Irregular ovarian activity, body condition and behavioural differences are associated with reproductive success in female eastern black rhinoceros (*Diceros bicornis michaeli*)

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A B S T R A C T

Ex situ populations of endangered species such as the black rhinoceros play an important role in global conservation strategies. However, the European captive population of eastern black rhinoceros is performing sub-optimally, with growth rates and genetic viability limited by low birth rates and high reproductive skew. We investigated several intrinsic differences between parous and nulliparous females that may underlie differences in reproductive success, including ovarian cyclicity, adrenal activity, behaviour and body condition. Faecal samples were collected from 39 females (17 parous, 15 nulliparous and 7 pre-reproductive) at 11 zoological institutions, every other day for between 4 months and 6 years. Progestagen metabolite concentration indicated that although all non-pregnant females exhibited ovarian activity, irregular cyclicity was common. Longer cycles (>40 days) were more common in nulliparous females and periods of acyclicity observed more often in females that had not bred for at least 7 years. Even when endocrine data indicated clear ovarian activity, overt behavioural signs of oestrus were not always apparent, particularly among nulliparous females. Faecal glucocorticoids did not differ between parous and nulliparous females, although did differ according to individual temperament. More unpredictable temperaments were associated with higher glucocorticoids, and nulliparous females tended to be rated as more unpredictable. Finally, nulliparous females had higher body condition scores than parous females. This is the first comprehensive survey of the reproductive physiology of this European captive population, and highlights a number of intrinsic differences related to parity, which may underlie differences in reproductive success among captive female black rhinoceros.

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1. Introduction

The European captive population of eastern black rhinoceros (*Diceros bicornis michaeli*) currently consists of around 10% of the global population of this critically endangered subspecies. However this population is only marginally self-sustaining and 49% of reproductive-age females are yet to reproduce (Biddle and Pilgrim, 2011, 2012, 2013). Recent analyses illustrate that low birth rates and high reproductive skew not only limit growth, but also the long-term genetic viability of this population (Edwards, 2013). Similarly, inconsistent rates of reproduction are amongst the factors that limit the sustainability of captive populations of

black rhinoceros in North America (Carlstead et al., 1999; Foose and Wiese, 2006). To create genetically healthy, self-sustaining *ex situ* populations of this species, it is essential to investigate why differences in reproductive success occur, so that reproductive skew can be minimised and overall birth rates improved.

In situ populations of eastern black rhinoceros have a much lower level of female reproductive skew than in captive populations (Edwards, 2013), suggesting that some aspect of captivity could be involved in differential reproductive success. However, nulliparous females are distributed across multiple institutions, often alongside parous females (Edwards, 2013), which suggests that differences in husbandry or physical conditions between institutions may not fully explain reproductive skew. Although variation in reproductive success could merely reflect differences in mating opportunities, there may be underlying physiological differences that could lead to variance in reproductive rates.

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A number of studies have been conducted both *in situ* (Brett et al., 1989; Garnier et al., 2002; MacDonald et al., 2008) and *ex situ* (Berkeley et al., 1997; Czekala and Callison, 1996; Graham et al., 2001; Hindle et al., 1992; Radcliffe et al., 2001; Ramsay et al., 1987; Schwarzenberger et al., 1993, 1996) to establish basic reproductive parameters in female black rhinoceros, such as characterising reproductive cyclicity and pregnancy determination. Brown et al. (2001) revealed that cyclicity was quite erratic in the North American captive population, with longer and shorter cycles than normal, as well as periods of acyclicity observed. This suggests that population growth rates may be limited by underlying issues with reproductive function. However, the potential causes of irregular cyclicity and their effects on fecundity in black rhinoceros have not yet been fully determined, nor has the prevalence of these different cycle types been investigated in the European captive population.

Ovarian activity can be disrupted by increased adrenal activity as a result of perceived stressors experienced in captivity (Hodges et al., 2010). In captive white rhinoceros, non-cycling females were found to have higher variability in faecal glucocorticoid concentration than cycling females (Carlstead and Brown, 2005), and in captive black rhinoceros, differences in adrenal activity have been associated with mortality (Carlstead and Brown, 2005). However, to investigate the role of potential stressors in differential reproductive success, we also need to take into account the behavioural adaptations involved in coping with stress (Wielebnowski, 2003), as these may moderate how an individual may perceive and respond to such challenges (Sapolsky, 1994).

Natural diets are often difficult to replicate in captivity, with alternatives often over nutrient-rich (Berkeley et al., 2011; Dierenfeld, 1997). In a range of wildlife species, unsuitable diets in captivity lead to higher than ideal body mass (Clauss et al., 2009; Goodchild and Schwitzer, 2008; Mustonen et al., 2009; Vester et al., 2008), and in captive female elephants body mass is related to a higher prevalence of acyclicity (Freeman et al., 2009). Furthermore, in the horse, obesity and related metabolic conditions are associated with altered oestrous cycle duration (Fitzgerald et al., 2003; Sessions et al., 2004), the presence of anovulatory follicles (Vick et al., 2006) and altered follicle development (Sessions-Bresnahan and Carnevale, 2014). Over-condition has also been suggested as a possible contributory factor in reproductive issues in the white rhinoceros (Berkeley et al., 2011). The black rhinoceros is predominantly a browsing species, and overfeeding of unsuitable energy-rich, roughage-low food types in captivity can lead to obesity (Clauss and Hatt, 2006), which may in turn impact reproductive function.

To facilitate captive breeding programmes, it also is important to understand behavioural cues associated with receptivity (Lindburg and Fitch-Snyder, 1994), as they can play a key role in successful mating. Captive black rhinoceros breeding pairs are often housed separately, and only introduced during oestrus. Although several behaviours associated with oestrus have been described, behaviours in the absence of a male are often difficult to distinguish and can be highly variable (Fouraker and Wagener, 1996; Radcliffe et al., 2001). Even when a female is receptive, courtship can be very aggressive, and full oestrus may be preceded by mock charges and defensive displays (Hutchins and Kreger, 2006). Introductions can therefore be difficult to manage, and reduced fecundity could be the result of insufficient opportunities to mate.

This study used a multi-institutional approach to investigate different factors that could influence ovarian activity and reproductive success in captive eastern black rhinoceros. Firstly, the overall prevalence of irregular cyclicity in this population was determined, before comparing different cycle types between parous and nulliparous females. Secondly, a number of intrinsic factors were investigated and compared between parous and nulliparous females, namely adrenal activity, temperament, body condition and the behavioural expression of oestrus.

2. Methods

2.1. Study population

This study included 39 female eastern black rhinoceros at 11 zoological institutions across Europe, between the ages of 1 year 3 months and 40 years 9 months. This represents 89% of females in the European Endangered Species Breeding Programme (EEP) that had been at or approaching reproductive-age during the study period. All females were housed at the same institution as at least one male (Biddle and Pilgrim, 2011; Pilgrim, 2009). However, breeding management varies according to both individual behaviour and the facilities available at each institution. Compatible pairs or groups may be housed together continuously until conception occurs, or alternatively, pairs may only be introduced during oestrus (Pilgrim and Biddle, 2014).

The full reproductive history of each individual between their birth (or capture in the case of wild-caught founders) and the end of 2010 was determined from the European Association of Zoos and Aquaria (EAZA) studbook (Biddle and Pilgrim, 2011; Pilgrim, 2009). Individuals were categorised as follows. Firstly, females were categorised by their age, with those between the ages of 5–32 considered to be of breeding age ($N = 31$); females aged 5 years and under classed as immature ($N = 7$), and individuals aged 33 years and over classed as post-reproductive ($N = 1$). Females in the reproductive age class ($N = 31$) were then further categorised as parous ($N = 17$, age range 8–28 years) if they had ever produced a live calf, whereas those that had never produced a live calf were considered nulliparous ($N = 15$, age range 5–21 years). However, to distinguish between females that were currently breeding and those that may have bred previously but not reproduced for some time, parous females were further categorised according to whether they had produced a calf within the last 7 years. This timescale represents double the period during which a female would ideally have produced a subsequent calf based on the average inter-calving interval of this population of 3½ years (Biddle and Pilgrim, 2011; Pilgrim, 2009). Therefore breeding age individuals were also categorised as either (1) parous females that had produced a calf within the last 7 years ($P < 7$; $N = 11$; age range 8–28), (2) parous females that had not produced a calf within the last 7 years ($P > 7$; $N = 6$; age-range 18–27 years), and (3) nulliparous (NP).

2.2. Faecal sample collection and preparation

A total of 9743 faecal samples were collected over a sample collection period that ranged between 4 months and 6 years. Faecal samples were collected at least every other day from females across the monitoring period. In 18 non-pregnant females (5 parous and 13 nulliparous), samples were collected over at least 12 consecutive months, to investigate any seasonal differences in cyclicity patterns or glucocorticoid metabolite concentrations. Samples were collected by keepers as soon as possible after defecation, immediately frozen at $-20\,°C$ and stored before shipment to Chester Zoo, UK for analysis.

Hormone metabolites were extracted from faecal samples according to an established wet-weight shaking extraction method (Edwards et al., 2013). In brief, each sample was thawed, thoroughly mixed and weighed ($0.5\,g \pm 0.003\,g$), before adding 5 ml 90% methanol, vortexing and shaking overnight on an orbital shaker. Each sample was then vortexed and centrifuged for 20 min at $598g$. The supernatant was decanted, dried under air, re-suspended