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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences



**Reproductive functionality of common eland
(*Tragelaphus oryx*) bulls and its relationships
with animal morphological indicators, androgen
secretion patterns, and social rank**

DISSERTATION THESIS

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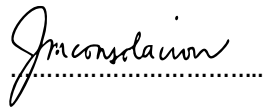
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Prague 2025

Declaration

I, Jerico M. Consolacion, hereby declare that I have finished this dissertation thesis entitled ***“Reproductive functionality of common eland (*Tragelaphus oryx*) bulls and its relationships with animal morphological indicators, androgen secretion patterns, and social rank”*** independently, except for the jointly authored publications that are included. In the case of such publication, my specific contributions have been clearly stated at the start of the relevant publication chapter. Furthermore, I confirm that proper acknowledgement has been provided within this thesis for any references made to the works of others, I also ensure that this work has not been, nor is it currently submitted for any other degree, to this or any other university. All information sources have been quoted and acknowledged by means of complete references.

In Prague 11.9.2025

A handwritten signature in black ink, reading 'Jmconsolacion', followed by a horizontal dotted line.

MSc. Jerico M. Consolacion

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Abstract

Game ranching is a significant economic contributor in southern Africa, yet limited scientific data exists on the reproductive management of commonly used ungulate species. This gap affects not only local game breeders but also the conservation of species in zoos and endangered populations. The common eland (*Tragelaphus oryx*) is widely used for both farming and ecotourism purposes, and serves as a model species in understanding the reproductive development, functioning, and thus management of captive antelope. This study explored the reproductive functionality and androgen secretion patterns of farmed common eland bulls and its relationships with factors such as animal morphological traits and social rank. Under current game management systems, these factors are difficult to control, and individual animal data is often lacking, confounding research studies. Six eland bulls (2–2.5 years old; 203 ± 20 kg) were used to investigate reproductive tract morphology and post-harvest epididymal sperm quality (Chapters 4 and 5), while 23 eland bulls (monitored between 10 to 24 months of age) were utilised for monitoring epiandrosterone faecal androgen metabolite secretion patterns (EA-fAM) (Chapter 6). Between 24–29 months old, eland bulls exhibited morphologically and histologically functional reproductive tracts with low asymmetry of their bilateral structures. Individual traits such as age, body weight, and social rank correlated the development of some testicular morphologies and thus may possibly be used for the selection of breeding males. Epididymal sperm recovery in eland bulls was feasible under basic field conditions without cooling or sperm media, with the best quality

obtained between 0–35 minutes post-slaughter. The use of physiological saline leads to metabolic deterioration due to lack of energy substrates and sperm defects *e.g* coiled tails, as expected, and future research will focus on collection and processing protocols for eland, including the use of different semen extenders for preservation. EA-fAM secretion patterns in eland bulls indicated a slight plateau around 2 years of age, and showed seasonal variation, and thus potentially optimal timing for breeding and semen collection. Individual variation is high and thus warrants further investigation. Future research should validate potential phenotypic selection indicators across a larger sample size, and under different environmental conditions.

Keywords: *Alternative farming species, faecal androgen metabolites, breeding selection, sperm quality, ungulates*

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List of abbreviations

AG – Accessory Gland

AIC – Akaike's Information Criterion

AL – Ampulla Length

ALH – Amplitude of the Lateral Head Displacement

ANOVA – Analysis of Variance

ARTs – Assisted Reproductive Technologies

ATP – Adenosine Triphosphate

*a** – Redness

AW – Ampulla Weight

BCF – Beat-Cross Frequency

BCS – Body Condition Score

BSE – Breeding Soundness Evaluation

BuW – Bulbourethral Gland Weight

*b** – Yellowness

CA – CALM

CASA – Computer Aided Semen Analyser

CBRA – Brazilian College of Animal Reproduction

CZU – Czech University of Life Sciences Prague

EA – Epi-Androsterone

EIA – Enzyme Immuno-Assay

EL – Epididymis Length

EV – Extracellular Vesicles

EW – Epididymis Weight
FA – Fluctuating Asymmetry
FAO – Food and Agriculture Organisation
fAM – Faecal Androgen Metabolites
GI – Gonadosomatic Index
GnRH – Gonadotropin-releasing Hormones
ID – Individual Identity
IUCN – International Union for Conservation of Nature
*L** – Lightness
LIN – Linearity
LH – Luteinising Hormone
LSD – Least Significant Differences
N – Sample size
PCA – Principal Component Analysis
P+Pr+SFL – Prepuce+ Penis+Sigmoid Flexure Length
P+Pr+SFW – Prepuce+ Penis+Sigmoid Flexure Weight
PL – Penis Sheath Length
PrL – Prostate Length
PrW – Prostate Weight
PUFA – PolyUnsaturated Fatty Acid
RE – REACTIVE
SC – Scrotal Circumference
SDG – Sustainable Development Goals
SFA – Saturated Fatty Acids

SFT – Society of Theriogenology
ST – Seminiferous Tubules
STC – Seminiferous Tubule Circumference
STT – Seminiferous Tubule Thickness
SVL – Seminal Vesicle Length
SVW – Seminal Vesicle Weight
SW – Scrotal Weight
TL – Testes Length
TS – Temperament Score
TW – Testes Weight
TWID – Testes Width
UQSMSP – University of Queensland Sperm Morphology
Standardisation Program
VAP – Average Path Velocity
VCL – Curvilinear Velocity
VIF – Variance Inflation Factor
VSL – Straight Line Velocity
WOB – Wobble
ZAR – South African Rand

CHAPTER 1

Introduction

1.1. General introduction

The utilisation of common eland (*Tragelaphus oryx*) has historically been practised in extensive production systems across southern Africa (Skinner, 1967) and later expanded to Eastern Europe in 1892, albeit on a small scale, with four bulls and four cows imported to Askaniya Nova Zoo in Ukraine (Treus & Lobanov, 1971). Nowadays, interest in utilising common eland as an alternative farming species has grown, due to their temperament, adaptability, and high-quality meat. The common eland population was estimated at around 136,000 in 2007, with individuals distributed across protected areas (50%) and private lands (30%), in South Africa (Furstenburg, 2016). The species is classified as "least concern" (~100,000 individuals) according to the IUCN (2024). This status is supported by the high genetic diversity in the population, contributing to population stability in zoological gardens and private breeding farms worldwide (Cetkovská et al., 2024).

The production potential of common eland has motivated the domestication efforts of common eland, due to their acceptable growth rates, favourable carcass yield and meat quality under marginal conditions, as well as their adaptability to diverse environments, reproductive efficiency, economic viability compared to livestock under marginal conditions, and population stability, as highlighted by the Food and Agriculture Organization (FAO,

1997; Laird et al., 2024). These traits are further supported by recent findings of Musa et al. (2021) on farmed common eland in the Czech Republic under semi-intensive conditions similar to those of cattle, which underscores their adaptability and flexibility to husbandry practices and climate. Given its resilience and production potential, the common eland (and game ranching in general) presents an opportunity to support the long-term objectives of the United Nations Sustainable Development Goals (SDGs), emphasising its role in sustainable development initiatives.

To support the production potential of common eland, optimisation of reproduction and breeding management is essential. In fact, several studies have focused on female eland, particularly in relation to oestrus and superovulation cycles (Penington, 2009) with assisted reproductive interventions having promising results. On the other hand, the breeding management of male eland remains largely overlooked, with limited studies available to support scientifically based breeding objectives. The research that exists is based on extremely limited sample sizes (often a single animal, or multiple animals of various ages), under conditions where factors such as age, nutrition, and other environmental factors cannot be controlled, or were not specified, and where individual animal information is lacking.

The current breeding criteria for common eland bulls primarily focus on phenotypic traits, with ranchers typically selecting breeding males, based on morphological characteristics, such as early physical maturity, larger body size, horn development (progeny are often for trophy hunting purposes), testicular size and conformation, and the timing of puberty onset (Khumalo, 2021). In bulls, reproductive performance plays a crucial role in shaping herd genetics, as traits are inherited from highly fertile and

early maturing individuals capable of servicing numerous females during the breeding season (Ohashi et al., 2011). To improve breeding management, efficiency, and overall production performance of common eland in captivity, evaluation of the reproductive tract development, semen and sperm characteristics, and hormonal secretion patterns is essential.

The detailed information of the reproductive biology of eland bulls also holds significant implications for advancing species conservation and enhancing captive breeding programs. Although knowledge in this aspect has gradually expanded, it remains relatively limited. Eland bulls are known to breed throughout the year when nutritional conditions are favourable, and they can mate with multiple females in natural settings (Skinner et al., 1971). Preliminary efforts in developing assisted reproductive techniques have provided initial insight into semen collection, cryopreservation, and artificial insemination, with promising results – albeit variable (Treus & Lobanov, 1971; Merilan et al., 1978; Bartels et al., 2001; Bissett & Bernard, 2005; Ros-Santaella et al., 2014). These advances underscore the importance of continued research into eland reproductive physiology and biotechnologies, especially given the species' production potential under optimised management conditions. As such, further research is needed to better understand the reproductive functionality of eland bulls, particularly at the point at which they would be considered for selection as breeding replacements or culled, and which is fundamental for the development of effective management and breeding strategies. This will also provide a valuable framework for establishing selection criteria for breeding bulls and lay the groundwork for the effective application of assisted reproductive technologies.

1.2. Aims of the thesis

In light of the increasing interest on the potential of common eland as an alternative farming species, this thesis aims to assess the reproductive functionality of common eland bulls and its relationships with several factors such as animal morphological traits, androgen secretion patterns, and social rank. Under extensive game management systems, these factors are challenging to control and individual animal data is often lacking, confounding research studies. This research specifically addresses the following objectives under controlled conditions, to:

- Characterise the morphology and bilateral symmetry of the reproductive tract in farmed common eland, and investigate its associations with secondary sexual characteristics, social hierarchy, and temperament at the age of initial selection for breeding or culling.
- Assess the post-harvest motility and morphological changes of spermatozoa following caudal epididymal recovery under basic field conditions, and their relationships with individual animal traits.
- Evaluate the influence of age and season on faecal androgen metabolite hormone secretion patterns and examine their associations with morphometric body traits.

1.3. Research questions

The present research seeks to answer the following questions:

- What is the basic reproductive physiological development, symmetry, and functionality of young eland bulls kept under semi-intensive farming conditions, at the typical age of breeding selection?
- What are the relationships between individual animal traits (such as age, body weight, horn dimensions, social hierarchy, and temperament) and reproductive development and functionality in young eland bulls?
- Do individual animal traits (such as age, body weight, horn dimensions, social hierarchy, and temperament) influence reproductive development and functionality in young eland bulls?
- Which individual animal traits may potentially serve as phenotypic indicators of reproductive development and functionality in young eland bulls?
- What is the effect of post-harvest recovery of eland epididymal sperm under basic field conditions (without cooling or supporting media) on viability, motility, and morphology?
- Will post-harvest timing intervals following the caudal epididymal recovery of spermatozoa affect sperm motility and its kinematics, viability, and head morphometry?
- What are the typical faecal androgen metabolite secretion patterns of eland bulls during sexual development?
- How do factors such as age and season influence faecal androgen metabolite secretion patterns in young eland bulls?

- Will age, body weight, and seasonal patterns affect the faecal androgen secretion patterns of young eland bulls?
- Are individual animal traits (body size, horn size, and temperament) and faecal androgen metabolite secretion patterns related?

1.4. Overview of the chapters

This thesis comprises of eight chapters. The first three chapters present the General Introduction, Literature Review, and General Methodology. These provide the background of the research, motivation and aims, and common methodology for the experimental work. This is followed by three research chapters, of which two have been published in Web of Science-indexed journals, whilst the third is currently unpublished.

The first study (Chapter 4 – **Consolacion et al., 2024**) of this thesis, entitled: “*Reproductive tract morphology and symmetry of farmed common eland (*Tragelaphus oryx*) bulls, and their relationship with secondary sexual traits and social rank*”, evaluates the reproductive functionality of eland bulls under semi-intensive conditions. The research was performed during the annual culling and routine management of the eland herd from the research facility, and individual animal information collected prior to slaughter were utilised (*i.e.*, social rank, temperament, body size, horn size, etc). Reproductive organs were evaluated from six eland bulls (203± 20 kg). Findings of this study were published in *Animal Reproduction Science* (<https://doi.org/10.1016/j.anireprosci.2024.107438>).

The second study (Chapter 5 – **Consolacion et al., 2025**) entitled “*Post-harvest motility and morphological changes of spermatozoa following caudal epididymal recovery in farmed common eland (Tragelaphus oryx) bulls*”, builds upon the work of Chapter 4. Immediately after slaughter, spermatozoa were harvested from the caudal epididymides of six eland bulls (203± 20 kg). The effect of post-timing intervals on the quality of eland epididymal spermatozoa were assessed, immediately after slaughtering under basic field conditions. The findings of this study were published in *Reproductive Biology* (<https://doi.org/10/1016/j.repbio2025.100997>).

The third study (Chapter 6 – **Consolacion et al., Unpublished Data**) entitled “*Effects of age and season on the faecal androgen metabolite hormone secretion patterns of common eland (Tragelaphus oryx) bulls, and their relationships with morphometric body traits*”, evaluates the secretion patterns of faecal androgen metabolites using an enzyme immuno-assay. Here, effects of animal age, morphometric body traits, and season are assessed. Such information may serve as a basis for the reproduction and breeding management of eland bulls.

The last three Chapters 7, 8, and 9 consist of the General Discussion, Conclusions and Recommendations, and References utilised throughout the thesis.

CHAPTER 2

Literature review

In southern Africa, the game ranching industry is a significant economic contributor, supporting more than 17,000 employment opportunities with a 0.068 return of investment, twice that of livestock-only farms in the same regions (Taylor et al., 2020; Saayman et al., 2018). Game ranching occupies less than 20%, or an equivalent of 17-20.5 million hectares, of South Africa's marginal agricultural land (Taylor et al., 2016). These economic attributes are associated with both consumptive and non-consumptive activities, which includes ecotourism (Muir et al., 2011), trophy hunting (Saayman et al., 2018), meat or biltong (a traditional dried meat product) hunting, culling (Van der Merwe et al., 2014), selling of live animals, and animal breeding (Taylor et al., 2020).

In terms of total annual revenue, it has been estimated that trophy hunting generates approximately ZAR (South African Rand) 13.6 billion (US 7.3 billion), and game breeding and live sales generates approximately ZAR 10 billion (US 5.4 billion) in South Africa (Cloete et al., 2015; Taylor et al., 2020). Game ranching and its associated activities are also considered as a driving factor in improving local food and job security, and supports conservation efforts (Bachmann et al., 2016). The profitability of these practices has encouraged game ranchers and farmers to adopt management strategies from the conventional livestock industry, including more precise

breeding methods to enhance production and maximise profits (Khumalo, 2021).

2.1. Game animal domestication, utilisation, and the production potential of common eland

Traditional game farming primarily revolves around trophy hunting, leisure activities, and live animal sales. However, with the increasing interest in the domestication of wild species, meat production has also emerged as an economic consideration (Hoffman & Wiklund, 2006). The practice of domesticating wild animals, including antelope, dates back to the African Stone Age around 2400 B.C. (Spinage, 1986). Historical records of this domestication are preserved on the tomb walls of Mereruka, a high-ranking official under Pharaoh Teti during Egypt's Sixth Dynasty, which document a breeder managing 1,135 gazelles (Spinage, 1986). Game farming with antelope contributes to livelihoods in southern Africa, and their meat, referred herein as "game meat" (Davies & Brown, 2008), provides a healthy food source, as it is known for its lower contents of energy and cholesterol, healthy fatty acid profiles, and high-quality proteins (Hoffman & Wiklund, 2006). The by-products and co-products, such as offal, also contribute towards local food security (Erasmus & Hoffman, 2017).

The utilisation of game species is a practical response to the growing need for quality proteins due to the increasing human population size, limited resources available for industrial livestock farming, and the complex of environmental issues associated with industrial farming, particularly in areas not suitable for extensive livestock production (Carruthers, 2008; Crooms et al., 2018). Game ranching in marginal areas thus contributes towards

climate-resilient agriculture due to the adaptability of different game animal species to specific environments, their ability to efficiently utilise of natural vegetation, their apparently lower dependence on water resources, and resistance to indigenous pests and diseases (Flack, 2013). Whilst meat from game species appeals to health-conscious consumers due to its unique taste and health properties, oftentimes it is more expensive than conventional meat sources (Hoffman & Wiklund, 2006). However, often offal and occasionally carcasses are donated to the local community when trophy hunting (for horns) is the priority. Given these attributes, game species present an economic potential as an alternative farming species, particularly in southern Africa (Carruthers, 2008) while also supporting the ongoing and long-term objectives of the United Nations Sustainable Development Goals (SDGs), such as SDG 2 – Zero Hunger (Kempen et al., 2023). Additionally, it may facilitate other SDGs, including SDG 1 – No Poverty, SDG 3 – Good Health, SDG 8 – Decent Work and Economic Growth, SDG 12 – Responsible Consumption and Production, and SDG 13 – Climate Action (Needham et al., 2023).

The common eland (*Tragelaphus oryx*) is among the most widely utilised antelope species in wildlife ranching and intensive breeding systems in southern Africa (Furstenburg, 2007). It has also been recognised as a potential candidate for domestication due to its exceptional meat quality (Posselt, 1963; Skinner, 1971). Taxonomically, eland belong to the Bovidae family, which includes cattle and other ruminant species, and are classified within the spiral-horned tribe, *Tragelaphini* (Blyth, 1863), alongside Sitatunga, Nyala, Bushbuck, Kéwel, Mountain Nyala, Lesser Kudu, and Greater Kudu (Estes, 1991). In general, eland are divided into two species: the common eland (*Tragelaphus oryx*) and the Derby (Giant) eland (*Tragelaphus derbianus*).

Further classification identifies five subspecies, which are distributed across Africa. The common eland consists of three subspecies – Livingstone, Cape, and Patterson – primarily found in southern Africa, while the Derby eland comprises two subspecies – Western and Central – classified as endangered by the IUCN (2024) (Posselt, 1963; Kingdon & Hoffmann, 2013).

The common eland population was estimated at approximately 136,000 individuals in 2007, with around 50% inhabiting protected areas and 30% residing on private lands in South Africa (Furstenburg, 2016). More recent assessments by the IUCN (2024) classify the species as “Least Concern”, with an estimated global population of around 100,000. This status is supported by the species' high genetic diversity, which has supported stable populations across zoological institutions and private breeding farms worldwide (Cetkovská et al., 2024). Owing to its ecological resilience and promising economic value, the common eland emerges as a compelling candidate for sustainable farming initiatives.

The suitability of a wild animal species for domestication is primarily determined by its production potential, which encompasses growth rates, adaptability to diverse environmental conditions, reproductive efficiency, economic viability, and population stability, as outlined by the Food and Agriculture Organisation (FAO, 1997; Laird et al., 2024). The common eland, alongside the fringe-eared oryx and African buffalo, has been recognised for its promising production traits (Thresher, 1980). Since their initial domestication, eland have predominantly been utilised in extensive production systems across southern Africa (Skinner, 1967). Their introduction to Eastern Europe dates back to 1892, albeit on a small scale, when four bulls and four cows were imported to Askaniya Nova

Zoo in Ukraine (Treus & Lobanov, 1971). Over time, selective breeding and genetic improvement led to the birth of 461 individuals from the foundation stock, for the purpose in enhancing their milk and meat quality (Treus & Kravchenko, 1968).

Between 1967 and 1972, their popularity expanded across central Europe, facilitated by private breeders and zoological institutions, most notably by Safari Park Dvůr Králové in the Czech Republic (Vágner, 1974). At the same park, the Czech University of Life Sciences Prague obtained a nucleus population of common eland and, in 2002, established an eland farm in Láň, Prague for research and educational purposes. More recently, interest in common eland farming has expanded among European breeders. In fact, this research facility has supplied breeding stocks to private breeders across the Czech Republic, Hungary, Germany, Switzerland, and Poland, accompanied by growing efforts to integrate eland into commercial production systems (Needham et al., 2023). This further highlighting the species' emerging role in diversified livestock enterprises.

Previous studies suggest that wild animals often reach marketable or economically harvestable sizes more quickly than domesticated species such as cattle and sheep on marginal land with poor quality natural vegetation (Reul, 1979). For instance, Thompson's gazelle and eland exhibit growth rates ranging from 0.06 to 0.33 kg per day, compared to 0.14 kg per day for poorly managed cattle under similar conditions in eastern African rangelands (Talbot et al., 1965). Additionally, common eland demonstrated favourable dressing percentages across age categories under enhanced husbandry and captive conditions, yielding better carcass quality than those reported in free-ranging individuals harvested in South

Africa or sub-adult beef cattle (Needham et al., 2019; 2022; Agriculture and Horticulture Development Board, 2022), indicating their potential for intensive production systems as well. Regarding nutritional value, eland meat is characterised by a favourable polyunsaturated/saturated fatty acids ratio (PUFA/SFA) ratio of 0.6 and low content of SFA, compared to cattle fed on the same diet (Bartoň et al., 2014), and thus may be considered a healthier option. Compared to beef, eland meat may have lower sensory scores for texture characteristics (tenderness, chewiness and fibrousness, and juiciness), flavour intensity, and overall acceptance than Fleckvieh bull meat under comparable intensive production systems and fed similar nutrition (Bartoň et al., 2014) likely due to its low intramuscular fat content. However, ageing has shown significant improvements in the tenderness of eland meat (Needham et al., 2020; 2022).

Common eland, and other wild African ungulate species, possess specialised water conservation mechanisms that enable them to be less dependent on water sources than cattle under similar conditions. In general, their thermoregulatory flexibility enhances oxygen extraction efficiency, which improves water utilisation (Taylor & Lyman, 1967). This adaptation is further supported by their exceptional renal and faecal water resorption systems, allowing them to sustain themselves using moisture from plants and shrubs. As a physiological response, water loss is minimised through mechanisms such as dry faeces, concentrated urine, lower metabolic rates, slow and deep breathing (Taylor & Lyman, 1967). This makes common eland highly water-efficient and independent of water sources (Estes, 1991), capable of tolerating heat stress up to 45°C, compared to domestic cattle (Retief, 1971; Furstenburg, 2007). Furthermore, the

eland's broad hoof structure facilitates more even weight distribution across the ground surface, thereby mitigating soil compaction and reducing trampling-induced degradation – particularly when compared to the narrower hooves of conventional livestock such as cattle (Taylor & Lyman, 1967; Cumming & Cumming, 2003).

In terms of feeding behaviour, common eland exhibit dietary flexibility, consuming a variety of forage types and demonstrating high tolerance for tannin-rich vegetation (Flack, 2013). This foraging strategy not only enhances the eland's ability to survive in nutritionally variable environments but also potentially contributes to a reduced greenhouse gas footprint. In contrast to cattle, which depend on high-energy, methane-intensive diets, eland are able to sustain body condition on lower-quality forage, producing less enteric methane as a result (Flack, 2013). Additionally, they can readily adapt to formulated cattle feed, further highlighting their nutritional versatility and suitability for intensive farming systems (Bartoň et al., 2014). Under feedlot production systems, common eland have demonstrated adaptability to routine husbandry and handling practices, which facilitated improved management efficiency and promote habituation (Musa et al., 2021).

Regarding reproductive efficiency, the common eland exhibits a significantly higher reproductive rate than domestic cattle, particularly *Bos indicus* breeds like the Afrikaner. This advantage is due to the combination of physiological and behavioural adaptations. As noted by Retief (1971) and further corroborated by Skinner & van Zyl (1969), eland have a shorter gestation period, averaging approximately 271 days, in contrast to the 284-day average as observed in cattle (Dittrich, 1972; Pappas, 2002). Moreover, eland display a reduced postpartum anoestrous

interval, resulting in a shorter calving interval period of around 354 days, thereby enabling females to conceive more rapidly following parturition. Female eland exhibit an oestrous cycle averaging approximately 21 days (Nowak, 1999; Posselt, 1963). A male-to-female sex ratio of 1:8–12 is considered optimal for achieving high conception rates in wild eland populations, particularly under nutritionally constrained conditions (Skinner et al., 1971; Furstenburg, 2016). Under extensive management systems, the recommended weaning age is around six months (Dittrich, 1972; Pappas, 2002). Notably, females reach sexual maturity earlier (typically at around two years of age) compared to males, which mature at approximately four years old (Hall, 1975; Underwood, 1981).

2.2. Breeding management and challenges for advanced reproduction techniques (ARTs) for common eland males

Ranchers frequently select eland for breeding, particularly in the context of trophy hunting, based on physical traits linked to early growth and the attainment of sexual maturity. These traits typically include greater body mass (Table 2.1), horn size (Figure 2.1), testicular dimensions and shape, as well as the age at which puberty begins (Khumalo, 2021). Currently, limited data exists on the breeding management for common eland bulls, whether they are managed in the wild, under extensive systems, or in captivity. Therefore, evaluating key phenotypic traits and reproductive parameters is essential, as they may serve as indicators to enhance production efficiency and fertility potential.

Table 2.1 Mean age-related growth rate for cape eland, adapted from Furstenberg (2016).

Age	Shoulder height (cm)		Live body mass (kg)	
	Female	Male	Female	Male
Birth	45	55	25	32
1 month	65	75	52	56
3 months	90	110	85	93
6 months	108	137	155	165
9 months	120	140	165	182
1 year	135	157	207	224
2 years	140	160	300	326
3 years	145	164	350	474
4 years	148	167	410	650
5 years	150	170	450	740
6 years	150	170	460	780

In cattle, a bull breeding soundness evaluation (BBSE) is a crucial tool for categorising individual bulls based on their breeding potential (Driscoll et al., 2009; Lone et al., 2017) and oftentimes is used to assess the reproductive viability of dairy and beef cattle. One key parameter evaluated over longitudinal periods is scrotal circumference (SC), a phenotypic trait with high heritability and moderately correlated with body weight in, for example, Nellore bulls (0.2968–0.5485) (Oliveira et al., 2021). Whilst this approach may also be applicable to eland bulls (although, not investigated) it only provides a general assessment of reproductive status, lacking consideration of essential indicators of fertility potential. Additionally,

establishing such an evaluation protocol requires large sample sizes, under various conditions, and a long period of monitoring to establish accurate data. On the other hand, a comprehensive assessment of reproductive tract morphology is an essential starting point to understand the reproductive physiology of a species and may be used as a basis for understanding development and functionality. Key criteria include position, shape, dimensions, symmetry, consistency, and anatomical relationships with other organs (Ohashi et al., 2011).

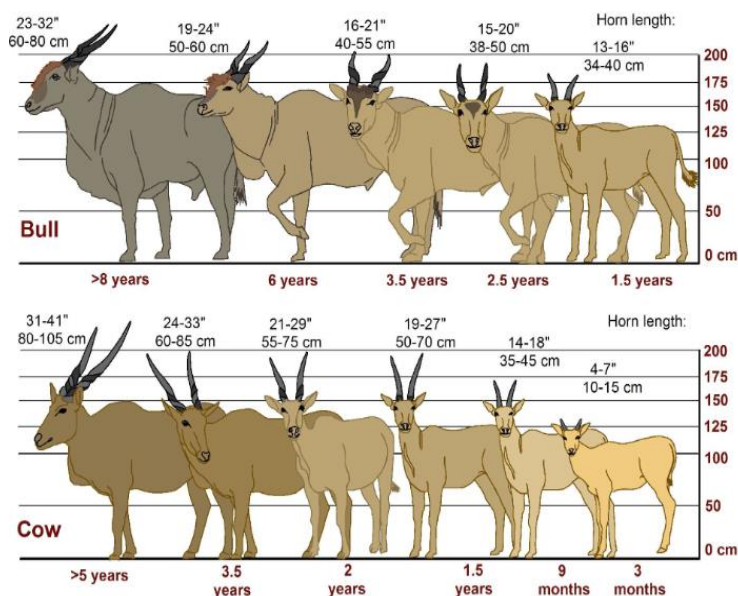


Figure 2.1. Body and horn growth according to the age of cape eland. Source: Furstenberg (2007).

Due to its phylogenetic proximity to bovine species, the reproductive morphology and function of eland bulls

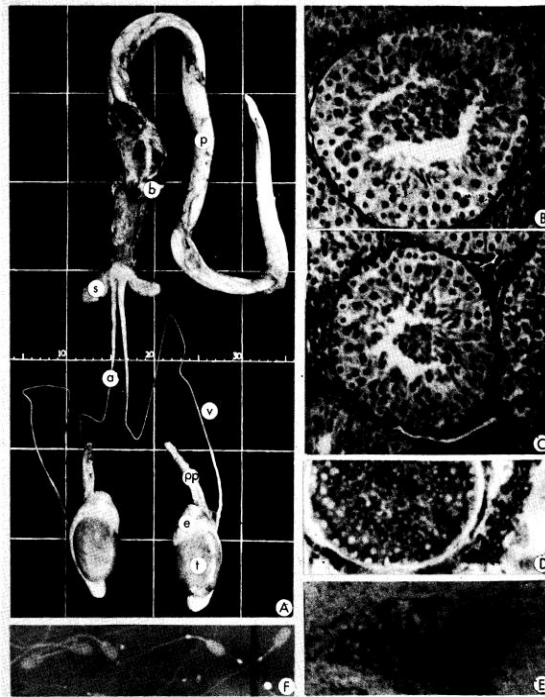


Figure 2.2. Dissected reproductive tracts of one eland bull. Source: Skinner et al. (1971).

- A. *The reproductive tract of the eland bull is depicted, illustrating the testes (t), epididymides (e), pampiniform plexus (pp), vas deferens (v), ampullae (o), seminal vesicles (s), bulbourethral glands (b), and penis (p). Scale indicated in centimetres.*
- B. & C. *Testicular cross-sections at 256× magnification, stained with Delafield's haematoxylin and chromotrope 2R. Variation in seminiferous tubule size between eland 1 (B) and eland 3 (C); however, an active spermatogenic cycle was consistently observed in both.*
- D. *Testicular cross-section stained with Sudan block, highlighting Leydig cells at 256× magnification.*

- E. *Unfixed frozen testis section incubated for 3 hours to demonstrate 5 α -3 β -hydroxysteroid dehydrogenase activity within the interstitial tissue (256 $\times$ magnification)*
- F. *Spermatozoa collected from the cauda epididymis, stained with nigrosin-eosin, exhibiting prominent distal droplets.*

closely resemble those of domesticated cattle (Figure 2.2; Skinner et al., 1971) and buffalo (Tonizza de Carvalho et al., 2014). Glover (1972) described a similar course and distribution of the testicular artery in eland, steinbok, and other domesticated ruminants. The artery runs through a helical region surrounding the pampiniform plexus, extending to the tunica albuginea of the testis, where it branches dichotomously into two primary arteries, ensuring efficient testicular blood flow. Additionally, Skinner et al. (1971) conducted a detailed dissections of eland bull reproductive tracts at the S.A. Lombard Nature Reserve in South Africa (Figure 2.2), where they observed that eland bulls reproduce year-round, with minimal seasonal variations of their sperm production.

Treus and Lobanov (1971) conducted the first recorded sperm collection from male common eland for artificial insemination trials in domestic cows. However, the experiment was unsuccessful, and no detailed methodology or findings were reported. Later, Merilan et al. (1978) pioneered the first cryopreservation of eland sperm, employing electroejaculation under general anaesthesia to collect fresh semen samples from an adult eland bull weighing 635 kg. Before further semen processing, basic semen and sperm characteristics of eland bulls were documented. Generally, the semen appeared milky-white, with a total collected volume of 3.5 ml, displaying vigorous wave and eddy formation and 65% progressive motility at room temperature. In addition, Merilan et al. (1978)

reported comparative size measurements of eland and Holstein spermatozoa (Table 2.2) and later expanded their analysis to include the sperm dimensions from other selected *Artiodactylid* species (Table 2.3; Merilan et al., 1982). Whilst collection of sperm via electroejaculation or artificial vagina has numerous benefits (*i.e.*, no need to slaughter the animal, repeated collection, collection of sperm with seminal fluid), this is often unfeasible and unsafe for game species not habituated to handling and requires sedation at least, especially when a larger number of animals are to be evaluated.

Table 2.2. Comparative dimensions (μ) of spermatozoa (mean $\pm$ SD; 20 cells) between common eland (collected via electroejaculation) and Holstein cattle (collected via artificial vagina), adapted from Merilan et al. (1978).

Cell region	Eland	Holstein
Head		
Width	6.33 $\pm$ 0.29	5.43 $\pm$ 0.48
Length	9.33 $\pm$ 0.33	9.84 $\pm$ 0.53
Midpiece	14.57 $\pm$ 0.65	13.18 $\pm$ 0.80
Tail (principal part and endpiece)	39.84 $\pm$ 1.62	47.31 $\pm$ 1.55
Total cell length	63.75 $\pm$ 1.41	70.33 $\pm$ 1.25

SD: Standard deviation

Merilan et al. (1978) investigated the cryopreservation of eland semen (collected via electroejaculation) by incorporating an egg yolk-citrate extender with 6% glycerol, followed by cooling to 4°C and freezing in 0.5-mL straws under liquid nitrogen vapor. This process resulted in 35% post-thaw motility (rather poor),

compared to the 65% progressive motility of the fresh semen prior to freezing, suggesting that semen extenders and processing protocols adapted from domestic bulls are potentially applicable to eland bulls. However, these results are not directly comparable to standard protocols typically applied to beef cattle, where a minimum of 40% progressive motility (post-thawed) is generally adequate for artificial insemination purposes (Kudratullah et al., 2024).

Building on this, Bartels et al. (2001) successfully produced an eland calf through artificial insemination using epididymal-collected sperm extended in Triladyl®, cooled to 4°C, stored in 0.25-mL straws, and frozen in liquid nitrogen vapour, achieving 55% post-thaw motility. Gestation of the calf was prolonged by two months beyond the expected 255–284 days and the researchers did not provide further details for this deviation. Prolonged gestation may be observed in wildlife species and under captive breeding, often resulting from a combination of biological, environmental, and management-related factors (Sontakke et al., 2009). Therefore, careful consideration of these factors is essential, as they can significantly influence reproductive success and offspring viability. Various semen extenders have been utilised and evaluated in other antelope species as part of assisted reproductive interventions aimed at enhancing reproductive performance. In greater kudu, the use of a Tris-citrate-based extender containing 20% egg yolk and 7% glycerol resulted in a post-thaw motility rate of 65%, with slightly improved sperm motility following thawing at room temperature (Schmid et al., 1997). Similarly, Triladyl® extender was applied to samples from four sitatunga bulls that yielded post-thaw motility ranging from 22% to 42%, compared to initial motility levels of 40% to 80% (Perez-

Table 2.3. Comparative characteristics of spermatozoa (mean $\pm$ S.D.; 20 cells) from selected species of *Artiodactylids*, adapted from Merilan et al. (1982).

Parameters	Banteng	Lesser Kudu	White-tailed Gnu	Sable Antelope	Blesbok	Impala
Total length (μ)	72.26 $\pm$ 5.69	68.86 $\pm$ 2.29	62.28 $\pm$ 4.62	62.63 $\pm$ 2.83	63.80 $\pm$ 2.69	62.14 $\pm$ 2.1
Head:						
Length (μ)	10.20 $\pm$ 0.38	9.58 $\pm$ 9.43	8.04 $\pm$ 0.41	6.79 $\pm$ 0.57	7.00 $\pm$ 0.55	9.40 $\pm$ 0.47
Width (μ)	5.47 $\pm$ 0.33	5.82 $\pm$ 0.18	5.75 $\pm$ 0.18	3.94 $\pm$ 0.45	5.01 $\pm$ 0.51	5.93 $\pm$ 0.21
Length: width ratio	1:0.54	1:0.61	1:0.72	1: 0.58	1:0.72	1:0.63
Midpiece length (μ)	14.22 $\pm$ 1.09	14.12 $\pm$ 0.95	11.74 $\pm$ 1.13	12.19 $\pm$ 0.41	11.26 $\pm$ 0.49	10.83 $\pm$ 0.5
Tail length (μ)	47.84 $\pm$ 5.20	45.19 $\pm$ 2.15	42.50 $\pm$ 4.46	43.64 $\pm$ 2.81	45.58 $\pm$ 2.56	41.93 $\pm$ 2.0

SD: Standard deviation

Garnelo et al., 2000). A TEST-yolk buffer incorporating 6% glycerol was also tested in bongo antelope and giant eland, though with limited success – recording post-thaw motility rates are below 40% and 30%, respectively (Schmid et al., 1997; Bartels et al., 2001). Despite these efforts, no standardised semen extender has yet been established for optimal sperm preservation in antelope species, including the common eland.

To investigate the effect of prolonged cold storage, Bissett and Bernard (2005) collected eland spermatozoa from the caudal epididymides after 4–5 hours of transportation, using fresh testes obtained postmortem and stored at 4°C in a portable refrigerator during transportation. This method has been successfully utilised with other animal species as a practical approach for collecting and preserving gametes from genetically superior animals that are endangered, often culled, targeted for trophy hunting, or lost to sudden death, particularly in southern Africa, where game ranches are frequently located in remote areas with limited access to reproduction laboratory facilities. As a result, sperm quality deteriorated over time, with reduced viability and motility after 108 hours, alongside increased membrane damage and high incidence of sperm defects due to the cold-storage conditions. Despite these limitations, epididymal sperm retrieval proved to be a viable method for gamete collection and preservation, offering potential applications in eland production and efforts to conserve the endangered Western Giant eland (Bissett & Bernard, 2005).

2.2.1. Understanding sperm biology and assessing sperm quality for the application of ARTs

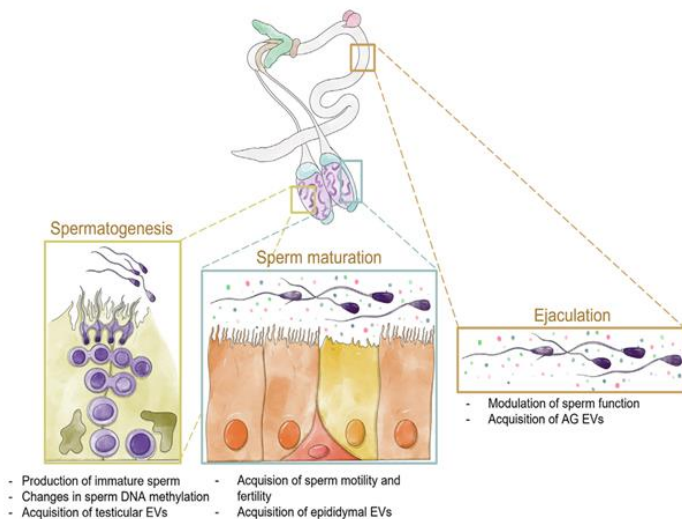


Figure 2.3. Sequential events to produce functional sperm, as illustrated for bull cattle. Source: Fernandez-Fuertes (2023). Abbreviations: EV-extracellular vesicles; AG-accessory glands.

Sperm undergo complex maturation cycles within the testes and epididymis (Figure 2.3). Spermatogenesis occurs in the seminiferous tubules (ST), followed by spermiation, during which immature spermatozoa interact with distinct luminal environments as they move through the excurrent ducts (Hess, 2002). The seminiferous tubules connect to the rete testis, a network of cuboidal epithelial structures, which then link to efferent ducts, varying between, *e.g.*, 4 to 20 tubules depending on the species (Robaire et al., 2006). These ducts converge into the epididymis, a single coiled duct whose length differs by species (Hinton et al., 2011). The epididymis consists of four anatomical regions: the initial segment, head (caput), body (corpus), and tail (cauda), each organised into lobules

separated by connective tissue septa (Robaire et al., 2006; Turner et al., 2003). Within the epididymis, spermatozoa undergo biochemical modifications, including progressive functional and molecular changes that enhance their fertilisation potential (Bork et al., 1988).

The most noticeable change in the epididymis is the acquisition of motility by spermatozoa. In the caput region, sperm exhibit irregular and asymmetric flagellar movement, which gradually becomes symmetrical, with wave propagation enhancing motility until reaching the cauda region, where forward progression mechanisms are fully developed (Dacheux & Dacheux, 2014). This motility is largely dependent on intracellular cAMP, produced by adenylyl cyclase, and regulated through protein phosphorylation pathways, including protein kinase A, A-kinase anchor proteins, and other associated proteins (Esseltine & Scott, 2013). Within the epididymal lumen, sperm progressively increase intracellular cAMP levels, enhancing metabolic capacity and ATP production as they move from the corpus to the cauda (Inskeep & Hammerstedt, 1982; Pariset et al., 1985). At this stage, mature spermatozoa can be harvested without seminal fluids or collected during ejaculation, where they combine with seminal fluids from accessory glands, including the seminal vesicle, bulbourethral gland, and prostate gland.

The structural and functional characteristics of sperm serve as essential tools for assessing reproductive potential across animal species, enabling the monitoring of reproductive cycles and fertility indicators. This approach has proven beneficial and is increasingly being applied to wildlife species, supporting efforts in biodiversity conservation. Regarding sperm motility, eland sperm was previously evaluated using a 0-to-5-point scoring system, which relies on subjective observation of general

movement under an electron microscope (Merilan et al., 1978). However, with advances in technology, computer-aided semen analysis (CASA) emerged in the mid-1980s, revolutionising sperm evaluation (Valverde & Valverde, 2018). The CASA system integrates a microscope equipped with a heating stage, a negative phase contrast optical device, and a video camera, connected to a computer with specialised software for various types of sperm analysis. The system captures and processes real-time movement, serving as a database for comprehensive semen quality assessment (Soler et al., 2018).

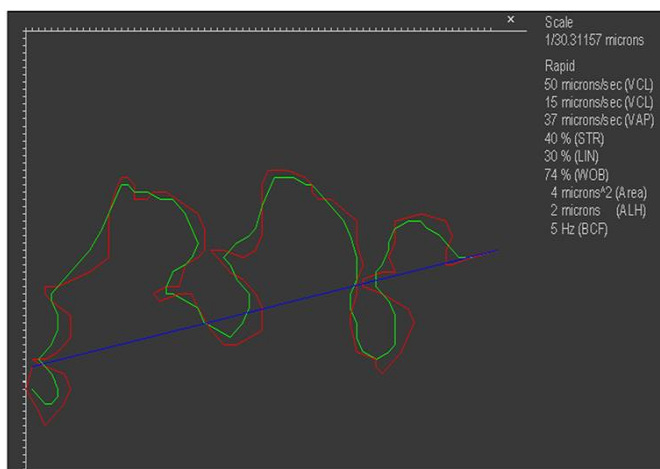


Figure 2.4. Example of sperm kinematic parameters obtained from ISAS®v1 CASA system. Source: Soler et al. (2018). Legend: Red line shows curvilinear velocity (VCL), blue line indicates straight line velocity (VSL) and green line shows the average path velocity (VAP).

CASA provides an objective method for evaluating sperm motility, extending its analysis to sperm trajectory

and kinematics, which provides an indication of potential fertilising ability for both natural and artificial insemination (Soler et al., 2018). Regarding sperm kinematics, the system measures the distance travelled by the sperm head over a given acquisition period (Gallagher et al., 2019). This assessment includes three parameters related to sperm motion velocity [curvilinear velocity (VCL), average path velocity (VAP), and straight line velocity (VSL)] three parameters evaluating velocity ratios (Straightness (STR) = $VSL/VAP \times 100$; Linearity (LINR) = $VSL/VCL \times 100$, Wobble/Oscillation = $VAP/VCL \times 100$), and two parameters characterising sperm wobble (ALH amplitude of the lateral head displacement (ALH) and beat-cross frequency (BCF), these are illustrated in Figure 2.4.

Sperm kinematic parameters offer detailed insights into spermatozoa motility characteristics, which are critical for successful fertilisation and play a pivotal role in predicting sperm survivability following cryopreservation. Specifically, VCL reflects the kinetic energy as a requirement for spermatozoa to travel to the female reproductive tract and fertilise the oocyte; however, insufficient VCL values are also associated with the damage of the spermatozoa's plasma membrane (Chianese & Pierantoni, 2021) and disruption of mitochondrial function (Ferramosca & Zara, 2014). VSL provides fundamental sperm functional properties (Purnawan et al., 2025), and it also indicates the propulsive movement of spermatozoa as affected by any changes of the ionic environment (Nagy et al., 2015). VAP serves as a key predictor of sperm viability, particularly following cryopreservation, and is frequently utilised in the classification of sperm into functional subpopulations based on motility profiles (Utami et al., 2025). In terms of the sperm velocity ratios such as LIN, STR, and WOB, they indicate the maximum efficiency of the

sperm's ability to reach the oocyte regarding direction of movement and optimal swimming patterns, highlighting the balance between stability and flexibility of spermatozoa movement (Broekhuijse et al., 2011; Valverde et al., 2019). Regarding sperm characterisation parameters, ALH is associated with hyperactivity movement of the sperm, an essential process for capacitation process, where the sperm undergoes a series of biochemical changes to improve their fertilising ability (Saravia et al., 2007). BCF is used to monitor the efficiency of energy use of the spermatozoa (Kumar et al., 2016). However, results of the sperm kinematic parameters from CASA vary according to the breed, age, collection techniques, sperm media, freezing protocols, and the software settings used (Soler et al., 2018). The common software settings in assessing cattle semen (*i.e.*, Belgian Blue cattle) are detailed in Table 2.4; Seifi-Jamadi et al., 2020).

Table 2.4. CASA software settings (IVOS-II, Hamilton Thorne Inc, Beverly, USA) for cattle semen, adapted from Seifi-Jamadi et al. (2020).

Parameter	Parameter function	Value
Frame rate (Hz)	Image capture	60
Number of frames captured	Image capture	30
Minimum contrast	Cell detection	20
Minimum cell size (pixels)	Cell detection	5
Straightness cut-off (%)	Progressive cell detection	80
Fast VAP cut-off ($\mu\text{m/s}$)	Progressive cell detection	50
Low VAP cut-off ($\mu\text{m/s}$)	Static cell detection	20
Statis VAP cut off ($\mu\text{m/s}$)	Static cell detection	5
Low VSL cut-off ($\mu\text{m/s}$)	Static cell detection	2

Additionally, sperm head morphometry assessment is incorporated into CASA's features, to identify any deviations in head size, as these may influence motility and the ability to navigate the female reproductive tract (Yániz et al., 2015) (Figure 2.5). For instance, a study of Kondracki et al. (2005) revealed that larger sperm head dimensions in cattle (Black and White Breed in Poland) were linked to specific morphological anomalies, and that fluctuations in head size might be associated with chromatin structure irregularities- factors that can adversely affect fertility. Sperm head morphometry is one of the important parameters used for the assessment of sperm quality for several bull cattle breeds, *e.g.* Limousine and Simmental (Gebrina et al., 2024). Based on the general classifications of sperm head morphometry across animal species, variations in sperm head width modulate the hydrodynamic efficiency of spermatozoa during their migration through the luminal environment, ultimately influencing fertilisation capacity (Maroto-Morales et al., 2016).

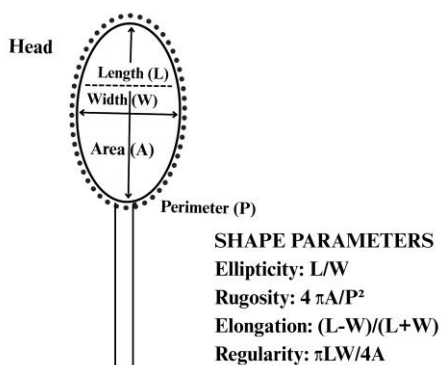


Figure 2.5. Sperm head indicating morphometric parameters, and their shape indices.

Shape indices (ellipticity, elongation, rugosity/roughness, and regularity) obtained from sperm head dimensions further describe sperm morphology and its associations with sperm movement ability (Kruger et al., 2004). According to the Tygerberg's strict criteria (Kruger et al., 2004), ellipticity reflects the degree to which the sperm head assumes an elongated, narrow, or conical form, whereas elongation represents the deviation from roundness, quantifying head slenderness. Rugosity measures surface irregularity and is indicative of amorphous or aberrant head contour. Regularity is characterised with symmetry and uniformity of the sperm head, often associated with a pyriform (pear-shaped) morphology. Furthermore, spermatozoa with elongated heads tend to exhibit enhanced motility, likely due to reduced fluid resistance (Garcia-Vazquez et al., 2016). Additionally, during mating, the sperm cells of mature Brahman breed (57.12 ± 14.50 months old) exhibit a more elongated, elliptical, and regular shape, with a positive influence on the linearity and straightness of sperm movement (Araya-Zúñiga et al., 2024).

The Society for Theriogenology (SFT) has established minimum sperm quality thresholds for breeding soundness evaluation (BSE) in dairy and beef bulls, which may also serve as reference benchmarks for common eland. According to SFT guidelines, acceptable semen parameters include $\geq 30\%$ progressive motility and $\geq 70\%$ morphologically normal spermatozoa (Koziol & Armstrong, 2018). In contrast, the Brazilian College of Animal Reproduction (CBRA) adopts a more stringent criterion, requiring $\geq 60\%$ motility, and evaluates sperm morphology based on a comprehensive defect classification system, distinguishing between total, major, minor, and specific abnormalities rather than relying solely on the

proportion of normal cells (CBRA, 2013). In 2018, the University of Queensland Sperm Morphology Standardisation Program (UQSMSP) revised threshold values for sperm morphology and abnormalities in beef cattle, following the criteria established by Fordyce et al. (2006); abnormal categories and their respective upper thresholds include: proximal droplets (PD) ($\leq 20\%$), midpiece abnormalities (MP) ($\leq 30\%$), loose heads and principal piece defects (HT) ($\leq 30\%$), pyriform heads (PY) ($\leq 20\%$), knobbed acrosomes (KA) ($\leq 30\%$), vacuoles and teratoids (VT), including DNA condensation defects ($\leq 30\%$), and swollen or absent acrosomes (SA) ($\leq 30\%$). While sperm with non-conception-affecting abnormalities are classified as 'normal', subcategories provide further granularity, such as distinctions among flat, beaded, and indented knobbed acrosomes, and multiple vacuolation profiles (Perry, 2017).

With the introduction of CASA, the post-thaw epididymal sperm kinematic patterns of selected ungulate species have been characterised (after cryopreservation using Biladyl containing 7% glycerol) and assessed to develop species-specific assisted reproductive technologies (ARTs) for *ex situ* conservation (see Table 2.5 for the summary of the sperm kinematic results; Chatiza et al., 2012). Despite the inclusion of sperm head morphometry assessment in CASA, there is currently no existing data for common eland sperm utilising this technique, but data exists for Friesian and Angus cattle, which may serve as reference points for eland bulls (Table 2.6).

Table 2.5. Comparison of post-thawed epididymal sperm kinetics (collected 3 hours post-mortem) of selected ungulate species (all considered sexually mature), adapted from Chatiza et al. (2012).

Sperm kinematics	Springbok (n=38)	Impala (n=26)	Blesbok (n=42)
Total motility (%)	70	58	85
Progressive motility (%)	13	17	46
Non-progressive motility (%)	57	41	39
Curvilinear velocity ($\mu\text{m/s}$)	65	115	150
Straight line velocity ($\mu\text{m/s}$)	40	35	85
Average path velocity ($\mu\text{m/s}$)	65	55	105
Linearity (%)	32	33	56
Straightness (%)	61	65	80
Oscillation (%)	51	51	69
Amplitude of lateral head displacement (μm)	3.6	3.7	3.3
Beat cross frequency (Hz)	16	17	20

Recently, Pintus et al. (2024) reported morphometric parameters for common eland ($n = 10$ bulls; ≤ 30 months old; 227.50 ± 9.44 kg) spermatozoa harvested from the caudal epididymides, collected from the same research facility in Czech Republic, and analysed using ImageJ software: head width ($6.00 \pm 0.05 \mu\text{m}$), head length ($9.71 \pm 0.09 \mu\text{m}$), head perimeter ($25.04 \pm 0.14 \mu\text{m}$), head area ($45.77 \pm 0.43 \mu\text{m}^2$), head ellipticity (1.62 ± 0.02),

Table 2.6. Comparison of sperm head morphometry and shape indices of selected cattle breeds species using CASA.

Parameters	<i>Vicente-Fiel et al., 2013</i>	<i>van der Horst et al., 2021</i>
	Friesian bulls	Angus bulls
Head area (μm^2)	32.11 ± 0.32	45.05 ± 4.04
Perimeter (μm)	23.74 ± 0.12	21.04 ± 1.11
Length (μm)	8.99 ± 0.06	9.58 ± 0.47
Width (μm)	4.54 ± 0.03	4.83 ± 0.23
Ellipticity	1.99 ± 0.02	1.99 ± 0.10
Elongation	0.33 ± 0.00	0.33 ± 0.02
Rugosity	0.72 ± 0.00	1.28 ± 0.10
Regularity	1.00 ± 0.00	0.81 ± 0.03

SEM: Standard error of the means (Friesian); SD: Standard deviation (Angus)

midpiece length ($14.57 \pm 0.14 \mu\text{m}$), principal plus terminal piece length ($47.19 \pm 0.37 \mu\text{m}$), flagellum length ($61.76 \pm 0.47 \mu\text{m}$), and sperm length ($71.48 \pm 0.50 \mu\text{m}$). These results were comparable to the ejaculated sperm samples evaluated by Merilan et al. (1978) (see Table 2.2), particularly with respect to head width, head length, midpiece length, and overall sperm length. However, it is important to note that the samples obtained by Merilan et al. (1978) and Pintus et al. (2024) were obtained using different collection methods and under varying management conditions (extensive vs. intensive), as well as across different age categories (mature vs. immature bulls). Under intensive feeding regimes, eland bulls may reach puberty as early as 15 months old, potentially earlier compared those under extensive management systems

(Ros-Santaella et al., 2014). As such, generating sperm quality data from a standardised male cohort across age classes under controlled conditions is crucial for characterising age-related changes in reproductive function and establishing baseline reproductive information. These insights provide a basis for identifying factors that influence male reproductive development and performances.

2.3. Potential effects of selected animal traits and environmental factors on the reproductive biology of common eland

Understanding factors influencing reproductive development is key for breeders to accurately assess and select high-quality males. Traits like age, body condition, hormone patterns, and social behaviour not only affect reproduction but may also serve as phenotypic indicators of reproductive status. Although the common eland is known to breed year-round in both wild and captive conditions (Skinner et al., 1971; Musa et al., 2021), several reproductive factors remain poorly understood and have yet to be fully described. One critical aspect in reproduction and breeding management for livestock production is the age of an individual (Kipper et al., 2017). In immature males, semen quality may be poor, with dysfunction of the epididymides and accessory glands, and low androgen levels, which collectively influence reproductive anatomy – such as lower testicular blood flow and constriction of the seminiferous tubules compared to mature males (Halvaei et al., 2020). To date, there are no available scientific data regarding the sequential reproductive developmental of eland bulls and its relationship with individual animal traits like age under controlled conditions. Therefore, early

assessment of these factors in relation to age and body growth-related changes is crucial, as it may serve as a valuable indicator of physiological development and fertility potential (Menegassi et al., 2019).

Typically, age affects the development of accessory glands and testicular tissue, as shown in the subsequent examples for different bovine species. Previous studies on Holstein bulls have demonstrated a gradual increase in semen volume from puberty to sexual maturity, with an average increment of approximately 0.5 mL per year, continuing until around four to seven years of age (Murphy et al., 2018; Taylor et al., 1985). In Nili-Ravi buffalo, lumen formation begins by 12 months old, accompanied by a restriction of intertubular spaces due to seminiferous tubule enlargement, whilst at 18 months old, both round and elongated spermatids are visible and a complete lumen formation with active spermatogenesis within the seminiferous tubules at 24 months old (Ahmad et al., 2010). These developmental patterns are critical for monitoring the reproductive functionality of male species. Although such information remains unavailable for wild common eland bulls, initial histological examination of the testes in captive males reported increased activity of Sertoli cells and spermatogenesis as early as 15 months old (Ros-Santaella et al., 2014; conducted in CZU Common Eland Research Facility). Therefore, age at puberty should be taken into account during the initial selection of breeding bulls or for culling decisions in common eland used for production, as routinely performed in commercial beef production systems, in order to reduce generation intervals with higher genetic gains (Brito, 2021). In the absence of testicular histology assessment, most cattle raisers utilised the minimum guideline for age and scrotal circumference measurement set by the Society of Theriogenology (SFT)

(Table 2.7; Koziol and Armstrong, 2018). Thus, the relationship between age, physiological development, and reproductive development need to be considered together when assessing the potential of common eland bulls for breeding during selection (typically prior to sexual maturity).

Table 2.7. Minimum guideline for age and scrotal circumference as basis for breeding sound evaluation (beef and dairy bulls), adapted from Koziol and Armstrong (2018).

Age (months)	Scrotal circumference (cm)
≤15	30
15 to 18	31
18 to 21	32
21 to 24	33
≥24	34

Moreover, Mekasha et al. (2007) noted that the reproductive potential of domestic animals is closely associated with body weight and body condition scores however, this association generally depends on nutrition. The manipulation of nutritional requirements of bulls during prepubertal and pubertal periods is also practised in dairy and beef production. Early onset of puberty in bulls is regulated by the hypothalamic–pituitary–testes axis, where optimal nutrition stimulates the hypothalamic gonadotropin releasing hormone (GnRH) pulse generator, enhancing luteinising hormone (LH) secretions and testicular development (Brito et al., 2007). With optimal nutrition, puberty is hastened by 1-1.5 months and increases testicular mass at 12-18%, at around 1.5 years old in cattle (crossbreds of Angus × Charolais), particularly

during the peripubertal stage, and resulted in larger testes (scrotal circumference and testis weight) (Brito et al., 2007). A similar trend has been observed in common eland under captive breeding conditions, where enhanced nutritional support has led to the earlier onset of puberty at 15 months of age (Ros-Santaella et al., 2014), compared to the 18–24 months as typically reported in extensive production systems in southern Africa (Du Toit, 2005). The interaction between nutrition and body development is also manifested in testicular development. Osinowo et al. (1981) demonstrated a strong correlation between body size and testicular growth in Bunaji bulls, which directly influenced sperm concentration, counts, semen volume, and sperm motility and morphology. Similarly, Coulter and Foote (1977) observed that Holstein bulls with larger testes and greater testicular mass had greater body weights with higher sexual activity—an effect attributed to the anabolic influence of androgens (Bratte et al., 1999). Thus, nutrition is also an important factor to consider when establishing baseline reproduction-related information for a species.

Seasonal variation is another critical factor influencing reproductive physiology. Fluctuations across seasons have been shown to affect hormonal profiles, sexual behaviour, sperm morphology, and overall fertility (Gerlach & Aurich, 2000), underscoring the need for further investigation in common eland bulls. Understanding these seasonal effects is essential for breeding management, refining the timing of semen collection, storage, and the application of assisted reproductive technologies in eland bulls (Morais et al., 2002).

The temperament of breeding males plays a crucial role in reproductive management, as it influences sexual behaviour and handling ease – key criteria for selecting viable bulls for both natural mating and artificial

insemination programs (Francolino da Silva et al., 2025). This is particularly relevant for eland bulls in captive settings, where their wild origin may affect how they respond to specific husbandry and breeding practices (Pennington et al., 2013). Temperament is highly established as an evaluation factor and is regarded as a criteria for the selection of cattle bulls (Valente et al., 2017). For instance, Nellore bulls are known for their more agitated and excitable temperament (Silveira et al., 2008), and thus often managed and selectively bred with an emphasis on enhancing handling ease and overall productivity. Francolino da Silva et al. (2025) reported the average cortisol and testosterone concentrations in crossbred Nellore × Santa Gertrudis bulls across different age groups and temperament type – calm versus reactive – as indicators of behavioural traits (Table 2.8). This study has demonstrated that temperament did not influence testosterone production and sexual precocity of crossbred bulls. However, a higher cortisol production indicated a lower reproductive performance related to sperm morphology and reduced vesicular gland development with greater animal reactivity. Highly reactive bulls also were reported to exhibit lower efficiency under extensive pasture-based production systems, typically lower total weight compared to calm crossbred bulls (Francolino da Silva et al., 2025). Thus, for game species which have not undergone intensive selection for temperament over time, like domesticated livestock species, temperament is an important selection criterion to consider, as well as its effects on reproduction.

In common eland, Musa et al. (2024) found that temperament during handling significantly affects blood biochemical profiles, even in habituated individuals, and thus may have an impact on endocrine secretion patterns.

However, the secretion patterns of faecal androgen metabolites (fAM) *i.e.* epiandrosterone have only been performed in common eland males under the scope of immunocastration. Monitoring androgen secretion patterns may give insight into the effects of various factors such as animal traits, environment, and social behaviour, as well as their relationships with reproduction in common eland males. Understanding the role of social hierarchy in reproduction in common eland is crucial, especially under captive conditions, as Bissett and Bernard (2005) postulated that subordinate kudu bulls exhibit suppressed testosterone levels together with reduced sperm quality. Although social rank is a dynamic trait that provides valuable insights into the social structures and management needs for captive eland bulls, inclusion as a selection criterion for breeding requires careful consideration and long-term monitoring as social rank may fluctuate over time.

Table 2.8. Average serum concentrations of cortisol and testosterone between calm and reactive at different age of crossbred bulls (Nellore x Santa Gertrudis), adapted from Francolino da Silva et al. (2025).

Age (Months)	Cortisol (ng/ml)			Testosterone (ng/ml)		
	CA	RE	SEM	CA	RE	SEM
12	12.4	52.34	15.98	13.09	10.91	2.51
14	12.3	55.6	16.41	16.5	8.59	4.64
16	5.56	17.27	4.5	9.22	4.17	2.48
18	4.24	12.18	2.81	11.56	7.42	3.17
20	a	a	a	44.89	29.12	7.55

^a not detectable; SEM: standard error of the mean; CA: calm; RE: reactive

In conclusion, despite the ecological value and production potential of the common eland, standardised reproductive management guidelines remain limited. Existing data are often derived from diverse production systems, lacking control of confounding factors, with limited sample sizes and without long-term scientific monitoring or validation. This gap poses significant challenges for establishing baseline information for assessing reproductive parameters – such as reproductive tract development and structural symmetry, and normal sperm quality traits – critical for the accurate selection of optimal breeding bulls and their subsequent management for fertility (Utt, 2016; Abdollahi-Arpanahi et al., 2017) as well as the effective application of assisted reproductive technologies (Butler et al., 2020). In addition to the challenge of defining key reproductive parameters, methods and techniques specific to eland sperm, such as collection procedures, analytical software settings, sperm media formulations, and standard reference values, remain underdeveloped and unstandardised. Bridging this gap in reproductive functionality is critical in designing targeted husbandry practices that accommodate the specific needs of different classes of eland herd, thereby maximising their farming value. Furthermore, investigating the relationships between factors such as age, body development and condition, androgen secretion patterns, behaviour and reproductive development and functioning provides important baseline information for future research objectives, and may contribute to other game conservation goals.

CHAPTER 3

General methodology

3.1. Study area description and animal husbandry

The research studies for this thesis were performed at the Common Eland Research Facilities of the Czech University of Life Sciences Prague (CZU) in Lány, Czech Republic (50°7'41" N, 13°57'31" E). The research facility is managed by the Department of Animal Science and Food Processing of the Faculty of Tropical Agrisciences, CZU. Experimental procedures were approved by the Czech University of Life Sciences Prague Animal Welfare and Clearance Committee (Permit: CZU 20/19). The facilities are accredited by the Czech Republic Ministry of Agriculture (Permits: 63479_2016-MZE-17214; MZE-13122/2022-13114). Additionally, the facilities maintain the permission to slaughter on-site under the supervision of a state veterinarian (SVS/2018/100483-S), according to the amended Czech Veterinary Care Act No. 166/1999.

The facility is located at an altitude of 421 m above sea level, in a cold temperate climate, and fully humid with relatively cool summers, according to Köppen-Geiger classification (Musa et al., 2024). The facility is equipped with two paddocks (2.5 ha each) and a barn (230m²) with deep litter system (straw bedding). The paddocks are enclosed with wooden poles (3m in height) and at the bottom, an electric fence line has been installed to avoid animals from damaging the wooden fence. The herd is generally kept in the barn from December to April (winter-

spring season) to meet their thermoneutral requirement, the barn itself maintains 2 to 10°C with the aid of generated heat from the animals and deep litter straw bedding system. Inside the barn, each pen has a total size of 18m × 7.5 m. The pens are separated by a passageway, which serves as a feeding alley, as commonly utilised in cattle feedlot systems.

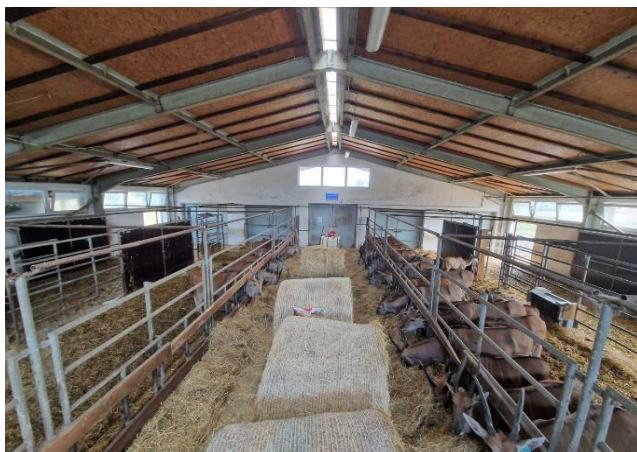


Figure 3.1. Current setup (mix-age-sex-herd system) at the Common Eland Research Facilities.

Currently, the facilities maintain up to 50 individuals for research and teaching purposes in a mixed-sex-and-age system (Figure 3.1). All experimental animals utilised in this research are European-all-born. The common eland herd was established in 2002 from a nucleus stock of five animals (originally from the tropical transboundary areas of Kenya, Uganda, Tanzania, Cameroon, and West Africa, and transported to Europe between 1967 and 1972) from Safari Park, Dvůr Králové, Czech Republic (Vagner, 1974). Since then, more than 353

animals have been born from this foundation stock. To improve genetic diversity, pedigree is monitored and the exchange of breeding stock with local zoos is performed regularly. The animals breed all-year-round and thus calving occurs throughout the year as well, but most frequently in spring. Annual culling is also conducted to manage its population size and adhere to the capacity of the facilities.

In terms of feeding, animals receive corn silage, lucerne haylage mix, and meadow hay *ad libitum*. Supplementation of oat grains and commercial pelleted cattle (19.05% crude protein) is provided based on the number of animals in the system, their body condition, and if weaners are present. Feed samples are collected monthly, and their nutritional composition is analysed. The average nutritional composition of the feed ingredients is provided in Table 3.1. Access to mineral blocks (as a supplement year-round) and an unlimited supply of water were also provided.

3.2. Experimental design and data collection

Since 2019, the animals at the facilities are handled every month, following a standardised data collection procedure. At the same year also, newly born calves are ear-tagged with electronic chips which store data related to their identification and growth, *i.e.*, weight, and average daily gain per month, and enter the monitoring system since birth. Older animals have been habituated to the handling system over time (Musa et al., 2021).

Table 3.1. Nutrient contents of different feed ingredients.

	Compound feed	Pasture	Hay	Silage
Crude protein (%)	19.05	21.54	10.28	4.57
Moisture (%)	10.20	8.55	15.31	58.42
Fat (%)	2.37	4.60	2.75	
Fibre (%)	7.04	18.44	20.86	
Starch (%)	28.64	14.43	15.64	
ADF (%)				14.04
NDF (%)				26.00
Ash (%)	7.22	7.47	6.82	2.91
Ca (%)	1.24	1.11	0.92	0.93
P (%)	0.64	0.64	0.13	0.07
K (%)	0.51	1.35	1.07	0.65
S (<i>ppm</i>)	694	3176	289	219
Si (<i>ppm</i>)	3,402	30,632	8,870	9,833
Mn (<i>ppm</i>)	45	90	99	98
Cu (<i>ppm</i>)	15	19	4	15
Fe (<i>ppm</i>)	366	3265	278	178
Zn (<i>ppm</i>)	120	158	42	26
Mo (<i>ppm</i>)	6	11	14	15

Legend: ADF-Acid detergent fibre; NDF-Non-detergent fibre; Source: Musa, 2024

A setup of raceway and squeeze chute system was developed and installed adjacent to the barn (A and B) (Figure 3.2; Musa et al., 2021) for routine handling and collection of biological samples from eland. The raceway

has a measurement of 11.60m × 1.05m × 1.85m, with four compartments (A-first section and entry from the barn), B (second section), C, D) divided by three galvanised sliding doors. To ensure low-light conditions in the corridor and to avoid the eland from jumping, the raceway was covered by a roof. Within the corridor walls, a narrow section (horizontal slit) was cut from the wall to allow handlers to monitor animals in the raceway. Within the third section (C), a platform on load bars and ear-tag panel reader were installed for the automatic identification of animals and measurement of animal body weight (TW-1 Weigh Scale G02601, Gallagher, Hamilton 3240, New Zealand). All data is recorded by the system, and manually by handlers. The final section (D) enters into the squeeze restraint chute. The squeeze chute system is composed of two rotating padded doors (1.50m long) and they are adjustable vertically according to the animal height (above the shoulder of the animal), to ensure that only the neck and head are accessible. Thick plastic curtains were also installed to close the upper portion of the restraint box to prevent eland from jumping when entering the squeeze chute. When the animal is inside of the chute, two doors are wheeled closed towards the direction of the animal by manual pushing. Serrated clips also were installed to allow door adjustments (squeezing) when restraining the animal (Musa et al., 2021).

Routine handling is performed each month at the research facilities, and data collection includes body weight and body condition scoring (BCS) based on the palpation method initially developed by Audigé et al. (1998) for red deer and adapted to common eland (Dearman, 2023). A flexible tape was used to measure the horn sizes (length, twisted length, and base circumference) and neck circumferences (Musa et al., 2021). A temperament scoring

(Table 3.2) was used following the adapted methods of Schütz et al. (2016) as used for red deer, and Parhama et al. (2019), as used for beef cattle. Temperament scoring was gathered by a single trained individual throughout the study (Parhama et al., 2019).

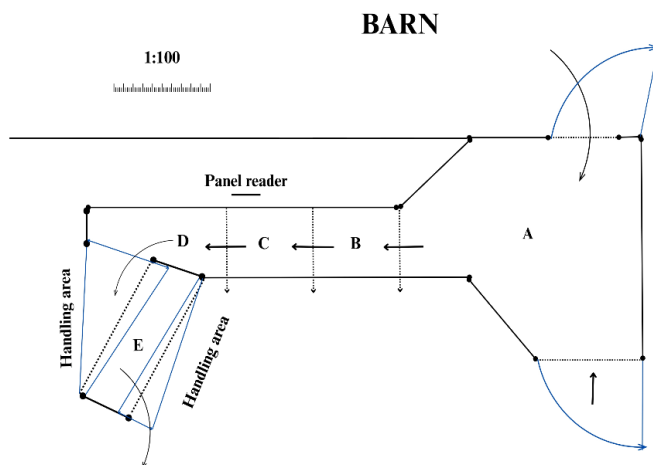


Figure 3.2. Layout of the raceway and squeeze chute system developed for common eland at the CZU Common Eland Research Facilities (Source: Musa et al., 2021).

Direct observation was applied for the monthly behavioural monitoring since video recordings did not yield adequate animal identification of all animals involved in every interaction. The observer was trained to identify and record behavioural interactions exhibited within the common eland herd. The recording of social interactions was based on the “all occurrences sampling”, adapting the methods of Altmann (1974). Interactions were grouped into affiliative, dominant-not-aggressive, and dominant-aggressive (Table 3.3; Musa et al., 2024), and subsequent

dominant interactions were coded, and each agonistic encounter (winner and loser) was recorded (Ceacero et al. 2007). A database (using Microsoft Excel Software) was created to store all the recorded eland herd behavioural observations before further processing for statistics.

Table 3.2. Temperament indicators used to assess the temperament of farmed eland during routine handling procedures, adapted from Musa et al. (2021).

Parameters	Categories	Description	Score
Entering the system	Voluntary	The animal walks towards the raceway without the handlers' interference.	0
	Lured	The animal is lured with a treat or sound cue.	1
	Forced	The animal is forced by handler using tapping stick.	2
State before and within the raceway	Calm	The animal does not make frequent eye contact and is not agitated.	0
	Nervous	The animal is agitated and uneasy.	1
	Panicked	Jumping, backwards and forward movement, or aggressive toward the handler.	2
Use of tapping sticks to trigger forward movement	Yes/No	This entails if a stick was used at each of the four areas of the corridor to touch/tap the animals to move into the next sections.	0/1

Movement through the system	Walking	The animal walks calmly through the raceway.	0
	Running	The animal seems to be tense and trots from one section to another.	1
	Jumping	The animal is jumping and moving forwards and backwards	2
Vocalization	None		0
	Low		1
	Medium		2
	High		3
State in the squeeze chute	Calm	The animal remains calm while being measured, and biological samples are being collected.	0
	Nervous	The animal is tense, uneasy, and trying to lie down.	1
	Panicked	The animal is trying to jump or pushing its head/horns towards the handler.	2
Chute exit	Walking	The animal calmly walks out of the chute.	0
	Running	The animal trots off out of the chute.	1
	Jumping	The animal jumps out and trots off with high speed.	2

Table 3.3. Ethogram indicating the social interaction between common eland, adapted from Musa et al. (2024).

Categories	Behaviour	Description
Affiliative	Grooming	Grooming of one individual to another, or each other (in that case, the interaction is counted twice)
	Flehmen	Lip curl in response to urination by another individual
	Mounting	Mount another individual in a non-mating context
Dominant (not aggressive)	Passing	Sudden alertness/moving away when another animal is passing by
	Threatening	With head or horns on approaching, without contact with the threatened individual
Dominant (aggressive)	Pushing	Pushing another animal to displace it with any other part of the body other than the horns
	Horning	Pushing another animal to displace it with the horns
	Wrestling	Locking/clashing of horns with certain force and alacrity

Furthermore, biological samples are taken each month, including faecal sampling. Individual faecal samples were collected directly from the rectum of each animal. Samples were stored in Ziplock bags and transported using an icebox to the Laboratory of Animal Sciences at the Faculty of Tropical AgriSciences, CZU Prague. Collected faeces were freeze-dried using lyophilisation for 72 hours, pulverised, and sieved (1 mm sieve) to remove undigested material. All samples were labelled and sealed in Ziplock

bags, sorted (by month and year), and stored in a large box under room temperature conditions until further analysis. A total of 152 faecal samples from twenty-three eland bulls, collected between 10 to 24 months old for each individual, were analysed (**Chapter 6**). Samples were selected every second month, between 2020 and 2024.

In the autumn period of 2022, annual culling was done as part of the routine management practices to maintain appropriate herd size and sex ratio, as males are typically selected for breeding or culled at this age. A total of six eland males (2–2.5 years old; 203 ± 20 kg) were culled within the handling system, using a captive bolt followed by thoracic exsanguination, at the research facility. Eland males had unlimited access to water and hay for the 12 hours prior to slaughter. Biometric data was collected immediately before slaughter, including body weight (TW-1, Gallagher Group Ltd, Hamilton, New Zealand), body condition score, horn size (base circumference, length, twist), and neck circumference. The eland bulls were slaughtered at 30-minute intervals, and data collection was further divided into regular time intervals to manage all activities. The reproductive tracts were recovered immediately, to assess the reproductive tract morphology and its symmetry, as well as their relationships with secondary sexual traits and social rank (**Chapter 4**). The testes were immediately processed to assess the post-harvest motility and morphological changes of spermatozoa following caudal epididymal recovery (**Chapter 5**).

3.3. Data processing and statistical analysis

All data collected from the routine monthly handling and reproductive experiments were stored and processed in Excel, and statistical analyses were performed in IBM® SPSS® Statistics 29 (IBM, Armonk, New York) and R version 4.3.1 (R Core Team, 2024). Further details are provided in the subsequent Research Chapters 4, 5, and 6.

CHAPTER 4

Reproductive tract morphology and symmetry of farmed common eland (*Tragelaphus oryx*) bulls, and their relationship with secondary sexual traits and social rank

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Reproductive tract morphology and symmetry of farmed common eland (*Tragelaphus oryx*) bulls, and their relationship with secondary sexual traits and social rank

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4.1. Abstract

Common eland bulls are important game ranching animals in southern Africa, for tourism, breeding/live sales, trophy hunting, and game meat production. Interest has grown in their production potential, intensifying animal husbandry and breeding approaches. However, little scientific information is available regarding the intensive

management of this species, including information regarding scientifically based selection criteria for breeding bulls. Often, simple phenotypic traits are utilised unless high-value game animals are being bred, with horn and body size often being the only traits considered for breeding bulls. This study provides basic information about the reproductive tract morphology, symmetry, and their relationships with secondary sexual traits, social rank, and temperament in common eland. Six eland males (2–2.5 years old; 203 ± 20 kg) were utilised for the study. Social rank, body weight and body condition score, temperament score, and horn size were determined before the animals were culled, and their reproductive tracts were collected for morphometric and histological evaluation. Results indicate relatively low asymmetry in bilateral reproductive tract components. Individual traits of eland bulls such as age, body weight, and social rank correlated the development of some testicular morphologies and thus may possibly be used in zoos, game ranching, and commercial production for the selection of breeding males, but not temperament and horn length, which should be carefully evaluated. Whilst this study provides baseline information from a limited sample size, further studies should incorporate the assessment of testicular hemodynamics, as well as the effects of factors such as season and androgen secretion patterns over a wider age range of common eland males and populations to provide further information for the management practices of breeding common eland bulls.

Keywords: *Alternative farming species, breeding selection, game ranching, temperament, testes, testicular morphology*

4.2. Introduction

The wildlife ranching industry is a significant economic contributor in southern Africa, supporting more than 17,000 employment opportunities (almost twice that of livestock-only farms in the same regions) (Saayman et al., 2018) improving local food security, and contributing towards conservation (Bachmann et al., 2016). This economic potential is mostly attributed to trophy hunting and game breeding and live sales (Taylor et al., 2020) with a total annual revenue of around ZAR 13.6 billion (US 7.3 billion) and ZAR 10 billion (US 5.4 billion), respectively (Cloete et al., 2015). Such lucrative practices have motivated game ranchers/farmers to implement practices from the livestock sector and seek more precise approaches to breeding management to improve production and profits (Khumalo, 2021).

The application of intensive and selective breeding has been increasingly integrated into game ranching practices to produce superior animals for live game sales and trophy hunting (Taylor et al., 2020). This is particularly true for high-value species that are intensively supported with pedigree records and often genetic data, such as the African buffalo (*Syncerus caffer*), sable antelope (*Hippotragus niger*), roan antelope (*Hippotragus equinus*), as well as colour variants, such as black impala (*Aepyceros melampus*) and golden wildebeest (*Connochaetes taurinus*) (Taylor et al., 2020). However, breeding other hunted game species still primarily focuses on phenotypic traits. Ranchers often base their criteria for selection for breeding male animals (usually for trophy hunting) on morphological measurements related to early development/reaching of physical maturity such as larger body size, bigger horns, testicular size and conformation, and the timing of the onset of puberty (Khumalo, 2021). In

cattle, for example, scrotal circumference (SC) has been used as a phenotypic trait in breeding management, which has high heritability, and moderate correlations with body weight (0.2968–0.5485) (Oliveira et al., 2021). However, there is little scientific information regarding the relationships between these parameters for African game species like the common eland, which is important when indirect phenotypic measurements are utilised for breeding selection. Furthermore, baseline information and characterisation of the reproductive morphology and development (including the symmetry thereof) of many African game species is missing but is essential for understanding the impacts of other environmental factors (such as climate or managerial) on reproductive performance. The principle of symmetry among the bilateral or paired reproductive structures also deserves to be considered in such context since symmetry is known to be an honest phenotypic signal of the genetic quality of an animal (Moubayidin and Ostergaard, 2015).

The common eland (*Tragelaphus oryx*) is one of the most widely distributed and of the most popular game species in southern Africa for trophy hunting and meat production. Its population was previously estimated at 136,000 and can be found both in protected areas (50 %) and private lands (30 %) of South Africa (Furstenburg, 2016). Moreover, it is widely distributed internationally, both in zoological gardens and private breeding farms. Due to its adaptability (Pappas, 2002), temperament (Musa et al., 2021), and production potential (*i.e.*, high carcass meat yields, high-quality and healthy meat) (Barton et al., 2014; Needham et al., 2022) the common eland has been recommended for domestication as an alternative species (compared to cattle) for meat production in regions of poor vegetation and low water availability. In Europe, common

eland have been used for production purposes as early as 1892, and are farmed for meat and milk production, albeit at a small scale (Treus & Kravchenko, 1968; Treus & Lobanov, 1971).

However, there is little information about the breeding management of common eland bulls, either in wild/extensive or captive conditions. Common eland can reproduce throughout the year under favourable conditions, but a lower sex ratio (the typical “wild” population ratio is 1 male: 8–12 females) is recommended to ensure a favourable conception rate during some periods of the year when nutrition may be limiting (Skinner et al., 1971; Furstenburg, 2016). Whilst sexual maturity is said to occur around 4–5 years old in the wild (Posselt, 1963), a preliminary study in captive eland found an overall increase in efficiency of spermatogenesis and Sertoli cell functionality of the common eland bulls examined in this study were at around 15 months old, which suggests an early onset of puberty in captivity (Ros-Santaella et al., 2014) where nutrition and other environmental factors are optimal. Under current game ranching conditions in southern Africa, individual animal records are lacking, which not only complicates more directional management but also the collection of scientific data, where confounding factors cannot be controlled.

Therefore, the aim of this study was to provide basic information of common eland bulls’ reproductive morphology, symmetry, and their relationships with a range of animal characteristics, including secondary sexual traits, social rank, and temperament which are often used as markers in selecting bulls for breeding. Common eland serve as a model species for such parameters, information of which may contribute to the continuous conservation efforts of endangered antelope species, such as the Derby

eland (*Tragelaphus derbianus*), and provide baseline information for the application of advanced reproductive technologies and breeding management of this species.

4.3. Materials and methods

4.3.1. Animals, live, and post-mortem data collection

This research study was conducted at the Common Eland Research Facilities of the Czech University of Life Sciences Prague (CZU) in Lány, Czech Republic (50 ° 7'41" N, 1357'31" E). The facilities are accredited by the Czech Republic Ministry of Agriculture (Permit: 63479_2016-MZE-17214). The facilities maintain up to 50 individuals for research and teaching purposes. The research facility utilises common eland as a model antelope species, and as an alternative farming species with climate resilient traits. The overall aims are to understand the physiological plasticity of the species, their husbandry and welfare requirements under captive conditions, and to serve as a validation herd for other studies under extensive (or otherwise varying) conditions. Furthermore, their production potential is also considered. Whilst sample size of animals available for slaughter at a given timepoint is somewhat limited, it offers a unique environment compared to what is currently available under large extensive herds in southern Africa, as each animal has pedigree information and monthly individual records from birth until it exits the facilities. All environmental and husbandry conditions are further standardised. Experimental procedures were approved by the Czech University of Life Sciences Prague Animal Welfare and Clearance Committee (Permit: CZU 20/19). Further details

of the routine practices regarding animal husbandry, nutrition, handling, and slaughter procedures are reported in Needham et al. (2020) and Musa et al. (2021). Routine handling is performed each month at the research facilities, and data collection includes body weight and body condition scoring (BCS) based on the palpation method developed by Audigé et al. (1998) for red deer and adapted to common eland (Dearman, 2023), horn size (length, twisted length, and base circumference), temperament score (TS, based on the behavioural response during handling; Musa et al., 2021), and social rank, based on routine monitoring of agonistic interactions and calculated through the inconsistencies and strength of inconsistencies method (I&SI) (Schmid and de Vries, 2013).

Six eland males (2–2.5 years old; 203 ± 20 kg) were culled within the handling system, using a captive bolt followed by thoracic exsanguination, at the research facilities. The facilities maintain the permission to slaughter on-site under the supervision of a state veterinarian (SVS/2018/100483-S), according to the amended Czech Veterinary Care Act No. 166/1999. The selection of the animals followed routine management practices to maintain appropriate herd size and sex ratios, as males are typically selected for breeding or culled at this age. Eland males had unlimited access to water and hay for the 12 hours prior to slaughter. Biometric data was collected immediately before slaughtering, including body weight (TW-1, Gallagher Group Ltd, Hamilton, New Zealand), body condition score, horn size (base circumference, length, twist), and neck circumference. All this baseline information regarding individual characteristics (age, body weight, BCS, TS, horn sizes, neck circumference, and social rank) were used for correlation analyses with the studied reproductive parameters.

4.3.2. Reproductive tract morphological measurements

After the exsanguination and complete bleeding of each animal, the length of the penis sheath was measured with a flexible tape measure, from the tip of the prepuce until the end of the sheath where it disappears within the inguinal area. Then, a sterile scalpel was used to remove the entire scrotum and testes by making a circumferential incision around the dorsal neck of the scrotum at the level of the abdominal wall. The testes and scrotum were then weighed (scrotum + testes). The testes were then removed from the scrotum, and the epididymis was removed together with other tissues before weighing (testes weight; Kern and Sohn GmbH kb 2000–2 N; D-72336, Balingen, Germany) and size measurement (testes length and width) with a digital calliper (Z22855, Milomex Ltd, United Kingdom). The gonadosomatic index was calculated from the mass of both testes divided by body weight and multiplied by 100. Asymmetry of the bilateral structures was calculated using the formula below:

$$\% \text{ Asymmetry} = \frac{\text{Absolute value (Left-Right)}}{\text{Average (Left+Right)}}$$

After the morphometric measurements, the testes were cross-sectioned (*i.e.*, they were cut in half, through the center of each testis, cutting along the latitudinal axis perpendicular to the longitudinal axis) for CIELab surface colour assessment (Needham et al., 2019; Lealiifano et al., 2011) using a calibrated colourimeter (CM-2500D, Minolta, Osaka, Japan; aperture size of 8 mm including the specular component and 0 % UV; illuminant/observer of D65/10°; zero and white calibration). Variations in testicular colour

may indicate differences in activity, blood flow, and functioning. Three measurements per sample were taken at three different positions on the cut surface of each testis. Results are reported according to the coordinates of the CIELAB colorimetric space, L^* (lightness), a^* (redness), and b^* (yellowness). The L^* values (or coordinates) in the CIELAB colour space range from 0 (black) to 100 (white), whilst the a^* axis ranges from green (negative values) to magenta (positive values), and the b^* axis ranges from blue to yellow (negative values towards blue and positive values towards yellow).

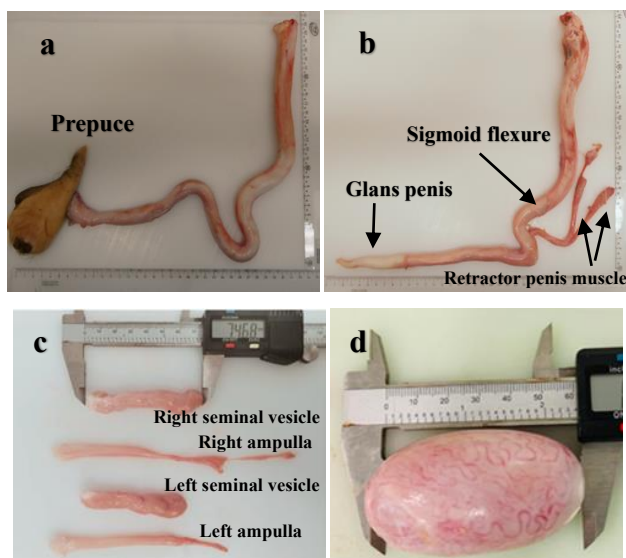


Figure 4.1 Images of the (a) prepuce + penis (glans + sheath) +sigmoid flexure, (b) penis (glans to root of penis), (c) accessory glands, and (d) testis of young eland (*Tragelaphus oryx*) bulls.

The remainder of the reproductive tract was dissected from the pelvis during processing at the abattoir of the Institute of Animal Science, Prague, Czech Republic, after approximately 1 h of transportation in a refrigerated truck (4 °C). The accessory glands were dissected free from the penis, and the section from the prepuce to the end of the sigmoid flexure (at the root of the penis) was further dissected. The length of the prepuce, penis sheath, and sigmoid flexure were measured (prepuce + penis + sigmoid flexure length; Fig. 4a) and then weighed (Kern and Sohn GmbH kb 2000-2 N; D-72336, Balingen, Germany). The lengths of the accessory glands were measured using the calliper (Figure 4.1c) and then they were weighed.

4.3.3. Histological analyses

Tissue samples from the mid-section of each testis, ampulla, seminal vesicle, prostate gland, and bulbourethral gland were taken and preserved in 10 % buffered formaldehyde in preparation for histological assessment (Bai et al., 2017). Subsequently, fixed tissue samples were processed, embedded in paraffin wax, sectioned at a thickness of 4 µm, and stained with hematoxylin and eosin.

Images of the stained tissue samples were captured using 0.28 µm/px (2560 × 1920 pixels; NIS Element Software and 40× magnification, Nikon Eclipse E600, Japan). 100 seminiferous tubules per testis were measured for tubule circumference and epithelium thickness using NIS-Elements AR 3.2 (Nikon Instruments Europe B.V., Amsterdam, Netherlands). Histological assessment was conducted to assess the ampulla, seminal vesicle, prostate gland, and bulbourethral gland.

The following variables were collected from the reproductive tract morphologies and histological measurements of common eland bulls: penis sheath length (PL); penis+prepuce+sigmoid flexure weight (P+Pr+SFW); penis+prepuce+sigmoid flexure length (P+Pr+SFL); ampulla weight (AW); ampulla length (AL); seminal vesicle weight (SVW); seminal vesicle length (SVL); prostate weight (PrW); prostate length (PrL); bulbourethral gland weight (BuW); scrotal weight (SW); testes weight (TW); testes length (TL); testes width (TWID); gonadosomatic index (GI); seminiferous tubule thickness (STT); seminiferous tubule circumference (STC); epididymis weight (EW); epididymis length (EL); lightness (L^*); redness (a^*); and yellowness (b^*).

4.3.4. Statistical analyses

All the statistical analyses were performed in IBM SPSS v. 29.0. The normality of distribution of the data was confirmed using the Shapiro-Wilk test. Paired-student t-test was used to discard differences between the left and right structures. Thus, the average between both measurements was used in further analyses. Descriptive statistics (mean, standard deviation, and coefficient of variation) were used for the characterisation of the reproductive tract. Pearson correlations were calculated to understand the associations among the studied reproductive tract morphology variables, as well as among these variables and the characteristics of the animals (age, TS, weight, neck, horns, body condition, and social rank). Heatmaps illustrating the correlations of the studied variables were generated using the *ggplot* package in R (R Core Team, 2022).

Table 4.1. Morphological characterisation of the reproductive tract of six farmed common eland (*Tragelaphus oryx*) bulls, and the differences between the left and right portions.

Reproductive tract morphology	n	Mean $\pm$ SD	c.v. %	L-R comparison
Penis sheath length (cm) ¹	6	27.92 $\pm$ 3.11	11.1	
Prepuce + penis + sigmoid flexure weight (g)	6	150.92 $\pm$ 28.01	18.6	
Prepuce + penis+ sigmoid flexure length (cm) ²	6	53.67 $\pm$ 9.60	17.9	
Ampulla weight (g)	6	3.46 $\pm$ 1.19	34.5	t=0.104; p=0.921
Ampulla length (mm)	6	86.57 $\pm$ 25.65	29.6	t=-0.450; p=0.672
Seminal vesicle weight (g)	6	6.49 $\pm$ 1.71	26.4	t=0.607; p=0.570
Seminal vesicle length (mm)	6	50.09 $\pm$ 14.51	25.0	t=0.887; p=0.715
Prostate gland weight (g)	4	0.53 $\pm$ 0.25	47.6	
Prostate gland length (mm)	4	56.21 $\pm$ 41.15	73.2	
Bulbourethral gland weight (g)	6	2.43 $\pm$ 2.55	104.9	
Scrotal weight (g)	6	210.98 $\pm$ 34.77	16.5	
Testes weight (g)	6	42.67 $\pm$ 5.37	12.6	t=1.284; p=0.255
Testes length (mm)	6	55.23 $\pm$ 5.03	9.1	t=1.590; p=0.173

Testes width (mm)	6	33.43 ± 2.17	6.5	t=-0.844; p=0.417
Gonadosomatic index (%)	6	0.072 ± 0.01	18.1	
Seminiferous tubules thickness (µm)	6	32.43 ± 3.25	10.0	t=1.485; p=0.188
Seminiferous tubules circumference (µm)	6	325.76 ± 26.58	8.2	t=1.105; p=0.311
Epididymis weight (g)	6	10.01 ± 1.47	14.7	t=0.237; p=0.822
Epididymis length (mm)	6	108.33 ± 8.99	8.3	t=0.237; p=0.822
<i>Testes colour</i>				
<i>L</i> *	6	65.34 ± 1.35	2.1	t=0.630; p=0.556
<i>a</i> *	6	2.55 ± 0.68	26.6	t=0.938; p=0.391
<i>b</i> *	6	13.18 ± 0.75	5.7	t=0.047; p=0.964

Legends for Table 4.1: L-left; R-right; c.v. % = coefficient of variation percentage, ¹from the tip of the prepuce until the end of the sheath where it disappears within the inguinal area, ²from the tip of the prepuce to the root of the penis

4.4. Results

The biometric data collected immediately before slaughtering (mean ± SD) is as follows for the six animals; body weight: 203 ± 20 kg, body condition score: 3.17 ± 0.16, horn base circumference: 23.43 ± 2.63 cm, length: 52.63 ± 1.00 cm, and twist: 64.54 ± 0.53 cm, and neck circumference: 60.50 ± 3.45 cm. No gross abnormalities

were observed/palpated and the reproductive tract's appearance is similar to that of domestic bovines. No abnormalities were found during the microscopic morphology of all the analysed tissues (testes, ampulla, seminal vesicles, prostate, and bulbourethral glands) from the histological evaluations (examples from one animal are shown in Figure 4.2). A description of the reproductive tract morphology of the young common eland bulls is shown in Table 4.1. Student paired-T-test analyses indicated that the morphology of all the bilateral reproductive organs was not statistically different ($P > 0.05$). Such uniformity between left and right tracts of the reproductive morphology was further supported by the relatively low asymmetry observed in the bilateral structures, except for the redness (a^*) of the testicular tissue samples, which was 33.7 % (Table 4.2).

Numerous correlations were found for the morphology of the reproductive tract of the common eland bulls (Figure 4.3). Regarding the testicular parameters, the scrotum+testes weights from eland bulls were positively correlated with trimmed testes weights (SW vs TW; $r = 0.853$; $P = 0.031$) and seminal vesicle weights (SW vs SVL; $r = 0.872$; $p = 0.024$). Testes weight was also positively correlated with testes length and width (TW vs TL; $r = 0.841$; $P = 0.036$; TW vs TWID; $r = 0.838$; $P = 0.037$). A significant positive correlation between testes length and the length of ampulla was found (TL vs AL; $r = 0.837$; $P = 0.038$). However, testes length was negatively correlated with bulbourethral gland weight (TL vs BuW; $r = -0.848$; $P = 0.033$). The epididymides weight was positively correlated to both seminal vesicle (EW vs SVW; $r = 0.819$; $P = 0.046$) and ampulla (EW vs AW; $r = 0.859$; $P = 0.028$) weights.

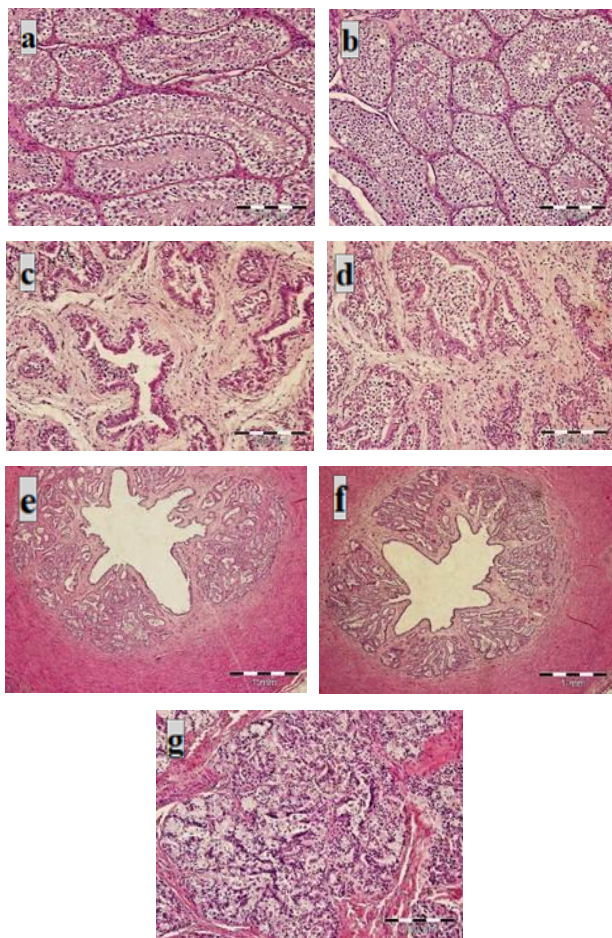


Figure 4. 2. Tissue cross-sections with H & E staining of the right (a) and left (b) testes (200 × magnification), right (c) and left (d) seminal vesicles (200 × magnification), right (e) and left (f) ampulla (40 × magnification), bulbourethral gland (g) (200 × magnification) from the same farmed common eland (*Tragelaphus oryx*) bull.

Table 4.2. Asymmetry (%) between the left and right portions in the morphology of the reproductive tract of six farmed common eland (*Tragelaphus oryx*) bulls.

Reproductive Tract	n	Asymmetry %
Ampulla weight	6	8.8
Ampulla length	6	8.7
Seminal vesicle weight	6	16.4
Seminal vesicle length	6	10.7
Testes weight	6	5.2
Testes length	6	4.2
Testes width	6	8.4
Seminiferous tubules thickness	6	6.9
Seminiferous tubules circumference	6	5.4
Epididymis weight	6	10.7
Epididymis length	6	12.3
<i>Testes colour</i>		
<i>L*</i>	6	2.7
<i>a*</i>	6	33.7
<i>b*</i>	6	6.0

In terms of testicular colour, the testes width was strongly associated with the degree of testes redness cut surface colour (TWID vs *a**; $r = 0.934$; $P = 0.006$). Testes redness (*a**) was significantly and positively correlated with the thickness of the seminiferous tubule (*a** vs STT; $r = 0.818$; $P = 0.047$). The degree of testes yellowness was negatively correlated with seminiferous tubule circumferences (STC vs *b**; $r = -0.855$; $P = 0.030$). Whilst both seminiferous tubule thickness and circumference

were positively correlated with each other (STT vs STC; $r = 0.818$; $P = 0.047$).

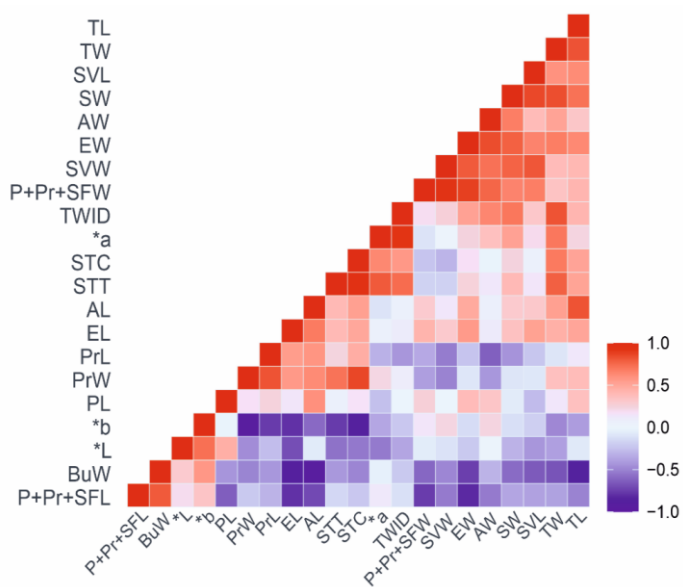


Figure 4.3. Heat map illustrating the Pearson correlations among the reproductive tract morphological characteristics of six common eland (*Tragelaphus oryx*) bulls. Red to purple colouration of the boxes indicates negative and positive correlations (r values between 1 to -1) with the following variables: Penis sheath length (PL); Prepuce+Penis+Sigmoid flexure weight (P+Pr+SFW); Prepuce+Penis+Sigmoid flexure length (P+Pr+SFL); Ampulla weight (AW); Ampulla length (AL); Seminal vesicle weight (SVW); Seminal vesicle length (SVL); Prostate weight (PrW); Prostate length (PrL); bulbourethral gland weight (BuW); Scrotal weight (SW); Testes weight (TW); Testes length (TL); Testes width (TWID); Seminiferous tubule thickness (STT); Seminiferous tubule circumference (STC); Epididymis weight (EW); Epididymis length (EL); Lightness (L^*); redness (a^*); and yellowness (b^*).

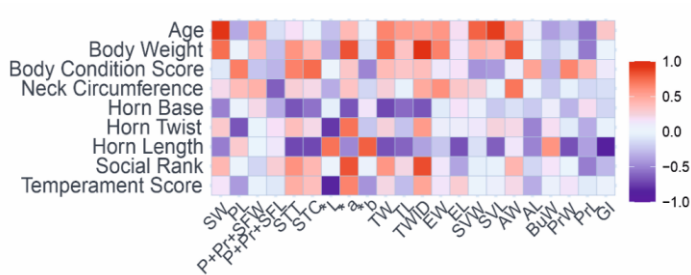


Figure 4.4. Heat map illustrating the Pearson correlations among the reproductive tract morphology of six common eland (*Tragelaphus oryx*) bulls and different animal characteristics. Red to purple colouration of the boxes indicates negative and positive correlations (r values between 1 to -1) with the following variables: Penis sheath length (PL); Prepuce+ Penis+Sigmoid flexure weight (P+Pr+SFW); Prepuce+ Penis+Sigmoid flexure length (P+Pr+SFL); Ampulla weight (AW); Ampulla length (AL); Seminal vesicle weight (SVW); Seminal vesicle length (SVL); Prostate weight (PrW); Prostate length (PrL); bulbourethral gland weight (BuW); Scrotal weight (SW); Testes length (TW); Testes length (TL); Testes width (TWID); Gonadosomatic index (GI); Seminiferous tubule thickness (STT); Seminiferous tubule circumference (STC); Epididymis weight (EW); Epididymis length (EL); Lightness (L^*); redness (a^*); and yellowness (b^*).

Regarding the remaining reproductive tract and accessory glands, the combined weights of the prepuce+penis+sigmoid flexure were positively correlated with the epididymides weight (P+Pr+SFW vs EW; $r = 0.899$; $P = 0.015$), seminal vesicle weight (P+Pr+SFW vs SVW; $r = 0.945$; $P = 0.004$), and bulbourethral gland weight (P+Pr+SFL vs CW; $r = 0.817$; $P = 0.047$). The bulbourethral gland weight was negatively correlated with the epididymides weight (BuW vs EW; $r = -0.865$; $P = 0.026$) and ampulla weight (BuW vs AW; $r = -0.911$; $P = 0.012$). Both the length and weight of the seminal vesicles were significantly positively correlated with each other (SVL vs SVW; $r = 0.825$; $P = 0.043$).

Moreover, individual animal characteristics showed several correlations with some of the reproductive tract variables (Figure 4.4). The age of the eland bulls was strongly correlated with the combined weights of the scrotum and testes (Age vs SW; $r = 0.931$; $P = 0.007$) and seminal vesicle length (Age vs SVL; $r = 0.941$; $P = 0.011$). Also, body weight was noted to have a strong correlation with testes width (Body weight vs TWID; $r = 0.959$; $P = 0.002$) and the degree of testes redness (Body weight vs a^* ; $r = 0.842$; $P = 0.036$), and ampulla weights (Body weight vs AW; $r = 0.817$; $P = 0.047$). The social rank of the eland bulls was positively correlated with the degree of testes redness (Social rank vs a^* ; $r = 0.838$; $P = 0.037$) and testes width (Social rank vs TWID; $r = 0.861$; $P = 0.028$). The temperament score of the animals was negatively correlated with the degree of testes lightness (TS vs L^* ; $r = -0.829$; $P = 0.041$). A negative correlation exists between horn length and gonadosomatic index (Horn length vs GI; $r = -0.872$; $P = 0.023$).

4.5. Discussion

This study aimed to provide baseline information regarding the reproductive morphology and symmetry of common eland bulls, and their relationships with secondary sexual traits, social rank, and temperament. The reproductive morpho-functional characteristics of the common eland bulls are categorically similar to those of bovines (cattle) due to the phylogenetic proximity of their taxa (Tonizza de Carvalho et al., 2014). The reproductive tract (Figure 4.1) of common eland bulls are made up of the scrotal sac, testes, epididymis, spermatic ducts, penis, preputial sheaths, and sigmoid flexures. Also, the ampulla and accessory sex glands (seminal vesicles, prostate, and

Cowper's glands; histomicrographs are shown in Fig. 4.2) are found within the abdominal cavity and pelvis.

Despite domestication efforts for common eland, there are little to no detailed bull selection criteria for breeding purposes based on scientific data. The basic criteria for selecting breeding males include the evaluation of reproductive organs such as their position, shape, dimension, and symmetry (Tonizza de Carvalho et al., 2014). Such selection protocols for breeding males are well-established in, *e.g.*, cattle, buffalo, and small ruminants. Thus, this study was conducted to describe the reproductive morphology of young eland bulls ready for selection and identify possible indicators that could be utilised, as well as their relationships with other important managerial factors, such as social rank and temperament, to support the integration of informed breeding practices into wildlife management, game ranching, zoos, and commercial production enterprises.

Generally, the available literature provides suggestions regarding the age and sex ratios of common eland for breeding management (Furstenburg, 2016), but as common eland are popular for trophy hunting, horn size and conformation, as well as body size are important parameters determining their economic value. Thus, the selection of breeding males has focused on this as a major selection criterion, but under more intensive breeding systems like on farms, early physical development and sexual maturity together with a calm temperament can be of high importance as well. Some data exists regarding the testes and accessory glands morphology of common eland (Table 4.3); however, the age range of the animals used in this previous study was between 3 and 10 years old, and thus, it reports a wide variation in these parameters (Skinner et al., 1971). Furthermore, other confounding

factors, such as nutritional level, are impossible to control under the current extensive game ranching practices. Although there is a limited sample size, under the uniform conditions of the CZU Common Eland Research Facilities and the narrow age range of the selected animals (24 – 29 months old), it is possible to see a more accurate representation of baseline reproductive data for bulls at the point of scrutiny for any breeding selection. According to a preliminary study conducted at the same research facilities (Ros-Santaella et al., 2014), the common eland males examined in this study were likely in the reproductive developmental phase between puberty and sexual maturity, which is supported by the comparative data of Skinner et al. (1971) who reports a greater weight and size range of the testes and accessory glands parameters. The testes weights (without scrotum but not trimmed of other tissues) are comparatively smaller, even at the lower range of the data from Skinner et al. (1971) (differences in dissection methods can also impact this). However, the upper ranges of the data for the other parameters (seminiferous tubule diameter, seminal vesicles weight, and bulbourethral gland weight) of the common eland in the present study are similar to the lower data ranges reported by Skinner et al. (1971) likely from the 3-year-old bulls.

No differences were found among the bilateral or paired reproductive tracts (comparable results were noted in several farmed animal species; (Pathak et al., 2014)), together with a relatively low asymmetry between left and right structures. Fluctuating asymmetry is a random and minor deviation from perfect symmetry in paired traits, commonly used as an indication of the stability of an organism from the effects of genetic and environmental perturbations during ontogeny (Benderlioglu et al., 2010). A relatively high asymmetry in a^* (redness) of the testicular

tissues was observed. Redness positively correlated with the thickness and circumference of the seminiferous tubules and testes width, body weight and social rank; and the highest asymmetry values were observed in the animals of 2 years of age ($n = 3$), while it was low for those of 2.5 years old. Thus, this asymmetry seems greater in still-developing animals with higher variability in the intensity of testicular blood flow (Auclair, 1993), which increases during pubertal development (Joffre, 1973) or may just reflect asymmetrical maturation.

The correlations between the reproductive tract parameters were as expected. Prepuce+penis+sigmoid flexure and epididymides weights, as well as testes length, were positively correlated with the weights and lengths of the ampulla, seminal vesicles, and bulbourethral gland. This finding is expected to cater for the capacity of the different cell-secreting activities and fluidity (with essential nutrients) for the motility and fertilising ability of the spermatozoa (Eissa, 1980). Some exceptions existed for the bulbourethral gland, which was generally negatively correlated with epididymides and ampulla weights. This is likely due to the primary functions of the bulbourethral gland, which is directly responsible for the secretion of glycoproteins during sexual stimulation and serves as a lubricant for the semen (Tonizza de Carvalho et al., 2014). Also, it secretes mucus-like fluid that neutralises the acidity of the urine residue in the urethra as well as in the vagina and provides lubrication in the tip of the penis during copulation (Chughtai et al., 2005). The current results also showed strong and positive correlations of testes width and the degree of testes redness with the thickness of the seminiferous tubules. On the contrary, negative correlations were found between the degree of testes yellowness and seminiferous tubule circumferences in this

study. This may indicate changes in testicular hemodynamics and spermatogenesis and thus reproductive functionality (De Girolamo et al., 2003; Sertoli, 2018). The relationships between instrumental testes colour and changes in testicular hemodynamics as an indication of testes functioning requires further examination, particularly in a larger sample size. Investigation into the heritability of these traits may provide further information regarding the possible utilization of these traits for breeding selection, as biometric traits of the testis in cattle are considerably heritable such as scrotal circumference (0.18–0.81) (Paneto et al., 2002), length of both testes (0.37) and their width (0.31-right; 0.28-left) (Silveira et al., 2012).

Table 4. 3. Summary of the reproductive morphology of common eland (*Tragelaphus oryx*) bulls from a previous study of Skinner et al. (1971) vs the present data set from six eland bulls.

Parameters	Skinner et al. (1971)	Current Data
Liveweight (kg)	294 - 496	184 - 239
Approximate age (years)	3 - 10	2.0 - 2.5
Testes weight (g)	150.7 - 288.0	35.5 - 48.8
Seminiferous tubule diameter (μm)	159.2 - 204.9	88.2 - 126.1
Epididymis weight (g)	26.2 - 46.4	7.8 - 11.2
Seminal vesicles weight (g)	11.7 - 33.4	4.1 - 8.6
Ampulla weight (g)	10.5 - 21.9	2.2 - 5.3
Bulbourethral gland weight (g)	2.7 - 15.6	0.4 - 7.2

Secondary sexual traits are also considered in this study as important determinants of reproductive success and the basis of any changes in the reproductive behaviour of common eland bulls (Emlen, 2008). It includes age, body weight, horn sizes and the effects of social rank of the bulls within the herd. Most of the literature suggested direct effects of age and body weight on the reproductive tract development (Mysterud et al., 2001), which are known to have medium to high positive genetic correlations with one another in other species (Dias et al., 2008). A similar pattern is observed in the present study under controlled conditions (same nutrition, handling, and husbandry practices): positive correlations existed for body weight and social ranking with testes weight, width, and redness, as would be expected. On the other hand, temperament had negative correlations with testes lightness. This is an interesting result since a calm temperament is a trait commonly sought during breeding selection (Chang et al., 2020). Moreover, it suggests that social-hierarchy structures were commonly observed with dominant and subordinate male structures within the eland herd and considerably suppressing testosterone levels as well as sperm quality traits (Bisette & Bernard, 2005). Such reproductive phenomena exist in African buffalo and greater kudu (*Tragelaphus strepsiceros*) (Brown et al., 1991; Schiewe et al., 1991). However, this aspect should be verified with the seasonal and sequential monitoring of hormonal (*e.g.* androgens and testosterone) patterns of eland bulls. Surprisingly, negative correlations were found between horn length and the gonadosomatic index. Horn size is a key factor during intra-sexual conflicts (Robinson et al., 2008), and early horn growth is considered an honest signal of reproductive quality (Willisch et al., 2015), but the current dataset shows the opposite trend. The results thus suggest that different investment strategies in pubertal

individuals may exist towards the development of the horns or the maturation of the reproductive organs, but this should be further studied with a higher sample size and a wider range of ages, and in different eland populations.

4.6. Conclusion

This study supports that age, body weight, and social rank influence the reproductive tract morphological development of common eland bulls may be used as possible selection criteria, but temperament and horn length should be carefully considered when selecting breeding bulls. Such data should be validated within larger populations of common eland. Further exploration into the heritability and possibility of utilising these phenotypic traits as selection criteria for breeding males should be considered. Further studies focusing on the relationships between these traits and seasonal effects, androgen secretion patterns, testicular hemodynamics, and spermatozoa production should also be considered to provide further information for the management practices of common eland bulls which will benefit breeders.

CHAPTER 5

Post-harvest motility and morphological changes of spermatozoa following caudal epididymal recovery in farmed common eland (*Tragelaphus oryx*) bulls

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Post-harvest motility and morphological changes of spermatozoa following caudal epididymal recovery in farmed common eland (*Tragelaphus oryx*) bulls

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5.1. Abstract

The caudal epididymal recovery of spermatozoa has been utilised in game animal species to preserve genetically superior material after trophy hunting or slaughter, due to the difficulties of handling wild animals. Furthermore, the potential application of assisted

reproductive techniques using harvested spermatozoa may contribute towards maintaining genetic diversity in isolated captive populations internationally. However, this technique requires clear and ideally simple protocols for the collection and handling of gametes after the death of the male animals, as the point of harvest is usually remote in game ranching. Therefore, this research aimed to investigate the effects of time after harvesting sperm under basic field conditions from the caudal epididymides of farmed common eland bulls in terms of their sperm motility and morphological changes. The relationship among these sperm quality parameters was also explored. Six bulls (2-2.5 years old; 203 ± 20 kg) were slaughtered, and their epididymal sperm were harvested and assessed for sperm motility and kinematics at minutes 0, 35, 70, 100, together with sperm viability, sperm head morphometry, and morphology at minutes 0, 20, 40, 60, 80 using CASA. Sperm quality sharply declined after 35 minutes of slaughter, but no effects were seen in sperm head morphometry. This study brings the first information regarding the quality of sperm retrieval from the cauda epididymides and sperm quality under physiological conditions in a uniform age group of eland. Future work should consider the effect of animal age and other individual animal traits, as well as the use of various media and extenders.

Keywords: *Antelope, caudal epididymides, computer assisted semen analyser, post-harvest time, sperm quality*

5.2. Introduction

Maintaining the genetic diversity of wildlife (game) animal species is crucial for adaptation (Reed and Frankham, 2003; Frankham, 2005) evolutionary potential, and conservation (Cousins et al., 2010) given that with higher rates of gene flow insufficiency, inbreeding and genetic drift are inevitable (Grobler et al., 1996) particularly in small and fragmented populations like zoos, private farms, or commercial breeding farms located far from the origin of the species, where access to new genetics is limited (Grobler et al., 2018). Assisted reproductive technologies (ARTs) have been utilised to maintain genetic diversity within small and isolated populations of animal species when natural breeding is not possible, with varying degrees of success (Monfort, 2014; Asa, 2016). Furthermore, ARTs eliminate the need for animal transportation between zoos or farms for breeding purposes and help prevent the spread of animal diseases (Wildt, 2003; Swanson, 2006; Roth & Swanson, 2018). Whilst the collection of semen from live animals is preferred for ARTs, the harvesting of spermatozoa from the caudal epididymides post-mortem is practised when such collection is not feasible or when a male animal of high value or genetic potential dies, allowing their genetic material to be preserved (Kaabi et al., 2003). Whilst this proves highly valuable for species under threat of extinction, this technique also supports wildlife biodiversity and game farming/ranching opportunities (Butler et al., 2005; Galarza et al., 2017; Russo et al., 2019). In southern Africa, most game ranching areas are remotely located, and animal movement is expensive and complicated, especially in areas with infectious disease risks (*i.e.*, red zones). Culling is commonly practised to maintain animal numbers, which presents an opportunity

to collect and store genetic material. However, the post-mortem collection of genetic material from such animals needs to be done remotely in the field, usually under hot environmental conditions in southern Africa, with limited or no access to laboratory equipment and materials (or even electricity), necessitating the development of simple but effective field techniques.

The currently available published data on sperm retrieval in common eland involves a limited sample size per uniform age groups (*e.g.*, one animal, using electroejaculation; (Merilan et al., 1978)), and harvesting and processing usually took place after an extended period post-mortem in the case of retrieval from the cauda epididymides, *e.g.*, after four hours of slaughter, with sperm quality changes evident due to cold temperatures (Bissett & Bernard, 2005). Established protocols from other bovine and domesticated bull species have demonstrated that samples collected immediately after slaughter and stored at 4 to 5°C yield viable sperm cells capable of surviving up to 24 hours post-harvest (Vieira et al., 2013). Thus, sperm retrieval from caudal epididymides of eland bulls with genetic interest requires immediate collection and processing, which is difficult to achieve in field conditions, especially when cooling is not an option. Consequently, it is essential to investigate the effect of timing of sperm retrieval that may still yield material with adequate sperm quality parameters for cryopreservation under highly simplified conditions.

Therefore, the aim of this research was to investigate the effects of post-harvest timing on the quality of sperm retrieved from the caudal epididymides of farmed common eland bulls after slaughter, under basic field conditions, in terms of motility and morphological changes. The relationship of these sperm quality parameters was

also explored, as this is yet to be described in eland and may provide a better understanding of their sperm functionality and fertility potential as the basis for developing adequate handling and semen processing techniques as well as guiding the field collection of such samples and suitable application of semen extenders.

5.3. Materials and Methods

5.3.1. Animals and slaughtering

Six common eland males (2-2.5 years old; 203 ± 20 kg) were slaughtered during routine herd management at the Common Eland Research Facilities of the Czech University of Life Sciences Prague (CZU) in Lány, Czech Republic ($50^{\circ}7'41''$ N, $13^{\circ}57'31''$ E) under the supervision of a state veterinarian (SVS/2018/100483-S), and according to the amended Czech Veterinary Care Act No. 166/1999. The animals were slaughtered during the autumn period of 2022. The herd reproduces all-year round, through all seasons. The accredited facilities, experiments, and procedures were authorised by the Czech Republic Ministry of Agriculture (Permit: 63479_2016-MZE-17214) and further approved by the Czech University of Life Sciences Prague Animal Welfare and Clearance Committee (Permit: CZU 20/19). The description of the research facilities and other standardised management practices, *e.g.*, animal husbandry, nutrition, slaughtering, and recording of the growth and other animal traits, are further described in Needham et al. (2020), Musa et al. (2021; 2024), and Consolacion et al. (2024).

Prior to slaughtering within the handling system, the animals were provided unlimited access to water and

feed, and immediately before slaughter, live body weights were recorded using a TW-1 Gallagher weighing scale (Gallagher Group Ltd, Hamilton, New Zealand). The eland bulls were slaughtered at 30-minute intervals, and data collection was further divided into regular time intervals to manage all activities. Detailed information regarding the reproductive tract morphology of these animals and its relationship with social rank, temperament, body and horn size of these animals are provided by Consolacion et al. (2024).

5.3.2. Spermatozoa harvesting and sampling conditions

Immediately after exsanguination and confirmation of death, the scrotum with testes was removed from the animal, and the epididymides were immediately dissected from the testes and processed in the field. The epididymides were blotted dry using clean paper towels to avoid hematic contaminations before incision. A tissue sample of 1 g was cut from the tip of the left cauda epididymis. This was done to standardise the amount of tissue sample taken per animal. The tissue sample was placed in a 5 ml sterile Eppendorf tube and 1 ml of physiological saline solution (maintained at 35°C to simulate *in vivo* conditions; (Agustina et al., 2017)) was added to each tube. This simple and easy-to-prepare solution was utilised to maintain osmotic conditions without any other added substrates and considering basic field conditions where access to materials is limited. However, further studies should consider identifying suitable media for the common eland. The tissue was subsequently gently macerated within the tubes using sterilised surgical scissors. The sample tubes were then placed in a water bath (maintained at 32 – 35°C). Sperm

sub-samples were taken from these tubes over time for sperm quality evaluation.

5.3.3. Sperm concentration, motility, and kinematics

A computer-aided sperm analyser (CASA) using a Microptic Sperm Class Analyzer was utilised in the field to assess the spermatozoa over time [SCA version 6.5.0.9; software settings were as follows: frame rate (50 frames per second), chamber depth (20 μm), calibration value (0.477 $\mu\text{m}/\text{pixel}$), grid distance (100 μm), static (10 $\mu\text{m}/\text{s}$), slow-medium (25 $\mu\text{m}/\text{s}$), rapid ($> 50 \mu\text{m}/\text{s}$), progressive (STR) $>70\%$]. Sub-samples were taken from the prepared tubes at time intervals of 0, 35, 70, and 100 minutes after slaughter. Aliquots of 10 μl of the diluted epididymal spermatozoa samples were transferred to Leja slides (IMV Technologies) and maintained on a heating stage at 37°C. The spermatozoa were evaluated for total motility, progressive motility, non-progressive motility, immotile sperm, and kinematics such as curvilinear velocity, straight-line velocity, average path velocity, linearity, straightness, oscillation, amplitude of lateral head displacement, and beat cross frequency (Farrell et al., 1998). Sperm concentration was measured just at time 0. One eland bull was finally not included in the analysis this dataset for sperm kinematic assessment due to the poor initial sample quality.

5.3.4. Sperm head morphometry, viability, and morphology

At post-mortem intervals of 0, 20, 40, and 80 minutes, another aliquot of 10 μl was taken from the diluted samples, placed on a microscope slide, and stained with

nigrosin (5%) and eosin (1%) to prepare a smear (three replicates were made per sample per time interval). Due to logistical limitations for sample processing, the same time points could not be followed as the previous measurements. Eosin-nigrosin staining was used due to its simplicity to apply in the field, so that sperm viability can be assessed, and because it has been widely employed for sperm head morphometric evaluations in other species (Kondracki et al., 2017; Gacem et al., 2021). Subsequently, the smeared slides underwent analyses using CASA (Microptic Sperm Class Analyzer (SCA) version 6.5.0.67) at the Sperm Laboratory of Sanidad y Biotecnología, University of Castilla- La Mancha, Albacete, Spain. Protocol advised by the manufacturer for cattle was followed, as no validated methodology currently exists for common eland. The Microptic SCA is equipped with a Labophot-2 (Nikon, Tokyo, Japan) microscope (x40 bright-field objective) and video camera (CCD AVC-D7CE; Sony Corporation, Tokyo, Japan) and connected to Pentium 950 MHz processor (Maroto-Morales Ramon et al., 2010). The analysis software settings were as follows: calibration value (0.21), frame size (150 pixels), and grid distance (10 μ m). During analysis, some spermatozoa appeared unrecognisable to the software system since SCA automatically captured and processed the images. These incorrectly captured spermatozoa, *e.g.* irregular sperm heads and edges, were manually corrected. Images were captured from multiple fields (at least 40) on each slide and a total of 200 randomly selected spermatozoa were analysed per sample per time point. Analyses included head area, perimeter, head width, head length, acrosome percentage, and the derived parameters such as ellipticity (L/W), rugosity ($4\pi A/P^2$), regularity ($\pi LW/4A$), elongation $(L-W)/(L+W)$, and roundness (Carrillo et al., 2007) (Figure 5.1).

Sixteen thousand and nine hundred forty-six spermatozoa were observed, using the samples across all collection time intervals to assess, the percentage of normal vs abnormal sperm, as well as the percentage of abnormalities of the head (tapered, pyriform, amorphous, small, and detached), midpiece/neck (bent and thin neck), cytoplasmic droplets (proximal, central, excess, and distal), and tails (short, bent, and coiled), calculated based on the total sperm counted (Bissett & Bernard, 2005). Sperm viability was calculated as the number of live sperm counted as a percentage of the total sperm counted per animal.

5.3.5. Statistical analysis

All the statistical analyses were performed in SPSS ver. 29.0 (IBM, SPSS, USA). Shapiro-Wilks' tests were used to assess the normality of the data. Descriptive statistics (frequency) was used for the epididymal spermatozoa morphology data. Spearman's rank correlation coefficients were established between the sperm quality parameters at collection time point 0. One-way analysis of variance (ANOVA) with Least Significant Differences (LSD) approach was applied to test the influence of collection time intervals on the sperm motility and morphometric variables.

5.4. Results

5.4.1. Correlation of sperm quality parameters

Sperm concentration was $395 \pm 256 \times 10^6/\text{ml}$, which was measured just at time 0. Numerous correlations were found among the epididymal sperm quality parameters at

this initial time point (see supplementary material Table SM1 <https://doi:10.1016/j.repbio.2025.100997>): the total motility percentage was positively correlated with percentage progressive motility and average path velocity ($\rho = 0.900$; $P = 0.037$). The percentage of progressive motility was positively correlated with curvilinear velocity ($\rho = 0.900$; $P = 0.037$), average path velocity ($\rho = 1.000$; $P = 0.001$), amplitude of lateral head displacement ($\rho = 0.900$; $P = 0.037$), sperm head width ($\rho = 0.975$, $P = 0.005$) and negatively with the percentage of non-progressive motility and immotile sperm ($\rho = -0.900$; $P = 0.037$). The opposite was found for the percentage of non-progressive motility, *i.e.*, negative correlations between curvilinear velocity ($\rho = -0.982$; $P = 0.003$), average path velocity ($\rho = -0.900$; $P = 0.037$), amplitude of lateral head displacement ($\rho = -1.000$; $P = 0.001$), beat cross frequency ($\rho = -0.9008$; $P = 0.0037$), and sperm head width ($\rho = -0.872$, $P = 0.054$). Curvilinear velocity and average path velocity were positively correlated with each other (curvilinear velocity vs average path velocity; $\rho = 1.000$; $P = 0.001$), and both parameters were also positively correlated to the amplitude of lateral head displacement of the spermatozoa (curvilinear velocity vs amplitude of lateral head displacement; $\rho = 1.000$, $P = 0.001$; average path velocity vs amplitude of lateral head displacement; $\rho = 0.900$; $P = 0.037$). Beat cross frequency was positively correlated with curvilinear velocity and amplitude of lateral head displacement ($\rho = 0.900$, $P = 0.037$). And both linearity and straightness were positively correlated with each other ($\rho = 1.000$, $P = 0.001$).

In terms of the sperm head morphology, the sperm head area was further positively correlated with sperm head length ($\rho = 0.928$; $P = 0.001$) and sperm head perimeter ($\rho = 0.943$; $P = 0.005$), both of which were positively correlated with each other ($\rho = 0.986$; $P = 0.001$). Sperm

head width was positively correlated with curvilinear velocity ($\rho = 0.872$; $P = 0.054$), average path velocity ($\rho = 0.975$; $P = 0.005$), amplitude of lateral head displacement, and beat cross frequency ($\rho = 0.872$; $P = 0.054$). The sperm elongation was positively correlated with ellipticity ($\rho = 0.971$, $P = 0.001$) and both were also positively correlated with percentage of sperm viability (elongation vs % sperm viability; $\rho = 0.971$, $P = 0.001$; ellipticity vs % sperm viability; $\rho = 0.886$, $P = 0.019$). The regularity of the sperm head was positively correlated with roundness $\rho = 0.884$, $P = 0.035$) and both parameters were positively correlated too with oscillation (oscillation vs regularity; $\rho = 0.949$, $P = 0.014$; oscillation vs roundness; $\rho = 0.894$, $P = 0.041$). Sperm head rugosity was negatively correlated with regularity (rugosity vs regularity; $\rho = -0.884$, $P = 0.035$), roundness (rugosity vs roundness; $\rho = -1.000$, $P = 0.001$), and oscillation (rugosity vs oscillation; $\rho = 0.894$, $P = 0.041$).

5.4.2. Effect of collection time intervals on eland epididymal sperm quality parameters and characterisation of sperm abnormalities

Total sperm motility, immotile sperm, average path velocity, and beat cross frequency were significantly affected by the collection time intervals at 100 mins after slaughter, compared to 0 mins ($P \leq 0.01$). Moreover, a significant decrease in progressive motility and curvilinear velocity was found from 70 to 100 mins ($P \leq 0.01$) (Table 5.1). The percentage of viable sperm also decreased ($P \leq 0.01$; Table 2) within the period between 0 min to 20 min collection time, but after that, no further significant differences were seen. No significant differences were found for the sperm head morphometric parameters regardless of the collection time intervals (Table 5.2; Figure

Table 5.1. Means $\pm$ SD of the sperm motility and kinematics of caudal epididymal recovered sperm from five common eland (*Tragelaphus oryx*) bulls over various collection time intervals immediately after slaughter.

Parameters	Collection time intervals (mins) immediately after slaughter				F	P-value
	0	35	70	100		
Total motility (%)	92.16 ^a ± 6.35	64.85 ^{ab} ± 29.40	52.96 ^{ab} ± 23.37	35.87 ^b ± 29.39	4.443	0.019
Progressive motility (%)	57.45 ^a ± 15.97	37.99 ^{ab} ± 24.38	22.10 ^{bc} ± 14.41	12.39 ^c ± 16.19	5.888	0.007
Non-progressive motility (%)	34.51 ± 12.28	27.08 ± 9.48	30.28 ± 13.77	23.48 ± 15.93	0.644	0.598
Immotile sperm (%)	7.84 ^a ± 06.36	34.93 ^{ab} ± 32.84	47.61 ^{ab} ± 23.44	64.09 ^b ± 29.34	4.457	0.019
Curvilinear velocity (µm/s)	73.30 ^a ± 23.97	56.17 ^{ab} ± 19.83	42.23 ^{bc} ± 10.38	30.86 ^c ± 14.82	5.177	0.011
Straight line velocity (µm/s)	19.69 ± 4.80	17.81 ± 6.97	14.10 ± 6.96	08.92 ± 11.12	1.848	0.179
Average path velocity (µm/s)	40.93 ^a ± 11.94	31.96 ^{ab} ± 12.53	22.82 ^{ab} ± 10.13	15.09 ^b ± 14.69	4.057	0.025
Linearity (%)	33.00 ± 6.76	30.99 ± 6.91	30.29 ± 8.79	20.51 ± 17.07	1.346	0.295
Straightness (%)	52.19 ± 6.50	51.62 ± 8.07	54.55 ± 7.37	43.48 ± 14.52	1.249	0.325
Oscillation (%)	59.37 ± 5.13	56.32 ± 6.17	51.41 ± 10.75	39.05 ± 22.03	2.406	0.105
Amplitude of lateral head displacement (µm)	3.43 ± 0.92	2.61 ± 0.77	2.03 ± 0.36	2.00 ± 1.24	2.885	0.068
Beat cross frequency (Hz)	5.79 ^a ± 0.85	5.33 ^a ± 1.67	4.29 ^a ± 0.94	2.34 ^b ± 1.72	6.371	0.005

^{abc} Within a row, means without a common superscript differ at $P < 0.01$

Table 5.2. Means $\pm$ SD of sperm viability and head morphometric parameters of caudal epididymal recovered sperms of six common eland (*Tragelaphus oryx*) bulls in various collection time intervals immediately after slaughter.

Parameters	Collection time intervals (mins) immediately after slaughter					F	P-value
	0	20	40	60	80		
Viable sperm (%)	56.12 ^a ± 9.89	34.02 ^b ± 10.11	29.31 ^b ± 13.59	31.41 ^b ± 10.85	26.94 ^b ± 9.66	6.988	0.001
Head area (µm ²)	89.19 ± 2.41	89.12 ± 02.67	88.54 ± 02.13	89.17 ± 1.44	89.77 ± 2.78	0.208	0.931
Perimeter (µm)	30.64 ± 0.67	30.82 ± 0.60	30.52 ± 0.54	30.54 ± 0.54	30.47 ± 0.52	0.353	0.840
Width (µm)	7.597 ± 0.142	7.573 ± 0.148	7.555 ± 0.147	7.592 ± 0.107	7.535 ± 0.152	0.202	0.935
Length (µm)	13.63 ± 0.39	13.69 ± 0.28	13.62 ± 0.36	13.61 ± 0.34	13.56 ± 0.32	0.082	0.987
Acrosome percentage (%)	55.502 ± 0.52	55.503 ± 0.83	55.460 ± 0.98	55.660 ± 0.71	55.590 ± 0.70	0.067	0.991
Ellipticity	1.797 ± 0.054	1.805 ± 0.040	1.803 ± 0.061	1.798 ± 0.057	1.808 ± 0.057	0.047	0.996
Rugosity	1.198 ± 0.023	1.182 ± 0.056	1.198 ± 0.025	1.205 ± 0.031	1.230 ± 0.069	0.912	0.472
Regularity	0.913 ± 0.012	0.915 ± 0.026	0.915 ± 0.008	0.912 ± 0.012	0.908 ± 0.020	0.162	0.956
Elongation	0.285 ± 0.015	0.290 ± 0.009	0.285 ± 0.016	0.287 ± 0.015	0.287 ± 0.015	0.137	0.967
Roundness (µm)	0.838 ± 0.018	0.835 ± 0.015	0.842 ± 0.019	0.835 ± 0.023	0.833 ± 0.023	0.170	0.952

^{ab} Within a row, means without a common superscript differ ($P < 0.01$)

Table 5.3. Morphology of caudal epididymal sperm collected from six common eland (*Tragelaphus oryx*) bulls, calculated as a percentage of the total number of epididymal spermatozoa evaluated (n=16,946 sperm). Samples collected for all time intervals after harvesting were evaluated.

Parameters	Calculated %
Normal Sperm	35.31
Abnormalities:	
Head	
Tapered	0.04
Pyriform	0.15
Amorphous	0.07
Small	0.47
Detached	2.74
Neck	
Bent	1.16
Thin	0.05
Cytoplasmic droplets	
Proximal	0.45
Central	0.17
Excess	0.02
Distal	0.54
Tail	
Short	0.11
Bent	12.66
Coiled	46.07

5.1). The descriptive statistics for the frequency of normal vs abnormal sperm morphology of the eland samples are provided in Table 5.3.

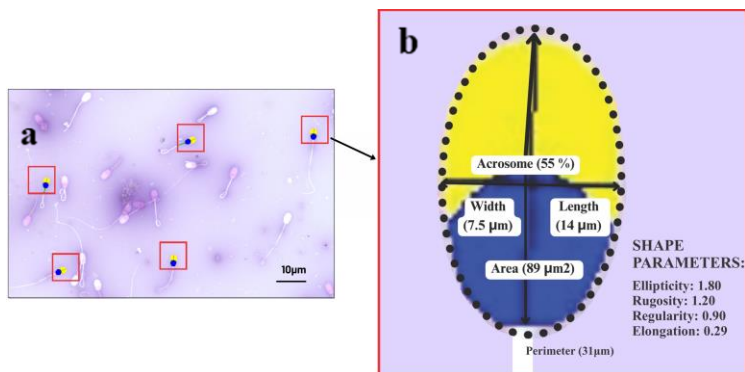


Figure 5. 1. Micrographs (a) of eland epididymal spermatozoa in eosin-nigrosin stains and (b) average values of different sperm head morphometrics (10 μm) of six common eland (*Tragelaphus oryx*) bulls using Microptic Sperm Class Analyser (SCA) version 6.5.0.67.

5.5. Discussion

The retrieval of spermatozoa from the caudal epididymides allows for the utilisation of genetic material following the sudden death of breeding stock or for the preservation of endangered species (Hansen, 2020; Bergstein-Galan et al., 2018). The collection protocol is difficult to manage under most of the current game ranches in, *e.g.*, southern Africa, due to their remoteness and access to reproductive laboratories in these areas. Thus, providing a simple species-specific collection and processing methodology will aid in successfully responding to situations where genetic material needs to be preserved in

the field and will provide baseline information for refining further sperm processing techniques for ARTs.

A previous study involving the retrieval of common eland using electro-ejaculation ($n = 1$; 635 kg bull) reported a progressive motility of 65% in raw, undiluted semen at 37°C (Merilan et al., 1978). After the addition of an egg yolk-citrate-glycerol extender and freezing for six months in liquid nitrogen, a post-thaw motility of 35% and a survival rate of over 50% were reported. This study also reported that the eland sperm obtained were morphologically similar to those of Holstein cattle obtained using an artificial vagina (only 20 sperm cells were compared per species) but had shorter and wider sperm heads, as well as shorter tails (head width: $6.33 \pm 0.29 \mu\text{m}$, head length: $9.33 \pm 0.33 \mu\text{m}$, midpiece length: $14.57 \pm 0.65 \mu\text{m}$, tail length: $39.84 \pm 1.62 \mu\text{m}$, total sperm cell length: $63.75 \pm 1.41 \mu\text{m}$). Within the present study, young eland sperm had slighter larger head widths ($7.6 \pm 0.16 \mu\text{m}$) but longer head lengths ($13.8 \pm 0.29 \mu\text{m}$) than reported by Merilan et al. (1978). Additionally, the eland sperm head area sizes in the present study were approximately $89 \mu\text{m}^2$ which is relatively higher compared to Friesian cattle ($38.08 \mu\text{m}^2$) (Vicente-Fiel et al., 2012). Whilst these variations in sperm head dimensions are species-specific, they can also be attributed to the slide preparation, type of staining reagents, temperature, and type and quality of the equipment used (Rijsselaere et al., 2004). Therefore, future studies may include additional factors, *e.g.* different staining agents, to describe the effect of these on the sperm measurements and provide comparative data for eland bulls.

Eland ($n = 9$; age unknown) sperm collected from the cauda epididymides at 4-5 hours post-mortem had a reported 82% progressive motility and approximately 70% viable sperm (Bissett & Bernard, 2005). Within the present

study, an average progressive motility of $57.45 \pm 15.97\%$ and sperm viability of $56.12 \pm 9.89\%$ was reported at 0 min collection time from young eland bulls (2-2.5 years old; 203 ± 20 kg live weight). It is acknowledged that the simple choice of media affected sperm quality; however, other factors, such as reproductive maturity, also contribute towards these measures.

Whilst attainment of sexual maturity of wild-living eland bulls is reported as approximately 4 to 5 years (Posselt, 1963), more recent data from captivity has shown that eland bulls already showed an increase in efficiency of spermatogenesis and Sertoli cell functionality at approximately 15 months of age (Ros-Santaella et al., 2014). Thus, the bulls in the present study were likely between puberty and attaining sexual maturity, and whilst the comparison with the bull utilised by Merilan et al. (1978) would indicate that they utilised a far more mature animal based on its body weight, the comparison with the study of Bissett and Bernard (2005) is limited since the age and weight range of the animals utilised is missing. Age is a factor that should be carefully considered in future research on this topic. During cold storage (4°C) of the samples collected by Bissett and Bernard (2005) the motility, percentage of live sperm (~ 70 to 40%), and the percentage of sperm with normal morphology (~ 38 to 20%) decreased significantly over collection time; particularly the percentage occurrence of sperm with distal cytoplasmic droplets. Furthermore, there was a high percentage of mid-piece defects, likely due to cold shock, and damaged acrosomal membrane integrity ($\sim 90\%$), significantly affecting fertilising ability (Bissett & Bernard, 2005). When sperm are exposed to cold shock, similar changes occur as those seen during capacitation and acrosome reactions, damaging the cell membrane when prolonged cold storage

is experienced (Jones et al., 1979; Aalseth et al., 1985; Bartels et al., 2001)). There is currently no information regarding the acceptable eland sperm mobility and viability ranges that are required to fertilise oocytes; however, based on the extrapolation of data from other species (*Oryx dammah*, *Bos frontalis gaurus*, and *Cervus eldii thamin*) that require a 55–70% sperm motility and 60–75% viability to fertilise oocytes, Bissett and Bernard (2005) hypothesised that eland sperm maintained at 4 °C could retain their fertilising ability in the epididymis for up to 48 h after death.

The present results show a decline in sperm motility and viability over collection time intervals, most notably between 0 and 35 minutes, which would be improved by utilising different sperm media, the effects of which should be studied next to identify suitable media for eland sperm. However, there were no significant changes in sperm morphology, which may indicate some benefit in utilising this protocol compared to cold storage. Compared to Bissett and Bernard (2005), few defects were seen in the sperm mid-piece in the present study. Typically, sperm head defects primarily occur during spermiogenesis within the testes (Comizzoli et al., 2006), whilst tail defects are both of testicular and epididymal origin (Nothling, 1984; Barth & Oko, 1989). Maturation (morphologically and functionally) takes place whilst the sperm moves through the epididymides, such as the displacement of cytoplasmic droplets from a proximate to a distal position (Axner et al., 1999). The primary sperm defects in the present study were coiled tails, which the media and preparation of the smears likely influenced (Yániz et al., 2015). Even though no significant changes in sperm morphology collection time were reported in the present study, considering the effect of different sampling points would provide further

information on the maturation process throughout the reproductive tract of common eland. Sperm head morphology may also be affected by disruption of their sperm membrane functions (Marco-Jimenez et al., 2006), as likely experienced in the samples of Bissett and Bernard (2005). Furthermore, these changes are more noticeable after the application of any semen extenders and subsequent cryopreservation.

Information regarding common eland sperm motility and kinematics is currently unavailable in the literature, and no standard reference ranges are available according to animal age. Thus, the present study provides the first information regarding common eland sperm motility, kinematics, and sperm head morphometry over different collection time intervals immediately after slaughtering in the field, as assessed by CASA. The decline in most of the caudal epididymal sperm kinetic parameters over the various collection time intervals was as expected since the sperm media (physiological saline solution) lacked nutrients and other diluents and further resulted in rapid loss of intracellular cAMP (adenosine 3,5'-cyclic monophosphate) levels, reduction of metabolic activity and ATP production (Hoskins et al., 1974; Dacheux and Paquignon, 1980; Inskeep & Hammerstedt, 1982; Pariset et al., 1985) as well as oxidative damage (Chatiza et al., 2012; Mir et al., 2012). The comparison of additives and extenders should be investigated to maintain the motility and viability of eland sperm.

Correlation analyses among the various sperm quality parameters are as expected to some extent. The age range of eland bulls in this study suggests that their sperm would be functional with fertility potential. The sperm kinematic parameters such as average path velocity, amplitude of lateral head displacement, and beat cross

frequency were positively correlated with sperm head width, which highlights the importance of sperm head development for their motility (Chatiza et al., 2012; Puri et al., 2024). These parameters are often used to estimate *in vitro* fertilisation outcome (Puri et al., 2024; Verstegen et al., 2002), and to assess changes related to hyperactivation and capacitation (Kondracki et al., 2020). Regarding sperm head morphology, the sperm head width was positively correlated with sperm head length and sperm head perimeter (both of which were positively correlated with each other), and thus, wider sperm heads (in both area and perimeter) suggest better sperm movement dynamics as supported by the positive correlations between oscillation and sperm head shape indices *e.g.*, regularity and roundness. This should be further evaluated as the preparation and processing of smeared slides may influence these findings.

For future research, the influence of other aspects such as social structure, social rank, testosterone production and sperm quality need to be considered, as studies on other African ungulates have shown that dominant African buffalo bulls produce more testosterone than submissive ones and that sperm motility in kudu (*Tragelaphus streptoceros*) males in large mixed-sex herds is lower than in bachelor males (Schiewe et al., 1991; Brown et al., 1991). Considering the social system of the species (Groves et al., 2011), it might be expected that the social environment of eland males in captivity may also affect sperm quality.

5.6. Conclusion

Eland epididymal sperm retrieval between 0 to 35 minutes immediately after slaughtering using simple media

is possible for collecting and processing of spermatozoa and can be applicable in remote areas either from culling practices or sudden death of individuals. However, other critical handling conditions necessary for further processing should be evaluated, as epididymal sperm are without seminal fluid, which directly influences the characteristics for subsequent processing. Additionally, standard reference values for these sperm quality parameters are needed in common eland. Whilst this study has provided basic information on the quality of epididymal sperm from eland bulls collected immediately after slaughter, the present findings should be further validated with a larger sample size and wide ranges of ages to further understand their relationships with animal traits that might be used for breeding animal selection, together with the use of different sperm media. The effect of season should be considered for future work.

CHAPTER 6

Effects of age and season on the faecal androgen metabolite hormone secretion patterns of farmed common eland (*Tragelaphus oryx*) bulls, and their relationships with morphometric body traits

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Effects of age and season on the faecal androgen metabolite hormone secretion patterns of farmed common eland (*Tragelaphus oryx*) bulls, and their relationships with morphometric body traits

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6.1. Abstract

The production potential of farmed common eland (*Tragelaphus oryx*) necessitates consideration of key performance traits including temperament, reproductive efficiency, growth rate, feed conversion, and meat quality, traits that are often linked to androgen secretion dynamics. However, data on androgen profiles in eland bulls under

controlled conditions is not available, impeding informed reproductive and management strategies. As such, this study was undertaken to characterise the androgen secretion patterns of common eland bulls through long-term monitoring using epiandrosterone faecal androgen metabolite enzyme immunoassays (EA-fAM EIA). A total of 152 faecal samples were collected bi-monthly from 23 eland bulls, from 10–24 months of age. Individual traits such as age, body weight, body condition scores, horn sizes, and temperament and their effects on EA-fAM were explored. Results reveal a plateau in EA-fAM concentrations around 2 years of age among the eland bulls, suggesting a possible transition between puberty and initiation of sexual maturity, which is earlier than initially postulated for common eland under extensive management systems in southern Africa. The EA-fAM patterns in common eland may exhibit some degree of seasonality, with late summer and early autumn being a potentially optimal period for mating and sperm collection activities. However, these findings should consider other genetic, physiological and behavioural data to better understand the substantial individual variability in EA-fAM concentrations.

Keywords: *epiandrosterone, faecal androgen metabolites, hormonal secretion patterns, reproductive management*

6.2. Introduction

Hormonal secretion patterns are a critical component in understanding the development, health, physiological challenges, and welfare status of animals (Evans et al., 2022), contributing towards informed management strategies. This is particularly relevant for antelope species in captivity such as the common eland (*Tragelaphus oryx*), which is currently also undergoing domestication, a process that began as early as 1848 (Jelliman, 1913). Economically, the common eland holds considerable value in wildlife ranching activities, including trophy hunting and meat production in southern Africa (Taylor et al., 2020), and is increasingly recognised as a viable alternative farming species in the face of growing global demand for high-quality animal protein and the challenges posed by climate change to conventional agricultural systems (Cromsigt et al., 2018). The common eland generally exhibit better adaptability to hot and semi-arid environments compared to traditional domestic ruminant livestock species, like cattle, under the same conditions (Posselt, 1963; Skinner, 1967). However, they have also shown adaptability to a range of environmental and management conditions, also thriving under semi-intensive farming conditions in the Czech Republic, Hungary, Germany, Switzerland, and Poland.

The production potential of common eland under intensive farming systems requires careful consideration of several factors, including temperament, reproductive efficiency, growth rate, feed efficiency, and meat quality (Haskell et al., 2014). In general, reproductive functionality in livestock species is closely linked to androgen production and secretion patterns (Mystrud et al., 2004). However, no published data currently exist on the androgen secretion profiles of eland bulls, presenting a significant gap in

understanding their reproductive physiology and investment strategies. Whilst basic reproductive management guidelines exist for eland in extensive systems in southern Africa, such as age of sexual maturity, male:female ratio, etc, no thorough scientific data exists to support breeding management objectives, especially under semi-intensive farming conditions. Initial data has shown that reproductive development is accelerated under these conditions, with eland bulls as young as 2–2.5 years old demonstrated reproductive tract and testicular morphology indicative of functional maturity earlier than their wild counterparts, which typically reach reproductive maturity between 4 and 6 years of age (Ros-Santaella et al., 2014; Consolacion et al., 2024). Elucidation of androgen secretion patterns, and their physiological relationships, is critical for improving the reproductive management and performance of captive eland males. These hormonal insights may serve as critical reference points for optimising eland management strategies within the growing interest of private European breeders and commercial production systems as well as baseline data for future research studies and other conservation efforts.

Common eland bulls are considered aseasonal and polygynous, exhibiting diverse reproductive behaviours during their breeding season. Among their secondary sexual characteristics, *Flehmen* response (lip-curling behaviour) is commonly observed (Pappas, 2002), alongside *Swahili* or *Ukali* displays, which serve as reproductive investments to attract females and intensify male–male competition (Benoit, 2008). Vocalisations are frequently used during courtship and mounting (Benoit, 2008), while the castanet-like clicking of the knees represents a unique acoustic signal associated with fighting ability and dominance-typically exhibited by bulls with

greater body mass and larger dewlaps (Bro-Jorgensen & Dabelsteen, 2008). Furthermore, social rank may affect the opportunity to participate in mating, and dominant bulls may suppress testosterone levels and sperm quality in subordinate males, as postulated in kudu bulls (Bissett & Bernard, 2005). Reproductive traits are largely mediated by androgens, which play a critical role in driving phenotypic expression in male mammals. As such, androgen-influenced traits – such as scrotal circumference, testicular function, body mass, musculature, and horn development – are frequently used as biomarkers for assessing reproductive fitness (Fuxjager et al., 2018).

While androgens are commonly assessed in serum obtained via blood sampling (Kamgang et al., 2020), alternative matrices such as urine and faecal samples offer practical, non-invasive approaches (when collected without animal contact) for evaluating testicular activity in free-roaming wildlife species (Hodges et al., 2010; Schwarzenberger et al., 2013). The measurement of faecal androgen metabolites (fAM) is applicable when assessing male reproductive functionality and secondary traits, offering insights into aspects such as reproductive seasonality, tusk development, and reproductive cues (Pereira et al., 2005; Ostner et al., 2008). Additionally, fAM quantification has contributed to understanding seasonal breeding patterns, social hierarchy, aggressiveness, and sexual behaviours in male ungulates, including roan antelope (*Hippotragus niger*) and bison (*Bison bison*) (Lincoln et al., 1977; Patton et al., 2001). This method has also been employed in domestic livestock, such as cattle and pigs, to support welfare assessments and reproductive monitoring (Möstl et al., 2023). In captive game species, like the common eland at CZU Common Eland Research Facilities, faecal androgen metabolite monitoring may

provide insights into the relationships between androgen secretion patterns and other factors, such as animal age, season, nutrition, animal behaviour, etc under conditions where confounding factors may be controlled, thereby providing important baseline information.

This research study was thus undertaken to determine the secretion patterns of faecal androgen metabolites in farmed common eland bulls, with the aim of deepening our understanding of their reproductive development across age and season, focusing on the period surrounding puberty. The findings are expected to offer valuable insights for future reproductive management and ongoing research goals.

6.3. Materials and Methods

6.3.1. Animal history, study area, management, and experimental set-up

The herd of common eland maintained at the Czech University of Life Sciences Prague (CZU) Research Facilities in Lány, Czech Republic (50°7'41" N, 13°57'31" E). The research facilities are certified by the Czech Republic Ministry of Agriculture (Permit: MZE-13122/2022-13114). Husbandry practices and environmental conditions were standardised in accordance with approved experimental protocols, as authorised by the Animal Welfare and Clearance Committee of the Czech University of Life Sciences Prague (Permit: CZU 20/19).

The Research Facility was established in 2002 from a founding stock of five animals. These individuals were originally captured from transboundary tropical regions –

including Kenya, Uganda, Tanzania, Cameroon, and West Africa – and transported to Europe between 1967 and 1972, where they were first housed at Safari Park Dvůr Králové (Vágner, 1974). Since its establishment, the herd has produced a total of 353 offspring. To maintain and improve genetic diversity, regular exchanges of breeding animals with local zoological institutions are undertaken. Annual population regulation through culling is also conducted. Currently, the facility maintains a population of 40-50 (maximum) individuals, primarily for research and educational purposes. The aim of the facility is to investigate the production potential of common eland as an alternative livestock species, serving both as a model organism and as validation of findings from earlier studies conducted under extensive farming conditions. Since 2019, intensive data collection efforts have focused on various scientific domains – including animal welfare, growth performance, and meat quality-to further understand the feasibility and utility of the species in farming systems. Each animal has a complete record of its pedigree and monthly individual data from birth until departure from the facility, through live sales or culling. Details of routine management procedures, including husbandry, nutrition, and handling, are described in Chapter 3, as well as by Needham et al. (2020) and Musa et al. (2021).

This study utilised 23 common eland males that were maintained in a mixed-sex-age herd structure designed to replicate natural social dynamics, including the presence of females, as observed in free-ranging populations (Hillman, 1987; Wirtu et al., 2004). Data and samples were collected between 10 and 24 months of age. This setting also aligns with the species' year-round breeding behaviour (Musa et al., 2024) and supports previous findings on early sexual maturity (as early as 15

months) in common eland males housed at the same research facility (Ros-Santaella et al., 2014). These conditions were considered critical in evaluating patterns of faecal androgen metabolite secretion and the development of primary and secondary sexual traits, including sperm characteristics, in common eland

Biometrical data was systematically collected during routine monthly handling at the research facility, as described in Chapter 3. Recorded parameters included body weight, body condition score (BCS) – as assessed following the established protocols (Audigé et al., 1998; Dearman, 2023) and horn measurements (length, twisted length, and base circumference). Additionally, temperament score (TS) was evaluated using a validated behavioural scoring system for common eland (Musa et al., 2021). Comprehensive details regarding the handling procedures, sampling techniques, and faecal collection protocols are available in Chapter 3, and Musa et al. (2021).

6.3.2. Faecal collection, sampling, processing, and steroid extraction

Individual faecal samples were collected directly from the rectum of each animal and placed in Ziplock bags for transport in an icebox. This allowed for full control over animal identification and sample collection, ensuring sample quality was not compromised. Upon arrival in the Laboratory of Animal Sciences at the Faculty of Tropical AgriSciences CZU Prague, samples were freeze-dried via lyophilisation (L10-55 PRO HSC, GREGOR Instruments, Czech Republic) for 72 hours, pulverized, and sieved through a 1 mm sieve to eliminate undigested material. For faecal androgen metabolite (fAM) analysis, a total of 152 samples were selected, originating from 23 common eland

bulls, from 10 to 24 months of age (*i.e.*, aged 289–735 days). Sampling was conducted bimonthly from 2020 to 2024, ensuring longitudinal coverage of individual developmental stages.

Steroid extraction was conducted following a modified protocol based on Sarmah et al. (2017). Approximately 0.100–0.110 g of dried faecal powder per sample was measured using a digital analytical balance (Z741087 Kern Analytical Balance, Merck) and extracted with 3 mL of 80% ethanol. The suspension was vortexed for 15 minutes and subsequently centrifuged at 1,500 rpm for 10 minutes. A 1 mL aliquot of the resulting supernatant was transferred to Eppendorf tubes, evaporated using a drying oven (Universal Oven UF450, Memmert GmbH & Co. KG) at 60°C, and stored at room temperature until further analysis. Prior to conducting the enzyme immunoassay, all samples were reconstituted in 0.8 mL of 80% ethanol and 0.2 mL of distilled water, followed by vortexing, as described by Palme and Möstl (1993).

6.3.3. Faecal androgen metabolites enzyme immunoassay analysis (fAM-EIA)

An epi-androsterone (EA) enzyme immunoassay (EIA) was employed in this study for the analysis of faecal androgen metabolites, following prior biological validation and confirmation of its suitability for use in common eland bulls (Musa et al., 2021). Detailed protocols for the EA-EIA procedures, including the characterisation of assay components were adapted from the methods of Ganswindt et al. (2002) and Palme and Möstl (1993): EA-EIA was performed using a Goat anti-Rabbit IgG with EA (100%), 5 α -androstane-3,17-dione (800%), 5 α -androstane-3 α -ol-17-one (40%), and 4-androstene-3,17-dione (15.6%). For

hormone quantification, 50 µl aliquots of EA standards range: 1.9–500 picogram (pg), quality control samples, and appropriately diluted faecal extracts were dispensed in duplicate into the wells of a microplate. Subsequently, 50 µl of biotinylated EA label and antibody were added to each well, followed by overnight incubation at 4 °C. After incubation, plates were placed into a microplate washer (four washing cycles with a washing buffer solution), and then 150 µl (equivalent to 20 ng) of streptavidin-peroxidase conjugate was added per well, and then the plate was incubated in the dark for 30 minutes. Plates were rewashed prior to the addition of 150 µl of peroxidase substrate solution, and colour development was allowed for 30–60 minutes. A 50 µl of 4N H₂SO₄ was used to stop the enzymatic reaction then, optical density was measured at 450 nm and 650 nm. Serial dilutions of faecal extracts were generated with the use of displacement curves parallel to the standard calibration curves, confirming assay specificity and matrix compatibility.

6.3.4. Statistical analysis

All statistical analyses were conducted in R version 4.3.1 (R Core Team, 2024). The sampling dates were parsed and converted to day-of-year using the lubridate package (Grolemund & Wickham, 2011). Exploratory graphics, including scatterplots, histograms, boxplots, and LOESS-smoothed trends, were generated with ggplot2 (Wickham, 2016). Pairwise correlations and variance inflation factors (VIF) were computed via GGally (Schloerke et al., 2023) and car packages (Fox & Weisberg, 2019), respectively. To address strong collinearity among body-size metrics (body weight, neck circumference, and horn length), principal component analysis (PCA) were performed using the

FactoMineR package (Lê et al., 2008) on a mean-imputed dataset produced by missMDA (Josse & Husson, 2016). The first principal component (Size_PC1) accounted for >80% of the total variance and was used as a univariate size covariate. Fixed-effect multicollinearity was reassessed post-PCA via VIF. Linearity and homoscedasticity were evaluated with residual vs. fitted plots and the Breusch-Pagan test (lmtest; Zeileis & Hothorn, 2002). Normality of residuals was examined via QQ-plots. Influence diagnostics (Cook's distance) were used to inspect leverage points.

Faecal androgen metabolite concentrations (androgen ng/g) were modelled as a function of age in days, Size_PC1, horn base diameter, body condition score, temperament score, and seasonal day-of-year. Age effects were captured using a 3-degree natural spline (splines package; Venables & Ripley, 2002) to allow non-linear growth trajectories, while seasonality was modelled with a 4-degree B-spline. Individual identity (ID) was included as a random intercept to account for repeated measures. Two random-effects structures (random intercept only vs. random intercept plus linear age slope) were compared using maximum likelihood estimation in lme4 (Bates et al., 2015). Model selection was based on Akaike's Information Criterion (AIC).

For the selected mixed model (random intercept structure), scaled residuals were simulated with the DHARMA package (Hartig, 2021) to test for uniformity, dispersion, and zero-inflation. Marginal and conditional R^2 were computed using MuMIn (Barton, 2020). *P*-values for fixed effects were obtained via the Satterthwaite approximation implemented in lmerTest (Kuznetsova et al., 2017) and confirmed with Type III Wald chi-square tests from car.

Estimated marginal effects for age, seasonality, and size were visualised with `visreg` (Breheny & Burchett, 2017) to display non-linear spline fits on the androgen concentration. All figures were finalised with `ggplot2`.

6.4. Results

Table 6.1. Fixed-effect estimates from the selected mixed-effects model predicting faecal androgen metabolite concentrations (ng/g).

Predictors	Estimates	CI	P-value
(Intercept)	8.02	-4.63 – 20.68	0.212
Age days [1st degree]	5.21	-1.79 – 12.21	0.143
Age days [2nd degree]	-10.61	-24.16 – 2.93	0.124
Age days [3rd degree]	-2.95	-10.68 – 4.78	0.452
Size PC1	0.71	-1.48 – 2.90	0.522
Horn base (cm)	-0.28	-1.32 – 0.77	0.598
BCS	1.13	-1.67 – 3.92	0.426
Temperament score	-3.15	-13.65 – 7.34	0.553
CalendarDate [1st degree]	3.29	-3.13 – 9.70	0.313
CalendarDate [2nd degree]	4.99	-0.69 – 10.68	0.085
CalendarDate [3rd degree]	13.93	8.07 – 19.80	<0.001
CalendarDate [4th degree]	6.69	-0.24 – 13.61	0.058

A linear mixed-effects model was fitted to 151 androgen measurements from 23 male eland bulls (aged 289–735 days), using a 3-df natural spline for age, a 4-df B-

spline for day-of-year, Size_PC1, horn-base diameter, body condition score (BCS), and temperament score as fixed covariates, plus a random intercept for individual. Model selection by AIC favoured the intercept-only random-effects structure ($\Delta\text{AIC} > 2$).

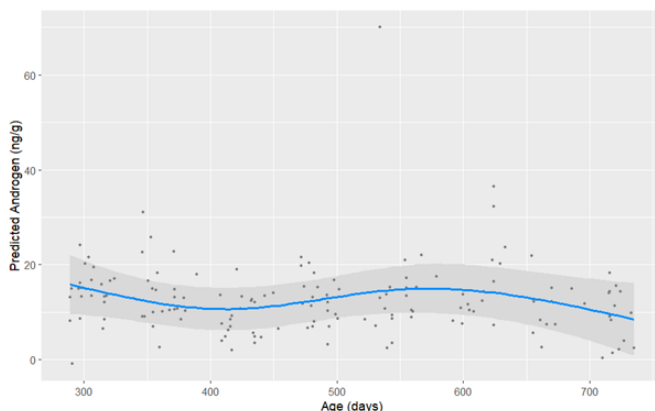


Figure 6.1. Observed faecal androgen metabolite concentrations and model-predicted age trajectory. Points are raw data; solid line is the 3-df natural spline fit ($\pm 95\%$ CI shaded).

Fixed effects showed a pronounced non-linear age trajectory (Wald $\chi^2_3 = 12.4$, $P = 0.006$), with faecal androgen metabolite levels rising steeply in young adults before plateauing (Figure 6.1). Seasonal variation in day-of-year was also significant (Wald $\chi^2_4 = 18.7$, $P < 0.001$), peaking around Julian day 250 (Figure 6.2). In contrast, overall size (Size_PC1; $\beta = 0.71 \pm 0.98$, $P = 0.522$), horn-base diameter ($\beta = -0.28 \pm 0.77$, $P = 0.598$), body condition ($\beta = 1.13 \pm 3.92$, $p = 0.426$), and temperament ($\beta = -3.15 \pm 7.34$, $P = 0.553$) were non-significant (all $P > 0.01$; Table 6.1). The final model explained 19% of total variance via fixed effects

alone (marginal $R^2 = 0.189$) and 49% when including individual differences (conditional $R^2 = 0.488$), indicating substantial between-animal variability (Figure 6.3).

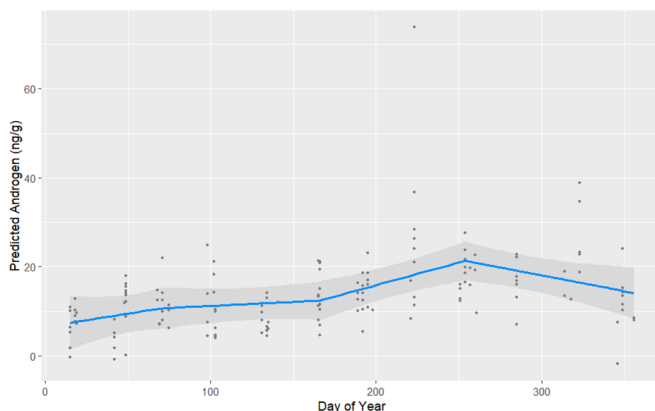


Figure 6.2. Model-predicted seasonal cycle of faecal androgen metabolite concentrations across day-of-year (4-df B-spline; $\pm 95\%$ CI).

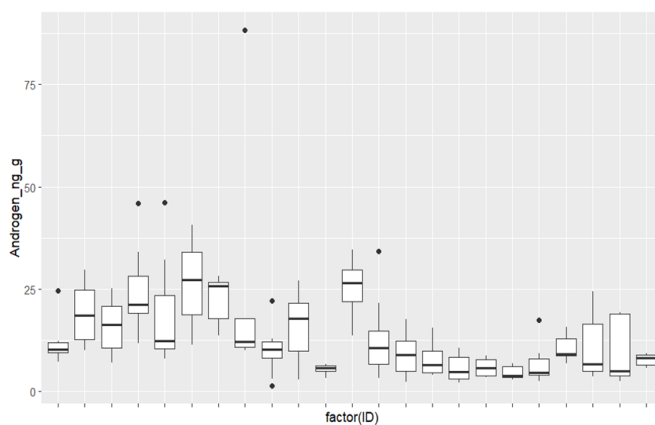


Figure 6.3. Boxplot of faecal androgen metabolite concentrations (ng/g) by individual animal ID, showing medians, quartiles, and outliers.

6.5. Discussion

Reproductive function in males across a wide range of taxa is predominantly regulated by androgens – a class of steroid hormones that includes testosterone – which play critical roles in initiating spermatogenesis, driving the morphological development of reproductive organs, and mediating the expression of secondary sexual characteristics and associated behavioural patterns (Weinbauer et al., 2010; Preston et al., 2012; Charlier et al., 2013; Hull & Rodriguez-Manzo, 2004). To monitor androgenic activity while minimising animal welfare concerns and stress during sample collection, non-invasive methods have been developed. Although blood sampling remains a conventional approach (Schwarzenberger & Brown, 2013), alternative matrices such as urine and faeces are increasingly employed for hormonal assessment. In particular, quantification of faecal androgen metabolites (fAM) using enzyme immunoassay (EIA) techniques has proven effective in characterising testicular endocrine activity in several ungulate species, and is particularly valuable for non-invasive monitoring under extensive systems or for free-living individuals (Mooring et al., 2004). On the other hand, if one is able to handle the animal (and they are habituated to this process to reduce stress), gentle rectal collection of fresh faecal samples ensures sample freshness (*i.e.*, no degradation of metabolites before collection) and reliability in terms of animal identification and timing of collection. Despite this limitation, faecal hormone metabolites provide a better indication of the current hormone concentrations, as blood often provides a shorter-term, and more variable, indication of hormone concentrations (*i.e.*, snapshot *vs* the bigger picture). Often, and especially with blood testosterone levels, concentrations are greatly affected by individual animal

factors, time of withdrawal, and vulnerable to repeated blood sampling – this oftentimes leads to stress responses of animals due to physical discomfort, and may increase the risk of infection if hygiene protocol is not followed, potentially confounding hormone levels and yielding low temporal resolution in monitoring results (Palme, 2019).

Importantly, concentrations of gonadal steroid hormones and their metabolites in faeces have demonstrated strong correlations with circulating plasma hormone levels (Desaulniers et al., 1989) but may also offer a more integrated representation of an individual's daily hormonal secretion patterns compared to isolated blood samples (Pelletier et al., 2003). In this context, the present study was undertaken to establish fAM profiles in common eland maintained under a semi-intensive management system, as a model antelope species for domestication based on their productive attributes. The common eland stands out as a particularly promising candidate for domestication, owing to its exceptional meat quality and consistent population stability across diverse geographic regions (Barton et al., 2014; Needham et al., 2022; Cetkovská et al., 2024). Furthermore, they are also a model species for the critical endangered Western Giant Eland, from which management strategies may be extrapolated from.

Previous investigations into fAM concentrations in common eland have been conducted primarily under captive conditions. However, these assessments have predominantly focused on fAM dynamics in relation to immunocastration (Musa et al., 2021), which has been incorporated into the management of game species, particularly in zoos. Epiandrosterone (EA), an androgenic metabolite, was validated as the most reliable indicator of

testicular endocrine activity following successful biological validation in common eland males (Musa et al., 2021).

In the present study, the predictive modelling reveals a non-linear age-related trajectory of EA-fAM concentrations in young eland bulls (Figure 6.1). This finding is consistent with the endocrine regulation of testicular development by the hypothalamic-pituitary-axis, which exhibits distinct phases of activity between puberty and sexual maturity (McGowan et al., 2018). This is particularly true upon considering the age of the onset/timing of puberty. Such information is pivotal for reproductive tract morphological development and optimising reproductive management strategies in individual bulls (Duittoz & Kenny, 2023). However, detailed data on this trajectory remain lacking for common eland bulls. In cattle, *Bos taurus* bulls generally attain puberty between 8 and 10 months of age, depending on their maturity type (Lunstra et al., 1978) and may be suitable for breeding during their yearling phase (Higdon et al., 2000). In contrast, *Bos indicus* bulls typically reach pubertal onset later, around 16 to 18 months, with recommended breeding initiation occurring after 24 months of age (Trocóniz et al., 1991; Vale et al., 1997). Additionally, in European-derived breeds, testicular development proceeds gradually until approximately 6 months of age, followed by a distinct acceleration in growth leading up to puberty, which generally occurs between 9 and 12 months old (Rawlings et al., 2008). Therefore, age at the onset of puberty is crucial for appropriate management practices of individual bulls. The existing literature suggests that age of common eland bulls when sexually mature is typically reached between 18 and 24 months of age (Du Toit, 2005), with breeding viability generally observed from approximately four years old under extensive management systems (Furstenberg,

2016). In contrast, the present data indicates that the onset of puberty in common eland bulls under semi-intensive management systems may occur considerably earlier – potentially around 10 months old (approximately ~ 300 days) or even earlier, based on initial hormonal developmental observations at the commencement of the data collection. This supports the work of Ros-Santaella et al. (2014) who observed physical indications of puberty in farmed common eland at an earlier age. This observation is further substantiated by a rising trend in EA-fAM concentrations around 450 days of age (Figure 6.1), which coincides with increased Sertoli cell activity and the progression of spermatogenesis observed at approximately 15 months, in other eland males at the same research facility (Ros-Santaella et al., 2014).

These hormonal trends are further corroborated by statistically significant positive correlations between age (at 24–29 months) with body weight (203 ± 20 kg) with several indicators of reproductive tract development, including combined scrotal and testicular mass, seminal vesicle weight, testicular width, ampulla weight, and testicular vascularization (Consolacion et al., 2024; Chapter 4). Simultaneously, these physical attributes correspond with the presence of viable spermatozoa, as described in Consolacion et al. (2025) (Chapter 5). Complementing these findings, longitudinal endocrine monitoring in the current study revealed a plateau in EA-fAM concentrations at around 720–735 days of age (around 2 years), suggesting stabilisation of testicular activity at the potential onset of sexual maturity in eland bulls under semi-intensive system. This estimated onset of sexual maturity is earlier than reported in other literature and other physiological factors, as well as mating success should be considered to corroborate this (Du Toit, 2005).

The findings of this study also clearly demonstrate a pronounced seasonal influence on EA-fAM concentrations in young eland bulls. A marked shift was observed around Julian day 250 – corresponding to early September, which aligns with the period between the late summer and the onset of autumn in the Czech Republic, where the research facility is located. Increased EA-fAM concentrations during late summer suggests seasonal activation of androgenic activity, likely driven by improved pasture quality and its stimulatory effect on the hypothalamus-pituitary-testes axis (HPT) (Short, 1987). Despite that, the eland bulls still breed year-round (Skinner et al., 1971; Musa et al., 2024), but this data supports a potentially optimal timing for organising mating and collecting sperm samples for processing. This trend is similarly observed in European cattle breeds, where breeding and other reproductive interventions are done effectively during late summer and early autumn (Borisenkov et al., 2004). However, environmental factors – such as photoperiod, rainfall, and temperature – should be taken into account, as they may offer valuable insights into androgen fluctuations and their role in modulating reproductive function. Variations in photoperiod influence melatonin synthesis in the pineal gland, which in turn affects the HPT axis. This regulation impacts the secretion of gonadotropin-releasing hormone (GnRH) and luteinising hormone (LH), thereby influencing testosterone production and overall testicular activity (Akar et al., 2024). However, the eland bulls examined in this study are of a relatively young age and this should be further considered over a longer period, as the eland mature further. The effect of photoperiod should also be further investigated in common eland, including its effects on sperm quality, as it may indicate a potentially optimal timing for organising mating and collecting sperm samples for further processing. Due to the distinct photoperiodic

conditions of the European temperate climate, similar data should be collected within southern Africa for comparison.

Of all factors investigated, a considerable inter-individual variability in EA-fAM concentrations was observed among the eland bulls. Many factors may influence this, one of which may be their social rank within the herd. This phenomenon warrants further investigation, as Bissett and Bernard (2005) have proposed that dominant individuals in eland bulls may exert social suppression of testosterone production, as similarly documented in other ungulates, such as kudu bulls.

Body size, body condition score, horn base diameter, and temperament – were not significantly associated with EA-fAM concentrations. This outcome is unexpected, given that these traits are often considered critical for breeding bull selection in game species. Typically, androgens play a central role in promoting the development of traits, such as testicular function, somatic growth, musculature, and horn morphology (Fuxjager et al., 2018). However, androgenic influence is modulated by additional parameters, including genetic predisposition, nutritional status, and environmental stimuli, which can directly impact the activation and responsiveness of the hypothalamic–pituitary–testicular (HPT) axis (Duittoz and Kenny, 2023). However, these findings support Consolacion et al. (2024) (Chapter 4) who reported no positive correlations between reproductive tract development and horn length and temperament in young eland bulls. Accordingly, caution should be done when relying on these secondary sexual traits for phenotypic selection purposes, and more data is required before reliable phenotypic indicators may be established for common eland bulls.

6.6. Conclusion

This study demonstrates that EA-fAM monitoring provides valuable insight into androgen hormone secretion patterns in common eland bulls. This study indicated a peak in EA-fAM concentrations around 2 years of age, indicating possible exit of puberty and initiation of sexual maturity, which is earlier than previously considered in extensive systems in southern Africa. Furthermore, common eland may exhibit some degree of seasonality, with late summer, early autumn being a potentially optimal period for mating and sperm collection activities. However, these findings need to be corroborated with further research, considering other important physiological data. Furthermore, considering the high individual variation in EA-fAM concentrations, this aspect needs to be detangled to understand the main drivers of this.

CHAPTER 7

General discussion

Understanding the reproductive functionality of male animal species is essential for effective breeding and fertility management, particularly in common eland, that are also often found in isolated populations outside of their area of origin (*i.e.*, zoos and private breeders). This approach specifically includes evaluating the development and structure of the reproductive organs, androgen secretion patterns, and the relationship between these, secondary sexual traits, and other important animal traits. Existing data are limited by confounding factors and small sample sizes, and are thus often inadequate to provide systematic baseline information for the optimisation of common eland reproductive performance. Therefore, this present study aimed to assess the reproductive functionality of young common eland bulls to improve their management, supporting the ongoing domestication efforts, and to serve as a potential model species for the conservation efforts of the endangered Western Giant Eland in Senegal. The findings present novel firsthand data on the reproductive functionality of farmed common eland bulls and are also the first to employ long-term monitoring of individuals with full life history and pedigree data, fAM monitoring of androgen secretion patterns, and CASA to provide a more comprehensive evaluation of reproductive status. The results of this study may contribute to the establishment of selection criteria and management strategies for breeding bulls and serve as a guideline for the

application of assisted reproductive technologies among the growing interest of European private breeders, zookeepers, and commercial farmers.

In general, the reproductive tract of young eland bulls closely resembled that of domesticated cattle, likely due to their phylogenetic relatedness (Tonizza de Carvalho et al., 2014). All structures may be considered morphologically and histologically functional with no gross abnormalities (**Chapter 4**). A low asymmetry also existed among the bilateral structures of their tracts. Furthermore, this was reflected in the sperm quality of the epididymal sperm recovered from these bulls (**Chapter 5**). This degree of reproductive development is further supported by the EA-fAM secretion patterns (**Chapter 6**), of which these same six intact bulls (experimental bulls from **Chapter 4**) were also included (prior to culling).

Despite the complementary findings and biological associations observed in the reproductive tract development of common eland bulls, several underlying factors warrant further investigation to establish robust guidelines for utilising these traits as selection criteria in breeding programs and to support their production potentials as an alternative farming species. A common selection trait being used is the age at the onset of puberty (Duittoz & Kenny, 2023), which is critical for implementing appropriate management practices of individual bulls that may possibly be selected as future breeder bulls in a herd. The current data suggests that onset of puberty in common eland bulls under semi-intensive management may occur as early as 10 months (~300 days), based on initial hormonal profiles. EA-fAM concentrations subsequently increase at around 450 days (**Chapter 6**), coinciding with enhanced Sertoli cell activity and spermatogenesis as observed at ~15 months old (450 days old) (Ros-Santaella

et al., 2014). Of course, age and body weight play an integral role in reproductive development in common eland, as indicated by the numerous significant positive correlations between reproductive tract parameters and age and body weight in young eland bulls.

The secretion patterns of EA-fAM exhibited a non-linear relationship with the age of an animal, which is expected, as other factors such as season have an influence on hormones mediated by the HPT axis (Short, 1987). In this study, a degree of seasonal patterns of EA-fAM concentrations were observed in young eland bulls. A distinct peak is observed around Julian day 250 (early September). This period is a transition from late summer to early autumn in the Czech Republic and appears to coincide with enhanced pasture conditions. However, additional research is needed with a focus on age-associated endocrine secretion patterns during and after sexual maturation, as well as the effects of seasonal photoperiodic cues on the reproductive functioning (*i.e.*, sperm quality) of eland bulls. This is required under both European and southern African conditions.

The monitoring of EA-fAM secretion patterns in this present study also reveals a higher inter-individual variability, indicating to some extent that each individual eland bull may have different physiological mechanisms for androgen production and utilisation. The metabolic pathway of androgens is complex, and affected not only by testicular production but also by the adrenal gland. Further characterisation of this pathway may also provide insight into individual differences and factors that impact fAM concentrations (Duittoz & Kenny, 2023; Ågmo, 2024). This finding may explain some of the negative (and/or non-significant) correlations between the reproductive tract parameters with traits such as horn size (**Chapter 4**).

Therefore, a systematic and longitudinal assessment of secondary sexual characteristics, alongside other potential phenotypic selection traits, together with EA-fAM secretion patterns and semen quality monitoring may provide further information essential for effective selection criteria (and their normal ranges) in the reproductive and breeding management of eland bulls.

Assessing sperm quality in eland bulls remains a critical aspect of reproductive research, particularly given the current lack of comprehensive studies examining eland sperm characteristics under controlled conditions (**Chapter 5**). Although electro-ejaculation and the use of an artificial vagina are the recommended techniques for semen research protocols, this represents significant challenges regarding their use in game species. Their use in animals that are not trained for collection, or require anaesthesia for collection, poses several ethical considerations and permission necessary for scientific research, as well as the need for veterinary intervention and specialised equipment. Nevertheless, the post-harvest recovery of eland epididymal sperm under basic field conditions presents a potentially viable and practical approach, particularly given the current challenges of conserving genetically superior eland bulls in extensive systems across southern Africa, where field-based culling, geographic remoteness, and limited access to reproductive facilities prevail. Furthermore, the present study yielded substantial data that post-harvest recovery of eland epididymal sperm under basic field conditions – without cooling or supporting media – is feasible. It further provides preliminary data on sperm viability, motility, and morphology, which are critical for validating reproductive tract development, assessing androgen profiles, and

informing the application of appropriate assisted reproductive technologies.

As anticipated, a progressive decline in eland epididymal sperm quality was observed with increasing post-harvest time intervals except for the sperm head morphometry; however, this was not as drastic as initially expected. The observed decline in sperm quality is attributed to the use of basic physiological saline, which lacks essential energy substrates and is further compounded by the absence of seminal fluid in the epididymides. These factors, along with a high incidence of coiled tails indicating potential pH issues, confirms this metabolic deterioration (Soler et al., 2018). Whilst the use of physiological saline allows for the basic assessment of sperm, a systematic approach to identifying suitable evaluation protocols and the use of different extenders in common eland needs to be conducted. These may include enhancements in sperm preservation media, improved temperature regulation, adjusted osmotic conditions, improved staining techniques, and precise software calibration in CASA systems to advance sperm quality assessment and cryopreservation efficiency. Nonetheless, the findings confirm that the testes of eland bulls aged 24–29 months appear to be reproductively functional.

This present study also supports previous conclusions that eland sperm exhibit morphological similarities to Holstein cattle (Merilan et al., 1978). However, eland sperm evaluated by Merilan et al. (1978) were characterised by shorter, broader heads and shorter tails (head width: $6.33 \pm 0.29 \mu\text{m}$; head length: $9.33 \pm 0.33 \mu\text{m}$; midpiece length: $14.57 \pm 0.65 \mu\text{m}$; tail length: $39.84 \pm 1.62 \mu\text{m}$; total length: $63.75 \pm 1.41 \mu\text{m}$). Sperm from young eland in the present study exhibited slightly wider ($7.6 \pm 0.16 \mu\text{m}$) and significantly longer

heads ($13.8 \pm 0.29 \mu\text{m}$) compared to the measurements reported by Merilan et al. (1978). However, standardised reference ranges specifically developed for assessing sperm quality in common eland have yet to be established.

CHAPTER 8

General conclusion and recommendations

This dissertation investigates the reproductive functionality of common eland bulls, aiming to support their production potentials by providing baseline information that may be used for establishing guidelines in selecting eland bulls for reproduction and breeding purposes.

Based on the findings from this study, the following overall conclusions and recommendations are presented:

a. The reproductive tracts of farmed eland bulls examined in this study were morphologically and histologically functional, with no gross abnormalities, and low asymmetry existed in their bilateral structures. Young bulls at this age (24–29 months) may thus be at a suitable point physiologically for breeding selection.

b. The status of age, body weight, and social hierarchy of eland bulls in this study positively correlated with the development and functionality of some reproductive tract structures, but not horn size and temperament. These parameters may be further evaluated and considered if they may be reliable breeding selection tools or not.

c. Post-harvest recovery of eland epididymal sperm under basic field conditions – without cooling or supporting media – is feasible. Post-harvest timing intervals significantly influence epididymal sperm quality, marked by declining from initial to 35 mins post-slaughter.

Whilst a decline in sperm quality due to the use of basic physiological saline lacking energy substrates and seminal fluid was experienced, as expected, morphological changes seen with cold storage were avoided.

d. Typical EA-fAM secretion patterns in eland bulls during sexual development display fluctuating levels in early stages, followed by elevated concentrations approaching puberty. Despite year-round breeding, seasonal secretion patterns occur, which may affect *e.g.*, semen quality.

Lastly, the following recommendations are listed below for future research endeavours:

a. Potential phenotypic trait should be validated across a larger sample size of common eland, under different conditions, to assess their reliability. Investigating the heritability of important reproductive traits in common eland also warrants further research. Additionally, studies examining the associations between these traits and factors like seasonal variation, androgen metabolite profiles, testicular hemodynamics, and spermatogenesis are essential to further understand these relationships and provide informed effective management strategies and enhance breeding outcomes for eland bulls.

b. Critical handling conditions for epididymal sperm must be evaluated due to their influence on post-harvest characteristics. Standard reference values for sperm quality in common eland are lacking, and while this study offers foundational data, further validation is needed across broader age ranges and larger samples. Future research should also explore trait associations, alternative sperm media, and seasonal effects to support breeding selection and improve management strategies.

c. Physiological and behavioural data should be explored together with monitoring the EA-fAM secretion patterns in common eland males, due to the high individual variation in eland EA-fAM concentrations, this aspect requires deeper investigation to elucidate the underlying factors. The effect of photoperiod needs to be further considered, especially after sexual maturity, and under different localities (*i.e.*, European vs southern African).

Overall, this present study supports the ongoing domestication efforts of common eland as an alternative farming species. Specifically, it provided preliminary reproductive information of eland bulls under semi-intensive conditions *i.e.* early onset of puberty as supported by well-developed reproductive tract morphology, viable epididymal sperm quality, and EA-fAM secretion patterns. However, larger sample sizes, different production conditions, and other confounding factors should be considered for future work in order to establish a criteria for eland bull selection for reproduction and breeding purposes, and application of several assisted reproductive technologies to improve its production.

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