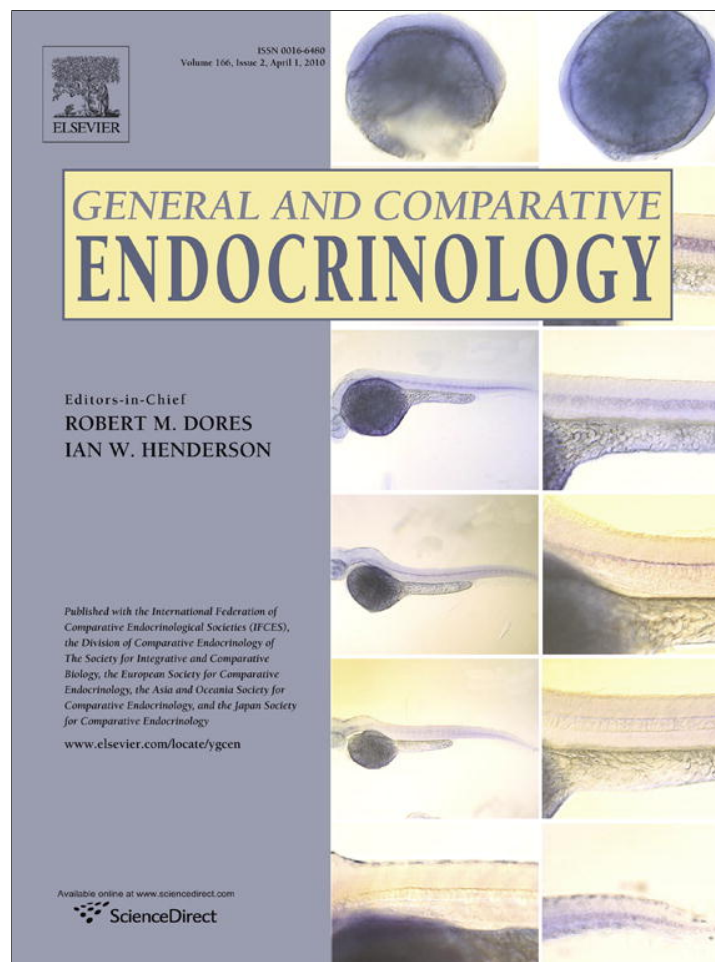


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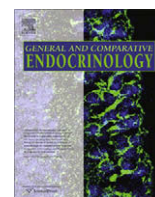
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Endocrine patterns of the estrous cycle and pregnancy of wildebeest in the Serengeti ecosystem

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ABSTRACT

Despite the importance of the western white-bearded wildebeest (*Connochaetes taurinus mearnsi*) to the Serengeti–Mara ecosystem, surprisingly little is known about the reproductive physiology of this keystone species. A longitudinal, non-invasive endocrine study was conducted on female wildebeest captured from the Serengeti–Mara migration and maintained for ~16 months in large fenced enclosures within the species' natural range. An intact bull was introduced to a female subgroup ($n = 5$), while remaining females ($n = 10$) were unexposed to a male. Fecal progesteragen patterns reflected ovarian activity and pregnancy. In non-pregnant animals, luteal and inter-luteal baseline progesteragen values differed ($p < 0.001$) over time, thereby allowing identification of recurrent estrous cycles. The average durations of the luteal phase, estrous cycle, gestation, and post-partum anestrus were 14.3 ± 0.5 , 22.6 ± 1.0 , 240.8 ± 11.7 , and 104.1 ± 15.6 d, respectively. Annual reproductive patterns indicated a distinctive period of ovarian activity that extended from 13 May through 3 December (203.5 ± 29.9 d) with all unmated females displaying from one to 14 estrous cycles. Progesteragens were higher ($p < 0.001$) in pregnant ($n = 4$) than non-pregnant ($n = 10$) cows. These data (1) reveal the value of fecal hormone monitoring for establishing the first ever endocrine profiles of female wildebeest in semi-free-living conditions in their native range, and (2) indicate that the species is a seasonal breeder that is polyestrous and a spontaneous ovulator.

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1. Introduction

The Serengeti ecosystem encompassing the great plains of southern Kenya and northern Tanzania is comprised of the world's largest aggregation of large land mammals. This includes ~1.25 million western white-bearded wildebeest (*Connochaetes taurinus mearnsi*), the smallest race of common wildebeest (Estes, 1991). The species is water dependent, with both mating and calving highly synchronized over a 2–3 weeks period. The rut occurs from May to July, at the end of the wet season, and parturition then typically occurs from January to March, at the beginning of the long rainy period (Talbot and Talbot, 1963; Watson, 1967; Sinclair, 1977a; Tanzania Wildlife Conservation Monitoring, 2000). Predation on neonates is limited by birth synchrony as well as excellent mobility displayed by precocious 'follower' calves (Estes, 1991).

Despite the highly synchronous rutting and birthing peaks, it is unknown if female wildebeest are truly seasonal breeders, mono- or polyestrous, or induced or spontaneous ovulators. Additionally, there is no information on estrous cycle characteristics or reproductive endocrine patterns for the species. Based upon estimations

of population mating and calving peaks, gestation has been estimated to range from 8 to 9 months (Talbot and Talbot, 1963; Estes, 1991). The duration of gestation has been calculated in several other Alcelaphinae species including: black wildebeest (*Connochaetes gnou*, 9 months, Skinner et al., 1973); blesbok (*Damaliscus dorcas phillipsi*, 8 months, Marais and Skinner, 1993); and red hartebeest (*Alcelaphus buselaphus*, 8 months, Skinner et al., 1973). Other than this information, there is a lack of fundamental data on reproductive physiology of Alcelaphinae with the exception of blesbok, which have been shown to be seasonally polyestrous (Marais and Skinner, 1993). Substantially greater basic knowledge has been generated for other wildlife species comprising the Bovidae subfamilies, including Antilopinae (Loskutoff et al., 1990; Skinner et al., 2001; Penfold et al., 2005), Bovinae (Fogwell, 1999), Caprinae (Goodrowe et al., 1996), and Hippotraginae (Asa et al., 1996; Sempéré et al., 1996; Thompson et al., 1998; Morrow et al., 1999). The common feature within these taxa is the significant variation in physiological function between species, thereby reinforcing the need to characterize each species of interest because ungulates have evolved substantially different reproductive mechanisms.

Non-invasive assessments of hormonal metabolites in excreta have allowed understanding of the physiology of reproduction

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for many wildlife species that never could have been studied otherwise (Monfort, 2003; Schwartz and Monfort, 2008). A major advantage of fecal monitoring is the ability to collect repeated samples to study animal endocrine patterns longitudinally without disturbance or stress (Monfort, 2003; Adkins-Regan, 2005; Schwartz and Monfort, 2008). Among the Bovidae family, longitudinal fecal progesterone profiles have been characterized in the scimitar-horned oryx (*Oryx dammah*, Morrow and Monfort, 1998), sable antelope (*Hippotragus niger*, Thompson and Monfort, 1999), bison (*Bison bison*, Kirkpatrick et al., 1996) and moose (*Alces alces*, Monfort et al., 1993). In the only study utilizing endocrine monitoring in wildebeest, Mduma (1996) analyzed fecal progesterone to ascertain pregnancy rates in the wild population. Single fecal samples from pregnant females were obtained from culled females during months 4 to 8 of gestation, whereas single fecal samples from non-pregnant animals were obtained from females found in proximity to newborn calves ~3 weeks following the birth peak. He found that fecal progesterone concentrations in non-pregnant females were one-half that observed in pregnant females.

The present study applied non-invasive endocrine techniques to characterize ovarian function in wildebeest maintained in captivity within their natural range. This design allowed for repeated sampling from known individuals to follow longitudinal patterns over the course of a year in both mated and unmated females. This is the first study designed to validate the use of non-invasive progesterone monitoring for establishing normative reproductive-endocrine parameters in wildebeest.

2. Experimental methods

2.1. Study area

The study area was located within the Grumeti Game Reserve, in the Western part of the Serengeti ecosystem (33° 30' to 34° E and 1° 30' to 2°30' S). The Grumeti Game Reserve adjoins the Western corridor of Serengeti National Park north of the Grumeti River, and is leased from the Tanzanian government as a hunting concession by the Singita Grumeti Reserves. The Reserve is within the natural range of the western white-bearded wildebeest. The ecosystem has been well-described (see McNaughton, 1985) and is characterized by a wet season that occurs from November through June with short, dry periods in January and February, but rainfall patterns are highly variable. The dry season runs from approximately June through October (Norton-Griffiths et al., 1975). Habitat types include closed-canopy woodland, deciduous and semi-deciduous thorn tree savanna, and plains of short and medium grasslands (McNaughton, 1985).

2.2. Animals, captures, husbandry, and habituation

Eighteen female wildebeest were captured from the main, migratory population as it passed through the Grumeti Game Reserve in early November. Fifteen pregnant females without calves and 3 juvenile, non-pregnant females (~2 years of age) were captured. Animals were darted from a vehicle using capture darts containing etorphine hydrochloride (Immobilon, 2–3 mg/animal; Novartis, South Africa) combined with xylazine hydrochloride (Rompun, 50–70 mg; Bayer HealthCare, USA) administered i.m., according to successful anesthetic protocols developed by Serengeti National Park veterinarians. The capture team was led by the Tanzanian Wildlife Research Institute (TAWIRI) veterinarian. Anesthetized animals were fitted with plastic ear tags for individual identification, and transported by truck (~0.5 to 2 h) to the study site.

During an initial habituation period of ~5 weeks, the cows were held in a 30 × 60 m boma divided into two units (approximately

30 × 30 m each) connected by a sliding door that could be operated from outside the enclosure. The animals were limited to one side of the boma at a time to allow for the contralateral side to be cleaned and for fresh feed to be supplied daily. An observation platform outside the boma allowed the wildebeest to be readily observed without disturbance. All animals were exposed to natural fluctuations in climate and photoperiod and allowed to graze on native grasses. Grazing was supplemented as needed with grasses cut from neighboring habitat as well as a feed mix of maize meal, cottonseed cake, and salt. Water, mineral licks, and supplemental feed were available *ad libitum* throughout the course of study.

Wildebeest were gradually exposed to the presence of researchers, initially by approaching from upwind without direct visual contact, but by exposing them to consistent vocalizations from the researchers to facilitate recognition. As the animals became habituated, as evidenced by no behavioral disruptions, exposure to the researchers became progressively more direct, culminating with an ability to remain within 10 m of the animals while collecting behavioral data, without any apparent changes in animal behavior.

The boma was contained within a large 500 × 500 m (25 hectare) fenced enclosure designed to accommodate the dietary needs (4.54 kg daily dry matter intake; Kreulen, 1975; Murray, 1995) of more than 17 lactating cows. Habituation was judged successful ~5 weeks after capture, and animals were released from the boma into the larger enclosure. All cows were left in the 25-hectare enclosure throughout the calving season and until the youngest calf was 2 weeks of age and the Serengeti veterinarian deemed it safe to dart and transport the animals. At the end of March 2003, all wildebeest were anesthetized and transported to one of three newly constructed enclosures, each 100 × 100 m. This move was necessitated by a concurrent long-term study on reproductive synchrony. Calves remained with dams, and adults and juveniles were randomly distributed amongst the three groups ($n = 5, 5, 5$). At the beginning of May 2003, a territorial bull from a resident population was introduced to one of the three groups, using the same capture protocol described for females.

Throughout the 1 year study interval (March 2003 through February 2004), the Serengeti veterinarian was consulted on any potential health issues of the study animals. Members of the local community were employed as guards to protect the wildebeest from predation by leopards and lions. Of the 18 cows initially captured, three died during the course of study. Because death occurred early in the study for two cows, data from these individuals were excluded. The remaining cow died ~8 months after study onset, and data collected while this animal was healthy was included in the analysis. Another female maintained with a bull also was excluded as she was estimated (from oral examination, overall poor body condition, lack of appetite, and lethargy) to be > 11 years of age at capture. At the conclusion of the study, all wildebeest were released into surrounding native habitat in accordance with the veterinarian's recommendations.

2.3. Fecal sample collection and hormone extraction

Fecal samples were collected from each cow at least once every 3 days by following movements of the herd within the enclosures, for a total of 3907 samples (range, 118–158 samples per individual). Each fresh fecal sample from a known wildebeest (based on ear tag and horn shape/size) was collected immediately after defecation and placed in a plastic zip bag. Each bag was marked with animal number, identity of the collector, date, and time of collection. Samples were stored in a thermos on ice (<3 h) and then stored in a propane-powered freezer (–20 °C) until extraction.

Fecal extractions were conducted on site in a field laboratory with a diesel generator powering necessary equipment. Each sam-

ple was thawed, homogenized, and 0.25 g of feces (wet weight) was weighed in a glass test tube (16 × 125 mm) and briefly vortexed with ~5 ml of 100% ethanol. Samples then were boiled for 30 min in a water bath, any evaporated ethanol replenished, and each tube centrifuged for 30 min at 2500 rpm. Resulting supernatant was poured into a clean glass test tube, dried completely under compressed air, rehydrated with 1 ml ethanol and then vortexed for 60 s. Finally, 0.85 ml of each extract was pipetted into a labeled 12 × 75 mm plastic test tube, dried completely, capped, and stored at room temperature for up to 6 months until analysis (Monfort et al., 1993).

2.4. Hormone assay

Fecal progesterone metabolites were analyzed using a broad spectrum pregnane enzyme immunoassay with antiserum (monoclonal pregnane CL425 1:10,000 dilution) provided by Coralie Munro (University of California, Davis, CA, USA). Parallel displacement curves were obtained by comparing serial dilutions of pooled wildebeest extracts with standard preparations. Fecal extracts were diluted in dilution buffer (1:50–1:300) and assayed in duplicate. The antiserum cross-reacts with 4-pregnen-3,20-dione (100%), 4-pregnen-3 α -ol-20-one (188%), 4-pregnen-3 β -ol-20-one (172%), 4-pregnen-11 α -ol-3,20-dione (147%), 5 α -pregnan-3 β -ol-20-one (94%), 5 α -pregnan-3 β ,20-dione (64%), 5 α -pregnan-3,20-dione (55%), 5 β -pregnan-3 β -ol-20-one (12.5%), 5-pregnan-3,20-dione (8%), 4-pregnen-11 β -ol-3,20-dione (2.7%), and 5 β -pregnan-3 α -ol-20-one (2.5%) (Graham et al., 2001). Sensitivity of the assay at maximum binding is 0.78 pg/well. Reverse-phase high-performance liquid chromatography (HPLC) as described by Monfort et al. (1991) revealed four immunoreactive fecal metabolites; one co-eluted with progesterone, whereas the other three were unknown immunoreactive progesterones.

2.5. Recording of mating and parturition

As part of a parallel study, all wildebeest were observed within their respective bomas for a minimum of 2.5 h per cow per week for a total of ~43 h of behavioral monitoring per week. Mating (defined as successful mounting and copulation), parturitions and suckling behavior were recorded *ad libitum*.

2.6. Statistical analysis

For each female, a 3 days running average progesterone value was calculated and used for the following analyses, unless otherwise stated. One day was subtracted from the date of each sample to compensate for the lag time between steroid hormone production and excretion, which is ~12 to 24 h for ruminants (Morrow and Monfort, 1998). All statistical analyses were performed using SigmaStat (2004, v. 3.11, Systat Software, Inc.), and graphs generated using SigmaPlot (2004, v. 9.0, Systat Software, Inc.).

For non-pregnant females, luteal phase and baseline progesterone values were differentiated using an iterative process (Graham et al. 1995; Brown et al., 1996). Baseline values were determined by calculating the mean plus 1.5 standard deviations (Graham et al. 1995; Morrow et al., 1999). Any value exceeding this criterion was considered elevated and removed from the set of nadir values. The process then was repeated until no values exceeded the criterion. Progesterone concentrations that were elevated for three or more data points (i.e., 9 days) were classified as 'luteal.' To compensate for apparent seasonal shifts in baseline values, any progesterone elevations in non-pregnant animals lasting for nine or more data points (i.e., 27 days; based on the mean cycle length of the raw data, which was ~26 days) were re-evaluated using the described iterative process, this time with a cut-off criterion

of 0.75 standard deviations. Data points not classified as luteal using the above criteria were considered baseline. Differences between baseline and luteal values were analyzed by two-way analysis of variance with Holm–Sidak method of pair-wise comparison.

Estrous cycle duration was calculated as the number of days from the first elevated progesterone value in one cycle to the first elevated progesterone value in the subsequent cycle, and the durations for all cycling individuals were averaged. Average luteal phase duration was calculated using all luteal phases of all animals. The duration of post-partum anestrus in females that gave birth was calculated as the average number of days from parturition until the first luteal rise. Any additional periods when progesterone values remained at baseline for 7 or more data points (i.e., 21 days) were considered anestrus. Average duration of gestation was calculated from observed mating and parturitions, and confirmed by sustained elevations in fecal progesterone concentrations.

To compare longitudinal progesterone excretion patterns during gestation, values throughout pregnancy were compared among pregnant females using Pearson product moment correlation. To compare average progesterone concentrations among pregnant individuals, samples collected during gestation were compared using Kruskal–Wallis one-way analysis of variance on ranks. Subsequent pair-wise multiple comparisons used Dunn's method. Progesterone concentrations in pregnant versus non-pregnant cows were compared using a Student's *t*-test. A range of progesterone concentrations also was established for each category (pregnant and non-pregnant) utilizing all samples (rather than 3 days averages) collected during gestation and the same time period in non-pregnant cows.

3. Results

3.1. Estrous cycle and gestational traits

Fecal progesterone profiles in non-pregnant females unexposed to a bull revealed cyclical patterns over time (Fig. 1), including clear differences ($p < 0.001$) in average baseline (267.6 ± 4.5 ng/g) and luteal (522.6 ± 9.1 ng/g) phase concentrations (Fig. 2). However, in 45 pair-wise comparisons, overall, baseline and luteal phase progesterone concentrations differed significantly in 44, 42, and 44% pairs of females ($p < 0.05$). Of the 10 study females

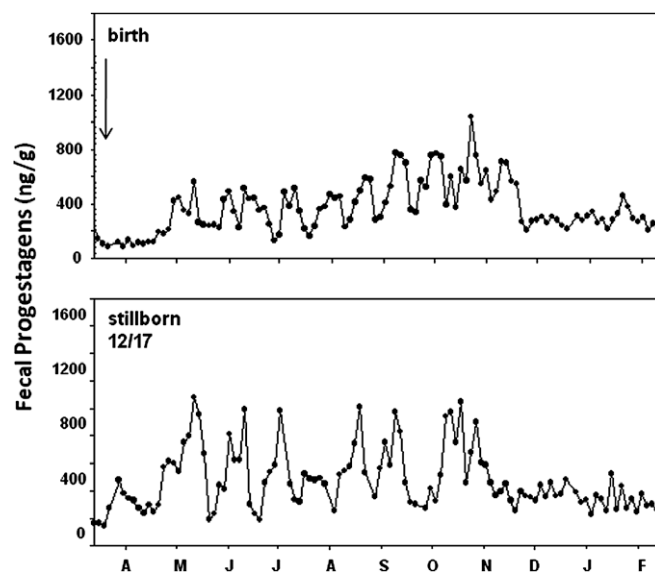


Fig. 1. Longitudinal fecal progesterone profiles in two representative non-pregnant western white-bearded wildebeest.

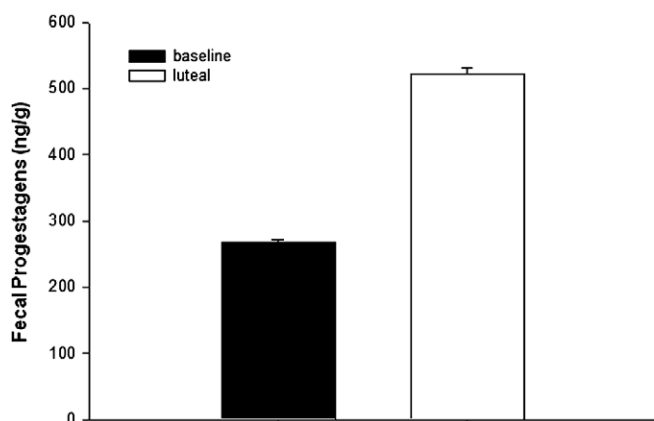


Fig. 2. Baseline ($n = 853$) and luteal ($n = 329$) fecal progesterone concentrations (mean \pm SEM) in non-pregnant western white-bearded wildebeest ($p < 0.001$).

unexposed to a bull, all produced at least one estrous cycle (range, 1–14) characterized by a temporal rise and fall in progesterone that also was indicative of spontaneous ovulation. Eight of these 10 cows displayed some degree of consecutive cycling (maximum, 13 consecutive estrous cycles). The luteal phase averaged 14.3 days of a 22.6 days estrous cycle, or 63.3% of the estrous cycle duration. The average gestation ($n = 4$) was 240.8 ± 11.7 d. Fifteen of the cows were pregnant at capture, and the first luteal phase occurred an average of 102.4 ± 17.7 days after parturition (average date, 13 May) (Table 1). For non-pregnant females, estrous cycling was observed for an average of 203.5 ± 29.9 days (average date of cessation, 3 December). There was no evidence of 'short' or attenuated cycling. Rather the last full estrous cycles appeared to occur in November and were followed by a significant interval of ovarian quiescence (Fig. 1).

3.2. Mating, parturition and suckling behavior

All four of the cows housed with the bull became pregnant during the study (Table 2). One of these was observed mating with the bull once, one mated on 2 consecutive days, and no copulation was observed in a third cow that nonetheless produced a calf. A fourth was observed to copulate on 5 June, which apparently failed to result in conception because re-mating occurred on 2 consecutive days beginning 15 July. Parturition was observed for three of the pregnant cows mating in late May/early June. The fourth cow, which copulated in mid-July, produced a calf after the conclusion of the study.

Table 1
Estrous cycle and pregnancy characteristics in western white-bearded wildebeest held *ex situ* within the native Serengeti ecosystem range.

Event	Mean \pm SEM		No. of occurrences
	Duration (days)	Date (\pm days)	
Post-partum anestrus	102.4 ± 17.7	—	11
Resumption of estrous cycling	—	5/13/03 \pm 15.2	14
Last estrous cycle	—	12/3/03 \pm 13.7	10
Consecutive estrous cycling	203.5 ± 29.9	—	10
Individual estrous cycle	22.6 ± 1.0	—	51
Luteal phase	14.3 ± 0.5	—	76
Gestation	240.8 ± 11.7	—	4

All unmated cows with surviving calves ($n = 5$) resumed estrous cycling while still suckling their calves. An additional 3 unmated cows lost their calves during the course of the study (one stillborn and two predated by leopard), but did not resume cycling immediately following the loss. Of the mated cows with calves ($n = 3$), all discontinued suckling upon introduction of the bull (6 days post introduction). For 2 of these cows, cycling did not resume until after the introduction and cessation of suckling, but one of these cows resumed estrous cycling prior to—and continued to suckle until—the male was introduced.

3.3. Progesterone levels in pregnant versus non-pregnant wildebeest

Progesterone excretion profiles indicated that two of the four pregnancies were preceded by a non-conceptive cycles (Fig. 3B). For conceptive cycles, fecal progesterone concentrations rose (from ~ 300 to >700 ng/g) shortly following the last observed copulation and declined (to ~ 400 ng/g) the day before or day of parturition (Table 2). Average fecal progesterone concentrations for individual pregnant cows ranged from 734.4 ± 19.8 to 1066.5 ± 40.8 ng/g (Table 3). While all females excreted elevated fecal progesterone concentrations throughout gestation, some animals experienced more marked fluctuations between 3 days average data points, leading to a lack of correlation ($p > 0.05$) among individuals in longitudinal excretion profiles (Fig. 3A and B). Nonetheless, in pair-wise comparisons average gestational fecal progesterone concentrations were similar ($p > 0.05$) in three of four cows (Table 3). The range in fecal progesterone concentrations for pregnant and non-pregnant cows during the sampling interval was 74.1–1718.4 and 74.7–1248.3 ng/g, respectively. Despite this overlap, fecal progesterone in pregnant cows (903.3 ± 69.8 ng/g) were elevated ($p < 0.001$) compared to non-pregnant cows (374.8 ± 30.6 ng/g) sampled across the same time interval (Fig. 4).

4. Discussion

Non-invasive fecal progesterone monitoring was useful for establishing reproductive-endocrine traits in the western white-bearded wildebeest. Our study design was novel in that a fractional subset of the Serengeti wildebeest population was removed from the wild and maintained in a 'natural' environment adjacent to their free-living conspecific counterparts. This strategy ensured that resulting data were normative and uncompromised by traditional zoological captivity while still allowing controlled observation and frequent fecal sampling. This study details, for the first time, longitudinal assessment of estrous cyclicity in western white-bearded wildebeest. Although there was substantial individual variation in average progesterone concentrations, baseline and luteal concentrations were readily distinguished. Results revealed that wildebeest are seasonal, polyestrous, spontaneous ovulators. Although only a few cows were exposed directly to a bull, these females all became pregnant within weeks of each other, consistent with the high fecundity and reproductive synchrony noted in this species, and allowing quantification of gestational parameters.

The average estrous cycle duration (~ 23 days) was similar to estrous cycle lengths reported for other Bovidae, including Antilopinae (gerenuk, *Litocranius walleri walleri*, 19 days, Penfold et al., 2005; suni, *Neotragus moschatus zuluensis*, 21 days, Loskutoff et al., 1990), Bovinae (water buffalo, *Bubalus bubalis*, 20 days, Kenai and Shimizu, 1986; domestic cattle, 21 days, Thatcher, 1999; African buffalo, 23 days, Sinclair, 1977b), Hippotraginae (sable antelope, 24 days, Thompson et al., 1998; Arabian oryx, 24 days, Sempéré et al., 1996; scimitar-horned oryx, 25 days, Morrow et al., 1999) and Caprinae (musk oxen, *Ovibus moschatus*, 20 days,

Table 2

Observed mating and parturition in four western white-bearded wildebeest held *ex situ* within the native Serengeti ecosystem range.

Animal	Date of observed mating	Rise in fecal progestagens following observed copulation (ng/g)	Date of parturition	Fecal progestagens 1 day pre-partum (ng/g)
NT	May 29, 2003 May 30, 2003	245.1 → 1344.3	February 4, 2004	372.9
15	None	—	February 3, 2004	428.3
16	June 5, 2003 July 16, 2003	526.3 → 729.9	February 24, 2004	Not available
18	June 5, 2003	364.5 → 1305.0	February 5, 2004	390.8

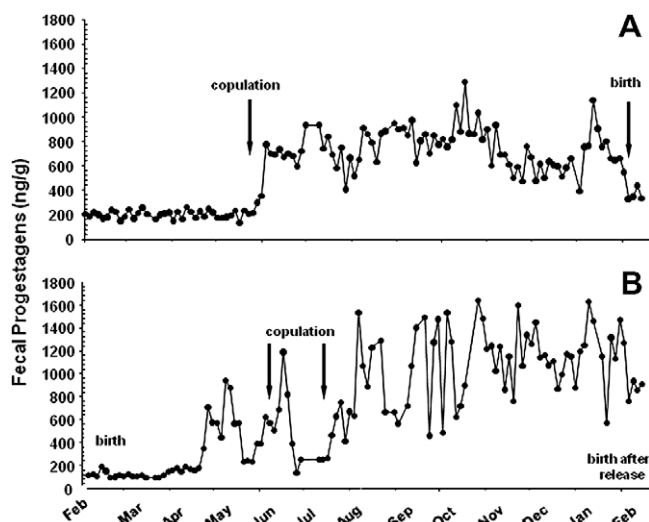


Fig. 3. Longitudinal fecal progestagen profiles of two representative, pregnant western white-bearded wildebeest.

Table 3

Average gestational fecal progestagen concentrations in four western white-bearded wildebeest held *ex situ* within the native Serengeti ecosystem range^a.

Animal	Median gestational fecal progestagens (ng/g)	Mean gestational fecal progestagens (ng/g) ± SEM	No. of samples
NT ^a	731.1	734.4 ± 19.8	80
15 ^b	986.5	923.0 ± 40.2	74
16 ^b	1,133.4	1,066.5 ± 40.8	63
18 ^b	1,031.2	1,003.2 ± 38.9	71

^a Cow NT had different median and mean gestational progestagen ($p < 0.05$) from counterparts.

^b Values did not differ from each other ($p > 0.05$).

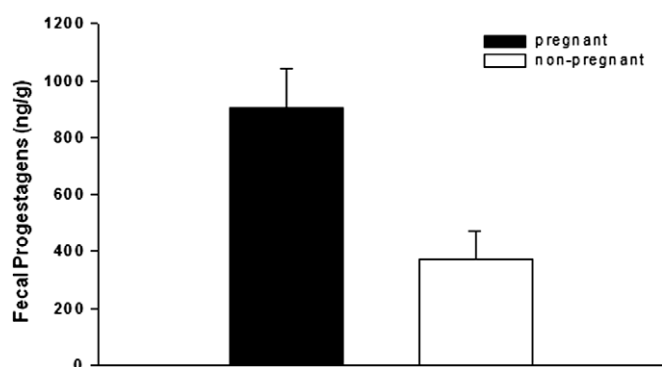


Fig. 4. Fecal progestagen concentrations (mean ± SEM) in pregnant ($n = 4$) versus non-pregnant ($n = 10$) western white-bearded wildebeest ($p < 0.001$).

Rowell and Flood, 1988; Dall's sheep, 18 days, Goodrowe et al., 1996).

The occurrence of recurring cyclic ovarian activity in cows—even while completely isolated from males—suggests that environmental cues are sufficient for annually up-regulating ovarian activity in wildebeest cows. This concurs with findings in other Bovidae (Sempéré et al., 1996; Goodrowe et al., 1996; Thompson et al., 1998; Morrow et al., 1999; Skinner et al., 2001), but differs from the closest related species studied to date, the blesbok. Marais and Skinner (1993) found that female blesbok held in the presence of a male were polyestrous, whereas those in isolation remained anestrus until the introduction of a male. Although male presence has been shown in a number of ungulates (Brooks and Cole, 1970; Knight and Lynch, 1980; Iason and Guinness, 1985; Verme et al., 1987; Signoret, 1990; Sempéré et al., 1996; Hosack et al., 1999) to have an effect on female reproductive function—ranging from advancement of the date of first estrus to ovulation-inducement—the difference between wildebeest and blesbok is surprising, given that the species have similar social systems. Like the Serengeti-Mara wildebeest population, blesbok were formerly migratory, forming mobile aggregations with territorial males who courted females as they crossed their territories (Estes, 1991). However, over-hunting and fencing has resulted in a forced change in blesbok life history from migratory to mostly sedentary, and this may partially explain the differences observed between the two species. It is important to note that although we have demonstrated that seasonal up-regulation of the hypothalamic-pituitary-gonadal axis occurs in the absence of males, this does not negate the possible influence of male factors in synchronizing female reproduction.

Wildebeest cows are not only seasonal, but appear to display tighter reproductive synchrony than would be expected based upon environmental seasonality alone. The evolutionary benefit of this approach has been attributed to the species having a 'follower' calf strategy that increases neonate survival through predator swamping and predator confusion (Estes, 1976; Rutberg, 1987). It is unlikely that synchronous reproduction in wildebeest evolved from an aseasonal breeding pattern, but rather a seasonal reproductive pattern that optimized resource availability was likely adjusted to yield a high degree of reproductive synchrony. The most likely explanation is that an ancestral 'hider' calf system evolved into the 'follower' strategy exemplified in the wildebeest today (Estes, 1991).

Understanding the proximate causes of seasonal reproductive onset have been the subject of numerous studies, with a primary emphasis on photoperiod (see review, Bronson, 1985). Indeed day-length has been identified as the major factor in the timing of seasonal breeding in black wildebeest and red hartebeest in South Africa (Skinner et al., 1973). However, in the latitudinal range of the western white-bearded wildebeest, daylight duration varies by only 20 min annually, which suggests that photoperiod alone is unlikely to modulate seasonal gonadal activity in Serengeti wildebeest (Sinclair, 1977a). Skinner and Skinner (2001) identify daylength as the most likely cue in mesic adapted seasonal breed-

ers, but acknowledge that this effect may be negated near the equator. Rutberg (1987) suggests that changes in rainfall and available nutrition are likely involved in modulating ovarian activity in equatorial species. In the wildebeest's native range the wet season typically occurs from November through May, with optimal quality grazing coinciding with energetically demanding lactation (Watson, 1967; Norton-Griffiths et al., 1975; Estes, 1991). Resumption of ovarian activity may require that a female achieve a threshold of nutritional quality and/or body condition, or it is possible that there are qualitative differences in certain compounds in newly growing vegetations (e.g., as suggested for rodents after seasonal rains; Leirs et al., 1994). For our wildebeest study, grazing was supplemented in order to assure the health of the study animals. Although we cannot be certain that this did not affect reproductive fitness, local grasses were utilized to reflect normal, seasonal vegetative quality. Thus, the onset of ovarian function towards the end of the rainy season (May), with cessation after the dry season (December), suggests that nutrition may have an important role in regulating ovarian cyclicity, although the influence of daylength was not addressed in this study and cannot be dismissed.

Copulations observed in the present study occurred during baseline troughs in progesterone excretion (i.e., the presumptive follicular phase) and were followed by a rise in progesterone excretion that reflected the onset of the luteal phase (Edqvist and Stabenfeldt, 1989). These results are typical of other ungulate species for which female receptivity has been observed to coincide with troughs in fecal progesterone excretion, including moose (Monfort et al., 1993), scimitar-horned oryx (Morrow and Monfort, 1998), sable antelope (Thompson and Monfort, 1999) and gerenuk (Penfold et al., 2005).

One of the mated cows—a young nulliparous female—experienced two non-conceptive cycles. The second cycle occurred following the introduction of the bull and was preceded by observed mating behavior. The impact of female age and experience on reproductive success would be interesting to explore, given a larger sample of mated females. It is important to note, however, that wildebeest in the wild have a reported three week calving peak and 80–100% conception rate (Estes, 1991; Mduma, 1996; Mduma et al., 1999). Although our findings confirm that unmated wildebeest are polyestrous, it is unlikely that wild females experience consecutive non-conceptive estrous cycling in the range reported here (i.e., 13 cycles).

The average duration of gestation (approximately 241 days) was slightly shorter than previously published estimates (9 months, Talbot and Talbot, 1963; 8–8.5 months, Estes, 1991), but similar to that reported for other Alcelaphinae (blesbok, approximately 240 days, Marais and Skinner, 1993; black wildebeest, 9 months, Skinner et al., 1973; red hartebeest, 8 months; Skinner et al., 1973). As would be expected, longitudinal pregnancy profiles were characterized by: a rise in progesterone excretion immediately following observed mating, continued production of progesterones throughout gestation, and a precipitous drop coincident with parturition. Despite individual variation, all cows showed a marked increase in progesterone concentrations during pregnancy and progesterones in pregnant cows exceeded levels quantified in non-pregnant individuals. Direct quantitative comparisons of progesterone excretion in this study with the results obtained by Mduma (1996) are not possible because different extraction protocols and assay methodologies were used. However, average fecal progesterone concentrations during pregnancy were at least twice non-pregnant concentrations in both studies. In the current study, substantial overlap in the ranges of hormone concentrations between these two states suggests that fecal progesterones must be assessed in multiple fecal samples to increase the accuracy of pregnancy diagnosis using this method (Monfort et al., 1993). Nonetheless, fecal monitoring of progesterone excretion

is a potentially valuable tool for pregnancy diagnosis in free-living wildebeest.

Progesterone excretion profiles post-parturition suggested that wildebeest ovaries were quiescent for ~3.5 months. Although lactating during this time, these cows also continued to nurse after resumption of ovarian cycling, suggesting that suckling and/or lactation alone does not suppress ovarian activity. In fact, one cow had given birth to a stillborn calf, while two additional cows lost their calves during the post-partum anestrus period and none of these cows resumed cycling earlier than the cows whose calves survived. This finding contrasts with domestic cattle, in which lactational status determines the duration of post-partum anestrus (range, 21–60 days, Fogwell, 1999). Because domestic cows may cycle year-round, quick resumption of ovarian activity after calf loss may lead to increased reproductive fitness. On the contrary, return to ovarian activity too early in the wildebeest may result in a birth outside the calving peak, markedly decreasing the chance for calf survival (Estes, 1991). Although post-partum anestrus also appears independent of lactation in gerenuk (Penfold et al., 2005) and Arabian oryx (Sempéré et al., 1996), these species appear to breed in the wild without regard to season (Stewart, 1962; Estes, 1991). The brevity of anestrus for these species (gerenuk: 20 days, Penfold et al., 2005; Arabian oryx: 18 days, Sempéré et al., 1996), is <20% of the 100 days interval we observed in the wildebeest. The protracted post-partum anestrus for the wildebeest may be necessary for uterine repair and involution, but more likely it is reflecting the evolution of differing mechanisms to re-set ovarian activity between seasonal and aseasonal breeders. Our observations support this as estrous cyclicity reinitiated precisely in May, following the seasonal pattern normally observed in the free-living population.

In conclusion, we found substantial value in characterizing the fundamental biology of the wildebeest by creating an 'ex situ, controlled' environment within the species' natural range. This allowed precise characterizations of temporal seasonality, the reproductive cycle, type of ovulation, duration of gestation, post-partum anestrus and resumptive onset of ovarian activity. Such measurements would have been logistically challenging, if not impossible, in a purely free-living situation, especially because serial tracking of marked individuals for protracted time intervals is not feasible. Likewise, traditional zoos do not maintain significant herds of this species and, even if available, results may have been confounded due to public disturbance, limited animal enclosure space, and an inability to replicate normal environmental cues that are likely essential for modulating reproductive function in this species. In summary, the fundamental knowledge derived from this study provides a sound foundation for future studies designed to understand the physiological basis of reproductive control and synchrony in one of the largest aggregations of large land mammals on earth—the Western white-bearded wildebeest of the Serengeti–Mara ecosystem.

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References

- Adkins-Regan, E., 2005. *Hormones and Animal Social Behavior*. Princeton University Press, Princeton.
- Asa, C.S., Houston, E.W., Fischer, M.T., Bauman, J.E., Bauman, K.L., Hagberg, P.K., Read, B.W., 1996. Ovulatory cycles and anovulatory periods in the addax (*Addax nasomaculatus*). *Journal of Reproduction and Fertility* 107, 119–124.
- Bronson, F.H., 1985. Mammalian reproduction: an ecological perspective. *Biology of Reproduction* 32, 1–26.
- Brooks, P.H., Cole, D.J., 1970. The effect of the presence of a boar on the attainment of puberty in gilts. *Journal of Reproduction and Fertility* 23, 435–440.
- Brown, J.L., Wildt, D.E., Wielebnowski, N., Goodrowe, K.L., Graham, L.H., Wells, S., Howard, J.G., 1996. Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal steroids. *Journal of Reproduction and Fertility* 106, 337–346.
- Edqvist, L.E., Stabenfeldt, G.H., 1989. Clinical reproductive endocrinology. In: Kaneko, J.J. (Ed.), *Clinical Biochemistry of Domestic Animals*, fourth ed. Academic Press, San Diego.
- Estes, R.D., 1976. The significance of breeding synchrony in the wildebeest. *East African Wildlife Journal* 14, 135–152.
- Estes, R.D., 1991. *Behavior Guide to African Mammals*. University of California Press, Berkeley.
- Fogwell, R., 1999. Cattle. In: Knobil, E., Neill, J.D. (Eds.), *Encyclopedia of Reproduction*, vol. 1. Academic Press, San Diego.
- Goodrowe, K.L., Smak, B., Presley, N., Monfort, S.L., 1996. Reproductive, behavioral, and endocrine characteristics of the Dall's sheep (*Ovis dalli dalli*). *Zoo Biology* 15, 45–54.
- Graham, L.H., Goodrowe, K.L., Raeside, J.I., Liptrap, R.M., 1995. Non-invasive monitoring of ovarian function in several felid species by measurement of fecal estradiol-17 β and progesterone. *Zoo Biology* 14, 223–237.
- Graham, L., Schwarzenberger, F., Möstl, E., Galama, W., Savage, A., 2001. A versatile enzyme immunoassay for the determination of progestagens in feces and serum. *Zoo Biology* 20, 227–236.
- Hosack, D.A., Miller, K.V., Ware, L.H., Mashburn, K.L., Morrow, C.J., Williamson, L.R., Marchington, R.L., Monfort, S.L., 1999. Stag exposure advances the LH surge and behavioral estrus in Eld's deer hinds after CIDR device synchronization of estrus. *Theriogenology* 5, 1333–1342.
- Iason, G.R., Guinness, R.E., 1985. Synchrony of oestrus and conception in red deer (*Cervus elaphus* L.). *Animal Behavior* 33, 1169–1174.
- Kenai, Y., Shimizu, H., 1986. Changes in plasma concentrations of luteinizing hormone, progesterone and oestradiol-17 β during the periovulatory period in cyclic swamp buffalo (*Bubalus bubalis*). *Animal Reproduction Science* 11, 17–24.
- Kirkpatrick, J.F., McCarthy, J.C., Gudermuth, D.R., Shideler, S.E., Lasley, B.L., 1996. An assessment of the reproductive biology of Yellowstone bison (*Bison bison*) subpopulations using noncapture methods. *Canadian Journal of Zoology* 74, 8–14.
- Knight, T.W., Lynch, P.R., 1980. Source of ram pheromones that stimulate ovulation in the ewe. *Animal Reproduction Science* 3, 133–136.
- Kreulen, D.K., 1975. Wildebeest habitat selection on the Serengeti plains, Tanzania in relation to calcium and lactation: a preliminary report. *East African Wildlife Journal* 13, 297–304.
- Leirs, H., Verhagen, R., Verheyen, W., 1994. The Basis of Reproductive Seasonality in Mastomys Rats (*Rodentia: Muridae*) in Tanzania. *Journal of Tropical Ecology* 10, 55–66.
- Loskutoff, N.M., Raphael, B.L., Nemeč, L.A., Wofe, B.A., Howard, J.G., Kraemer, D.C., 1990. Reproductive anatomy, manipulation of ovarian activity and non-surgical embryo recovery in suni (*Neotragus moschatus zuluensis*). *Journal of Reproduction and Fertility* 88, 521–532.
- Marais, A.L., Skinner, J.D., 1993. The effect of the ram in synchronization of oestrus in blesbok ewes (*Damaliscus dorcas philipsi*). *African Journal of Ecology* 31, 255–260.
- McNaughton, S.J., 1985. Ecology of a grazing ecosystem, the Serengeti. *Ecological Monographs* 55, 259–294.
- Mduma, S., 1996. *Serengeti Wildebeest Population Dynamics, Regulation, Limitation and Implications for Harvesting*. PhD thesis, University of British Columbia.
- Mduma, S.A.R., Sinclair, A.R.E., Hilborn, R., 1999. Food regulates the Serengeti wildebeest: a 40-year record. *Journal of Animal Ecology* 68, 1101–1122.
- Monfort, S.L., 2003. Non-invasive endocrine measures of reproduction and stress in wild populations. In: Holt, W.V., Pickard, A.R., Rodger, J.C., Wildt, D.E. (Eds.), *Reproductive Science and Integrated Conservation*. Cambridge University Press, Cambridge, U.K.
- Monfort, S.L., Arthur, N.P., Wildt, D.E., 1991. Monitoring ovarian function and pregnancy by evaluating excretion of urinary oestrogen conjugates in semi-free-ranging Przewalski's horses (*Equus przewalskii*). *Journal of Reproduction and Fertility* 91, 155–164.
- Monfort, S.L., Schwartz, C.S., Wasser, S.K., 1993. Monitoring reproduction in captive moose using urinary and fecal steroid metabolites. *Journal of Wildlife Management* 57, 400–407.
- Morrow, C.J., Monfort, S.L., 1998. Ovarian activity in the scimitar-horned oryx (*Oryx dammah*) determined by faecal steroid analysis. *Animal Reproduction Science* 53, 191–207.
- Morrow, C.J., Wildt, D.E., Monfort, S.L., 1999. Reproductive seasonality in the female scimitar-horned oryx (*Oryx dammah*). *Animal Conservation* 2, 261–268.
- Murray, M.G., 1995. Specific nutrient requirements and migration of wildebeest. In: Sinclair, A.R.E., Arcese, P. (Eds.), *Serengeti II: Research, Management and Conservation of an Ecosystem*. The University of Chicago Press, Chicago.
- Norton-Griffiths, M., Kerlocker, D., Pennycuik, L., 1975. The patterns of rainfall in the Serengeti ecosystem, Tanzania. *East African Wildlife Journal* 12, 249–271.
- Penfold, L.M., Monfort, S.L., Wolfe, B.A., Citino, S.C., Wildt, D.E., 2005. Reproductive physiology and artificial insemination studies in wild and captive gerenuk (*Litocranius walleri walleri*). *Reproduction, Fertility and Development* 17, 707–714.
- Rowell, J.E., Flood, P.F., 1988. Progesterone, oestradiol-17 β , and LH during the oestrous cycle of muskoxen (*Ovibos moschatus*). *Journal of Reproduction and Fertility* 84, 117–122.
- Rutberg, A.T., 1987. Adaptive hypothesis of birth synchrony in ruminants: An interspecific test. *American Naturalist* 130, 692–710.
- Schwartz, M.K., Monfort, S.L., 2008. Genetic and endocrine tools for carnivore surveys. In: Long, R.A., MacKay, P., Ray, J.C., Zielinski, W.J. (Eds.), *Noninvasive Survey Methods for North American Carnivores*. Island Press, Washington, DC.
- Sempéré, A.J., Ancrenaz, M., Delhomme, A., Greth, A., Blanvillain, C., 1996. Length of oestrous cycle and gestation in the Arabian oryx (*Oryx leucoryx*) and the importance of the male presence for the induction of post-partum oestrus. *General and Comparative Endocrinology* 101, 235–241.
- Signoret, J.P., 1990. Chemical signals in domestic ungulates. In: MacDonald, D.W., Muller-Schwarze, D., Natynczuk, S.E. (Eds.), *Chemical Signals in Vertebrates 5*. Oxford University Press, Oxford.
- Sinclair, A.R.E., 1977a. Lunar cycle and timing of mating season in Serengeti wildebeest. *Nature* 267, 832–833.
- Sinclair, A.R.E., 1977b. *The African Buffalo*. Chicago University Press, Chicago.
- Skinner, D.C., Richter, T.A., Malpaux, B., Skinner, J.D., 2001. Annual ovarian cycles in an aseasonal breeder, the springbok (*Antidorcas marsupialis*). *Biology of Reproduction* 64, 1176–1182.
- Skinner, J.D., Skinner, D.C., 2001. Significance of seasonal breeding in arid adapted antelope in Southern Africa. In: Prakash, I. (Ed.), *Ecology of Desert Environments*. Scientific Publishers, India.
- Skinner, J.D., Van Zyl, J.H.M., Van Heerden, J.A.H., 1973. The effect of season on reproduction in the black wildebeest and red hartebeest in South Africa. *Journal of Reproduction and Fertility* 19 (Suppl.), 101–110.
- Stewart, D.R.M., 1962. The Arabian oryx (*Oryx leucoryx pallas*). *East African Wildlife Journal* 1, 103–118.
- Talbot, L.M., Talbot, M.H., 1963. The wildebeest in Western Masailand, East Africa. *Wildlife Monographs* 12, 8–88.
- Tanzania Wildlife Conservation Monitoring (TWCM), 2000. *Status and Trend of the Migratory Wildebeest in the Serengeti Ecosystem*. Tanzania Wildlife Conservation Monitoring/Frankfurt Zoological Society, Arusha, Tanzania.
- Thatcher, W.W., 1999. Ruminants. In: Knobil, E., Neill, J.D. (Eds.), *Encyclopedia of Reproduction*, Vol. 4. Academic Press, San Diego.
- Thompson, K.V., Monfort, S.L., 1999. Synchronization of oestrous cycles in sable antelope. *Animal Reproduction Science* 57, 185–197.
- Thompson, K.V., Mashburn, K.L., Monfort, S.L., 1998. Characterization of estrous cyclicity in the sable antelope (*Hippotragus niger*) through fecal progestagen monitoring. *General and Comparative Endocrinology* 112, 129–137.
- Verme, L.J., Ozoga, J.J., Nellist, J.T., 1987. Induced early estrus in penned white-tailed deer does. *Journal of Wildlife Management* 51, 54–56.
- Watson, R.M., 1967. *The Population Ecology of Wildebeest (Connochaetes taurinus albojubatus) in the Serengeti*. Ph.D. Dissertation, Cambridge University.