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Assessment of reproductive traits in male gemsbok (*Oryx gazella*)

(Bovidae, Hippotraginae)

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Little is known about the reproductive biology of the *Oryx* genus, which includes threatened or even extinct species. In this study, we provided several indices of spermatogenesis as well as epididymal sperm parameters of an adult male gemsbok. Furthermore, we provided the morphometrics of the seminiferous tubules and spermatozoa, with a total of sixteen morphological traits described. It is remarkable that a large percentage of sperm cells showed normal morphology as well as a small variation in sperm size, similar to the values found in other polygynous ungulates. Overall, the spermatogenesis and the morphometry of the seminiferous tubules of the gemsbok are similar to those of other ungulates. The data reported in this work are described for the first time in this species, and can be used as a reference for zoo and wildlife researchers and practitioners.

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Introduction

The genus *Oryx* de Blainville, 1816 belongs to the Bovidae family (subfamily: Hippotraginae) and includes large ungulates inhabiting the most arid and hottest areas of Africa and the Arabian Peninsula. Their most remarkable functional adaptations to desert environments include the ability to use heterothermy in order to reduce water loss (Ostrowski et al. 2003) and the ability to tolerate long periods of water and food scarcity (Ostrowski et al. 2006). The genus *Oryx* comprises one species already extinct in the wild, the scimitar-horned oryx (*Oryx dammah* Cretzschmar, 1827), and others that are rapidly disappearing such as the Arabian oryx (*Oryx leucoryx* Pallas, 1777; vulnerable), the fringe-eared oryx (*Oryx beisa callotis* Thomas, 1892; vulnerable), and the common beisa oryx (*Oryx beisa beisa* Rüppell, 1835; near threatened) (IUCN 2017). On the other hand, the gemsbok (*Oryx gazella* Linnaeus, 1758) is the only species within this genus classified as least concern (IUCN 2017).

The gemsbok is a sexually monomorphic and polygynous species that may breed at any time of the year (Estes 1991). Females may conceive when they are 2 years old, whereas males reach sexual maturity in their sixth year (Estes 1991). Concerning the male reproductive behaviour, two reproductive tactics have been described in the gemsbok: territorial and non-territorial or bachelor males (Dieckmann 1980, Estes 1991). Courtship and mating are found to be largely a privilege of territorial males and are rarely performed by bachelor males (Dieckmann 1980).

Because of its taxonomic proximity to the endangered relatives and it having a not endangered IUCN status, the gemsbok represents an excellent model for the development of assisted reproductive techniques within the *Oryx* genus (Durrant et al. 2011). Surprisingly, there is still limited knowledge concerning the reproductive biology of this species, and the few data available are mostly focused on the female.

The aim of this report is to broaden the knowledge concerning the reproductive biology of the male

gemsbok. An extensive overview of the testicular structure and functions is provided together with epididymal sperm analyses. To our knowledge, with the exception of the sperm head morphometry, the data provided in this study are described for the first time in this species.

Materials and methods

One sexually-mature gemsbok male (8 years old) was culled at the Dvůr Kralové Zoo (Dvůr Kralové nad Labem, Czech Republic) according to Act no. 246/1992 Coll. of the Czech Republic on the protection of animals against cruelty and Directive 2010/63/EU on the protection of animals used for scientific purposes. The testes, within the scrotum, were removed and transported at room temperature to the laboratory. All reagents were purchased from Sigma-Aldrich (Prague, Czech Republic), unless otherwise stated.

Table 1. Testicular parameters in gemsbok. SD = standard deviation.

Assessed parameters	Mean ± SD
Spermatogenic cells	
Spermatogonia (%)	1.85 ± 0.95
Primary spermatocytes (%)	20.83 ± 0.96
Secondary spermatocytes (%)	0.55 ± 0.04
Round spermatids (%)	35.22 ± 6.43
Elongated spermatids (%)	18.62 ± 5.35
Spermatozoa (%)	22.93 ± 2.95
Seminiferous tubules	
Tubular diameter (µm)	239.70 ± 23.10
Epithelium height (µm)	73.87 ± 10.84
Tubular area (µm^2)	44 624.10 ± 8460.24
Epithelium area (µm^2)	38 429.82 ± 7650.17
Lumen area (µm^2)	6194.29 ± 2600.19

Testicular mass, cytology, and histology

Testis mass was recorded to the nearest 0.1 g using an electronic balance (EK-600G, LTD, Japan). The samples for testicular histology and cytology were prepared as described previously (Pintus et al. 2015). For the testicular cytology, at least 200 spermatogenic and Sertoli cells were evaluated per each testis using bright-field microscopy (Nikon Eclipse E600 Tokyo, Japan; 100 x objective; Fig. 1A). Then, we estimated the percentage of spermatogenic cell subtypes (Table 1) and several parameters that define the spermatogenic activity and the Sertoli cell efficiency: Sertoli cell index (the percentage of Sertoli cells per total germ cells), meiotic germ cell loss (the ratio of round spermatids to primary spermatocytes), post-meiotic germ cell loss (the ratio of elongated spermatids to round spermatids), overall germ cell loss (the ratio of elongated spermatids to total germ cells), and Sertoli cell functionality (the ratio of round spermatids to Sertoli cells). For assessing the morphometry of the seminiferous tubules, round tubular cross-sections (Fig. 1B) were photographed using a digital camera (Digital Sight DS-Fi1, Nikon, Japan) under bright-field microscopy (20× objective). The seminiferous tubule measurements were assessed using the ImageJ software (NIH, USA) with a digital drawing tablet (Wacom, USA). We measured the following tubule traits: the diameter (major and minor axes), the areas of three sections (i.e. the area of the seminiferous tubule, epithelium, and lumen), and the height of the seminiferous epithelium (in four different regions corresponding to the major and minor axes of each tubule). A total of 50 tubular cross-sections were measured.

Sperm analyses

Sperm samples were recovered from the epididymal caudae and suspended in 0.5 ml of PBS solution. Sperm concentration was assessed using a Bürker chamber. The integrity of the sperm head membrane was assessed using the eosin/nigrosin staining (Minitube, Tiefenbach, Germany), whereas the integrity of the flagellum

Table 2. Morphometry of epididymal spermatozoa in gemsbok. SD = standard deviation; CV = coefficient of variation.

Assessed parameters	Mean ± SD	Range	CV (%)
Head width (µm)	4.39 ± 0.20	3.96–4.66	4.60
Head length (µm)	7.13 ± 0.21	6.74–7.66	2.94
Head area (µm^2)	24.55 ± 1.26	22.12–27.01	5.12
Head perimeter (µm)	18.35 ± 0.42	17.71–19.16	2.31
Proximal midpiece width (µm)	0.91 ± 0.08	0.75–1.09	8.61
Distal midpiece width (µm)	0.73 ± 0.07	0.58–0.89	9.26
Midpiece length (µm)	11.54 ± 0.29	10.99–12.17	2.55
Principal piece length (µm)	43.57 ± 0.64	41.83–45.02	1.46
Terminal piece length (µm)	3.36 ± 0.50	2.40–4.29	14.85
Total flagellum length (µm)	58.46 ± 0.96	56.95–60.24	1.64
Sperm length (µm)	65.59 ± 0.87	64.34–67.40	1.32

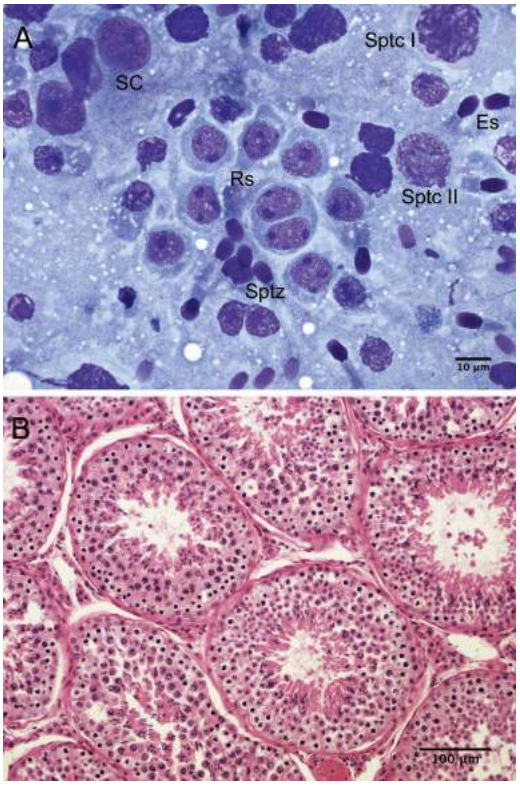

Fig. 1. A. Testicular cytology in gemsbok (Hemacolor staining, Merck, Darmstadt, Germany). Abbreviations: **SC**, Sertoli cell; **Sptc I**, primary spermatocyte; **Sptc II**, secondary spermatocyte; **Rs**, round spermatid; **Es**, elongated spermatid; **Sptz**, spermatozoon. **B.** Testicular histology in gemsbok (haematoxylin and eosin staining).

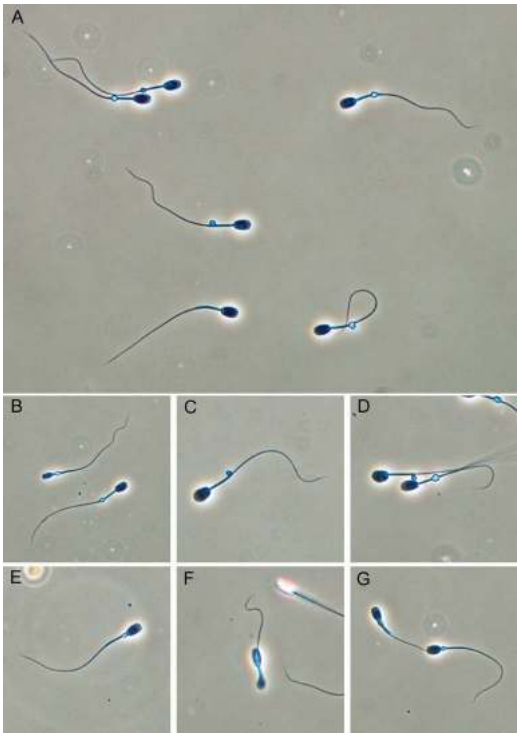

Fig. 2. Gemsbok epididymal spermatozoa. **A.** Sperm cells with normal morphology. **B–G.** Sperm abnormalities: **B.** microcephalic; **C.** macrocephalic; **D.** abnormally shaped head; **E.** proximal cytoplasmic droplet; **F.** bent midpiece; **G.** abnormal midpiece development.

membrane was assessed by the hypo-osmotic swelling test. The evaluation of the membrane integrity of the sperm head and flagellum was performed under bright field or phase-contrast microscopy, respectively (40× objective). A small aliquot of sperm sample was recovered from the cauda of both epididymides and fixed in 2 % glutaraldehyde-0.165M cacodylate/HCl buffer (pH 7.3) in order to evaluate the sperm morphology, morphometry, and the acrosomal status under phase-contrast microscopy (40× objective). For the evaluation of the sperm membrane integrity, morphology, and acrosomal status, 200 spermatozoa were assessed. Sperm morphometry was assessed on 25 spermatozoa using the ImageJ software (NIH, USA) as previously described by Ros-Santaella et al. (2014). A total of 11 sperm morphometry parameters were measured (Table 2).

Descriptive statistics were calculated using the SPSS 20.0 software (SPSS Inc, USA). Data are shown as mean ±SD.

Results

The mean testis mass was 94.0 ± 6.2 g (98.3 g and 89.6 g for the right and left testis, respectively). The histological and cytological testicular parameters are shown in Table 1. The main testicular indices were: Sertoli cell index, 4.65 ± 1.18 %; meiotic germ cell loss, 1.68 ± 0.23; post-meiotic germ cell loss, 0.56 ± 0.24; overall germ cell loss, 0.19 ± 0.05; and Sertoli cell functionality, 7.97 ± 0.46.

The epididymal sperm concentration was 2.29×10^9/ml. The percentage of sperm cells with intact acrosomes was 93 %, whereas the percentage of sperm head and flagellum membrane integrity were 67 % and 89 %, respectively. Examples of a normal sperm phenotype and the main sperm abnormalities are shown in Figure 2. The percentage of sperm abnormalities was small (11.5 %) and equally distributed in the sperm head (5.5 %) and flagellum (6 %). The most common defects were head abnormalities (5.5 %), proximal cytoplasmic droplets (3 %), and midpiece defects (2 %) (Fig. 2B–G). Regarding

the sperm morphometry, the proportion of each structure in relation to the total sperm length was: head length, 10.87 %; midpiece length, 17.59 %; principal piece length, 66.43 %; and terminal piece length, 5.12 %. Measurements of the sperm head and flagellum are shown in Table 2.

Discussion

This report provides a broad dataset concerning the morphological and quantitative assessment of the testicular and epididymal sperm parameters in an adult male gemsbok. We found that the combined testes mass was 187.9 g, which is considerably greater than the values reported in other oryx species such as the fringe-eared oryx (37.9 g; Anderson et al. 2004), the scimitar-horned oryx (53.4 g; Anderson et al. 2004), and the Arabian oryx (37.0 g; Eljarah et al. 2012). The reason for this great difference in testes mass is most likely the result of the variation of body size within the *Oryx* genus, with the Arabian oryx being the smallest (mean adult male body mass: 85 kg; Eljarah et al. 2012) and the gemsbok the largest species (mean adult male body mass: 176 kg; Estes 1991). Concerning the testicular cytology and according to previous studies in alpacas (*Vicugna pacos* Linnaeus, 1758; Stelletta et al. 2011) and red deer (*Cervus elaphus* Linnaeus, 1758; Pintus et al. 2015), we found that round spermatids are the most abundant cells, whereas secondary spermatocytes were the rarest. In contrast, Leme & Papa (2010) found that elongated spermatids are the most abundant spermatogenic cells in horses (*Equus ferus caballus* Linnaeus, 1758). Similar to our results, secondary spermatocytes are rarely observed in cytological smears of stallions, probably because of their short lifespan (Leme & Papa 2010). The proportion of the spermatogenic cell subtypes is also within the range described in alpacas (Stelletta et al. 2011) and red deer (Pintus et al. 2015), but not in horses (Leme & Papa 2010). Although the testicular indices overall are within the range previously described in red deer (Pintus et al. 2015), our findings seem to indicate that in the male gemsbok the germ cell loss occurs mostly during meiosis than during spermiogenesis. In this way, from the theoretical 4, on average only 1.7 spermatids arise from a single primary spermatocyte. In contrast, in other ungulates like goats (*Capra aegagrus hircus* Linnaeus, 1758; Leal et al. 2004) and red deer (Pintus et al. 2015), on average 2.8 and 2.7 round spermatids respectively arise from each primary spermatocyte. Our results also show that in the gemsbok more than half of the round spermatids develop into elongated spermatids, a value slightly greater to that found in red deer during the breeding season (0.56 vs. 0.43 for gemsbok and red deer, respectively; Pintus et al. 2015). Nevertheless, larger datasets are needed before drawing any firm conclusion. Histological analysis revealed that the mean diameter and the height of the seminiferous tubules in the adult gemsbok are similar to the values described in other ungulates such as goat (237 µm and 78 µm, respectively; Leal et al. 2004) and wild boar (*Sus scrofa* Linnaeus, 1758; 251 µm and 75 µm, respectively; Almeida et al. 2006).

Our data relative to normal sperm morphology and acrosome integrity are within the range reported by Roth et al. (1998, 1999) in the scimitar-horned oryx. In agreement with Downing Meisner et al. (2005), the sperm head has a wide oval shape with a rounded posterior region. However, the size of the sperm head in our study is a little bit greater compared to the range reported by Downing Meisner et al. (2005) in this species, probably because of the protocols used for electron microscopy (e. g., sample dehydration). On the other hand, we found that the sperm head and principal piece lengths are similar to the fringe-eared oryx (Anderson et al. 2005). In spite of this single case study, it is worth highlighting the small percentage of sperm abnormalities (11.5 %) and the small coefficient of variation of the total sperm length (1.32 %) found in the gemsbok. In domestic artiodactyls, where the artificial selection selects for males with the greatest reproductive traits, at least 70 % morphologically normal spermatozoa are required for a male to be classified as a satisfactory potential breeder (suids: Sancho & Vilagran 2013; bovids: Sathe & Shipley 2014; Hopper & King 2015). Moreover, the small percentage of sperm abnormalities and the small variation in sperm size are similar to the values found in other non-domestic polygynous artiodactyls (bovids: Santiago-Moreno et al. 2007; cervids: Ros-Santaella et al. 2015). These sperm traits are probably an evolutionary consequence of their polygamous mating system, given that males from monogamous species tend to show great intra-male variation in sperm size and frequent sperm morphological abnormalities (Van der Host & Maree 2014).

In conclusion, our results indicate that the reproductive biology of the male gemsbok is overall similar to that described in other ruminants, suggesting that the application of assisted reproductive techniques is feasible in this species. Because of the taxonomic proximity to its endangered relatives, the gemsbok could be successfully used as a model for optimizing assisted reproductive biotechnologies to preserve other oryx species from extinction.

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