THE MORPHOLOGY AND MORPHOMETRY OF THE MALE REPRODUCTIVE SYSTEM OF RUFOUS SENGI

(Elephantulus rufescens).

BY:

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UNIVERSITY OF NAIROBI.

2009
DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other university.

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DEDICATIONS

This work is dedicated to the sengis, whose relatives were sacrificed to make this work a success.
ACKNOWLEDGEMENTS

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SUMMARY

The Morphology of the male reproductive system of rufous sengi was studied using light and electron microscopy while the testicular morphometry was studied using stereological techniques. The system consisted of cylindrical-shaped testes, genital ducts, accessory sex glands and the penis.

The testes were intra-abdominal, located just caudal to the kidneys and comprised of a parenchyma bound by tunica albuginea. The parenchyma was composed of the seminiferous tubules and the interstitial tissue; the former being more predominant than the later and exhibiting complete spermatogenesis. The interstitial tissue occurred either between the seminiferous tubules, mainly in relatively larger spaces formed when three or four seminiferous tubules approximate one another or beneath the tunica albuginea. The Leydig cells were generally polyhedral with irregular nuclei and had numerous lipid droplets within their cytoplasm but, in cases where the interstitial tissue made extensions into narrow spaces between adjoining seminiferous tubules, the Leydig cells therein were elongate with rod-shaped nuclei.

The testicular arteries branched off from renal arteries and ran caudo-laterally to the testis without convolutions or intimate association with the vein. The testicular veins also followed a straight course, without pampiniform plexuses. These animals had separate right and left caudal vena cavae which received ipsilateral testicular and renal veins. After receiving the renal veins, the left caudal vena cava crossed to the right side to join the right one to form a common caudal vena cava which then extended cranially up to the right atrium.
The genital ducts comprised of the rete testis, efferent ductules, epididymis, ductus deferens and the urethra. The rete testis was made up of interconnecting channels located outside the testicular parenchyma while the efferent ductules connected the rete testis to the caput epididymis. The epididymis consisted of a highly coiled duct organized into three topographic regions; the caput, corpus and cauda epididymis. The caput epididymis was applied on dorso-lateral border, extending from cranial to the caudal pole of the testis. The corpus epididymis extended caudally from the caput to a position between the pelvic urethra and the rectum where it joined the cauda epididymis. The cauda epididymis was organized into a pear-shaped mass, located in a somewhat lateral position between the rectum and the pelvic urethra. The caput and corpus epididymis were lined by a tall pseudostratified columnar epithelium while the cauda epididymis was lined by cuboidal or low columnar epithelium.

The ductus deferens was short, connecting the cauda epididymis to the pelvic urethra. The urethra consisted of two parts; the pelvic and the penile urethra. The pelvic urethra, surrounded by a thick muscular coat, extended from the neck of the urinary bladder to the bulb of the penis and received the ductus deferens, uterus masculinus and the ducts of the accessory sex glands. The penile urethra extended from the bulb to the tip of the penis.

The accessory sex glands consisted of the prostate and bulbourethral glands but without vesicular glands. The prostate gland was made up of several paired lobes organized into two groups; the cranial and the caudal group of lobes, also referred to as the cranial and the caudal prostates respectively. The cranial
prostate consisted of lobes organized around the neck of urinary bladder and included the ventral, latero-dorsal and the medio-dorsal lobes. The caudal prostate consisted of a single pair of lobes located dorsal to the pelvic urethra. The bulbourethral gland consisted of a pair of medio-laterally flattened glands found laterally at about the junction between the pelvic urethra and the bulb of penis.

The mean reference volume of the sengi testis was $0.089 \pm 0.003 \text{ cm}^3$, 98.3% of which was constituted by the parenchyma and the rest being contributed by the capsule. The seminiferous tubules occupied 90.94% of the testicular parenchyma, while the interstitial tissue, on the other hand, occupied about 9.07%. Out of this total interstitial tissue volume, 7.6% was contributed by the subcapsular interstitial tissue.

The morphology of the male reproductive system of the sengi and the pattern of testicular blood vessels was generally similar to that of the African elephant and the rock hyrax, supporting the existence of close phylogenetic relationships between these animals as earlier suggested. Additionally, the pattern of testicular blood vessels suggest that they play no role in testicular thermoregulation. The separate left and right caudal vena cava is most likely due to retention of embryonic pattern to adulthood. The morphometric data on the testis confirmed, with quantification, that the parenchyma of sengi’s testis is predominated by the seminiferous tubules with little interstitial tissue.
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1.1 GENERAL DESCRIPTION OF SENGIS

Sengis, also known as elephant shrews, are small mainly insectivorous mammals found only in Africa (Kingdon, 1974, 2001; Nowak, 1999).

1.1.1 External morphology

These animals have characteristically long, tubular and flexible snouts that taper distally, comparable to those of elephants. Their snouts, which are very sensitive, are movable in a circular manner and have nostrils at their distal ends (Kingdon, 1974, 2001; Nowak, 1999). They have long and slender tails with glands, the sub-caudal glands, on the undersurface near the base. Their ears and eyes are well developed and the legs are long and slender; back legs being longer than the front ones. The feet have five toes except in the genus *Petrodromus* in which the toes are four. Most soft furred sengis are brownish or grey in colour while the giant ones are more colourful with a chequered coat, metallic yellow rumps or reddish orange forequarters and black rumps (Kingdon, 1974, 2001; Rathbun, 1976; Nowak, 1999).

1.1.2 Phylogeny

Sengis or elephant shrews are actually not shrews, and are only distantly related to elephants. They are among the mammals that have had a long
history of misunderstood ancestry. They are not closely related to the members of the order insectivora under which they were earlier classified. They have also mistakenly been associated with ungulates, primates and lagomorphs (Van Valen, 1967; McKenna, 1975).

It has been shown that sengis, along with tenrecs and golden moles don’t belong to the order insectivora (Simons et al., 1991; Springer et al., 1997; Liu et al., 2001). With the use of molecular phylogenetic analysis, it has also been demonstrated that eye lens crystallins of sengis show close similarities to those of paenungulates (African elephants, sea cows and hyraxes) (de Jong et al., 1981, 1993) and that their DNA sequences closely resemble those of paenungulates, tenrecs, golden moles, and aardvarks; all these animals apparently being indigenous to Africa (de Jong et al., 1981, 1993; Springer et al., 1997; Stanhope et al., 1998; Carter, 2001; Liu et al., 2001; Murphy et al., 2001; van Dijk et al., 2001; Arnason et al., 2002; Asher et al., 2003; Carter et al., 2004). These findings strongly supported common ancestry for these animals, and on this basis, their grouping together in a super-ordinal clade called Afrotheria was suggested.

The use of the common name “sengi” as opposed to “elephant shrew” has found preference in order to differentiate the species in this order (Macroscelidea) from the true shrews (family Soricidea) in the order Insectivora and also to point out that they are not elephants (Rathbun, 2005; Rathbun and Kingdon, 2006).
Sengis belong to the order Macroscelidea, which has a single family (Macroscelididae), two subfamilies (Macroscelidinae and Rhynchocyoninae) (see table 1), four genera and fifteen species (Corbet and Hanks, 1968; Kingdon, 1974, 2001; Rathbun, 1976; Corbet, 1995; Nowak, 1999; Myers, 2000).

The genus *Elephantulus* has, with support from cladistic analysis, been known to be a monophyletic group although this has been based on very few synapomorphies (Corbet, 1995). With the use of molecular techniques, it has now been shown that this genus is diphyletic, *E. rozeti* being more different. In fact, these molecular based phylogenies indicated that *E. rozeti* is closer to *Petrodromus tetradactylus* than it is to other species in the genus *Elephantulus* (Douady et al., 2003).

More recently, a potentially new species of giant sengi (genus *Rhynchocyon*) found in Udzungwa mountains of Tanzania was described based on camera trap images, visual sightings and voucher specimens. This potentially new species, unlike the other species in the genus, is diurnal and comparatively larger in size (Rovero et al., 2008).
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<td>E. brachyrhynchus</td>
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<td>Petrodromus</td>
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Table 1. A table showing the grouping of sengi species into subfamilies and genera. The potentially new species from Tanzania is not included.
1.1.3 Distribution

These animals are found only in Africa, distributed throughout most of the east, central, and southern Africa, with an isolated species in north-west Africa. Centres of diversity are within eastern and southern Africa (Kingdon, 1974; Nowak, 1999; Rathbun, 2005). *Macroscelides* species are restricted to southern Africa, while *Rhynchocyon* and *Petrodromus* occur in central and eastern Africa. *Elephantulus* is the most widespread genus, with one isolated species, *E. rozeti* in north-west Africa (Kingdon, 1974, 2001; Nowak, 1999; Rathbun, 2005). In Kenya, sengis are found in Arabuko Sokoke forest, various parts of Taita Taveta district and many other parts of the Coast Province.

1.1.4 Habitat

Sengis live in a variety of habitats, including plains, savannah, bushy country, rocky outcrops, desert and tropical forests (Kingdon, 1974, 2001; Rathbun, 1976; Nowak, 1999). Soft-furred sengis (sub-family Macroscelidinae) are mainly found in shaded but dry environments while giant sengis (sub-family Rhynchocyoninae) favour moist habitats (Kingdon, 2001). Sengis' reliance on cover, shelter or burrows varies, usually residing in burrows that have an emergency exit, ground depressions, rock crevices, under the logs in the forest, crevices of termite mounds and some may use old rodent burrows (Nowak, 1999; Kingdon, 2001). They construct a network of paths which are regularly cleared of litter in the case of *Elephantulus* species (Kingdon, 1974, 2001). Paths of *Petrodromus tetradactylus* have broken appearance, such that
they have regularly spaced "landing pads" of cleared bare earth created as a result of their bounding locomotion (Nowak, 1999; Kingdon, 1974, 2001; Jennings and Rathbun, 2001).

1.1.5 Behaviour and activity patterns

Sengis are mainly diurnal, but are nocturnal during hot weather, moonlit nights and when harassed by diurnal predators (Nowak, 1999). Soft-furred species may normally be crepuscular, showing some activity both in the day and at night (Rathbun, 1976). They exhibit grooming behaviour such as scratching and licking and in a typical mammalian fashion, they stretch and yawn after rest or sleep (Rathbun, 1976). Although vocalization is not common (Rathbun, 2005), species in the genus *Elephantulus* and *Rhynchocyon* squeak while *Petrodromus* make cricket-like calls (Nowak, 1999). Most species foot-drum (*Petrodromus* and *Elephantulus* species) or tail-flap (*Petrodromus* and *Rhynchocyon*) in stressful situations (Rathbun, 2005). They mark their territories with secretions from sternal and sub-caudal scent glands (Kingdon, 1974).

1.1.6 Food habits

Invertebrates form the main diet of sengis, especially the ants and termites. The other food items include earthworms, beetles, millipedes, centipedes, spiders and a number of members of the order *Orthoptera* (grasshoppers, crickets). Soft-furred species supplement their invertebrate diet with small fruits, seeds and green plant matter (Rathbun, 1976). Sengis posses a
functional caecum, something not found in most insectivorous mammals (Jones, 2002).

These mammals mainly forage in the leaf litter during which they walk slowly pocking their noses in and out of the litter, flushing out the insects. Some perform soil excavation and ingestion of food is mainly done with a flick of the tongue, which can be extended beyond the nose (Rathbun, 1976).

1.1.7 Breeding

These animals exhibit monogamy, forming pairs of a male and a female which live in a common territory, each defending the territory from other members of its own sex. Exception is found in some species of *Elephantulus* that may live in small colonies (Nowak, 1999). The rufous sengis breed throughout the year, with the gestation period of two months, giving birth to 1 – 2 precocial young ones (Rathbun, 1976; Awaad, 2002). In South Africa, the rock sengi (*Elephantulus myurus*) has been reported to exhibit seasonal variation in seminiferous tubule diameter, being lower in the months of April to June compared to the rest of the year (Woodall and Skinner, 1989).

1.1.8 Adaptations

Sengis have long and narrow tongues that enable them to gather small food items rapidly. Their functional caecum is most likely used for water resorption as a means of conserving the scarce resource. They have powerful hind limbs that aid in making rapid bounding locomotion away from predators. Their
vocalization and foot drumming serves as deterrence to predators and also acts as an alarm call. Giving birth to highly precocial young ones is an adaptation to minimize susceptibility of their offsprings to predators. Besides territory marking, the musky products from their sub-caudal glands serve to make them unsavory to many potential carnivorous predators. Soft-furred species in particular have coat colours that match with the local soils, and this aids in camouflage (Kingdon, 2001).

1.1.9 Predation and conservation

The main predators include birds, snakes, monitor lizards and mongoose (Kingdon, 1974, 2001; Rathbun, 1976; Awaad, 2002). The coastal people, for example the Giriama in the Kenyan coast, hunt them for food (Kingdon, 1974; Rathbun, 1976). Most of the species are not endangered but a few are threatened due to habitat destruction (Kingdon, 2001; Arkive web, 2004). The endangered species are; *Rhynchocyon chrysopygus*, *R. petersi* and *E. revoilii*, while the vulnerable ones are; *R. circnei*, *M. proboscideus*, *E. edwardii* and *E. rupestris* (I.U.C.N - SSC Afrotheria Specialist Group, 2002). The rufous sengi is generally not threatened (FitzGibbon et al., 2008).
CHAPTER 2

LITERATURE REVIEW

2.1 THE MALE REPRODUCTIVE SYSTEM

The male reproductive system consists of gonads, genital ducts, accessory glands and the copulatory organ (the penis). Studies on the reproductive system of the sengis are generally scarce, focusing mainly on the female with the male system remaining largely ignored. In females, studies focused on the placenta and fetal membranes of *Elephantulus* (van der Horst, 1950; Oduor-Okelo et al., 1980), *Rhynchocyon chrysopygus* (Oduor-Okelo, 1984, 1985) and *Petrodromus tetradactylus* (Oduor-Okelo et al., 2004).

Gross anatomical description of the male reproductive system in some species of sengi dates way back to 1852 (Tripp, 1970). A study by Tripp (1970) on the male reproductive system of three species in the genus *Elephantulus* (*E. myurus*, *E. intufi* and *E. rozeti*) and *Petrodromus tetradactylus* did not delve into detailed structural examination probably because the focus of the study was on the female.

2.1.1 The testis

Testes or male gonads are usually paired organs whose sizes, shapes and locations vary among various groups of mammals. They develop retroperitoneally in the dorsal wall of the abdominal cavity and then migrate (descend) in majority of mammals, into the scrotum while in others, the
testiconda, they are retained within the abdomen. In the sengis, testes are intra-abdominal, suspended just caudal to the kidneys (Asdell, 1964; Woodall, 1995). They are generally small, with a mean mass for both of them being 0.08g in *E. myurus* during the months of October to December (Woodall, 1995). This is only 12% of the average testis weight for a mammal of this size, perhaps only equaled by *Notomys ssp.* and the Gorilla (*Gorilla gorilla*) (Woodall, 1995).

A mammalian testis generally consists of a parenchyma enclosed by a capsule. In scrotal mammals, the testicular capsule consists of an outer layer of visceral peritoneum (epiorchium), a middle tight fibromuscular layer or tunica albuginea and an inner vascular layer also called tunica vasculosa (Middendorff et al., 2002). Connective tissue septae (septuli testis) connects tunica albuginea with the mediastinum testis within which are the rete testis. The septuli testis divides the organ into lobules and forms support for passage of blood vessels, lymphatics and nerves to the interior of the organ.

The position and degree of development of the mediastinum testis varies in different species of vertebrates (Dhingra, 1977; Fawcet, 1986). Like the mouse, porpoise and fin whale, sengi have no fibrous mediastinum (Stoch, 1954; Dhingra, 1977).

The testicular parenchyma, divided into lobules by septuli testis, consists of seminiferous tubules and the interstitial tissue. The relative volume occupied
by either of these show variation among various groups and species of mammals (Fawcett et al., 1973; Onyango et al., 1993).

2.1.1.1 The seminiferous tubules

The seminiferous tubules are highly convoluted tubes connected to the rete testis at both ends by short straight segments called the tubuli recti, whose epithelia are devoid of germ cells (Jones et al., 1979; Fawcett, 1986). The wall of seminiferous tubules consists of the seminiferous epithelium and the boundary tissue (lamina propria). The seminiferous epithelium in turn consists of two types of cells; the somatic or supportive (Sertoli) and the germ (Spermatogenic) cells, which are partially enveloped by the former.

The Sertoli cell is a tall polymorphic cell that rests on basal lamina and spans the entire height of the epithelium. It sends ramifications that partially envelop the spermatogenic cells (Courot et al, 1970; Russell et al., 1990). Closer to the base of the epithelium, contacts between neighbouring Sertoli cells form tight junctions that constitute the most important component of the blood-testis barrier (Dym and Fawcett, 1970; Fijak and Meinhardt, 2006) and divide the seminiferous epithelium into two compartments; the basal and adluminal compartments (Dym and Fawcett, 1970). Contained in the basal compartments are the spermatogonia, preleptotene spermatocytes (Dym and Fawcett, 1970) and also leptotene and zygotene spermatocytes (Fijak and Meinhardt, 2006) while the more advanced spermatocytes and spermatids are contained in the adluminal compartment. The role of these inter-Sertoli cell
tight junctions that compartmentalize the seminiferous epithelium is to restrict passage of substances from the testicular interstitial tissue and, indeed from the blood, to the adluminal compartment of the seminiferous epithelium.

The Sertoli cell nucleus is generally basal in location, irregular and sometimes has deep indentation(s) (Courot et al., 1970; Russell et al., 1990). The cytoplasm has abundant rough endoplasmic reticulum, well developed Golgi apparatus, mitochondria with tubular cristae, clusters of ribosomes and fairly prominent microtubules. Contributions of Sertoli cells towards the functions of seminiferous epithelium are both enormous and important. Besides protection of the advanced germ cells through the formation of blood-testis barrier, the Sertoli cells play many other roles, some of which include; maintenance of structural organization and integrity of seminiferous epithelium, metabolic and morphological support of germ cells, secretion of fluid, hormones and androgen binding proteins, phagocytosis of residual bodies and degenerating germ cells, and co-ordination of spermatogenesis (Courot et al., 1970; Russell et al., 1990; Bardin et al., 1993). During the embryonic life, Sertoli cells play an important role of directing the genitalia of genetically male fetus towards masculinization through production of Mullerian inhibiting substance/hormone.

The germ cell component of seminiferous epithelium consists of several generations of spermatogenic cells. These cells include the spermatogonia, spermatocytes and spermatids. Spermatogonia are situated at the basal compartment of the seminiferous epithelium with one surface flattened on the basal lamina (Russell et al., 1990), and are of several types (Meistrich and van
Beek, 1993; Dym, 1994; de Rooij, 2001). The major types of spermatogonia are type A, Intermediate and type B spermatogonia. Type A spermatogonia have several subsets (de Rooij, 2001) which can be grouped as undifferentiated type A and differentiating type A spermatogonia (Meistrich and van Beek, 1993; Meachem et al., 2001). Among the undifferentiated type A spermatogonia are the spermatogonial stem cells from which all other cellular elements of spermatogenic lineage arise, through the process of spermatogenesis. With advances in stem cell research, focus on spermatogonia has grown remarkably leading to enormous progress in development of tools and techniques for research and life applications of spermatogonial stem cells; principal among them being the spermatogonial/germ cell transplantation (Ogawa et al., 1999; McLean et al., 2001; Meachem et al., 2001; Gosden and Nagano, 2002; Shinohara et al., 2001, 2002, 2006; Oatley et al., 2002; Matzuk, 2004; Dobrinski, 2006; Hill and Dobrinski, 2006; Ryu et al., 2007).

It is often difficult to differentiate between the various types of spermatogonia (Russell et al., 1990). Generally, subsets of differentiating spermatogonia may be distinguished by the amount of flakes of heterochromatin lying along the inner surface of nuclear envelope. On that basis, type A spermatogonia, which often have a visible nucleolus, have pale stained nuclei with granular or dust-like chromatin. They essentially lack these flakes of chromatin, whose amount increases with differentiation through intermediate to type B spermatogonia (Russell et al., 1990; Meistrich and van Beek, 1993).
increases with differentiation through intermediate to type B spermatogonia (Russell et al., 1990; Meistrich and van Beek, 1993).

Spermatogonia that arise from stem cell spermatogonia (a subset of type A spermatogonia) proliferate through mitosis to giving rise to successive stages of spermatogonial lineage, the intermediate and type B spermatogonia. As spermatogenesis proceeds, the spermatogonia move away from the basal lamina and, together with their nuclei, take up a rounded form. The most mature type B spermatogonia divide to produce primary spermatocytes, which then undergo the first meiotic division to produce secondary spermatocytes (Russell et al., 1990). Secondary spermatocytes undergo a rapid second meiotic division to produce spermatids. Through several phases of spermiogenesis, spermatids differentiate to form spermatozoa (Russell et al., 1990; Clermont et al., 1993). Spermatids, the products of second meiotic division of spermatocytes, are antigenically different from the rest of the body cells and are therefore highly immunogenic. These cells are, however, not attacked by the body's immune system due to the physical protection offered by the restrictive blood-testis barrier (Dym and Fawcett, 1970) and also due to immunotolerance imparted by very high testicular levels of androgens (Head and Billingham, 1985; Fijak and Meinhardt, 2006).

The lamina propria (boundary tissue) forms the outermost layer of the seminiferous tubule and it separates it from the intertubular tissue. It is made up of connective tissue, fibroblasts and myoid (peritubular contractile) cells. (Courot et al., 1970; Steinberger and Steinberger, 1977; Fawcet, 1986;
Russell et al., 1990; Junqueira and Carneiro, 2003). Besides maintaining structural integrity of the seminiferous tubules (Maekawa et al., 1996), contractions of myoid cells, which are regulated by Sertoli cells (Santiemma et al., 1996; Tripiciano et al., 1996), facilitate transport of sperms along the seminiferous tubule. Myoid cells also influence the secretion of ABP and other substances by the Sertoli cell (Tung and Fritz, 1980; Zwain et al., 1993).

2.1.1.2 The interstitial tissue

The interstitial tissue occupies the spaces between the seminiferous tubules. It is composed of Leydig cells, connective tissue, macrophages, blood vessels and lymphatics (Fawcett et al., 1973; Connell and Connell, 1977; Yee and Huston, 1983; Russell et al., 1990). In addition, infrequent fibroblasts, a few lymphocytes, occasional mast cells and some relatively undifferentiated cells of mesenchymal origin also occur (Fawcet, 1986).

Organization and quantities of various testicular interstitial tissue components show variations among various mammalian species (Fawcett et al., 1973). In some, like the African elephant and the hyrax which are also true testiconda, the interstitial tissue has compact clusters of lipid-rich Leydig cells scattered in abundant loose areolar tissue (Fawcett et al., 1973; Neaves, 1973). Other species such as the zebra, domestic boar, warthog and the naked mole rat have very large volume of interstitial tissue (Fawcett et al., 1973). In the non-breeding naked mole rat, Heterocephalus glaber, there is extreme predominance of the interstitial tissue such that the seminiferous tubules are
The Leydig cells are large polyhedral epithelioid cells that occur as clusters and sometimes singly in the intertubular tissue of the testis. It has a single eccentric spherical or ovoid nucleus with one or up to three eccentric nucleoli (Hooker, 1970). It also has chromatin granules distributed mainly towards the periphery of the nucleus. Typical of steroid secreting cells, the cytoplasm of Leydig cells has abundant agranular endoplasmic reticulum, mitochondria and lipid droplets. The numbers and sizes of Leydig cells show interspecies differences (Hooker, 1970). Leydig cells produce most, if not all, of the testicular androgens, principally the testosterone most of which usually binds to a Sertoli cell secreted protein, the androgen binding protein (ABP), which facilitates crossing of testosterone from interstitial tissue into the seminiferous tubules. Although required locally for maintenance of spermatogenesis, testosterone usually hyperconcentrates within the testis to levels far much higher than needed for this purpose. It has since been hypothesized that high intratesticular testosterone greatly contribute to the immune privilege of the testis via local immunosuppression (Head and Billingham, 1985; Fijak and Meinhardt, 2006) thereby imparting immunotolerance of antigenically unique spermatids and spermatozoa.

The testicular macrophages are confined to the interstitial tissue in close association with the Leydig cells (Yee and Huston, 1983; Russell et al., 1990). Besides the immunological role (Wei et al., 1988), these cells also produce paracrine factors that act on Leydig cells to influence their differentiation (Mendis-Handagama and Ariyaratne, 2001; Chen et al, 2002) and stimulates
testosterone production (Sun et al., 1993; Kern et al., 1995; Hutson et al., 1996). The Leydig cells on the other hand exert a paracrine negative feedback to regulate testicular macrophage secretion (Lukyanenko et al., 2002).

2.1.1.3 The testicular blood supply

In scrotal mammals, each testicular artery generally arises from the abdominal aorta and exits from the abdominal cavity through the inguinal canal to reach the respective testis via the spermatic cord (Crouch, 1969; Setchel, 1970; Nickel et al., 1981). The testicular vein, on the other hand, exits from the testis then approximately retraces the course of the artery to drain into the caudal vena cava. In some species, the right and left testicular veins show asymmetry where the right vein drains into the caudal vena cava and the left one into the renal vein (Greene, 1969; Crouch, 1969; Kent, 1978). Along the spermatic cord, the testicular vein forms a network, the pampiniform plexus, which is intimately associated with the highly convoluted testicular artery (Crouch, 1969; Greene, 1969; Nickel et al., 1981). In ascrotal mammals such as the monotremes, edentates, cetaceans and chiropterans, the testicular arteries run from the abdominal aorta to the testis while the veins run from the testes to either the caudal vena cava (Setchel, 1970; Kallen, 1977; Rommel et al., 1991, 1994) or the renal veins (Short et al., 1967; Setchel, 1970).

The mammalian caudal vena cava is usually a single large median vein which starts caudally after the union of the two common iliac veins at the level of distal lumbar vertebrae then extends cranially up to the right atrium, receiving all the veins draining the pelvic and abdominal viscera, kidneys and gonads
included (Kent, 1978; Nickel et al., 1981; Walker and Liem, 1994; Moore and Persaud, 2003). It develops from the cardinal venous network that comprises the veins that drain the hind limbs, the tail and the caudal parts of the trunk of a mammalian embryo. This network consists of the three pairs of veins that develop at different times during embryonic life, namely; the caudal (posterior) cardinal, subcardinal and supracardinal veins (Kent, 1978; Noden and de Lahunta, 1985; Walker and Liem, 1994; Sadler, 1990; Moore and Persaud 2003). With further development, these primordial veins undergo a series of changes that, with incorporation of the right hepatic vein (proximal segment of the right vitelline vein), generally results in the formation of the definitive single median large vein, the caudal vena cava, which starts caudally after the union of the two common iliac veins at the level of distal lumbar vertebrae and then extend cranially up to the right atrium (Kent, 1978; Nickel et al., 1981; Walker and Liem, 1994; Moore and Persaud 2003). Based on contributions by the primordial veins, the caudal vena cava can be considered to be made of four segments, namely; the hepatic, pre-renal, renal and post-renal segments, derived from the right hepatic, right subcardinal, sub-supracardinal anastomosis and the right supracardinal veins respectively.

2.1.2 The genital ducts

The mammalian genital ducts, also referred to as the excurrent duct system, consists of a series of ducts linking the testis, in particular the seminiferous tubules to the exterior. They therefore convey sperms and semen to the
female reproductive tract with parts that play a role in storage and maturation of sperms.

2.1.2.1 The rete testis

Rete testis consists of a network of channels usually found within mediastinum testis, connected by tubuli recti to both ends of each seminiferous tubule. They are lined by a single layer of squamous epithelial cells with irregular nuclei and paucity of organelles (Jones et al., 1979; Fawcett, 1986). In some animals, occurrence of a single flagellum on each epithelial cell has been reported (Jones et al., 1979; Aire and Soley, 2003). The rete testis form a link between the tubuli recti and the efferent ductules and the lining epithelial cells are capable of uptake of substances from the lumen for disposal by their lysosomal system (Aire and Soley, 2003). The androgen-rich rete testis fluid, which differs from that of the seminiferous tubules (Aire and Soley, 2003), stimulates oxygen uptake by spermatozoa (Voglmayr and White, 1979) and is required for normal functioning of the efferent ductules (Gray et al., 1983). The sengi, like the mouse, porpoise and fin whale, have superficial rete testis confined to a small area of the testicular capsule (Stoch, 1954; Dhingra, 1977).

2.1.2.2 The efferent ductules

Efferent ductules emerge from rete testis, and then exit from the testis by perforating the tunica albuginea to join the caput epididymis. They are lined by a simple low columnar or cuboidal epithelium composed of principal and
ciliated cells with occasional leucocytes (Hotter and Greenberg, 1978; Jones et al., 1979; Jones and Brossman, 1981; Oke et al., 1988). The non-ciliated cells which are also referred to as the principal or absorptive cells, have basal ovoid nuclei, apical microvilli and can be classified into three types; type I, II and III. Type I cells are thought to serve as stem cells for both types II and III. Type II cells have PAS-positive granules while the type III have PAS-negative vacuoles (Goyal et al., 2000). Available reports on sengis show that efferent ductules are lined by a low columnar epithelium (Stoch, 1954).

2.1.2.3 The epididymis

The mammalian epididymis is made up of a single highly convoluted tube/duct which forms a compact mass that is topographically divided into three regions; the caput, corpus and cauda epididymis. On finer details, the epididymis can further be divided into several regions, the number of which varies between species (Hoffer and Greenberg, 1978; Jones et al., 1979; Goyal and Williams, 1991). It is lined by a pseudo-stratified columnar epithelium with at least two basic cell types; the principal cells, which are tall columnar cells with long branched microvilli often referred to as stereocilia (Fawcett, 1986; Junqueira and Carneiro, 2003) and the basal cells. The other cell types, whose presence within epididymal epithelium show variation between species and regions of epididymis are the intraepithelial leucocytes, apical cells and clear cells (Hoffer and Greenberg, 1978; Goyal and Williams, 1991; Jones et al., 1979). The epididymis performs the functions of transportation, storage, and maturation of spermatozoa (Orgebin-Crist, 1969; Setiadi et al., 1997; Jones, 1999). Its role
as a site for maturation of spermatozoa requires inputs from various hormones including the androgens and oxytocin (Tekpetey et al., 1989; Veeramachaneni and Amann, 1990; Oduma et al., 1994; Sirivaidyapong et al., 2001).

The epididymis of sengis is long, extending from the cranial pole of the testis to the pelvic urethra just caudal to the urinary bladder (Stoch, 1954; Woodall, 1995). The caput extends from the cranial to the caudal pole of the testis then continues caudally as a coiled corpus epididymis. The corpus epididymis terminates at the cauda epididymis, which consists of a coiled tube located between the rectum and the urethra (Tripp, 1970; Woodall and Skinner, 1989).

2.1.2.4 The ductus deferens

The mammalian ductus deferens is generally a long tubular distal continuation of cauda epididymis with a narrow lumen that has longitudinal folds, lined by a pseudostratified columnar epithelium and surrounded by a thick muscular coat. In scrotal mammals, it forms part of the spermatic cord. In many species such as the rat, the distal part of the ductus deferens thickens and dilates into ampulla which has a thick and extremely folded mucosa. Beyond the ampulla, the ductus deferens decreases in diameter and thickness before terminating at the dorsal wall of the pelvic urethra, either independently or after joining with the excretory duct of the vesicular gland to form ejaculatory duct (Hamilton and Cooper, 1978). In the sengis, the ductus deferens is short and has a highly muscular wall (Woodall and Skinner, 1989; Woodall, 1995) and connects the cauda epididymis to the pelvic urethra.
2.1.2.5 The urethra

The urethra is a relatively large duct characterized by longitudinal mucosal folds with a dual role of conveying both the urine and semen. It can be divided into two parts; the pelvic and penile urethra (Fawcett, 1986; Pinheiro et al., 2003). The pelvic urethra extends from the neck of the urinary bladder to the bulb of the penis and it can further be divided into two parts. The proximal segment of the pelvic urethra surrounded by the prostate gland and which receives ejaculatory ducts is called the prostatic urethra while the distal part extending from the caudal pole of the prostate gland to the bulb of the penis is the membranous urethra. The penile urethra, also referred to as the foamy urethra extends from the bulb of the penis to the tip.

The pelvic urethra is generally made up of the mucosa surrounded by a thick muscular coat while the penile urethra is surrounded by corpus cavernosum urethrae (corpus spongiosum). The prostatic urethra is lined by transitional epithelium while the membranous and penile urethra are lined by pseudostratified columnar epithelium (Fawcett, 1986; Pinheiro et al., 2003). The mucous membrane of the urethra also has recesses (lacunae of Morgagni) into glands of litre which are especially well developed on the dorsal surface of penile urethra.

2.1.3 The accessory sex glands

In mammals, there is interspecies variation in the organization and structure of the accessory sex glands as well as the constituent gland types. These glands
include the vesicular, prostate, and bulbourethral (Cowper’s) glands. The available information indicates that sengis have the prostate and bulbourethral glands but lack the vesicular gland (Tripp, 1970).

### 2.1.3.1 The prostate gland

The gross arrangement of the adult prostate gland shows great variation among various groups of mammals. In some animals, for example the primates, the prostate gland does not show obvious lobation but in others like the rodents, it is made up of distinct lobes which show morphological, physiological and chemical differences (Brandes, 1974; Burger et al., 2005; Suzuki et al., 2007). In the rat, the prostate gland is composed of three pairs of anatomically distinct lobes and a coagulating gland, which is also known as the anterior prostate. These lobes are; the ventral, dorsal, and lateral lobes (Dahl et al., 1973; Brandes, 1974; Suzuki et al., 2007). The lobes are made up of alveolar glands lined by a simple columnar epithelium and each acinus is surrounded by a fibromuscular coat (Dahl et al., 1973). The epithelial cells show regional differentiation of cytoplasm into five regions namely; the apical pole, Golgi zone, supranuclear, nuclear and basal regions (Brandes, 1974). The luminal borders of the cells have microvilli which are well developed into brush border in the lateral lobe (Dahl et al., 1973).

The prostate gland has been studied in a number of sengi species, including *Elephantulus myurus, Elephantulus intufi, and Elephantulus rozeti* (Stoch, 1954; Tripp, 1970) albeit without any ultrastructural details. The prostate gland
of sengis is lobated and the organization and the number of lobes seem to differ between species (Tripp, 1970; Woodall, 1995). Based on available information, it is apparent that the prostate gland of rufous sengi has not been studied.

2.1.3.2 The bulbourethral gland

In sengis, a pair of bulbourethral (Cowper’s) gland is connected to the dorsal surface of the urethra just caudal to the prostate glands by long ducts (Tripp, 1970; Woodall, 1995). They are alveolar glands lined by squamous to columnar epithelium. These glands are surrounded by a thick capsule of striated muscles that project inwardly to divide the gland into lobules (Tripp 1970; Woodall, 1995). Seminal vesicles are absent in sengis (Tripp, 1970).

2.1.4 The uterus musculinus

The uterus musculinus in sengis, as in many other animals, is a blind ending rudiment of the Mullerian duct situated between vasa deferentia and shows species variation. In *E. rozeti*, it is single-lobed and contains many leucocytes while in *E. myurus, E. intufi and Petrodromus*, it is bilobed with a lumen filled with eosinophilic secretion in *E. myurus* (Tripp 1970). The Uterus musculinus is lined by a tall columnar epithelium (Woodall, 1995) and it is connected to the urethra by a narrow duct, the vagina musculinus, which is non-patent in *E. intufi* (Tripp, 1970).
2.1.5 The penis

In the sengi, the penis is very long, lacks baculum (Tripp, 1970) and runs cranially under the ventral skin to emerge just caudal to the sternum (Woodall, 1995). This extreme elongation of the penis under the abdominal skin is not found in other mammals (Woodall, 1995). When flaccid, the penis of *E. myurus* is 5 cm long and 1 mm wide while that of *P. tetradactylus*, is 5.5 cm long and 2 mm wide (Tripp, 1970). The distal half of the penis lies in a preputial sheath, hence, it is termed the glans penis (Woodall, 1995). Sengis lack preputial glands (Tripp, 1970).

The distal end of the glans penis has a structure that is characteristic of each genus. It is tri-lobed in the genus *Petrodromus*, has a distinct “collar” in the genus *Macroscelides*, a serrate ridge in *Rhynchocyon* and it is bi-lobed in the genus *Elephantulus* except in *E. rozeti* (Tripp, 1970; Woodall, 1995; Douady et al., 2003). *E. rozeti*, unlike other members of the genus *Elephantulus*, has a tri-lobed glans penis that resembles that of *Petrodromus*; lateral lobes being directed forward as a fork-shaped structure whilst being connected to the central shaft by a membrane (Tripp 1970; Douady et al., 2003). The structure of the glans penis agrees with molecular studies in suggesting close relationships between *E. rozeti* and *Petrodromus tetradactylus*. 
Morphometry is the quantification of parameters of morphological structures while stereology, which is one of the morphometric techniques, is a body of mathematical methods relating three-dimensional parameters defining a structure to two-dimensional measurements obtainable on sections of the structure. Using stereology, parameters of the structure being studied such as size/volume, length, surface area and number can be measured (Mayhew, 1991). Design-based, hence assumption-free stereology has become the technique of first choice whenever three-dimensional structural quantities need to be extrapolated from planar measurements performed on two-dimensional slice images (Mayhew, 1992; Mayhew and Gundersen, 1996). In design-based stereology, measurements of the structure are based on sound sampling designs and not on the shape or geometry of the structure under investigation. Model-based stereology, on the other hand, takes into account the shape/geometry of the structure under study.

Stereology has been used to quantify various testicular components in a number of animal species including the hamster (Sinha-Hikim et al., 1988), young and old men (Petersen and Pakkenberg, 2000), donkeys and mules (Neves et al., 2002) and the domestic cat (Franca and Godinho, 2003). Although it has been pointed out that sengis have little interstitial tissue in their testes with few interstitial cells, quantification of these parameters in the rufous sengi has not been done.
2.3 AIMS AND OBJECTIVES

Morphological information on various parts of the male reproductive system of the rufous sengi is scanty and, moreover, the little that is available does not provide detailed account of histological and ultrastructural analysis from which reliable inferences can be made. This study aims to bridge this gap by providing detailed information on histological and ultrastructural characteristics of the male reproductive system of this animal.

In addition, this study aims at providing stereological measurements of testicular parameters of this animal in order to clarify information from previous studies on the qualitative evaluation of the testis, which reported that testes of sengis have little interstitial tissue with few interstitial cells. This study therefore aims to illustrate and quantify these parameters in the rufous sengi.

2.4 JUSTIFICATION

Sengis can be used as animal models for studying spermatogenesis in true testiconda. In this regard therefore, it is necessary that the structure of the testis, epididymis and the rest of the male reproductive tract is studied in details to provide the basic information and probably the leads upon which studies on spermatogenesis could be based.

Reproductive parameters such as placentation (Carter, 2001; Carter et al., 2004), spermatogenic cycle (Ojoo et al., 2005), sperm morphology (Jamieson, 1995; Robson et al., 1997) and accessory sex glands (Bedford et al., 1998)
are increasingly finding use in phylogenetic studies. It is apparent that in sengis, the structure of the male reproductive system is a very important feature in suggesting their phylogenetic relationships (Douady et al., 2003). This study therefore provides important morphological information that can be used to determine the phylogenetic ranking of sengis alongside other mammals. When compared with available morphological data from other species in the super order Afrotheria, morphological details from this study can offer further support to the classification of sengis under super-order Afrotheria as suggested by molecular phylogeneticists.

Information on spermatogenesis and testicular function in the African elephant and many other larger, late maturing animals is scanty, mainly because of the high costs involved in carrying out such studies, the long time it takes for testis to mature, lack of models for such studies and their restricted use for research particularly the African elephant, due to high conservation attention they receive in many countries, Kenya being in the forefront. Through xenografting of testis fragments of late maturing animals such as primates (Honaramooz et al., 2004) and cat (Snedaker et al., 2004) into early maturing animals such as mice, or transplantation of spermatogonia into seminiferous tubules of appropriate early maturing hosts (Ogawa et al., 1999; Honaramooz et al., 2002; Shinohara et al.; 2006), these limitations have been bypassed. From these studies, it has been shown that xenografting of either fresh or cryopreserved neonatal testis fragments and spermatogonial transplantation results into early maturation and onset of donor based spermatogenesis. From
studies on spermatogonial transplantation, it was noted that the success in colonization and establishment of donor-based spermatogenesis depended on phylogenetic closeness between the donor and the host (McLean et al., 2001). Owing to their similarity in testes location and their emergent relatively close phylogenetic relationship, sengis could serve as excellent models to be used as early maturing and handy recipients or hosts for elephant testis fragments or spermatogonia. This study therefore serves to stimulate interest and focus towards sengis as possible models for studying the male reproductive biology of African elephants.
CHAPTER 3

MATERIALS AND METHODS

3.1 ANIMALS

Two batches each consisting of five male rufous sengis (*Elephantulus rufescens*) were trapped in the months of July 2007 and May 2008 from the plains around Voi town of Taita district in Kenya, where these animals occur in abundance. The animals were then transported to the laboratory in the Department of Veterinary Anatomy and Physiology at Chiromo campus of the University of Nairobi, where the study was conducted. Experts, from the National Museums of Kenya (Mammalogy section) and from the Institute of Primate Research (IPR), were consulted for confirmation of the species of the animal under study and for identification of the parasites that infested the body wall and viscera of these animals respectively.

3.2 ANAESTHESIA AND FIXATION

Each animal was anaesthetized using diethyl ether then weighed. The abdominal and thoracic cavities were then opened along the ventral midline after which the heart was localized.

Intra-cardial perfusion was done through a cannulated left ventricle connected to a fixative container raised to attain a pressure head of 120cmH₂O and the perfusate drained through a slit made on the right atrium. Before perfusion with the fixative, the animal was first flushed with physiological saline through
the canula on the left ventricle. The saline was allowed to perfuse by gravitational force to clear the blood from the tissues. It was then replaced with the fixative, 2.5% glutaraldehyde in 0.1 M phosphate buffer in all but two animals in the first batch where saline was replaced by 10% formalin. These fixatives were allowed to perfuse by gravitational force for 30 minutes. After perfusion, the male reproductive organs were each dissected out and further immersed into the respective fixative.

3.3 TISSUE PROCESSING

Tissues from glutaraldehyde fixed animal specimens were processed for epon embedding while those fixed with formalin were processed for paraffin wax embedding.

Tissues for epon embedding were cut into smaller pieces of approximately 1 mm³, and for each tissue, three such smaller pieces (blocks) were selected randomly and further processed for electron microscopy. The selected tissue blocks were rinsed in phosphate buffer and then post-fixed in 1% osmium tetroxide in saline for two (2) hours after which they were rinsed with 0.1 M phosphate buffer. The specimens were then dehydrated in ascending concentrations of ethanol, three changes of fifteen minutes each, starting with 50%, 70%, 80%, 90% and finally in absolute ethanol. The dehydrated specimens were cleared using two, thirty-minute changes of propylene oxide, infiltrated with epoxy-resin mixture and finally embedded in epoxy-resin.
mixture with accelerator. Tissues were then kept overnight in an oven for curing.

Tissues for histology were dehydrated in a series of graded concentrations of ethanol, cleared with methyl benzoate then infiltrated and embedded in molten paraffin wax.

### 3.3.1 Tissue processing for electron microscopy

The epon embedded tissue blocks were first used to obtain semi-thin sections using Reichert® ultra-microtome with glass knives. The sections were then stained with toluidine blue and examined under a light microscope to select the area of interest /rich area (Hayat, 1970). Some analyses were also done using these semi-thin, toluidine blue stained sections. Ultra-thin sections were obtained from the selected rich area using Reichert® ultra-microtome, picked with copper grids, stained with 5% uranyl acetate and counterstained with 0.5% lead citrate. These sections were again rinsed in saline, allowed to dry and examined under a Philips 201C Transmission Electron Microscope.

### 3.3.2 Tissue processing for histology

Paraffin wax embedded tissue blocks were mounted on wooden blocks and 5μm thick sections obtained using a Leitz Wetzlar® rotary microtome. The sections so obtained were mounted on microscope slides, deparaffinized then rehydrated by passing the sections through descending concentrations of ethanol (100%, 90%, 70%, 50%, 20%) and finally into water. The rehydrated
sections were stained with Periodic Acid Schiff (PAS) (Drury et al., 1967) and then examined under Zeiss Axioskope® light microscope. The photographs were taken either using Zeiss MC 80 DX camera (Zeiss, Germany) connected to Zeiss Axioskope® light microscope or through the eyepiece of the above mentioned light microscope using Kodak® C743 zoom digital camera.

3.4 Testis morphometry

Morphometric analysis was conducted using the right testes of the five animals trapped in the month of May 2008.

3.4.1 Sampling and processing of testes for morphometry

The reference volume \([V(\text{ref})]\) of each testis was estimated using Scherle (water displacement) method (see Makanya et al., 2004) and the body mass - normalized testis volumes obtained by calculating the \(V_{\text{ref}} : \text{body mass ratio}\). Each testicle was cut into four slabs of approximately equal thickness, each of which was further cut into two equal slices (Fig. 1). Three slices were selected at random and each further cut into half slices. From the half slices, three were selected at random and processed for epon embedding (see 3.3 above).

The selected epon-embedded quarter slabs from each testis, named blocks 1 to 3, were serially sectioned and an average of five semi-thin sections picked from each embedded quarter slab at an interval of not less than five serial sections. All the sections picked were stained with toluidine blue then mounted with cover slips. From each embedded quarter slab, two stained sections were
selected at random and examined under a light microscope at X200 magnification. From the selected sections, two fields were selected at random, each of which was photographed through the eyepiece using Kodak® C743 zoom digital camera. The photographs were then uploaded to a computer as jpg images. Each of the images was opened with corel photopaint® X3 computer software which displayed the image on a monitor and set to a standard width of 150mm.

Stereological techniques were used to estimate the relative densities of various testicular components at light microscopy using point counting method where a grid of 5mm squares was generated and superimposed on the image (Howard and Reed, 1998; Neves et al., 2002). Intersections between the grid lines were considered as points and those lying or hitting on the entire surface of the section’s image, also called the reference space \([\Sigma pi(ref)]\), were counted. The points were then grouped according to the testicular structures they hit, that is, those hitting on the seminiferous tubules \([\Sigma pi(ST)]\), interstitial tissue \([\Sigma pi(IT)]\), sub-capsular interstitial tissue \([\Sigma pi(sIT)]\), and tunica albuginea \([\Sigma pi(t.al)]\).

The respective volume densities, \(Vv()\), were then estimated using the equations below:

\[
Vv(ST) = \Sigma pi(ST) / \Sigma pi(ref)
\]

\[
Vv(IT) = \Sigma pi(IT) / \Sigma pi(ref)
\]
\[ V_V(sIT) = \frac{\Sigma pi(sIT)}{\Sigma pi(ref)} \]

\[ V_V(t.al) = \frac{\Sigma pi(t.al)}{\Sigma pi(ref)} \]

The absolute volumes of the seminiferous tubules, \( V(\text{St}) \), and the interstitial tissue, \( V(\text{It}) \), were calculated using values for the volume densities and the reference volume, \( V(\text{ref}) \) as follows;

\[ V(\text{ST}) = V_V(\text{St}) \times V(\text{ref}) \]

\[ V(\text{IT}) = V_V(\text{It}) \times V(\text{ref}) \]

\[ V(sIT) = V_V(sIT) \times V(\text{ref}) \]

\[ V(t.al) = V_V(t.al) \times V(\text{ref}) \]

The body mass – normalized volumes of various testicular components were also obtained by calculating the ratio between the absolute volume of each component to the body mass.
Whole testis cut into four approximately equal slabs

Each slab divided into two equal slices

Three slices selected at random and each divided into half slices

Three half slices selected.

Selected half slices embedded

Sections obtained from each block

Fig. 1. A schematic diagram showing the sampling protocol for obtaining morphometric data from the sengi testes.
CHAPTER 4

RESULTS

4.1 General observations

The species diagnosis of trapped animals was confirmed at the mammalogy section of the National Museums of Kenya and the representative picture of the animals is shown in Figure 2. All the animals trapped were generally in good body condition weighing 41.8 ± 1.5 g (Table 2) and with no obvious signs of illness except moderate tick infestation.

Externally, there was no sign or evidence of the presence of the scrotum and the only part of the male reproductive system visible was the prepuce in the ventral abdominal wall. The male reproductive organs consisted of the testes, the genital ducts made up of the epididymis, ductus deferens and the urethra, the accessory sex glands and the penis (Fig. 3).
<table>
<thead>
<tr>
<th>Animal number</th>
<th>Body mass (grams)</th>
<th>Testis Vref (cm³)</th>
<th>TV:BM (cm³ g⁻¹)</th>
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<tr>
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<td>0.0967</td>
<td>0.0025</td>
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<tr>
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<td>0.08</td>
<td>0.0018</td>
</tr>
<tr>
<td>3</td>
<td>45.3</td>
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<td>0.0018</td>
</tr>
<tr>
<td>4</td>
<td>40.6</td>
<td>0.09</td>
<td>0.0022</td>
</tr>
<tr>
<td>5</td>
<td>38.2</td>
<td>0.093</td>
<td>0.0024</td>
</tr>
<tr>
<td>MEAN</td>
<td>41.8</td>
<td>0.089</td>
<td>0.002</td>
</tr>
<tr>
<td>SEM</td>
<td>1.5</td>
<td>0.003</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Table 2. A table showing body masses, testicular reference volumes (Vref) and body mass – normalized testis volumes (TV:BM) of rufous sengis captured in May, 2008.
4.2 MORPHOLOGY

4.2.1 The testes

The testes were intra-abdominal, located caudo-lateral to the kidneys (Fig. 4) and suspended from the dorsal body wall by mesorchium. Their shapes were roughly cylindrical with their long axes oriented cranio-caudally. Between the cranial and caudal poles, the testes measured an average of 1.1 cm. On their dorso-lateral borders, caput epididymides were attached and extended the whole length of the testis (Fig. 5).

On histological examination, the testicular parenchyma was bound by a tight capsule, the tunica albuginea, and comprised predominantly of seminiferous tubules interspersed by the interstitial tissue (Figs. 6A and B). In one incidence, the left testis was markedly smaller in size compared to the right one and on examination of toluidine blue-stained semi-thin sections, it was composed of an apparently compact parenchyma with various patches of varied colourations and patterns with outlines of different colours (Figs. 7A and B). The surface of this testis had a single layer of fibroblast-like cells beneath which was a more or less uniform blue coloured zone with a few greenish patches. In the central part, the patches tended to form more of circular patterns which had apparently denser outlines and cores with vacuolations. Several fibroblasts were also observed within the parenchyma.
4.2.1.1 The seminiferous tubules

The seminiferous tubules were generally circular in cross-section (see Fig. 6A) and lined by a complex seminiferous epithelium (Fig. 6B) composed of two cell types; the germ (spermatogenic) cells at various stages of development (Figs. 8 and 9) and the somatic Sertoli cells (Fig. 9). The Sertoli cell appeared as a tall columnar branched cell resting on a basement membrane, and generally spanning the entire thickness of the epithelium. Its branches provided ramifications that surrounded the germ cells within the epithelium. The nucleus was irregular in shape with deep indentations and located towards the base (Fig.10) while the cytoplasm showed presence of numerous vesicles (Fig. 11).

Type A spermatogonia rested on the basal lamina with their long axes parallel to the tubular wall (Figs.10 and 12). These cells had pale cytoplasm with numerous mitochondria. Other generations of spermatogenic cells were also observed, including the primary spermatocytes and spermatids (see Figs. 8 and 9). Adjacent cap phase spermatids with nuclei characteristically capped with acrosomal vesicles containing acrosomal granules and prominent nucleoli were joined by cytoplasmic bridges (Fig. 13). The cytoplasm of these spermatids exhibited prominent rough endoplasmic reticulum concentrated towards the developing tail and mitochondria arranged peripherally along the cell membrane. The seminiferous epithelium was surrounded by the boundary tissue with a single layer of myoid cells (see Figs. 9, 10 and 12).
4.2.1.2 The interstitial tissue

The interstitial tissue generally occurred in the interstices between the seminiferous tubules, particularly at the angle where three or four seminiferous tubules came to approximate each other (see Figs. 6A and B). Frequently, thin strips of this tissue made extensions into narrow spaces between two adjoining tubules. Furthermore, a substantial amount of interstitial tissue, herein referred to as subcapsular interstitial tissue, was found distributed beneath the tunica albuginea (Fig. 14).

The interstitial tissue was mainly composed of Leydig cells, blood vessels and connective tissue and cells (Figs. 15-17). Leydig cells were more or less evenly distributed in the interstitial spaces and not all showed obvious preference for close association with blood vessels (Fig. 15). Generally, they had irregular nuclei with prominent nucleoli and abundant lipid droplets in the cytoplasm (Fig. 16). Within the narrow strips of interstitial tissue extending between two adjoining seminiferous tubules, the Leydig cells were narrow and elongated with rod-shaped nuclei (Fig. 17).

4.2.1.3 Testicular blood supply pattern

Testes received their arterial blood supply from the testicular arteries which branched off, almost perpendicularly, from the renal arteries (Fig. 18A). Each artery followed a straight course in a caudo-lateral direction to the respective testicle without exhibiting any convolutions or close association with any vein. (Figs. 18 A and B).
Immediately after exiting from the testis, the testicular vein received two branches; one on the cranial aspect from the caput epididymis and the other on the caudal aspect from the corpus epididymis. This vein then followed a straight course, without forming a pampiniform plexus or showing intimate association with testicular artery, to the ipsilateral caudal vena cava (Figs. 4 and 19 A and B). Rufous sengi had a separate left and right caudal vena cava, each receiving the ipsilateral testicular vein and, further cranially, the ipsilateral renal vein (Figs. 4 and 19 A and B). After receiving the renal vein, the left caudal vena cava crossed to the opposite side to join the right one forming the common caudal vena cava which then extended cranially to eventually drain into the right atrium.
Fig. 2. A photograph of the rufous sengi, *Elephantulus rufescens*, taken after anaesthesia. Notice the characteristic long tubular snout. Bar = 10 mm.
Fig. 3. A photograph showing various components of the male reproductive system of the rufous sengi, which include; the testis (T), caput epididymis (CE), corpus epididymis (BE), cauda epididymis (CdE), ductus deferens (Dd), prostate glands (P), bulbourethral glands (Bu), bulb of penis (Bp), penis (Pe) and preputial sheath (PS). The urinary bladder (UB) is also shown. Bar = 10 mm.
Fig. 4. A photograph of the exposed abdominal cavity of rufous sengi showing the caudo-lateral position of the testis (T) in relation to the kidneys (K) and the *in situ* arrangement of the paired caudal vena cavae (CC), each receiving ipsilateral testicular (TV) and renal (R) veins. Notice the adrenal gland (asterisk) lying cranio-medial to the kidney. Bar = 5 mm.
Fig. 5. A photograph of the left testis (T) of rufous sengi with caput epididymis (CE) intimately applied on its dorso-lateral border. Notice the corpus epididymis (BE) extending from the caput epididymis. Bar = 5 mm.
Figs. 6 A and B. Photomicrographs of the testis of rufous sengi. A: The testicular parenchyma, comprising of the seminiferous tubules (ST) interspersed by the interstitial tissue (arrows), is bound by the tunica albuginea (arrowheads). B: A higher magnification photomicrograph showing the seminiferous epithelium (SE) lining the seminiferous tubules and the tubule lumen (L). Bars: A = 27.8 μm; B = 6.9 μm. Toluidine blue.
Figs. 7 A and B. Photomicrographs showing the parenchyma of atrophic testis from one of the male rufous sengis.

A: The parenchyma with patches (asterisks) and outlines (arrows) of varied colourations.

B: The core of one of the patches with vacuolations.

Bars: A = 25 μm; B = 12.5 μm. Toluidine blue.
Fig. 8. A photomicrograph of the seminiferous epithelium of rufous sengi’s testis showing various stages of spermatogenic cells, including the primary spermatocytes (SPC), round spermatids (SPT), and a spermatozoon (arrow). Note the desquamated spermatogenic cell (arrowhead) in the lumen (L) and also a portion of the interstitial tissue (IT). Bar = 3.5 μm. Toluidine blue.
Fig. 9. An electron micrograph showing a tall Sertoli cell (S) with several ramifications (arrows) enveloping the spermatogenic cells. The spermatogenic cells shown are at various stages of development, and include the spermatocytes (SPC) and round spermatids (SPT). The boundary tissue (BT) is also shown. Bar = 2.7 μm.
Fig. 10. An electron micrograph showing a Sertoli cell nucleus (SCN) located basally closer to the boundary tissue (BT). The nuclear envelope is irregular with deep indentations (arrow). The type A spermatogonium (SGA) and primary spermatocytes (SCT) are also shown. Bar = 1.3 μm.
Fig. 11. An electron micrograph of a Sertoli cell cytoplasm with numerous vesicles (asterisks). Nu = Nucleolus of a Sertoli cell; SCT = Spermatocytes. Bar = 1.4 μm
Fig. 12. An electron micrograph showing type A spermatogonium (SGA) of the rufous sengi. The cell has a pale cytoplasm and the long axis is parallel to the tubular wall constituted by the boundary tissue (BT). Notice a portion of the Sertoli cells with irregular nucleus (SCN) surrounding the spermatogonium, and the myoid cell (arrowhead) within the boundary tissue (BT). Bar: = 1.5 μm.
Fig.13. An electron micrograph showing two cap phase spermatids joined by a cytoplasmic bridge (IB). The nucleus (N) has a prominent nucleolus (Nu) and the mitochondria (arrows) are arranged along the cell membrane. The rough endoplasmic reticulum (ER) is in close association with the developing tail (T). Note the developing acrosome (A) with acrosomal granule (asterisk) and a closely associated aggregate of materials (arrowheads). Bar = 1.0 μm.
Fig. 14. A photomicrograph of rufous sengi's testis showing the subcapsular interstitial tissue (arrowhead) located just beneath the tunica albuginea (TA), with Leydig cells and blood vessels (BV). SE = seminiferous epithelium; L = lumen of seminiferous tubule. Bar = 3.5 μm. Toluidine blue.
Fig. 15. A high magnification photomicrograph showing sengi's testicular interstitial tissue with evenly distributed Leydig cells (arrowheads) some of which do not show obvious intimate association with the blood vessel (BV). Portions of seminiferous epithelia (SE) lining the adjacent seminiferous tubules are also shown. Bar = 3.3 μm. Toluidine blue.
Fig 16. An electron micrograph showing a Leydig cell with an irregularly shaped nucleus (N) that has a prominent nucleolus (Nu) and a cytoplasm with several lipid droplets (LD). Notice the boundary tissue (BT), a fibroblast (F) and a blood vessel (BV) with its lining endothelial cell (E). Bar = 1 μm.
Fig. 17. An electron micrograph showing a Leydig cell (LC) interposed between two adjacent seminiferous tubules. This cell is long and narrow with an elongated cylindrical nucleus (N), lipid droplets (asterisks) and mitochondria (arrows). Notice the myoid cell (arrowhead) within the boundary tissue (BT), type A spermatogonium (SGA) and spermatocytes (SCT) within the seminiferous epithelium (SE). Bar = 1.4 µm.
Fig. 18 A and B. A photograph (A) and a schematic diagram (B) showing the testicular arteries (TA) of rufous sengi branching off from the renal arteries (RA), which in turn branched off from the abdominal aorta (A). Note the straight course of the testicular arteries. The left adrenal gland (asterisk) lying cranio-medial to the left kidney and a stump of the ureter (U) from the same kidney are also shown. Bar = 5 mm.
Figs. 19 A and B. A photograph (A) and a schematic diagram (B) showing the ventral view of paired caudal vena cava (CC) of rufous sengi, each receiving ipsilateral testicular vein (TV) and renal vein (R). Upon receiving the renal veins, the left caudal vena cava crosses to the opposite side to join the right one to form a common caudal vena cava (C). The tributaries of the testicular vein; one (open arrowheads) from the caput epididymis (CE) and the other (closed arrowheads) from the corpus epididymis (BE), are shown. Notice the adrenal glands (asterisk) lying on the cranio-medial border of the kidney (K). Bar = 5 mm.
4.2.2 The genital ducts

4.2.2.1 The rete testis

The rete testis formed a network of channels (Fig. 20) located in a small area outside the confines of the testicular capsule (Fig. 21) and are lined by attenuated simple squamous or cuboidal epithelium. Within their lumina, spermatozoa and some immature germ cells were frequently observed. At the junction with efferent ductules, the epithelium of the rete testis abruptly changed to simple columnar (Figs. 21 and 22).

4.2.2.2 The efferent ductules

The efferent ductules consisted of several tubules connecting the rete testis to the initial part of caput epididymis. These tubules were bound by a loose connective tissue capsule, shared with the caput epididymis. They were lined by a simple cuboidal or low columnar epithelium (Fig. 23) with two cell types; the ciliated and non-ciliated cells (Fig. 24).

The non-ciliated cells had microvilli on their apical borders, and their profiles, from the apical border to the basal lamina, were almost always clearly defined. Their nuclei occupied most of the basal third of the cells, and their shapes ranged from oval to cylindrical and sometimes “inverted comma”-shaped (Fig 24). The ciliated cells, on the other hand, had prominent cilia projecting into the lumen from the apical surface. Their nuclei, with two or more nucleoli, were round to oval in shape and located in the apical half of the cell (Fig. 24).
In the apical cytoplasm of these cells, basal bodies corresponding to the cilia and also mitochondria were observed.

4.2.2.3 The epididymis

The epididymis was relatively long and topographically divided into three regions; the caput, corpus and cauda epididymis (Fig. 25). The caput epididymis formed an elongated mass extending from the cranial to the caudal pole, along the dorso-lateral border of the testis, to which it was attached by a peritoneal fold (see Fig. 5). The corpus was long and the most slender portion of the epididymis, running from the caudal pole of the testis to a position around the distal part of the neck of the urinary bladder and ventral to the rectum. The cauda epididymis was roughly a pear-shaped mass that extended caudally from the corpus epididymis and located between the rectum and the pelvic urethra. Commonly, the distal right corpus epididymis crossed over to the left side and vice versa, hence shifting the position of the right cauda epididymis to the left side and vice versa (Fig. 26). Each ductus deferens in this case crossed over, back to the original side before joining the pelvic urethra.

The caput epididymis was surrounded by a fibrous capsule, and in cross-sections, it displayed numerous tubular profiles between which was an inter-tubular interstitium of loose connective tissue with blood vessels (Fig. 27). Surrounding and closely applied to each tubular profile was a prominent dense connective tissue coat with several concentric layers of fibroblasts and blood...
vessels. Generally, the tubules were lined by a tall pseudostratified columnar epithelium resting on a prominent basement membrane.

The epithelium of caput epididymis had two typical cell types; the principal and basal cells (Fig. 28). The principal cells were very tall columnar cells which extended the entire thickness of the epithelium with basal ovoid nuclei oriented parallel to the cells’ long axes and one or two prominent nucleoli. The supranuclear cytoplasm had minute dark bodies reminiscent of secretory granules. The principal cells in the cranial most part of the caput epididymis were taller than those of other portions and their apical surfaces exhibited blebbing into the lumen (Fig. 28). Caudally, there was gradual reduction in the height of the principal cells with the disappearance of the apical blebbing. The basal cells, on the other hand, were confined to the base of the epithelium, lying on and parallel to the basal lamina.

The corpus epididymis was lined by a pseudostratified epithelium mainly made up of the principal and basal cells. The height of the epithelium was lower compared to that of the caput epididymis and on the luminal surface, the principal cells had very long microvilli (stereocilia). The tubular profiles and their lumina generally had ovoid outlines.

The cauda epididymis was generally placed somewhat laterally between the rectum dorsally and the pelvic urethra ventrally (Fig. 29). In majority of these animals, portions of the cauda epididymis extended dorsally to lie lateral to the distal part of the rectum (Fig. 30). It was enclosed by a somewhat translucent
capsule that allowed visualization of the underlying coils of the cauda epididymal duct.

On histological examination, the cauda epididymis, enclosed by a loose connective tissue capsule, consisted of numerous spermatozoa filled tubular profiles, (referred to as tubules), interspersed with an interstitium composed of loose connective tissue, blood vessels and connective tissue cells (Figs. 31 and 32). Each tubule was, in turn, closely surrounded by a dense connective tissue coat with several concentric fibroblasts (Figs. 32 and 33). Besides the spermatozoa, the lumina of the tubules also had occasional large ovoid masses (Fig. 32). Each tubule was lined by a low pseudostratified columnar epithelium, which on histological sections appeared foamy due to the presence of vacuoles and rested on a basement membrane. On the apical surface, the epithelial cells had numerous microvilli that formed a brush border. The epithelium was made up of two cell types, the principal and basal cells.

The principal cells had basal lobulated nuclei, numerous microvilli at their apical surfaces and numerous round vacuoles of various sizes distributed mainly at the apical cytoplasm but also occasionally in perinuclear region (Fig. 33). The basal cells were confined to the base of the epithelium interposed between basal or basolateral surfaces of principal cells and the basement membrane with their long axes parallel to the basement membrane onto which they laid (Fig. 33).
The lumen of cauda epididymis had many spermatozoa whose heads had variations in morphology. In general, the heads of spermatozoa in the lumen of cauda epididymis had spatulate dark stained nuclei that almost completely filled their heads, capped by long acrosomes (Fig. 34). The concavity of the implantation fossa was asymmetrically located on the posterior pole of the nucleus so that one edge (or margin) of the fossa was taller and extended more posteriorly than the other (Fig. 35). The proximal centriole was closely associated with the implantation fossa, mainly located towards the shorter edge of the fossa. The tails had midpieces each containing a sheath of circumferentially oriented mitochondria inwardly lined by columns of outer dense fibers. Several sections through various regions of the spermatozoa tails had cytoplasmic droplets with several vesicles of different sizes attached to them (Fig. 36). A number of spermatozoa had apparent morphological abnormalities in their heads including bulbous heads (Fig. 37). The lumen of cauda epididymis also had amorphous dark staining materials that appeared to engulf one or more spermatozoa tails and occasionally the heads (Fig. 38).

4.2.2.4 The ductus deferens

The ductus deferens was a very short duct that extended caudo-ventrally from the cauda epididymis to penetrate the muscular coat that surrounded the pelvic urethra, and ran caudally within the boundaries of this coat before joining the urethral canal (Figs. 3 and 39). It was lined by a pseudostratified columnar epithelium which, in turn, was surrounded by a very thick wall made up of dense connective tissue and smooth muscle cells organized into three
concentric strata that merged without clear demarcations (Fig. 40). The innermost of these layers, which appeared darker than the others, laid immediately outer to the basal lamina of the epithelium. It was composed of a number of parallel layers of connective tissue with cells arranged concentrically around the epithelium. The middle layer was much thicker and composed of smooth muscle tissue whose elements were arranged haphazardly. The outermost layer, which contained numerous blood vessels of varying diameters, was made up of parallel concentric connective tissue elements that formed an envelope around the deferent duct as a whole. The epithelium frequently exhibited apical effusions into the lumen (Fig. 41) and the apical cytoplasm of the epithelial cells had numerous vacuoles that made the epithelium appear foamy.

Between the deferent ducts was a blind ending uterus masculinus which was apparently not divided into horns (see Fig. 39). It had a wide lumen which occasionally contained spermatozoa and some pink staining material. It was lined by a simple columnar epithelium.

4.2.2.5 The urethra

The urethra extended from the urinary bladder to the tip of the penis and it was divided into two parts; the pelvic and the penile urethra. The pelvic urethra was almost completely surrounded by the accessory sex glands, particularly the prostate gland (see Fig. 3) and extended from the neck of the urinary bladder to the bulb of the penis from where it continued as the penile
urethra (Fig. 42). The pelvic urethra was composed of a lumen lined by transitional epithelium, a loose connective tissue stroma surrounding the epithelium and a very thick outer muscular coat made up of several concentric layers of striated muscle fibres (see fig. 39). The outer coat of striated muscle fibres appeared to get more developed distally where it also, through its dorsal border, incorporated the distal ductus deferens, uterus musculinus and excretory ducts of the prostate glands. Just after incorporation within the boundary of the urethral muscular coat, the stromal tissue around the deferent ducts and uterus musculinus appeared distinctly separate from that around the urethra and the prostatic ducts (Fig. 43) but distally, these stromal tissues completely merged (Fig 44).

Within the confines of the urethral muscular coat, the urethral duct was accompanied laterally by the prostatic ducts and dorsally by the deferent ducts and the uterus musculinus (see Figs. 39, 43 and 44) all of which eventually joined it at various levels, with the deferent ducts and the uterus masculinus joining obliquely at about the same point (Fig. 45).
Fig. 20. A photomicrograph showing channels of the rete testis (R) of rufous sengi, with numerous round spermatids (arrows). A spermatozoon (arrowhead) is also shown. Bar = 5 μm. Toluidine blue.
Fig. 21. A photomicrograph of the extra-testicular rete testis of rufous sengi, with its lumen (L) lined by a simple low cuboidal epithelium (E1) which abruptly changes to columnar type (E2) at the junction with the efferent ductules (arrows). Notice a portion of the testis parenchyma with seminiferous tubule (ST) beneath the tunica albuginea (TA). Bar = 12.5 μm. Toluidine blue.
Fig. 22. A photomicrograph showing the junction between the rete testis and the efferent ductules (arrow). At this junction, the squamous or cuboidal epithelium (E1) lining the rete testis abruptly changes to columnar epithelium (E2) lining the efferent ductules.

Bar = 5 μm. Toluidine blue.
Fig. 23. A photomicrograph of the efferent ductule, lined by a low columnar epithelium surrounded by a dense connective tissue coat (Ct) with blood vessels (arrows). Notice the inter-tubular interstitium (In) of loose connective tissue. Bar = 5 μm. Toluidine blue.
Fig. 24. An electron micrograph of the epithelium lining the efferent ductule. The ciliated cell (C) has round nucleus (N1) and cilia (Ci) on its apical border while the non-ciliated cell (NC) has 'inverted comma'-shaped nucleus (N2) and microvilli (Mv) on its apical border. Notice the mitochondria (arrowheads) and the basal bodies (arrows) in the sub-apical cytoplasm of the ciliated cell. Bar = 0.6 μm.
Fig. 25. A photograph of the epididymis of the rufous sengi showing the topographic regions: the caput (CE), corpus (BE) and cauda epididymis (CdE). Notice a portion of the ductus deferens (DD) extending from the cauda epididymis. Bar = 5 mm.
Fig. 26 A photograph showing the criss-crossed cauda epididymides. The right cauda (CdR) is on the left side while the left one (CdL) on the right side. The right (closed arrow) and the left (open arrows) corpora epididymides also criss-cross as they join their respective cauda epididymides. The right ductus deferens (closed arrowhead) descends from the corresponding cauda epididymis and crosses to the right side to join the pelvic urethra (pU). The converse is true about the left ductus deferens. Notice the ureters (open arrowheads) joining the urinary bladder (UB). Bar = 3 mm.
Fig. 27. A low magnification photomicrograph of the caput epididymis. It is composed of several epithelium-lined tubular profiles (T) interspersed by an interstitium of loose connective tissue (asterisks) with blood vessels (arrows). The whole organ is enclosed by a loose connective tissue capsule (arrowheads). Bar = 25 μm. Toluidine blue.
Fig. 28. A photomicrograph of the cranial caput epididymis. The epithelium consists of very tall columnar principal cells (P) with basal ovoid nuclei (n) and basal cells (closed arrowheads). The principal cells have dense bodies (arrows) in their apical cytoplasm and their apical surfaces show blebbing (open arrowheads) into the lumen (L). Notice the connective tissue coat (Ct) that closely surrounds the duct and interstitium with blood vessel (asterisk). Bars = 5 μm. Toluidine blue.
Fig. 29. A photograph of some of the pelvic organs of the male rufous sengi *in situ*. The cauda epididymis (CdE) is situated somewhat laterally between the rectum (R) and the pelvic urethra (pU). Notice the bulbourethral gland (BU), the bulb of the penis (BP) and a portion of the urinary bladder (UB). Bar = 2.5 mm.
Fig. 30. A dorsal view photograph showing the disposition of cauda epididymis (CdE) within the pelvic cavity. The cauda epididymides are situated lateral to the distal part of the rectum (R) and covered by a translucent capsule that allows the underlying coils of the cauda epididymal duct to be visualized. Notice the bulbourethral gland (BU). Bar = 2.5 mm.
Fig. 31. A photomicrograph of the cauda epididymis showing several spermatozoa-filled tubular profiles (T) interspersed by an interstitium (In) of loose connective tissue. Bar: = 25 μm. Toluidine blue.
Fig. 32. A high magnification photomicrograph of the cauda epididymis. The epithelium (E) has numerous apical vacuoles (arrowheads) that make it appear foamy and it is outlined by a dense connective tissue coat (Ct). The lumen (L) is filled with spermatozoa and contains an ovoid mass (asterisk). Notice the blood vessel (BV) in the intertubular interstitium (In). Bar = 5 μm. Toluidine blue
Fig. 33. An electron micrograph showing the epithelium of cauda epididymis. The principal cells have basal lobulated nuclei (N), vacuoles (V) in apical cytoplasm and apical microvilli (Mv). The basal cell (arrow) is confined to the base of the epithelium. Notice the lumen (L) with spermatozoa (open arrowheads) and the connective tissue coat (CT) that closely surrounds the epithelium, with fibroblasts (asterisks). Bar = 2.5 μm.
Fig. 34. An electron micrograph showing a sagital section through anterior portion of the head of a spermatozoon. The head has a spatulate nucleus (N) anteriorly capped by an acrosome (asterisk). Notice the transverse sections of spermatozoa tails (Pp) neighbouring the head. Bar = 0.4 μm.
Fig. 35. An electron micrograph showing a sagittal section through the head and the proximal tail of a spermatozoon. The nucleus (N) is capped by an acrosome (asterisk) and has an asymmetrical implantation fossa (arrowhead). The proximal centriole (open arrow) lies close to the shorter margin of the implantation fossa. The midpiece has a mitochondrial sheath (arrows) Bar = 1.1 μm.
Fig. 36. An electron micrograph of the cauda epididymal spermatozoon with its tail attached to a cytoplasmic droplet (CD) with vacuoles (asterisks). Notice the longitudinal section through the spermatozoon head with spatulate nucleus (N) capped by an acrosome (A). Mp = midpiece. Bar = 0.9 μm.
Fig. 37. An electron micrograph showing a spermatozoon from the lumen of cauda epididymis, with abnormal head morphology ("bulbous head") (arrow). Notice the spermatozoon with a normal spatulate head (arrowhead).  Bar = 1.4 μm.
**Fig. 38.** An electron micrograph showing an amorphous dark staining material (D) observed within the lumen of cauda epididymis. This amorphous material appeared to engulf spermatozoa parts (*arrows*). Notice the normal spermatozoon (*arrowheads*). Bar = 2 μm.
Fig. 39. A photomicrograph of a cross-section through the pelvic urethra showing the deferent ducts (asterisks) running within the confines of urethral muscular coat (MC) before joining the urethral duct (U). The prostatic ducts (arrowheads) and the uterus masculinus (UM) are also shown. Bar = 100 μm. Toluidine blue
Fig. 40. A photomicrograph of the ductus deferens showing the epithelium (E), surrounded by a thick coat apparently organized into; a sub-epithelial connective tissue layer (A), a thick muscular layer (B) and an outer connective tissue layer (C) with blood vessels (arrowheads). L = Lumen. Bar = 25 μm. Toluidine blue.
Fig. 41. A photomicrograph showing the epithelium (E) of the ductus deferens, composed of cells with vacuoles (arrows) in the apical cytoplasm which make them appear foamy and foldings (asterisks) into the lumen (L). The layer (A) immediately beneath the epithelium with its components running parallel around the epithelium is also shown. Notice the spermatozoa (S) in the lumen. Bar = 5 μm. Toluidine blue.
Fig. 42. A photograph showing the pelvic urethra (PU) of the rufous sengi connected cranially to the neck of urinary bladder (UB) and caudally to the bulb of the penis (BP). The accessory sex glands and the deferent ducts have been removed. Bar = 5 mm.
Fig. 43. A photomicrograph of a cross-section through the proximal pelvic urethra after incorporation of deferent duct and uterus masculinus within the boundary of the urethral muscle (MC). Notice that the stroma around the urethral duct (U) and the prostatic ducts (arrows) appear separate from that surrounding deferent ducts (asterisk) and uterus masculinus (UM). Bar = 200 μm. PAS.
Fig. 44. A photomicrograph of a cross-section through the middle pelvic urethra showing merger of stromal tissues surrounding the deferent duct (asterisks) and uterus masculinus (arrowhead) with that surrounding the urethral duct (U) and the prostatic ducts (arrows). Notice the thick wall of urethral muscle (MC). Bar = 50 μm. PAS.
Fig. 45. A photomicrograph of a cross-section through the distal pelvic urethra showing the deferent ducts (arrowheads) joining the urethral duct (U). Notice the uterus masculinus (asterisk) in close association with the deferent ducts, and the prostatic ducts (arrows) joining the urethral duct. All these ducts run within the boundary of a thick wall formed by the urethral muscle (MC). Bar = 50 μm. PAS.
4.2.3 The accessory sex glands

The accessory sex glands of the rufous sengi were clustered around both the pelvic urethra, to which they were connected, and the urinary bladder (Fig. 46). They included the multi-lobated prostate gland and a pair of bulbo-urethral (Cowpers) gland.

4.2.3.1 The prostate gland

The prostate gland was made up of two distinct sets of glands namely; the cranial and the caudal prostate glands (Fig. 46). The cranial prostate gland was brownish red in colour with its lobes arranged around the neck of the urinary bladder and the proximal part of the pelvic urethra. The caudal prostate, on the other hand, consisted of a single whitish-cream pair of lobes arranged along the dorso-lateral surface of the middle and distal parts of the pelvic urethra.

4.2.3.1.1 The cranial prostate

The cranial prostate consisted of three paired lobes; a single ventral and two dorsal lobes. The ventral lobes were situated ventro-lateral to the neck of the urinary bladder and proximal pelvic urethra (Fig. 47). They were compound branched tubulo-alveolar glands lined by simple columnar epithelium that rested on a basement membrane. The glandular tubules were interspersed with an interstitium made up of very loose connective tissues.
The dorsal lobes consisted of dorso-medial and dorso-lateral lobes, the former being applied to the dorso-lateral surface of the distal neck of urinary bladder and proximal pelvic urethra and the latter lying on and completely covering the lateral surface of the former (Fig. 47). Histologically, these two lobes were generally similar both being compound tubulo-alveolar glands lined by a simple columnar epithelium and between the glandular units was an interstitium of loose connective tissue. Generally, the epithelial cells showed weak staining and in sections stained with toluidine blue, there was an apparent pattern of alternating light and dark epithelial cells (Figs. 48 A and B). The cells had relatively large centrally located spherical or ovoid nuclei (Fig. 49) and their cytoplasm showed characteristic bleb formation which appeared to be cast off into the lumen as spherical globules (Figs. 48 and 49). Vesicles were observed in the perinuclear cytoplasm but absent in the blebs.

4.2.3.1.2 The caudal prostate

The caudal prostate consisted of a single and relatively large paired gland situated dorsolateral to the pelvic urethra (see Fig. 46). It was a branched tubular gland lined by a simple columnar epithelium resting on a basement membrane (Fig. 50). Between the secretory units was an extremely loose connective tissue with scanty formed elements.

The epithelium of this gland was made up of columnar cells which had apical cytoplasms filled with round dark granules (Figs. 50 and 51) and basal lobulated nuclei with marginated chromatin (Fig. 52). The basal and
perinuclear cytoplasm had an extensive rough endoplasmic reticulum mainly consisting of parallel cisternae with several dilatations.

4.2.3.2 The bulbourethral gland

The bulbourethral gland occurred as a pair of medio-laterally flattened glands connected to the dorsal surface of the urethra at about the entry of the pelvic urethra into the bulb of the penis (see Figs. 29 and 46). Along the mid-line of the lateral surface was a vein (see Fig. 29) which, at acute angles, received several tributaries at more or less regular intervals. This vein, together with its tributaries, gave the lateral surface of this gland a leaf-like appearance.

On histological sections, this gland was covered externally by a thick capsule made up of several layers of striated muscles (Figs. 53) that made extensions inwardly into the gland. It was mainly made up of compound tubulo-alveolar glandular units lined by a simple tall columnar epithelium (Fig. 54). The connective tissue between the glandular units apparently had elastic fibres. In toluidine blue stained semi-thin sections, the lumina had some round pink staining materials that appeared like crystals (Figs. 53 and 54).
Fig. 46. A dorsal view photograph showing the accessory sex glands of the male rufous sengi clustered around the pelvic urethra and the neck of the urinary bladder (UB). The prostate gland consists of several paired lobes generally grouped into two (2) as; a dark cranial group (cP) and a lighter caudal group (cdP). The bulbourethral gland (BU) consists of a single pair of glands on the dorso-cranial surface of the bulb of penis (BP). Bar = 2.5 mm.
Fig. 47. A low magnification micrograph of a cross section through the proximal pelvic urethra (asterisk) surrounded by the cranial prostate gland, constituted by the ventral (V), dorso-lateral (LD) and dorso-medial (MD) lobes. The ductus deferens (Dd) is also shown. Bar = 100 μm. PAS.
Fig. 48. Low (A) and high (B) magnification photomicrographs of the dorso-lateral lobe of cranial prostate. The alveoli are lined by a simple columnar epithelium (E) interspersed with an interstitium (In) of loose connective tissue. The epithelial cells show apical blebbing (asterisks) and an apparent pattern of alternating dark (closed arrows) and light (open arrows) cells. The lumen (L) has spherical globules (arrowhead) that result from casting off of apical blebs of epithelial cells. Note the dense connective tissue coat (Ct) enveloping each alveolus.

Bars: A = 8.3 μm; B = 3.0 μm. Toluidine blue.
Fig. 49. An electron micrograph showing epithelial cells of dorso-lateral lobe of the cranial prostate. The cells have central ovoid nuclei (N), vesicles (arrowheads) in perinuclear cytoplasm and show blebbing (BL) of apical cytoplasm. L = lumen of the gland. Bar = 2.5 μm.
Fig. 50. A section of the caudal prostate showing a portion of secretory unit lined by a low columnar epithelium (E) and externally surrounded by a connective tissue coat (arrow) with fibroblasts (arrowheads). L = Lumen. Note the dark granules in the apical cytoplasm. Bar = 2.8 μm. Toluidine blue.
Fig. 51. An electron micrograph showing the epithelium of the caudal prostate. The epithelial cells have basal lobulated nuclei (N) and dark spherical secretory granules (SG) that fill the apical and most of the perinuclear cytoplasm. 

L = lumen of the gland.  
Bar = 2.5 μm.
Fig. 52. An electron micrograph showing basal and perinuclear portion of an epithelial cell of the caudal prostate. The nucleus (N) has margined chromatin (arrowhead) and deep indentations (arrows). The cytoplasm has well developed endoplasmic reticulum (ER) in the basal and perinuclear regions and Golgi apparatus (G) in the supranuclear region. Notice the secretory granules (SG) in the supranuclear region. Bar = 0.6 μm.
Fig. 53. Photomicrographs of the bulbourethral gland showing branched tubuloalveolar glandular units (asterisks) and a muscular coat (MC) covering the gland. Note the crystal-like materials (arrow) in the lumen (L). Bar = 50 μm. Toluidine blue.
Fig. 54. A higher magnification of the bulbo-urethral gland of rufous sengi showing a glandular unit lined by tall columnar epithelial cells (E) with basal nuclei (N). Beneath the epithelium is an elastic tissue (arrowhead). Note the pink staining crystal-like materials (arrows) in the lumen (L). Bar = 3.0 μm. Toluidine blue.
4.3 MORPHOMETRY

The mean body mass of the animals used for morphometric study was 41.8±1.5 grams (see table 2). The mean reference volume of the right testes of the animals used for morphometric analyses was 0.089 ± 0.003 cm³ with a mean body mass-normalized testis volume of 0.002 ± 0.0002 (see table 2). The mean volume densities of the various components of the testis are summarized in table 3 and Fig. 55. A mean of about 1.7% of testis volume was contributed by the testicular capsule, the tunica albuginea, with the other 98.3% contributed by the parenchyma. The mean volume density of the testis parenchyma occupied by the seminiferous tubules and interstitial tissue (including the sub-capsular interstitial tissue) was 89.4% and 8.9% respectively. These translated to approximate mean absolute volumes of 0.0793 ± 0.003 cm³ and 0.008 ± 0.001 cm³ (Table 4). About 7.6% of the total interstitial tissue was found beneath the tunica albuginea, and was referred here as sub-capsular interstitial tissue (sIT). This sIT represented about 0.68% of the total testis volume. The mean body mass normalized volumes of the seminiferous tubules and the interstitial tissue was 0.002 ± 0.0001 and 0.0002 ± 0.00003 respectively (Table 5).
<table>
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<th>Vv(ST)</th>
<th>Vv(IT)</th>
<th>Vv(sIT)</th>
<th>Vv(tal)</th>
<th>Total</th>
</tr>
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<td>0.0688</td>
<td>0.0039</td>
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<tr>
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<td>0.0735</td>
<td>0.0073</td>
<td>0.0172</td>
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<tr>
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<tr>
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<td>0.0055</td>
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<tr>
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<td>0.0824</td>
<td>0.0068</td>
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<tr>
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<td>0.0074</td>
<td>0.0012</td>
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**Table 3.** A table showing volume densities (Vv) of various testicular components including the seminiferous tubules (ST), interstitial tissue (IT), sub-capsular interstitial tissue (sIT) and the tunica albuginea (tal).
Fig. 55. A chart showing mean proportions of various testicular components in the rufous sengi. ST = seminiferous tubules; IT = interstitial tissue; sit = sub-capsular interstitial tissue; t.al = tunica albuginea.
<table>
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<th>V(ST)</th>
<th>V(IT)</th>
<th>V(sIT)</th>
<th>V(t.al)</th>
<th>Total</th>
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<td>0.0015</td>
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<td>± SEM</td>
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<td>± 0.0008</td>
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<td>± 0.0001</td>
<td>± 0.003</td>
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</tbody>
</table>

Table 4. A table showing absolute volumes (V) of various testicular components including the ST, Interstitial tissue (IT), sub-capsular interstitial tissue (sIT) and the tunica albuginea (t.al).

All values are in cm³.
<table>
<thead>
<tr>
<th>Animal No.</th>
<th>ST : BM</th>
<th>IT : BM</th>
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</tr>
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<td>5</td>
<td>0.0022</td>
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<tr>
<td><strong>MEAN ± SEM</strong></td>
<td><strong>0.002 ± 0.0001</strong></td>
<td><strong>0.0002 ± 0.00003</strong></td>
</tr>
</tbody>
</table>

Table 5. A table showing body mass normalized volumes of the seminiferous tubules (ST : BM) and interstitial tissue (IT : BM) in the rufous sengi.
4.5 Miscellaneous findings

These animals had diffuse nodules on the inner surface of the body wall and occasionally on the viscera (Fig. 56). Each of these nodules contained a whitish-cream worm-like organism. Based on morphology, these parasites were diagnosed as larvae of the genus *Porrocaecum*. These larvae were also found freely roaming in the abdominal cavity.
Fig. 56. A photograph of the inner surface of a portion of abdominal wall of rufous sengi, with numerous nodules (arrows). A portion of the intestine (asterisk) is also shown. Bar = 5 mm.
5.1 General remarks

Rufous sengis are abundantly found in areas around Voi town in Taita District. All the animals trapped were generally healthy, but with moderate tick infestation.

5.2 The testes

The testes of rufous sengi are intra-abdominal as earlier reported in all other members of the order macroscelidea and indeed members of the new super-order Afrotheria (Stoch, 1954; Tripp, 1970; Neaves, 1980; Jones and Brosnan, 1981; Jones and Holt, 1981).

Generally, the testes of vertebrates develop in a cranio-medial position relative to the kidneys (Browder et al., 1991; Orth, 1993; Walker and Liem, 1994). In most vertebrates, the testes maintain more or less the same position all the way into adulthood but in a majority of mammals, they descend into a sac-like structure outside the abdominal cavity called the scrotum. In the rufous sengi, the testes, like those of other true testicondas, maintain their intra-abdominal location into adulthood. However, unlike the lower vertebrates where the testes are positioned more or less cranio-medial to the kidneys (Kent, 1978; Walker and Liem, 1994) and the African elephant whose testes lie ventro-medial to the kidneys (Gaeth et al., 1999), the testes of the rufous sengi, like
those of the rock hyrax, lie caudo-lateral to the kidneys. The caudal positioning of sengi testes relative to the kidneys could have been due to some degree of descent during embryonic life or due to the ascent of the kidneys as reported to occur in the humans (Moore and Persaud, 2003). It is unclear why the testes of sengis lies laterally against the body wall but in this study, it is speculated that this lateral positioning may be a consequence of lateral shift of the testes during development in an attempt to find a relatively cooler site. This position, however, may not offer as much cooling as that offered by the scrotum.

The testes of rufous sengi were more or less cylindrical in shape and on histological sections, the parenchyma was predominated by the seminiferous tubules between which were thin rims of interstitial tissue. This finding is in agreement with earlier reports that sengis have very little interstitial tissue (Asdell, 1964; Fawcett et al., 1973). This is also similar to what has been reported in the African elephant and the rock hyrax (Fawcett et al., 1973).

It has been shown that in scrotal mammals, slightly lower than core body temperatures offered by the scrotum with complementary input from the pampiniform plexuses, are necessary for successful spermatogenesis. Transposition of the testes of scrotal mammals into the abdomen resulted in incomplete spermatogenesis (Bedford et al., 1982) and it has been demonstrated that one cause of this is germ cell apoptosis in response to elevated temperatures (Yin et al., 1998). Like in other true testicondas, seminiferous tubules of the rufous sengi exhibited complete spermatogenesis
in spite of the intra-abdominal location of the testes and the absence
structures or features that may help cool the testes such as the pampiniform
plexuses. It is therefore intriguing how complete spermatogenesis is achieved
by testes in these animals at high intra-abdominal temperatures. In this
regard, a number of arguments can be advanced. In this study, the location of
the testes is speculated to offer some cooling. Another possibility is that these
animals may have evolved some biochemical mechanisms that allow
spermatogenesis to proceed at high abdominal temperatures. This then
implies that in these animals, temperature is not critical for spermatogenesis
but to sperm storage. This latter suggestion finds a strong morphological
backing in these animals provided by the marked descent of the cauda
epididymis, as much in agreement with what Bedford, (1978a) reported.

It has been hypothesized that scrotal mammals have evolved a mechanism
whereby at certain periods, body temperatures fall, allowing spermatogenesis
to proceed during these intermittent periods of lowered body temperature
(Glover and Sale, 1968; Bedford et al., 1982). It has been found that sengis
(Lovegrove et al., 2001; Mzilikazi et al., 2002) and perhaps all other afrotheres
(Scantlebury et al., 2008) exhibit heterothermy and torpor. This therefore
suggests that spermatogenesis in sengis and other Afrotherians is
temperature-sensitive and proceeds intermittently during the periods of torpor.

The boundary tissue (lamina propria) that surrounds each seminiferous tubule
is present in all mammals and several birds and contains one or more layers
of myoid cells depending on species (Maekawa et al., 1996; Aire and Ozegbe,
2007). In the rufous sengi, the boundary tissue contained a single layer of myoid cells. This is similar to what is found in rats, mice and hamsters (Maekawa et al., 1996).

Like the general scenario in mammals, the interstitial tissue of rufous sengi was distributed between the seminiferous tubules. It was however observed in this study that substantial amounts of interstitial tissue with Leydig cells also occurred beneath the tunica albuginea. Such a disposition of the interstitial tissue beneath the testicular capsule has not been described or highlighted in many available reports on testicular morphology and it is probably a unique feature of Macroscelids.

Mammalian Leydig cells are generally polyhedral with round or ovoid nuclei (Hooker, 1970; Fawcett, 1986). In the rufous sengi, Leydig cells generally had irregular nuclei and in addition, elongated cells with rod-shaped nuclei extended into narrow spaces between adjoining seminiferous tubules. It is therefore plausible to suggest that, besides their general polyhedral shape and ovoid nuclei, Leydig cells can vary their shape and that of their nuclei to conform and fit into the available space. Similar to what has generally been reported in mammals, the structure of Leydig cells of rufous sengi conform to that of steroid secreting cells, having abundant lipid droplets, numerous mitochondria and well developed smooth endoplasmic reticulum (Fawcett, 1986).
5.3 Testicular blood supply

Mammalian testicular arteries generally branch off from abdominal aorta while the veins drain into either the caudal vena cava (Setchel, 1970; Kallen, 1977; Nickel et al., 1981; Rommel et al., 1991, 1994) or renal veins (Short et al., 1967; Setchel, 1970). The branching off of testicular arteries from the renal arteries exhibited by the sengi, and their straight course to the testes, is typically an avian feature where the testicular arteries branch from the cranial renal arteries (King and McLelland, 1984). This feature has also been reported in the rock hyrax (Glover and Sale, 1968; Glover, 1973) and the African elephant (Short et al., 1967; Setchel, 1970; Gaeth et al., 1999), both of which belong to the superorder Afrotheria. It appears that, among mammals, this pattern is rather unique to the Afrotherians (Glover and Sale, 1968; Setchel, 1970; Gaeth et al., 1999) suggesting that it is possibly a derived trait. The straight course of the testicular vein without exhibiting pampiniform plexuses is similar to what has been reported in the rock hyrax, the elephant and the dolphin implying that this is a feature common to all true testicondas (Short et al., 1967; Glover and Sale, 1968; Setchel, 1970; Gaeth et al., 1999).

The testicular blood supply plays an important role in the mammalian testicular thermoregulation through arterio-venous countercurrent heat exchangers. In scrotal mammals, such a countercurrent heat exchanger is formed along the spermatic cord by the close association between the highly convoluted and
branched testicular artery and the pampiniform plexus of the testicular vein (Setchel, 1970, 1991). In cetaceans, which are true testiconda, the arteriovenous countercurrent heat exchanger is formed by the juxtaposition between the testicular arterial plexus and the subcutaneous vein from the peripheral surface of the dorsal fin and flukes (Rommel et al., 1991, 1994). These countercurrent heat exchangers permit the cooling of arterial blood, hence, the testis; a requirement for successful spermatogenesis. The straight course of testicular blood vessels observed in the sengi, without exhibiting any sign of intimate association between the testicular arteries and testicular or other veins, suggests that blood supply play no significant role in testicular thermoregulation in these animals. This appears to be a common phenomenon among the Afrotherians as exhibited by the rock hyrax (Glover and Sale, 1968) and the African elephant (Short et al., 1967; Gaeth et al., 1999). Spermatogenesis in these animals therefore, either proceeds at high abdominal temperatures or there could be other thermoregulatory mechanisms such as those suggested in 5.2 above.

The pattern of caudal vena cavae in the sengi, where the right and left caudal vena cavae are separate and only unite after receiving the renal veins, is similar to what has been reported in Megabats (Kallen, 1977) and the ringed seal (Smidtaka et al., 2008). This could have resulted from unique developmental events shared by Megachiropterans, Phocids and Macroscelideans. This feature is most probably a result of the persistence of the post renal portion of the left supracardinal vein, which usually disappears
in other mammals (Kent, 1978; Noden and de Lahunta, 1985; Walker and Liem, 1994; Moore and Persaud, 2003); a phenomenon reported to occur in dogs (Miller et al., 1964; Nickel et al., 1981) and humans (Moore and Persaud, 2003) as an aberration.

5.4 Genital ducts

5.4.1 The rete testis

The rete testis of rufous sengi is comprised of interconnected epithelium-lined channels in conformity with the typical mammalian and avian feature (Ladman and Young, 1958; Aire, 1982). Like the mouse, porpoise and fin whale, the sengi have extratesticular rete testis (Stoch, 1954; Dhingra, 1977). Similar to the report on the guinea pig (Ladman and Young, 1958), the rete testis of rufous sengi was lined by a squamous to cuboidal epithelial cells.

5.4.2 The efferent ductules

Efferent ductules connect the rete testis to the epididymis and are generally lined by a simple cuboidal or low columnar epithelium with ciliated and nonciliated cells (Hoffer and Greenberg, 1978; Jones et al., 1979; Jones and Brosnan, 1981; Oke et al., 1988). These general features of mammalian efferent ductules were also observed in the rufous sengi. Basal cells have been reported to occur in some species, including the elephant (Jones and Holt, 1981). These cells were absent in the rufous sengi, similar to the goat and the bull (Goyal et al., 2000).
The non-ciliated (principal) cells have basal ovoid nuclei, vacuoles in sub-apical cytoplasm and microvilli on their apical surfaces (Oke et al., 1988). Classification of non-ciliated cells into three types based on occurrence of PAS-positive granules and PAS-negative sub-apical vacuoles (Goyal et al., 2000) was however not demonstrated in the efferent ductules of rufous sengi, as in the African elephant (Jones and Holt, 1981).

5.4.3 The epididymis

The mammalian epididymis consists of a single elongated highly convoluted duct organized as a compact mass covered by a tough layer of fibrous tissue and divided into three topographic regions; the caput, corpus and cauda epididymis (Oke et al., 1988; Schimming and Vicentini, 2001). In scrotal mammals, it is usually applied on the epididymal border of the testis.

In the rufous sengi, the epididymis was relatively long extending far caudally beyond the testis with distinct topographic division into caput, corpus and cauda epididymis. It was made up of a single highly coiled duct enclosed by a fibrous capsule but because of the convolutions, several portions of the duct, each of which appeared as a distinct tubule, were captured during microtomy, creating an impression of many tubules bundled together, as it does in other mammals.

Most reports on epididymis mainly give details on the organization of its epithelium with little focus on other structures constituting its wall and the tissue between the coils of the duct. A report by Schimming and Vicentini
(2001) described all the components of the wall of epididymal duct in the dog, which included the epithelium that lay on a basal membrane, surrounded by a dense connective tissue coat or periductal stroma, consisting of several layers of collagen with elongated cells. Between the coils of the duct was a loose connective tissue referred to as intertubular interstitium. In the rufous sengi, the epididymal duct, just like what was reported in the dog (Schimming and Vicentini, 2001), consisted of an epithelium surrounded by a coat of dense connective tissue, the periductal stroma, containing elongated cells thought to be fibroblasts and blood vessels of varying sizes. Outer to periductal stroma was intertubular interstitium made up of loose connective tissue with blood vessels. The relatively high density of blood vessels in the sengi epididymis found both in the periductal stroma and intertubular interstitium conforms to the general picture of mammalian epididymis as a highly vascular organ (Fawcett, 1986).

The caput epididymis of rufous sengi was applied on dorso-lateral surface of the testis extending its entire length. In relation to the testis, the predisposition of the caput epididymis of rufous sengi is similar to that of the entire epididymis in the scrotal mammals. It was lined by a tall pseudostratified columnar epithelium with principal and basal cells. The apical cells observed in epididymal epithelium of some animals (Hoffer and Greenberg, 1978; Marengo and Amann, 1990; Goyal and Williams, 1991) were not observed in this study and this is similar to what has been reported on the giant rat (Oke et al., 1988) and the rabbit (Jones et al., 1979). Like the guinea pig (Hoffer and
Greenberg, 1978) and the goat (Goyal and Williams, 1991), clear cells were also not observed in the epididymal epithelium of rufous sengi. In the proximal portion, just after transition from efferent ductules, there was a dramatic increase in the height of principal cells with reduction in the luminal diameter. In conformity to the general morphological pattern in mammals, these cells had long microvilli. Caudally through the caput and corpus epididymis, there was gradual decrease in the height of principal cells with an increase in the luminal diameter. The apical blebbing of the epithelial principal cells exhibited in the proximal portion of the sengi caput epididymis is a common feature in many mammalian species (Saez et al., 2003) and it was initially thought to be artifacts of tissue fixation but later proven to be a tool of apocrine secretion (Aumuller et al., 1999; Hermo and Jacks, 2002; Baska et al., 2008). This unique apocrine secretion achieved through detachment of apical blebs, then referred to as epididymosomes, play a role of transferring epididymis-secreted proteins to sperm plasma membrane (Saez et al., 2003; Frenette et al., 2005; Baska et al., 2008; Sullivan, 2008; Girouard et al., 2009) thus fostering sperm maturation during epididymal transit and storage (Frenette et al., 2006; Sullivan, 2008).

Cauda epididymis is the sperm storage site in mammals, rufous sengis included as illustrated by high density of spermatozoa within the lumina of their cauda epididymal ducts. In scrotal mammals, the cauda epididymis is closely attached to the extremitas caudata of the testis. Generally, cauda epididymis tends to descend further than the testis; both in scrotal mammals
and true testiconda (Bedford, 1978a). In scrotal mammals, the cauda epididymis tends to extend beyond the extremitas caudata of the testis and is applied on a more exposed part of the scrotum that has a corresponding skin surface devoid of hair (Bedford, 1978a). In the study by Bedford (1978b), it was observed that surgical placement of epididymis connected to a normal scrotal testis into the abdomen does not affect fertility but shortens the storage time within cauda epididymis. Based on these, it was argued that epididymis, particularly the cauda epididymis requires lower temperatures for proper functioning as sperm storage duct. In the rufous sengis, the cauda epididymis was farther away, caudally, from the testis. This is similar to what has been reported in other sengi species studied (Stoch, 1954; Woodall and Skinner, 1989; Woodall, 1995) and confirms that in the absence of testicular descent, the cauda epididymis detaches from the testes then proceeds to markedly descend to a lateral position between the distal rectum and the pelvic urethra. This predisposition closely places the cauda epididymis against a part of the pelvic wall that more or less corresponds with the broad sacrotuberal ligament. This location, most probably offer temperatures lower than those in the abdomen. The position of the sengi’s cauda epididymis relative to that of the testis therefore shows that in these animals, the requirement for lower temperatures are more critical for the functioning of the cauda epididymis than it is for the testis. The same has also been demonstrated in other animals, including those with scrotal and ascrotal testes (Bedford, 1978a). It is therefore plausible to suggest, in complete agreement with Bedford (1978a), that the epididymis is a prime mover in the descent of mammalian testes.
Similar to observations on rock sengi (Stoch, 1954), the criss-crossing of cauda epididymides so that the right cauda is located on the left side and vice versa was rather common in the rufous sengi. There is however no significance that can be attached to this arrangement.

Characteristic of typical mammalian cauda epididymis, the epithelium of cauda epididymis in the rufous sengi consists of the principal and basal cells. The presence of numerous microvilli in the apical surface and numerous vacuoles in the supranuclear cytoplasm of principal cells illustrates the significant role the cauda epididymis plays in fluid reabsorption.

The lumen of cauda epididymis of rufous sengi was densely packed with spermatozoa, reminiscent of sperm storage role characteristic to all mammals. In the marsupials, at least the brush-tailed possum, the cytoplasmic droplets are mainly detached from the spermatozoa before they reach the cauda epididymis (Temple-Smith, 1984). The possum therefore have very few spermatozoa that bear cytoplasmic droplets within the lumen of cauda epididymis. In the eutherian mammals, on the other hand, the detachment of cytoplasmic droplets from spermatozoa mainly takes place in the cauda epididymis (Temple-Smith, 1984). The presence of numerous spermatozoa that bear cytoplasmic droplets within the lumen of the cauda epididymis of rufous sengi indicates that little or no detachment of cytoplasmic droplets takes place before the spermatozoa reach the cauda epididymis, reflecting what is generally known in eutherian mammals (Temple-Smith, 1984). In agreement with earlier reports on sengis (Woodall, 1995), the spermatozoa of
rufous sengi have spatulate nuclei capped with long acrosomes. Occurrence of few spermatozoa with abnormal morphology in the cauda epididymis of the sengi is a reflection of the general mammalian characteristic (Fawcett, 1986; Bernard, 2005).

Rare instances of occurrence of non–germ cell contents within the lumen of the epididymis, some foreign to the body such as coccidia (Hrudka et al., 1983), has previously been reported. In the bonnet monkey (Macaca radiata), two or more sperm tails were surrounded within a single membrane by amorphous material of cytoplasmic origin after treatment with gossypol (Kalla et al., 1986). The occurrence of dark staining masses that appeared to engulf spermatozoa in the cauda epididymis of wild rufous sengis is not only unique but also interesting. These masses could be cytoplasmic, resulting from merger of several cytoplasmic droplets and degenerated spermatozoa with probable inputs from the residues of epididymosomes.

5.4.4 The ductus deferens

In scrotal mammals, the ductus deferens is a long duct connecting cauda epididymis to the pelvic urethra. It is usually lined by a pseudostratified columnar epithelium surrounded by a thick wall with striated muscles. In the rufous sengi, just like what has been reported in other sengis (Stoch, 1954; Woodall and Skinner, 1989; Woodall, 1995), the ductus deferens was much shorter and was lined by a pseudostratified columnar epithelium. The epithelial cells had numerous vacuoles on their apical cytoplasm suggesting that they
play a role in fluid reabsorption. The principal cells of the ductus deferens play an apocrine secretory role (Andonian and Hermo, 1999; Andonian et al., 2002), a feature exhibited in the sengi by apical effusions of the principal cells. The thick muscular wall that surrounds the epithelium of this duct in mammals has also been reported to occur in other sengi species studied as well (Stoch, 1954; Woodall, 1995). The current study confirms that the same feature is also exhibited by the rufous sengi.

5.4.5 The uterus masculinus

The uterus masculinus of rufous sengi was apparently not divided into horns, similar to what has been reported in *Elephantulus rozeti* (Tripp, 1970). The uterus masculinus doesn’t play a role in conveyance of spermatozoa neither does it store. The presence of sperms within its lumen therefore was most likely as a result of reflux.

5.4.6 The urethra

The demarcation between the pelvic and the penile urethra, marked by the junction between the former and the bulb of the penis was distinctively clear in the sengi. Like the Mongolian gerbil (Pinheiro et al., 2003), the pelvic urethra of rufous sengi had a thick muscular coat of striated muscles that surrounded it like a belt. This urethral muscular coat incorporated the distal part of the ductus deferens through its dorsal border as has been reported in *Elephantulus myurus* (Stoch, 1954), and also the uterus masculinus and the prostatic ducts. These ducts ran caudally along the urethral ducts before
eventually joining it. The transitional epithelium lining the urethra of rufous sengi conforms to the general mammalian feature.

5.5 The accessory sex glands

The accessory sex glands of mammals consist of the vesicular, prostate and bulbo-urethral (Cowper's) glands. Similar to earlier reports on sengis (Stoch, 1954; Tripp, 1970), this animal under study lacked the vesicular glands. There are variations in the gross structure of the prostate glands between various groups of mammals (Brandes 1974). In rodents, the prostate gland is made up of several distinct lobes, a feature not obviously exhibited in primates. In a number of sengi species studied (Stoch, 1954; Tripp, 1970), the prostate gland, like that of the rat, was lobated. In the report by Stoch (1954) on Elephantulus myurus, the lobes of the prostate gland were grouped apparently according to their position relative to the other lobes and the pelvic urethra as; posterior dorsal, middle dorsal, inner anterior dorsal, outer anterior dorsal and anterior ventral. In agreement with reports on other sengi species, the prostate gland of the rufous sengi comprised of several lobes which were grouped using the criterion of Stoch (1954), but in addition, their gross appearance, mainly the colour was also considered. On this basis therefore, the lobes of the prostate gland were divided into two groups where, all the brown-red coloured lobes, which were placed cranially around the distal part of the neck of the urinary bladder were grouped together and referred to as cranial group or the cranial prostate. The large cream-white coloured caudally placed lobe constituted the caudal prostate or caudo-dorsal prostate. Additionally, the
naming of various lobes of sengi prostate in this study differs from that used in previous studies in the sense that the outer and the inner anterior dorsal lobes in the previous report (Stoch, 1954) correspond to the latero-dorsal and medio-dorsal lobes respectively while the posterior dorsal lobe corresponds to the caudo-dorsal lobe. The reason for this variation is that this study takes cognizance of the anatomical disposition of quadrupeds hence replacing the terms anterior and posterior with cranial and caudal respectively.

Histologically, the two dorsal lobes of the cranial prostate were similar while the ventral lobe showed similarities to the caudo-dorsal prostate. This is similar to what was reported in *Elephantulus myurus* (Stoch, 1954). The epithelial cells of the dorsal lobes of the cranial prostate exhibited apical blebs which are cast off into the lumen confirming apocrine secretion. This feature agrees with what has previously been reported about the prostate gland in other mammalian species (Nicander et al., 1974; Aumuller and Adler, 1979; Kachar and da Silva, 1980). The cast off apical blebs, then referred to as prostasomes, become one of the components of semen that make contributions to the post-testicular maturation, protection and capacitation of sperms in the female reproductive tract (Saez et al., 2003). The caudo-dorsal lobe of rufous sengi had very scanty intertubular stromal tissue closely reflecting what was observed in *Petrodromus* (Tripp, 1970) and *Elephantulus myurus* (Stoch, 1954; Tripp, 1970).

The bulbouretral gland of rufous sengi was medio-laterally flattened with a leaf-like appearance. Similar to the general mammalian structure, the bulbo-
urethral gland of rufous sengi was covered by a thick wall made up of striated muscles that extended into the interior of the gland. The same has also been reported in *Elephantulus myurus* (Stoch, 1954). The pink staining materials observed in the lumen of this gland have also been reported to occur in *Elephantulus myurus* (Stoch, 1954). These materials could be the equivalent of prostatic concretions observed in the prostate gland particularly in old men (Fawcett, 1986).

5.6 Morphometry

Point counting stereological technique was used to estimate volume densities of various testicular components. Using the same technique, Franca and Godinho (2003) found that in the domestic cat, seminiferous and inter-tubular tissues accounted for 88.2 ± 1.2% and 11.8 ± 1.2% of the testis parenchyma respectively. Woodall and Skinner (1989) reported that in *Elephantulus myurus*, the densities of the interstitial tissue varied between 16% and 21% during winter and spring/summer respectively. In the rufous sengi, testicular parenchyma accounted for approximately 98.3% of the testicular volume, with the other 1.7% being contributed by the capsule. Seminiferous tubules occupied 90.94% of the testicular parenchyma, which translates to 89.4 ± 0.8% of the total testis volume (the testicular capsule included). The interstitial tissue on the other hand occupied about 9.07% of the parenchyma or 8.9 ± 0.82% of the total testis volume, with 7.6% of its volume being contributed by the subcapsular interstitial tissue. These morphometric data therefore serve to confirm that sengis have little interstitial tissue as has earlier been reported.
(Asdell, 1964). The volume densities of testicular components in the rufous sengi reported apparently differ from what has been reported on *Elephantulus myurus* (Woodall and Skinner, 1989). This could have resulted from the difference in the techniques used to estimate the various volume densities.

5.7 **Morphology-based phylogeny**

Sengis have for a long time been considered to belong to a family within the order insectivora but were later placed in their own order, Macroscelidea, using morphology (Simons et al., 1991; Symonds, 2005) with the backing of molecular techniques (Stanhope et al., 1998). Molecular phylogenetics have further suggested the existence of close phylogenetic relationship between the sengis and a number of other animals belonging to different endemic African mammalian orders, namely; tenrecidae (tenrecs), paenungulata (African elephants, sea cows and hyraxes), tubulidentata (aadvarks) and chrysochloridae (golden moles) (de Jong et al., 1993; Springer et al., 1997; Stanhope et al., 1998; Liu et al., 2001; Murphy et al., 2001; van Dijk et al., 2001). Earlier, before application of molecular biology in phylogenetics, morphological studies did not offer support for such a diverse African clade (Stanhope et al., 1998; Symonds, 2005). In recent years, a few studies have attempted to shed more light on the issue of Afrotheria phylogeny from a morphological point of view (Woodall, 1995; Carter et al., 2004; Zack et al., 2005). To clarify this issue of Afrotheria monophyly from a morphological angle, the structure of the male reproductive system could, perhaps, be the key.
The sengi share several morphological features in the reproductive system with other members of the new superorder Afrotheria. Most notable among the shared features is the possession of intra-abdominal testes. The predisposition of sengis' testes relative to the kidneys compares well with those of the rock hyrax (Glover and Sale, 1968) and the dugong (Marsh et al., 1984). The pattern and the course of testicular blood vessels in the rufous sengi is similar to that reported in the rock hyrax (Glover and Sale, 1968) and the African elephant (Gaeth et al., 1999). Together with the unique pattern of the caudal vena cava, which is probably common to all Afrotherians, the course of the testicular blood vessels could be of great phylogenetic importance.

The gross structure of the epididymis of sengis with marked descent of cauda epididymis is similar to what has been reported in the rock hyrax (Glover and Sale, 1968) and the African elephant (Jones et al., 1979; Jones and Brosnan, 1981). These similarities exhibited by sengis and other Afrotherians in their male reproductive systems most probably indicate that these animals share a recent common ancestor and thus supporting their grouping together under the suggested super-order Afrotheria.

5.8 Miscellaneous

Members of the genus Porrocaecum whose larvae were found either freely roaming within the abdominal cavity or within diffuse nodules on the body wall muscles and viscera are usually intestinal parasites of birds (Digiani and
Sutton, 2001). Their larvae use arthropods, mainly the earthworms, as intermediate hosts and sengis become infested when they feed on arthropods. It is surprising that with such a heavy infestation by these larvae, the animals did not exhibit any sign of illness or distress. This could suggest that these animals acted as hosts to these larvae for a long time during which mechanisms were evolved to tolerate the infestation.

5.9 Conclusion and recommendations

The gross anatomical features of the reproductive system in the male rufous sengi generally resembled those of other sengis studied. It also shows great similarities to that of the African elephant and the rock hyrax. The origin and the course of testicular blood vessels also resemble what has been reported in the African elephant and the rock hyrax. These results may therefore offer support to earlier reports on molecular biology that suggested the existence of close phylogenetic relationships between sengi and these animals, the basis upon which they were grouped together under the new super-order Afrotheria. However, to have a complete picture of this cladistic grouping, studies on other members of this superorder are recommended.

On histological and ultrastructural analysis, the various components of the male reproductive system of the rufous sengis exhibited the basic mammalian features. Their testes exhibited complete spermatogenesis in spite of their intra-abdominal location. The mechanisms through which sengis and other testicondas are able to achieve complete spermatogenesis are yet to be clear.
Further research into this area is therefore necessary in an attempt to uncover these mechanisms and the possible contribution of torpor exhibited by sengis towards the success of spermatogenesis at high intra-abdominal temperatures.

The separate left and right caudal vena cavae observed in this study is rather unique. This feature is most likely a result of retention of embryonic pattern of caudal vena cava to adulthood. In order to clearly understand how this pattern develops, further embryological studies are required. This pattern could also be of great phylogenetic importance, and in that light, studying the pattern in the other members of the super-order Afrotheria and, in deed, in other testicondid mammals is necessary.

The adult *Porrocaecum* whose larvae were found in the sengi are usually avian parasites whose larvae use arthropods such as the earthworms as intermediate hosts. These invertebrate intermediate hosts form the largest portion of sengis’ diet, and the sengis are on the other hand preyed upon by some birds. It is therefore plausible to conclude that these worms’ larvae invade the sengi’s organs after ingestion of infected intermediate host (arthropod) thus using the sengi as a paratenic host. Further parasitological studies are however necessary so as to identify the species of these worms and to reveal the interrelationships between them, the intermediate host, the sengi and the definitive host (the birds). Studies into the general biology of these worms and their possible effects on the general health and reproductive fitness of sengis are also recommended. Epidemiologic studies may also be
required to outline the variations in the prevalence between various regions where sengis are found and between various species of sengi.
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