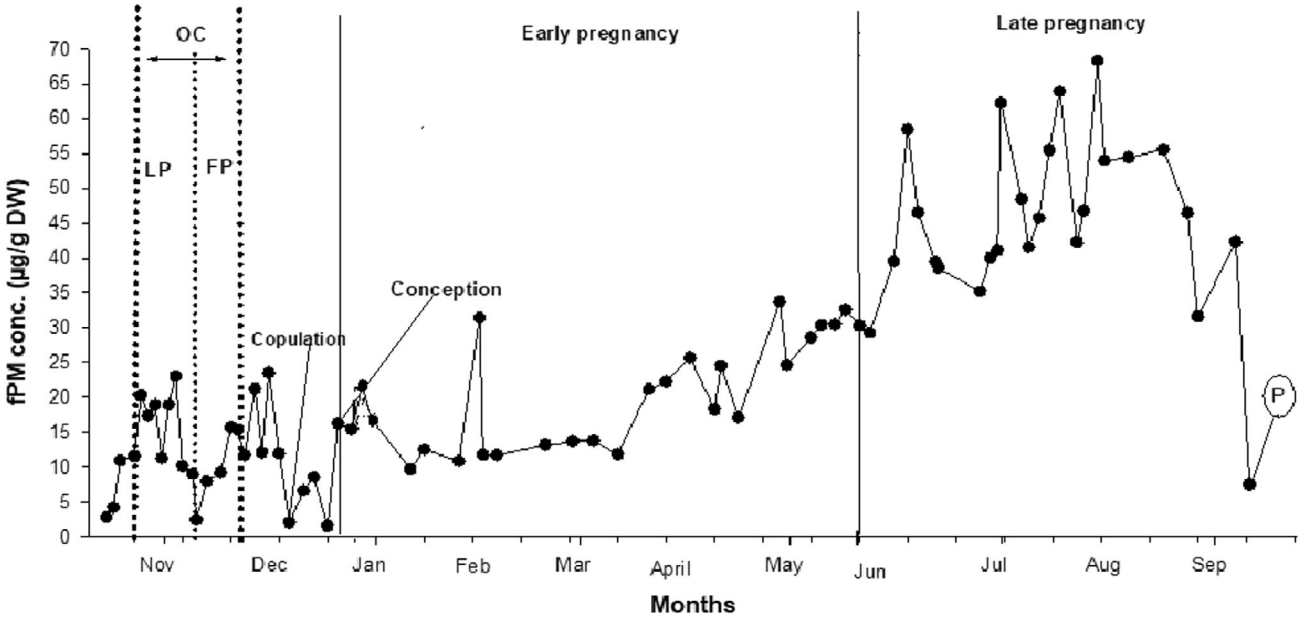

Figure 3 Longitudinal profile of faecal progestogen metabolite concentrations during oestrous cycle and pregnancy in female roan antelope. FP, follicular phase; LP, luteal phase; OC, oestrous cycle; P, parturition.

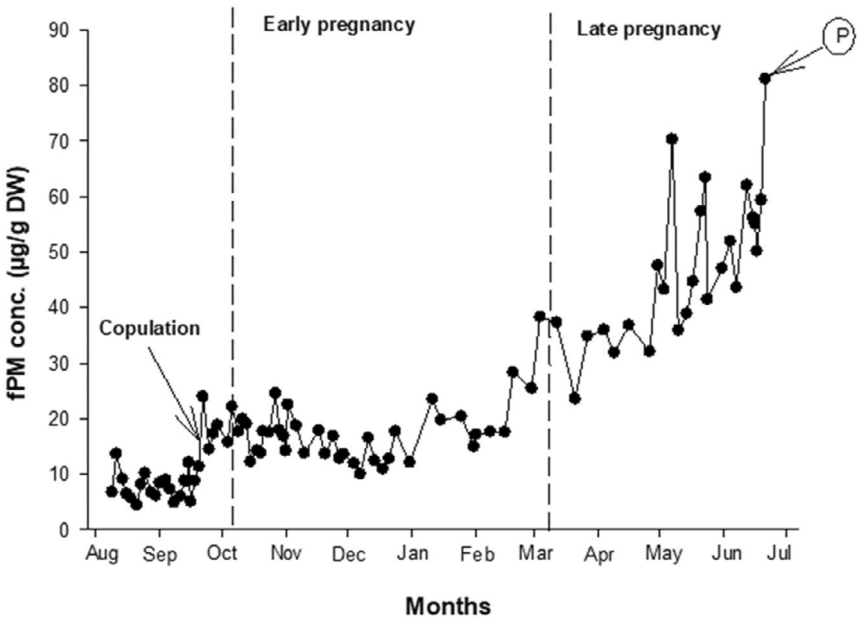

Figure 4 Longitudinal profiles of faecal progestogen metabolite concentrations of a multiparous roan female (that did not show an apparent oestrous cycle) during pregnancy (F5). P, parturition.

Discussion

In the present study, non-invasive methods for measuring faecal oestrogen and progestogen metabolite concentrations in female roan antelope were successfully established. This is the first time that faecal hormone metabolite monitoring has been used to assess ovarian activity in this species. The oestrous cycle, pregnancy as well as post-partum ovarian activity were characterised via respective endocrine correlates.

Faecal progestogen metabolite patterns indicate that the oestrous cycle length in roan antelope is between 16 and 39 days, with a mean length of 31 days; whereas the mean length of the luteal and follicular phases were approximately 17 and 15 days respectively. The length of the cycle is similar to the

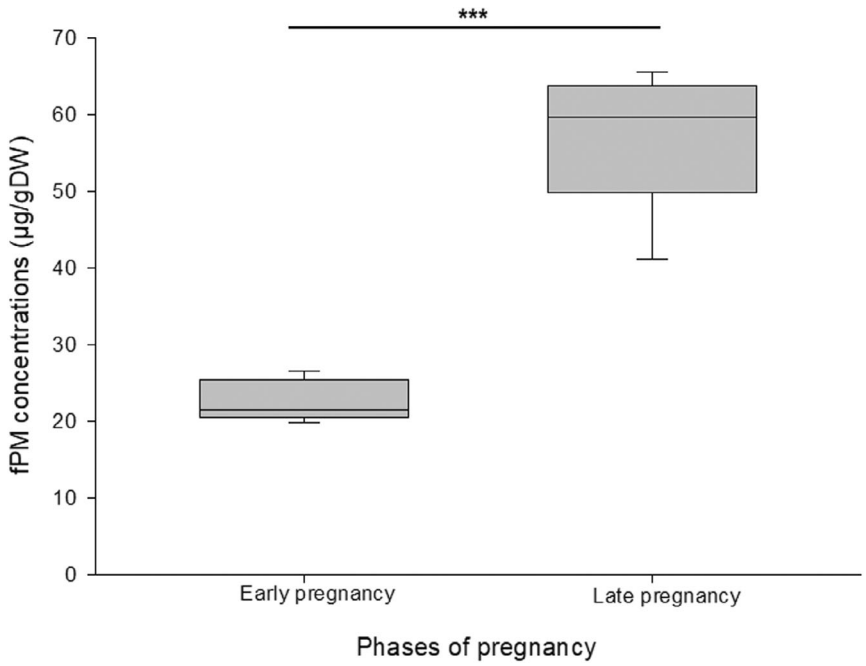

Figure 5 Individual concentrations faecal progestogen metabolite across the different phases of pregnancy in female roan antelope. Boxes show the median value, the 10th, the 25th, the 75th and the 90th percentiles of the values; ***: $P < 0.001$.

32 days oestrous cycle reported for the addax (*Addax nasomaculatus*; Asa et al., 1996), but longer than the oestrous cycle length reported for a number of other antelope species, including the closely related sable antelope (24 days; Thompson et al., 1998), as well as the scimitar-horned oryx (23 days; Morrow et al., 1999). Also, the length of the luteal phase is comparable to the 18 days reported for the sable antelope (Thompson et al., 1998; Thompson et al. 1999), and 17 days for the scimitar-horned oryx (Bowen & Barrell, 1996). However, the follicular phase is longer than that of other hippotragine species, which ranges from 5 to 8 days (Asa et al., 1996; Ostrowski et al., 2005; Thompson et al., 1998) or that of other bovids species such as cattle (4–6 days). The differences in oestrous cycle length and in the length of phases between roan antelope and the other hippotragines found in the current study may be attributed to the post-partum state of the females monitored. In dairy cattle, for example, Sakaguchi et al. (2004) showed that cyclicity returns to normal after the second post-partum ovulation. Hinshelwood et al. (1982) have also reported short oestrous cycles of less than 17 days post-partum in cattle, and attributed this change to the post-partum state of the females. Similar results have been reported in addax (Asa et al., 1996), cattle (Hanzen, 1986; Webb et al., 1980) and marsh deer (Polegato et al., 2018). Hanzen (1986) discussed that premature luteolysis, low FSH levels after parturition, failure of the follicle to ovulate or a reduction of the GnRH-induced LH surge may be contributing factors to the origin of these short luteal phases.

As observed in other ungulate species (Asa et al., 1996; Khonmee et al., 2014; Ostrowski et al., 2005; Polegato et al., 2018; Thompson et al., 1998), hormonal profiles of oestrous cycles showed higher fPM concentrations during the luteal phase compared to the follicular phase. However, fEM profiles of the female roan antelope did not show significant increases in the follicular phase when compared to the luteal phase. A similar pattern has been reported in species such as the okapi (*Okapia johnstoni*, Kusuda *et al*. 2006), with no increase in fEM concentrations during oestrous cycles, but increase during pregnancy. Hence, to optimise reproductive rates, roan antelope breeding programmes can quantify fPM concentrations to monitor oestrous cyclicity. Herein, females monitored were in the post-partum state, their fEM profile showed high levels before the first copulation or first cycle. This could be a carry-over of fEM concentrations from the parturition as high oestrogens levels have been reported in other ungulates species such as cattle (*Bos taurus*) and Buffalo (*Bubalus bubalis*; Henricks et al., 1972; Eissa et al., 1995). In this study, copulation was shown not to occur at the later period of the follicular phase. In goats and cattle, the follicular phase of the cycle corresponds to the period of development of the ovulatory follicles that will be produced at the end of the follicular phase, even though follicle development begins during the preceding luteal phase (Fatet et al., 2011; Forde et al., 2011). Therefore, the occurrence of sexual behaviour during the early follicular phase indicates that the last stages of dominant follicle development occur during the late stage of the luteal phase. This pattern of follicular development during the preceding luteal phase is also true for giraffe (Lueders et al., 2009).

The average length of pregnancy in the roan antelope was approximately 280 days and ranged between 264 and

307 days. This is comparable to the 268–286 days interval previously reported for this antelope by Dittrich (1974) and the 276–287 days interval reported by Joubert (1976), obtained through behavioural observations. The intercalving period recorded in this study was slightly above the 296–339 days intercalving period previously reported by Joubert (1976), or the estimate of 320 days observed by Dittrich (1974) for this species. It was also longer than the interval reported for other hippotragine species including the Arabian oryx (285 days; Sempéré et al., 1996) and the scimitar-horned oryx (231 days; Morrow et al., 1999). The females monitored during this study were captive, and the periods for breeding synchronised for all females by the reserve management. Therefore, management interventions may have influenced the length of the intercalving periods. The impact of captivity is also seen in the addax, which was reported to be a seasonal breeder in the wild, while females kept in captivity were observed to give birth at any month of the year (Densmore & Kraemer, 1986).

Similar to Arabian oryx (Ostrowski et al., 2005) and other ungulate species such as the Mohor gazelle (*Gazella dama mhorr*; Pickard et al., 2001) pregnancy in female roan antelope was marked by a progressive increase in fPM levels up until parturition. Similar results have been also found for the forest musk deer (*Moschus berezovski*; Wang et al., 2016). At the early stage of pregnancy, fPM levels were higher compared to those respective concentrations during the luteal phase. These findings suggest that fPM monitoring can be used to diagnose pregnancy in female roan antelope. Ostrowski et al. (2005) revealed similar results for the Arabian oryx, as they demonstrated that for this species, pregnancy detection via fPM monitoring was reliable from 2.5 months of pregnancy. One of the monitored pregnant females in this study had a late miscarriage and results indicate low levels of fPM concentrations comparable to other females at the same stage of pregnancy. As it is known that progesterone is the main hormone responsible for the maintenance of pregnancy in most mammals (Gomes & Erb, 1965), and a respective suppression may lead to pregnancy interruption (Thorburn et al., 1977), this result suggests that adequate progestogen concentrations are necessary for the maintenance of pregnancy in the roan antelope as well.

Faecal progesterone metabolite concentrations at parturition remained above baseline, but were low compared to respective fPM concentrations 72 h before parturition in this study. Similar patterns have been reported in the Mohor gazelle (Pickard et al., 2003) and the forest musk deer (Wang et al., 2016). In cattle, progesterone levels were observed to slowly decrease during the final 3–4 week prior to parturition, then rapidly declining within 2 days of parturition (Hanzen, 1986; Smith et al., 1973).

The interval time from parturition to the first oestrous cycle is an important reproductive parameter, especially within a captive breeding context where high reproductive rates are targeted. In ungulates, an increase in progesterone levels is considered as an indicator of luteal activity (Schirar et al., 1989; Schwartz et al., 1995). This study showed an increase in fPM concentrations after parturition between 14 and 42 days post-partum in female roan antelope. This interval is close to the 18 days reported in captive Arabian oryx (Asa et al., 1996). The results of this study partly corroborate the findings of Joubert (1976) who reported that female roan antelope usually enter the first oestrous cycle between 2 and 4 weeks following parturition, but are slightly different from the findings of Dittrich (1974), which ranged between 1.5 and 3.5 months. In cattle, this interval ranges from 14 to 69 days, depending on whether the female is being milked or suckled (McDougall et al., 1995; Rajamahendran & Taylor, 1990; Rawlings et al., 1980; Savio et al., 1990; Webb et al., 1980). Previous studies on ungulates have shown that factors such as suckling (Hinshelwood et al., 1982; Webb et al., 1980), photoperiod (Llewelyn et al., 1992; Santos et al., 2009; Savio et al., 1990), nutrition (Hinshelwood et al., 1982; Santos et al., 2009; Savio et al., 1990), exposure to a male (Ferreira-Silva et al., 2017; Gifford et al., 1989) and parity (Hinshelwood et al., 1982; Santos et al., 2009; Tanaka et al., 2008) may influence the interval from post-partum up to the return of oestrous cyclicity. The females monitored in this study were suckled, exposed to males, and calving occurred during the southern hemisphere winter. Hence, the differences in the resumption of the ovarian activity may have been influenced by these parameters. Further investigations are therefore needed to verify the influences these parameters on the return of the ovarian activity after parturition in female roan antelope, as the knowledge will assist breeding facilities in establishing management practices.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Longitudinal profile of fEM and fPM concentrations profiles during a regular oestrous cycle in a nulliparous female (F33). The solid black line with black dots represents the fEM profile and the solid dark grey line with white dots represents the fPM profile. The *x*-axis represents the number of days the cycle.

Figure S2. fEM and fPM concentrations profiles during the oestrous cycle in a nulliparous female (F30). The black line represents fEM profile and the blue line represents fPM profile.

Table S1. Median faecal oestrogen metabolite concentrations (ng/g DW) during oestrous cycle.

Table S2. Faecal progestogen metabolite concentrations (µg/g DW) during oestrous cycle in female roan antelope.

Table S3. Faecal progestogen metabolite concentrations (µg/g DW) during the different stages of pregnancy.

Table S4. fPM concentrations in post-partum females during and after calving.