THE VASCULATURE OF THE MALE REPRODUCTIVE ORGANS OF THE OSTRICH (*STRUTHIO CAMELUS*)

by

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DECLARATION

I hereby declare that the work presented here is my original work, to the best of my knowledge. The work has never been published or submitted for a degree in any University. The University of Pretoria reserves the right of permission for duplication of the whole thesis or in part thereof.

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M. Z. J. Elias

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SUMMARY

THE VASCULATURE OF THE MALE REPRODUCTIVE ORGANS OF THE OSTRICH (*STRUTHIO CAMELUS*)

by

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Promotor: Prof. Dr. T. A. Aire
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The ostrich industry is a small, important section of the economy in the Republic of South Africa. The demand for ostrich products is high in the world, but the ostrich industry faces some production problems, one of which is the low fertility of commercially farmed ostriches.

This study aimed to determine the vasculature of the reproductive system in the male ostrich and determine the kind of blood-testis and blood-epididymis barriers, since there is no published information regarding this aspect. Thus 85 pre-pubertal and adult sexually active male ostrich were studied. Arterial supply, venous drainage, microvasculature of the male reproductive system, and blood-testis and blood-epididymal barriers, were determined in this specie, for the first time.

Results show that the pattern of the arterial blood supply to the reproductive organs of the male ostrich is similar to that of the domestic fowl and pigeon. However, few highlighted, distinctive, features were noted, the communication between the cranial renal arteries and middle renal arteries via collateral circulation.

The pattern of the venous drainage of the reproductive organs of the male ostrich, was similar to that described in the domestic fowl. However, important differences were the fusion between the caudal renal veins and the existence of a unique caudal median vein that had the caudal mesenteric vein as tributary.

The epithelial structure in the proximal efferent ducts was consistent with fluid absorption. The fluid absorbed in the efferent ducts is harvested also by capillary lymphatics situated in the interstitium.

Both the cranial and caudal segments of *Ductus deferens* and the *Receptaculum ductus deferentis* displayed one vascular networks beneath the tubular epithelium and other capillaries, venules, arterioles and collecting veins were in the periphery. Arterioles and collecting veins were also under the serosa. Subepithelial capillaries, in those segments, lacked fenestrations.

The spongy structure, in the root of phallus, exhibited several blood vessels in the strands. The capillaries and the venules displayed fenestrations. The erectile tissue in the phallic sulcus had the same features like the spongy in phallus. The spongy is comparable to corpus paracloacal vascular body present in other avian species and it should be called *lymphobulbus phalli*, it is the main source of lymph for phallus erection in the male ostrich.

There are similarities in the microvasculature pattern between ostrich and domestic fowl, but there are some differences in the ostrich:

(i) Absence of fenestrations in the capillaries of testis,

(ii) Very simple and random distribution of stromal blood capillaries, arterioles and venules in the epididymis,

(iii) Endothelial fenestrations, only in the blood capillaries close to proximal efferent ducts,

(iv) Isolated lymphatic capillaries in the epididymis, occasionally, presented endothelial fenestrations fitted with a membranous diaphragm,

(v) Existence of three vascular networks surrounding the tubule of deferent duct and receptaculum, that were determined on the base of the distribution, location and size of the vessels.

The blood-testis barrier of the ostrich is positioned: in (i) the capillary endothelium in the interstitium, between adjacent seminiferous tubule, (ii) the boundary tissue, and (iii) between spermatogonia and spermatocytes, and spermatogonia and Sertoli cells, and finally, (iv) between Sertoli cells. The occluding junctional complexes between the various tissue or cellular elements are mainly tight junctions.

The blood-epididymal barrier in the ostrich is revealed by participation of the endothelium of the capillaries and venules in the epididymal region, as lanthanum is trapped there, it does not appear beyond that point.

The blood-testis and blood-epididymal barriers are similar to those described for the domestic rooster.

CHAPTER 1

GENERAL INTRODUCTION

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1. OVERVIEW AND HISTORICAL BACKGROUND

The ostrich has been utilized by man since ancient times and the utilitarian, spiritual and decorative value of these birds has been exploited throughout the centuries. There are even indications that these birds may have been kept in captivity by the Egyptians (Holtzhausen & Kotzé 1990). Ostrich feathers, for example, were considered a symbol of justice by the Egyptians (Grzimek 1972) and were also used as fans and head-dresses. Roman and Greek generals, as well as Medieval knights, decorated their helmets with ostrich feathers as symbols of rank and status. In the late nineteenth century ostriches were exclusively hunted in South Africa for their feathers in order to supply the world's fashion markets. Feathers were so valuable that their earnings ranked fourth behind gold, diamonds and wool (Smit 1964; Holtzhausen & Kotzé 1990). This practice, although on a limited scale and utilizing farmed birds, continues today. Ostriches were also hunted for their meat, fat and skin by the Arabs and the San, the fat being much sought after for its medicinal and nutritional properties. The eggshells (Fig. 1.7) were used by the San for carrying water and for making beads, whereas a religious role was reserved for the shells in Egyptian temples and Coptic Christian churches in Ethiopia (Anonymous 1952).

The ostrich industry has experienced fluctuating fortunes since the domestication and breeding of these birds was initiated in 1850. The farming of ostriches was a direct result of the decline in wild ostrich populations due to hunting and was facilitated by the introduction of lucerne farming and wire fencing in South Africa (Smit 1964; Holtzhausen & Kotzé 1990). However, during the late nineteenth century droughts and epidemic diseases severely affected ostrich farming (Smit 1964; Osterhoff 1979) and a collapse of the ostrich

industry occurred in 1914 due to the effects of the First World War and campaigns against the use of ostrich feathers overseas. This resulted in a reduction of semi-domesticated ostriches from a million birds in 1914 (Holtzhausen & Kotzé 1990) to 32 000 in 1930 (Smit 1964). After the Second World War the ostrich industry began a steady consolidation of its activities under the auspices of the Klein Karoo Landboukoöperasie. The recovery of the industry was based on the utilization of the entire bird (feathers, fresh meat, dried meat [biltong], eggs and skin), of which the tanned skin became the most valuable product (Fig. 1.6). A number of commercial slaughter facilities were established throughout South Africa including the Little Karoo (Western Cape), Eastern Cape Province and Gauteng, at which tens of thousands of birds are slaughtered annually (Figs. 1.2 – 1.5) Ostriches also play an important role in the tourist industry (Spotlight 1994) particularly in the Oudtshoorn district of the Little Karoo (Fig. 1.1).

Ostrich farming for the production of meat, skin and feathers has become a global phenomenon and these birds are raised in numerous countries including Namibia, Zimbabwe, Israel, the United States of America, Australia, Japan (Hastings 1991; Mellet 1993), France (Kimminau 1993), Argentina (Shanawany 1995), Brazil (Lopes, Ferreira & Ferreira 2005), Germany (Wöhr & Erhard 2005), Saudi Arabia (Haydar 2005), China (Zhang 2005), India (Selvan, Sivakumar, Veeramani & Prabakaran 2008) and Spain (Carbajo & Lopez 2008). Despite the world-wide distribution of this industry, South Africa remains the major producer of ostrich products (Fig. 1.6) (Deeming 1999) and birds are commercially slaughtered at a number of facilities throughout the country (Figs. 1.2-1.5).

2. JUSTIFICATION

The ostrich industry continues to play an important role in the economy of South Africa, offering employment to thousands of people, and contributing to the development and growth of tourism. The world-wide demand for ostrich products has shown a steady increase (Kimminau 1993) and, despite periodic fluctuations in the global economy, the demand for ostrich products remains high. This has placed additional pressure on ostrich

producers to meet the increased demand. Although the ostrich industry is well established, it continues to experience production problems, resulting in fewer birds than anticipated reaching slaughter age. In addition, the reported low hatchability of artificially-incubated eggs (Burger & Bertram 1981; Deeming 1999; Malecki & Martin 2005) is a further complicating factor. It has been suggested that the fertility of male ostriches is key to the success of increased production, particularly in respect of their potential role in artificial insemination programmes. Although a considerable body of knowledge is available on the male reproductive tract of the ostrich, (Duerden 1912; Berens Von Rautenfeld 1977; King 1981; Budras & Meier 1981; Cho, Brown & Anderson 1984; Bezuidenhout 1986; Soley 1990; Fowler 1991; Soley 1992; Soley & Els 1992; Soley 1997; Soley & Groenewald 1999; Aire & Soley 2000; Aire & Soley 2003; Soley, Van Wilpe, Aire & Ozegbe 2005; Ozegbe, Aire & Soley 2006 a, b; Elias, Soley & Aire 2008a; Elias, Soley, Aire & du Plessis 2008b), large gaps still exist on the basic reproductive biology of this commercially important bird. Against this background, an investigation into the vascular system of the male reproductive organs of the ostrich was designed with a view to contributing baseline data on this currently poorly understood aspect of the male reproductive tract.

3. LITERATURE REVIEW

3. 1. Basic structure of the male reproductive tract of birds

The male reproductive tract of birds comprises paired testes (*testes*), the epididymis (*epididymides)* and deferent ducts (*Ducti deferentis*). The phallus, which lies in the proctodeum of the cloaca, also forms part of the reproductive tract, although it is not structurally connected directly with the above components.

a. The testes

The paired testes in birds are intra-abdominal. They are generally bean-shaped but display a variety of forms in some species, for example in swifts where they are vermiform in appearance. Each testis has a cranial extremity (*Extremitas cranialis)*, a caudal extremity

(*Extremitas caudalis)*, a free border (*Margo liber)* and an epididymal border (*Margo epididymidis)*. The testes are suspended by peritoneal folds on either side of the midline in the dorsal part of the coelomic cavity (Gray 1931; Gray 1937; Lake 1981; Nickel, Schummer & Seiferle 1977). They vary in size from 10-20mm in length in the domestic fowl (Lake 1981) to 160mm in the ostrich during the breeding season (Bezuidenhout 1986). The left testis is generally larger than the right (Gray 1937; King 1975).

The size of the testis increases greatly during periods of sexual activity, following an increase in the diameter and length of the seminiferous tubules and an increase in the number of the interstitial cells of Leydig (Temple 1974). In the fowl, for example, they increase to between 25–60mm in length and double their diameter (Lake 1981). In seasonal birds, the testes undergo cyclical variations in size (Artoni, Orsi, Carvalho, Vicentini & StefaninI 1999). The colour of the testes also varies according to the degree of sexual activity, from white in active testes, to a darker, dull grey, light brown or black colour in the inactive state. The testes of the emu are highly pigmented irrespective of season (Cho *et al*. 1984; Fowler, 1991; Malecki, Martin, O'Malley, Meyer, Talbot & Sharp, 1998).

The avian testis is encapsulated by a *Tunica albuginea* which is lined externally by a mesothelium representing the visceral layer of the peritoneum. No distinct septa project into the testis parenchyma, as occurs in mammals (Samuelson 2007) and the organ is therefore not lobulated; there is also no *Mediastinum testis* (Lake 1957). The *Parenchyma testis* of sexually active birds is composed of thousands of *Tubuli seminiferi* (Baumel 1975; Nickel *et al.* 1977). The seminiferous tubules begin blindly, anastomose, and form a complex tubular network (Lake 1957). They are lined internally by the germinal (seminiferous) epithelium, and surrounded externally by peritubular/interstitial tissue. Spermatogenesis takes place in the seminiferous epithelium. Leydig cells, which occur within the vascular interstitial connective tissue, occupy the intertubular spaces of the testis, and secrete androgens (Baumel 1975; Nickel *et al.* 1977). In seasonally-breeding birds the Leydig cells involute during the sexually quiescent period, but in the sexually active phase the cells become much larger.

In the domestic fowl, turkey, guinea-fowl and duck, the seminiferous tubules open into the *rete testis* (Tingari 1971a; Aire 1979a). The channels or lacunae that form the rete testis network are located mainly outside the testis, in the epididymis (Tingari 1971a). However, Budras & Sauer (1975) in the chicken, Aire (1979a, 1982) in the Japanese quail, Aire (1982, 2007) in the guinea fowl and Aire & Soley (2003) in the ostrich, report that the small initial part of the rete testis is intratesticular. In some species of birds the epididymis appears to be relatively simple in structure and in the Common Tern (*Sterna hirundo)*, for example, the seminiferous tubules open directly into the efferent ductules (Traciuc 1967). In the Jackdaw (*Corvus monedula*), the *rete testis* is apparently absent (Traciuc 1969).

b. The epididymis

The epididymis has been described as an elongated, spindle-shaped enlargement running along most of the length of the dorso-medial border of the testis (Bailey 1953; Lake 1971), and which forms a small organ enclosed in an extension of the *Tunica albuginea* of the testis. The cranial extremity of the epididymis of the domestic fowl is embedded in the adrenal capsule (Gray 1937; Lake 1957). The main components of this organ are the extra-testicular *Rete testis*, the efferent ducts, the connecting ducts and the epididymal duct (Baumel 1975; Hess, Thurston & Biellier 1976; Aire 1979a,b; Stefanini & Orsi 1999). The *Rete testis* extends from the medial border of the testis to the adjacent aspect of the epididymis (Gray 1937; Lake 1957; Lake 1981) where it forms the extra-testicular *Rete testis*. The avian epididymis is not sub-divided into a head, body and tail, as the efferent ducts into which the *Rete testis* open, arise throughout most of the length of the testis dorso-medially. However, in the Jackdaw, the efferent ducts may be exclusively concentrated at the caudal extremity of the testis (Traciuc 1969). The number of efferent ducts varies between species. There are five in Common tern (*Sterna hirundo)* and ten in Jackdaws (*Corvus monedula)* (Traciuc 1967, 1969). The connecting duct in several birds is a short duct linking the efferent ducts with the epididymal duct, and has the same type of epithelial lining as the latter duct (Aire 1979a). The Ductus epididymidis begins at the cranial extremity of the medial border and continues to the caudal extremity of the epididymis, from where it is continued caudally by the deferent duct. The epididymis in seasonally breeding birds undergoes cyclical changes (Bailey 1953; Traciuc 1969; Aire 2002a,b).

c. The *Ductus deferens*

The deferent duct is serpentine, compactly convoluted, and runs caudally with the ureter, together with which it enters the wall of the cloaca on the dorsal aspect of the urodeum. The duct ends in the barrel-shaped *Receptaculum ductus deferentis* that opens into the urodeum via the *Papilla ductus deferentis* (Lake 1971; Baumel 1975; Aire 1979b; McCracken, Kainer & Spurgeon 1999). In the sexually active domestic fowl, the duct is full of semen, whereas in the sexually inactive bird it is reduced in diameter and contains a watery secretion (Lake 1981).

d. The accessory sex organs

In birds there are no structures or organs that correspond to the mammalian accessory sex glands (*Glandula bulbourethralis, Glandula vesicularis, Ampulla ductus deferentis* and *Prostata*). However, the highly convoluted termination of the deferent ducts in passerine birds has incorrectly been referred to as the *Vesicula seminalis* (Nickel *et al.* 1977). This region is now known as the *Seminal glomus* (Aire 2007).

e. The avian phallus

Studies on the phallus have been conducted in domestic anseriform, galliform and ratite birds (Müller 1838 quoted by King 1981). In *Gallus* the phallus lies in the ventral midline of the *proctodeum*, immediately caudo-medial to the papilla of the deferent duct. It consists of a median phallic body and paired lateral phallic bodies. A lymphatic fold lies between the lateral phallic body and the papilla of the deferent duct. Lymphatic channels in the phallus are linked to those of the vascular bodies and lymphatic folds. Although not forming part of the phallus itself, the paracloacal vascular body, *Corpus vasculare paracloacale,* is the source of the lymph which erects the male phallus in the drake and domestic fowl (Kudo, Sugimura & Yamano 1975; Sugimura, Kudo & Yamano 1975; Knight 1984; Sasaki, Nishida, Fugihara & Mochizuki 1984). Therefore, in the domestic Anseriforms and Galliforms, the mechanism of erection is lymphatic (King 1981). Two varieties of avian phallus are recognized: the *phallus protrudens,* found in ratites and anseriform birds and the *phallus non-protrudens,* found in galliform species (King 1981). A detailed description of the functional morphology of the ostrich phallus is provided in section 3.5.d. below.

3. 2. Basic pattern of blood supply to the avian male reproductive tract

The basic pattern of the blood supply to the gonads and genital tract of male birds has been described by Nishida (1964) in the chicken, Bhaduri, Biswas & Das (1957) in the pigeon, and by Okamoto, Vollmerhaus, Roos, Waibl & König (1992) in the Japanese quail. Baumel (1975) has also published a diagram of the blood supply to the abdominal wall and viscera of the chicken in which the appropriate blood vessels of the reproductive organs conform to those described by Nishida (1964). Kurihara & Yasuda (1975a) described the arterial supply of the kidneys of the male fowl which included a description of the branches of the cranial and middle renal arteries that supply the testes, the cranial aspect of the deferent duct and the middle deferent duct. Kurihara & Yasuda (1975b) also described the venous system of the kidneys in the domestic fowl and described aspects of the vascular drainage of the cranial and middle portions of the deferent duct.

a. The testes

Nishida (1964), Kremer & Budras (1990) and Okamoto *et al.* (1992) described both the arterial supply and venous drainage of the gonads of the domestic fowl, Peking drake and Japanese quail, and observed that the *Arteria testicularis* arises from the *Arteria renalis cranialis,* and that the *Arteria testicularis accessoria* arises directly from the aorta. Both the testicular and accessory testicular arteries supply the testis, entering at the cranial half of the medial border of each testis. However, the presence of an *Arteria testicularis accessoria* is inconsistent, being absent in some birds. The *Venae testiculares* drain the testes, and empty into the caudal vena cava laterally.

Variations in the arterial supply to the testis of the fowl have been observed (Nishida 1964; Okamoto *et al.* 1992): variant 1 demonstrates two *Arteriae testiculares accessoria* arising directly from the aorta on either side; variant 2 is the occurrence of only one *Arteria testicularis accessoria* arising directly and separately from the aorta on the left side; in variant 3, the *Arteria testicularis accessoria* arises directly from the aorta on the right side; variant 4 demonstrates one *Arteria testicularis accessoria* on either side, each vessel arising from a common stem together with the testicular artery of that side; and variant 5 has a similar pattern to that of variant 4, except that one of the testicular accessory arteries arises directly from the aorta.

It has also been noted that the blood vascular system of the testis of the fowl does not show a pattern suited to a thermo-regulatory mechanism such as that found in mammals, as the testes are intra-abdominal and there is no pampiniform plexus (Lake 1981).

b. The epididymis

The *Arteria ramus epididymales* arises from the A*rteria testicularis* and supplies the epididymis (Nishida 1964).

c. The *Ductus deferens*

The *Ductus deferens* receives blood from*:* (1) the *Rami (Rr.) ureterodeferentiales cranialis* that arise from the *Arteria renalis cranialis*, at the dorso-medial aspect of the caudal pole of the testis; (2) the *Rr. ureterodeferentiales medii* that arise from the ventral part of the *Arteria ischiadica* via the *Arteria renalis media,* and (3) the *Rr. ureterodeferentiales caudales* that arise from the *Arteria pudenda* which lies dorso-lateral to the cloaca (Nishida 1964; Okamoto *et al.* 1992).

The *Venae ureterodeferentiales craniales* drain the cranial part of the *Ductus deferens* into the *Venae testiculares,* while the *Venae ureterodeferentiales medii* drain the middle part of the *Ductus deferens* into the *Vena renalis caudalis.* The *Venae ureterodeferentiales caudales* drain the caudal part of the *Ductus deferens* into the *Vena pudenda* (Nishida 1964).

d. Cloacal structures

The paracloacal vascular body, rudimentary copulatory organs, and cloaca receive their blood supply from branches (*Arteriae bursocloacales)* of the *Arteria pudenda* (Knight, Lucas & Ringer 1969; Kudo *et al.* 1975; Fugihara, Nishiyama & Nakashima 1976; Knight 1984; Okamoto *et al.* 1992). The *Vena pudenda* and *Venae cloacales* collect the venous blood from the above structures, and empty it into the *Vena iliaca interna* (Knight 1984).

3. 3. The microvasculature of the avian male reproductive tract

The microvasculature of the male reproductive organs of the domestic fowl has been studied by Nishida (1964), Nakai, Hashimoto, Kitagawa, Kon & Kudo (1988), Knight (1970), Sugimura *et al.* (1975), Kudo *et al.* (1975) and Gunawardana & Scott (1978). Nakai *et al.* (1988) found the interstitial tissue of the rete testis to be poorly endowed with an irregularly arranged capillary network emanating from several branches of the testicular artery and vein. In the rete testis the capillary network was rarely fenestrated. Peritubular capillary networks encircled individual efferent ducts. These capillaries were located just beneath the epithelium and were fenestrated. The microvasculature of the *Ductus deferens and Receptaculum ductus deferentis* also forms a prominent peritubular vascular network.

Knight (1970) has described the arterial and venous blood vessels of the phallus and lymph fold (*Plica lymphatica*) in the fowl. He reported that the basic units of the paracloacal vascular body were the glomera that have reteform tufts of capillaries. Sugimura *et al.* (1975) and Gunawardana & Scott (1978) noted the presence of fenestrated blood capillaries in the *Glomera corporis vascularis paracloacalis* of the same species. The vascularized bodies consist of blood capillaries which arise from branches of the *Arteria pudenda* and are drained by the *Vena pudenda*, as described by Nishida (1964) and Bhaduri *et al.* (1957). The microvasculature of the paracloacal vascular body in the guinea fowl (*Numida meleagris*) was investigated by Sasaki *et al.* (1984); it was composed of capsular, peripheral and internal lymphatic sinuses, trabeculae and capillary cords containing blood vessels. Arterioles, venules, collagen fibres, fibroblasts and nerve endings were present in the interstitial tissue of the capillary cords.

3. 4. Blood-testis (BTB) and blood-epididymis (BEB) barriers

The blood-testis-barrier (BTB) is a barrier composed of specialised junctional complexes between adjacent Sertoli cells near the base of the seminiferous epithelium (Dym & Fawcett 1970) that exclude large molecules and other substances from entering the tubules (Gilula, Fawcett & Aoki 1976; Setchell 1980). It is also considered that, in addition to the Sertoli cells,

the components of the peritubular tissue, as well as the blood vessels in the interstitial tissue, also form part of the BTB (Fawcett, Leak & Heidger 1970; Neaves 1977; Setchell & Waites 1975). The BTB has been extensively studied in numerous mammalian species (see Chapter VI).

The BTB is designed to: (a) prevent contact between the blood and the interior of the seminiferous tubule for the maintenance of a favourable spermatogenic environment within the seminiferous tubules (Hinton 1985; Waites & Gladwell 1982; Toyama, Maekawa & Yuasat 2003), (b) prevent contact between sperm auto-antigens and the blood, (c) protect the lumen of seminiferous tubules against sperm-specific antibodies, (d) protect developing germ cells against environmental toxins and mutagens, and (e) maintain the structural integrity of the seminiferous epithelium and tubules (Waites & Gladwell 1982). It partitions the seminiferous epithelium into basal and adluminal compartments (Dym & Fawcet 1970), and preserves and enables endocrinological substances formed in the seminiferous tubules to reach the distal portion of the male genital tract where they are absorbed (Setchell & Waites 1975).

The BTB has been extensively studied in numerous mammalian species (see Chapter VI) and its existence has been noted in the avian testis and information provided on the structure of intercellular junctions between elements of the seminiferous epithelium and between the contractile cells of the peritubular tissue (Cooksey & Rothwell 1973; Rothwell 1975; Pfeiffer & Vogl 1993; Aire 1997). The use of tracers has also been applied to determine the extent of the blood-testis barrier in the domestic fowl (Osman, Ekwall & Plöen 1980; Bergmann & Schindelmeiser 1987; Weber *et al.* 1988; Pelletier 1990), and duck Pelletier (1990).

A similar blood-tissue barrier, the blood-epididymis barrier (BEB), has also been reported in the epididymis (Lopez, Fuentes, Retamal & de Souza 1997). Tight junctions (*Zonula occludens*) (Suzuki & Nagano 1978a,b; Friend & Gilula 1972) separate the epididymal lumen from the blood (Hoffer & Hinton 1984) and form the BEB. Thus, the epididymal luminal fluid is different from blood plasma (Turner 1979; Turner & Howards 1985). The BEB protects the maturing spermatozoa from potentially harmful external factors (Friend & Gilula 1972; Hinton 1985) in a similar fashion to that described for the BTB.

Studies on the BEB have been performed in several mammals, including rats (Suzuki & Nagano 1978b; Cooper & Waites 1979; Hinton & Howards 1981; Turner, Giles & Howards 1981; Hoffer & Hinton 1984; Agrawal & Hoffer 1989), hamsters (Howard, Jesse, & Johnson 1976; Turner, Cochran & Howards 1981; Turner, D'Addario & Howards 1983; Turner & Howards 1985), rabbits (Flickinger 1975), and stallions (Lopez *et al.* 1997). A review on the role of the BEB has also been published (Hinton & Palladino 1995).

Studies on the avian epididymis reported the presence of junctional complexes at the ad-luminal aspect of the epithelial cells surrounding the excurrent ducts (Tingari 1971a,b; Budras & Sauer 1975; Bellamy & Kendal 1985; Aire 2002a,b). The BEB has also been demonstrated in the domestic fowl using horse-radish peroxidase (Nakai *et al.* 1988; Nakai & Nasu 1991). The tracer was observed to leave the subepithelial capillaries by various routes, pass into the intercellular spaces between adjoining epithelial cells and be stopped from entering the lumen of the excurrent ducts by the apical junctional complexes.

Although some ultrastructural studies on the ostrich testis (Soley 1990, 1992, 1997; Soley *et. al.* 2005) and epididymis (Budras & Meier 1981; Aire & Soley 2003; Ozegbe *et al.* 2006a,b; Elias *et al.* 2008b) have supplied morphological data on the junctional complexes present in these components of the reproductive tract, no specific information on the extent of the BTB and BEB in this species has been presented.

3. 5. Basic structure of the male reproductive tract of the ostrich

a. The testis

The testes of the ostrich are located intra-abdominally, where they are situated on either side of the caudal vena cava and lie ventral to the cranial division of the kidneys (Bezuidenhout 1986; Soley 1992), as has been described for other birds. The general morphology of the ostrich testis has been reported by Duerden (1912), Cho *et al.* (1984), Bezuidenhout (1986), Soley (1990), Fowler (1991), Soley (1997) and Soley & Groenewald (1999). The testes of sexually immature birds are rod-shaped or finger-like, while in sexually active adults they

appear oval. The size of the testis varies with age and the phase of the reproductive cycle (Duerden 1912; Soley 1992).

Various studies have detailed the histological and ultrastructural features of the ostrich testis (Soley 1990, 1992, 1997; Soley and Groenewald 1999; Elias *et al.* 2008a). The testis is enclosed by a well-developed *Tunica albuginea* which is covered by the visceral layer of the *Tunica vaginalis*. Numerous blood vessels are present, particularly beneath the peritoneal lining, and nerves and profiles of the rete testis are also observed. A number of fine septa run from the capsule between the highly convoluted seminiferous tubules and unite with the peritubular tissue in the interstitium. Occasionally, large, well-vascularised septa are seen although the testis lacks lobulation (Soley 1992, 1997). The interstitial connective tissue supports the seminiferous tubules and accommodates some blood and lymphatic vessels, as well as groups of Leydig cells (Soley 1990, 1992). The seminiferous epithelium is composed of regularly spaced Sertoli cells between which lie the various stages of the developing germ cells. The seminiferous tubules terminate in the *Rete testis* which in turn join the *Ductuli efferentes* in the epididymis (Budras & Meier 1981; Soley & Els 1992; Aire & Soley 2003).

b. The epididymis

The epididymis lies on the dorso-medial surface of the testis (Bezuidenhout 1986; Soley 1992) and comprises a cranial *Appendix epididymidis,* followed caudally by the main part which is attached to the testis along most of its length. It is continued caudally by the deferent duct. The embryological development and structure of the epididymis has been described in the ostrich by Meier (1979) and Budras & Meier (1981). They observed that the *Rete testis*, proximal efferent ducts and distal efferent ducts developed from the mesonephros, while the epididymal duct developed from the Wolffian duct. The epididymis of the ostrich is similar to that of various other birds (Aire 2007) in consisting of several ducts, namely, the *Rete testis*, proximal and distal efferent ducts, connecting ducts and the *Ductus epididymidis* (Soley & Els 1992; Aire & Soley 2000; Aire & Soley 2003; Ozegbe, Aire & Soley 2006 a, b) (for further details see Chapter IV).

c. The deferent duct

The *Ductus deferens* of the ostrich is a caudal continuation of the epididymis. It begins as a relatively straight tube, but then becomes extremely convoluted as it runs caudally, alongside the ureter, in the vicinity of the dorsal midline. Near the cloaca, the duct becomes straight and forms the *Pars recta ductus deferentis,* which then forms the barrel-shaped *Receptaculum ductus deferentis* that opens into the urodeum (Duerden 1912; Bezuidenhout 1986; Fowler 1991; Soley 1992; Soley & Groenewald 1999). This duct is clearly visible in sexually mature birds during the breeding season due to its obvious wavy appearance (Soley & Groenewald 1999).

d. The phallus

The phallus of the ostrich has been described by Duerden (1912), [Müller 1836 and Gerhardt 1933 quoted by King (1981)] and Fowler (1991). The ostrich phallus is of the intromittent type, and lacks an internal cavity. It is connected to the ventral wall of the cloaca and comprises two main parts: the *Basis phalli* and *Apex phalli* [Müller 1836 and Gerhardt 1933 quoted by King (1981)]. Structurally, the phallus is composed of a pair of fibrous bodies, a *Sulcus phalli*, a median elastic vascular body and a pair of phallic muscles. In adults, the flaccid phallus is about 20 cm long and about 40 cm long, when erect [Gerhardt 1933 quoted by, King (1981)]. It does not possess a urethra, and does not have a urinary function.

In the ostrich, the erection of the phallus is a function of the lymphatic system (Berens von Rautenfeld 1977) and is effected by a large lymphobulbus phallus on either side of the seminal groove at the dorsal aspect of the organ. The lymphatic system of the phallus is linked to the general somatic lymphatic system and, hence, to the general systemic circulation (Berens von Rautenfeld 1977).

3. 6. Blood supply to the reproductive tract of the male ostrich

Reports on the blood supply to the reproductive tract of the ostrich are sparse and often incidental, with most information being supplied by Bezuidenhout (1999) who reported that the testicular arteries arose from the cranial renal arteries. This is in agreement with the

findings in the domestic fowl (Nishida 1964) and the pigeon (Bhaduri *et al.* 1957). Additionally, Bezuidenhout (1999) reported that the testicular artery may arise directly from the aorta caudal to the renal arteries, which contrasts with the situation in the domestic fowl (Nishida 1964), and that additional testicular arteries can also arise directly from the aorta. According to Bezuidenhout (1999), the blood from the testis is drained into the azygos vein and caudal vena cava through unnamed tributaries.

4. BENEFITS ARISING FROM THE STUDY

a. The study will contribute to a better understanding of ostrich reproduction by providing new information on the pattern of vascularization of the reproductive system of the male ostrich.

b. The study will enhance efforts to increase the fertility and productivity of ostriches under commercial farming conditions.

c. The study will contribute to the field of comparative avian anatomy and stimulate interest in the reproductive physiology of ratites.

5. AIMS OF THE STUDY

Based on the various considerations outlined above, this study aims to:

a. Describe the basic gross anatomical pattern of the arterial blood supply and venous drainage of the male reproductive tract and determine possible variations in the general pattern.

b. Determine the distribution and morphological characteristics of the microvasculature of the reproductive organs, and explore the relationship between the blood vessels and the particular part of the reproductive tract they are associated with.

c. Determine the nature of the blood-testis and blood-epididymis barriers.

6. REFERENCES

AIRE, T. A. 1979a. The epididymal region of the Japanese quail *(Coturnix coturnix japonica). Acta Anatomica,* 103: 305-312.

AIRE, T. A. 1979b. Micro-stereological study of the avian epididymal region. *Journal of Anatomy,* 129: 703-706.

AIRE, T. A. 1982. The rete testis of birds. *Journal of Anatomy*, 135: 97-110.

AIRE, T. A. 1997. The structure of the interstitial tissue of the active and resting avian testis. *Onderstepoort Journal of Veterinary Research,* 64: 291-299.

AIRE, T. A. 2002a. Cyclical reproductive changes in the non-ciliated epithelia of the epididymis of birds. *Anatomia, Histologia & Embryologia,* 31: 113-118.

AIRE, T. A. 2002b. Morphological changes in the efferent ducts of the epididymis during the main phases of the reproductive cycle of birds. *Journal of Morphology,* 253: 64-75.

AIRE, T. A. 2007. Anatomy of the testis and male reproductive tract, in *Reproductive Biology and Phylogeny of Birds Vol 6A.* Edited by B. G. M. Jamieson. Jersey: Science Publishers. pp. 37-113.

AIRE, T. A. & SOLEY, J. T. 2000.The surface of the epithelial lining of the ducts of the epididymis of the ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia*, 29**:** 119-126**.**

AIRE, T. A. & SOLEY, J. T. 2003. The morphological features of the rete testis of the ostrich *(Struthio camelus). Anatomia & Embryologia,* 207**:** 355-361.

ANONYMOUS. 1952. Anecdotes of ostriches. *Bentley's Miscellany*. 31**:** 491-506.

ARTONI, S. M. B., ORSI, A. M., CARVALHO, T. L. L., VICENTINI, C. A. & STEFANINI, M. A. 1999. Seasonal morphology of the domestic quail (*Coturnix coturnix japonica*) testis. *Anatomia, Histologia, Embryologia,* 28: 217-220.

BAILEY, R. E. 1953. Accessory reproductive organs of male fringillid birds: seasonal variations and response to various sex hormones. *The Anatomical Record,* 115: 1-20.

BAUMEL, J. J. 1975. Heart and blood vessels, in *The Anatomy of Domestic Animals,* edited by R. Getty. Philadelphia: Saunders Company. pp. 1968-2009.

BELLAMY, S. J. & KENDAL, M. D. 1985. The ultrastructure of the epithelium of the *ductuli efferentes testis* in the common starling (*Sturnus vulgaris*). *Journal of Anatomy*, 140: 189-203.

BERENS VON RAUTENFELD, D. 1977. Mitteilungen zur künstlichen Besamung, Geschlechts- und Altersbestimmung beim Strauß (*Struthio camelus australis,* GURNEY). *Der Praktische Tierarzt,* 5/77: 359-364.

BERGMANN, M. & SCHINDELMEISER, J. 1987. Development of the blood-testis barrier in the domestic fowl (*Gallus domesticus*). *International Journal of Andrology,* 10: 481-488.

BEZUIDENHOUT, A. J. 1986. The topography of the thoraco-abdominal viscera in the ostrich *(Struthio camelus). Onderstepoort Journal of Veterinary Research,* 53: 111-117.

BEZUIDENHOUT, A. J. 1999. Anatomy, in *The Ostrich. Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp. 13-50.

BHADURI, J. L., BISWAS, B. & DAS, S. K. 1957. The arterial system of the domestic pigeon *(Columba livia* Gmelin). *Anatomischer Anzeiger*, 104: 1-14.

BUDRAS, K. D. & SAUER, T. 1975. Morphology of the epididymis of the cock (*Gallus domesticus*) and its effect upon the steroid sex hormone synthesis. I. Ontogenesis, morphology and distribution of the epididymis. *Anatomia & Embryologia,* 148**:** 175-196.

BUDRAS, K. D. & MEIER, U. 1981. The epididymis and its development in ratite birds (ostrich, emu, rhea). *Anatomia & Embryologia,*162**:** 281-299.

BURGER, A. E. & BERTRAM, B. C. R. 1981. Ostrich eggs in artificial incubators: Could their hatching success be improved? *South African Journal of Science*, 77: 188-189.

CARBAJO, E. & LOPEZ, J. F. 2008. HACCP approach to the monitoring of potential hazards in ostrich processing: microbial testing of ostrich products. *Proceedings of the 23rd World's Poultry Congress. World Poultry Science Journal*, 64: 668.

CHO, P; BROWN, R. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3: 133-144.

COOKSEY, E. J. & ROTHWELL, B. 1973. The ultrastructure of the Sertoli cell and its differentiation in the domestic fowl (*Gallus domesticus*). *Journal of Anatomy,* 144: 329-345.

COOPER, T. G. & WAITS, G. M. H. 1979. Steroid entry into rete testis barrier. *Journal of Endocrinology*, 65: 195-206

DEEMING, D. C. 1999. Introduction, in *The ostrich. Biology, Production and Health*. Edited by D. C. Deeming. London: CABI Publishing. pp. 1-11.

DUERDEN, J. E. 1912. Experiments with ostriches - XX. The anatomy and physiology of the ostrich. C - The Internal Organs. *South African Agricultural Journal:* 1-27.

DYM, M. & FAWCETT, D. W. 1970. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biology of Reproduction*, 3: 308-326.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2008a. Intercellular junctions in the ostrich *(Struthio camelus)* testis. *Proceedings of the 38th Conference of the Anatomical Society of Southern Africa,* Kruger Park, South Africa: 97.

ELIAS, M. Z. J., SOLEY, J. T., AIRE, T. A. & DU PLESSIS, L. 2008b. Membrane specialization in the efferent ducts of the ostrich epididymis. *Proceedings of the Microscopy Society of Southern Africa, 46:* 76.

FAWCETT, D. W., LEAK, L. V. & HEIDGER, P. M. 1970. Electron microscopy observations on the structural components of the blood-testis barrier. *Journal of Reproduction and Fertility (Supplement)*, 10: 105-122.

FLICKINGER, C. J. 1975. Fine structure of the rabbit epididymis and *vas deferens* after vasectomy. *Biology of Reproduction*, 13: 50-60.

FOWLER, M. E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22: 204-227.

FRIEND, D. S. & N. B. GILULA, 1972. Variations in tight and gap junctions in mammalian tissue. *Cell and Biology,* 53: 758-776.

GILULA, N. B., FAWCETT, D. W. & AOKI, A. 1976. The Sertoli occluding junctions and gap junctions in mature and developing mammalian testis. *Developmental Biology,* 50: 142-168.

GRAY, J. C. 1931. The anatomy of the male genital ducts in the fowl. *The Anatomical Record,* 51: 88.

GRAY, J. C. 1937. The anatomy of the male genital ducts in the fowl. *Journal of Morphology,* 60: 393-405.

GRZIMEK, B. 1972. The Ratites, in *Grzimek's Animal Life Encyclopedia Vol 7*. Edited by B. Grzimek B. New York: Van Nostrand Reinold. pp.89-109.

GUNAWARDANA, V. K. & SCOTT, M. G. A. D. 1978. On the structure of the vascular body in the domestic fowl. *Journal of Anatomy,* 127: 447-457.

HASTINGS, M.Y**.** 1991. A history of ostrich farming—Its potential in Australian agriculture, in *Recent Advances in Animal Nutrition Australia*, Edited by D.J. Farrell. Armidale: University of North England.pp.292-297.

HAYDAR, R. A. 2005. Egg production, nutrition and heat effect on bird performance in Saudi Arabia. *Proceedings of the 3rd International Ratite Science Symposium of the World's Poultry Science Association & XII World Ostrich Congress:* 272-276.

HESS, R. A., THURSTON, R. J. & BIELLIER. 1976. Morphology of the epididymal region and ductus deferens of the turkey (*Meleagris gallopavo*). *Journal of Anatomy,* 122: 241-252.

HINTON, B. T. 1985. The blood-epididymis barrier, in *Male Fertility and its Regulation*. Edited by T. J. Lobl & E. S. E. Hafez. Lancaster: MPT Press Limited. pp. 371-393.

HINTON, B. T. & PALLADINO, M. A. 1995. Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. *Microscopy Research and Technique*, 30: 67-81.

HINTON, B. T. & HOWARD, S. S. 1981. Permeability characteristics of the epithelium in the rat caput epididymis. *Journal of Reproduction and Fertility*, 63: 95-99.

HOFFER, A. P. & HINTON, B. T. 1984. Morphological evidence for a blood-epididymal barrier and the effects of gossypol on its integrity. *Biology of Reproduction,* 30**:** 991-1004.

HOLTZHAUSEN, A. & KOTZÉ, M. 1990. *The ostrich. C. P*. Nel Museum, Oudtshoorn.

HOWARD, S. S., JESSE, S. J. & JOHNSON, A. L. 1976. Micropuncture studies of the blood-seminiferous tubule barrier. *Biology of Reproduction*, 14: 164-269.

KIMMINAU, K. M. 1993. Introducing the ostrich. *Continuing Education,* 14: 459-467.

KING, A. S. 1975. Urogenital System, in *Sisson and Grossman's, The Anatomy of the Domestic Animals.* Edited by R. Getty. Philadelphia: W. B. Saunders Company. pp.1919-1935.

KING, A. S. 1981. Phallus, in *Form and Function in Birds.* Edited by A. S. King & J. McLelland. London: Academic Press. pp.107-147.

KNIGHT, C. E. 1970. The anatomy of structures involved in the erection-dilution mechanism in the male domestic fowl. PhD thesis, Michigan State University.

KNIGHT, C. E. 1984. Anatomy of the *corpus vasculare paracloacale* of the male turkey. *Poultry Science,* 63**:** 1883-1891.

KNIGHT, C. E., LUCAS, A. M. & R. K. RINGER. 1969. Anatomy of structures involved in the production of seminal fluid in the chicken. *Poultry Science,* 48**:** 1830-1831.

KREMER, A. & BUDRAS, K. D. 1990. Zur Blutgefassversorgung des Hodens beim Pekingerpel (*Anas platyrhynchos, L*.). Makroskopische, lichtmikroscopische und rasterelektronenmikroskopische Untersunchungen. *Anatomischer Anzeiger*, 171:73-87.

KUDO, N., SUGIMURA, M. & YAMANO, S. 1975. Anatomical studies of *corpus paracloacalis vascularis* in cocks. *Japanese Journal of Veterinary Research,* 23: 1- 10.

KURIHARA, S. & M. YASUDA. 1975a. Morphological study of the kidney in the fowl. I. Arterial system. *Japanese Journal of Veterinary Science,* 37**:** 29-47.

KURIHARA, S. & YASUDA, M. 1975b. Morphological study of the kidney in the fowl. II. Renal portal and venous systems. *Japanese Journal of Veterinary Science,* 37: 363- 377.

LAKE, P. E. 1957. The male reproductive tract of the fowl. *Journal of Anatomy,* 91: 116-129.

LAKE, P. E. 1971. The male in reproduction, in *Physiology and Biochemistry of the Domestic Fowl.* III. Edited by D. J. Bell & B. M. Freeman. London: Academic Press. pp. 1411-1447.

LAKE, P. E. 1981. Male genital organs, in *Form and Function in Birds.* Edited *by* A. S. King & J. McLelland. London: Academic Press. pp.1- 61.

LOPES, M. A. E, FERREIRA, C. S. A. & FERREIRA, A. J. P. 2005. Identification of enteric bacteria from ostrich and their use as a comparative exclusion product in the control of enteritis. *Proceedings of the 3rd International Ratite Science Symposium of the World's Poultry Science Association & XII World Ostrich Congress:* 223-228.

LOPEZ, M. I.; FUENTES, P.; RETAMAL, C. & DE SOUZA, W. 1997. Regional differentiation of the blood-epididymis barrier in stallion (*Equus caballus). Journal of Submicroscopic Cytology and Pathology,* 29: 353-363.

MALECKI, I.A., MARTIN, G.B., O'MALLEY, P.J., MEYER, G.T., TALBOT, R.T. & SHARP, P.J. 1998. Endocrine and testicular changes in a short-day seasonally breeding bird, the emu (*Dromaius novaehollandiae*), in southwestern Australia. *Animal Reproduction Science* 53: 143-155.

MALECKI, I. A. & MARTIN, G. B. 2005. Fertility in ratites. *Proceedings of the 3rd International Ratite Science Symposium of the World's Poultry Science Association & XII World Ostrich Congress.* 45-51.

McCRACKEN, T. O, KAINER, R. A. & SPURGEON, T. L. 1999. The chicken *(Gallus gallus domesticus),* in *Spurgeon's Color Atlas of Large Animal Anatomy.* Philadelphia: Lippincott Williams & Wilkins. pp. 127-141.

MEIER, U. 1979. Zur Genese von Rete testis und Nebenhoden bei den Laufvögeln Strauß, Nandu und Emu - Eine makroskopische, licht- und elektronenmikroskopische Untersuchung. Inaugural Dissertation, Freien Universität, Berlin.

MELLET, F. D. 1993. Ostrich production and products, in *Livestock Production Systems.* Edited by C. Maree & N. H. Casey. Agricultural Development Foundation. p. 187.

NAKAI, M. & NASU, N. 1991. Ultrastructural study on junctional complexes of the excurrent duct epithelia in the epididymal region in the fowl. *Journal of Veterinary Medicine Science,* 53: 677-677.

NAKAI, M., HASHIMOTO, Y., KITAGAWA, H., KON, Y. & KUDO, N. 1988. Microvasculature of the epididymis and *ductus deferens* of domestic fowls. *Japanese Journal of Veterinary Science,* 50: 371-381.

NEAVES, W. B., 1977. The blood-testis barrier, in *The Testis*. Edited by A. D. Johnson & W. R. Gomes. Vol. 4. New York: Academic Press. pp. 125-162.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Circulatory System, in *Anatomy of the Domestic Birds*. Berlin: Verlag Parey. pp. 85-107.

NISHIDA, T. 1964. Comparative and topographical anatomy of the fowl. XLII. Blood vascular system of the male reproductive organs. *Japanese Journal of Veterinary Science,* 26: 211- 221.

OKAMOTO, T., VOLLMERHAUS, B., ROOS, H., WAIBL, H. & KÖNIG, H. E. 1992. The arteries supplying the male reproductive organs of Japanese quails (*Coturnix coturnix japonica*) and their post-hatching development. *Anatomia, Histologia, Embryologia,* 21: 127-135.

OSMAN, D. I. 1978. The ultrastructure of rete testis and its permeability barrier before and after efferent ductuli ligation. *International Journal of Andrology*, 1**:** 357-370.

OSMAN, D. I., EKWALL, H. & PLÖEN, L. 1980. Specialized cell contacts and blood-testis barrier in seminiferous tubules of the domestic fowl (*Gallus domesticus*). *International Journal of Andrology,* 3: 553-562.

OSTERHOFF, D. R. 1979. Ostrich farming in South Africa. *World Review of Animal Production,* 15: 19-30.

OZEGBE, P. C., AIRE, T. A. & SOLEY, J. T. 2006a. The morphology of the efferent ducts of the testis of the ostrich, a primitive bird. *Anatomy & Embryology,* 211: 559-565.

OZEGBE, P., AIRE, T. A. & SOLEY, J. T. 2006b. The epididymal duct unit of the ostrich (*Struthio camelus*). *Proceedings of the 36th Annual Conference of the Anatomical Society of Southern Africa,* Golden Gate: 74.

PELLETIER, R. M. 1990. A novel perspective: The occluding zonule encircles the apex of the Sertoli cell as observed in birds. *American Journal of Anatomy,* 188: 87-108.

PFEIFFER, D. C. & VOGL, A. W. 1993. Ectoplasmic junctional specializations in Sertoli cells of the rooster and turtle: evolutionary implications. *The Anatomical Record,* 235**:** 33-50.

ROTHWELL, B. 1975. Designation of the cellular component of the peritubular boundary tissue of the seminiferous tubule in the testis of the fowl (*Gallus domesticus*). *British Poultry Science,* 16: 527-529.

SAMUELSON, D. A. 2007. Male reproductive system, in *Textbook of Veterinary Histology*. St. Louis: Saunders Company. pp. 419-441.

SASAKI, H., NISHIDA, T., FUJIMURA, H. & MOCHIZUKI, K. 1984. Vascular system of paracloacal vascular body in the guinea fowl (*Numida meleagris)*. *Japanese Journal of Veterinary Science,* 46**:** 425-435.

SELVAN, S.T., SIVAKUMAR, T., VEERAMANI, P. & PRABAKARAN, R. 2008. Growth performance of ostriches in India. *Proceedings of the 23rd World's Poultry Congress. World Poultry Science Journal,* 64:667.

SETCHELL, B. P. 1980. The functional significance of the blood-testis barrier. *Journal of Andrology,* 1: 1-10.

SETCHELL, B. P. & WAITES, G. M. H. 1975. The blood-testis barrier, in *Handbook of Physiology. Endocrinology. Male reproduction system. Vol. V*. Edited by R. O. Greep, E. B. Astwood, D. Hamilton, R. O. Greep & S. R. Geiger. Washington: American Physiological Society. pp. 143-172.

SHANAWANY, M. M. 1995. Recent development in ostrich farming. *World Animal Review,* 83: 3-8.

SMIT, D. J. van Z. 1964. Ostrich farming in the Little Karoo. *Bulletin No 358,* Department of Agricultural Technical Services, Pretoria: V & R Printers.

SOLEY, J.T. 1990. Ultrastructural features of the boundary tissue of the seminiferous tubule of the ostrich (*Struthio camelus*). *South African Journal of Science,* 86**:** 163.

SOLEY, J. T. 1992. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). PhD thesis, Faculty of Veterinary Science, University of Pretoria.

SOLEY, J. T. 1997. The morphology of the testicular capsule of the ostrich (*Struthio camelus*). *Proceedings of the Microscopy Society of Southern Africa,* 27**:** 109.

SOLEY, J. T. & ELS, H. J. 1992. The morphology of the proximal region of the ductuli efferentes in the ostrich testis. *Proceedings of the Microscopy Society of Southern Africa*, 22**:** 139-140.

SOLEY, J. T. & GROENEWALD, H. B. 1999. Reproduction, in *The Ostrich. Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp. 129-158.

SOLEY, J. T., VAN WILPE, E., AIRE, T. A. & OZEGBE, P. C. 2005. The morphology of the seminiferous tubules in three-day-old ostrich chicks. *Proceedings of the Microscopy Society of Southern Africa,* 35: 60.

STEFANINI, M. A. & ORSI, A. M. 1999. Caracteristicas morfologicas da região epididimaria do Pombo doméstico (*Columbia livia, L*). *Brazilian Journal of Veterinary Research in Animal Science,* 36: 2-12.

SUGIMURA, M.; KUDO, N. & YAMANO, S. 1975. Fine structures of *corpus paracloacalis* in cocks. *Japanese Journal of Veterinary Research,* 23: 11-16.

SUZUKI, F. & NAGANO, T. 1978a. Development of tight junctions in the caput epididymal epithelium of the mouse. *Developmental Biology,* 63: 321-334.

SUZUKI, F. & NAGANO, T. 1978b. Regional differentiation of cell junctions in the excurrent duct epithelium of the rat testis as revealed by freeze-fracture. *The Anatomical Record,* 191: 503- 520.

TEMPLE, S. A. 1974. Plasma testosterone titres during the annual reproductive cycle of starlings *(Sturnus vulgaris). General Comparative Endocrinology,* 22: 470-479.

TINGARI, M. D. 1971a. On the structure of the epididymal region and ductus deferens of the domestic fowl (*Gallus domesticus*). *Journal of Anatomy,* 109: 423-435.

TINGARI, M. D. 1971b. The fine structure of basal cells in the male reproductive tract of the domestic fowl. *Journal of Anatomy*, 110: 167-169.

TOYAMA, Y., MAEKAWA, M. & YUASAT, S. 2003. Ectoplasmic specializations in the Sertoli cell: new vistas based on genetic defects and testicular toxicology. *Anatomical Science International,* 78: 1-16.

TRACIUC, E. 1967. L'anatomie microscopique de l'epididyme chez *Sterna hirundo* L. *Anatomischer Anzeiger,* 121: 381-386.

TRACIUC, E. 1969. La structure de l' epididyme de *Coloeus monedula* (*Aves, Corvidae*). *Anatomischer Anzeiger,* 125: 49-67.

TURNER, T. T. 1979. On the epididymis and its function. *Investigation in Urology*, 16: 311-319.

TURNER, T. T. & HOWARDS, S. S. 1985. The tenacity of the blood-testis and blood-epididymal barriers, in *Male fertility and its regulation.* Edited by T. J. Lobl & E. S. E. Hafez. Boston: MPT Press Limited. pp. 383-393.

TURNER, T. T., COCHRAN, R. C. & HOWARDS, S. S. 1981. Transfer of steroids across the hamster blood-testis and blood-epididymal barriers. *Biology of Reproduction*, 25: 342-348.

TURNER, T. T., GILES, R. D. & HOWARDS, S. S. 1981. Effect of oestradiol valerate on the rat blood-testis and blood-epididymal barriers to [^{3}H]inulin. *Journal of Reproduction and Fertility*, 63: 355-358.

TURNER, T. T., D'ADDARIO, D. A. & HOWARDS, S. S. 1983. The transepithelial movement of ^{3}H-3-O- methyl-D-glucose in the hamster seminiferous and cauda epididymidal tubules. *Fertility and Sterility,* 40: 530-535.

WAITES, G. M. H. & GLADWELL, R. T.1982. Physiological significance of fluid secretion in the testis and blood-testis barrier, *Physiology Review,* 62:624-671.

WÖHR, A & ERHARD, M. 2005. Ostrich farming in Germany. *Proceedings of the 3rd International Ratite Science Symposium of the World's Poultry Science Association & XII World Ostrich Congress:* 145-156.

ZHANG, L. 2005. The growing ostrich industry in China. *Proceedings of the 3rd International Ratite Science Symposium of the World's Poultry Science Association & XII World Ostrich Congress:* 377-380.

6. FIGURES.

Figure 1.1. Flock of sub-adult male ostriches in the Oudtshoorn district, Western Cape Province.

Figure 1.2. Slaughter birds (12-14 months old) in a holding pen at the Krugersdorp abattoir.

Figure 1.3. De-feathered ostrich carcass arriving at the skinning station, Krugersdorp abattoir.

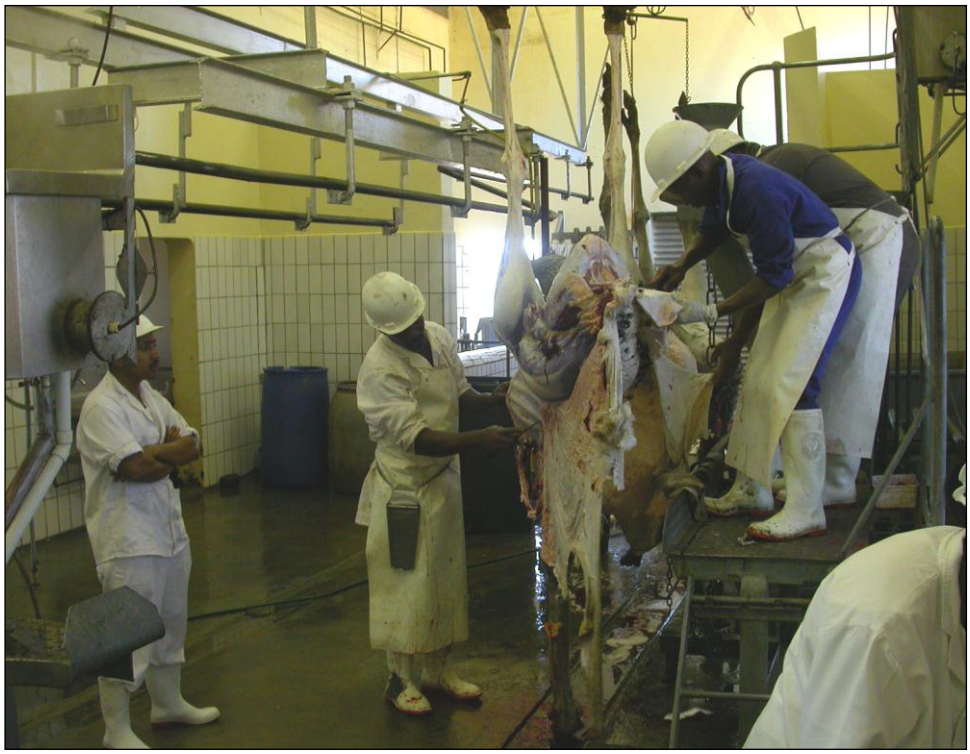

Figure 1.4. Ostrich carcasses being skinned at the Oudtshoorn abattoir, Western Cape Province.

Figure 1.5. Ostrich carcasses being moved to the cold-storage facility at the Krugersdorp abattoir.

Figure 1.6. Ostrich leather goods for sale at a shop in Port Elizabeth, Eastern Cape Province.

Figure 1.7. Ostrich eggshells on display in a shop in Port Elizabeth, Eastern Cape Province.

CHAPTER 2

MACROSCOPIC FEATURES OF THE ARTERIAL SUPPLY TO THE REPRODUCTIVE SYSTEM

1. INTRODUCTION

The ostrich industry currently forms a small but important component of the South African economy and has also become established in various other countries such as Namibia, Zimbabwe, Israel, Australia, the United States of America and parts of Europe (Deeming & Angel 1996; Deeming 1999). During recent years the world-wide demand for ostrich products has increased (Kimminau 1993; Deeming 1999) which has put pressure on ostrich breeders to supply an increasing number of slaughter birds for the market.

The increasing economic importance of the ostrich as a farmed bird, coupled with pressure for accelerated production, makes it imperative to fully understand the reproductive biology of this species, particularly in view of the reported low fertility and hatchability prevalent in the industry (Deeming &Ar 1999). While factors such as poor management, nutritional imbalances and the incorrect incubation of eggs, etc. play a role in this phenomenon, the fertility of male ostriches may also be a contributory factor. Very little information, however, is available on the basic reproductive biology of the male ostrich. Although some aspects of the structure of the male reproductive organs have been published (MacAlister 1864; Duerden 1912; Budras & Meier 1981; Cho, Brown & Anderson 1984; Bezuidenhout 1986, 1999; Fowler 1991; Soley 1992; Soley & Groenewald 1999; Aire, Soley & Groenewald 2003), no information is available on the vascularisation of these organs.

Although information on the vascularization of the male reproductive tract of the Japanese quail (Okamoto, Vollmerhaus, Roos, Waibl & König 1992), Peking drake (Kremer & Budras 1990) and domestic pigeon (Bhaduri, Biswas & Das 1957) has been provided, only that of

the domestic fowl has been described in any detail (Nishida 1964). As part of a study on the vascularisation of the male reproductive tract of the ostrich, this chapter describes the gross pattern of the arterial supply to the testis, epididymis, *Ductus deferens* and phallus. The terminology used is that of Nomina Anatomica Avium (Baumel, King, Breazile, Evans & Berge 1993).

2. MATERIALS AND METHODS

The torsos of 24 male ostriches with viscera intact, but which had been skinned and from which the limbs had been removed, were obtained from the Oryx abattoir in Krugersdorp, Gauteng and from the Klein Karoo abattoir in Oudtshoorn, Western Cape, South Africa. The specimens consisted of 16 sub-adult birds (12 to 14 months-old) and 8 sexually mature and active birds (three years and older).

The arterial system of 18 torsos (12 sub-adult and 6 sexually mature birds) was flushed free of blood by injecting physiological saline through the descending aorta, using a 50 ml syringe. Using the same technique, red Latex* was subsequently injected into the arteries via the same route. Severed arteries resulting from the slaughter process and which leaked latex were closed off using an artery forceps. The torsos were trimmed of excess tissue, immersion-fixed in a 10% formalin bath for a minimum period of 5 days, rinsed in running water for two days and then carefully dissected to expose the latex-filled arteries. The specimens were also utilized for a description of the morphological and topographical features of the reproductive tract.

The remaining six torsos (4 sub-adult and 2 sexually mature birds) were rinsed with physiological saline as described above but were injected with a polyester resin* via the

*(Plastomax Supplies CC, Pretoria, South Africa)

*(Plastomax Supplies CC, Pretoria, South Africa)

descending aorta. The specimens were subsequently kept in a cold room (approximately 4°C) for a number of days after which the tissue was macerated in a 20 – 30% sodium hydroxide solution until the resin-filled arteries were exposed.

The pattern of the arterial system was described and visually recorded using a Nikon Coolpix 4500 digital camera.

3. RESULTS

3.1. Basic morphological features and topography of the reproductive organs and kidneys

For proper perspective, the morphological features and topography of the reproductive organs and kidneys are briefly described. The anatomical features of the male reproductive organs and kidneys in the ostriches resembled those previously described for this bird (MacAlister 1864: Duerden 1912; Cho *et al.* 1984; Bezuidenhout 1986, 1999; Soley 1992; Soley & Groenewald 1999). The reproductive organs comprised the testis, epididymis and deferent duct which opened into the urodeum of the cloaca via a small papilla, as well as the phallus.

The testes were closely related topographically to the kidneys with which they shared some arteries. The kidneys displayed three divisions, namely cranial, middle and caudal. They lay immediately ventral to the synsacrum and stretched between the last vertebral rib and the middle of the pelvis. The medial aspects of the kidneys bordered the aorta which lay in the dorsal median plane. The ureters extended from the caudo-medial surfaces of the cranial division of the kidneys, and ran caudally, close to the midline accompanying the *Ducti deferentia*. They opened into the *urodeum* of the cloaca close to the papillae of the *Ducti deferentia*.

3.1.1. The testis

The intra-abdominal testes were situated on either side of the caudal vena cava, ventral to the cranial divisions of the kidneys, and caudal to the adrenal glands. They were attached to the body wall by a fold of mesentery and demonstrated a marked variability in size in the specimens examined.

The semi-adult birds displayed small rod-shaped testes which measured approximately 1.3 to 4.6cm in length and 0.5 to 0.8cm in width, while in the sexually mature birds they were oval structures approximately 7.6 to 14.2cm long and 5 to 7cm wide.

3.1.2. The epididymis

The epididymis was attached to the dorso-medial surface of the testis and in the sexually mature birds extended several centimeters caudal to the testis. It was divided into three parts: the cranial appendix (Fig. 2.1), the main part attached by connective tissue to the testis along most of its length, and the free caudal extremity which was continuous caudally with the *Ductus deferens*. In semi-adult birds the epididymis was poorly developed.

3.1.3. The deferent duct

The *Ductus deferens* was best identified in sexually mature and active birds. The *Ductus deferens* left the caudal part of the epididymis, first as a fairly straight tube and then as a highly convoluted tube that ran parallel to the ureter, near the midline. Close to the cloaca the duct became straight, forming the *pars recta Ductus deferentis,* and subsequently expanded into a spindle or barrel-shaped structure, the *Receptaculum ductus deferentis* that opened into the *urodeum* of the cloaca via the papilla of the *Ductus deferens*.

3.1.4. The phallus

The phallus was attached to the ventral wall of the cloaca and exhibited the typical anatomical features summarized by Soley and Groenewald (1999). In semi-adult birds (prepubertal), the flaccid phallus was approximately 1.2 to 2cm wide and 1.5 to 5.5cm long, whereas in the sexually mature adult birds it was approximately 3 to 6.9cm wide and 20 to 24cm long.

3.2. Arterial supply to the reproductive organs

3.2.1. The testis and epididymis

a. The testicular artery (*A. testicularis*)

The testis and epididymis were supplied by the testicular artery and, in some instances, one or more accessory testicular arteries. The testicular artery branched from the cranial renal artery which, in turn, originated from the cranial (thoraco-abdominal) segment of the descending aorta (*Aorta descendens*). This portion of the aorta ran mid-ventral to the spinal column and between the lungs, and terminated beneath the third lumbar vertebra. The left and right cranial renal arteries (*Aa. renales craniales*) arose from the ventro-lateral aspect of the cranial segment of the aorta where it passed between the cranial divisions of the kidney, approximately 3 to 5cm caudal to the last vertebral rib. When viewed from the ventral aspect, the cranial renal arteries were concealed by the overlying caudal *vena cava*.

The first branches (unnamed) of the cranial renal artery emerged approximately 1 to 2cm from the origin of the vessel and supplied the cranial division of the kidney (Figs. 2.1, 2.2A-D, 2.8). More distally, a variable number of testicular arteries (between 1 to 3) branched off from the cranial renal artery, running into the testis midway along its dorso-medial border (Figs. 2.1, 2.2A-D, 2.8). Small branches of the testicular arteries were observed to supply the epididymis. This pattern of blood supply to the testes whereby both the testicular artery

and cranial renal artery arose from a common trunk on both sides of the aorta, was present in 54.2% of the specimens and represented the general pattern observed.

b. The accessory testicular artery (*A. testicularis accessoria*)

In some instances one or more additional vessels were observed to arise directly from the aorta unilaterally or bilaterally and to supply the testes (Figs. 2.2A-D). These accessory testicular arteries were present in 45.8% of the specimens examined and were 0.15 to 0.4 cm in diameter and up to 6.5 cm in length. They originated approximately 1.1 to 1.8 cm cranial to the origin of the cranial renal artery before running ventro-laterally onto the dorso-medial surface of the testis.

Due to variations in the positioning (unilaterally or bilaterally) and number of the accessory testicular arteries, various architectural types based on the pattern of vascularization of the testes could be identified in the ostrich:

Type A: As for the general pattern (bilateral origin of the testicular and cranial renal arteries from a common branch of the aorta), but with a single accessory testicular artery on either side of the aorta (12.5% of specimens) (Fig. 2.2A).

Type B: As for the general pattern, but with a single accessory testicular artery on the right side only. No accessory vessels on the left side (25% of specimens) (Fig. 2.2B).

Type C: As for the general pattern, but with two accessory testicular arteries on the right side only. No accessory vessels on the left side (4.1% of specimens) (Fig. 2.2C).

Type D: As for the general pattern, but with a single accessory testicular artery on the right side from which branched the right cranial ureterodeferential branch (see below) (4.1% of specimens) (Fig. 2.2D).

3.2.2. The deferent duct (*Ductus deferens*)

Along its length the *Ductus deferens* was supplied by cranial, middle and caudal ureterodeferential branches emanating from various sub-divisions of the aorta (Fig. 2.1).

a. The cranial ureterodeferential branch (*R. ureterodeferentialis cranialis*)

The cranial ureterodeferential branch originated from the caudal aspect of the cranial renal artery where it crossed the ventral surface of the epididymis. This branch ran caudo-medially and supplied the cranial part of the *Ductus deferens* and adjacent ureter by means of smaller branches. It continued caudally on the ventro-medial surface of the ipsilateral kidney for a distance of 11 to 12.8 cm, running parallel to the aorta and *Ductus deferens* to which it was related medially, before joining the middle renal artery (Figs. 2.1, 2.8, 2.9).

This bilateral anastomosis between the cranial ureterodeferential branch and the middle renal artery effectively created a collateral arterial circulation to the *Ductus deferens* and was observed in all the specimens (Figs. 2.1,2.9). However, in one bird (4.1% of the specimens), the right cranial ureterodeferential branch was seen to originate from the right accessory testicular artery (Fig. 2.2D).

b. The middle ureterodeferential branch (*R. ureterodeferentialis media*)

One or two middle ureterodeferential branches were observed to originate from the middle renal artery (*A. renalis media*). These vessels ran ventro-laterally to supply the adjacent deferent duct and ureter (Figs. 2.1, 2.3 – 2.7, 2.9).

In 70.8% of the specimens the middle renal artery, together with the caudal renal artery (*A. renalis caudalis*), arose from a common trunk, the common renal artery (*A. renalis communis*) situated at the base of the sciatic artery (*A. ischiadica*) (Figs. 2.1, 2.9). The sciatic artery emerged bilaterally from the middle (sacrolumbar) segment of the aorta (Figs. 2.1, 2.3 – 2.7, 2.9) in the vicinity of, or just caudal to, the acetabulum and approximately at the caudal poles of the middle divisions of the kidneys. The middle segment of the aorta

ran ventral to the caudal lumbar vertebra, medially between the caudal divisions of the kidneys and into the sacral region where it continued caudally to form the caudal segment of the aorta.

A number of variations in this general pattern were, however, observed. In one specimen both the left and right common renal arteries arose from a single common trunk (*Truncus renalis communis*) emanating from the ventral surface of the aorta close to the origin of the sciatic arteries (Fig. 2.3). In 25.1% of the specimens the middle and caudal renal arteries on either side arose independently from the base of the sciatic artery (Fig. 2.4).

In some instances the arterial supply of the middle ureterodeferential branch was augmented by additional vessels. In one specimen the right *Ductus deferens* was additionally supplied by a right ventro-lateral arterial branch (accessory ureterodeferential branch) originating directly from the aorta just caudal to the origin of the sciatic arteries (Fig. 2.5). In another specimen both the left and right *Ductus deferentes* received accessory ureterodeferential branches which arose from a common accessory ureterodeferential trunk originating from the same region of the aorta as the single accessory vessel described above (Fig. 2.6).

c. The caudal ureterodeferential branches (*Rr. ureterodeferentiales caudales*)

Two to three caudal ureterodeferential branches were observed to split from the pudendal artery (*A. pudenda* – previously named the internal pudendal artery by Nishida, 1964 and Pintea, Constantinescu & Radu 1967) and to supply the *Ductus deferens* and ureter in the pelvic region via a variable number of thinner vessels (Figs. 2.1, 2.10). The pudendal artery, as well as the lateral caudal artery (*A. lateralis caudae* – previously named the external pudendal artery by Nishida 1964 & Pintea *et al*. 1967) originated from the internal iliac artery which branched from the caudal segment of the aorta (Figs. 2.1, 2.10). The lateral caudal artery supplied the lateral wall of the pelvic region.

In a single specimen the right *Ductus deferens* received a short branch from the right internal iliac artery. This branch originated approximately 2 cm proximal to the division of the internal iliac artery into the lateral caudal and pudendal arteries (Fig. 2.7).

3.2.3. Phallus (*Phallus*)

Between one to three relatively thin branches of the pudendal artery were observed to run caudo-ventrally towards the cloaca which they vascularized. Other branches continued towards the root of the phallus where they formed an arterial plexus or network (Figs. 2.1, 2.11).

4. DISCUSSION

Very few studies have provided information on the blood supply to the male reproductive organs in birds. The most comprehensive reports are those of Nishida (1964) in the domestic fowl (*Gallus domesticus*), Okamoto *et al.* (1992) in the Japanese quail and Kremer & Budras (1990) in the drake, and to a lesser extent, the study of Bhaduri *et al.* (1957) in the pigeon (*Columbia livia*). This information has been supplemented by miscellaneous data, mainly on the domestic fowl (Lake 1971; Kurihara & Yasuda 1975) and Peking drakes (Kremer & Budras 1990). The present study provides the first detailed description of the arterial supply to the male reproductive tract of the ostrich and adds to the limited comparative data available on birds in general.

The origin of the testicular artery from the cranial renal artery in the ostrich is similar to that reported in the domestic fowl (Nishida 1964; Kurihara & Yasuda 1975) pigeon (Bhaduri *et al.* 1957), Peking drake (Kremer & Budras 1990) and Japanese quail (Okamoto *et al.* 1992). This was considered to be the general pattern of arterial supply to the testes in the ostrich and occurred in 54.2% of the specimens examined (see Fig. 2.1). The incidence of this particular pattern in the fowl is reported to be 54.5% (18 out of 33 specimens examined, Kurihara & Yasuda 1975), and in the Japanese quail 85.6% (71 out of 83

specimens examined, Okamoto *et al.* 1992), and represents the type IV variation described by both Nishida (1964) and Kurihara & Yasuda (1975). The remarkable similarity in the incidence of this pattern in the fowl, Japanese quail and ostrich would seem to lend support to the suggestion that this is the general pattern observed in birds. Similarly, the presence of accessory testicular arteries and their variations in the ostrich are comparable to most of those described for the domestic fowl (Nishida 1964; Kurihara & Yasuda 1975). The type A variation described for the ostrich (single, bilateral accessory testicular arteries) is similar to the type I variation reported in the fowl (Nishida 1964; Kurihara & Yasuda 1975) and occurs, respectively, in 9% (Kurihara & Yasuda 1975) and 12.5% of fowls (Nishida 1964) and ostriches (this study) examined. Likewise, the type B variation in the ostrich (single accessory testicular artery on the right side only) is similar to the type III variation reported in the fowl (Nishida 1964; Kurihara & Yasuda 1975), although the reported incidence is higher in the ostrich (25%) than in the fowl (9% - Kurihara & Yasuda 1975). The type B variation has also been observed in the Peking drake (Kremer & Budras 1990). The type C and D variations reported in this study appear to be unique to the ostrich, since they have not been reported previously in birds. In the Japanese quail single or double accessory testicular arteries originating from the aorta and which run cranially or caudally to the testicular artery have also been described (Okamoto *et al*. 1992). The study of more specimens from a wider range of avian species would probably reveal a common range of variations in respect of the accessory testicular arteries. It is also not known why the accessory testicular artery described in the ostrich is more commonly present on the right side than on the left side.

The origin of a cranial ureterodeferential branch from the cranial renal artery (which supplies the cranial aspect of the *Ductus deferens*) is similar in the domestic fowl (Nishida 1964), Japanese quail (Okamoto *et al.* 1992) and the ostrich. However, the anastomosis observed between the caudal continuation of the cranial ureterodeferential branch and the middle renal artery in the ostrich has not been reported in other birds and may be a distinctive feature of this species.

In the ostrich, the middle segment of the deferent duct is supplied by the middle ureterodeferential branch/branches that originate from the middle renal artery. In the Japanese quail the middle ureterodeferential branch/branches also originate from the middle renal artery as well as from the caudal renal artery (Okamoto *et al.* 1992). In 70.8% of the specimens examined, the middle renal artery, together with the caudal renal artery, arises from a common trunk emanating from the base of the sciatic artery. In this study the common trunk is named the common renal artery (*A. renalis communis*). A similar situation is apparent in the domestic fowl although the common trunk referred to above is not named in any of the studies describing or illustrating this vessel (Nishida 1964; Siller & Hindle 1969; Kurihara & Yasuda 1975). Two variations in this pattern are evident in the ostrich. In one specimen both the left and right common renal arteries arose directly from the aorta via a common trunk. In 25.1% of the specimens the middle and caudal renal arteries originated independently from the sciatic artery. This variation is reported to be the standard pattern in the pigeon (Badhuri *et al.* 1957). The existence of occasional accessory vessels augmenting the supply of the middle ureterodeferential branch to the ureter and deferent duct in the ostrich has also been reported in the domestic fowl (Nishida 1964). However, the presence, albeit in one specimen, of a common accessory ureterodeferential trunk from the aorta that supplies both the left and right *Ductus deferens*, has not been reported in other birds.

The caudal segment of the *Ductus deferens* and ureter in the ostrich is vascularised by a number of caudal ureterodeferential branches emanating from the pudendal artery, which in turn arises from one of the terminal branches of the aorta, the internal iliac artery. The pattern of branching of the internal iliac artery in the male ostrich is basically similar to that reported in the domestic fowl (Nishida 1964; Pintea *et al*. 1967), pigeon (Bhaduri *et al.* 1957) and quail (Okamoto *et al.* 1992), although the nomenclature used is inconsistent. In one ostrich specimen the caudal region of the right *Ductus deferens* and ureter was additionally supplied by an arterial branch arising proximally from the right internal iliac artery. This variation has also been reported or illustrated in the domestic fowl by Nishida (1964) and in the pigeon by Bhaduri *et al.* (1957), although the terminology used in the latter description is confusing. In

the Japanese quail the caudal ureterodeferential arteries are reported to originate from the *A. pudenda* and the *A. caudae lateralis* (Okamoto *et al.* 1992).

In the male ostrich the cloaca and phallus are supplied by branches of the pudendal artery as previously reported for the domestic fowl (Nishida 1964; Lake 1971) and quail (Okamoto *et al*. 1992). The report that the cloaca is also supplied by branches of the external pudendal artery (lateral caudal artery) (Nishida, 1964) could not be confirmed in the present study. The network of arterial vessels located at the base of the phallus appears to be peculiar to the ostrich.

In conclusion, the pattern of the blood supply to the reproductive organs of the male ostrich is generally similar to that of the domestic fowl, pigeon and Japanese quail. A few noteworthy differences include:

- The origin of the right cranial ureterodeferential branch from the right accessory testicular artery in one specimen.
- The anastomosis observed between the caudal continuation of the cranial ureterodeferential branch and the middle renal artery.
- The presence, in one specimen, of accessory ureterodeferential branches from the aorta that supply both the left and right deferent ducts in the sacral region.
- The existence of an arterial plexus or network surrounding the root of the phallus.

5. REFERENCES

AIRE, T. A., SOLEY, J. T. & GROENEWALD, H. B. 2003.A morphological study of simple testicular cysts in the ostrich (*Struthio camelus*). *Research in Veterinary Science,* 74: 153-162.

BAUMEL, J. J., KING, A. S., BREAZILE, J. E., EVANS, H. E. & BERGE, J. C. V. (Eds.) 1993. *Handbook of avian anatomy. Nomina Anatomica Avium.* 2nd ed. Cambridge, Massachusetts: Nuttall Ornithological Club.

BEZUIDENHOUT, A. J. 1986. The topography of the thoraco-abdominal viscera in the ostrich (*Struthio camelus).Onderstepoort Journal of Veterinary Research,* 53: 111-117.

BEZUIDENHOUT, A. J. 1999. Anatomy, in *The ostrich, biology, production and Health.* Edited by D. C. Deeming. London: CABI Publishing.pp.13-50.

BHADURI, J. L., BISWAS, B. & DAS, S. K. 1957.The arterial system of the domestic pigeon *(Columba livia* Gmelin*). Anatomischer Anzeiger,* 104: 1-14.

BUDRAS, K. D. & MEIER, U. 1981. The epididymis and its development in ratite birds (ostrich, emu, rhea). *Anatomia & Embryologia,* 162: 281-299.

CHO, P., BROWN, R. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3: 133-144.

DEEMING, D. C. & ANGEL, C. R. 1996. Introduction to the ratites and farming operations around the world, in *Improving our understanding of ratites in a farming environment.* Edited by D. C. Deeming. Ratite Conference. Oxfordshire, UK. pp.1-4.

DEEMING, D. C. 1999. Introduction, in *The Ostrich, Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp.1-11.

DEEMING, D. C. & AR, A. 1999.Factors affecting the success of commercial incubation, in *The Ostrich, Biology, Production and Health.* Edited by D. C.Deeming. London: CABI Publishing. pp. 159-190.

DUERDEN, J. E. 1912. Experiments with ostriches - XX. The anatomy and physiology ofthe ostrich. The Internal Organs. *South African Agricultural Journal*, April/May: 1-27.

FOWLER, M. E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22: 204-227.

LAKE, P. E. 1971. The male in reproduction, in *Physiology and Biochemistry of the domestic fowl.* III. Edited by D. J. Bell & B. M. Freeman. London: Academic Press.pp.1411-1447.

KIMMINAU, K. M. 1993. Introducing the ostrich. *Continuing Education* 8**:** 459-467.

KREMER, A. & BUDRAS, K. D. 1990. Zur Blutgefassversorgung des Hodens beim Pekingerpel (*Anas platyrhynchos, L*.). Makroskopische, lichtmikroscopische und rasterelektronenmikroskopische Untersunchungen. *Anatomischer Anzeiger*, 171: 73-87.

KURIHARA, S. & M. YASUDA. 1975. Morphological study of the kidney in the fowl. I. Arterial system*. Japanese Journal of Science,* 37: 29-47.

MACALISTER, A. 1864.On the anatomy of the ostrich (*Struthio camelus*).*Proceedings of the Royal Irish Academy,* 9:1-24**.**

NISHIDA, T. 1964. Comparative and topographical anatomy of the fowl. XLII. Blood vascular system of the male reproductive organs. *Japanese Journal of Veterinary Science,* 26: 211-221.

OKAMOTO, T., VOLLMERHAUS, B., ROOS, H., WAIBL, H. & KÖNIG, H. E. 1992. Die Arterien der männlichen GeschlechtsorganeJapanischer Wacheln(*Coturnix coturnix japonica*) undihre Entwicklungnachdem Schlupf. *Anatomia, Histologia & Embryologia,* 21: 127-135.

PINTEA, V., CONSTANTINESCU, G. M. & RADU, C. 1967. Vascular and nervous supply of bursa of Fabricius in the hen.*Acta Veterinaria Academica Scientifica Hungarica,* 17: 263-268.

SILLER, W. G. & HINDLE. R. M.1969. The arterial blood supply to the kidney of the fowl. *Journal of Anatomy,* 104: 117-135.

SOLEY, J. T. 1992. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). PhD thesis, Faculty of Veterinary Science, University of Pretoria.

SOLEY, J. T. & GROENEWALD, H. B. 1999. Reproduction, in *The Ostrich, Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing.pp.129-158.

6. FIGURES

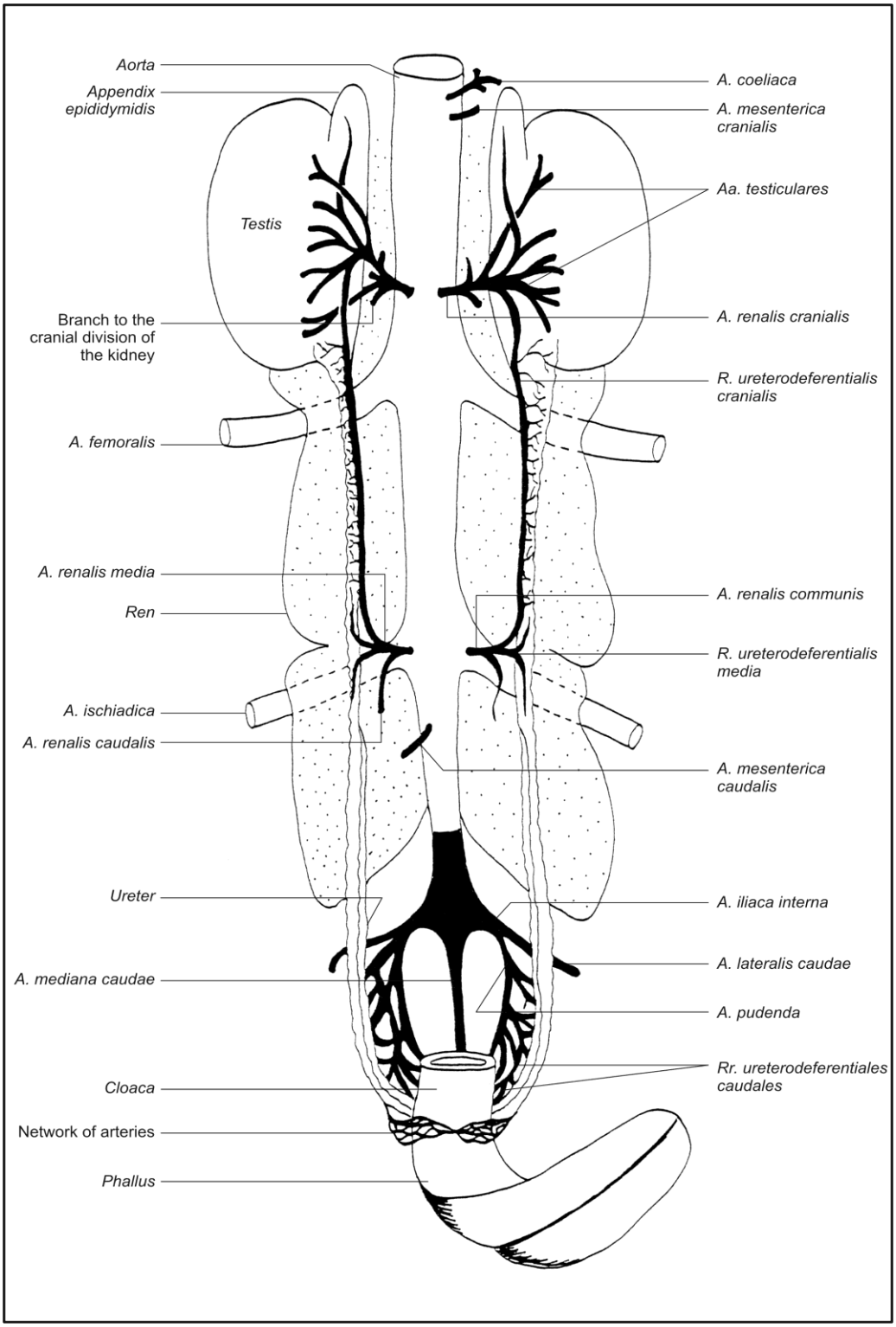

Figure 2.1. Diagrammatic representation of the arterial supply to the reproductive organs.Ventral view.

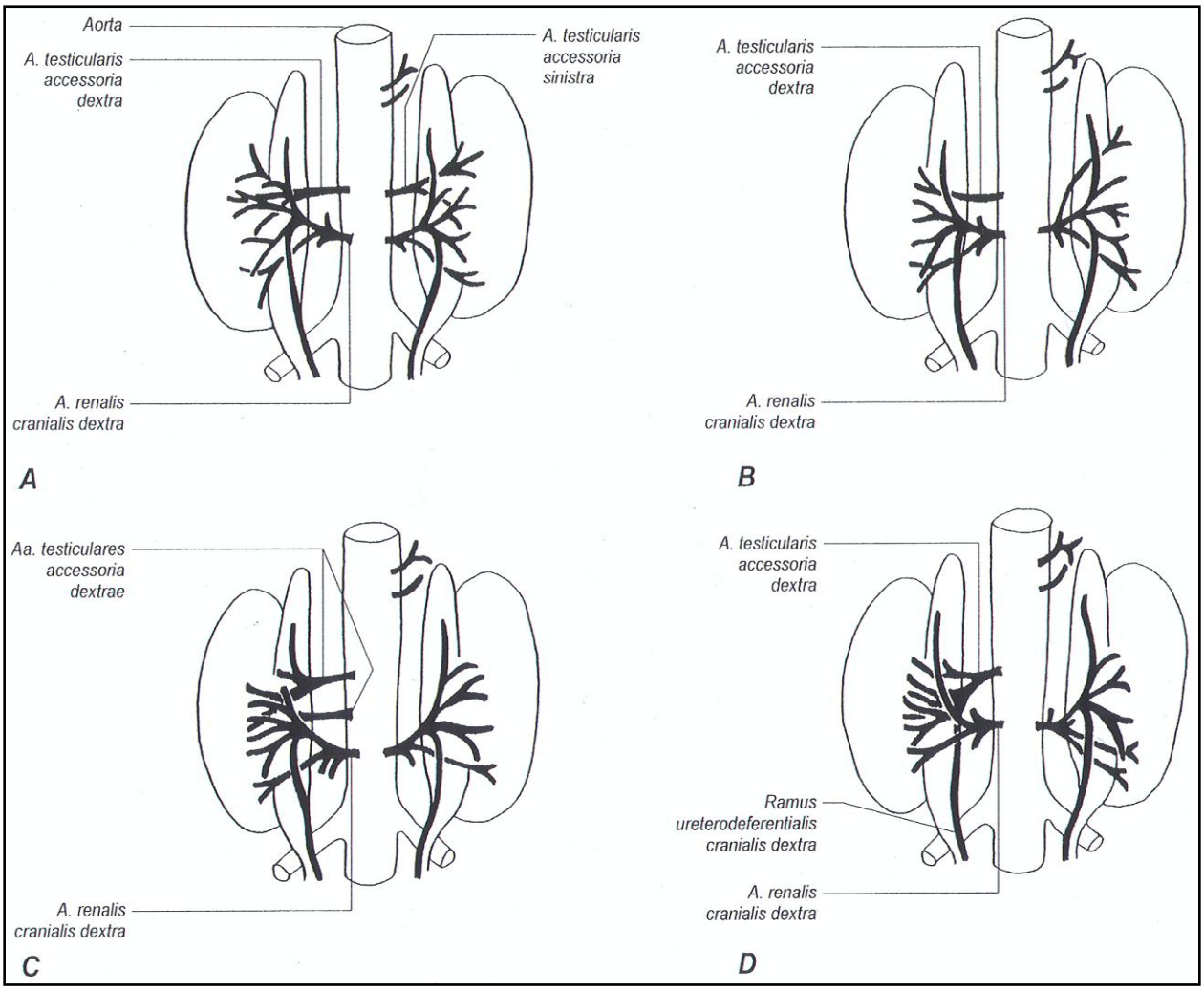

Figure 2.2. Diagrammatic representation of the variations in the pattern and origin of the accessory testicular arteries. Ventral view.

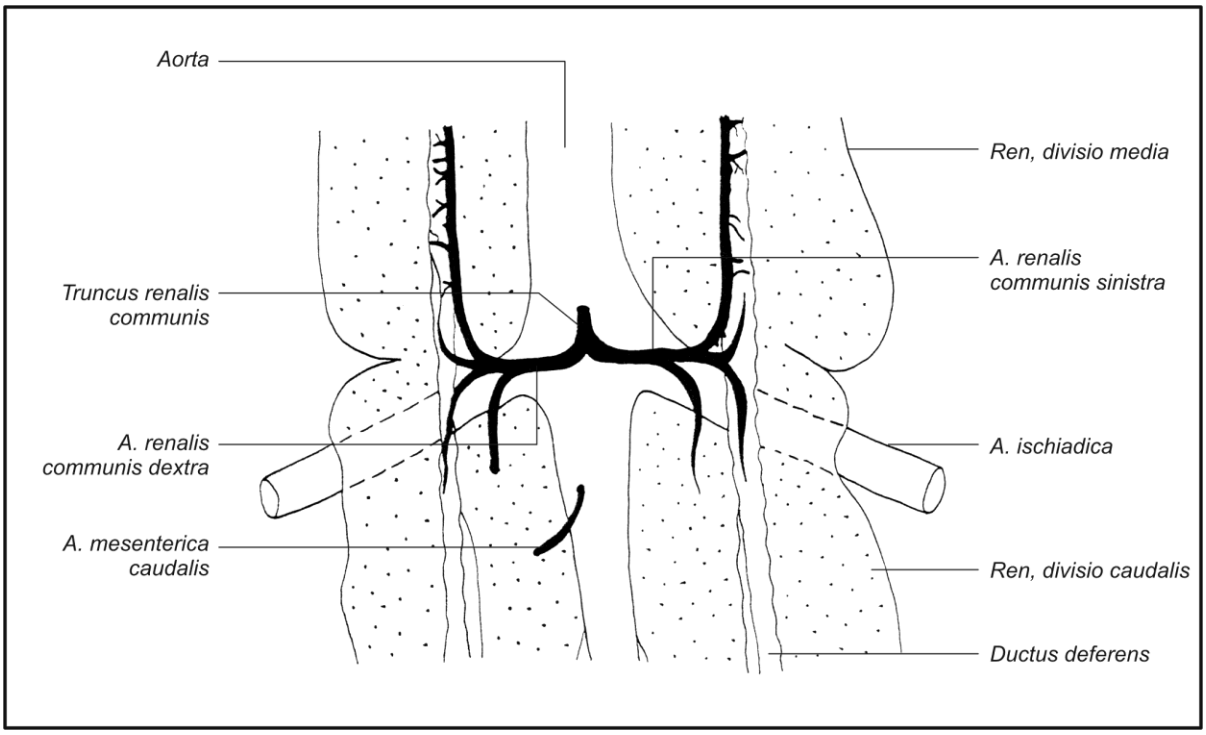

Figure 2.3. Ventral view of the sacrolumbar segment of the aorta showing the origin of the common renal arteries from a common renal trunk

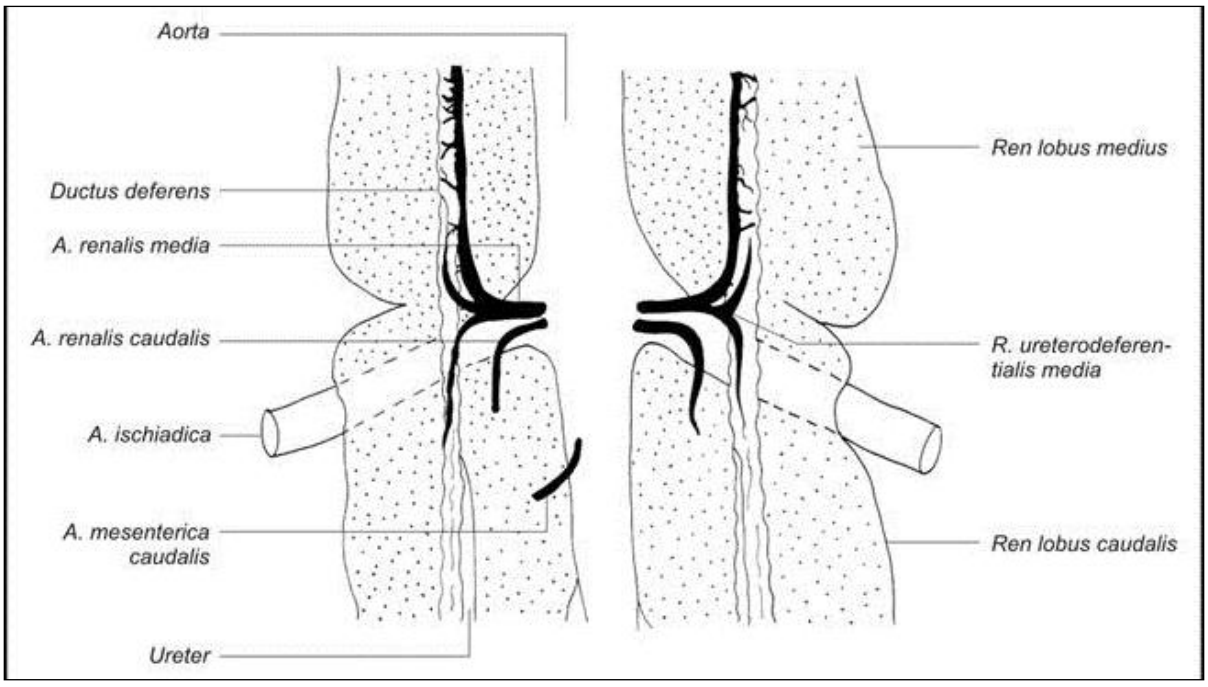

Figure 2.4. Ventral view of the sacrolumbar segment of the aorta showing the separate origin of the middle and caudal renal arteries from the sciatic artery.

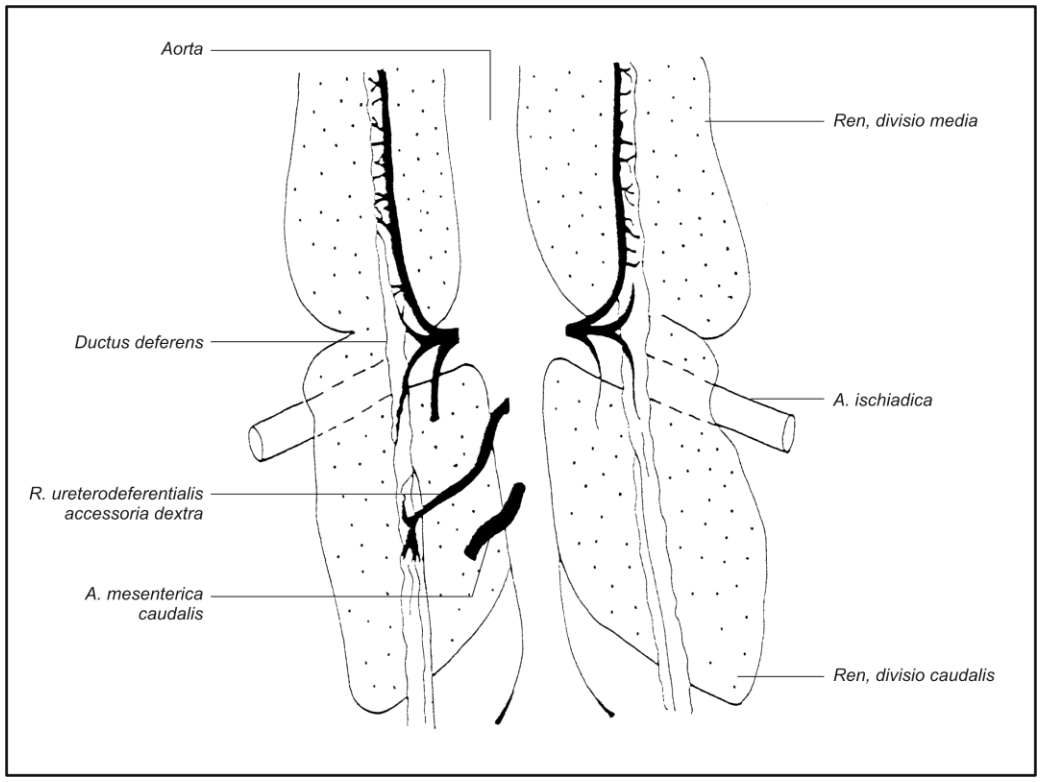

Figure 2.5. Ventral view of the sacrolumbar segment of the aorta showing the presence of a right accessory ureterodeferential branch from the aorta.

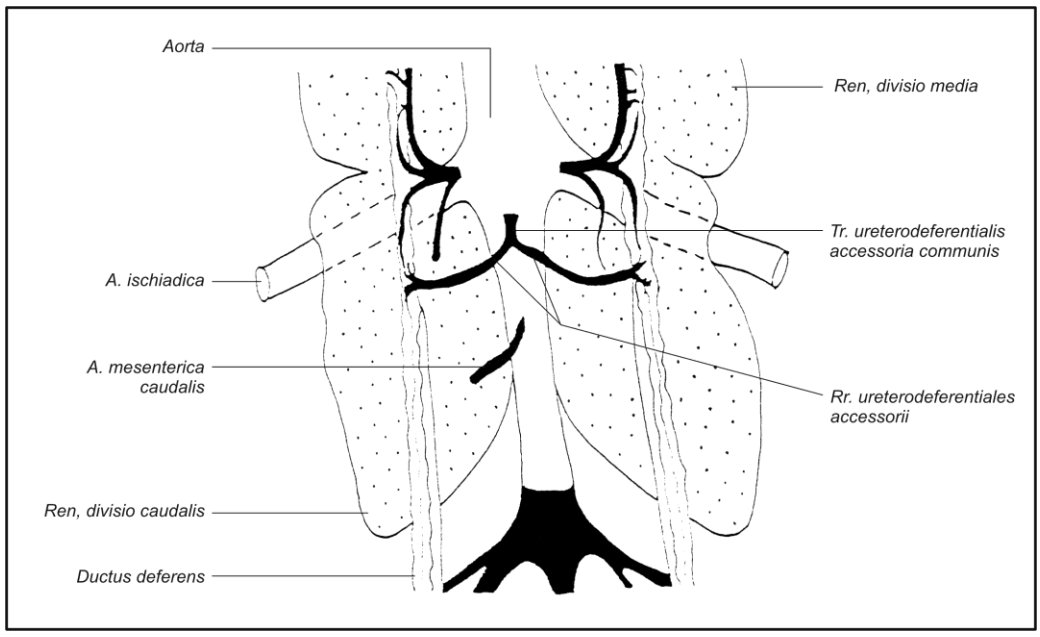

Figure 2.6. Ventral view of the sacrolumbar segment of the aorta showing the presence of a common accessory ureterodeferential trunk bifurcating into left and right accessory ureterodeferential branches.

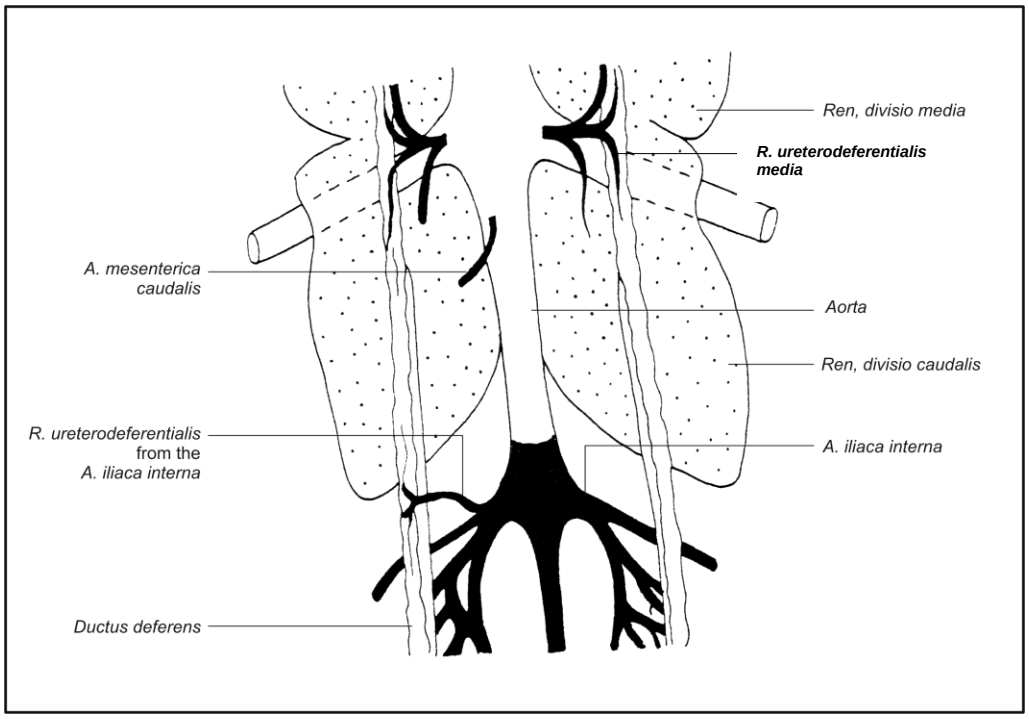

Figure 2.7. Ventral view of the sacrolumbar segment of the aorta showing the presence of an occasional ureterodeferential branch from the right common iliac artery to the right *Ductus deferens* and ureter.

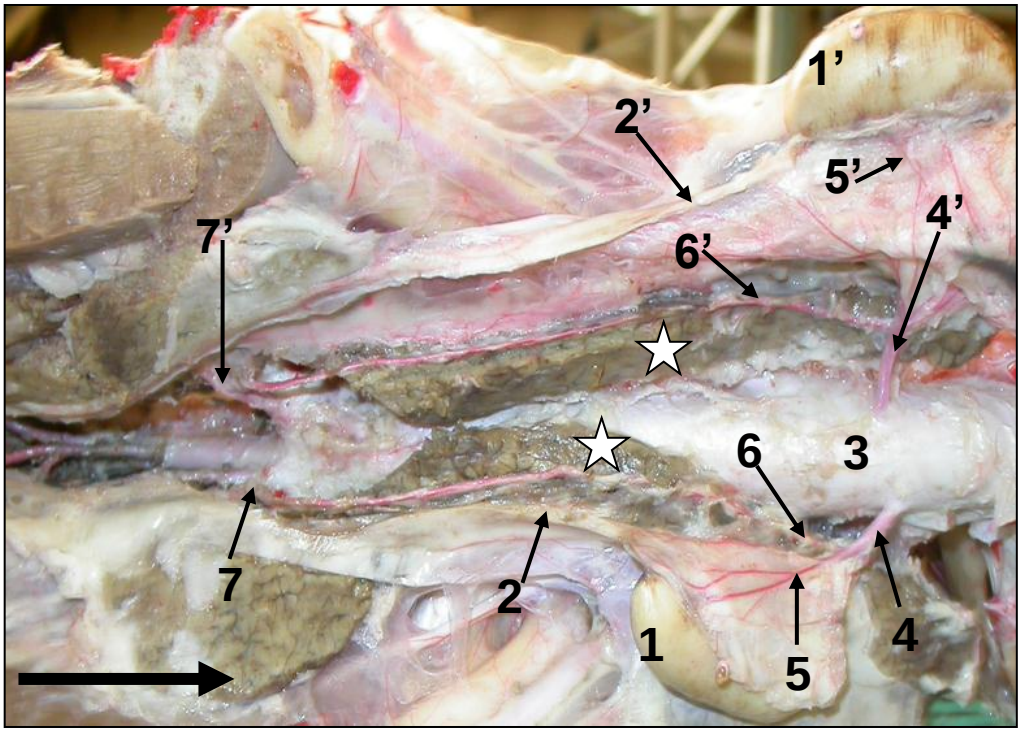

Figure 2.8. Ventral view of the thoracoabdominal and sacrolumbar segments of the aorta and its branches. Left and right testes (1,1'). Left and right deferent ducts (2, 2').Aorta (3). Left and right cranial renal arteries (4,4'). Left and right testicular arteries (5, 5').Leftand right cranial ureterodeferential branches (6, 6'). Left and right middleureterodeferential branches (7, 7'). Cranial divisions of kidney (white stars). Arrow bottom left indicates caudo-cranial direction.

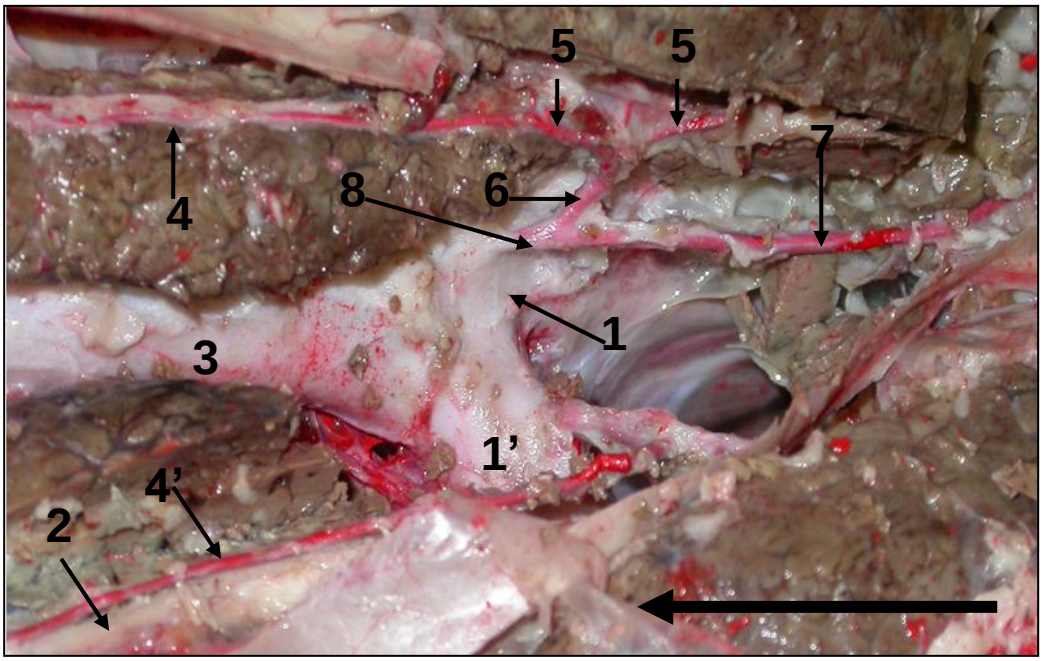

Figure 2.9. Ventral view of the sacrolumbar segment of the aorta showing the left common renal artery and its branches: The equivalent vessels on the right side are not exposed. Left and right ischiadic arteries (1, 1'). Right deferent duct and ureter (2).Aorta (3).Collateral circulation (left and right cranial ureterodeferential arteries) (4, 4'). Left middle ureterodeferential branches (5). Left middle renal artery (6).Left caudal renal artery (7).Left common renal artery (8) for (6 and 7). Arrow bottom right indicates caudo-cranial direction.

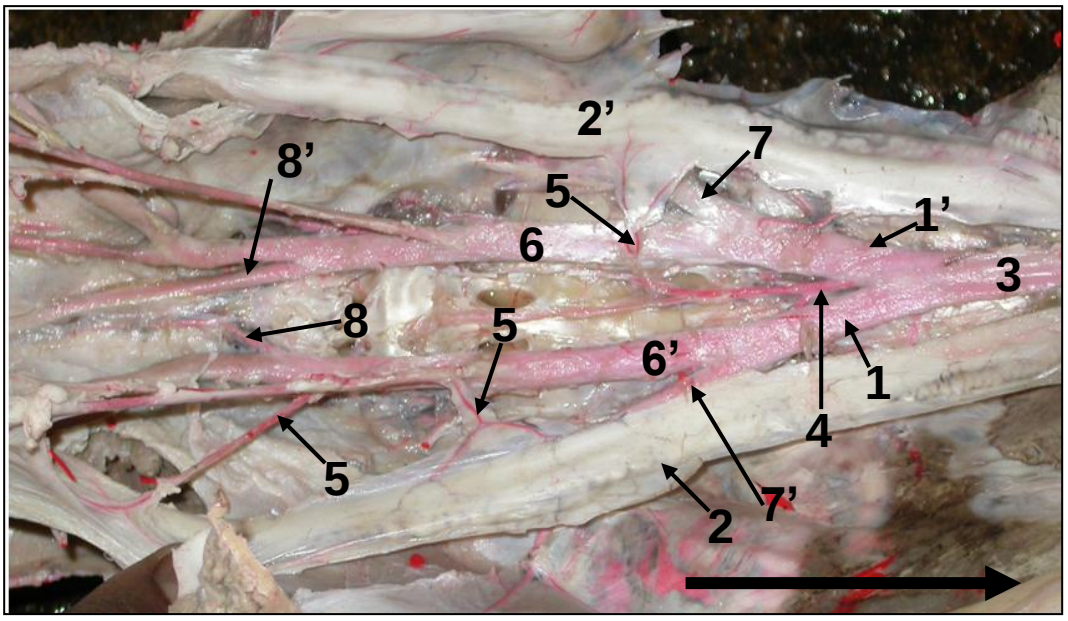

Figure 2.10. Ventral view of the pelvic segment of theaorta and its branches.Left and right internal iliac arteries (1, 1'). Left and right deferent ducts and ureters (2, 2'). Aorta (3). Caudal median artery (4).Caudal ureterodeferential branches (5). Left and right pudendal arteries (6, 6'). Left and right lateral caudal arteries (7,7'). Left and right cloacal arteries (8, 8'). Arrow bottom right indicates caudo-cranial direction.

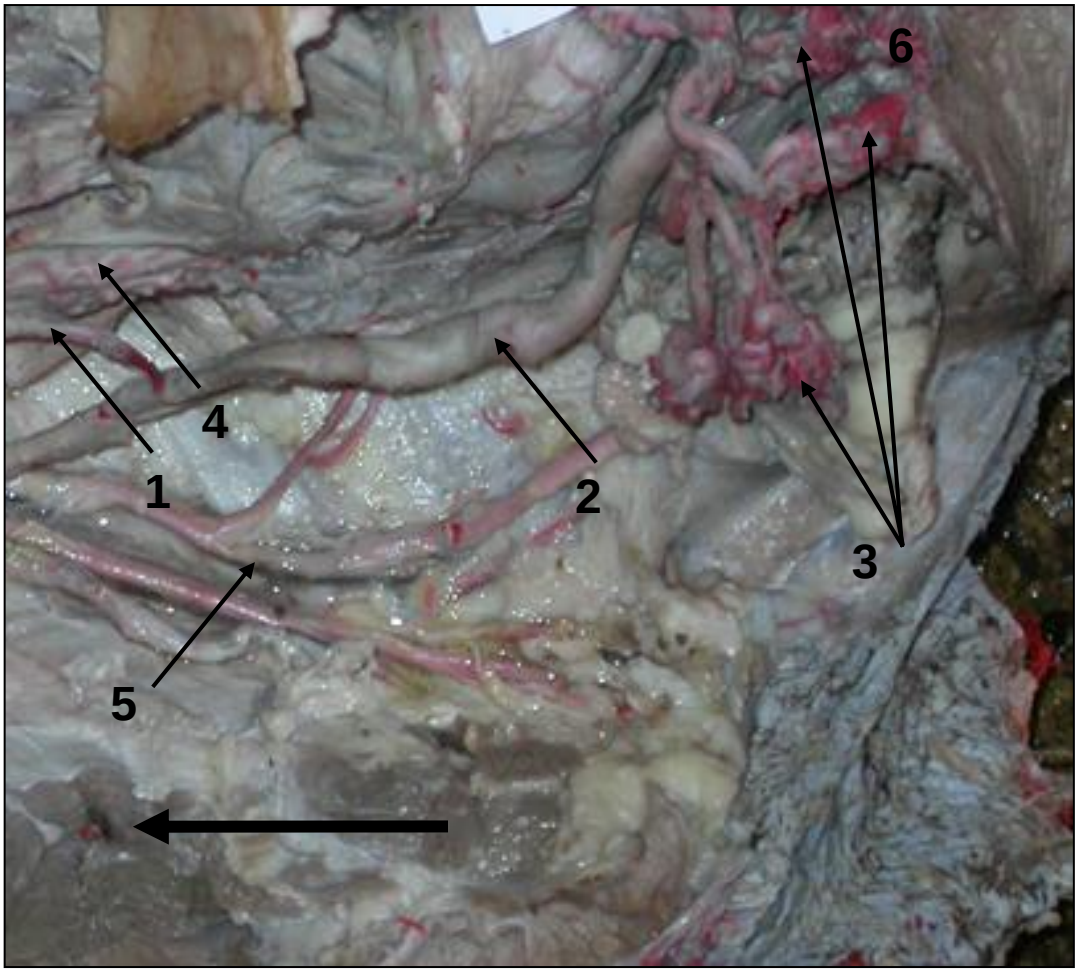

Figure 2.11. Ventro-lateral view of the pelvic region showing the network of arteries (3) at the base of the phallus.Caudal ureterodeferential branch (1).Branch of the pudendal artery to the base of phallus (2).Left deferent duct and ureter (4).Caudal median artery (5). Phallus (6). Arrow at bottom left indicates caudo-cranial direction.

CHAPTER 3

MACROSCOPIC FEATURES OF THE VENOUS DRAINAGE OF THE REPRODUCTIVE SYSTEM

1. INTRODUCTION

The vascular system of the male reproductive tract in birds has, in general, been poorly studied. The only comprehensive report of both the arterial supply and venous drainage is that of Nishida (1964) in the domestic fowl and of Kremer and Budras (1990) in the Peking drake. A general description of the arterial supply to the male reproductive organs of the pigeon has also been provided (Bhaduri, Biswas & Das 1957) and a more recent study (Elias, Aire & Soley 2007) has described the macroscopic features and variations in the arterial supply to the reproductive tract of the male ostrich. The venous system has received even less attention, with most of the information being supplied by Nishida (1964) and some incidental data being provided from a study on the renal portal and venous systems of the fowl kidney by Kurihara & Yasuda (1975). Descriptions of the venous system involving the male reproductive tract of birds presented in textbooks [eg. Baumel (1975), Lake (1981) and Baumel 1993] simply reflect the work of Nishida (1964) and Kurihara & Yasuda (1975). The only reference to drainage of the reproductive organs in the male ostrich is by Bezuidenhout (1999) who notes that “As the caudal vena cava passes cranially and ventrally it receives veins from the gonads, adrenals and surrounding tissues”.

In view of the lack of comparative data on the vascularization of the male reproductive tract of birds, and considering the need for a better understanding of the reproductive biology of the ostrich as a farmed animal, this chapter presents a description of the gross pattern of venous drainage in the male ostrich. Variations in the basic pattern are also described. The

terminology used is that of *Nomina Anatomica Avium* (Baumel, King, Breazile, Evans & Berge 1993).

2. MATERIALS AND METHODS

The torsos of nine male ostriches with viscera intact, but which had been skinned and from which the limbs had been removed, were obtained from the Oryx Abattoir in Krugersdorp, Gauteng Province and from the Klein Karoo Abattoir in Oudtshoorn, Western Cape Province, South Africa. The specimens comprised six sub-adult birds (12 to 14 months-old) and three sexually mature and active birds. The torsos were prepared as follows for latex injection and the formation of resin casts, respectively.

The venous system of five of the birds (four sub-adult and one sexually mature bird) was flushed free of blood by rinsing with physiological saline injected through the caudal vena cava (5 to 10 cm above the vessel's origin between the cranial poles of the testes), after which blue Latex[1]was subsequently injected into the veins via the same route using a 50 ml syringe. The torsos were trimmed of excess tissue and immersion fixed in a 10% formalin bath for a number of days. The fixed specimens were rinsed in running water for 2 days after which they were carefully dissected to expose the latex-filled veins. The pattern of venous drainage was described and digitally recorded with a Nikon 4500 Coolpix digital camera.

For the preparation of resin casts of the veins, four torsos (two sub-adults and two sexually active birds) were injected with a coloured resin[2] via the caudal vena cava using the same technique employed for the latex injections. Each specimen was subsequently kept for a number of days in a cold room before tissue maceration in a bath containing a 20-30 % sodium hydroxide solution. The corrosion casts obtained were carefully washed clean and the pattern of veins described and digitally recorded.

[1]RevertexChemicals Company®, Pretoria, South Africa

[2]Pigment Preparation Pty. Ltd. Pretoria, South Africa

3. RESULTS

3.1. Venous drainage of the testes

The testes were drained via testicular veins (*Venae testiculares*) that were variable in number (ranging from one to four for each testis) and point of termination (Figs. 3.1, 3.2A-C, 3.3). These veins were observed to arise from a well-developed venous network located in the testicular capsule (Fig. 3.3). Based on the relationship of the testicular veins to the caudal vena cava and the common iliac veins, the following variations were observed:

***Type A*:** In this variation, the testicular veins draining the right testis, the epididymis and its appendix, and the cranial aspect of the *Ductus deferens* and ureter, joined both the caudal vena cava (just cranial to its origin) and the right common iliac vein. The vessels draining the left testis and associated structures emptied exclusively into the left common iliac vein, caudal to the confluence of the two common iliac veins (Figs. 3.1, 3.2B, 3.3) This was the most common pattern noted and occurred in five (55.5%) of the specimens examined.

***Type B*:** This variation was observed in four (44.5%) of the specimens and was characterized by the right testicular veins draining only into the caudal vena cava, while the left testicular veins again emptied into the left common iliac vein (Figs. 3.2A, C).

As noted above, the testes displayed a well-developed venous network situated in the testicular capsule, particularly in the sexually mature birds, and from which the testicular veins emanated. Due to the complexity of this network and the formation of numerous tributaries, it was difficult in some specimens to accurately determine the exact number of testicular veins present. However, based on obvious connections between the testicular veins and the caudal vena cava and common iliac veins, four basic numerical patterns were observed.

Type *I*: In this variation at least four testicular veins were observed to drain the right testis while a similar number drained the left testis (Figs. 3.1,3.3). This was the most common pattern noted and occurred in three (33.4%) of the specimens examined.

Type *II*: In this instance three testicular veins (essentially cranial, middle and caudal vessels) drained the right testis and two testicular veins (cranial and caudal) the left testis (Fig.3.2A). This variation occurred in two (22.2%) of the specimens.

Type *III*: This type was characterized by the presence of two testicular veins draining the right testis and a single, large testicular vein draining the left testis. The latter was formed by a large number of branched tributaries (Fig. 3.2B). This variation was present in two (22.2%) of the specimens examined.

Type *IV*: This variation was also observed in two (22.2 %) of the specimens and consisted of a single testicular vein draining the right testis and two veins draining the left testis (Fig. 3.2C). Each of these vessels was composed of numerous tributaries.

It was observed in all specimens that a small vessel (effectively an additional testicular vein?) emanated from the venous network at the cranial aspect of each gonad and emptied into the adrenal vein. (Figs. 3.1, 3.2A-C, 3.3, 3.4). In the ostrich this vessel was prominent and well-developed on the left side of the body where it drained into the left common iliac vein. The adrenal vein was smaller on the right side where it emptied into the caudal vena cava. In addition to draining the cranial aspect of the testis, the adrenal vein drained part of the *Appendix epididymidis,* the adrenal gland and the cranio-lateral body wall of the thoraco-abdominal region. Very fine branches of the adrenal vein also drained the cranial division of the kidney.

The position and course of the caudal vena cava of the ostrich were similar to that described for the fowl (*Gallus domesticus*) (Nishida 1964). It was formed by the confluence of the right and left common iliac veins in the vicinity of the cranial poles of the testes, the adrenal glands and cranial divisions of the kidneys, where it lay almost directly below the aorta. In the material studied, the caudal vena cava varied between 2 to 4 cm in width, and 20 to 28 cm in length. It lay slightly to the right of the median plane and coursed obliquely cranio-ventrally (Figs. 3.1, 3.3) towards the right atrium of the heart.

3.2. Drainage of the *Ductus deferens*

The cranial aspect of each deferent duct was drained by the most caudal testicular vein through the cranial ureterodeferential vein (*Vena ureterodeferentialis cranialis*) (Figs. 3.1, 3.2A-C,3.3).

The middle portions of the deferent duct and ureter were drained by the middle ureterodeferential veins *(Venae ureterodeferentiales mediae).* These vessels were variable in number, size and point of entry along the caudal renal veins into which they emptied. The caudal renal veins also drained the caudal part of the cranial division and all of the middle and caudal divisions of the kidney as well as the ureter in those regions. The caudal renal veins were observed to fuse with each other at a level between the caudal and middle renal lobes (Figs. 3.1, 3.5, 3.6) to form a single vessel approximately 3 to 5 cm long, before again dividing cranially into two separate vessels. In all specimens studied, the middle ureterodeferential veins emptied into both the fused and separated parts of the caudal renal veins. The cranial continuation of the caudal renal veins drained ventro-caudally into the common iliac veins (Figs. 3.1, 3.3), while the femoral veins (*Vena femoralis*) entered the common iliac veins dorso-laterally. Each common iliac vein (*Vena illiaca communis*) emptied into the caudal vena cava in the vicinity of the cranial aspect of the testes.

The caudal part of the deferent duct and the cloaca were drained by the caudal ureterodeferential veins *(Venae ureterodeferentiales caudales)* to the pudendal veins and to the caudal portion of the internal iliac veins. Each pudendal vein *(Vena pudenda*), after receiving the caudal lateral vein (from the wall of the pelvic region) formed the internal iliac vein (Figs. 3.7, 3.8). The two internal iliac veins proceeded cranially and were connected by a transverse vessel, the interiliac anastomosis, 2 to 5cm caudal to the caudal pole of the kidney. The anastomosis was linked caudally to the caudal median vein (Figs. 3.6, 3.7). In three specimens (33.3%), tributaries from the caudal deferent duct, together with branches from the rectum and cloaca, were observed to drain into the caudal mesenteric vein (*Vena mesenterica caudalis*) in addition to the branches draining into the pudendal vein. The caudal mesenteric vein emptied into the caudal median vein (Fig. 3.7). In one of these specimens the caudal mesenteric vein, in addition to its connection to the caudal median vein, was linked

to the left internal iliac vein by a large unnamed vessel draining the wall of the rectum (Fig. 3.8).

3.3. Drainage of the phallus

A number of superficial tributaries displaying no particular pattern drained the mucosa, but not the substance of the phallus, (Figs. 3.1,3.9) to the pudendal vein. These vessels were relatively large (Fig. 3.9). A venous network of fine vessels was situated at the root of the phallus which also drained into the pudendal vein (Fig. 3.1).

4. DISCUSSION

The gross pattern of venous drainage of the male reproductive tract in the ostrich is basically similar to that described for the fowl (Nishida 1964). However, certain noteworthy variations were observed in the material studied which may reflect unreported differences between avian species.

In both the ostrich and fowl (Nishida 1964) the testicular veins arise from a well-developed network located in the testicular capsule. In the fowl both sets (left and right) of testicular veins are reported to empty into the corresponding common iliac vein and the caudal vena cava as illustrated in the sketch by Nishida (1964). In other studies on the fowl, however, the testicular veins are shown to empty only into the caudal vena cava (Nickel, Schummer & Seiferle 1977). A similar situation is also illustrated for the veins draining the single ovary of *Gallus* (Baumel 1993). In the Peking drake both the left and right testis were drained by two testicular veins which emptied into the caudal vena cava (Kremer & Budras 1990). In five of the ostriches studied, the drainage pattern of the right testis resembled that described by Nishida (1964) (testicular veins emptied into the right common iliac vein and the caudal vena cava) whereas the testicular veins from the left testis emptied exclusively into the left common iliac vein. In four birds, however, the testicular veins emanating from

the right testis drained only into the caudal vena cava, with those from the left testis again emptying into the left common iliac vein.

In the ostrich, therefore, the route of drainage of the right testicular veins reflects both patterns reported in the fowl. However, the exclusive drainage of the left testicular veins into the corresponding common iliac vein in all the ostrich specimens examined appears to be a unique feature and contrasts with the observation that in the ostrich the veins from the gonads empty into the caudal vena cava (Bezuidenhout 1999). The slightly more cranial positioning of the right testis reported in the ostrich (Soley 1992; Soley & Groenewald 1999) may provide an explanation for that drainage into both the right common iliac vein and the caudal vena cava or only into the caudal vena cava, and to the exclusive drainage of the more caudally positioned left testis into the left common iliac vein.

Little information is available in the literature regarding the numerical patterns of the testicular veins. The illustration of Nishida (1964) shows one large vein draining the right testis and three vessels draining the left testis, similar to the Type IV variation reported in the present study. As the authors did not have a full English translation of the text available, it is possible that a range of variations were described by Nishida (1964).The illustration by Nickel *et al.* (1977) demonstrates at least three veins draining both the left and right testes respectively which corresponds closely with the most common variation (Type I) seen in the ostrich involving four testicular veins draining each testis. These observations would suggest that a range of numerical variations is typical for the testicular veins of birds.

An interesting observation in the ostrich was the presence of a small vessel emanating from the venous network at the cranial aspect of each gonad and which drained into the adrenal vein. This drainage route for the testes has not been reported in the fowl although Baumel (1993) illustrates some *Vv. ovaricae* reaching the caudal vena cava via the adrenal vein. In the ostrich, the adrenal vein on the left side of the body drained into the left common iliac vein whereas that on the right side emptied into the caudal vena cava. In the fowl both the left and right adrenal veins reportedly drain into the caudal vena cava (Goodchild 1969;

Baumel 1993). Again, the unequal positioning of the ostrich testes may account for this phenomenon.

In the ostrich, drainage of the cranial and middle segments of the *Ductus deferens* parallels that described in the fowl (Nishida 1964).The cranial aspect of the *Ductus deferens* and ureter are drained by the cranial ureterodeferential veins to the most caudal testicular veins and from there to the common iliac veins or caudal vena cava. However, it is not clear whether the cranial ureterodeferential veins in the fowl (*Vv. ureto-deferentiales anteriores* – Nishida 1964) reach the common iliac veins independently or via the caudal testicular veins.

In both the ostrich and fowl, middle ureterodeferential veins (*Vv. ureto-deferentiales mediae* – Nishida 1964) drain the middle segments of the ductus deferens and ureter to the caudal renal veins (*V. renalis efferens* - Nishida 1964). A marked difference in the ostrich is that some of the middle ureterodeferential veins enter the fused portion of the left and right caudal renal veins, a situation lacking in the fowl as fusion of the caudal renal veins does not occur (Akester 1964; Kurihara & Yasuda 1975; Baumel 1993). Fusion of the caudal renal veins has only previously been reported in a single 5-day old chicken embryo (Miller 1903). Why this phenomenon should be a consistent feature in the ostrich, or what its functional significance is, remains unknown.

In six of the nine specimens, the caudal ureterodeferential veins in the ostrich were observed to drain into the pudendal vein and the caudal aspect of the internal iliac vein which formed the continuation of the former vessel. In three specimens, however, the caudal ureterodeferential veins were seen to drain into the caudal mesenteric vein in addition to the branches draining into the pudendal vein. In the fowl the caudal ureterodeferential veins (*Vv. ureto-deferentiales posteriors* – Nishida 1964) are shown draining only into the pudendal vein (*V. pudenda interna* – Nishida 1964). As a caudal mesenteric vein (*V. coccygomesenterica* - Nickel *et al*. 1977) has been described in the fowl (Nickel *et al.* 1977; Baumel 1993) it is possible that in this species branches of the caudal ureterodeferential veins do in fact empty into this vessel but have not yet been

described. It was also noted that in the ostrich the caudal mesenteric vein drained into the caudal median vein which in turn was linked to the interiliac anastomosis. In the fowl, both the caudal median vein and the caudal mesenteric vein appear to drain into the interiliac anastomosis (Baumel 1993, page 467), although alternative drainage patterns have also been described (Kurihara & Yasuda 1975; Baumel 1993).

The large tributaries draining the mucosa of the phallus to the pudendal vein in the ostrich have not been described in the fowl. This omission may be due to the relatively small size of the phallus and the attendant difficulties in revealing the blood vessels in this species.

In conclusion, the pattern of venous drainage of the reproductive organs of the male ostrich follows the basic pattern described in the domestic fowl by Nishida (1964). Although the differences observed between the ostrich and fowl are significant, they may simply reflect variations in the normal pattern of venous drainage of the reproductive tract of birds which could be verified by studying more specimens and more species.

5. REFERENCES

AKESTER, A. R. 1964. Radiographic studies of the renal portal system in the domestic fowl (*Gallus domesticus*). *Journal of Anatomy*, 98: 365-376.

BAUMEL, J. J. 1975. Heart and blood vessels, in *The anatomy of domestic animals*. Vol.2. Edited by R. Getty. Philadelphia: Saunders Company. pp. 1968-2009.

BAUMEL, J. J. 1993. Systema cardiovascular, in *Handbook of avian anatomy. Nomina Anatomica Avium.* 2nd ed. Edited by J.J. Baumel, A. S. King, J. E. Breazile, H. E. Evans & J. C. V.Berge. Cambridge, Massachusetts: Nuttall Ornithological Club, pp. 407-475.

BAUMEL, J. J., KING, A. S., BREAZILE, J. E., EVANS, H. E. & BERGE, J. C. V. (Eds.) 1993. *Handbook of avian anatomy. Nomina Anatomica Avium.* 2nd ed. Cambridge, Massachusetts: Nuttall Ornithological Club.

BHADURI, J. L., BISWAS, B. & DAS, S. K. 1957. The arterial system of the domestic pigeon *(Columba livia* Gmelin). *Anatomischer Anzeiger*, 104: 1-14.

BEZUIDENHOUT, A. J. 1999. Anatomy, in *The Ostrich.Biology, production and health.* Edited by D. C. Deeming. Cambridge: CABI Publishing. pp. 13-50.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2007. Macroscopic features of the arterial supply to the reproductive system of the male ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 36: 255-262.

GOODCHILD, W. M. 1969. The venous system of the adrenal glands of *Gallus domesticus*. *British Poultry Science,* 10: 183-185.

KREMER, A. & BUDRAS, K. D. 1990. Zur Blutgefassversorgung des Hodens beim Pekingerpel (*Anas platyrhynchos,*L.). Makroskopische, lichtmikroscopische und rasterelektronenmikroskopische Untersunchungen. *Anatomischer Anzeiger*, 171: 73-87.

KURIHARA, S. & YASUDA, M. 1975. Morphological study of the kidney in the fowl. II.Renal portal and venous systems. *Japanese Journal of Veterinary Science*, 37: 363-377.

LAKE, P. E. 1981. Male genital organs, in *Form and function in birds*. Edited by A.King & J. McLelland. London: Academic Press. pp. 1-61.

MILLER, A. M. 1903. The development of postcaval vein in birds. *American Journal of Anatomy*, 2: 283-298.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Circulatory system, in *Anatomy of the Domestic Birds*. Berlin: Verlag Parey. pp. 85-107.

NISHIDA, T. 1964. Comparative and topographical anatomy of the fowl. XLII. Blood vascular system of the male reproductive organs. *Japanese Journal of Veterinary Science, 26*: 211-221.

SOLEY, J.T. 1992. A histological study of spermatogenesis in the Ostrich (*Struthio camelus*). PhD thesis. University of Pretoria.

SOLEY, J. T. & GROENEWALD, H. B. 1999. Reproduction, in *The Ostrich. Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp. 129-158.

6. FIGURES

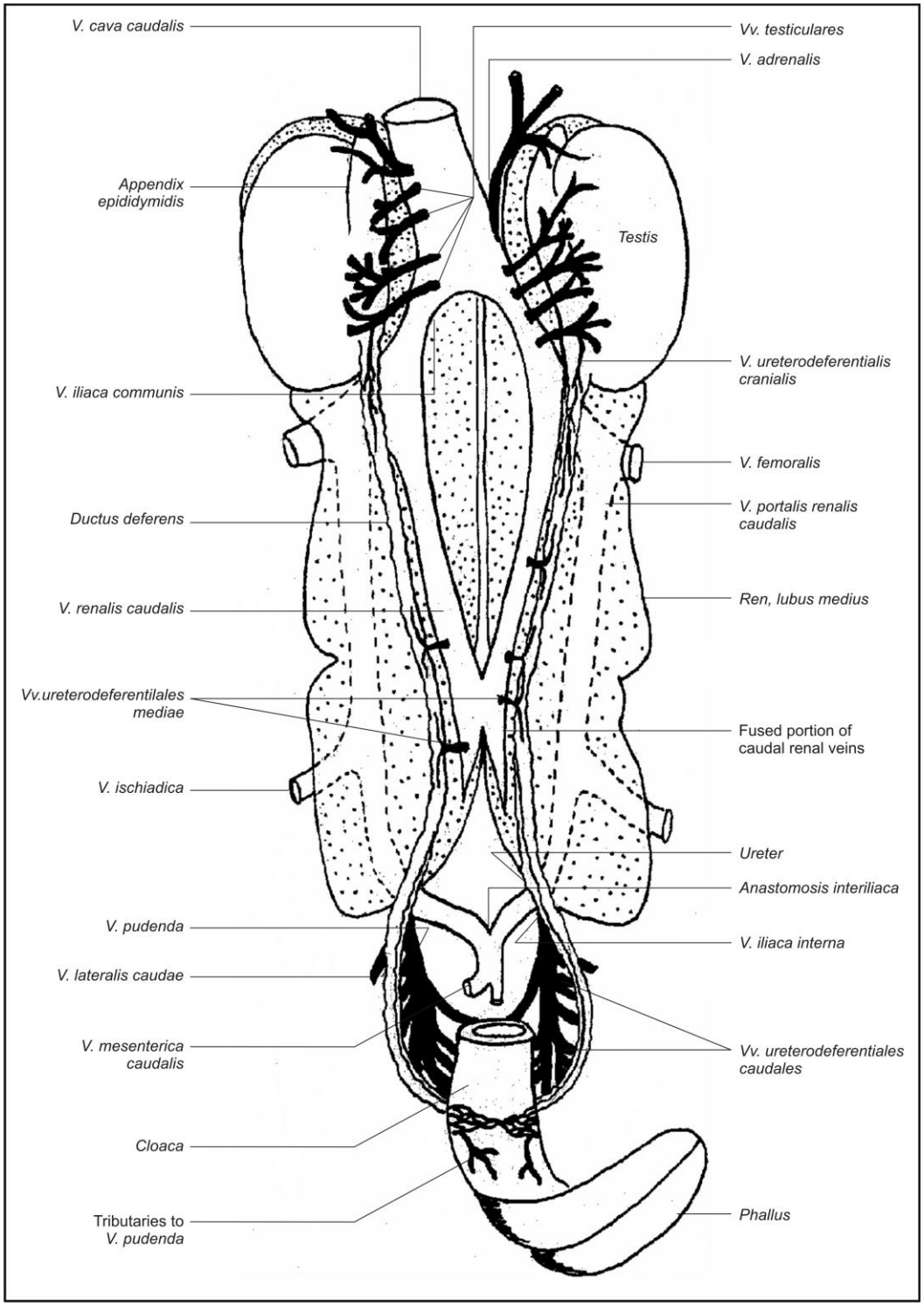

Figure 3.1. A diagrammatic sketch showing, in ventral view, the main veins draining the reproductive tract of the male ostrich. The testes are shown reflected laterally to illustrate the testicular veins and associated vessels.

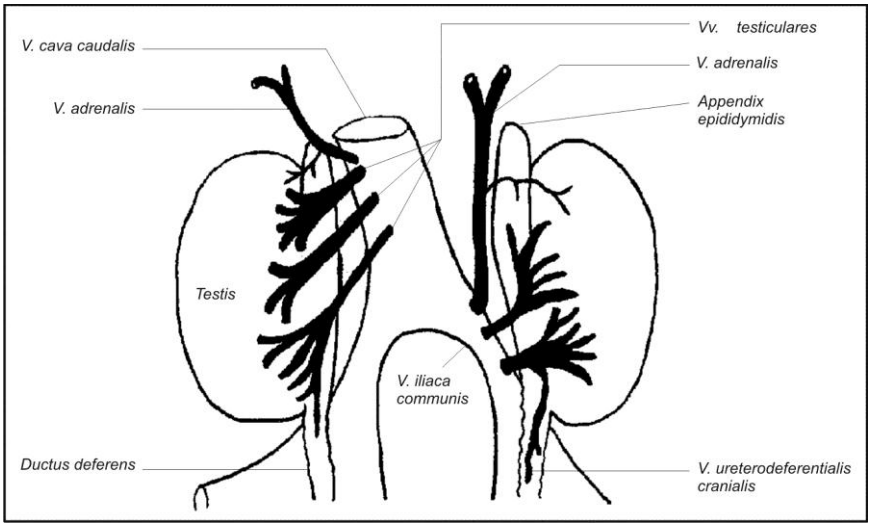

Figure 3.2A

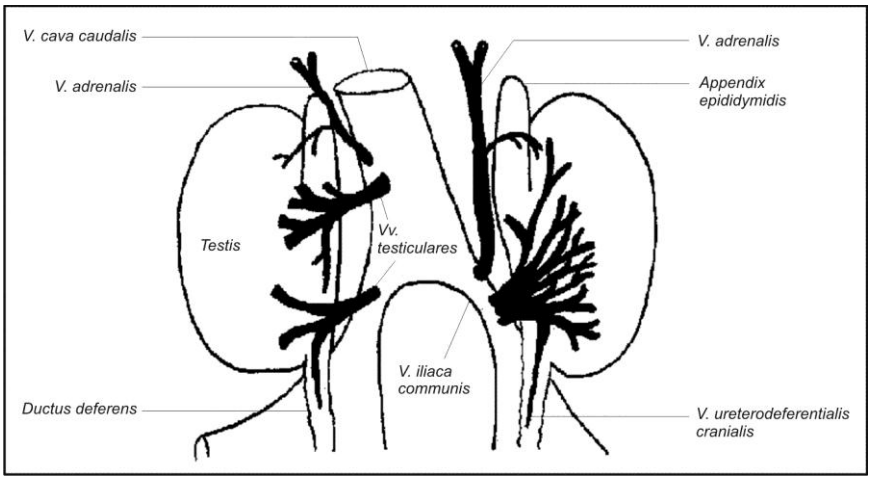

Figure 3.2B

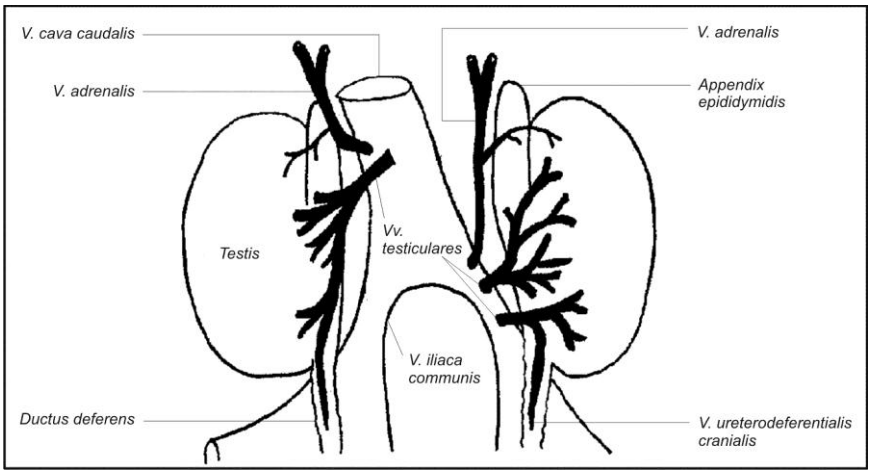

Figure 3.2C

Figure 3.2. A, B and **C.** Diagrammatic sketches showing, in ventral view, the main variations of the testicular veins in respect of their number and location.

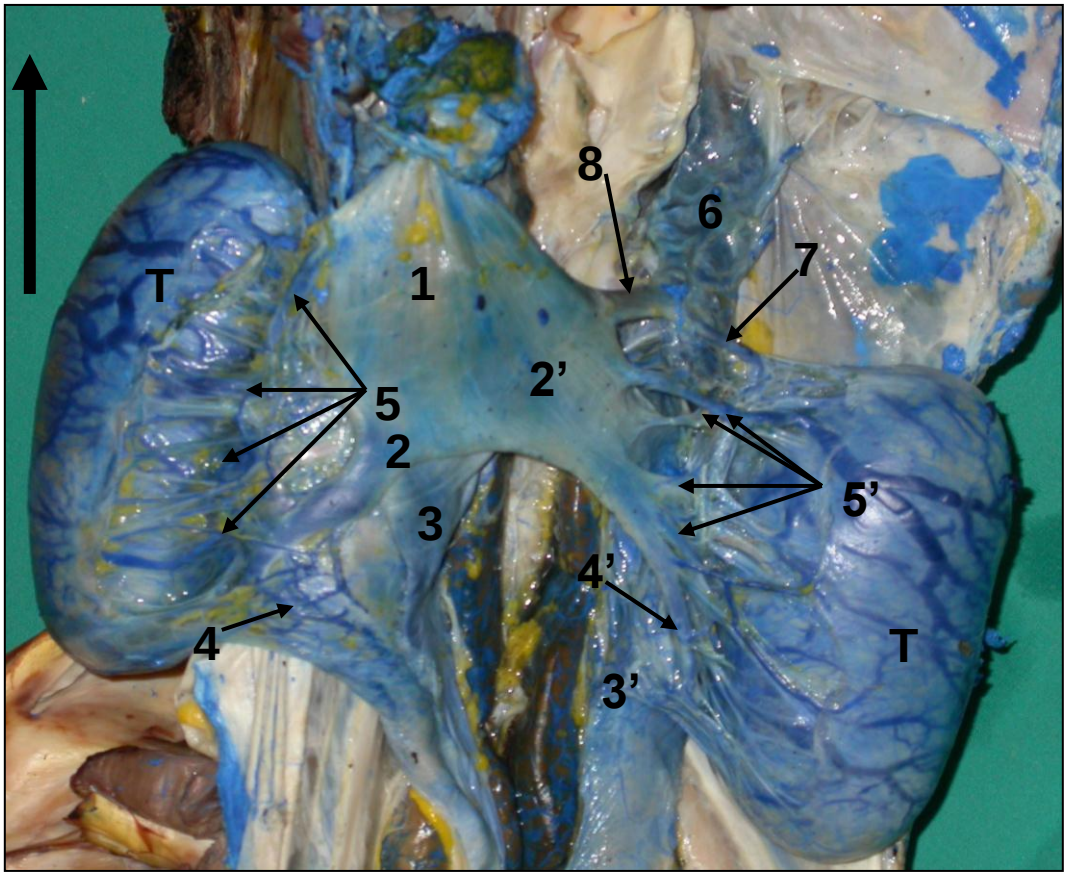

Figure 3.3. Ventral view of the reproductive tract of the ostrich illustrating the testes (T) and associated veins. The testes have been reflected laterally to expose more clearly the relevant veins. Note the extensive venous network within the testicular capsule. *V. cava caudalis* (1), *Vv. iliacae communes dextra et sinistra* (2, 2'), *Vv. renales caudales dextra et sinistra* (3, 3'), *Vv. ureterodeferentiales craniales dextra et sinistra* (4, 4'), *Vv. testiculares dextra et sinistra* (5', 5), *Appendix epididymidis* (6), Branch draining the cranial pole of the testis to the adrenal vein (7). *V. adrenalis sinistra* (8). Arrow at top left indicates caudo-cranial direction. Latex injection.

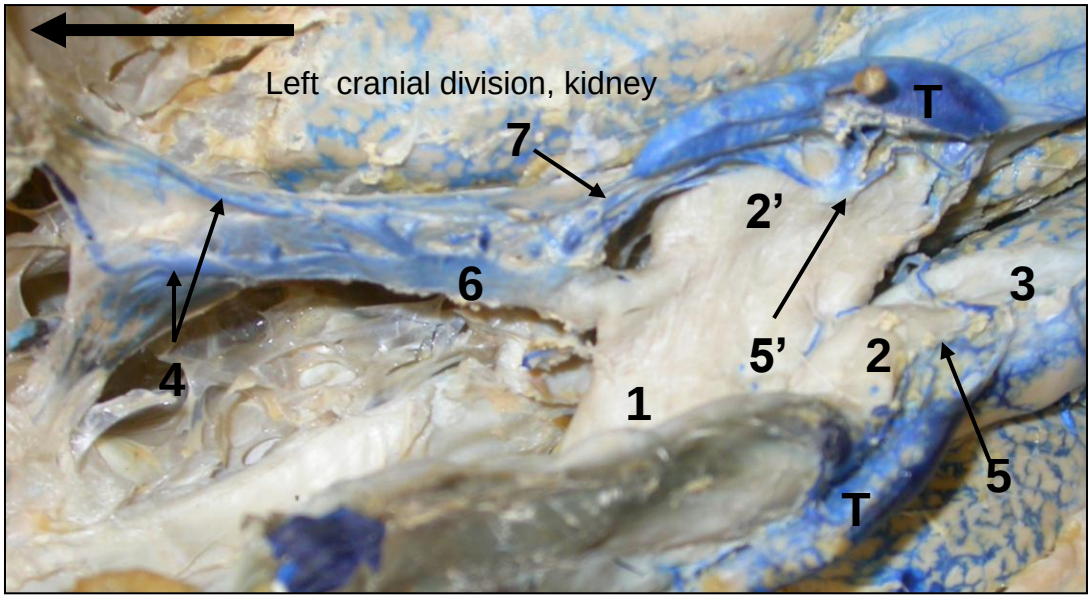

Figure 3.4. Ventral view of the thoraco-abdominal region of a pre-pubertal bird illustrating the testes (T) and associated veins. *V. cava caudalis* (1), *Vv. iliacae communes dextraetsinistra* (2, 2’), *V. renalis caudalis dextra* (3), *Vv. testiculares dextra et sinistra* (5’, 5), *V. adrenalis sinistra* (6). Note the branches from the latero-dorsal body wall (4) draining into the adrenal vein (6) as well as a testicular vein (7) that drains the left testis into the left common iliac vein via the adrenal vein. Arrow top left indicates caudo-cranial direction. Latex injection.

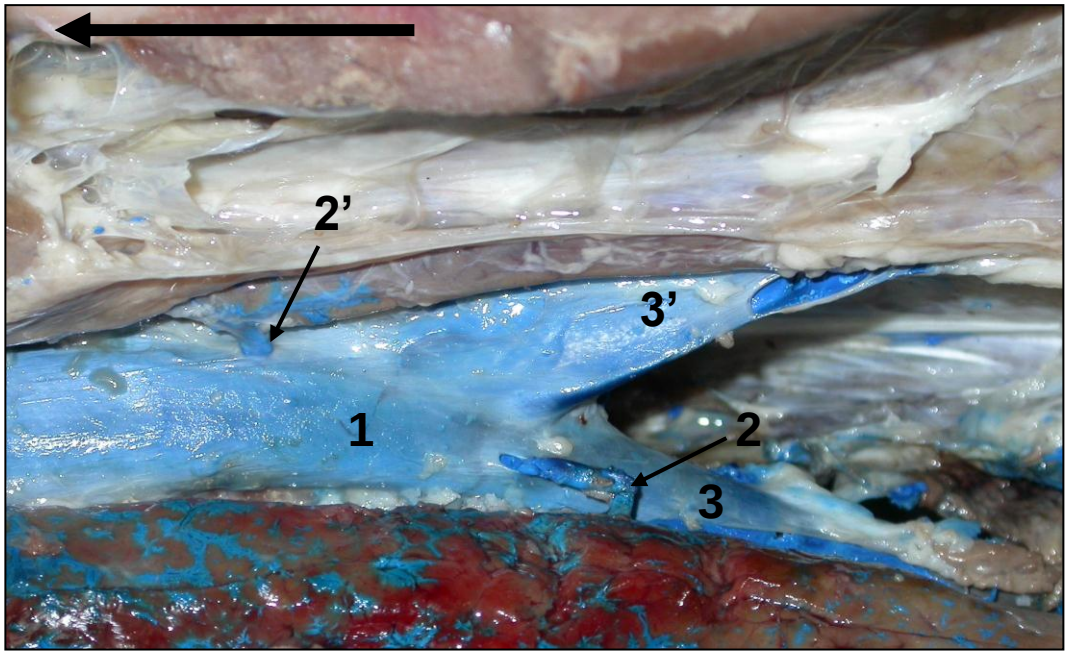

Figure 3.5. A ventral view of the middle segment of the reproductive tract showing the region of fusion between the left and right caudal renal veins (1), *Vv. ureterodeferentiales mediae dextra et sinistra* (2, 2’), *Vv. renales caudales dextra et sinistra* (3, 3’). Arrow top left indicates caudo-cranial direction. Latex injection.

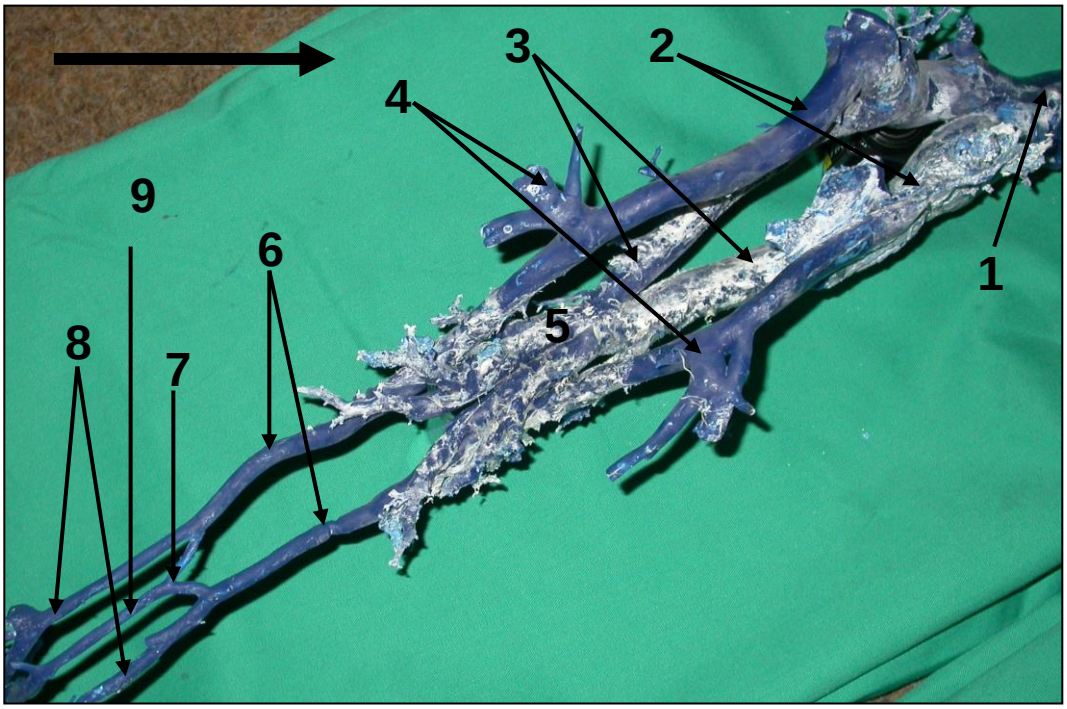

Figure 3.6. A corrosion cast (dorsal view) showing the main components of the venous system involved in drainage of the male reproductive tract: *V. cava caudalis* (1), *Vv. iliacae communes* (2), *Vv. renales caudales* (3). *Vv. ischiadicae* (4), fused portion of caudal renal veins (5), *Vv. portales renales caudales* (6), *anastomosis interiliaca* (7), *Vv. iliacae internae* (8) and *V. caudae medianae* (9). Arrow top left indicates caudo-cranial direction. Latex injection.

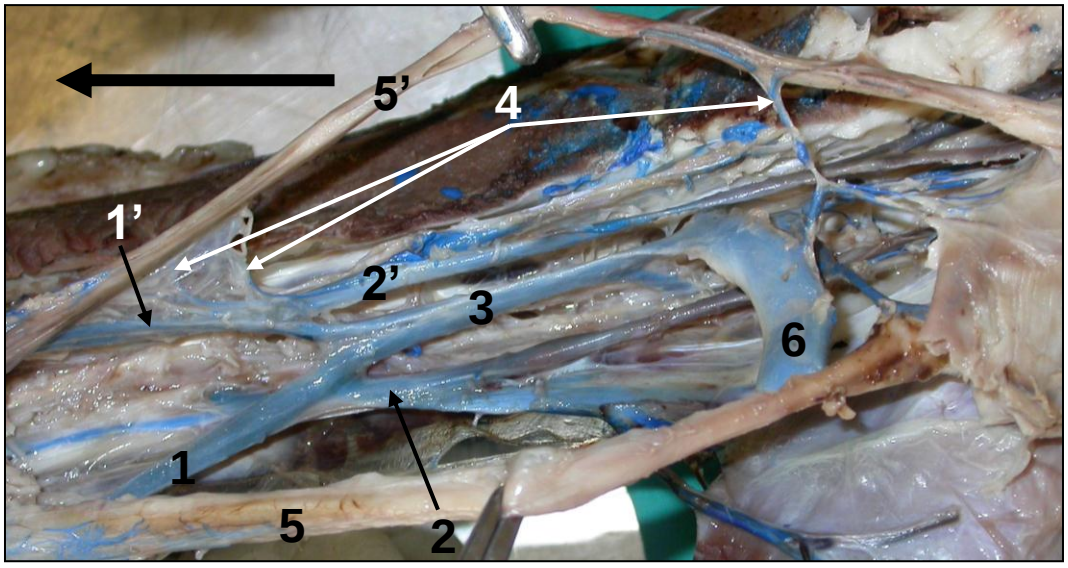

Figure 3.7. Ventro-lateral view of the pelvic region displaying the: *Vv. iliacae internae dextra et sinistra* (1, 1'), *Vv. pudendae dextra et sinistra* (2, 2'), *V.caudae medianae* (3), *Vv. ureterodeferentiales caudae* (4), *Ductus deferentes et ureteres dextrae et sinistrae* (5, 5'), *V. mesenterica caudalis* (6). Note the caudal ureterodeferential vein (4 – single white arrow) draining the ductus deferens (5) to the caudal mesenteric vein (6). Arrow top left indicates caudo-cranial direction. Latex injection.

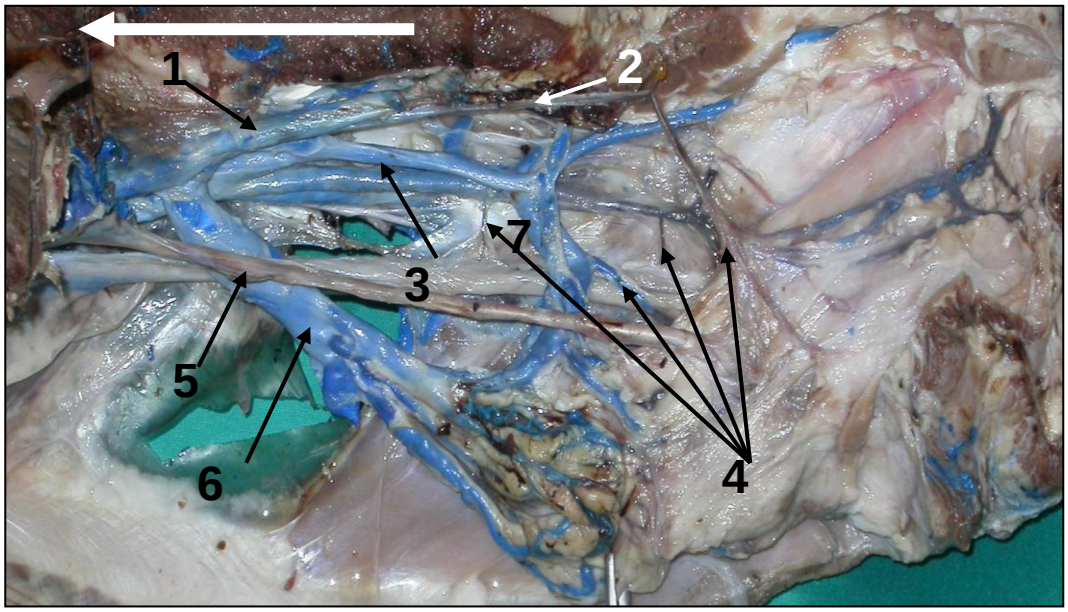

Figure 3.8. Ventro-lateral view of the pelvic region showing the:*V. iliaca interna sinistra* (1), *V. pudenda sinistra* (2), *V. caudae medianae* (3), *V. ureterodeferentialis caudalis* (4), *Ductus deferentes et ureteres sinistrae* (5). In this view the exact positioning of the veins in the pelvic region has been obscured to illustrate the anastomosis between the *V. mesenterica caudalis* (7) and the left internal iliac vein (1) via a large unnamed vein (6). Arrow top left indicates caudo-cranial direction. Latex injection.

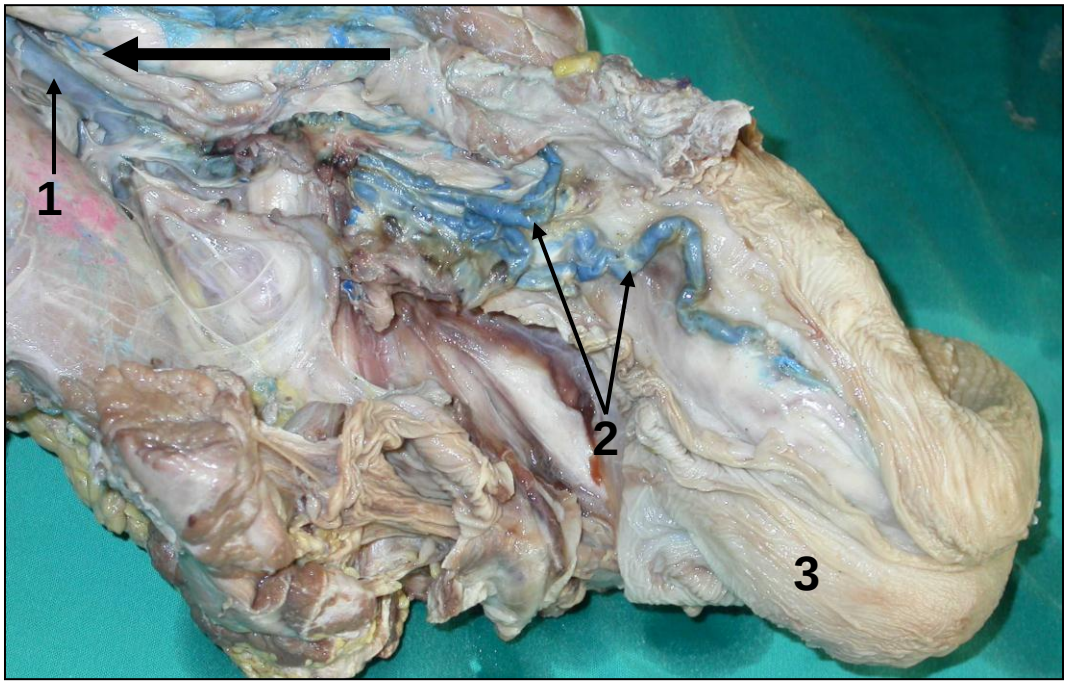

Figure 3.9. Ventro-lateral view of the phallus showing the *V. pudenda dextra* (1) receiving large, superficial tributaries (2) from the root and body of the *phallus* (3). Arrow top left indicates caudo-cranial direction. Latex injection.

CHAPTER 4

THE DISTRIBUTION AND STRUCTURE OF THE MICROVASCULATURE OF THE TESTIS AND EPIDIDYMIS

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1. INTRODUCTION

There are a number of reports on the distribution and morphological characteristics of the microvasculature of the testis and epididymis of mammals, particularly of rodents. Ladman & Young (1958) and Suzuki (1982), for example, noted the presence of fenestrated capillaries around the efferent ducts in the guinea pig and mouse, respectively. Similarly, a dense network of fenestrated capillaries has been observed to surround the initial segment of the *Caput epididymidis* of the mouse (Abe, Takano & Ito 1984). A sharp decrease in vascularity was reported between the initial segment and the C*auda epididymidis* in the rat (Markey &Meyer 1992). In the human testis, Kormano & Suoranta (1970) observed that the arteries in the testis run in both centripetal and centrifugal directions, giving rise to intertubular capillary networks they consider to be similar to those in the rodent testis. Suzuki & Nagano (1986) showed that, in the testis parenchyma, the capillaries formed a plexus that surrounded the seminiferous tubules.

Based on the distribution of blood vessels relative to the tubular elements of the testis and excurrent duct system in the mouse, Suzuki (1982) classified the peritubular vessels of the male reproductive tract into two fundamental types, namely, the *testicular type* (adjacent tubules share blood capillaries) and the *deferential type* (peritubular capillaries lie beneath the epithelium, encircling individual tubules).

In contrast, very little specific information is available on the microvasculature of the avian male reproductive tract. Lake (1957) simply reports that capillaries and arterioles of the testicular artery ramify throughout the intertubular tissue of the testis in the domestic fowl.

Other reports have documented the incidental observation of microvasculature in various segments of the avian reproductive tract, for example, in the domestic fowl (Lake 1957; Budras & Sauer 1975; Aire 1980, 1982, 1997), duck (Aire 1997), guinea fowl (Aire 1980, 1982, 1997), Japanese quail (Aire 1980, 1982, 1997), turkey (Hess, Thurston & Biellier 1976), and in some non-domestic species (starlings, red-billed quelea and puffin) (Barker & Kendal 1984). However, the microvasculature of the epididymis and deferent duct of the domestic fowl has been described in detail by Nakai, Hashimoto, Kitagawa, Kon & Kudo (1988).The rete testis of the domestic fowl displays a sparsely arranged network of blood vessels, whereas in the efferent ductules, peritubular capillary networks encircle the individual tubules in a cylindrical fashion. This arangment of the capillary network is maintained along the excurrent duct system. The sub-epithelial blood capillaries of the proximal and distal efferent ducts as well as the connecting ducts are fenestrated (Nakai *et al.* 1988).

Although the gross pattern of the arterial supply (Elias, Aire & Soley 2007) and venous drainage (Soley, Elias & Aire 2007; Elias, Aire & Soley 2008) of the male reproductive tract of the ostrich has been established, only limited, incidental information is available on the microvascularization of its testis and epididymis (Soley1992,1997; Soley & Groenewald 1999). This chapter reports on the distribution and ultrastructural features of the microvasculature of the testis and epididymis in the ostrich, using India ink injection and transmission electron microscopy (TEM), as investigative tools.

General structural features of the testis and epididymis

Comprehensive descriptions of the morphology of the testis (Soley 1990, 1992, 1997; Soley & Groenewald 1999) and epididymis (Budras & Meier 1981; Soley 1992; Soley & Els 1992; Aire & Soley 2000, 2003; Ozegbe, Aire & Soley 2006a, b) of the ostrich have been presented. A brief overview based on the available literature and on observations from the present study is presented to provide proper perspective on the distribution of blood vessels in the testis and epididymis.

The testis

The testis is enclosed by a thick capsule with a rich vascular supply seen macroscopically on the surface. The well-developed *Tunica albuginea* is covered by a serosa. A number of fine septa run from the capsule between the seminiferous tubules and unite with the peritubular tissue in the interstitium. Occasionally, large septa are seen although the testis lacks lobulation (Soley 1992, 1997). The interstitial connective tissue supports the seminiferous tubules and accommodates some blood and lymphatic vessels, as well as groups of Leydig cells (Soley 1990, 1992).

The epididymis

The epididymis comprises the rete testis, the proximal and distal efferent ducts, connecting ducts and epididymal duct (Budras & Meier 1981; Soley&Els1992, Aire & Soley 2003; Ozegbe *et al*. 2006a).

The *rete testis* consists of a small intratesticular component as well as intracapsular and extratesticular components that are lined by columnar (intratesticular) to cuboidal (extratesticular) epithelium. The epithelial cells possess short microvilli projecting from the apical surface of the cells (Aire & Soley 2000; Aire & Soley 2003), and an intricate interdigitation of adjacent lateral cell membranes is apparent. The cell contains many intermediate filaments in the vicinity of the basally positioned nucleus and close to the basal cell membrane, as well as a large heterogeneous lipid body in the supranuclear region of the cell (Aire & Soley 2003). The rete testis links the seminiferous tubules with the proximal efferent ducts (Budras & Meier 1981; Aire & Soley 2000; Aire & Soley 2003; Ozegbe *et al.* 2006a) and is confined to the lateral and dorsal aspects of the epididymis (Soley 1992).

The rete testis is succeeded by the efferent ducts, connecting and epididymal ducts. The proximal efferent ducts are lined by a simple high cuboidal or low columnar epithelium with a majority of non-ciliated cells (70%). These cells display a basally positioned round nucleus and numerous supranuclear electron-dense bodies. The distal efferent duct exhibits a simple cuboidal to columnar epithelium, with a predominance of ciliated cells devoid of

subapical dense bodies (Ozegbe *et al.* 2006a). The distal efferent ducts empty into the connecting ducts (Budras & Meier 1981; Aire & Soley 2003; Ozegbe *et al*. 2006a).

The connecting ducts display a pseudo-stratified columnar epithelium and are sparsely distributed throughout transverse sections of the epididymis (Ozegbe *et al.* 2006a). They have greater diameter and epithelial height than the rete testis and efferent ducts and reveal mucosal folds (Soley 1992; Aire & Soley 2000) which are longitudinal in orientation (Aire & Soley 2000). In most cells a single cilium projects into the ductal lumen together with numerous short, regular microvilli (Aire & Soley 2000).

The wide epididymal duct is convoluted and displays occasional large epithelial folds. It lies on the dorso-medial border of the epididymis and is continued distally as the deferent duct. The duct is lined by a pseudo-stratified, non-ciliated columnar epithelium, The luminal surface of the non-ciliated cells is adorned with short, regular microvilli. The supra-nuclear region of the cells displays a single large heterogeneous lipid droplet and a Golgi complex. Moderately abundant mitochondria, smooth endoplasmic reticulum, sparsely granulated endoplasmic reticulum, smooth walled vesicles and dense vesicles occur in the sub-nuclear region (Ozegbe, Aire & Soley 2006b). In light microscopy, the duct reveals mucosal folds (Soley 1990; 1992; Aire & Soley 2000; Ozegbe *et al.* 2006b).

The tubular profiles of the rete testis, proximal and distal efferent ducts, connecting ducts and epididymal duct are widely separated by dense connective tissue (Soley 1992; Aire & Soley 2000), unlike in other birds (Aire 1982, 2007).

Although the gross pattern of the arterial supply (Elias, Aire & Soley 2007) and venous drainage (Soley, Elias & Aire 2007; Elias, Aire & Soley 2008) of the male reproductive tract of the ostrich has been established, only limited, incidental information is available on the microvascularization of its testis and epididymis (Soley 1992,1997; Soley & Groenewald 1999). This chapter reports on the distribution and ultrastructural features of the microvasculature of the testis and epididymis in the ostrich, using India ink injection and transmission electron microscopy (TEM), as investigative tools.

2. MATERIALS AND METHODS

The torsos of 18 sexually mature and active male ostriches with their viscera intact, but which had been skinned and from which the limbs had been removed, were obtained from the Oryx abattoir in Krugersdorp, Gauteng and from the Klein Karoo abattoir in Oudtshoorn, Western Cape Province, South Africa. The vascular system of the reproductive tract of six ostriches was flushed free of blood by injecting physiological saline through the descending aorta. The aorta was carefully cut open to expose the origin of the cranial renal artery. This vessel was cannulated using a curved 18 gauge needle through which India ink was injected into the arterial system of the testis and epididymis. A similar technique was employed for injecting ink into the venous system in six other torsos, in this instance, using the testicular veins as the route of entry.

The testis and its adjoining epididymis were freed from the torsos in both groups of birds, selected areas were removed, trimmed into small blocks and immersion-fixed in 10% buffered formalin for a minimum period of 48 hours. The tissue blocks were conventionally processed for light microscopy, and histological sections were stained with Haematoxylin and Eosin (H & E). The distribution of the arteries and veins was studied and described using an Olympus BH-2 microscope and appropriate information was digitally recorded with a Nikon Coolpix 4500 digital camera. The diameter of the vessels was determined with the aid of a calibrated stage micrometer (GraticulesPyser-SGI Ltd., UK).

The remaining six torsos were prepared as follows for transmission electron microscopy (TEM). The vascular system of the reproductive tract was flushed free of blood, as described above, and the testis and epididymis were then perfused via the aorta with 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). Immediately following perfusion, small blocks of tissue were removed from the testes and epididymides and immersed in fresh fixative. The specimens were stored at 4ºC, prior to processing for TEM.

The tissue blocks were rinsed in 0.1M cacodylate buffer for 30 minutes and then post-fixed in 1% osmium tetroxide in the same buffer, for 1 hour. The blocks were subsequently

rinsed in the cacodylate buffer for 2 hours, dehydrated through a graded series of ethanol and substituted with propylene oxide (2 changes of 15 minutes each). Thereafter the specimens were infiltrated with a 2:1 propylene oxide:epoxy resin mixture for 1 hour and a 1:2 propylene oxide:epoxy resin mixture for 1-2 hours followed by embedding in 100% epoxy resin (Epon 815) overnight at 60ºC. Ultra-thin sections were cut, stained conventionally with uranyl acetate and lead citrate and viewed and photographed in a Philips CM10 transmission electron microscope, operated at 80 kV.

Tissue blocks of testicular and epididymal material immersion-fixed for a previous study (Soley 1992), from five ostriches, were also utilized for the ultrastructural description of the microvasculature.

3. RESULTS

3.1. Distribution of blood vessels

The distribution of arteries and veins could be readily visualized in histological sections of the testis and epididymis because of the presence of India ink in the lumen of the vessels.
The identification and naming of the vessels (capillaries, arterioles, arteries, venules and collecting veins) was based on the dimensional criteria set out by Rhodin (1974).

3.1.1. Blood vessels of the testis

In the testis, arterial vessels (capillaries and arterioles) were sparsely distributed in the *Tunica albuginea* of the testicular capsule. These vessels often showed a longitudinal orientation and appeared to be more concentrated towards the surface of the capsule (Figs. 4.1-2). Arterial vessels were more common in the interstitial tissue, especially at the interstices between 3 or more seminiferous tubules (Fig. 4.1) and larger septa carried arterioles. In some instances individual seminiferous tubules were almost entirely surrounded by arterial vessels. The interstitial arterial vessels demonstrated great variability in size, ranging from blood capillaries

to arterioles (up to 58µm in diameter). Some interstices demonstrated a greater concentration of blood vessels than others. In contrast, the venous network consisting of both venules and collecting veins (ranging up to 71.8µm in diameter) appeared to be concentrated in the testicular capsule. In addition to the well-developed network of large veins observed macroscopically within the testicular capsule (see Chapter 3), numerous variably-sized veins (including larger collecting veins) were observed in the *Tunica albuginea* (Fig. 4.3). These vessels were also longitudinally positioned. Only a few randomly distributed and variably-sized venous vessels were observed in the interstitial tissue (Fig. 4.3).

3.1.2. Blood vessels of the epididymis

The parenchyma of the epididymis contained relatively few arterial and venous vessels. When present, none of these vessels appeared to be specifically oriented in respect of any of the tubular elements (rete testis, proximal and distal efferent ducts, connecting ducts and epididymal ducts) of this part of the reproductive tract. However, occasionally, a few arterial vessels (ranging from 8 to 69µm in diameter) were specifically associated with the efferent ducts and rete testis (Figs. 4.8). Arterial vessels, venules and collecting veins (Fig. 4.9) were encountered in the connective tissue capsule surrounding the epididymis. As in the testis, the veins were larger (up to 93 µm in diameter) and more numerous than the arteries (Fig. 4.9).

3.2. Vascular ultrastructure

3.2.1. Blood Vessels of the testis

In contrast to the light microscopic observations, numerous blood vessels were observed sandwiched between adjacent seminiferous tubules and at the interstices when viewed by electron microscopy. Blood capillaries and larger vessels displayed similar morphological characteristics and it was difficult to distinguish between arterioles and venules as the endothelium of all vessels appeared to be surrounded by myofibroblasts and acellular elements of the interstitium (Figs. 4.4A, 4.5). Occasional lymphatic capillaries were seen in the interstitium (Fig. 4.4B). The endothelial cells rested on a prominent basal lamina (Fig.

4.6A). Very few pericytes (defined by their inclusion within the basal lamina of the blood vessel) were identified, although cells resembling pericytes were intimately associated with the endothelium of blood vessels, albeit at a discrete distance from the endothelial basal lamina (Figs. 4.6B, 4.7). The wall of capillaries in the testis interstitium was composed of a thin endothelial lining resting on a basal lamina. In most instances the endothelial cells were attenuated and formed complex interdigitations with adjacent cells by means of slender cytoplasmic processes. Numerous similar processes also extended into the lumen of the blood vessels (Figs. 4.6A,B, 4.7). Occasionally, the endothelium showed overlapping, with a number of junctional complexes being present (Fig. 4.6B). The cytoplasm of the attenuated portions of the endothelial cells was moderately electron-dense and homogeneous in nature, with only a few organelles being observed (Fig.4.6B, 4.7). However, organelles were concentrated in widened parts of the cells, particularly in the vicinity of the cell nucleus (Fig. 4.6A). The most numerous organelles were free and poly-ribosomes, short strands of rough endoplasmic reticulum, oval mitochondria, a small Golgi apparatus, lysosome-like structures and centrosomes. Very few pinocytotic vesicles were observed, and fenestrations were not seen (Figs. 4.6A,B, 4.7).

3.2.2. Blood vessels of the epididymis

The stroma of the epididymis displayed numerous blood capillaries, some arterioles and venules as well as some collecting veins. In the epididymal stroma, the blood capillaries and other vessels were randomly located in relation to the tubular elements (rete testis, efferent ducts, connecting duct and epididymal duct) of this region.

The blood capillaries varied in appearance. Some profiles revealed an attenuated endothelium (Fig. 4.11A-C), some were thick-walled (Fig.4. 10A,B), while others displayed a combination of both. Cytoplasmic organelles and inclusions such as dense bodies, micropinocytotic vesicles and mitochondria were observed in the wider parts of the endothelium. In some capillary profiles the endothelium appeared continuous (Fig. 4.10A, B) while in others, numerous fenestrations (Fig. 4.11A-D, 4.12A-B) were observed, particularly in capillaries that were close to the proximal efferent ducts. These capillaries

displayed an attenuated endothelium and were situated close (3.5 to 5µm) to the epithelial lining of the proximal efferent ducts. Numerous cytoplasmic processes projected from the endothelium into the lumen and large micropinocytotic vesicles (Fig.4.10A,B) were present in some profiles. Adjacent endothelial cells were connected by slender cytoplasmic processes displaying *Zonulae adherentes* (Fig. 4.10A). Blood capillaries were surrounded by a prominent basal lamina and, occasionally, by pericytes and fibroblasts.

Occasional lymphatic capillaries with an attenuated endothelium were present in the epididymal stroma, either adjacent to blood capillaries (Figs. 4.13A,B) or isolated within the stroma (Fig. 4.14A). In the thickened segments of isolated lymphatic capillaries several micropinocytotic vesicles were aggregated, presenting a foamy appearance (Figs. 4.14A, C). Attenuated regions of the isolated lymphatic capillary endothelium, in the epididymalstroma, occasionally, displayed fenestrations fitted with a membranous diaphragm (Figs. 4.14A,B).The endothelial cells of lymphatic capillaries exhibited cytoplasmic processes projecting into the lumen of the vessels (Fig. 4.13B).

4. DISCUSSION

Light microscopy revealed that in addition to the large veins found in the testicular capsule of the ostrich (Elias *et al.* 2008, see chapter 3), blood capillaries, arterioles, venules and veins of small calibre were present throughout the capsule, confirming the earlier reports by Soley (1992, 1997) that numerous arteries and veins were present, particularly beneath the peritoneal lining of the testis. In the domestic fowl, venous drainage of the testis is through a system of vessels that travel on the surface of the gonads (Nishida 1964; Lake 1981), a fact confirmed in the ostrich by the presence of India ink in these vessels, following ink injection via the testicular veins. The venous nature of the network of blood vessels observed in the avian testis, including the ostrich, poses the question as to whether it is of functional significance. The fact that birds do not have a pampiniform plexus (Lake 1981) has led to the suggestion that the close association between the avian testis and the abdominal air sacs may be instrumental in cooling the intra-abdominal testes (Cowles 1965). However,

contrasting evidence has been provided indicating that the air sacs do not cool the testes (Herin, Booth & Johnson 1960) in the rooster, and that the germinal epithelium has been adapted to function at a high body temperature (Béaupre, Tressler, Béaupre, Morgan, Bottje & Kirby 1997). Mezquita, Mezquita & Mezquita (1988) report a biochemical compound produced in the domestic fowl, that genetically enables spermatogenesis to occur at high body temperature.

The presence of blood vessels in the interstitial and peritubular tissue of the ostrich testis is a typical feature of avian species and has been described, for example, in the rooster (Lake 1957), drake, guinea fowl and Japanese quail (Aire 1997). Interstitial vessels have also been identified in the ostrich (Soley1992, Soley & Groenewald 1999, Aire 2007) although no information regarding their structure or distribution was provided. The observation that arterial vessels appeared to predominate in the interstitium, whereas venous vessels were concentrated in the testicular capsule, has not been specifically noted in previous studies. Based on the distribution of blood vessels it is established that the pattern of vascularization of the ostrich testis is of the *testicular type* (adjacent seminiferous tubules share blood capillaries) as described by Suzuki (1982) in the mouse. Characterization of the vascular pattern had previously not been described for any avian species.

Transmission electron microscopy (TEM) indicated that the interstitial blood vessels of the ostrich testis are of the continuous type (they lack fenestrations). The lack of fenestrations and the paucity of pinocytotic vesicles observed in the blood capillary endothelium would seem to suggest that an active physiological exchange between the interstitial blood vessels and the seminiferous tubules is not as pronounced as that reported in the excurrent duct system (with the exception of the rete testis) of birds (Rothwell & Tingari 1973). The absence of fenestrations does not necessarily indicate that the interstitial vessels are incapable of interstitial fluid uptake. The strategic positioning of these vessels close to and between adjacent seminiferous tubules as well as the attenuated nature of the endothelium would compensate for the lack of fenestrations in these vessels. In addition, the presence of lymphatic capillaries in the interstitium of the ostrich testis would function as pathways to carry interstitial fluid to the venous system as has been described in the Peking drake (*Anas*

platyrhynchos, L) (Kremer & Budras 1987) and in mammals (Dransfield 1945; Fawcett, Heidger & Leak 1969; Perez-Clavier, Harrison & Macmillan 1982).

The observation that the parenchyma of the ostrich epididymis displayed relatively few arterial and venous vessels is supported by the light microscopical study of the rooster epididymis by Tingari (1971) who described it as a “poorly vascularized structure”. In striking contrast, Nakai *et al.* (1988) describe the rooster epididymis as richly vascularized, and reports on the presence of an elaborate network of blood vessels arranged around the tubular elements of this region, with the exception of the rete testis. The few vascular structures seen by light microscopy in the ostrich epididymis displayed no specific orientation with respect to the tubular elements. However, TEM revealed numerous scattered blood capillaries, some of which were closely associated with the epithelial lining of the excurrent duct system, in particular the proximal and distal efferent ducts. The close association between blood capillaries and the lining of the efferent ducts was also noted by Nakai *et al.* (1988) in the rooster. The conflicting information on the relative density of the capillary networks reported in the study of Nakai *et el.* (1988) and Tingari (1971) in the rooster and the present observations in the ostrich may stem from the more graphic results obtained by the vascular cast technique additionally employed by Nakai *et al*.(1988).

The sparse distribution of blood vessels seen in the peritubular connective tissue of the rete testis of the ostrich is similar to that reported for the starling (*Sturnus vulgaris*), red-billed quelea (*Quelea quelea*), puffin (*Fratercula arctica*) (Barker & Kendal 1984) and rooster (Nakai *et al.* 1988), but contrasts markedly with the observations made in the rooster, Japanese quail, guinea-fowl and duck by Aire (1982).The equally sparse distribution of blood capillaries around the proximal and distal efferent ducts, connecting ducts and epididymal ducts in the ostrich also contrasts with the situation in the rooster which possesses a very well developed dense network of blood capillaries encircling all the elements of the excurrent duct system, with the exception of the rete testis (Nakai *et al.* 1988). As noted previously, the discrepancies regarding the relative number of blood vessels surrounding elements of the epididymis may reflect differences in technique rather than species-specific peculiarities.

The ultrastructural features of blood capillaries in the epididymis of the ostrich are similar to those reported in the rooster (Nakai *et al.* 1988). Although Nakai (1988) did not specifically report the presence of mitochondria and dense bodies points of similarity included a prominent basal lamina, few cytoplasmic processes, an attenuated endothelium, fenestrations (in the sub-endothelial capillaries of the efferent ducts, connecting ducts and epididymal ducts), micropinocytotic vesicles and the presence of pericytes (Nakai *et al.* 1988). It is noteworthy that the blood capillaries in the periductal tissue of the connecting duct and epididymal duct of the ostrich did not display any endothelial fenestrations. In mammals (mice) the epididymis presents fenestrations only in the initial segment (head) whereas the blood capillaries in other segments of the epididymis lack fenestrations (Suzuki 1982; Abe, Takano & Ito 1984). This is because the initial segment of the mammalian epididymis absorbs most of the fluid carried into the epididymis (Suzuki 1982, Suzuki & Nagano 1986).

The fenestrated capillaries located beneath the proximal efferent duct epithelium, together with the particular structure of this duct [presence of enlarged intercellular spaces, well developed sub-apical endocytic apparatus of apical tubules, endocytic vacuoles, coated pits, coated tubules and dense bodies in the non-ciliated cells (Aire & Soley 2000; Ozegbe *et al*. 2006a)] suggest the participation of both the epithelium and sub-epithelial blood capillaries in fluid absorption from the ductal lumen. Thus, the spermatozoal content of the duct becomes more concentrated in the connecting and epididymal ducts. This is in consonance with the situation in the rooster (Tingari 1971), turkey, domestic fowl, Japanese quail and guinea fowl (Aire & Josling 2000). The fluid present in the lumen of the efferent ducts is absorbed by epithelial cells, then carried into the blood capillaries, facilitated by the fenestrations in the endothelium, as in the initial segment of the epididymis of the mouse (Suzuki 1982; Abe et al.1984). Whereas in the ostrich fenestrated capillaries were restricted to vessels associated with the proximal efferent ducts, fenestrated capillaries reportedly surround all the tubular elements of the epididymis in the rooster with the exception of the rete testis (Nakai *et al.* 1988).

Lymphatic capillaries present in the ostrich epididymis, by virtue of their structural characteristics, apparently also participate in fluid absorption. In the mouse, the lymphatic networks are scarce in the initial segment but abundant in the cauda epididymidis (Hirai, Naito, Terayama, Ning, Miura, Shirakami & Itoh 2010).

In conclusion, the features described and discussed above, in the ostrich, are generally similar to those described for the rooster. However, the ostrich displays the following differential features:

(i) Arterial vessels were more common in the interstitial tissue, especially at the interstices between 3 or more seminiferous tubules. In contrast, the venous network appeared to be concentrated in the testicular capsule.

(ii) The epididymal stroma possesses blood capillaries and venules that lack a specific arrangement.

(iii) The capillary lymphatics in the epididymis, close to efferent ducts, occasionally present endothelial fenestrations fitted with a membranous diaphragm.

5. REFERENCES

ABE, K., TAKANO, H. & ITO, T. 1984. Microvasculature of the mouse epididymis, with special reference to fenestrated capillaries localized in the initial segment. *The Anatomical Record,* 209: 209-218.

AIRE, T. A. 1980. The ductuli efferentes of the epididymal region of birds. *Journal of Anatomy,* 130: 707-723.

AIRE, T. A. 1982. The rete testis of birds. *Journal of Anatomy,* 135: 97-110.

AIRE, T. A. 1997. The structure of the interstitial tissue of the active and resting avian testis. *Onderstepoort Journal of Veterinary Research,* 64: 291-299.

AIRE, T. A. 2007. Anatomy of the testis and male reproductive tract, in *Reproductive Biology and Phylogeny of Birds.* Edited by B. G. M. Jamieson. Jersey: Science Publishers. pp. 37-113.

AIRE, T. A. & JOSLING, D. 2000.Ultrastructural study of the luminal surface of the ducts of the epididymis of gallinaceous birds. *Onderstepoort Journal of Veterinary Research,* 67: 191-199.

AIRE, T. A. & SOLEY, J. T. 2000. The surface of the epithelial lining of the ducts of the epididymis of the ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 29: 119-126.

AIRE, T. A. & SOLEY, J. T. 2003. The morphological features of the rete testis of the ostrich (*Struthio camelus*). *Anatomy& Embryology,* 207: 355-361.

BARKER, S. G. E. & KENDAL, M. D. 1984. A study of rete testis epithelium in several wild birds. *Journal of Anatomy*, 138: 139-152.

BÉAUPRE, C.E., TRESSLER, C. J., BÉAUPRE, S. J., MORGAN, J. L. M., BOTTJE, W. C. & KIRBY, J. D. 1997. Determination of testis temperature rhythms and effects of constant light on testicular function in the domestic fowl (*Gallus domesticus*). *Biology of Reproduction*, 56: 1570-1575.

BUDRAS, K. D. & SAUER, T. 1975. Morphology of the epididymis of the cock (*Gallus domesticus*) and its effect upon the steroid sex hormone synthesis. I. Ontogenesis, morphology and distribution of the epididymis. *Anatomy& Embryology,* 148: 175-196.

BUDRAS, K. D. & MEIER, U. 1981. The epididymis and its development in ratite birds (ostrich, emu, rhea). *Anatomy& Embryology,* 162: 281-299.

COWLES, R. B. 1965. Hyperthermia, Aspermia, Mutation Rates and Evolution. *The Quarterly Review of Biology*, 40: 341-367.

DRANSFIELD, J. W. 1945. The lymphatic system of the domestic fowl. *The Veterinary Journal,* 101: 171-179.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2007. Macroscopic features of arterial supply to the reproductive system of the male ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 36: 255-262.

ELIAS, M. Z. J., AIRE. T. A & SOLEY, J. T. 2008. Macroscopic features of the venous drainage of the reproductive system of the male ostrich (*Struthio camelus*). *Onderstepoort Journal of Veterinary Research,* 75: 289-298.

FAWCETT, D., HEIDGER, P. M. & LEAK, L. V. 1969.Lymph vascular system of the interstitial tissue of the testis as revealed by electron microscopy. *Journal of Reproduction and Fertility.*19: 109-119.

HERIN, R. A., BOOTH, N. A. & JOHNSON. R. M. 1960. Thermoregulatory effects of abdominal air sacs on spermatogenesis in domestic fowl. *American Journal of Physiology*, 198: 1343-1345.

HESS, R. A., THURSTON, R. J. & BIELLIER, H. V. 1976.Morphology of the epididymal region and ductus deferens of the turkey (*Meleagris gallopavo*). *Journal of Anatomy*, 122: 241-244.

HIRAI, S., M., NAITO, M., TERAYAMA, H., NING, Q., MIURA, M., SHIRAKAMI, G. & ITOH, M. 2010. Difference in abundance of blood and lymphatic capillaries in the murine epididymis. *Medical Molecular Morphology*, 43: 37-42.

KORMANO, M. & SUORANTA, H. 1970.Microvascular organization of adult human testis. *The Anatomical Record*. 170: 31-40.

KREMER, A. & BUDRAS, K. D. 1987.Lymphsystem und lymph drainage imhoden des Pekingerpels (*Anas platyrhynchos, L*). *Anatomia, Histologia, Embryologia,* 17: 264-257.

LADMAN, A. J. & YOUNG, W. C. 1958.An electron microscopic study of the *ductuli efferentes* and *rete testis* of the guinea pig. *Journal of Biophysics, Biochemistry and Cytology*, 4: 219-226.

LAKE, P. E. 1957. The male reproductive tract of the fowl. *Journal of Anatomy,* 91: 116-129.

LAKE, P. E. 1981. Male genital organs, in *Form and Function in Birds.* Edited *by* A. S. King & J. McLelland. London: Academic Press. pp. 1-61.

MARKEY, C. M. & MEYER, G. T. 1992. A quantitative description of the epididymis and its microvasculature: an age-related study in the rat. *Journal of Anatomy*, 180: 255-262.

MEZQUITA, B., MEZQUITA, C. & MEZQUITA, J. 1998. Marked differences between avian and mammalian testicular cells in the heat shock induction and polyadenylation of Hsp70 and ubiquitin transcripts. *FEBS Letters*, 436: 377-386.

NAKAI, M., HASHIMOTO. Y., KITAGAWA, H, KON, Y, & KUDO, N. 1988.Microvasculature of the epididymis and *ductus deferens* of domestic fowls. *Japanese Journal of Veterinary Science,* 50: 371-381.

NISHIDA, T. 1964. Comparative and topographical anatomy of the fowl. XLII. Blood vascular system of the male reproductive organs. *Japanese Journal of Veterinary Science,* 26: 211-221.

OZEGBE, P., AIRE, T. A. & SOLEY, J. T. 2006a. The morphology of the efferent ducts of the testis of the ostrich, a primitive bird.*Anatomia, Embryologia & Histologia,* 211: 559-565.

OZEGBE, P., AIRE, T. A. & SOLEY, J. T. 2006b. The epididymal duct unit of the ostrich (*Struthio camelus*). *Proceedings of the 36th Annual Conference of the Anatomical Society of Southern Africa,* Golden Gate, South Africa: 74.

PÉREZ-CLAVIER, R., HARRISON, R.G. & MACMILLAN, E. W. 1982.The pattern of the lymphatic drainage of the rat epididymis. *Journal of Anatomy,* 134: 667-675.

RHODIN, J. A.G. 1974. Cardiovascular system, in *Histology. A text book and atlas.* New York: Oxford University Press. pp. 331-370.

ROTHWELL, B. & TINGARI, M. D. 1973.The ultrastructure of the boundary tissue of the seminiferous tubule in the testis of domestic fowl (*Gallus domesticus*). *Journal of Anatomy,* 114: 321-328.

SOLEY, J.T. 1990.Ultrastructural features of the boundary tissue of the seminiferous tubule of the ostrich (*Struthio camelus*). *South African Journal of Science,* 86: 163

SOLEY, J.T. 1992. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). PhD thesis. University of Pretoria.

SOLEY, J. T. 1997. The morphology of the testicular capsule of the ostrich (*Struthio camelus*). *Proceedings of the Microscopy Society of Southern Africa, 27:109.*

SOLEY, J. T. & GROENEWALD, H. B. 1999. Reproduction, in *TheOstrich. Biology, Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp. 29-158.

SOLEY, J. T. & ELS, H. J. 1992.The morphology of the proximal region of the *ductuli efferentes* in the ostrich testis. *Proceedings of the Microscopy Society of Southern Africa,* 22: 139-140.

SOLEY, J. T., ELIAS, M. Z. J. & AIRE, T. A. 2007. Variations in the gross pattern of venous drainage of the ostrich male reproductive tract: A comparison with the general avian model. *Proceedings of Ist Conjoint International Conference on Fertility, Anatomy and Morphological Sciences.* Lagos. Nigeria: 35-36.

SUZUKI, F. 1982. Microvasculature of the mouse testis and excurrent duct system. *American Journal of Anatomy,* 163: 309-325.

SUZUKI, F. & NAGANO, T. 1986. Microvasculature of the human testis and excurrent duct system. *Cell and Tissue Research,* 243: 79-89.

TINGARI, M. D. 1971. The fine structure of basal cells in the male reproductive tract of the domestic fowl. *Journal of Anatomy,* 110: 167-169.

6. FIGURES

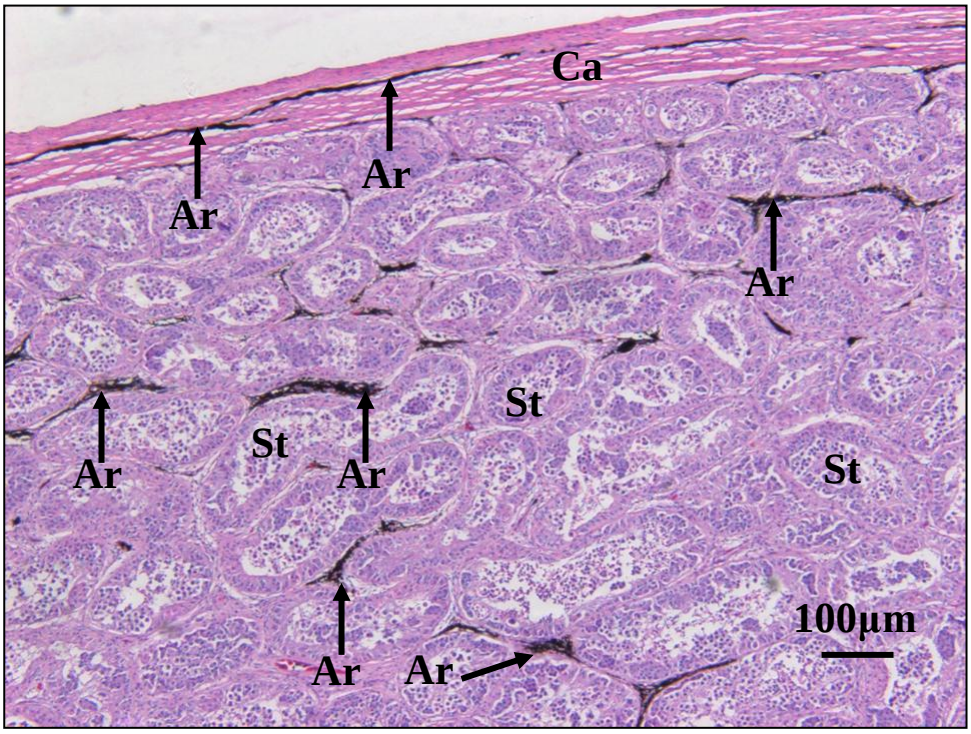

Figure 4.1. Light micrograph of the testis demonstrating the distribution of India Ink-filled arterial vessels (Ar) in the testicular capsule (Ca) and interstitial tissue between the seminiferous tubules (St).

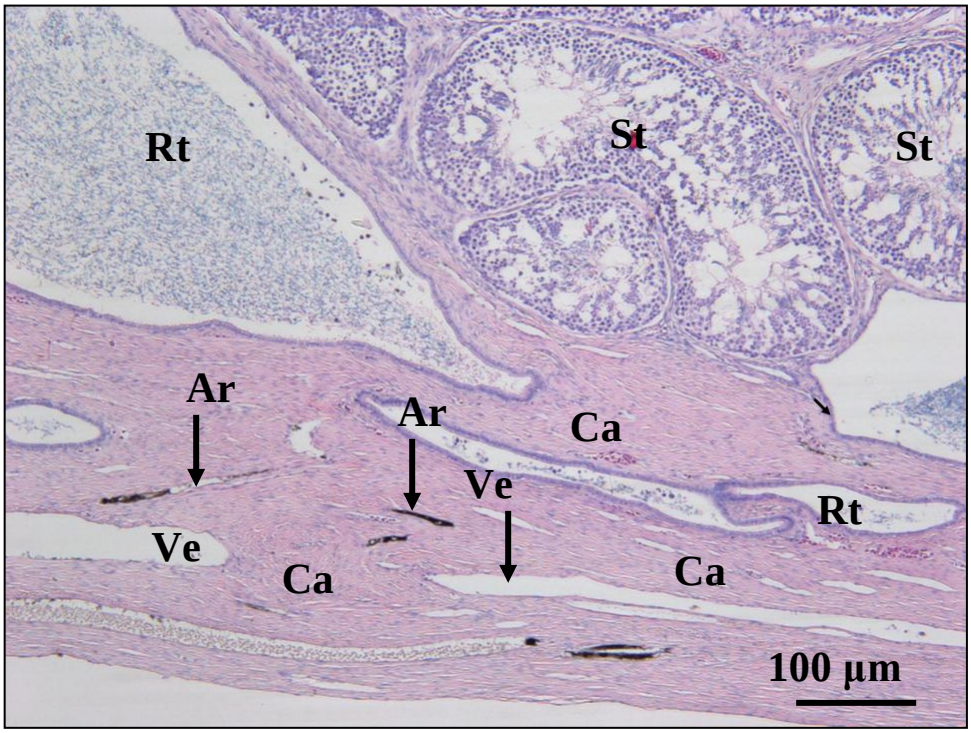

Figure 4.2. Light micrograph of the testicular capsule (Ca) illustrating arterial vessels (Ar) filled with India ink and empty venous vessels (Ve). Intracapsular elements of the rete testis (Rt) are visible. Seminiferous tubules (St) are visible.

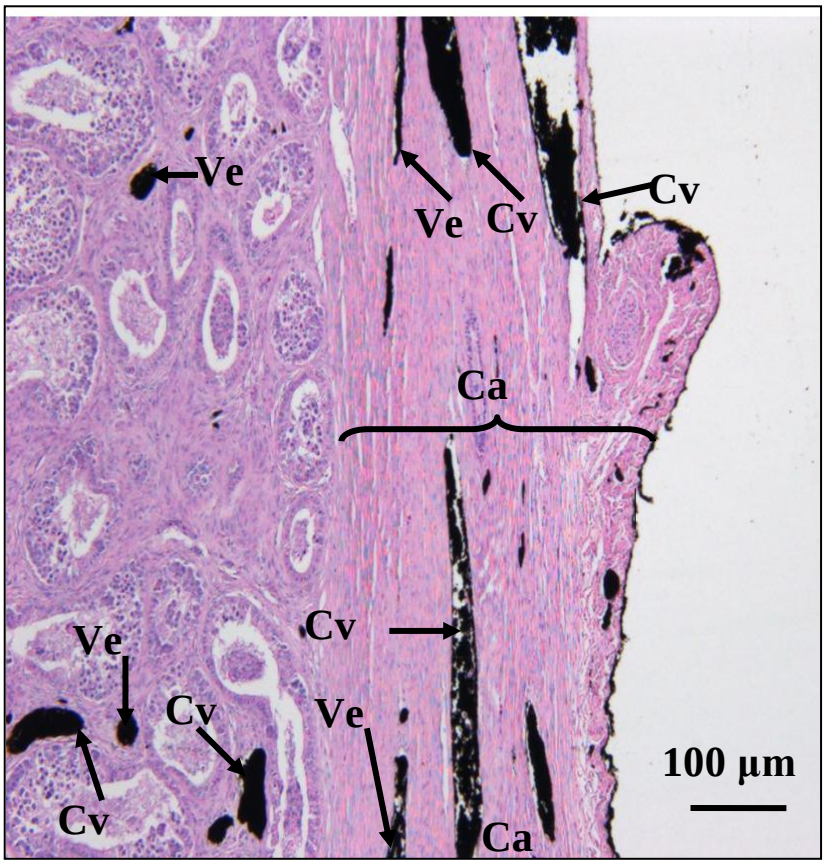

Figure 4.3. Light micrograph of the testis showing venules (Ve), as well as prominent collecting veins (Cv), filled with India ink in the testicular capsule (Ca) and in the interstitium of the testicular parenchyma.

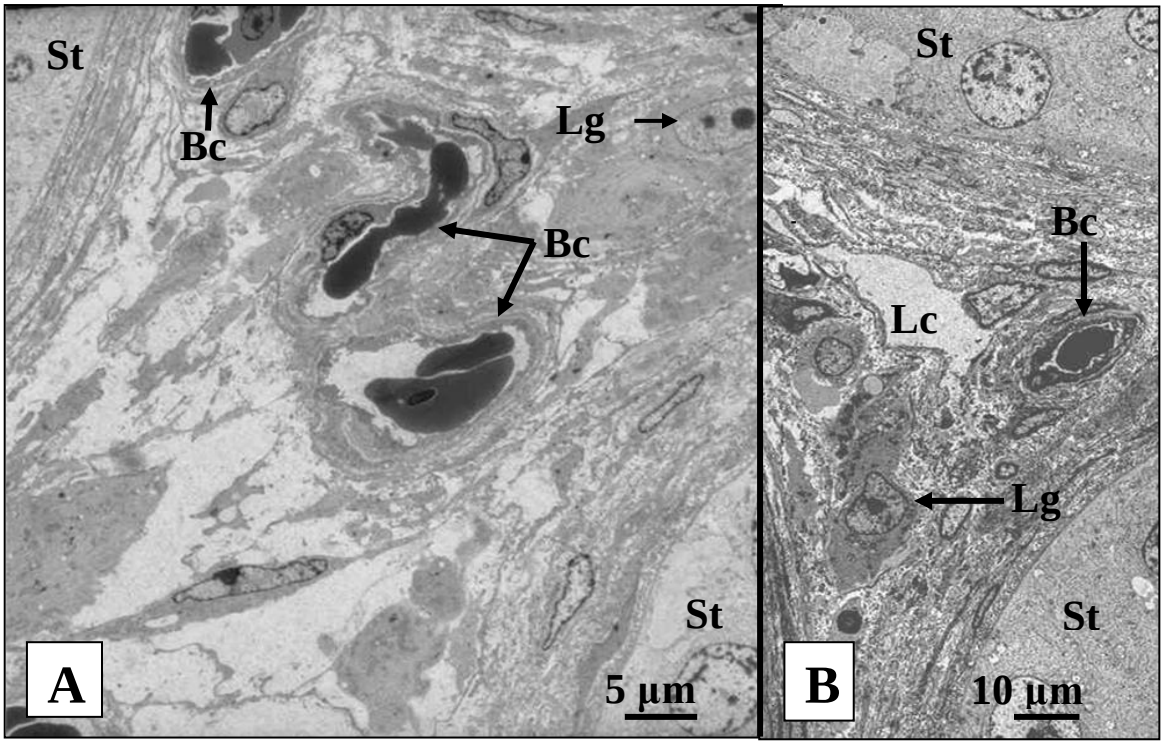

Figure 4.4. A & B. Electron micrographs of the testis interstitium displaying blood capillaries (Bc), Leydig cells (Lg) and a lymphatic capillary (Lc) in the angular spaces between adjacent seminiferous tubules (St).

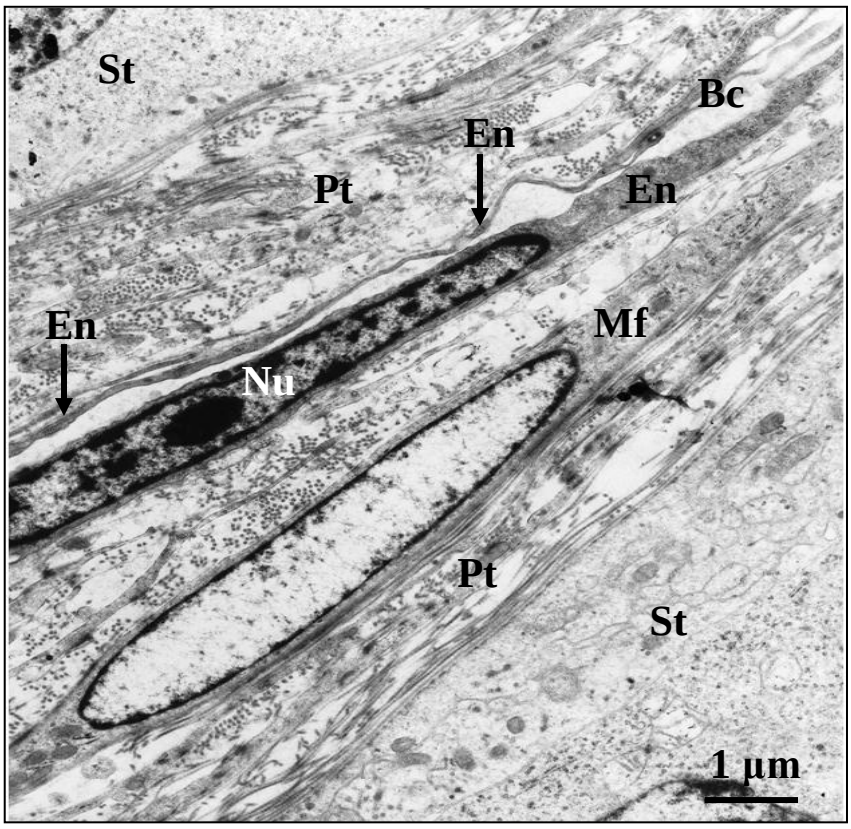

Figure 4.5. View of the peritubular tissue (Pt) between adjacent seminiferous tubules (St) showing a blood capillary (Bc), positioned midway between two seminiferous tubules. The blood vessel is longitudinally sectioned, and displays a prominent nucleus (Nu) and an attenuated endothelium (En), except at the pole of the nucleus. Myofibroblast (Mf).

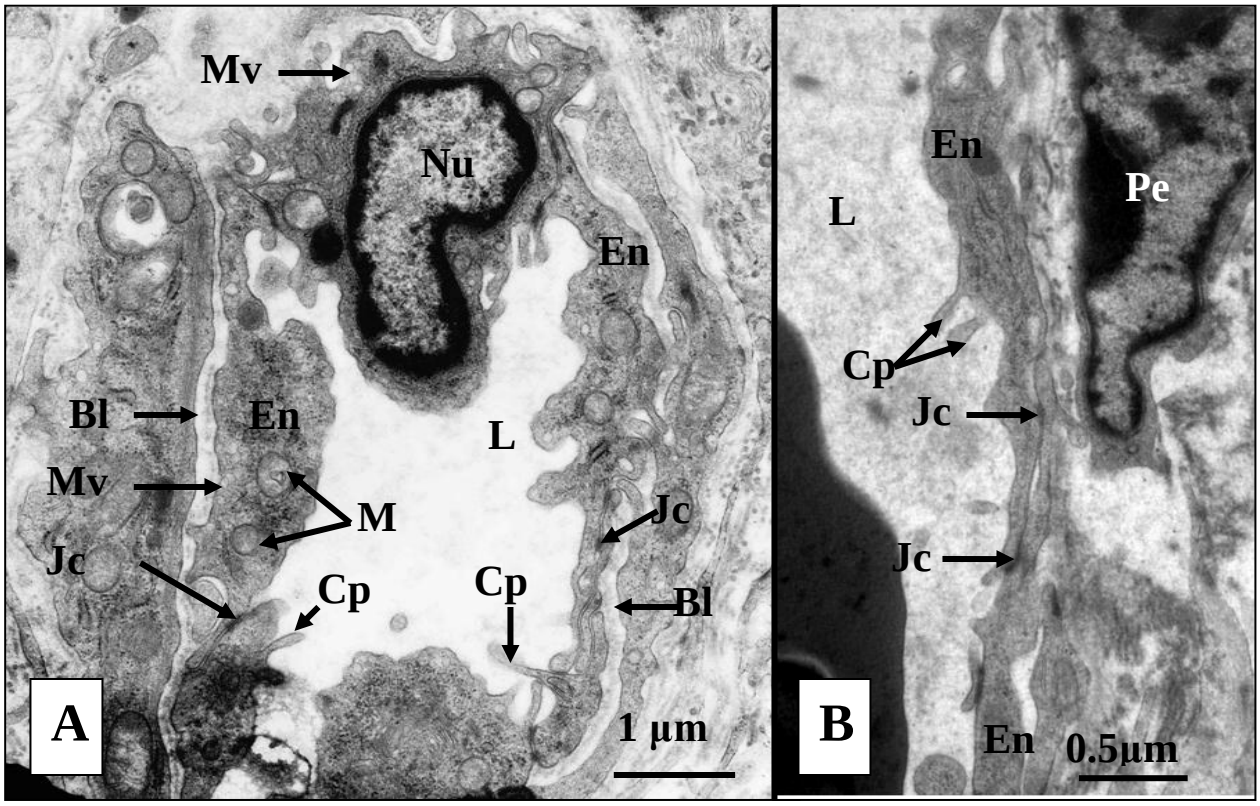

Figure 4.6. A. A blood capillary in the testis interstitium displaying a generally thickened endothelium (En) resting on a prominent basal lamina (Bl). Note the cytoplasmic processes (Cp) extending into the lumen (L), micropinocytotic vesicles (Mv), nucleus (Nu), junctional complexes (Jc) and mitochondria (M). **B**. A blood capillary showing extensive overlapping of the endothelium (En). Note the junctional complexes (Jc), cytoplasmic processes (Cp), the capillary lumen (L) and a presumptive pericyte (Pe).

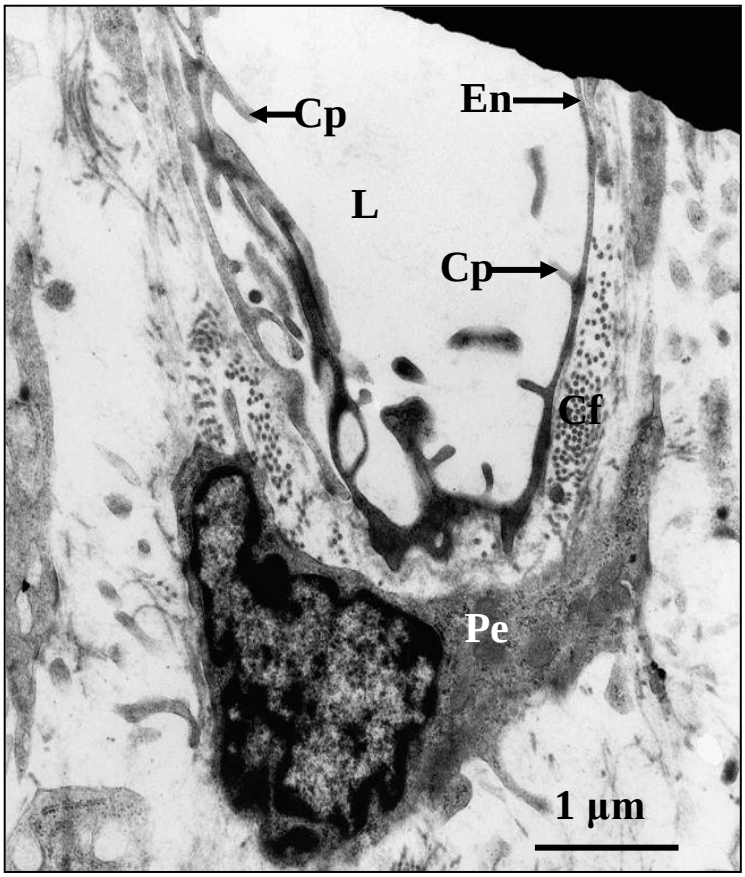

Figure 4.7. A blood capillary in the testis interstitium with attenuated endothelium (En) displaying cytoplasmic processes (Cp). A presumptive pericyte (Pe) is intimately associated with the capillary although separated from it by a layer of connective tissue, including bundles of collagen fibres (Cf). Capillary lumen (L).

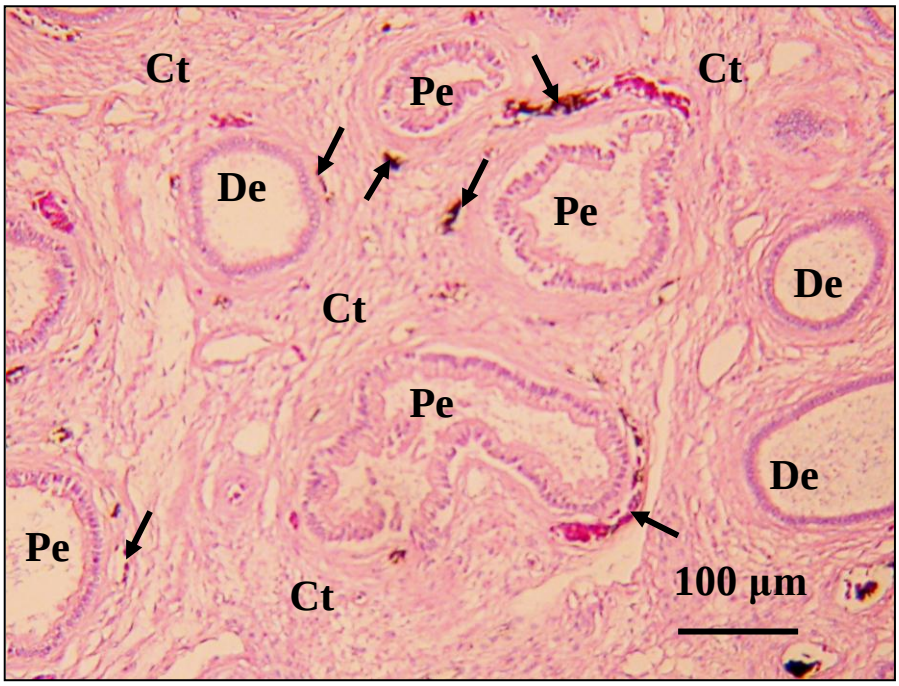

Figure 4.8. Light micrograph of part of the epididymis showing arterial vessels (arrows) filled with India Ink, proximal efferent ducts (Pe), distal efferent duct (De) and connective tissue (Ct).

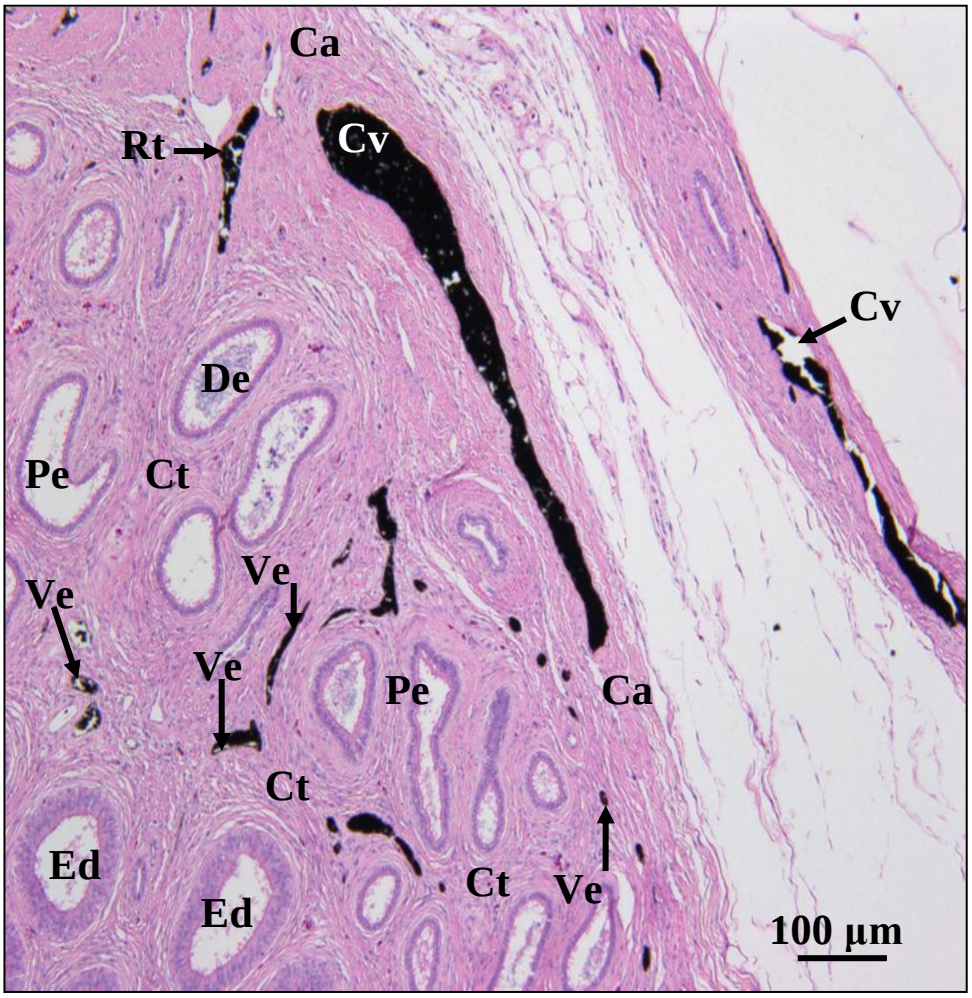

Figure 4.9. Light micrograph of the capsule (Ca) and adjacent tissue of the epididymis showing venules (Ve) and collecting veins (Cv) filled with India Ink. Note the arbitrary positioning of the blood vessels between the proximal efferent ducts (Pe), distal efferent ducts (De) and epididymal ducts (Ed). Periductal connective tissue (Ct).

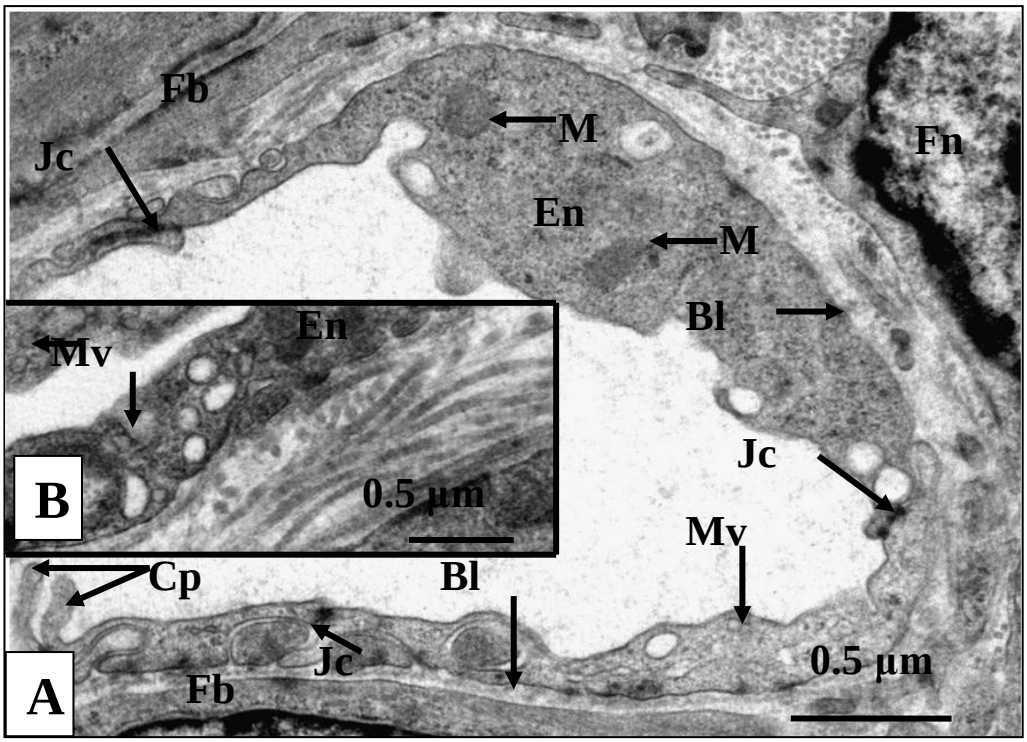

Figure 4.10. A and **B**. Transmission electron micrographs of vascular capillaries in the epididymal region. The blood capillary shows a continuous endothelium (En) which rests on a prominent basal lamina (Bl), and displays micro-pinocytotic vesicles (Mv), mitochondria (M), junctional complexes (Jc) and cytoplasmic processes (Cp). Fibro-blasts (Fb), fibroblast nucleus (Fn).

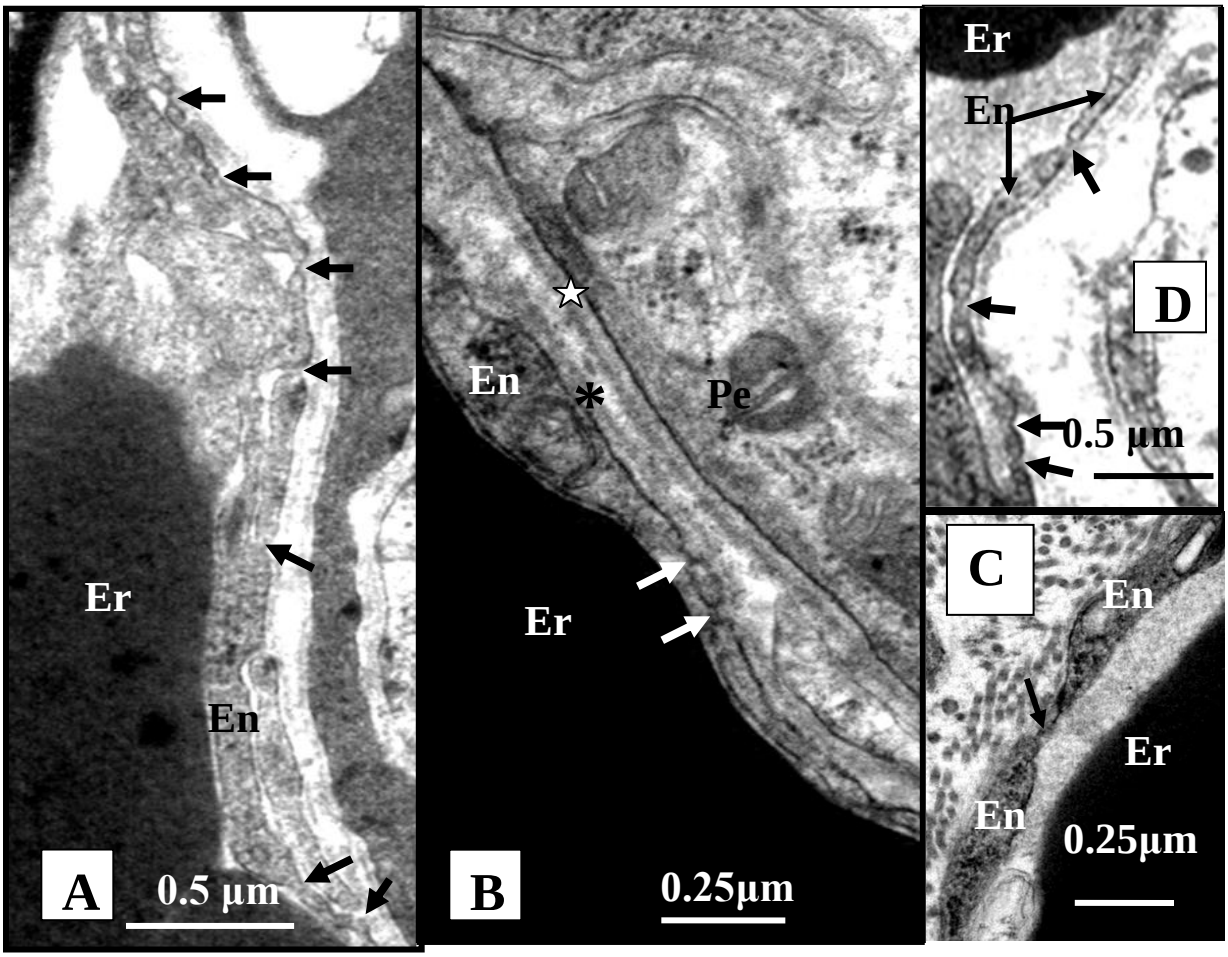

Figure 4.11. A, B, C and D. Transmission electron micrographs of vascular capillaries beneath the proximal efferent duct in the epididymis. Note the fenestrations (arrows) in the endothelium (En) and the presence of a membranous diaphragm spanning the fenestration. In Fig. B, note the basal lamina of both the endothelium (*) and the epithelial lining (white star) of the proximal efferent duct (Pe). Erythrocyte (Er).

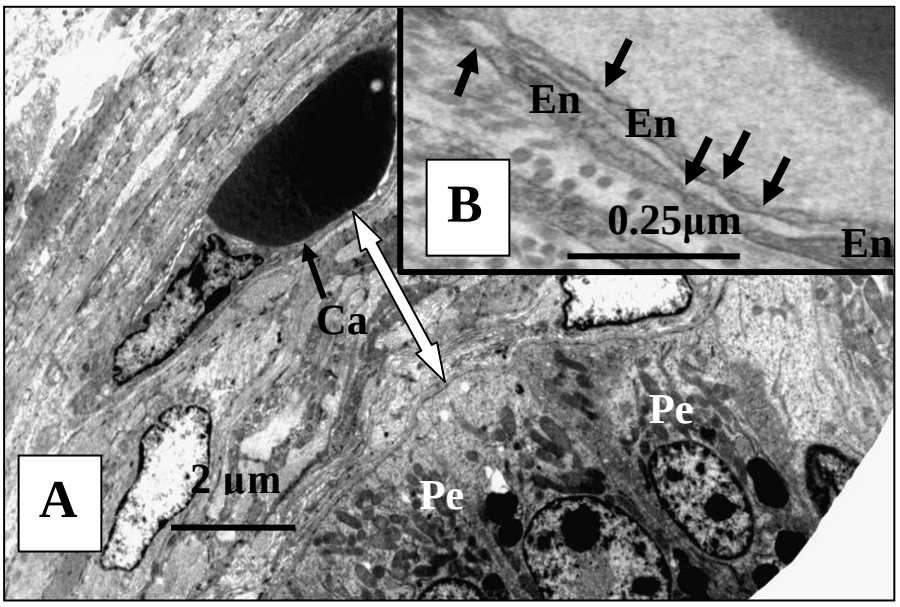

Figure 4.12. A. Electron micrograph of a blood capillary (Ca) in close proximity to a proximal efferent duct (Pe). Note the short distance between the wall of the blood vessel and the base of the duct epithelium (double headed arrow) **B**. Higher magnification of the capillary endothelium (En) showing an area of over-lapping endothelial cells (En) with fenestrations (thick arrows).

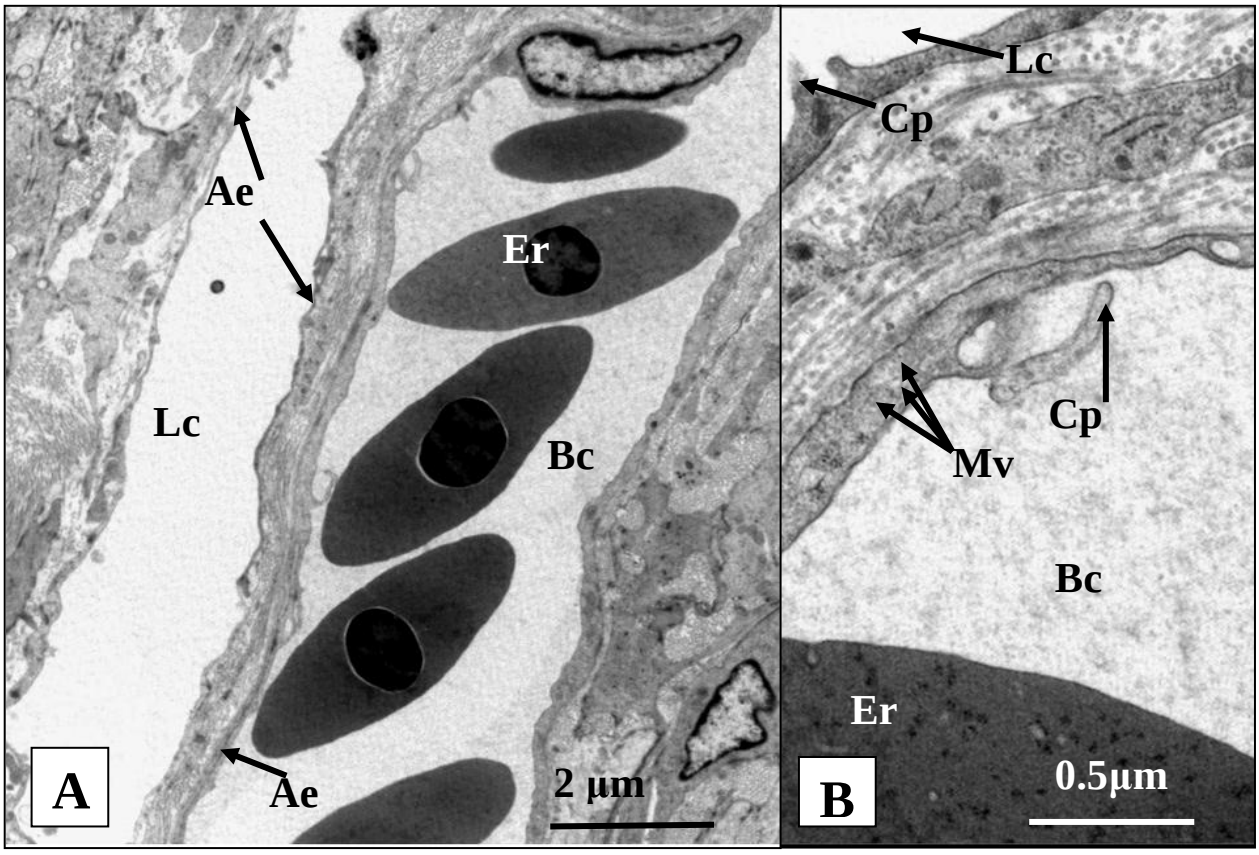

Figure 4.13. A and **B**. Transmission electron micrographs of a blood capillary (Bc) and an adjacent lymphatic capillary (Lc) in the epididymis at low (**A**) and high (**B**) magnifications. Both vessels demonstrate a continuous attenuated endothelium (Ae) and small cytoplasmic processes (Cp). The vascular capillary contains erythrocytes (Er) and the endothelium displays some micropinocytotic vesicles (Mv).

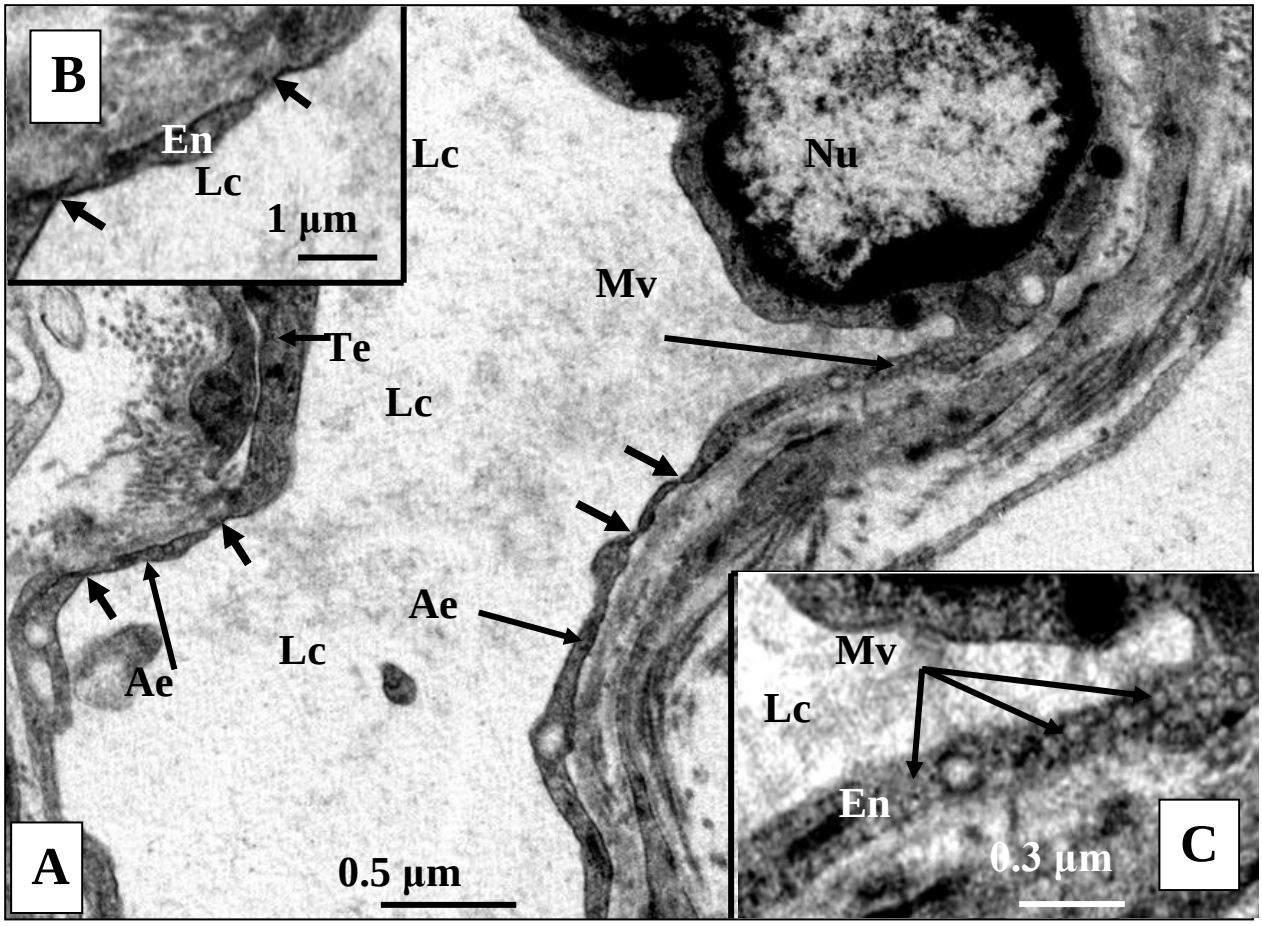

Figure 4.14. A, B and **C**. Transmission electron micrographs of an isolated lymphatic capillary (Lc) in the stroma of the epididymis showing attenuated (Ae) and thicker (Te) regions of the endothelium (En). The attenuated, but not thicker, regions display fenestrations (thick arrows) – see inset (B). Micropinocytotic vesicles (Mv) are visible, with some regions demonstrating a foamy cytoplasm (inset C).

CHAPTER 5

THE DISTRIBUTION AND STRUCTURE OF THE MICROVASCULATURE OF THE *DUCTUS DEFERENS, RECEPTACULUM DUCTUS DEFERENTIS* AND PHALLUS

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1. INTRODUCTION

The *Ductus deferens* is a direct continuation of the epididymal duct. It extends caudally and increases in diameter cranio-caudaly in sexually active birds (Lake 1981). Close to the cloaca, the *Ductus deferens* straightens out to form the *Pars recta ductus deferentis* that subsequently enlarges to form the *Receptaculum ductus deferentis.* The latter opens into the cloaca (*urodeum*), as the *Papilla ductus deferentis* (Marvan 1969 quoted by Lake 1981; Lake 1971).

The distribution and characterisation of the microvasculature of the *Ductus deferens* has received some attention in the domestic fowl (Nakai, Hashimoto, Kitagawa, Kon & Kudo 1988; Hess, Thurston & Biellier 1976). Nakai *et al*. (1988) reported the presence of a dense network of blood capillaries beneath the epithelium of the *Ductus deferens* of the rooster, with the subepithelial capillaries in the cranial segment being fenestrated. In the caudal segment of the deferent duct an additional peritubular network of sparsely arranged blood vessels was observed to be located in the smooth muscular layer (Nakai *et al*. 1988). Hess *et al*. (1976) described the presence of numerous blood capillaries close to the epithelial basement membrane of the deferent duct in the turkey whereas Lake (1957) reported the existence of tortuous subepithelial arterioles and venules in the fibrous connective tissue of the submucosa of the deferent duct papilla of the fowl and turkey. Additionally, Hess *et al*. (1976) described blood sinuses in the lamina propria of the deferent duct papilla in the fowl.

The gross morphological structure of the ostrich *Ductus deferens* (Duerden 1912; Soley & Groenewald 1999), *Receptaculum ductus deferentis, Papilla ductus deferentis* and *phallus* (Muller 1838 quoted by King 1981; Duerden 1912; King 1981; Fowler 1991) has been described. As in other birds, the *Ductus deferens* of the ostrich is a caudal continuation of the epididymal duct of the epididymis. It runs caudally in a wavy manner in sexually mature birds (Duerden 1912; Soley 1992; Soley & Groenewald 1999). The caudal part ends as a straight duct, the *Pars recta ductus deferentis* which opens into the barrel-shapped *Receptaculum ductus deferentis.* The latter projects into the urodeum of the cloaca as the papilla of the deferent duct, and represents the last portion of the deferent duct (Soley & Groenewald 1999). This part of the deferent duct has also been referred to as the ejaculatory duct in certain avian species, for example, the domestic fowl (Lake, 1957). The *Ductus deferens* and *Receptaculum ductus deferentis* are considered to function in the transportation of spermatozoa (Lake 1957; Clulow & Jones 1982, 1988) and in the storage of sperm available for ejaculation (Lake 1957; Clulow & Jones 1982, 1988). The role played by the luminal epithelium in the cranial segment of deferent duct in the domestic fowl in fluid absorption, has also been reported (Nakai *et al.* 1988).

Although the gross pattern of both the arterial supply and venous drainage of the male reproductive tract of the ostrich has been reported (Elias, Aire & Soley 2007; Soley, Elias &Aire 2007; Elias, Aire & Soley 2008) (see also Chapters 2 and 3), no information is currently available on the microvascularization of the above segments of the reproductive tract.

According to Aire (2007), the phallus is formed from closely related folds of tissue that derive from the ventral wall of the proctodeum in male birds. It is analogous to the mammalian penis, because it transfers ejaculated spermatozoa to the female reproductive organs. There are two types of phallus, the intromittent type that penetrates the female cloaca and non-intromittent type where penetration does not occur. The ostrich phallus is of the intromittent type. The gross anatomy of the ostrich phallus has been described by a number of authors (Duerden 1912; Berens von Rautenfeld 1977; King 1981; Fowler 1991), although very little histological information is available. The phallus is essentially composed of

two fibroelastic bodies, a phallic suclus, an elastic vascular body and one pair of muscles (King 1981).

Attention has been focussed on the mechanism of erection of the avian phallus which is achieved by the flow of lymph into the erectile tissue of the organ. In some birds, for example the drake and rooster, the source of lymph is reported to be the paracloacal vascular body (*Corpus vasculare paracloacale*) (King 1981). This body is located in the wall of the cloaca, is also reportedly present in several other avian species, and has been variably named by different authors. It has been referred to as the *Corpus vasculare paracloacale* in the turkey (Knight 1970), paracloacal vascular body in the guinea fowl (Sasaki, Nishida, Fujimura & Mochizuki1984), *Corpus paracloacalis vascularis* in the domestic fowl (Kudo, Sugimura&Yamano1975; Sugimura, Kudo & Yamano 1975; Yamano, Sugimura& Kudo 1977) and vascular body in the same species (Guanawardana & Scott 1978). It is referred to in Nomina Anatomica Avium (King 1993) as the *Corpus vasculare phalli*, *Glomera corporis vascularis phalli* and *Lymphobulbus phalli*, the latter term being used by Berens von Rautenfeld (1977) to describe the structure found in the ostrich.

Although King (1981) notes that no structure equivalent to the paracloacal vascular body has been identified in the ostrich, Berens von Rautenfeld (1977) has described a well-developed lymphatic system in this bird responsible for erection. It is reported to consist of a large lymphobulbus phallus positioned on either side of the seminal groove which is linked to the somatic lymphatic system. However, both King (1981) and Montgomerie & Briskie (2007) note that the lymphatic mechanism in the ostrich requires further investigation, particularly using modern techniques.

This chapter describes the distribution and structure of the microvasculature in the *Ductus deferens, Receptaculum ductus deferentis* and phallus of the ostrich, using India ink injection (light microscopy) and transmission electron microscopy (TEM).

2. MATERIALS AND METHODS

The materials and methods used for the preparation of the *Ductus deferens, Receptaculum ductus deferentis* and phallus were essentially the same as for the testis and epididymis as set out in Chapter 4. Additionally, the descending aorta was cannulated and India ink injected into the arterial system of 6 birds. In another 6 birds 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) was injected into the arterial system. For the study of the venous system, another six birds were injected with India ink through the caudal vena cava.

Samples for light microscopy of both the arterial and venous distribution of vessels were taken from the proximal segment of the deferent duct (collected 5 to 10cm caudal to the epididymis), from the distal segment (harvested approximately 5cm cranial to the receptaculum) and from the receptaculum. For the phallus, samples were taken from the root, the walls of the phallic sulcus and from the elastic vascular body, which is located in the ventral part of the body of the phallus. Samples from all the above regions were taken from the six glutaraldehyde-perfused reproductive tracts and additionally immersion fixed in fresh fixative prior to further processing. The preparation of samples for both light microscopy and transmission electron microscopy was the same as detailed in Chapter 4.

3. RESULTS

3.1. General morphological features

A brief overview of the histological structure of the *Ductus deferens* and phallus, based on observations from the present study, is presented to provide perspective on the distribution of blood vessels in this part of the reproductive tract.

The entire *Ductus deferens*is lined by a pseudo-stratified columnar epithelium resting on a basal lamina and supported by a prominent *Lamina propria*. The bulk of the duct wall consists

of a fibromuscular coat which is thicker in the distal segment. The proximal segment of the *Ductus deferens* (that part immediately caudal to the epididymis, and extending to the middle of the deferent duct) displays mucosal folds (Fig. 5.1). Although some folds are observed in the distal deferent duct (that part extending from the middle of the deferent duct to the receptacle of the duct), they are less prominent than those in the proximal region. Both segments of the *Ductus deferens* display a dense mass of spermatozoa uniformly distributed within the lumen (Figs. 5.1- 4).

The *Receptaculum ductus deferentis* revealed the same basic structure as the deferent duct and was also was lined by a pseudo-stratified columnar epithelium (Fig. 5.9).The epithelium of the receptaculum displayed mucosal folds with intervening crypts. The lumen of the receptaculum also contained a mass of spermatozoa (Fig. 5.9).The terminal part of the deferent duct , the papilla, protruded through the dorso-lateral aspect of the urodeum of the cloacal chamber (Fig. 5.11).The external surface of the papilla displayed folds (Figs. 5.12B, C) which were lined by a pseudo-stratified columnar epithelium. The lumen of the papilla was filled with a mass of spermatozoa. Both the inner and outer epithelial surfaces were supported by connective tissue.

The phallus was lined externally by a stratified squamous epithelium, and its root was surrounded ventro-laterally by a sponge-like tissue consisting of numerous lymph spaces traversed by connective tissue septa or struts (Figs. 5.16-17). The phallic sulcus was dorsally situated and originated close to the papilla of the deferent duct, extending to the tip of the organ. The walls of the sulcus were composed of erectile tissue and were lined externally by stratified squamous epithelium.

3.2. Structure and distribution of blood vessels

Blood vessels (blood capillaries, arterioles, arteries, venules and collecting veins) are named according to the dimensional criteria proposed by Rhodin (1974) (see Chapter 4).

3.2.1. Blood vessels of the *Ductus deferens*

a. Distribution

The entire *Ductus deferens* displayed the following basic vascular pattern: A network of blood capillaries surrounded the duct, lying just beneath the ductal epithelium (Figs. 5.1, 5.3). Some capillaries approached as close as 2µm to the base of the epithelium while individual capillaries were almost in contact with the epithelium (Fig. 5.6). The underlying *Lamina propria* extended for approximately 15µm from the layer of sub-epithelial capillaries and was separated from the outer fibromuscular coat by a network of arterioles (Fig. 5.1). The fibro-muscular coat exhibited an inner more compact layer and a looser outer layer consisting of tissue bundles. A sparse collection of capillaries and arterioles appeared in the fibro-muscular coat, particularly between the tissue bundles (Fig. 5.2). Larger arterioles were situated superficially beneath the serosa (Fig. 5.2). A limited number of venules were also obvious at the boundary between the lamina propria and the fibro-muscular layer (Fig. 5.4). Collecting veins were widespread within the fibro-muscular coat and a few were superficially situated in the subserosa (Fig. 5.4).

b. Ultrastructural features

Transmission electron microscopy (TEM) confirmed that both segments of the deferent duct displayed a single, well-developed capillary network immediately beneath the duct epithelium (Fig. 5.5). The blood capillaries revealed a thick, continuous endothelium resting on a prominent basal lamina. The endothelial cells interdigitated in a complex fashion with neighbouring cells and were attached by adhering junctions. The cytoplasm of the endothelial cells was moderately electron-dense and homogeneous, displaying only a few organelles and inclusions. These included strands of rough endoplasmic reticulum (RER), small to medium-sized electron-dense bodies that resembled lysosomes, occasional mitochondria, and a few pinocytotic vesicles. The endothelial cells, however, lacked fenestrations. A limited number of cytoplasmic projections jutted into the lumen of the vessels. Despite the fact that the tissue had been perfused, erythrocytes occupied the lumen in some of the vessels (Fig. 5.6).

Arterioles in the peritubular tissue of both segments of the *Ductus deferens* displayed a thick continuous endothelium which extended a few cytoplasmic processes into the vascular lumen. Overlapping of adjacent endothelial cell processes accompanied by adhering junctions was also observed. The cytoplasm contained limited numbers of the organelles described above, as well as pinocytotic vesicles and ocassional round or oval-shaped dense bodies. The endothelium lacked fenestrations, and rested on a thin basal lamina (Figs. 5.7A, B).

The collecting veins in both segments of the duct displayed a continuous thin endothelium (Fig. 5.8A) that rested on a basal lamina. The most striking cytoplasmic feature of the endothelial cells was the presence of numerous dense bodies of varying shape (Fig. 5.8B). Several pinocytotic vesicles and a few mitochondria were also present (Figs. 5.8B, C).The endothelial cells overlapped extensively and displayed typical adhering junctions (Fig. 5.8C). Apical cytoplasmic processes could be seen jutting into the lumen of the vessel. There were no endothelial fenestrations (Figs. 5.8B, C). The endothelium of the venules was continuous, generally thick, and showed extensive overlapping with adhering junctions coupling adjacent cells. The endothelial cytoplasm contained the standard organelles and a few pinocytotic vesicles. Cytoplasmic processes could be seen jutting into the lumen.

3. 2. 2. Blood vessels of the *Receptaculum ductus deferentis*

a. Distribution

The distribution of blood vessels in the *Receptaculum ductus deferentis was* similar to that observed in the distal deferent duct, and again characterised by the appearance of a single capillary network beneath the epithelium (Fig. 5.9). A network of arterioles lay between the lamina propria and the fibromuscular coat, sparse capillaries and arterioles were scattered throughout the fibromuscular coat, and larger calibre arterioles were situated superficially beneath the serosa. The distribution of the venules and collecting veins also displayed a similar pattern to that observed in both segments of deferent duct with collecting veins again appearing widespread throughout the fibromuscular coat.

b. Ultrastructural features

The endothelium of the capillaries located immediately beneath the lining of the *Receptaculum ductus deferentis* vessels was continuous (i.e. there were no fenestrations), and in most instances appeared relatively thick, although attenuated regions were occasionally apparent. A degree of overlapping of adjacent endothelial cells was observed, with adhering junctions providing linkage between the cells. Occasionally, the basal lamina protruded into cup-shaped depressions in the endothelial cells (Figs. 5.10A,B). The cytoplasmic features were similar to those seen in the *Ductus deferens*. A degree of sequestration of material from the lumen of the vessels was apparent, but not as pronounced as in the spongy tissue of the phallus (see below).

It was difficult to distinguish venules from capillaries, based solely on their structure. Both vessels displayed a single layer of endothelial cells lying on a basal lamina. The wider size of the venules and the presence of occasional peripheral fibroblasts differentiated them from blood capillaries. The collecting veins displayed a continuous non-fenestrated endothelium lying on a basal lamina. The endothelial cells overlapped and displayed adhering junctions, and cytoplasmic processes jutted into the lumen of the blood vessel. The cytoplasm of the endothelium contained some dense bodies (although these were not as numerous or prominent as in the deferent duct) and pinocytotic vesicles.

3. 2. 3. Blood vessels of the *Papilla ductus deferentis*

a. Distribution

The papilla of the deferent duct displays both external and inner surfaces. The inner surface was covered by a simple columnar epithelium but its outer epithelial lining (facing the cloaca) was simple columnar to pseudo-stratified (Figs. 5.12A, B) in nature, depending on the plane of section. Scant, but clearly visible, sub-epithelial blood capillaries lay 2 to 10µm beneath the epithelium. Very few arterioles were observed in the papilla (Fig. 5.12A, B), and they were predominantly located closer to the outer (cloacal) epithelium. Similarly, a paucity of venules and collecting veins was also observed. To what extent this

phenomenon reflected poor penetration of the India ink into this segment of the reproductive tract, could not be determined.

b. Ultrastructural features

The endothelium of the capillaries was thick, continuous and lacked fenestrations (Fig. 5.13). Adjacent endothelial cells revealed a degree of overlapping accompanied by a few adhering junctions. The normal complement of cytoplasmic organelles was observed together with a limited number pinocytotic vesicles. A few cytoplasmic processes jutted into the lumen. Arterioles displayed essentially the same structural features as the capillaries.

Both the venules (Fig. 5.14A) and collecting veins (Figs. 5.15A, B, C) in the papilla of the *Ductus deferens* displayed a continuous attenuated endothelium devoid of fenestrations. Adjacent endothelial cells occasionally overlapped and displayed adhering junctions. The cytoplasm revealed few organelles, scant cytoplasmic processes (Fig. 5.15A), a limited number of dense bodies (Fig. 5.15B) and ubiquitous pinocytotic vesicles (Fig. 5.14B).

3. 2. 4. Blood vessels of the phallus

a. Distribution

The root of the phallus was surrounded ventro-laterally by a sponge-like structure consisting of numerous large lymph spaces traversed by connective tissue septa or struts. The connective tissue struts were richly supplied with capillaries, relatively large arterial vessels (Fig. 5.16) as well as abundant venules and large collecting veins (Fig. 5.17A, B).

The connective tissue septa were randomly arranged and varied in size and shape, but all were characterised by an extensive network of blood vessels as indicated above. The variably sized intervening lymphatic spaces communicated with each other forming an extensive lymphatic reservoir. The sponge-like tissue was supplied by branches of the pudendal artery and drained by branches of the pudendal vein (see Chapters II and III).

The walls of the phallic sulcus were composed of closely packed, regularly spaced mucosal folds covered by a stratified squamous epithelium. The surface epithelium was supported by a relatively wide layer of connective tissue characterised by an extensive network of small lymph spaces. This well-developed layer of erectile tissue displayed a zone of smaller blood vessels, mainly capillaries, lying beneath the epithelium, whereas larger, randomly distributed arterioles and a few sparsely distributed venules were scattered throughout (Fig. 5.18). When compared to the spongy tissue at the root of the phallus, the erectile tissue lining the phallic sulcus presented relatively few capillaries and venules. Numerous lymphocytes were also a feature of this tissue. The lymph spaces communicated with larger spaces situated in a deeper zone of connective tissue. This zone resembled some of the features of the spongy tissue described above, although the septa were more substantial, forming a greater proportion of the tissue. Numerous capillaries, arterioles and some larger arterial vessels were prominent within the septa. Prominent accumulations of colloid-like substance were obvious in the larger lymph spaces indicating the presence of lymph (Fig. 5.18).

The elastic vascular body located in the ventral part of the body of the phallus, exhibited a few arterial vessels (arterioles and capillaries) that were sparsely distributed in the elastic connective tissue. The elastic vascular body was continuous with the fibrous body which was composed of collagen fibres and poorly vascularised. However, it did demonstrate an erectile potential by virtue of a low incidence of lymphatic channels. . The fibrous body did not exhibit blood vessels and contained collagen fibres, a few lymphatic channels (some of which contained lymph) and scant erectile tissue.

b. Ultrastructural features

The capillaries located in the connective tissue struts forming the spongy tissue at the base of the phallus (Figs. 5.19B, C) displayed both wide and attenuated endothelial profiles. The most obvious morphological feature was the presence of numerous wide fenestrations beneath which the basal lamina remained continuous (Fig. 5.19C). A diaphragm sealing the fenestrations could not be convincingly demonstrated in the material studied. Numerous cytoplasmic processes extended into the lumen from the surface of the

endothelium (Fig. 5.19C). In certain areas the cytoplasmic processes appeared to enclose and sequester luminal contents (Fig. 5.19C). During this process the cytoplasmic extensions trapped material from the lumen and interiorized it into the endothelium, resulting in the formation of pale vesicle-like structures (Fig. 5.19C). The endothelium was extremely irregular in profile, and in some instances was composed of more than one layer of overlapping squamous cells joined by apical adhering junctions. In addition to the vesicles, the endothelium displayed mitochondria, lysosomes, profiles of RER and free ribosomes. Arterioles were structurally similar in appearance to the capillaries but also revealed dense bodies. Internalisation of luminal material was also observed.

Venules were generally lined by an attenuated endothelium which was broken in places by wide fenestrations (Fig. 5.19A). A continuous basal lamina was again obvious. The endothelial cells displayed blunt luminal projections and regions of cellular overlap were marked by sparse adhering junctions. In addition to the standard cytoplasmic organelles, the cells also displayed occasional dense bodies as well as pinocytotic vesicles.

The vessels present in the erectile tissue lining the phallic sulcus exhibited features similar to those seen in the spongy tissue in respect of their basic structure and organelle content. However, the endothelial cells were markedly more intricate and irregular in design, displaying numerous interdigitating cytoplasmic processes, particularly in regions of overlapping cells. The capillary endothelium displayed numerous fenestrations as well as regions where apparent sequestration of luminal contents resulted in exceptionally attenuated stretches of endothelial cytoplasm. In both instances a well-developed, continuous basal lamina was apparent (Fig. 5.20).

4. DISCUSSION

This study provides the first information on the distribution and structure of the microvasculature of the *Ductus deferens*, *Receptaculum ductus deferentis* and phallus of the ostrich. Based on the results obtained it is clear that both the cranial and caudal segments of

the deferent duct of the ostrich display a sub-epithelial capillary network similar to that described in other birds such as the turkey (Hess *et al. 1976)* and chicken (Nakai *et al.* 1988). In addition, the ostrich demonstrated two more vascular networks, one between the *Lamina propria* and the fibromuscular coat and another stretching throughout the fibromuscular coat to the submucosa. In total, only two vascular beds have been described in the chicken (Nakai *et al.* 1988).The distribution of vascular networks along the *Ductus deferens* of the ostrich also differs from that of some mammals such as mouse and human, which have up to four vascular networks encircling the deferent duct (Ohtani & Gannon 1982; Suzuki &Nagano 1986). Based on the distinction between the testicular type and deferential type of vascular architecture recognised in the testis and excurrent ducts in man and mouse (Suzuki 1982; Suzuki & Nagano 1986), it is clear (and to be expected) that the arrangement of blood capillaries in the deferent duct of the ostrich is of the deferential type as only a single duct is supplied by a particular set of vessels. A fuller description and understanding of the three-dimensional picture of the vessels would have been obtained by scanning electron microscopy (SEM) of corrosion casts as shown by Nakai *et al*. (1988). However, the technical difficulties experienced in injecting fluids into the reproductive system of the ostrich, coupled with delays in obtaining material from the abattoir, made this currently impossible.

The vascular architecture in the deferent duct is correlated with the volume of blood required to satisfy the metabolic activity or increased calibre of the tubule (Suzuki 1982). It would therefore appear that the deferent duct of birds in general acts as a sperm conduit and plays a lesser role in the modification of its contents. This argument is further advanced by the observation in the present study that no fenestrations are found in the sub-epithelial capillary network of the deferent duct although they have been reported to occur in the endothelium of sub-epithelial capillaries of the cranial segment of the deferent duct in the rooster.

In mice, fenestrated blood capillaries occurred in parts of the reproductive system, where there was a high level of secretion or fluid absorption (Abe, Takano & Ito 1984). In the quail, Clulow & Jones (1988) have shown that the efferent ducts, which are fenestrated, are responsible for 85.8% of the fluid absorption, and the connecting ducts for only 6.5%. Whereas the efferent ducts in the ostrich also demonstrate fenestrated capillaries, their

absence in the deferent duct would again suggest that the role of this part of the excurrent duct system is restricted to the storage and transport of spermatozoa and does not have an absorptive function.

The presence of dense bodies in the endothelium of venules, collecting veins and arterioles of the deferent duct, receptacle of the deferent duct and even in the papilla of deferent duct, is an uncommon feature and has not previously been described in any other avian species. Rhodin (1974) reported structures similar to the dense bodies in the venules and arterioles of skeletal muscle in man and called them “specific endothelial granules”. According to Rhodin (1974), it had been speculated that those granules may contain procoagulative substances. Why only the ostrich displays these granules in this specific part of the reproductive tract remains unexplained, although it would be interesting to determine whether this is a particular feature of ratite species.

The spongy tissue that lies ventro-lateral to the root of the phallus in the ostrich is comparable to a concentration of tissue with similar structural features that has been referred to as the paracloacal vascular body in various avian species (see Introduction above). This spongy tissue in the ostrich has been termed the “great erectile cushion” by Boas (1891; quoted by King 1981) “*lymphobulbus phallus*” by Berens von Rautenfeld (1977) and “*lymphobulbus phalli*” by King (1993) in Nomina Anatomica Avium.

The paracloacal vascular body is considered to be an accessory organ of the *phallus*. It develops at puberty and transmits lymph from arterial vessels into the phallus, during tumescence (Fujihara, Nishiyama & Nakashima 1976). According to King (1981), earlier authors suspected the presence of the paracloacal vascular body in the ostrich, but they failed to provide adequate evidence of its existence. This report therefore is the first to localize, describe the gross and histological features of this organ, as well as describe its microvasculature.

In the rooster, the paracloacal vascular body is a small, red, oval structurelocated in the wall of the urodeum, close to the receptacle of the deferent duct and is covered by a connective

tissue capsule (Kudo *et al.* 1975). When viewed by light microscopy, the paraclocal vascular body reveals a capsule, trabeculae, capillary cords and lymphatic spaces. The lymphatic spaces are divided into peripheral lymphatic spaces, under the capsule, and internal lymphatic spaces in the parenchyma (Sugimura *et al.* 1975). The lymphatic channels or spaces are present close to groups of blood capillaries (Gunawardana & Scott 1978).The trabeculae contain one or two arterioles and venules. These features were verified in the present study of the spongy tissue of the ostrich phallus, except that the connective tissue element was not obviously divided into capillary cords and trabeculae. In the ostrich, larger blood vessels were found together with capillaries Scanning electron microscopy (SEM) of corrosion casts of the paracloacal vascular body confirmed the existence of anastomosing vascular cords, between which were lymphatic spaces (Sugimura *et al.* 1975).

The basic structure of the paracloacal body, namely, lymph-filled spaces separated by vascular-rich fibrous cords as described in the fowl (Sugimura *et al.* 1975; Gunawardana & Scott 1978) has also been confirmed in the turkey (Knight 1984) and in the guinea fowl (Sasaki *et al.* 1984). Although Sugimura *et al.* (1975) and Sasaki *et al.* (1984) observed only a few fenestrations in the blood capillary endothelium of the fowl and guinea fowl respectively, Gunawardana & Scott (1978) described numerous fenestrations without closing membranes in the rooster. A similar situation was observed in the ostrich therefore supporting a previously held view (Knight 1984) that fluid leaves the capillaries by diffusion rather than by active transport. It should be conceded, however, that the large numbers of pinocytotic vesicles noted in the ostrich blood vessels certainly suggests that active transport of fluid may play a supplementary role.

The specific morphological features of the spongy tissue described in this chapter as well as the particular distribution of abundant, variably-sized capillaries and venules with fenestrations, would suggest that this structure participates in the mechanism of erection of the phallus in the ostrich, as has been demonstrated in other avian species (Lake 1957; Kudo *et al*. 1975; Fugihara *et al.* 1976; Yamano *et al.* 1977; Gunawardana & Scott 1978; Sasaki *et al.* 1984). That the spongy tissue at the root of the ostrich phallus supplies the fluids for erection is based on the presence within the spongy tissue (*lymphobulbus phalli*) of lymphatic

vessels (lymph channels), as well as connective tissue strands with arterial vessels (capillaries and arterioles) and venous vessels (venules and collecting veins) that are richly supplied with fenestrations.

This study therefore confirms the existence of a paravascular cloacal body in the ostrich, and also that the mechanism of erection of the phallus in the ostrich is lymphatic as previously reported by Berens von Rauntenfeld (1977). The use of the term "*lymphobulbus phalli*" in respect of the ostrich phallus (King 1993) is thus supported.

The erectile tissue in the sulcus of the phallus exhibits the same morphological features as that of the *Lymphobulbus phalli*, although in the latter this tissue is more abundant, more complex, and is the main source of lymph for phallic erection in the ostrich. Additionally, there was no indication that the erectile tissue in the *Sulcus phalli* communicates with the elastic vascular body that lies ventro-caudal to the shaft of the phallus. In the present study the elastic vascular body did not reveal much erectile tissue, in contrast to earlier observations that the elastic vascular body contains an inner core of erectile tissue (Müller 1836, quoted by King 1981).

In conclusion, this chapter demonstrated that the epithelial lining of the *Ductus deferens* and *Receptaculum ductus deferentis* of the ostrich were encircled by a peritubular capillary network of the deferential type. As the blood capillaries in these segments of the reproductive tract were devoid of fenestrations, it is suggested that the deferent duct and its components are not involved in active fluid absorption, as are the efferent ducts (see Chapter IV). In the *lymphobulbus phalli* and erectile tissue of the phallic sulcus, the presence of many fenestrations in the capillaries, arterioles and venules reflect the potential for rapid fluid exchange consistent with the process of erection and detumescence. Additionally, the relatively loose arrangement of overlapping endothelial cells connected by only a few adhering junctions in these vessels would also assist in the rapid drainage of lymph from the lymph spaces to the blood circulation during detumescence.

5. REFERENCES

ABE, K., TAKANO, H. & ITO, T. 1984. Microvasculature of the mouse epididymis, with special reference to fenestrated capillaries localized in the initial segment. *The Anatomical Record*, 209: 209-218.

AIRE, T. A. 2007. Anatomy of the testis and male reproductive tract, in *Reproductive Biology and Phylogeny of Birds.* Edited by B. G. M. Jamieson. Jersey: Science Publishers. pp. 37-113.

BERENS VON RAUTENFELD, D. 1977. Mitteilungen zur künstlichen Besamung, Geschlechts- und Altersbestimmung beim Strauß (*Struthio camelus australis,* GURNEY). *Der Praktische Tierarzt,* 5: 359-364.

CLULOW, J. & JONES, R. C. 1982. Production, transport, maturation, storage and survival of spermatozoa in the male Japanese quail, *Coturnix coturnix*. *Journal of Reproduction and Fertility*, 64: 259-266.

CLULOW, J. & JONES, R. C. 1988. Studies of the fluid and spermatozoal transport in the extra testicular genital ducts of the Japanese quail. *Journal of Anatomy*, 157: 1-11.

DUERDEN, J. E. 1912. Experiments with ostriches - XX. The anatomy and physiology of the ostrich. C - The Internal Organs. *South African Agricultural Journal*, 1-27.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2007. Macroscopic features of arterial supply to the reproductive system of the male ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 36: 255-262.

ELIAS, M. Z. J., AIRE. T. A & SOLEY, J. T. 2008 Macroscopic features of the venous drainage of the reproductive system of the male ostrich (*Struthio camelus*). *Onderstepoort Journal of Veterinary Research,* 75: 289-298.

FOWLER, M. E. 1991.Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22: 204-227.

FUJIHARA, N., NISHIYAMA, H. & NAKASHIMA, N. 1976. Studies on the accessory reproductive organs in the drake. 2. Macroscopic and microscopic observations on the cloaca of the drake with special reference to the ejaculatory groove. *Poultry Science,* 55: 927-935.

GUNAWARDANA, V. K. & SCOTT, M. G. A. D. 1978. On the structure of the vascular body in the domestic fowl. *Journal of Anatomy,* 127: 447-457.

HESS, R. A., THURSTON, R. J. & BIELLIER, H. V. 1976.Morphology of the epididymal region and ductus deferens of the turkey (*Meleagris gallopavo*). *Journal of Anatomy,*122: 241-252.

KING, A. S. 1981. Phallus, in *Form and function in Birds.* Edited by A. S. King & J. McLelland. London: Academic Press. pp. 107-147.

KING, A. S. 1993. Apparatus urogenitalis, in Handbook of Avian Anatomy. Handbook of Avian Anatomy. *Nomina Anatomica Avium*. Edited by J. J.Baumel, A. S. King, J. E. Breazile, H. E. Evans & J. C. V. Berge. Cambridge: Academic Press. pp. 329-397.

KNIGHT, C. E. 1970. The anatomy of structures involved in the erection-dilution mechanism in the male domestic fowl. PhD thesis. Michigan State University.

KNIGHT, C. E. 1984. Anatomy of the *corpus vasculare paracloacale* of the male turkey. *Poultry Science,* 63**:** 1883-1891.

KUDO, N., SUGIMURA, M. & YAMANO, S. 1975. Anatomical studies of corpus paracloacalis vascularis in cocks. *Japanese Journal of veterinary Research,* 23: 1-10.

LAKE, P. E. 1957. The male reproductive tract of the fowl. *Journal of Anatomy,* 91: 116-129.

LAKE, P. E. 1971. The male in reproduction, in *Physiology and Biochemistry of the domestic fowl.* III. Edited by D. J. Bell & B. M. Freeman. London: Academic Press. pp. 1411-1447.

LAKE, P. E. 1981. Male genital organs, in *Form and Function in Birds.* Edited *by* A. S. King & J. McLelland. London: Academic Press. pp. 1-61.

MONTGOMERIE, R. & BRISKIE, J. 2007.Anatomy and Evolution of Copulatory Structures, in *Reproductive Biology and Phylogeny of Birds.* Edited by B. G. M. Jamieson. Jersey: Science Publishers. pp. 115-148.

NAKAI, M., HASHIMOTO.Y., KITAGAWA, H, KON, Y, & KUDO, N. 1988.Microvasculature of the epididymis and *ductus deferens* of domestic fowl. *Japanese Journal of Veterinary Science,* 50: 371-381.

OHTAN, G. & GANNON, B. 1982. The microvasculature of rat vas deferens: scanning electron and light microscopic study. *Journal of Anatomy,* 135: 521- 529.

RHODIN, J. A.G. 1974. Cardiovascular system, in *Histology. A text book and atlas.* New York: Oxford University Press. pp. 331-370.

SASAKI, H., NISHIDA,T., FUJIMURA, H. & MOCHIZUKI, K. 1984. Vascular system of paracloacal vascular body in the Guinea Fowl (*Numida meleagris)*. *Japanese Journal of Veterinary Science,* 46**:** 425-435.

SOLEY, J.T. 1992.A histological study of spermatogenesis in the ostrich (*Struthio camelus*). PhD thesis. University of Pretoria.

SOLEY, J. T. & GROENEWALD, H. B. 1999. Reproduction, in *The Ostrich. Biology,Production and Health.* Edited by D. C. Deeming. London: CABI Publishing. pp. 29-158.

SOLEY, J. T., ELIAS, M. Z. J. & AIRE. T. A. 2007. Variations in gross pattern of venous drainage of the ostrich male reproductive tract: A comparison with the general avian model. *Proceedings of Ist Conjoint International Conference on Fertility, Anatomy and Morphological Sciences.* Lagos. Nigeria: 35-36.

SUGIMURA, M.; KUDO, N. & YAMANO, S. 1975. Fine structures of *corpus paracloacalis* in cocks. *Japanese Journal of Veterinary Research,*23: 11-16.

SUZUKI, F. 1982. Microvasculature of the mouse testis and excurrent duct system. *American Journal of Anatomy,* 163: 309-325.

SUZUKI, F. & NAGANO, T. 1986. Microvasculature of human testis and excurrent duct system. *Cell and Tissue Research*, 243: 79-89.

YAMANO, S., SUGIMURA, M. & KUDO, N. 1977. The lymphatic system of the corpus paracloacalis vascularis and the second fold in the male domestic fowl. *Japanese Journal of Veterinary Research,* 25: 93-98.

6. FIGURES

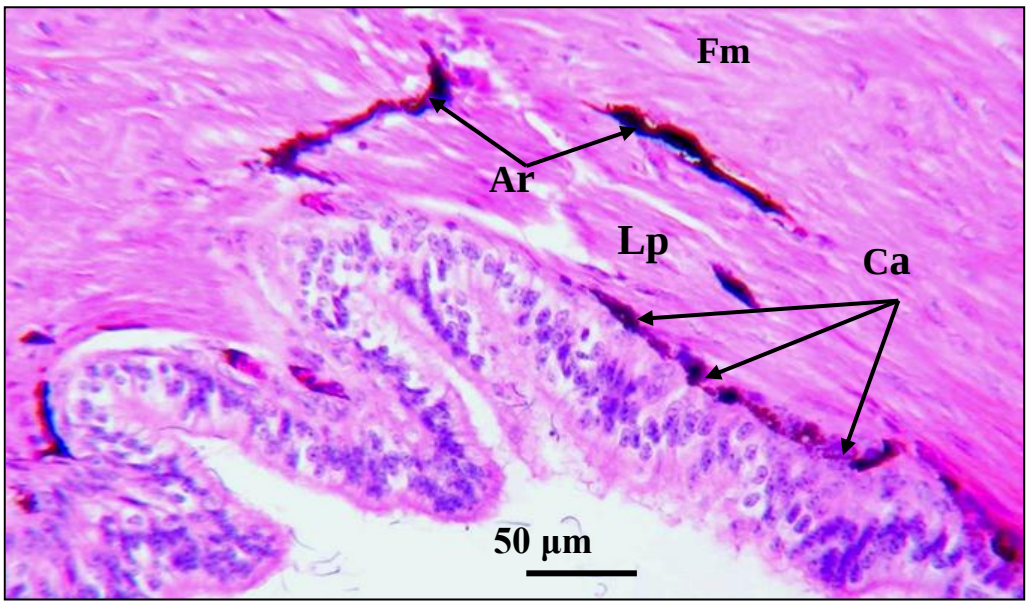

Figure 5.1. Proximal segment of the *Ductus deferens* showing blood capillaries (Ca) situated immediately beneath the pseudo-stratified columnar epithelium.Arterioles (Ar) filled with India ink lie between the lamina propria (Lp) and the fibromuscular coat (Fm).

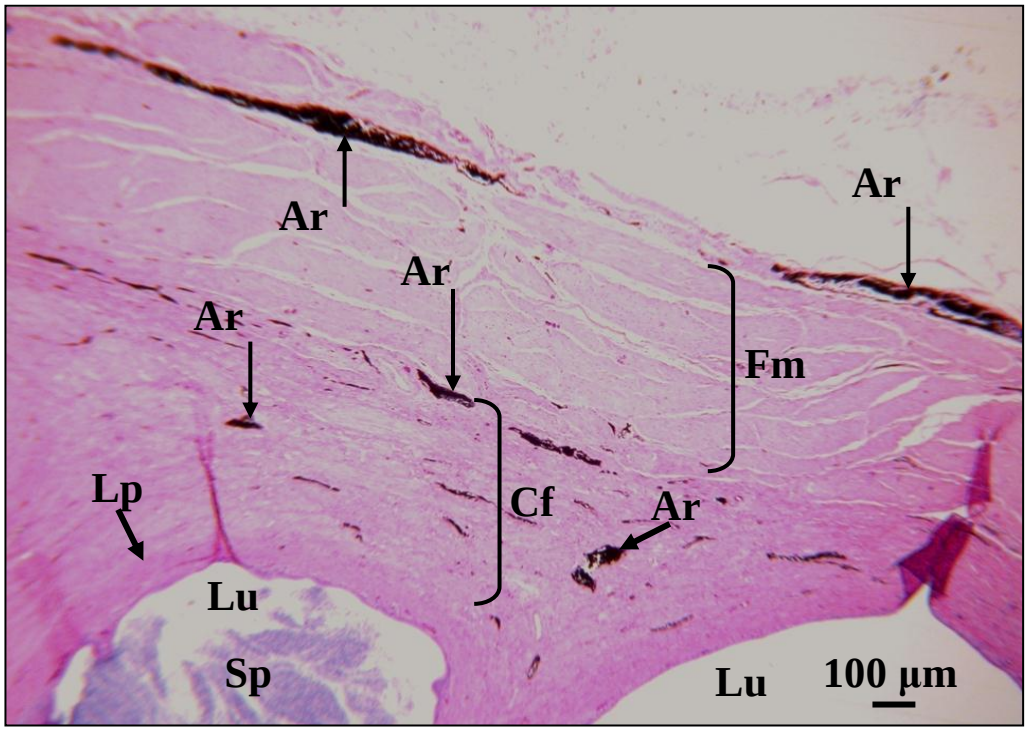

Figure 5.2. Distal segment of the *Ductus deferens* showing arterioles (Ar) scattered in the fibromuscular coat and larger arterioles located superficially beneath the serosa. Note the more loosely arranged outer fibromuscular coat (Fm), the inner more compact fibromuscular coat (Cf) and the lamina propria (Lp). The lumen (Lu) of the duct is filled with sperm (Sp).

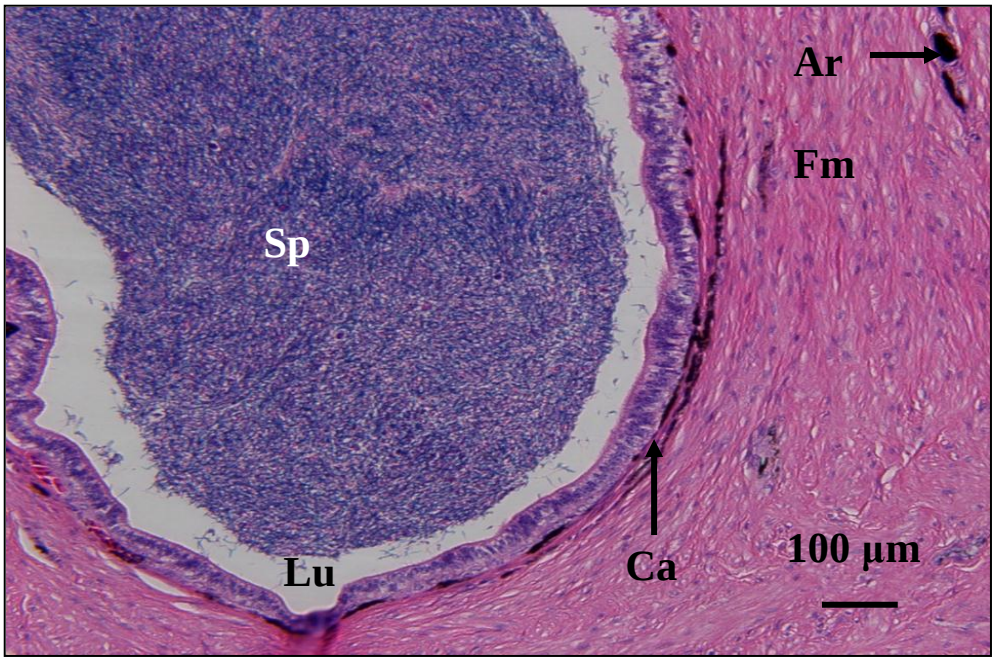

Figure 5.3. Distal *Ductus deferens* showing the location of blood capillaries (Ca) beneath the epithelium, and arterioles (Ar) within the fibromuscular coat (Fm). Note the mass of sperm (Sp) within the lumen (Lu).

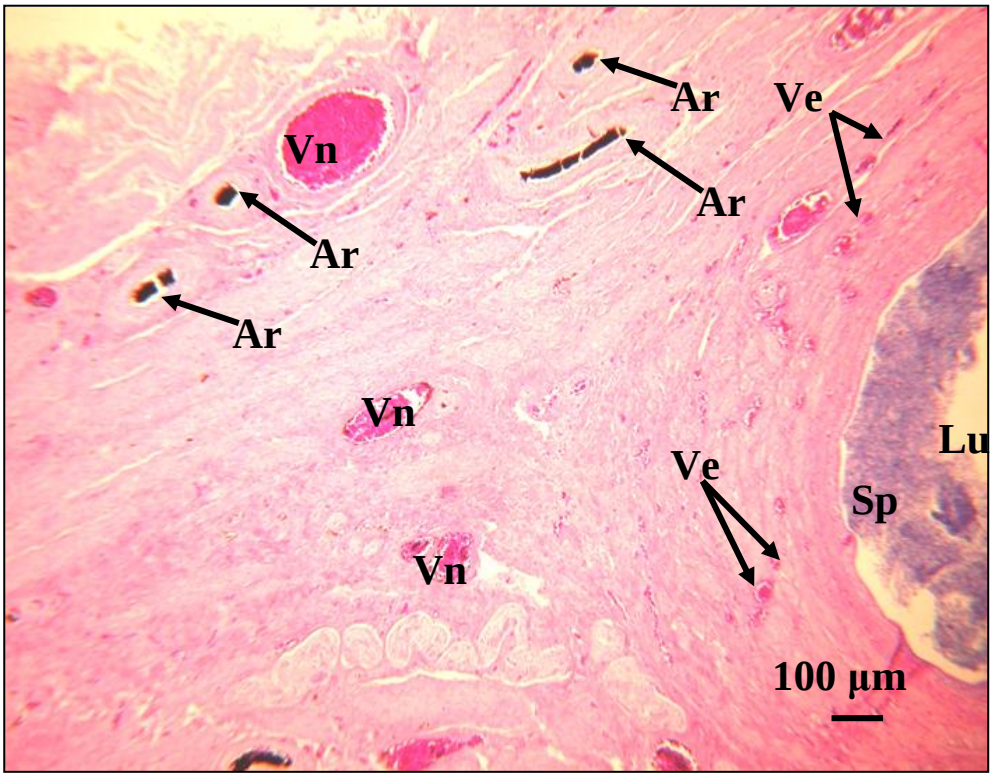

Figure 5.4. Distal *Ductus deferens* showing the distribution of venules (Ve) (demonstrated by a lack of India ink in the vessels), collecting veins (Vn) and arterioles (Ar, with India ink).Note the sperm (Sp) in the lumen (Lu) of the duct.

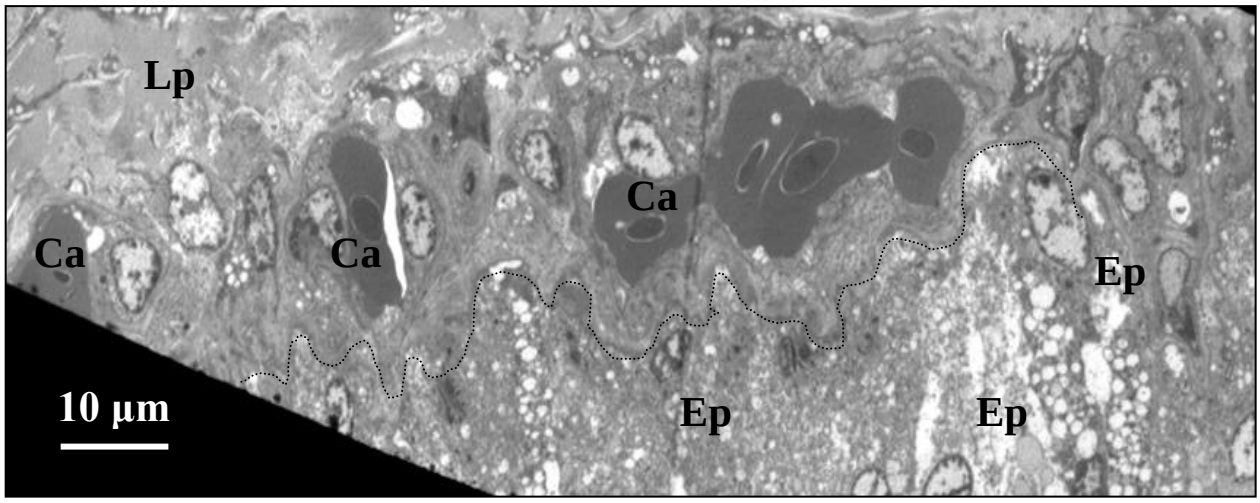

Figure 5.5. Low magnification TEM of a distal deferent duct showing the distribution of sub-epithelial capillaries (Ca) beneath the base of the duct (stippled outline). Duct epithelium (Ep), Lamina propria (Lp).

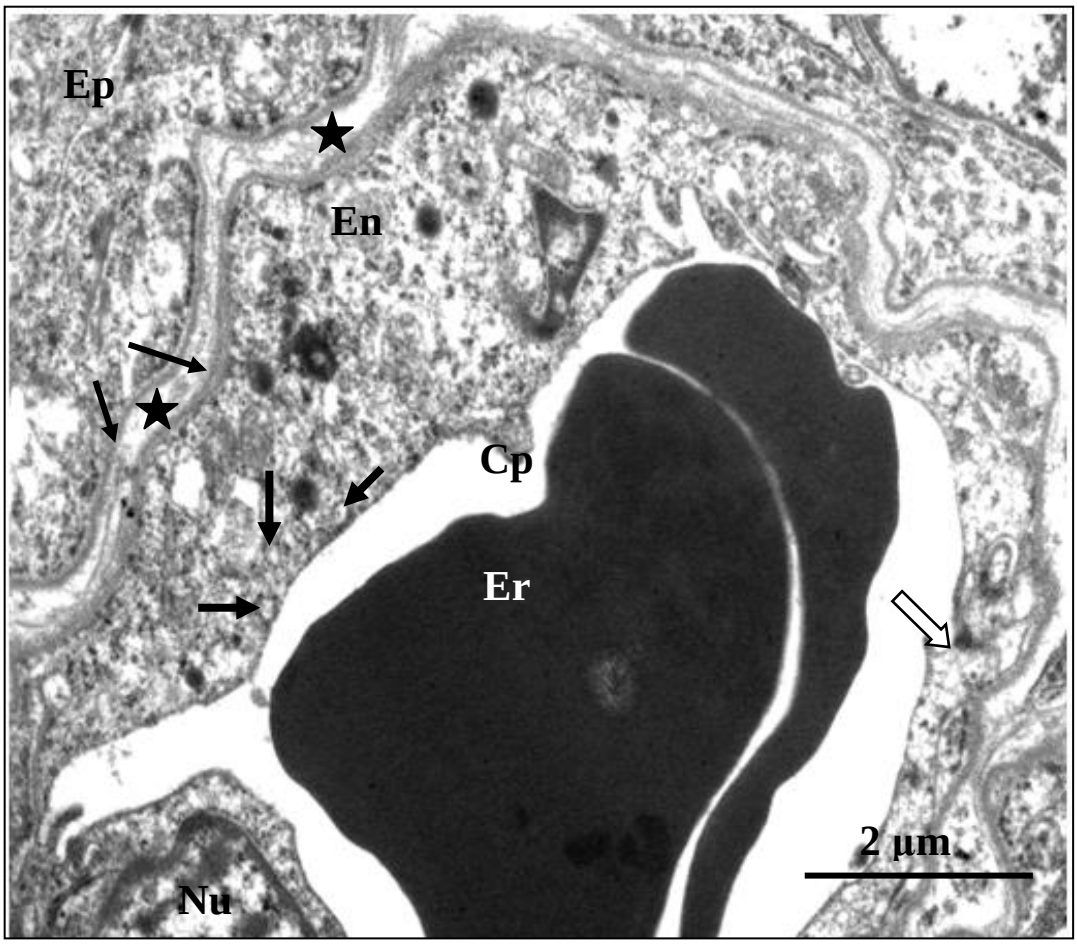

Figure 5.6. A sub-epithelial capillary beneath the distal deferent duct. Note the relatively wide endothelium (En), the nucleus (Nu) of an endothelial cell, erythrocytes (Er) in the vessel lumen, a cytoplasmic process (Cp), some pinocytotic vesicles (thick arrows), and the narrow space (stars) separating the basal laminae (thin arrows) of the endothelium and the adjacent duct epithelial cell (Ep). A junctional complex attaches two adjoining endothelial cells (open arrow).

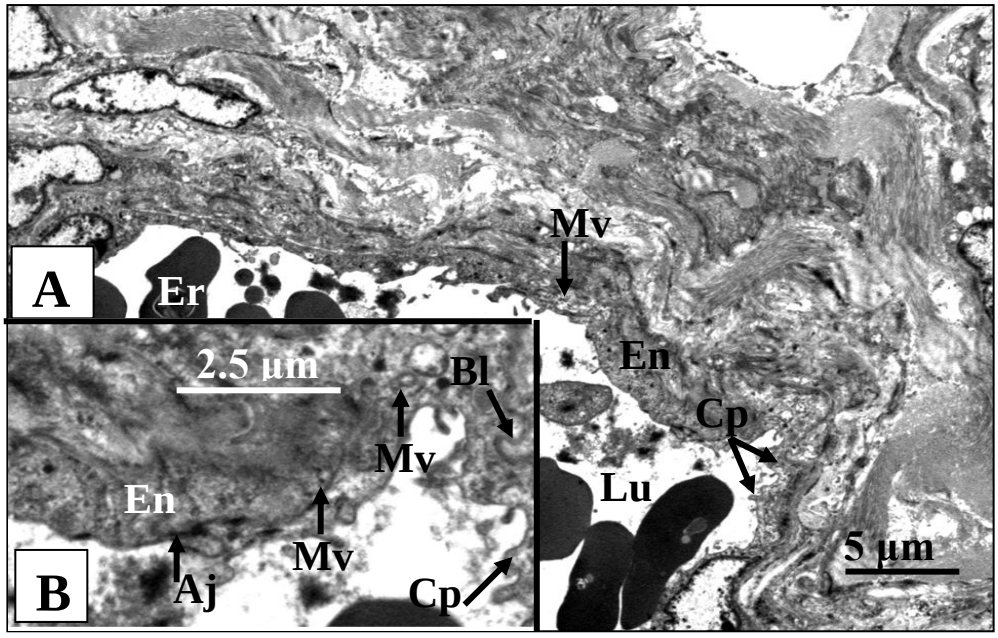

Figure 5.7. A. An arteriole in the distal *Ductus deferens* demonstrating a thick continuous endothelium (En). Note the pinocytotic vesicles (Mv), cytoplasmic processes (Cp) projecting into the lumen (Lu) and erythrocytes (Er). **B**. Magnification of part of the wall of the arteriole to show the basal lamina (Bl), pinocytotic vesicles (Mv), overlapping of adjacent endothelial (En) cells with an adhering junction (Aj) and cytoplasmic processes (Cp).

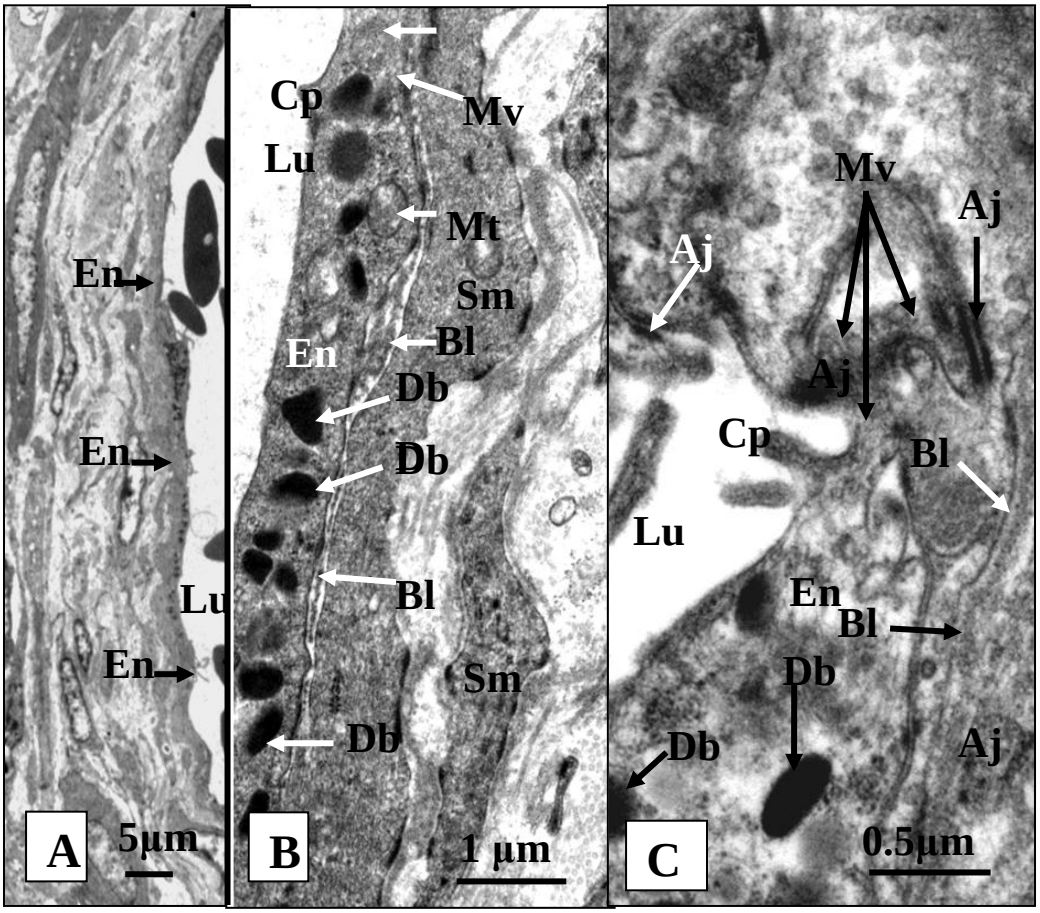

Figure 5.8. A. Low magnification of a collecting vein in the proximal part of the deferent duct showing the lumen (Lu) and the thin endothelium (En) typical of this vessel. **B.** Magnification of part of the collecting vein in A to show the endothelium (En) with numerous dense bodies (Db), some mitochondria (Mt), pinocytotic vesicles (Mv), the basal lamina (Bl) and a blunt cytoplasmic process (Cp) jutting into the lumen (Lu). Note the adjacent smooth muscle cells (Sm) with cytoplasmic densities and basal lamina material. **C.** A higher magnification of part of Fig 5.8A showing details of the endothelium (En) with overlapping cells. Note the adhering junctions (Aj), cytoplasmic processes (Cp) projecting into the lumen (Lu), pinocytotic vesicles (Mv), basal lamina (Bl) and dense bodies (Db).

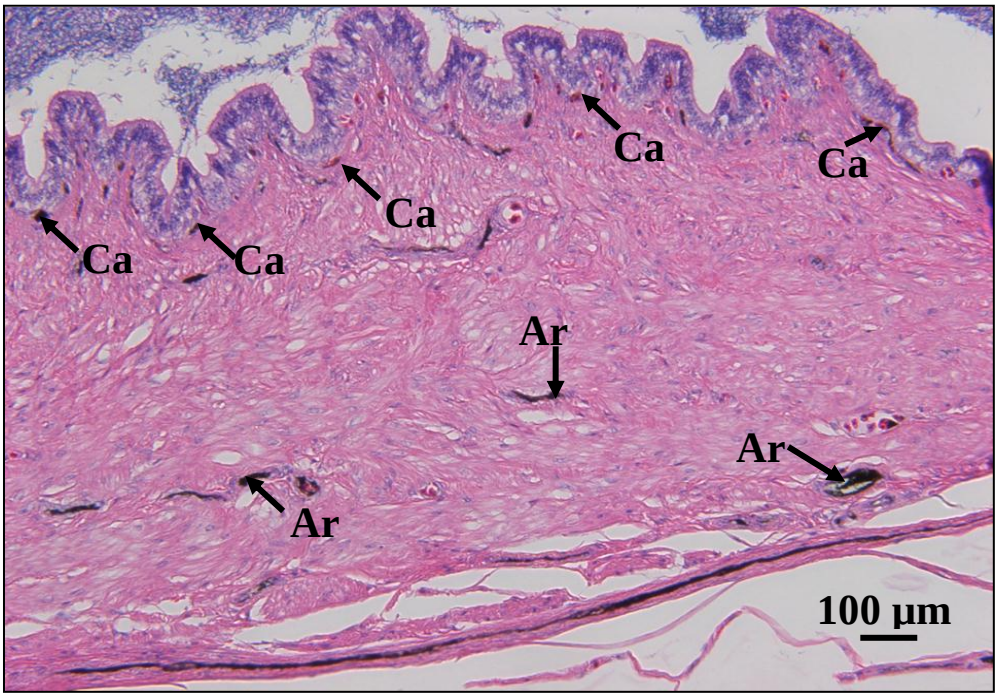

Figure 5.9. A light micrograph of the *Receptaculum ductus deferentis* showing the distribution of blood capillaries (Ca) close to the epithelium, while arterioles (Ar) lie closer to the surface of the fibromuscular coat.

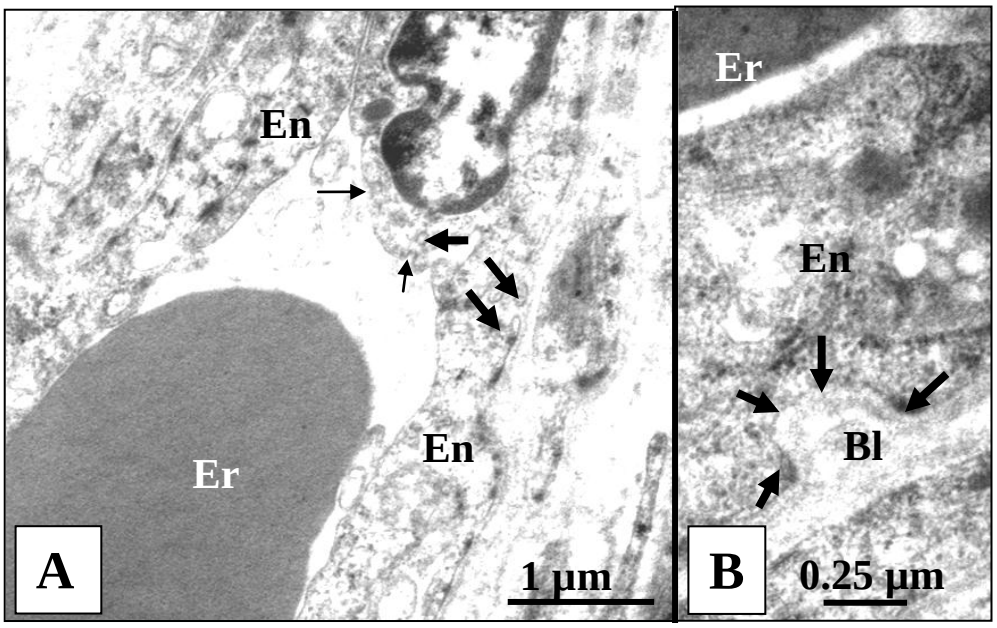

Figure 5.10. **A.** A sub-epithelial capillary in the receptacle of the deferent duct. Note the pinocytotic vesicles (thick arrows) in the endothelium (En) and the forming vesicles (small arrows). An erythrocyte (Er) occupies the lumen. **B**. Magnification of part of the capillary endothelium (En) showing the basal lamina (Bl) following the contours of a cup-like indentation of the endothelium (arrows).Erythrocyte (Er).

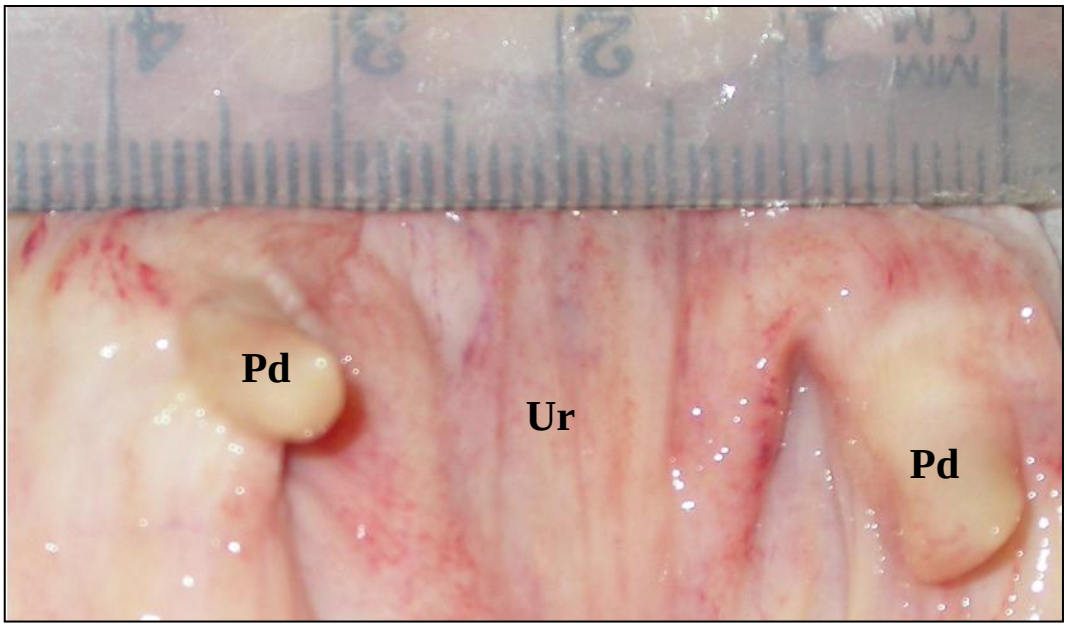

Figure 5.11. The *Papilla ductus deferentis* (Pd) projecting into the lumen from the dorso-lateral wall of the urodeum (Ur).

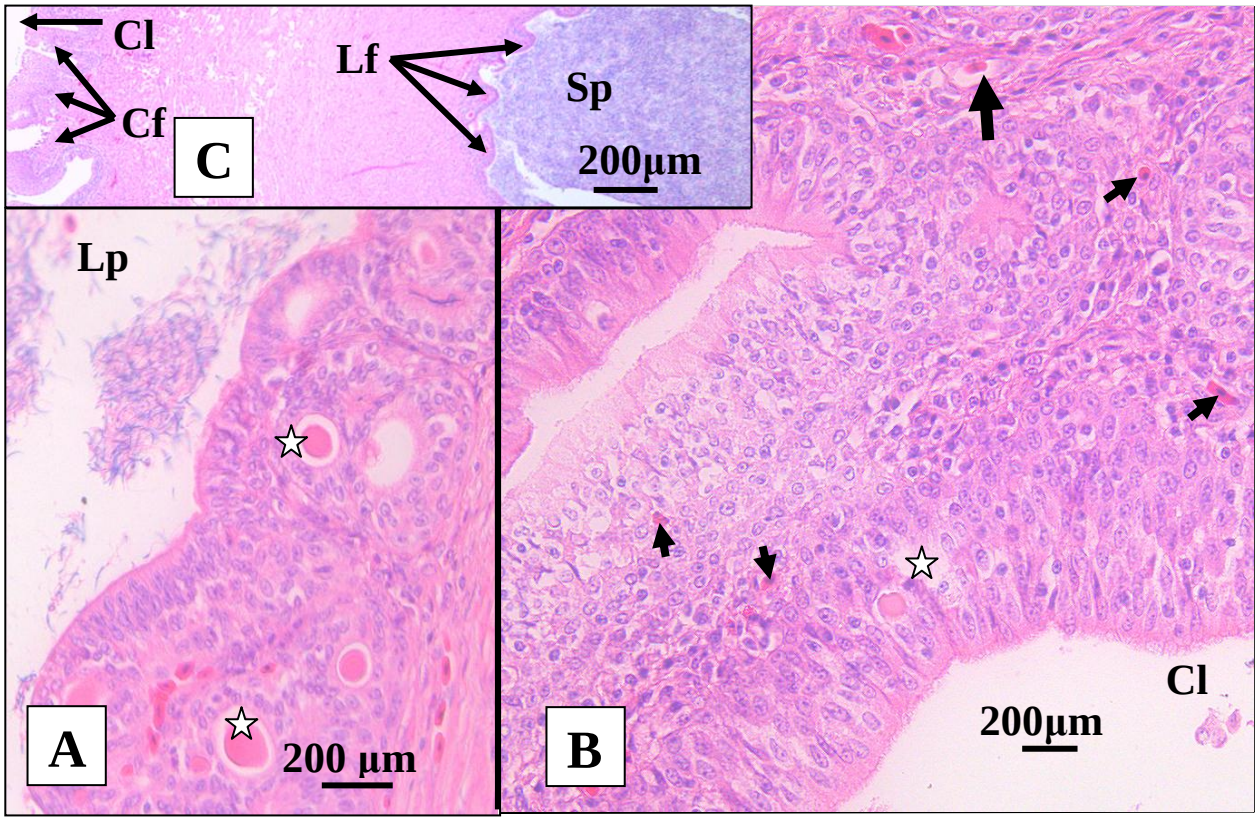

Figure 5.12. *Papilla ductus deferentis*. **A**. Inner mucosa, note the eosinophilic bodies in the wall of the papilla (stars).Papilla lumen (Lp). **B**. Outer (cloacal) mucosa displaying eosinophilic bodies in the epithelium (star).Capillaries (short arrows), arteriole (large arrow) and cloacal lumen (Cl). **C**. Cloacal folds (Cf) of the outer (cloacal) lining of the papilla and luminal folds (Lf) of the inner lining of the papilla. Note the mass of sperm (Sp) in the lumen of the papilla.

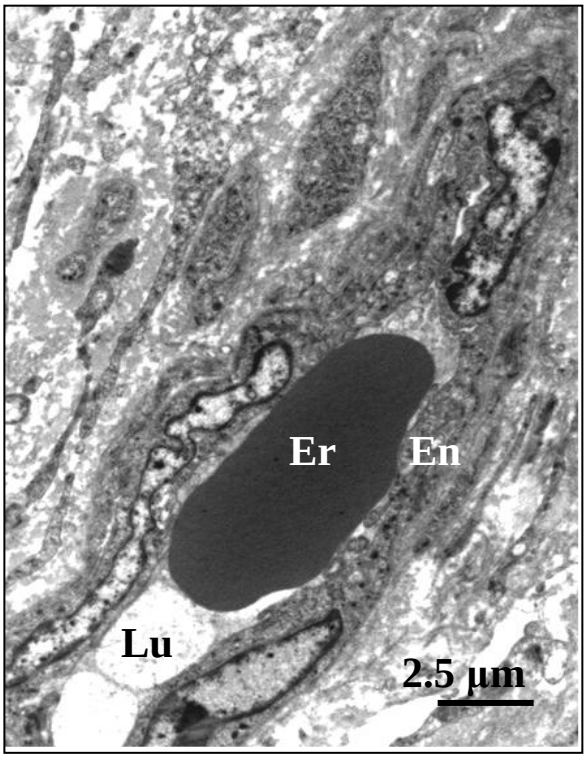

Figure 5.13. Electron micrograph of a typical sub-epithelial capillary in the papilla of the deferent duct. Note the relatively thick, continuous endothelium (En).An erythrocyte (Er) fills the lumen (Lu).

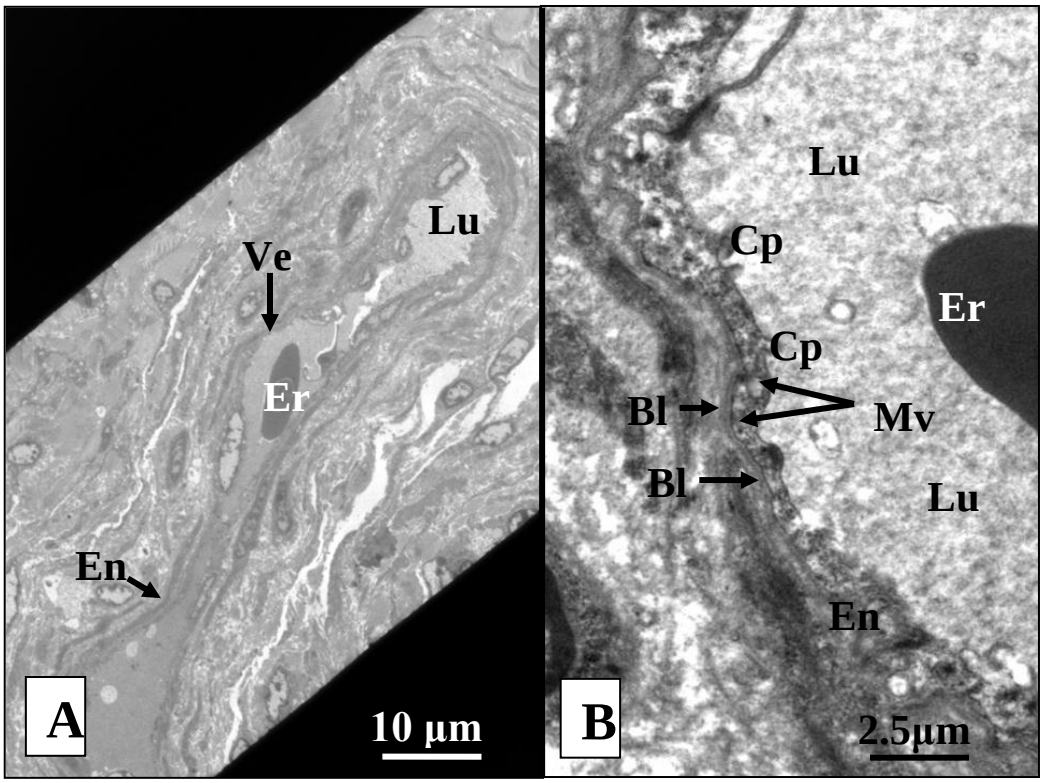

Figure 5.14. A. A low power electron micrograph of a venule (Ve) in the papilla of the deferent duct showing the thin endothelium (En), the lumen (Lu) with an erythrocyte (Er). **B**. A higher power view of Fig. 5.14A to show the thin continuous endothelium (En) resting on a basal lamina (Bl), pinocytotic vesicles (Mv) and cytoplasmic processes (Cp). The lumen (Lu) displays an erythrocyte (Er).

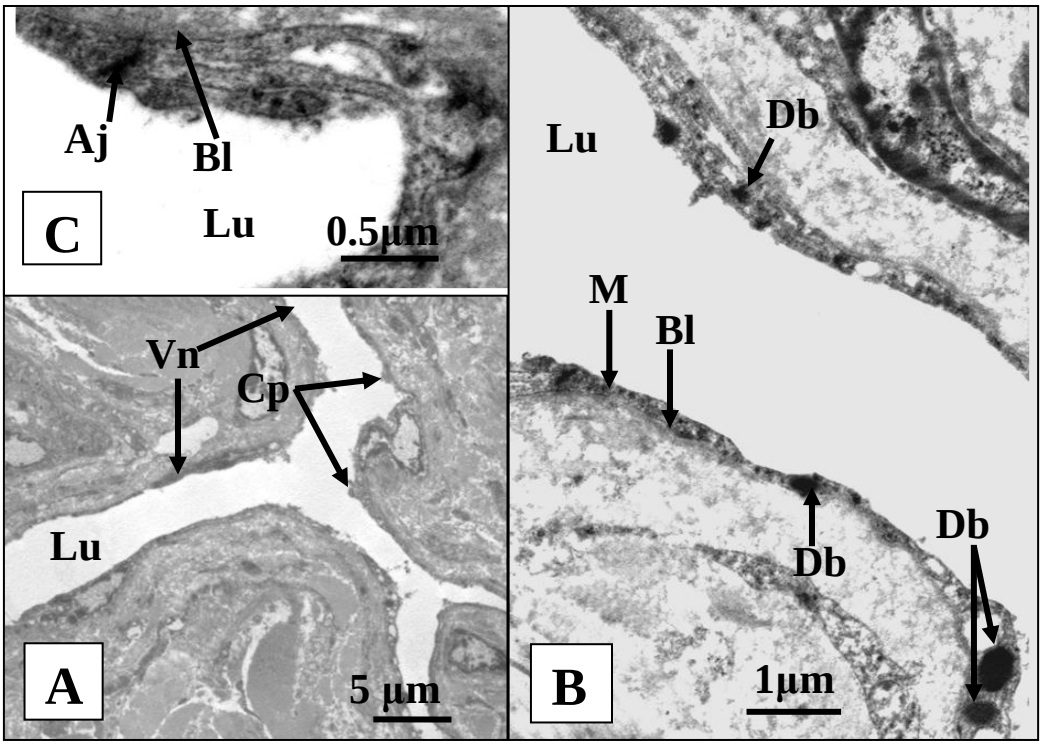

Figure 5.15. A. A low power electron micrograph of a collecting vein (Vn) in the papilla of the deferent duct, showing an empty lumen (Lu) and scant cytoplasmic processes (Cp). **B**. A higher magnification of a collecting vein that displays the endothelium with dense bodies (Db), and a few mitochondria (M). **C**. A region of overlapping endothelial cells connected by an adhering junction (Aj). Basal lamina (Bl), lumen (Lu).

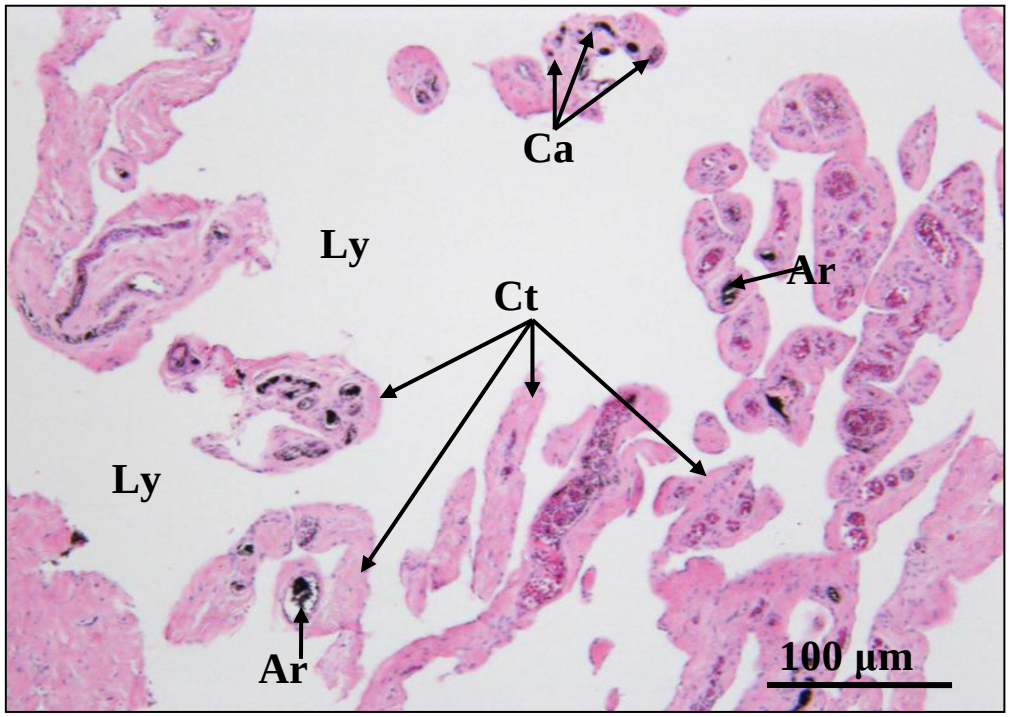

Figure 5.16. Light micrograph of the spongy tissue at the root of the phallus. Note the variably sized connective tissue struts (Ct) with blood capillaries (Ca) and arterioles (Ar) filled with India ink. Note the empty lymph spaces (Ly) between the struts.

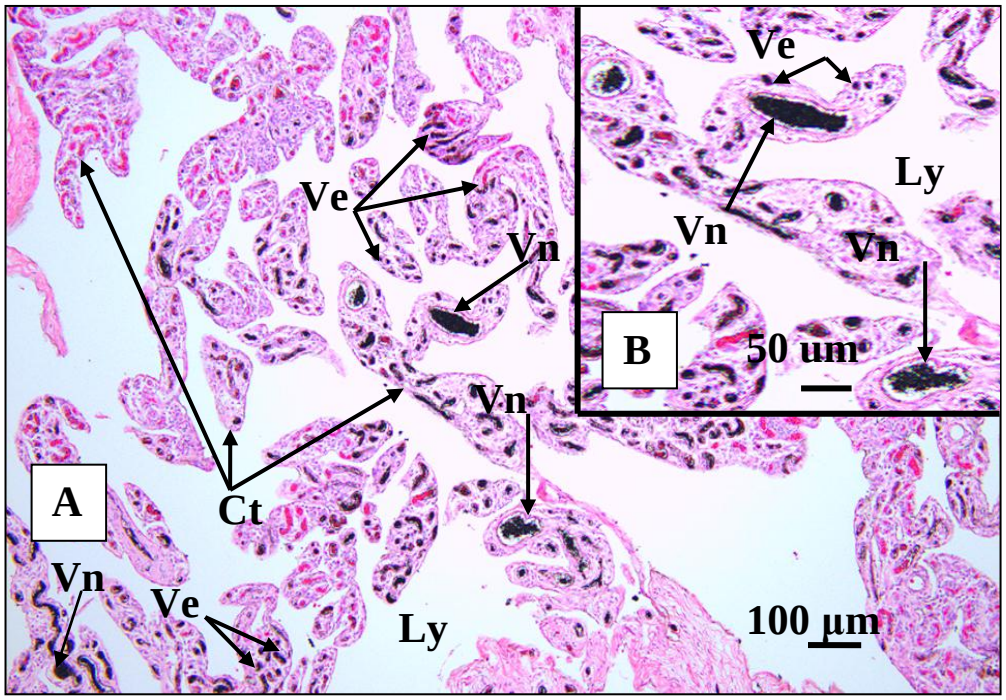

Figure 5.17. A. A region similar to that shown in Fig. 5.16 but with India ink injected into the venous system. Note the connective tissue struts (Ct) with abundant venules (Ve) and collecting veins (Vn) and the large intervening lymph spaces (Ly). **B**. A magnification of part of Fig. A showing tissue struts with venules (Ve) and collecting veins (Vn). Note the empty lymph spaces between the struts (Ly).

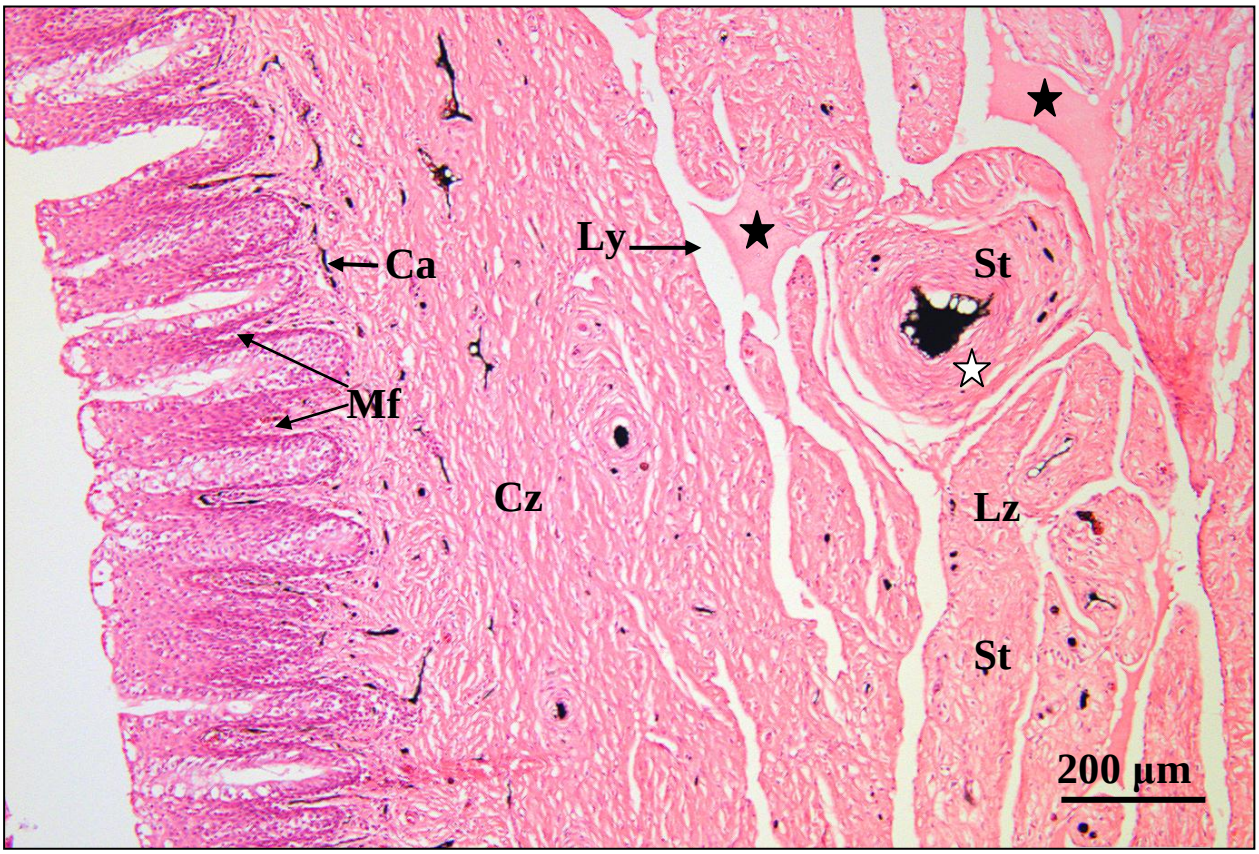

Figure 5.18. Light micrograph of the phallic sulcus. Note the regular mucosal folds (Mf) lining the surface, the more compact zone (Cz) with scattered capillaries (Ca) and the looser zone (Lz) with connective tissue struts (St) containing larger vessels (white star) as well as capillaries. Colloid-like substance (black stars) lies in the lymph spaces (Ly) between the struts. India ink injection.

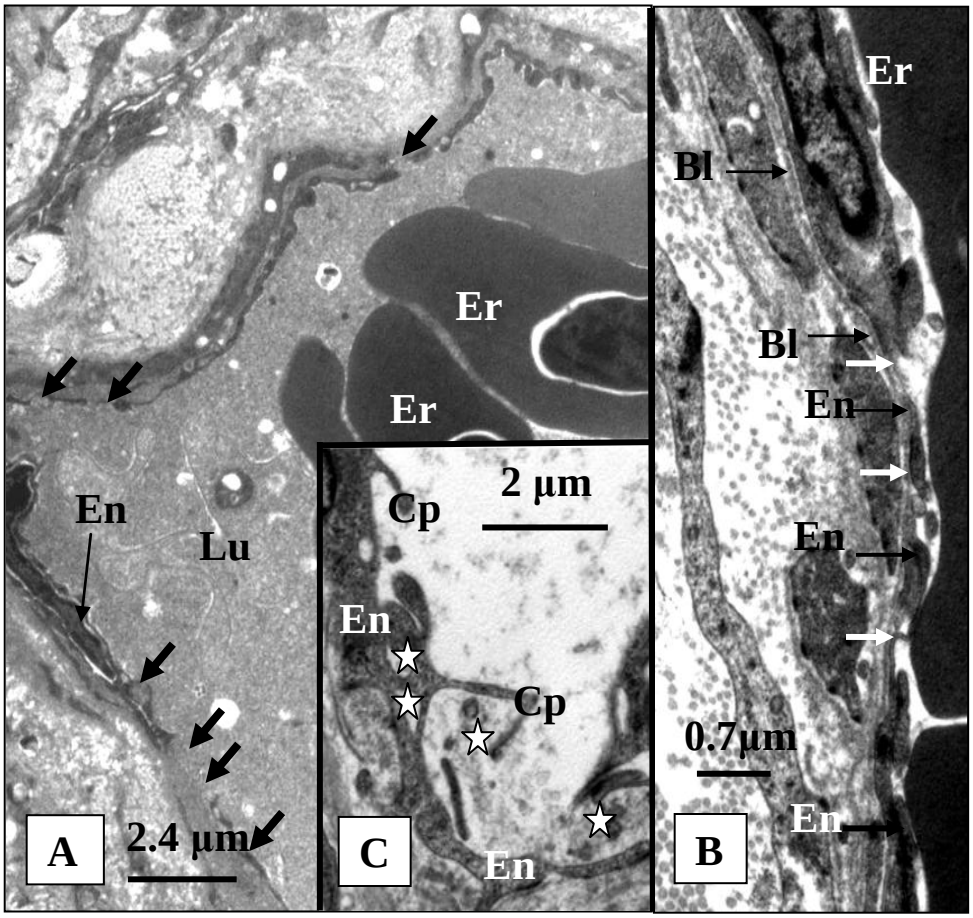

Figure 5.19. A. A venule in the spongy tissue at the root of the phallus displaying wide fenestrations (thick arrows) in the endothelium (En). Erythrocytes (Er) are packed in the vessel lumen (Lu). **B.** Longitudinal section of a capillary in the spongy tissue. The endothelium (En) is discontinued by wide fenestrations (white arrows) although the basal lamina (Bl) is continuous. **C.** A blood capillary displaying cytoplasmic processes (Cp) extending from the vessel wall (En) and which appear to be sequestrating luminal contents (stars).

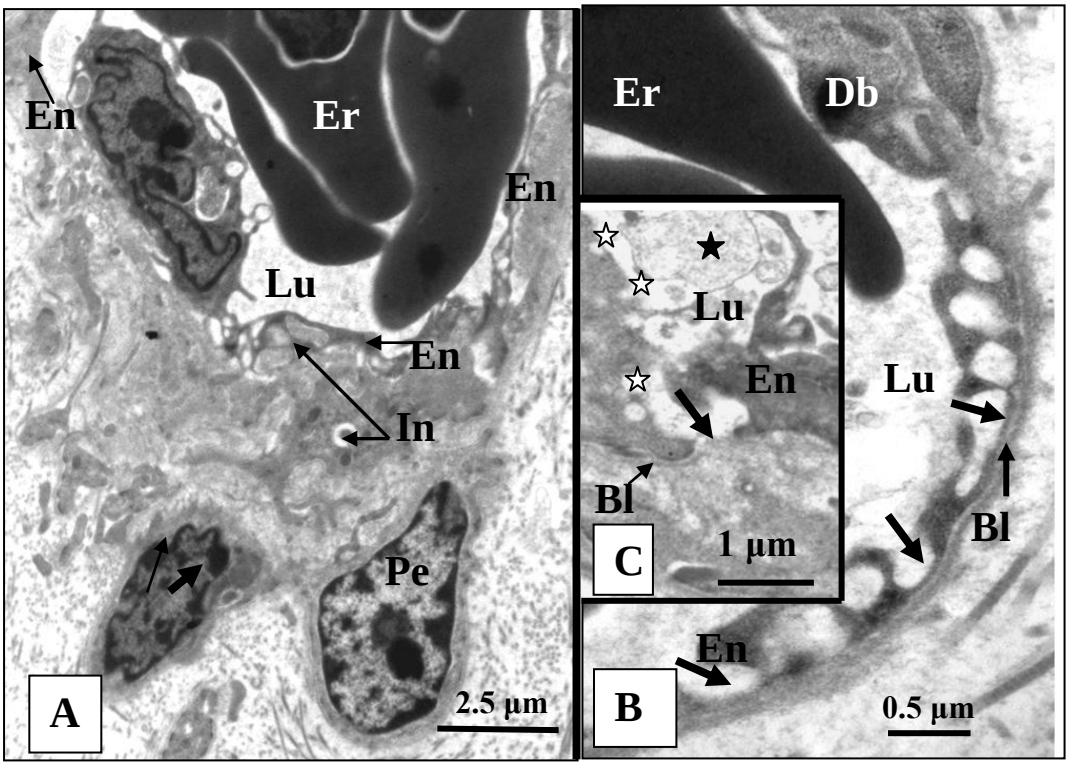

Figure 5.20. A. An electron micrograph of a blood capillary in the erectile tissue of the phallic sulcus demonstrating complex interdigitations of the endothelium (En).Internalised material (In), lumen (Lu), erythrocytes (Er), presumptive pericyte (Pe). **B**. A higher magnification of part of the capillary wall in Fig. 20 A. Note the apparent internalisation of luminal contents resulting in narrow strips of attenuated endothelium (short thick arrows) resting on a continuous basal lamina (Bl). Dense body (Db), Lumen (Lu), erythrocytes (Er). **Inset C**. A higher magnification of the capillary endothelium (En) showing cytoplasmic processesin the act of internalising luminal material (black star). An endothelial fenestration (short thick arrow) is apparent as well as formation of pinocytotic vesicles (stars).Lumen (Lu), basal lamina (Bl).

CHAPTER 6

THE BLOOD-TESTIS AND BLOOD-EPIDIDYMIS BARRIERS

1. INTRODUCTION

The blood-testis-barrier (BTB) is situated near the base of the seminiferous epithelium and is formed by specialised junctional complexes between adjacent Sertoli cells that exclude large molecules and other substances from entering the seminiferous tubules (Gilula, Fawcett & Aoki 1976; Setchell, Laurie & Fritz 1980). It has been suggested that, in addition to the Sertoli cells, the components of the peritubular tissue, as well as the blood vessels in the interstitial tissue, also form part of the BTB (Fawcett, Leak & Heidger 1970; Neaves 1977; Setchell & Waites 1975). The BTB is designed to prevent contact between the blood and the interior of the seminiferous tubule, maintain a favourable spermatogenic environment within the seminiferous tubules (Toyama, Maekawa & Yuasa 2003), prevent contact between sperm auto-antigens and the blood, protect the lumen of seminiferous tubules against sperm-specific antibodies, protect developing germ cells against environmental toxins and mutagens, maintain the structural integrity of the seminiferous epithelium and tubules (Waites & Gladwell 1982), partition the seminiferous epithelium into basal and adluminal compartments (Dym & Fawcet 1970), and retain endocrinological substances formed in the seminiferous tubules, thus allowing them to reach the distal portion of the male genital tract where they are absorbed (Setchell & Waites 1975) .

The permeability of the BTB can be influenced by ligation of the efferent ductules, autoimmune orchitis and vasectomy (Neaves 1977). Autoimmune orchitis can also develop as a result of trauma or disease (Bishop 1970), which can cause serious damage to spermatids, spermatocytes and spermatogonia, thus resulting in aspermatogenic lesions (Neaves 1977; Tung & Alexander 1977). The BTB can also be affected in some mammalian species by genetic factors (Toyama *et al.* 2003).

The BTB is absent in newborn mammals (Waites & Gladwell 1982) and in sexually immature birds (Bergmann & Schindelmeiser 1987). This barrier develops fully at puberty (Vitale, Fawcett & Dym 1973; Setchell *et al.*1980), although some components of the junctional complex, such as the tight junctions, exist before the blood-testis barrier becomes effective in the fowl (Bergmann & Schindelmeiser 1987).

The BTB has been extensively studied in several mammals, such as the rat (Dym & Fawcett 1970; Vitale *et al.* 1973; Aoki & Fawcett 1975; Nagano & Suzuki 1976; Gilula *et al.* 1976; Main & Waits 1977; Osman & Plöen 1978; Russel 1978; Turner, Giles & Howards 1981; Cavicchia & Sacerdote 1988; Webber, Turner, Tung & Russel 1988; Chung, Mruk, Mo, Lee & Cheng 2001; Noguchi, Toyama, Yuasat, Kikuchi & Kaneko 2002; Toyama *et al.* 2003; Yan & Cheng 2005), mouse (Nagano & Suzuki 1976; Ross 1977; Setchell *et al.* 1980), guinea pig (Johnson 1969, 1970; Fawcett *et al.* 1970; Pelletier, Nemirovsky, Calvert & Hugon 1981; Pelletier & Friend 1983, 1986), sheep (Setchell 1967; Setchell, Voglmayr & Waites 1969), monkey (Dym 1973; Dym & Cavicchia 1977, 1978), mink (Pelletier 1986, 1988; Pelletier & Shivers 1986), hamster (Howard, Jesse & Johnson 1976; Turner, D'Addario & Howards 1979; Turner, Cochran & Howards 1981), dog (Cornell 1978, 1980), rabbit (Sun & Gondos 1986), swine, bovine and goat (Osman 1978), and man (Plöen, Hagens, Ritzén & Narén 1976; Landon & Pryor 1981; Lackgren & Plöen 1984).

The existence of the BTB has been noted in the avian testis and information provided on the structure of intercellular junctions between elements of the seminiferous epithelium and between the contractile cells of the peritubular tissue (Cooksey & Rothwell 1973; Rothwell 1975; Pfeiffer & Vogl 1993; Aire 1997). The use of tracers has also been applied to determine the extent of the BTB in the domestic fowl (Osman, Ekwall & Plöen 1980; Bergmann & Schindelmeiser 1987; Pelletier 1990), and duck Pelletier (1990).

The blood-epididymis barrier (BEB) in mammals separates the lumen of the epididymis from the surrounding blood capillaries (Agrawal & Hoffer 1989). The tight junction or *Zonula occludens* (Suzuki & Nagano 1978a,b; Friend & Gilula 1972) forms the most important

component of the barrier (Hoffer & Hinton 1984). Epididymal luminal fluid therefore differs in composition from blood plasma (Turner & Howards 1985). The basic function of the barrier is to protect the maturing spermatozoa from outside influence (Friend & Gilula 1972; Hinton 1985).

Studies on the BEB have been performed in several mammals, including the rabbit (Flickinger 1975), hamster (Turner, D'Addario & Howards 1983), rat (Turner, Cochran & Howards 1981; Turner, Giles & Howards 1981; Hoffer & Hinton 1984; Agrawal & Hoffer 1989) and stallion (Lopez, Fuentes, Retamal & de Souza 1997). A review on the role of the BEB has been presented by Hinton & Palladino (1995).

The avian epididymis differs from that of mammals in that it represents a discrete organ housing the excurrent duct system of the reproductive tract (rete testis, efferent ducts, connecting ducts and epididymal duct). Studies on birds have reported the presence of junctional complexes at the apico-lateral aspect of the epithelial cells lining the various components of the epididymis (Tingari 1971a,b; Budras & Sauer 1975; Bellamy & Kendal 1985; Aire 2002). Additionally, the BEB in the domestic fowl has been determined using a tracer, lanthanum nitrate (Nakai, Hashimoto, Kitagawa, Kon & Kudo 1988; Nakai & Nasu 1991).

Although a number of studies have characterized the structural features of the ostrich testis (Soley 1990, 1992, 1997; Soley, van Wilpe, Aire & Ozegbe 2005; Elias, Aire & Soley 2008) and epididymis (Budras & Meier 1981; Aire & Soley 2000, 2003; Ozegbe, Aire & Soley 2006a, b; Elias, Soley, Aire & du Plessis 2008), very little specific information, with few exceptions (Soley 1992), has been published on the BTB and BEB in the ostrich. This chapter provides additional information on the components and extent of the BTB and BEB in this species based on conventional TEM and tracer studies using lanthanum nitrate. The basic architecture of the ostrich testis and epididymis is presented in Chapters 1 and 4.

2. MATERIALS AND METHODS

The torsos of 16 sexually mature and active male ostriches with viscera intact, but which had been skinned and from which the limbs had been removed, were obtained from the Oryx abattoir in Krugersdorp, Gauteng and from the Klein Karoo abattoir in Oudtshoorn, Western Cape, South Africa. The torsos were prepared as follows for fixation of the testis and epididymis for transmission electron microscopy (TEM). The vascular system of the reproductive tract of 10 ostriches was flushed free of blood by injecting physiological saline through the descending aorta using a 50 ml plastic syringe. The aorta was carefully cut open to expose the origin of the cranial renal artery. This vessel was cannulated using a curved 18 gauge needle through which the fixative (1% lanthanum + 2.5% glutaraldehyde in 0.1M cacodylate buffer pH7.4) was injected into the arterial system of the testis and epididymis. Immediately following perfusion, small blocks of tissue were removed from the testes and epididymis, immersed in fresh fixative (2.5% glutaraldehyde + 0.1M cacodylate buffer pH 7.4), and the specimens stored at 4°C prior to processing for TEM. The testes of six sexually mature ostriches were fixed as above but without lanthanum nitrate in the fixative and routinely processed for TEM.

The fixed tissue blocks, irrespective of the primary fixative used, were rinsed in 0.1M cacodylate buffer for 30 minutes and then post-fixed in 1% osmium tetroxide in the same buffer for 1 hour. The blocks were subsequently rinsed in the cacodylate buffer for 2 hours, dehydrated through a graded series of ethanols and substituted with propylene oxide (2 changes of 15 minutes each). Thereafter, the specimens were infiltrated with a 2:1 propylene oxide/epoxy resin mixture for 1 hour and a 1:2 propylene oxide/ resin mixture for 1-2 hours followed by embedding in 100% epoxy resin (Epon 815) overnight at 60ºC. Ultra-thin sections were cut, stained with lead citrate and uranyl acetate, then viewed and photographed in a Philips CM10 transmission electron microscope (Eindhoven, The Netherlands) operated at 80kV.

3. RESULTS

3.1. Intercellular junctions in the testis parenchyma

Examination of the testis samples not perfused with lanthanum nitrate revealed the range of junctional complexes previously reported for the ostrich (Soley 1992, Elias *et al.* 2008). Adhering (desmosome-like) junctions were present between the myofibroblasts of the peritubular tissue (Figs. 6.1A, B) and also between adjacent Sertoli cells and between Sertoli cells and spermatogonia. Fingers of Sertoli cell cytoplasm isolated the spermatogonia from the basal lamina of the seminiferous tubules and to which they were attached by rudimentary hemi-desmosomes (Fig. 6.2). In the region of the germinal epithelium above the spermatogonia, tight (occluding) junctions and adhering junctions were observed between adjacent Sertoli cells (Figs. 6.3A, B), and between Sertoli cells and spermatocytes (Figs. 6.4) and spermatids (Figs. 6.5A, B).

Weakly-developed adhering junctions were present between all germ cell progeny, and tight junctions were also observed between some primary spermatocytes. A single hemi-desmosome-like junctional complex was generally present between the apical tip of the forming acrosome (tightly covered by the plasmalemma) of early elongating spermatids and the surrounding Sertoli cell cytoplasm (Figs. 6.5A, B). This type of cell junction was extensive and was formed by a conspicuous density situated just beneath the plasmalemma of the Sertoli cell. Between some spermatids were isolated adhering junctions (Fig. 6.5C). No typical gap junctions were observed between myoid cells in the peritubular tissue or between the various components of the seminiferous epithelium.

The adhering junctions observed in the peritubular tissue (Figs. 6.1A, B), and between the cellular elements of the seminiferous tubules (Figs. 6.2 - 6.5), displayed similar morphological characteristics. Although variable in length these junctions demonstrated an empty intercellular space with an accumulation of electron-dense material on the cytoplasmic face of each cell membrane participating in the junction. The amount and

electron-density of the cytoplasmic material forming the junctions varied markedly. Oblique sections through the junctions presented as moderately electron-dense plaques.

Tight junctions were particularly obvious between Sertoli cells and between Sertoli cells and spermatocytes (Figs. 6.3A, 6.4). These junctions were formed by small points of contact between adjacent cell membranes thus forming focal tight junctions. The adluminal region of the seminiferous epithelium was also characterized by a series of attenuated cytoplasmic processes of Sertoli cells which were often seen to interdigitate in a complex manner (Fig. 6.3A). The tight junctions between Sertoli cells and germ cells were frequently associated with long subsurface cisternae of endoplasmic reticulum within the Sertoli cell processes (Fig. 6.4).

3.2. The blood-testis barrier (BTB)

Of the ten ostriches utilized for the BTB tracer study, only four allowed the solution with lanthanum nitrate to pass into the testis and epididymis. The reasons for this phenomenon are not currently understood.

In the testis parenchyma, lanthanum nitrate was observed to accumulate on the luminal surface of the endothelium of the interstitial blood vessels (Figs. 6.6A, B). In instances where endothelial cells overlapped, some tracer appeared to be trapped between the cells (Fig. 6.6B). Although much of the tracer appeared to be contained by the blood vessel endothelium, considerable amounts clearly crossed the capillary endothelial junctions (adhering junctions). Concentrations of lanthanum nitrate were seen between myoid cells separated by basal lamina-like material, between the layers of myofibroblasts forming the peritubular (boundary) tissue (Fig. 6.6A), as well as along the connective tissue face of the basal lamina of the seminiferous tubules where it accumulated in the form of staggered dense patches (Figs. 6.7,6.8).

Within the seminiferous tubule the tracer tended to accumulate between the Sertoli cells and the spermatogonia positioned at the base of the seminiferous epithelium. In some

sections, due to the method of fixation, the positions occupied by the spermatogonia were represented by empty pockets outlined by the tracer (Figs. 6.7, 6.8). The lanthanum nitrate was also observed in the intercellular space between primary spermatocytes (Fig. 6.8), but this appeared to be restricted to the more proximal aspect of the cells.

3.3. Junctional Complexes in the epididymis

Apicolateral junctional complexes (Figs. 6.9-13) were commonly observed in the various ducts of the epididymis. These junctional complexes are prominent membrane specialisations which are restricted in location to the apical aspect of the lateral cell membrane. They form a relatively small part of the total membrane length and are composed of three distinct regions. Immediately beneath the lumen are a series of focal tight junctions (*Zonula occludens*), followed by a short stretch of closely apposed membranes enclosing a narrowed intercellular space, and which constitutes an intermediate junction (*Zonula adherens*). The final region is variable in length and forms desmosomes (*Macula adherens*).

The cuboidal to columnar lining cells of the *Rete testis* were linked by apicolateral junctional complexes as described above (Fig. 6.9). Desmosome-like (adhering junctions randomly connected the lateral cell membranes of adjacent cells. As previously noted (Soley 1992; Ozegbe *et al*. 2006a; Elias *et al.* 2008), the apicolateral junctional complexes appeared similar in both the proximal and distal components of the efferent ducts and also occurred between both cell types (ciliated and non-ciliated cells) which make up the epithelial lining of the ducts (Figs. 6.10). The lateral cell membranes differ markedly between the proximal and distal efferent ducts. The proximal duct displayed long stretches of complex cytoplasmic interdigitations and enlarged intercellular spaces (Fig. 6.10), whereas in the distal duct adjacent cells simply abutted each other forming a consistently regular intercellular space (Fig. 6.11). Adjacent cells in both ducts were linked by desmosome-like structures which in appearance, however, resembled weakly developed adhering junctions. In both ducts the basal plasmalemma of all cells were attached to the underlying basal lamina by an extensive network of hemi-desmosomes.

The same complement of intercellular junctions was again obvious between the pseudo-stratified columnar cells of the connecting ducts (Fig. 6.12) and epididymal duct (Fig. 6.13) and displayed the same structural properties previously described.

3.4. The blood-epididymis barrier (BEB)

Examination of the blood vessels (capillaries and arterioles) in the epididymis of lanthanum nitrate-treated specimens revealed that the tracer was apparently unable to cross the vascular endothelium (Figs. 6.14-16). The lanthanum nitrate was clumped on the luminal surface of the vessels and was concentrated in areas where adjacent endothelial cells overlapped (Fig. 6.15, 6.16), presumably due to the blocking action of the adhering cell junctions present between the cells. Lanthanum nitrate was, therefore, not present in the peritubular tissue or in the lumen of any of the excurrent ducts of the testis examined.

4. DISCUSSION

The location and morphological features of the junctional complexes observed in the testis of the ostrich confirm, generally, the findings made previously in this species (Soley 1992; Elias *et al*. 2008). The desmosome-like junctions reported to be present between the myofibroblasts of the peritubular tissue in the ostrich (Soley 1992) were confirmed in this study although they were referred to as adhering junctions. The variety of adhering junctions reported in the fowl (Cooksey & Rothwell 1973; Rothwell 1975; Osman *et al.* 1980) and duck (Pelletier 1990) were not observed in the ostrich material studied, and only the subtype characterized by a condensation of electron-dense material along the cytoplasmic aspect of the apposed plasmalemmas was readily identified. This junction corresponded to the subtype “A” described by Osman *et al.* (1980).

Based on the distribution of the lanthanum nitrate tracer, it was clear that the endothelial lining of the interstitial blood vessels as well as the attendant basal lamina formed an important component of the BTB. As indicated by the accumulation of the tracer against the luminal surface of the endothelium, it was evident that the tracer was only capable of breaching the epithelial lining through the adhering junctions coupling adjacent endothelial cells. This was emphasized in instances where adjoining endothelial cells overlapped each other, trapping the tracer between them. Significant amounts of the tracer were also contained by the endothelial basal lamina which acted as a final barrier between the blood vessels and the surrounding boundary tissue. The fact that tight junctions were not observed between the endothelial cells in the present study is consistent with the observations by Fawcett *et al.* (1970) that the endothelium is not a barrier for blood, because it does not completely block the passage of macromolecules emanating from the blood vessels. It has also been demonstrated in the mouse that the blood capillary endothelium, lymphatic endothelium, and basal lamina are involved in the BTB (Neaves 1977). It has further been demonstrated that although the lymphatic endothelium constitutes an insignificant physical barrier, the interposed lymphatic spaces and channels are capable of diverting substances moving from the blood to the seminiferous tubules, limiting their appearance in the testicular interstitium (Fawcett *et al.*1970).

In the ostrich, significant amounts of lanthanum were trapped between the myoid cells and myofibroblasts making up the boundary tissue, indicating that a limited quantity of the tracer crossed the adhering junctions linking these cells. An important observation was that the basal lamina of the germinal epithelium appeared capable of concentrating pockets of the tracer along its connective tissue face, again restricting the amount of tracer entering the seminiferous tubules. These observations support the findings of Fawcett *et al.* (1970) and Neaves (1977) that in mammals the myoid cells constitute part of the BTB, preventing larger molecules from entering the seminiferous tubules (Neaves 1977). As revealed in this study, in birds, as in mammals, the endothelium of the blood capillaries located within the interstitial tissue, as well as the boundary tissue, including the basal lamina of the seminiferous tubules, act as the first component of the BTB (Waites & Setchell 1969 quoted by Dym & Fawcett 1970; Setchell & Waites 1975; Neaves 1977).

According to some reports (Cooksey & Rothwell 1973) there are no intercellular junctions between the Sertoli cells and spermatogonia in the fowl. In the present study adhering junctions were routinely observed between the two cell types as previously described in this species (Soley 1992) and also in the fowl and duck (Osman *et al.* 1980; Pelletier 1990). In addition, gap junctions (Pelletier 1990), as well as localized collections of dense material positioned within in the intercellular space (Osman *et al.* 1980), have been described between the spermatogonia and surrounding Sertoli cells. These cell junctions were not observed in the ostrich material studied, possibly due to the limitations imposed by routine transmission electron microscopy of plastic sections. It was clear, however, that the adhering junctions (and presumably also the gap junctions) allowed the penetration of the tracer (as noted in mammals – Friend & Gilula 1972) which was observed to surround the spermatogonia. This suggested that the lanthanum nitrate, although accumulating at the connective tissue face of the basal lamina of the seminiferous tubule, was able to cross this barrier and enter the intercellular space at the base of the germinal epithelium.

As in the fowl and duck (Osman *et al.* 1980, Pelletier 1990), the most common type of junction observed between all elements of the seminiferous epithelium was the adhering junction. In the ostrich these junctions were obvious between Sertoli cells, particularly in the region between the basal and adluminal compartments of the seminiferous epithelium. This region was also characterized by numerous focal tight junctions (see below) and complex interdigitations of the Sertoli cell membranes. The presence of inter-Sertoli cell adhering junctions in birds contrasts with the report that this type of junction is infrequently seen between Sertoli cells in mammals (Russell & Peterson 1985).

It is widely accepted that in mammals the major anatomical component of the BTB is the series of tight junctional complexes formed between adjacent Sertoli cells and which separate the basal (spermatogonia) and adluminal (spermatocytes) compartments of the germinal epithelium (Dym & Fawcett 1970; Fawcett *et al.*1970; Russell 1977, 1978; Neaves 1977; Mital, Hinton & Dufour 2011). In the ostrich, focal tight (occluding) junctions and adhering junctions were observed between adjacent Sertoli cells as well as between Sertoli cells and the surrounding germ cells above the level of the spermatogonia. This

pattern of Sertoli-Sertoli cell junctions (tight junctions and adhering junctions) was similar to that reported in the chicken and duck (Osman *et al.* 1980, Pelletier 1990). The role of the tight junctions described above in forming the BTB in birds is based on the observation that vascularly infused lanthanum nitrate does not penetrate beyond the layer of spermatogonia within the seminiferous epithelium (Osman *et al.* 1980). Pelletier (1990) concurred with this observation but additionally was of the opinion that the actual physical barrier was the product of a system of continuous tight junctions (Pelletier 1990). Only focal tight junctions were identified in the present study and in the chicken (Osman *et al.* 1980), presumably because conventional TEM was employed in these studies and not the freeze-fracture replication technique used by Pelletier (1990). In accordance with the above observations, and although the lanthanum nitrate-perfused ostrich tissue did not display optimal ultrastructural detail, it was clear that the tracer was restricted to the intercellular space around the spermatogonia and to a limited degree to the basal aspect of some primary spermatocytes below the region of focal tight junctions. The observation that the tracer sometimes extended for a limited distance beyond the layer of spermatogonia may indicate the existence of an intermediate compartment similar to that described in the fowl (Bergmann & Schindelmeiser 1987). Whereas the tight junctions effectively form the BTB, the interdigitation of Sertoli cell processes, coupled with the adhesion properties of the adhering junctions, would serve to stabilize the dynamically proliferating seminiferous epithelium.

A consistent observation in the ostrich was the presence of long subsurface cisternae of endoplasmic reticulum closely associated with the tight junctions. No obvious connection was apparent between the cisternae and the junctions and the distance between the two structures was variable. Pelletier (1990) reported a similar situation in the fowl and duck. Cytoplasmic organelles have also been seen in close proximity to tight junctions in mammals, although 7nm filaments (Dym & Fawcett 1970; Gilula *et al*. 1976; Pelletier & Friend 1983) are associated with the junctions in addition to the cisternae. For example, the intermittent tight junctions between neighbouring Sertoli cells in the rabbit are flanked by dense intermediate filaments and cisternae of rough endoplasmic reticulum arranged parallel to the membrane surfaces (Sun & Gondos 1986). However, Cooksey and Rothwell

(1973) refer to the cisternae in the fowl as “subsurface cisternal junctions” and consider them to be the structures linking adjacent Sertoli cells. This type of “junction” was also reported by Osman *et al*. (1980) to exclusively link Sertoli cells in the same species. In the ostrich the cisternae were not restricted to inter-Sertoli cell contact, but were also seen between Sertoli cells and spermatocytes and spermatids (see Fig. 6.4). Whether a particular functional association exists between the tight junctions and the cisternae remains undetermined.

Although clearly not part of the BTB, the extensive hemi-desmosome-like junctions attaching the rostral aspect of the acrosome of developing spermatids to the encompassing Sertoli cells were a prominent feature of the ostrich germinal epithelium. A similar phenomenon is apparent in the fowl where it has been classified as a distinct junction (Osman *et al*. 1980). Considering the anchoring role ascribed to hemi-desmosomes, this particular junction may act to stabilize the head of the developing spermatid relative to the surrounding cells.

Based on structural features and the penetration of vascularly-infused lanthanum nitrate, it is concluded that the BTB in the ostrich is composed of a number of components. These include the endothelial lining and basal lamina of the blood vessels; the cellular and acellular layers of peritubular connective tissue; the basal lamina of the seminiferous epithelium and, most prominently, the series of tight junctions located above the layer of spermatogonia. Although influenced by the limitations imposed by conventional TEM, this study showed that the Sertoli and germ cell elements of the testicular parenchyma of the ostrich are linked by intercellular junctions in a similar manner to that previously described in other avian species (Osman *et al.* 1980; Bergmann & Schindelmeiser 1987; Pelletier 1990). Whereas this study revealed similarities regarding the anatomical components of the BTB, it has been emphasized that the BTB is more complex in nature than a mere structural barrier and is also composed of a physiological component (determined by the permeability of the baso-lateral and apical membranes of the seminiferous epithelium to molecules) and an immunological component (through “the involvement of immunomodulatory factors and other immunoregulatory mechanisms”) (Mital *et al*. 2011).

The BEB provides a favourable environment for sperm maturation and survival, protection of maturing spermatozoa from outside influences and excludes many toxic metabolites and toxic environmental agents from entering the epididymal lumen (Hinton 1985).

The material examined in the present study revealed very limited penetration of lanthanum nitrate through the blood capillaries into the peritubular connective tissue, mainly due to the blocking of the tracer by the adhering junctions between adjacent endothelial cells. It is apparent, therefore, that the endothelium of the blood capillaries and arterioles in the epididymis functions as part of the BEB in a similar fashion to the role of blood vessels as part of the BTB (see above). However, it is clear from other studies in mammals and birds that tracer is able to pass across the blood vessel wall and pass between adjacent ductal epithelial cells until blocked by the adluminal apico-lateral junctional complexes (see Introduction). The failure of the lanthanum to pass beyond the endothelium may, in part, be due to the route chosen for injecting the tracer. It is possible that most of the lanthanum injected into the cranial renal artery goes directly to the kidney and that too little reaches the reproductive organs for ideal filling of the vascular bed in the epididymis. The time delay of a number of hours before obtaining the ostrich material from the abattoir is a further exacerbating factor.

This study confirmed the presence of apicolateral junctional complexes (tight junctions and adhering junctions) in the various ducts of the epididymis in the ostrich as previously described (Soley 1992). The junctional complexes were also similar to those reported in the fowl by Nakai & Nasu (1991). In the rat, as in the ostrich, the tight junctions in the duct epithelium form the ultimate structural component of the rat BEB (Hoffer & Hinton 1984). The *Zonulla occludens* also forms a strong barrier to the passage of substances from the ductal lumen into the intercellular space (Friend & Guilula 1972). The tight junctions in the epithelium of the epididymis are the mostly highly developed among the various epithelial cell contacts (Friend & Guilula 1972), and the BEB in some aspects appears to be a more formidable one than the blood-testis barrier (Turner & Howards 1985).

In conclusion, the BEB in the ostrich comprises the subepithelial blood capillaries and arterioles and, although not directly demonstrated in the current tracer study, the adluminal apico-lateral junctional complexes situated in the epithelium of the rete testis, efferent ducts, connecting ducts and epididymal duct. In order to provide definitive information on the true extent of the BEB in the ostrich, a technique for the reliable injection of tracer and/or fixative into the testis and epididymis will have to be devised.

5. REFERENCES

AGRAWAL, A. & HOFFER, A. P. 1989. Ultrastructural studies on the development of the blood-epididymis barrier in immature rats. *Journal of Andrology,* 10: 425-431.

AIRE, T. A. 1997. The structure of the interstitial tissue of the active and resting avian testis. *Onderstepoort Journal of Veterinary Research,* 64: 291-299.

AIRE, T. A. 2002. Cyclical reproductive changes in the non-ciliated epithelia of the epididymis of birds. *Anatomia, Histologia, Embryologia,* 31: 113-118.

AIRE, T. A. & SOLEY, J. T. 2000. The surface features of the epithelial lining of the ducts of the epididymis of the ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 29: 119-126.

AIRE, T. A. & SOLEY, J. T. 2003. The morphological features of the rete testis of the ostrich *(Struthio camelus). Anatomy & Embryology,* 207: 355-361.

AOKI, A. & FAWCETT, D. W. 1975. Impermeability of Sertoli cell junctions to prolonged exposure to peroxidase. *Andrology*, 7: 63-76.

BELLAMY, S. J. & KENDAL, M. D. 1985. The ultrastructure of the epithelium of the *ductuli efferentes testis* in the common starling (*Sturnus vulgaris*). *Journal of Anatomy*, 140: 189-203.

BERGMANN, M. & SCHINDELMEISER, J. 1987. Development of the blood-testis barrier in the domestic fowl (*Gallus domesticus*). *International Journal of Andrology,* 10: 481- 488.

BISHOP, D. W. 1970. Immunological responses of the testis, in *The testis*. Edited by Johnson, A. D., W. R. Gomes and N. L. Van Denmark. Vol. 3. New York: Academic

Press. pp. 41- 66.

BUDRAS, K. D. & SAUER, T. 1975. Morphology of the epididymis of the cock (*Gallus domesticus*) and its effect upon the steroid sex hormone synthesis. I. Ontogenesis, morphology and distribution of the epididymis. *Anatomy & Embryology,* 148**:** 175-196.

BUDRAS, K. D. & MEIER, U. 1981. The epididymis and its development in ratite birds (ostrich, emu, rhea). *Anatomy & Embryology,* 162**:** 281-299.

CAVICCHIA, J. C. & SACERDOTE, F. L. 1988. Topography of the rat blood-testis barrier after intratubular administration of intercellular tracers. *Tissue and Cell,* 20: 577-586.

CHUNG, N. P. Y., MRUK, D., MO, M., LEE, W. M. & CHENG, C. Y. 2001. A 22-amino acid synthetic peptide corresponding to the second extracellular loop of rat occludin perturbs the blood-testis and disrupts spermatogenesis reversibly in vivo. *Biology of Reproduction*, 65**:** 1340-1351.

COOKSEY, E. J. & ROTHWELL, B. 1973.The ultrastructure of the Sertoli cell and its differentiation in the domestic fowl (*Gallus domesticus*). *Journal of Anatomy,* 144: 329-345.

CORNELL, C. J. 1978. A freeze fracture and lanthanum tracer study of the complex junction between Sertoli cells of the canine testis. *Journal of Cell Biology*, 76: 57-75.

CORNELL, C. J. 1980. Blood-testis barrier formation and the initiation of meioses in the dog, in, *Testicular development, structure and function*. Edited by A. Steinberger & E, Steinberger. New York: Raven Press. pp. 71-78.

DYM, M. 1973. The fine structure of the monkey (Macaca) Sertoli cell and its role in maintaining the blood-testis barrier. *The Anatomical Record,* 175: 639-656.

DYM, M. & CAVICCHIA, J. C. 1977. Further observations on the blood-testis barrier in monkeys. *Biology of Reproduction,* 17**:** 390-403.

DYM, M. & CAVICCHIA, J.C. 1978. Functional morphology of the testis. *Biology of Reproduction,* 18: 1-15.

DYM, M. & FAWCETT, D. W. 1970. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biology of Reproduction*, 3: 308-326.

ELIAS, M. Z. J., SOLEY, J. T., AIRE, T. A. & Du Plessis. 2008. Membrane specialization in the efferent ducts of the ostrich epididymis. *Proceedings of the Microscopy Society of Southern Africa*, 47: 76.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2008. Intercellular junctions in the ostrich *(Struthio camelus)* testis. *Proceedings of the 38th Conference of the Anatomical Society of Southern Africa,* Kruger Park, South Africa*:* 97.

FAWCETT, D. W., LEAK, L. V. & HEIDGER, P. M. 1970. Electron microscopy observations on the structural components of the blood-testis barrier. *Journal of Reproduction and Fertility (Supplement)*, 10: 105-122.

FLICKINGER, C. J. 1975. Fine structure of the rabbit epididymis and vas deferens after vasectomy. *Biology of Reproduction*, 13: 50-60.

FRIEND, D. S. & GILULA, N. B. 1972. Variations in tight and gap junctions in mammalian tissue. Journal of *Cell Biology,* 53: 758-776.

GILULA, N. B., FAWCETT, D. W. & AOKI, A. 1976. The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testis. *Developmental Biology*, 50: 142-168.

HINTON, B. T. 1985. The blood-epididymis barrier, in *Male fertility and its regulation*. Edited by T. J. Lobl & E. S. E. Hafez. Dordrecht: MPT Press. pp. 371-393.

HINTON, B. T. & PALLADINO, M. A. 1995. Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. *Microscopy Research and Technique*, 30: 67-81.

HOFFER, A. P. & HINTON, B. T. 1984. Morphological evidence for a blood-epididymal barrier and the effects of gossypol on its integrity. *Biology of Reproduction,* 30**:** 991-1004.

HOWARD, S. S., JESSE, S. J. & JOHNSON, A. L. 1976. Micropuncture studies of the blood-seminiferous tubule barrier. *Biology of Reproduction*, 14: 164-269.

JOHNSON, M. H. 1969. The effect of cadmium chloride on the blood-testis barrier of the guinea pig. *Journal of Reproduction and Fertility*, 19: 551-553.

JOHNSON, M. H. 1970. Changes in the blood-testis barrier of the guinea-pig in relation to histological damage following iso-immunization with testis. *Journal of Reproduction and Fertility*, 22: 119-127.

LACKGREN, G. & PLÖEN, L. 1984. The morphology of the human undescendent testis with special reference to the Sertoli cell and puberty. *International Journal of Andrology,* 7: 23-38.

LANDON, G. V. & PRYOR, J. P. 1981. The blood-testis barrier in man of diverse fertility status: An ultrastructural study. *Virchows Archives (Pathology Anatomy),* 392: 355-364.

LOPEZ, M. L., FUENTES, P., RETAMAL, C. & de SOUZA, W. D. 1997. Regional differentiation of the blood-epididymis barrier in stallion (*Equus caballus). Journal of Submicroscopic Cytology and Pathology,* 29: 353-363.

MAIN, S. J. & WAITES, G. M. H. 1977. The blood-testis barriers and temperature damage to the testis of the rat. *Journal of Reproduction and Fertility*, 51: 439-450.

MITAL, P., HINTON, B.T. & DUFOUR, J.M. 2011. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biology of Reproduction*, 84: 851-858.

NAGANO, T. & SUZUKI, F. 1976. The postnatal development of the junctional complexes of the mouse Sertoli cells as revealed by freeze-fracture. *The Anatomical Record,* 185: 403-418.

NAKAI, M. & NASU, N. 1991. Ultrastructural study on junctional complexes of the excurrent duct epithelia in the epididymal region in the fowl. *Journal of Veterinary Medicine Science,* 53: 677-677.

NAKAI, M., HASHIMOTO, Y., KITAGAWA, H., KON, Y. & KUDO, N. 1988. Microvasculature of the epididymis and ductus deferens of domestic fowls. *Japanese Journal of Veterinary Science,* 50: 371- 381.

NEAVES, W. B., 1977. The blood-testis barrier, in *The testis*. Edited by A. D. Johnson & W. R. Gomes. Vol. 4. New York: Academic Press. pp. 125-162.

NOGUCHI, J., TOYAMA, Y., YUASAT, S., KIKUCHI, K. & KANEKO, H. 2002. Hereditary defects in both germ cells and the blood-testis barrier system in as-mutant rats: evidence from spermatogonial transplantation and tracer-permeability analysis. *Biology of Reproduction,* 67: 880-888.

OSMAN, D. I. 1978. The ultrastructure of rete testis and its permeability barrier before and after efferent ductuli ligation. *International Journal of Andrology*, 1**:** 357-370.

OSMAN, D. I. & PLÖEN, L. 1978. The terminal segment of the seminiferous tubules and the blood testis barrier before and after efferent ductule ligation in rat. *International Journal of Andrology,* 1: 235-249.

OSMAN, D. I., EKWALL, H. & PLÖEN, L. 1980. Specialized cell contacts and blood-testis barrier in seminiferous tubules of the domestic fowl (*Gallus domesticus*). *International Journal of Andrology,* 3: 553-562.

OZEGBE, P., AIRE, T. A. & SOLEY, J. T. 2006 a. The morphology of the efferent ducts of the testis of the ostrich, a primitive bird. *Anatomia, Embryologia, Histologia,* 211: 559-565.

OZEGBE, P., AIRE, T. A. & SOLEY, J. T. 2006 b. The epididymal duct unit of the ostrich (*Struthio camelus*). *Proceedings of the 36th Annual Conference of the Anatomical Society of Southern Africa.* Golden Gate. South Africa, 74.

PELLETIER, R. M. 1986. Cyclic formation and decay of the blood-testis barrier in the mink (*Mustela vison*), a seasonal breeder. *American Journal of Anatomy*, 175: 91-117.

PELLETIER, R. M. 1988. Cyclic modulation of Sertoli cell junctional complexes in a seasonal breeder: The mink (*Mustela vison*). *American Journal of Anatomy*, 183: 68-102.

PELLETIER, R. M. 1990. A novel perspective. The occluding zonule encircles the apex of the Sertoli cell as observed in birds. *American Journal of Anatomy,* 188: 87-108.

PELLETIER, R. M. & FRIEND, D. S. 1983. The Sertoli cell junctional complex: structure and permeability to Filipin in neonatal and adult guinea pigs. *American Journal of Anatomy,* 168: 213-228.

PELLETIER, R. M. & FRIEND, D. S. 1986. Sertoli cell junctional complexes in gossypol-treated neonatal and adult guinea pigs. *Journal of Andrology*, 7:127-139.

PELLETIER, R. M. & SHIVERS, R. R. 1986. Filipin-sterol complexes in breeding and non-breeding mink (*Mustela vison*) Sertoli cell junctional membranes. *Journal of Cell Biology,* 103: 365.

PELLETIER, R. M., NEMIROVSKY, M. S., CALVERT, R. & HUGON, J. S. 1981. Effects of immunization with Freund's complete adjuvant and isologous spermatozoa on the seminiferous epithelium and blood-testis barrier in guinea pigs.*The Anatomical Record*, 199: 197-211.

PFEIFFER, D. C. & VOGL, A. W. 1993. Ectoplasmic junctional specializations in Sertoli cells of the rooster and turtle: evolutionary implications. *The Anatomical Record,* 235: 33-50.

PLÖEN, L., HAGENS, L., RITZÉN, E. M. & NARÉN, S. 1976. Morphological demonstration of the blood-testis barrier in human testicular biopsies. *Journal of Ultrastructure and Research,* 57: 228.

ROSS, H. M. 1977. Sertoli-Sertoli junctions and Sertoli-spermatid junctions after efferent ductule ligation and lanthanum treatment. *American Journal of Anatomy*, 148: 49-56.

ROTHWELL, B. 1975. Designation of the cellular component of the peritubular boundary tissue of the seminiferous tubule in the testis of the fowl (*Gallus domesticus*). *British Poultry Science,* 16: 527-529.

RUSSELL, L. 1977. Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. *American Journal of Anatomy*, 148: 313-328.

RUSSELL, L.D. 1978. The blood-testis barrier and its formation relative to spermatocyte maturation in the adult rat: a lanthanum tracer study. *Anatomical Record*, 190: 99-111.

RUSSELL, L.D. & PETERSON, R.N. 1985. Sertoli cell junctions: Morphological and functional correlates. *International Review of Cytology,* 94: 177-211.

SETCHELL, B. P. 1967. The blood-testicular barrier fluid in sheep. *Journal of Physiology,* 189: 63-65.

SETCHELL, B. P. & WAITES, G. M. H. 1975.The blood-testis barrier, in *Handbook of physiology. Endocrinology. Male reproduction system*. Vol. V. Edited by R. O. Greep, E. B. Astwood, D. Hamilton, R. O. Greep & S. R. Geiger. Washington: American Physiological Society. pp. 143-172.

SETCHELL, B. P., VOGLMAYR, J. F. & WAITES, G. M. H. 1969. A blood-testis barrier restricting passage from blood into rete testis fluid but not into lymph. *Journal of Physiology*, 200: 73-85.

SETCHELL, B. P., LAURIE, M. S. & FRITZ, I. B. 1980. Development of the function of the blood-testis barrier in rats and mice, in *Testicular development, structure, and function.* Edited by A. Steinberger & E. Steinberger. New York: Raven Press. pp. 650-669.

SOLEY, J. T. 1990. Ultrastructural features of the boundary tissue of the seminiferous tubule of the ostrich (*Struthio camelus*). *South African Journal of Science,* 86: 163.

SOLEY, J. T. 1992. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). PhD thesis. University of Pretoria, Pretoria, South Africa.

SOLEY, J. T. 1997. The morphology of the testicular capsule of the ostrich (*Struthio camelus*). *Proceedings of the Microscopy Society of Southern Africa, 27:109.*

SOLEY, J. T., VAN WILPE, E., AIRE, T. A. & OZEGBE, P. C. 2005. The morphology of the seminiferous tubules in the three-day-old ostrich chicks. *Proceedings of the 35th Annual Conference of the Anatomical Society of Southern Africa*, East London: 60.

SUN, E. L. & GONDOS, B. 1986. Formation of the blood-testis barrier in the rabbit. *Cell and Tissue Research,* 243: 575-578.

SUZUKI, F. & NAGANO, T. 1978a. Development of tight junctions in the caput epididymal epithelium of the mouse. *Developmental Biology,* 63: 321-334.

SUZUKI, F. & NAGANO, T. 1978b. Regional differentiation of cell junctions in the excurrent duct epithelium of the rat testis as revealed by freeze-fracture. *The Anatomical Record,* 191: 503-520.

TINGARI, M. D. 1971a. On the structure of the epididymal region and *ductus deferens* of the domestic fowl (*Gallus domesticus*). *Journal of Anatomy,* 109: 423-435.

TINGARI, M. D. 1971b. The fine structure of basal cells in the male reproductive tract of the domestic fowl. *Journal of Anatomy*, 110: 167-169.

TOYAMA, Y., MAEKAWA, M. & YUASA, S. 2003. Ectoplasmic specializations in the Sertoli cell: new vistas based on genetic defects and testicular toxicology. *Anatomical Science International*, 78: 1-16.

TUNG, K. S. K. & ALEXANDER, N. J. 1977. Auto immune reaction, in *The testis.* Edited by A. D. Johnson & W. R. Gomes. Vol. 4. New York: Academic Press. pp. 491-516.

TURNER, T. T., D'ADDARIO, D. A. & HOWARDS, S. S. 1979. Effects of vasectomy on the blood-testis barrier of the hamster. *Journal of Reproduction and Fertility*, 55: 323-328.

TURNER, T. T., D'ADDARIO, D. A. & HOWARDS, S. S. 1983. The transepithelial movement of ^{3}H-3-O-methyl-D-glucose in the hamster seminiferous and caudal epididymidal tubules. *Fertility and Sterility*, 40: 530-535.

TURNER, T. T., COCHRAN, R. C. & HOWARDS, S. S. 1981. Transfer of steroids across the hamster blood-testis and blood-epididymal barriers. *Biology of Reproduction*, 25: 342-348.

TURNER, T. T., GILES, R. D. & HOWARDS, S. S. 1981. Effect of oestradiolvalerate on the rat blood-testis and blood-epididymal barriers to [^{3}H]inulin. *Journal of Reproduction and Fertility*, 63: 355-358.

TURNER, T. T. & HOWARDS, S. S. 1985. The tenacity of the blood-testis and blood-epididymal barriers, in *Male fertility and its regulation*. Edited by T. J. Lobl & E. S. E. Hafez. Dordrecht: MPT Press. pp.383-393.

VITALE, R., FAWCETT, D. W. & DYM, M. 1973.The normal development of the blood-testis barrier and the effects of clomiphene and estrogen treatment.*The Anatomical Record,* 176: 333-343.

YAN, H. H. N. & CHENG, C. Y. 2005. Blood-testis barrier dynamics are regulated by an engagement / disengagement mechanism between tight and adherens junctions via peripheral adaptors. *Cell Biology*, 102: 11722-11727.

WAITES, G. M. H. & GLADWELL, R. T. 1982. Physiological significance of fluid secretion in the testis and blood-testis barrier. *Physiology Review*, 62: 624-671.

WEBER, J. E., TURNER, T., TUNG, K. S. K. & RUSSEL, L. D. 1988. Effects of cytochalasin D on the integrity of the Sertoli cell (blood-testis) barrier. *American Journal of Anatomy*, 182: 130-147.

6. FIGURES

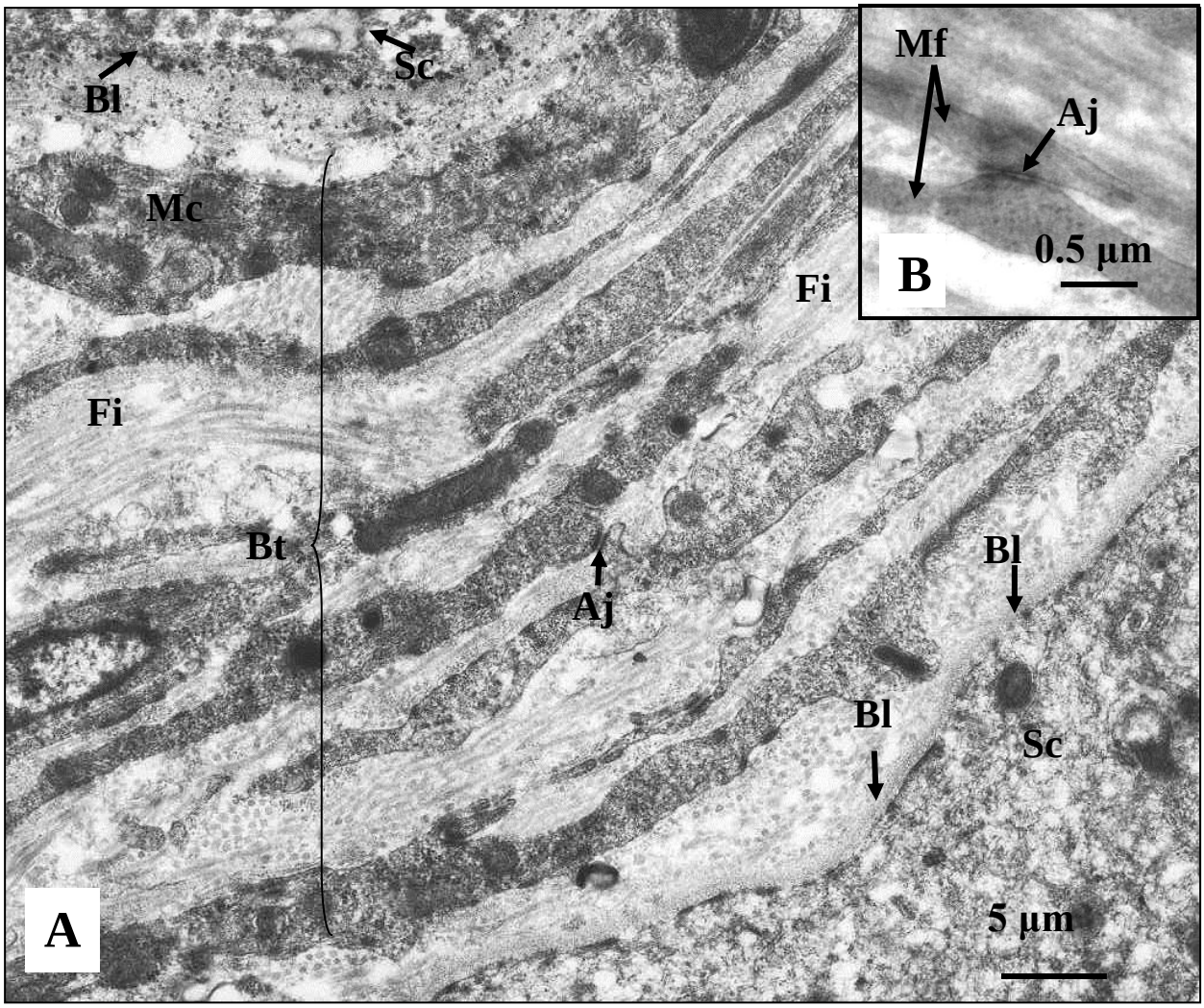

Figure 6.1. A. A survey transmission electron micrograph (TEM) showing the boundary tissue of the testis (Bt) composed of alternating layers of myofibroblasts and accelular lamellae. Adhering junction (Aj), Sertoli cells (Sc), basal lamina (Bl), myoid cell (Mc) and collagen fibres (Fi). Inset **B** shows an adhering junction (Aj) between adjacent myofibroblasts (Mf)**.**

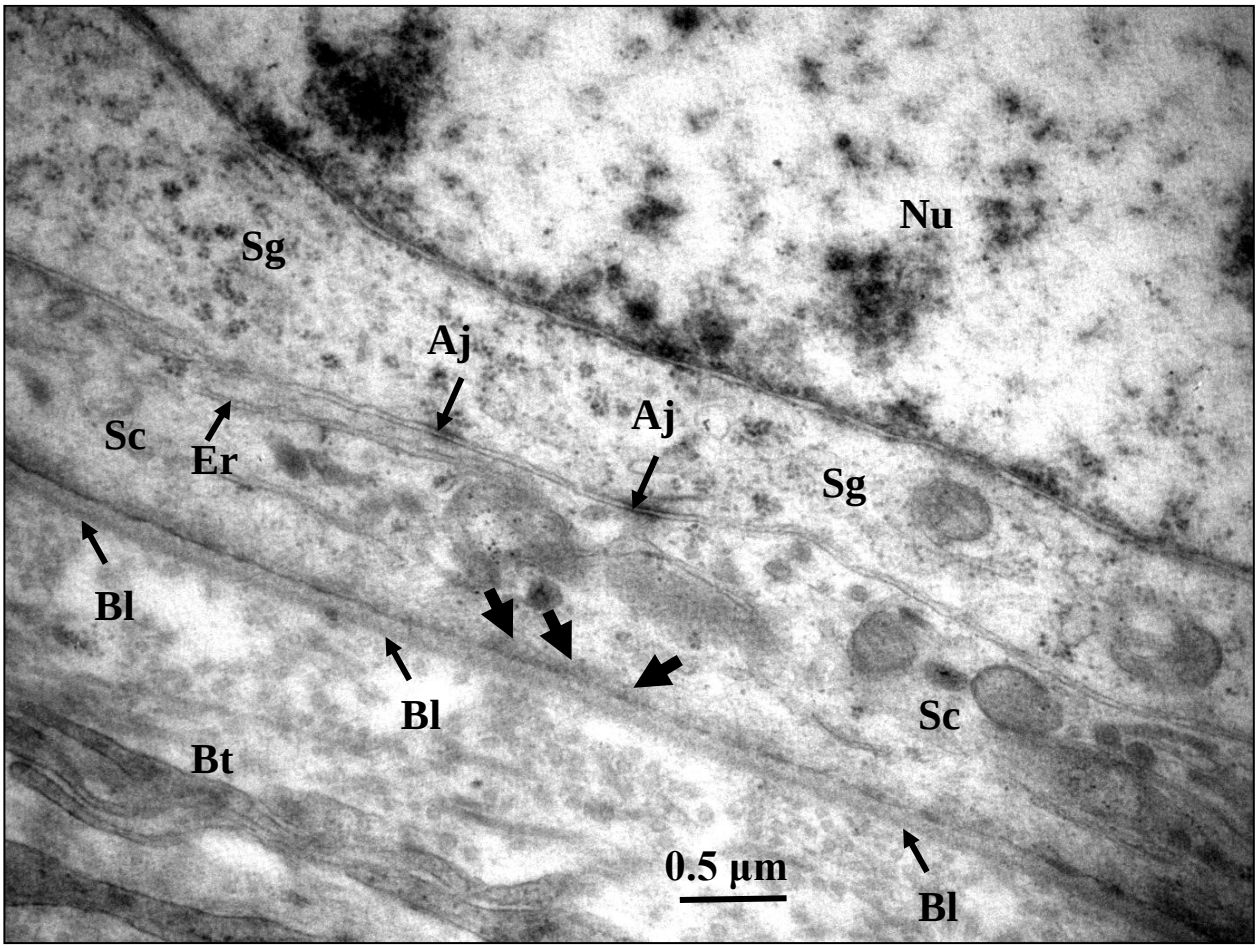

Figure 6.2. TEM demonstrating adhering junctions (Aj) between the cytoplasmic extension of a Sertoli cell (Sc) and a spermatogonium (Sg). Rudimentary hemi-desmosomes (thick arrows) attach the extension to the underlying basal lamina (Bl). Note the dilated subsurface cisternae of endoplasmic reticulum (Er) in the cytoplasm of the Sertoli cell and the nucleus of the spermatogonium (Nu). Boundary tissue (Bt).

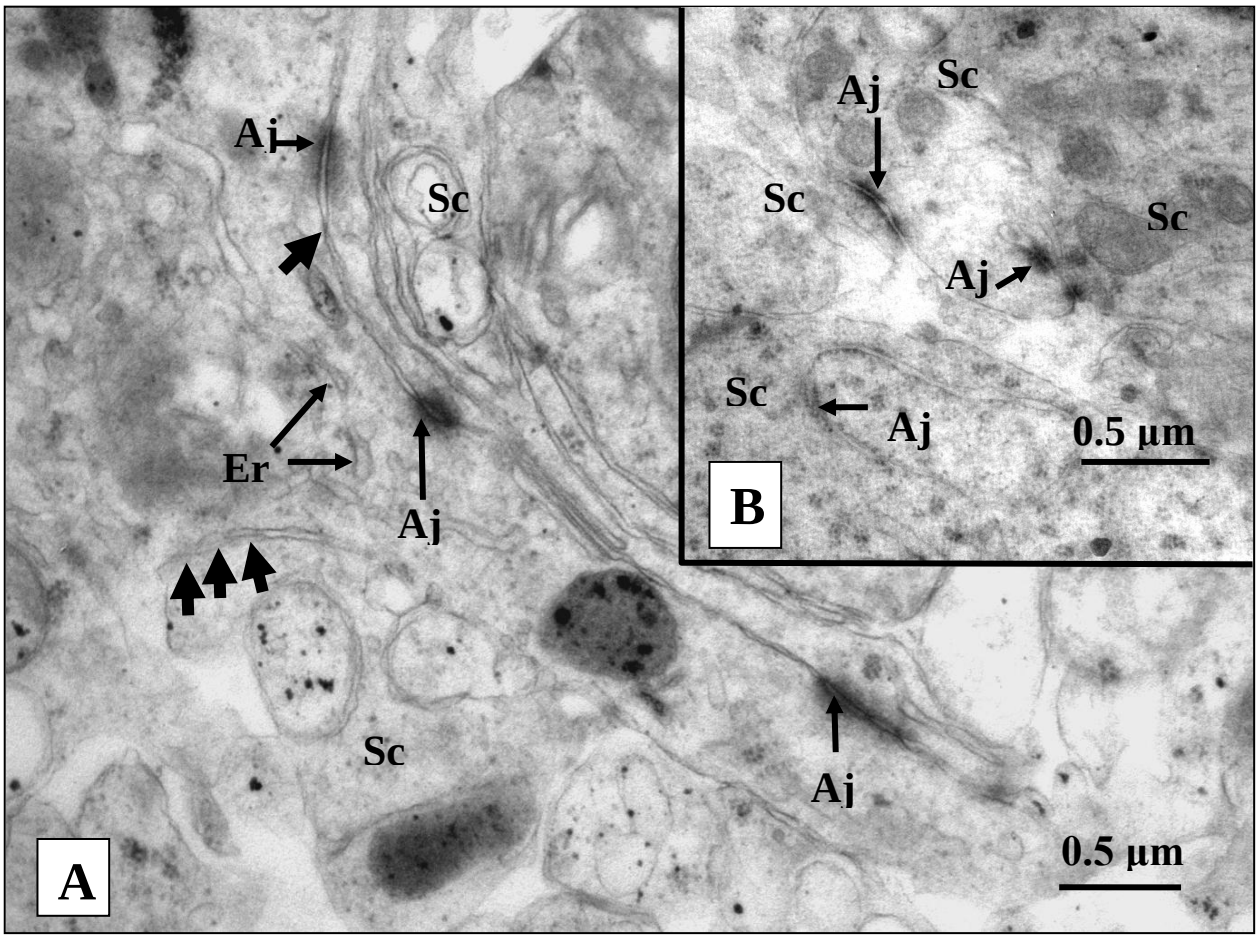

Figure 6.3. A. Adjacent Sertoli cells (Sc) in the adluminal region of the seminiferous epithelium exhibiting complex interdigitation of the cell membranes and a collection of both adhering junctions (Aj) and tight junctions (thick arrows). Note the profiles of smooth endoplasmic reticulum (Er). **B**. A similar region to that shown in Fig. 6.3A but demonstrating only adhering junctions (Aj) between the Sertoli cells (Sc).

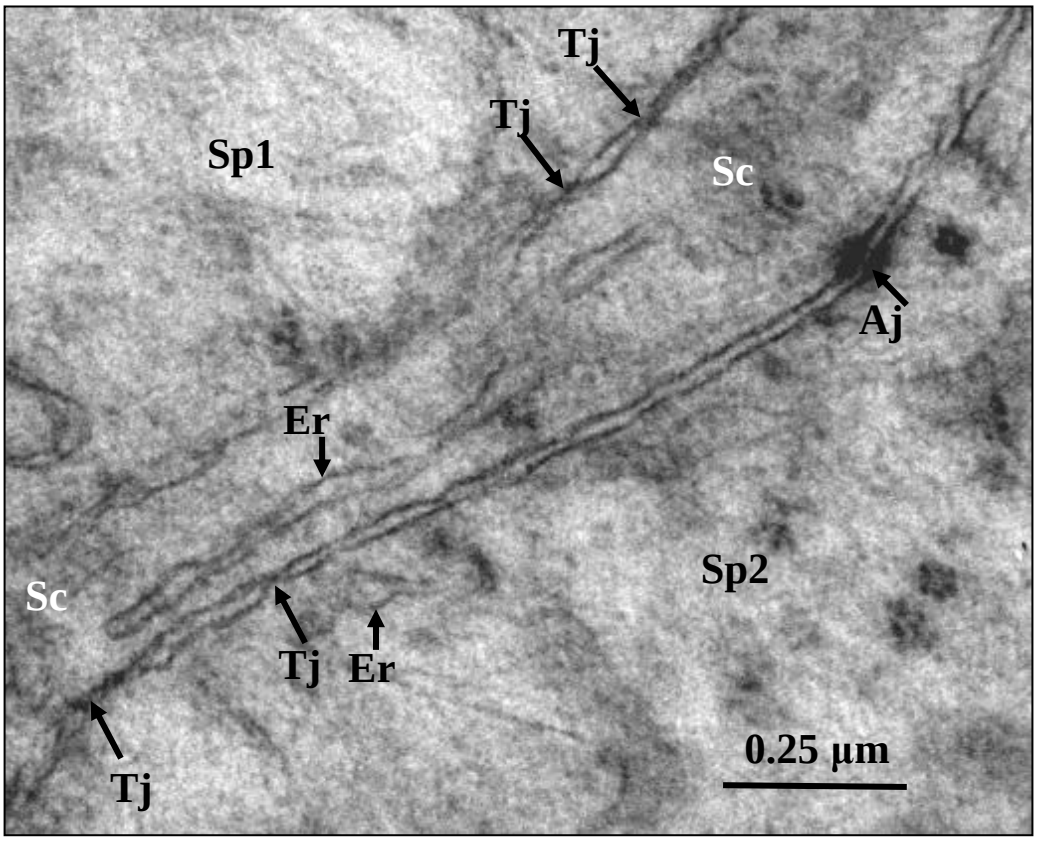

Figure 6.4. Focal tight (occluding) junctions (Tj) and adhering junctions (Aj) between a Sertoli cell (Sc) and two adjoining spermatocytes (Sp1 and Sp2). Note the dilated subsurface cisternae of endoplasmic reticulum (Er) in close proximity to the tight junctions in the cytoplasm of the Sertoli cell and one of the spermatocytes (Sp2).

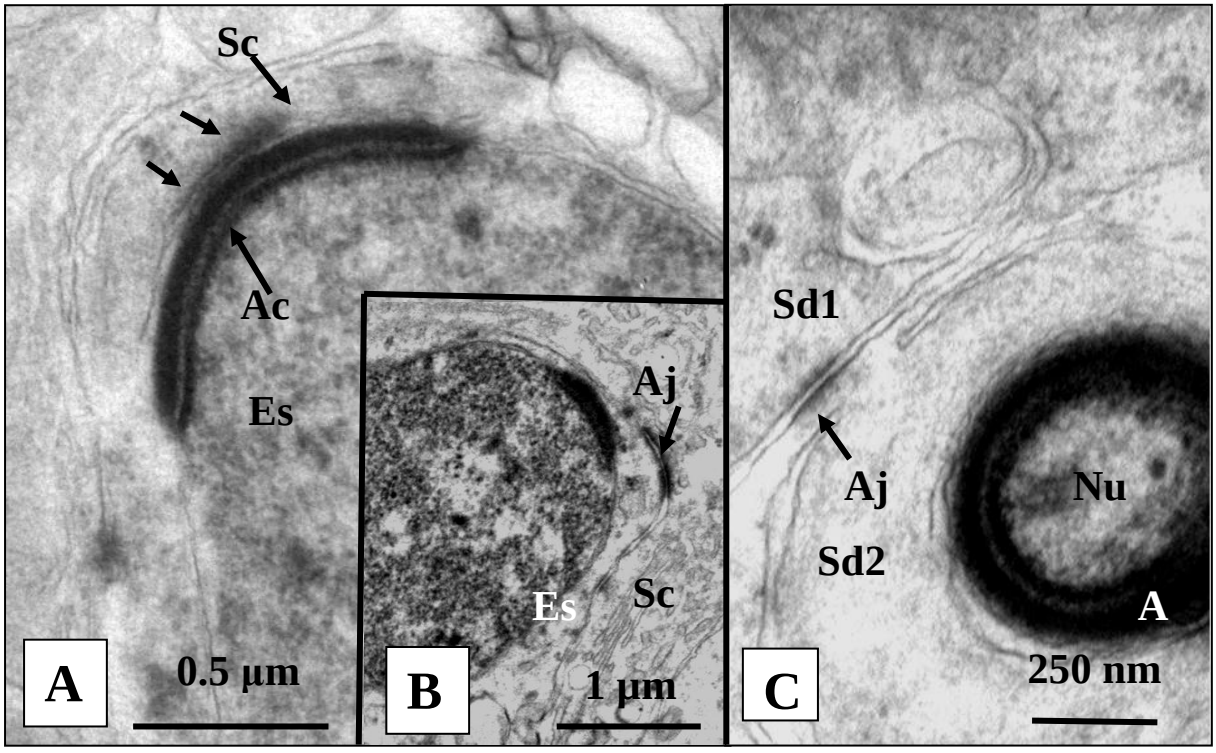

Figure 6.5. A. A hemi-desmosome-like junction (arrows) situated at the rostral aspect of the forming acrosome (Ac) of an early stage elongating spermatid (Es). The dense material is restricted to the Sertoli cell (Sc) cytoplasm. **B.** A series of adhering junctions (Aj) between a Sertoli cell (Sc) and a later stage elongating spermatid (Es). **C**. An adhering junction (Aj) between two adjoining spermatids (Sd1 and Sd2).Sd2 demonstrates a transverse section through the nucleus (Nu) and forming acrosome (A).

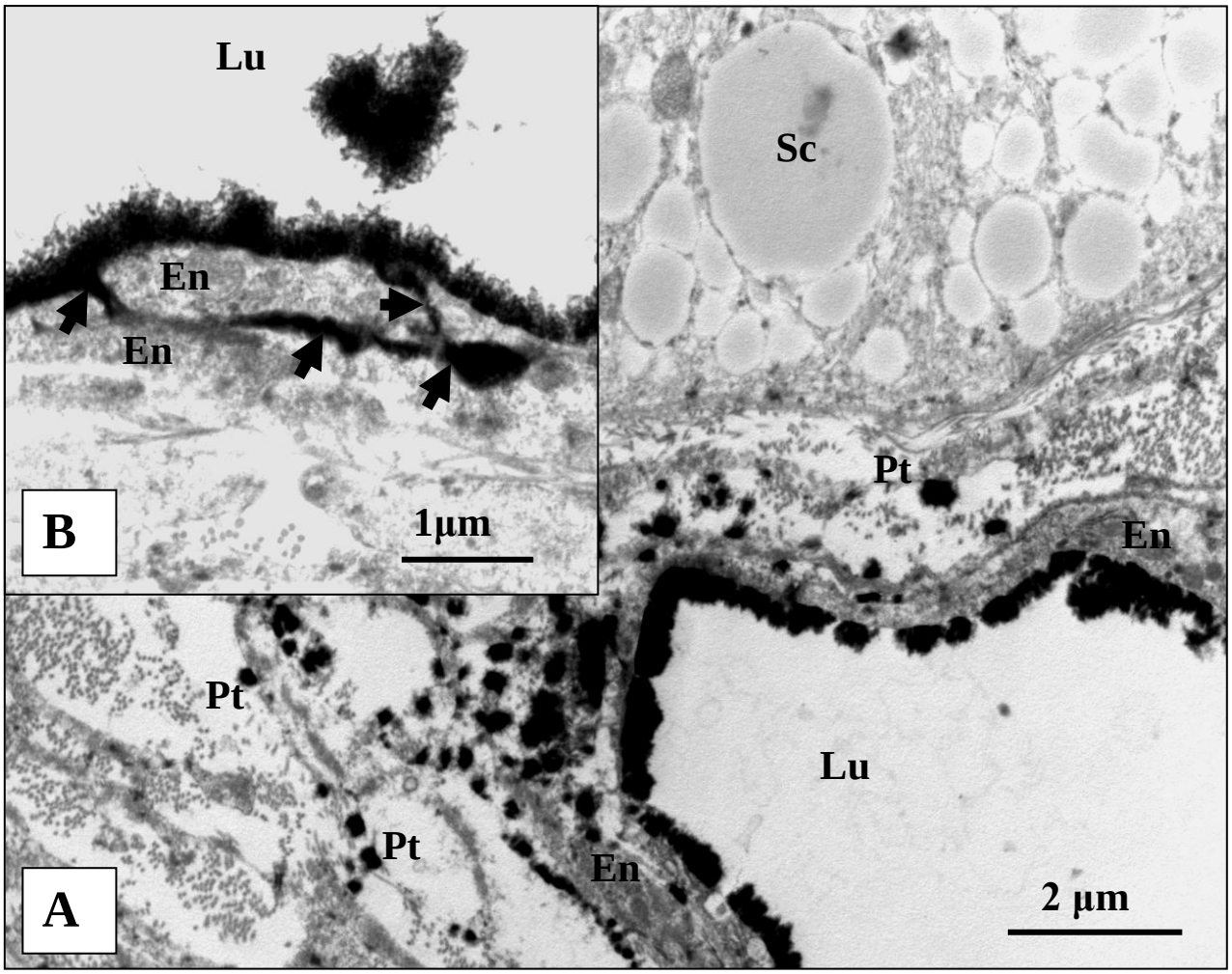

Figure 6.6. A. TEM of a blood capillary in the testis interstitium demonstrating the concentration of lanthanum nitrate on the luminal surface of the endothelium (En) and in the surrounding peritubular tissue (Pt). Capillary lumen (Lu), Sertoli cell (Sc) with lipid droplets. **B**. Part of the wall of an interstitial blood capillary showing overlapping processes of endothelial cells (En) with tracer (thick arrows) trapped between them. Note the concentration of the tracer on the luminal (Lu) surface of the endothelial cells.

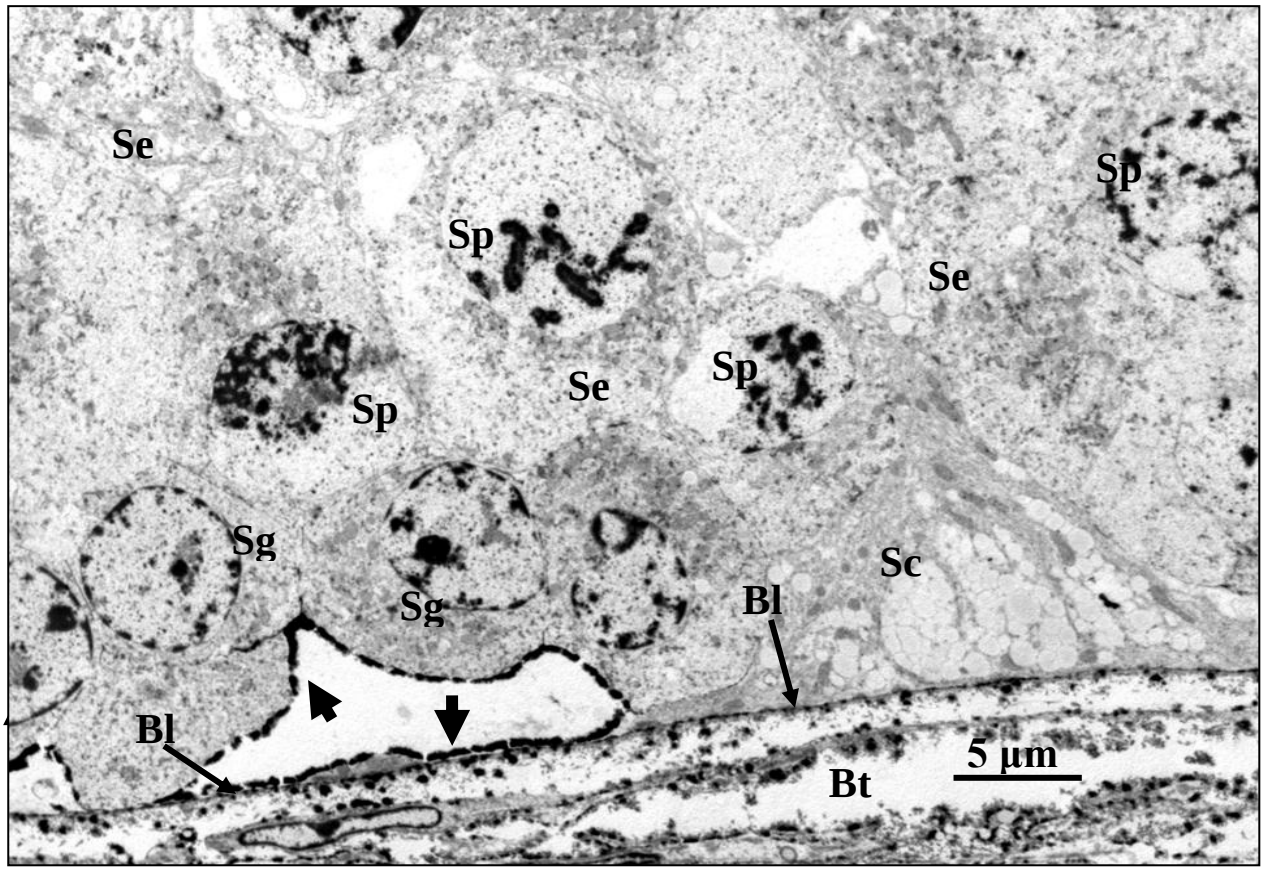

Figure 6.7. A low-power TEM illustrating the distribution of lanthanum nitrate in the boundary tissue (Bt) and base of the seminiferous epithelium (Se). Note the accumulation of the tracer against the basal lamina (Bl) and around a compartment vacated by a spermatogonium (thick arrows).Spermatogonia (Sg), Sertoli cell (Sc), Primary spermatocytes (Sp).

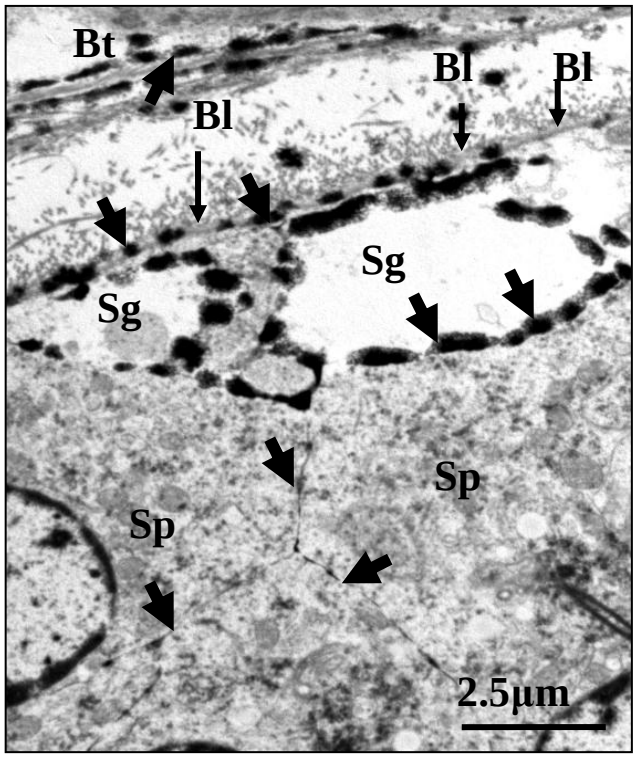

Figure 6.8. A TEM demonstrating localization of lanthanum nitrate (thick arrows) in the boundary tissue (Bt), the basal lamina (Bl) and around the perimeter of the basal compartment of the seminiferous epithelium. The structure of the spermatogonia (Sg) has not been preserved. Pockets of the tracer are also obvious between adjacent primary spermatocytes (Sp).

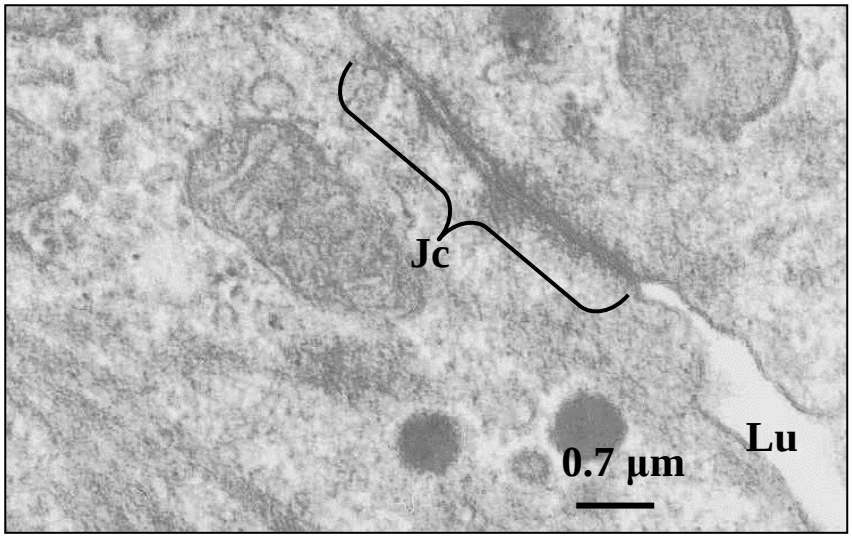

Figure 6.9. Transmission electron micrograph of two adjacent cells in the rete testis exhibiting a typical apical junctional complex (Jc).The plane of section does not allow identification of the individual components of the complex. Duct lumen (Lu).

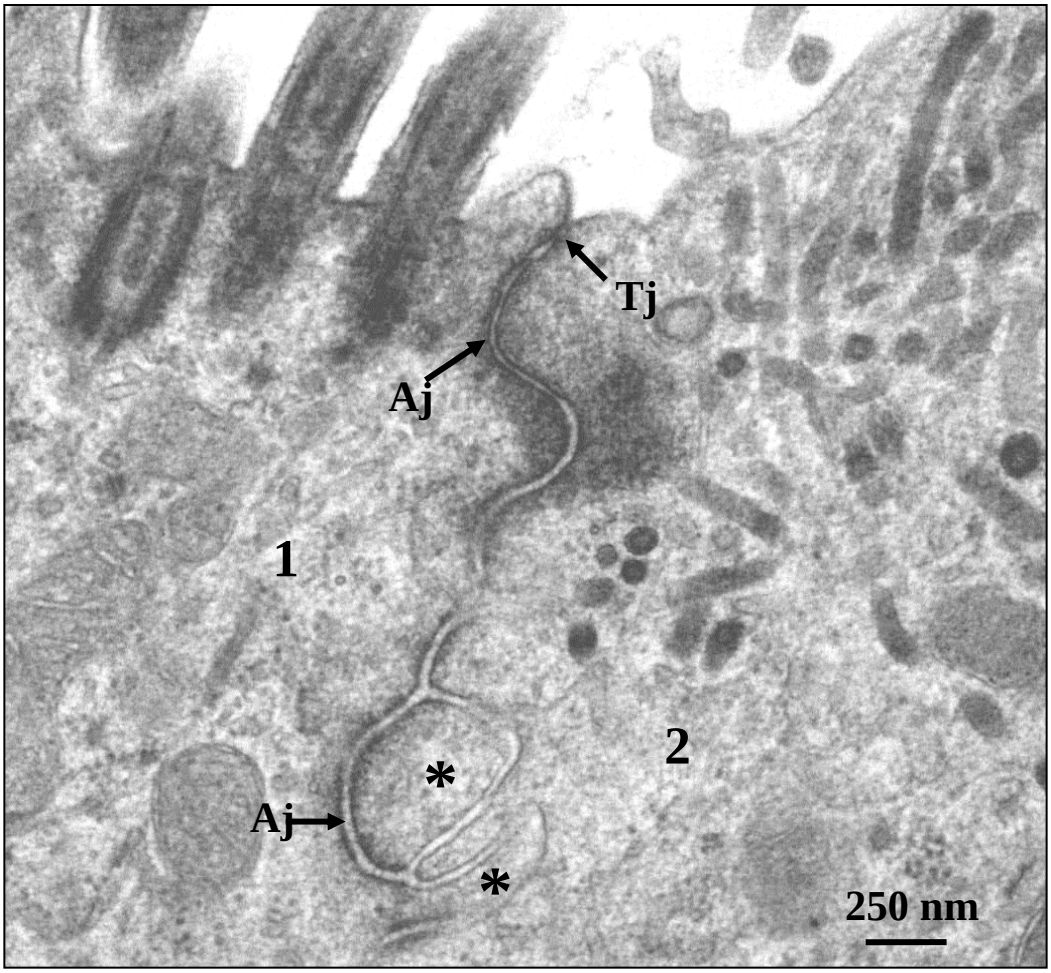

Figure 6.10. An apico-lateral junctional complex between a ciliated cell (1) and a non-ciliated cell (2) in the proximal efferent duct showing focal tight junctions (Tj) and adhering junctions (Aj). Note the interdigitating lateral cell membranes (asterisks).

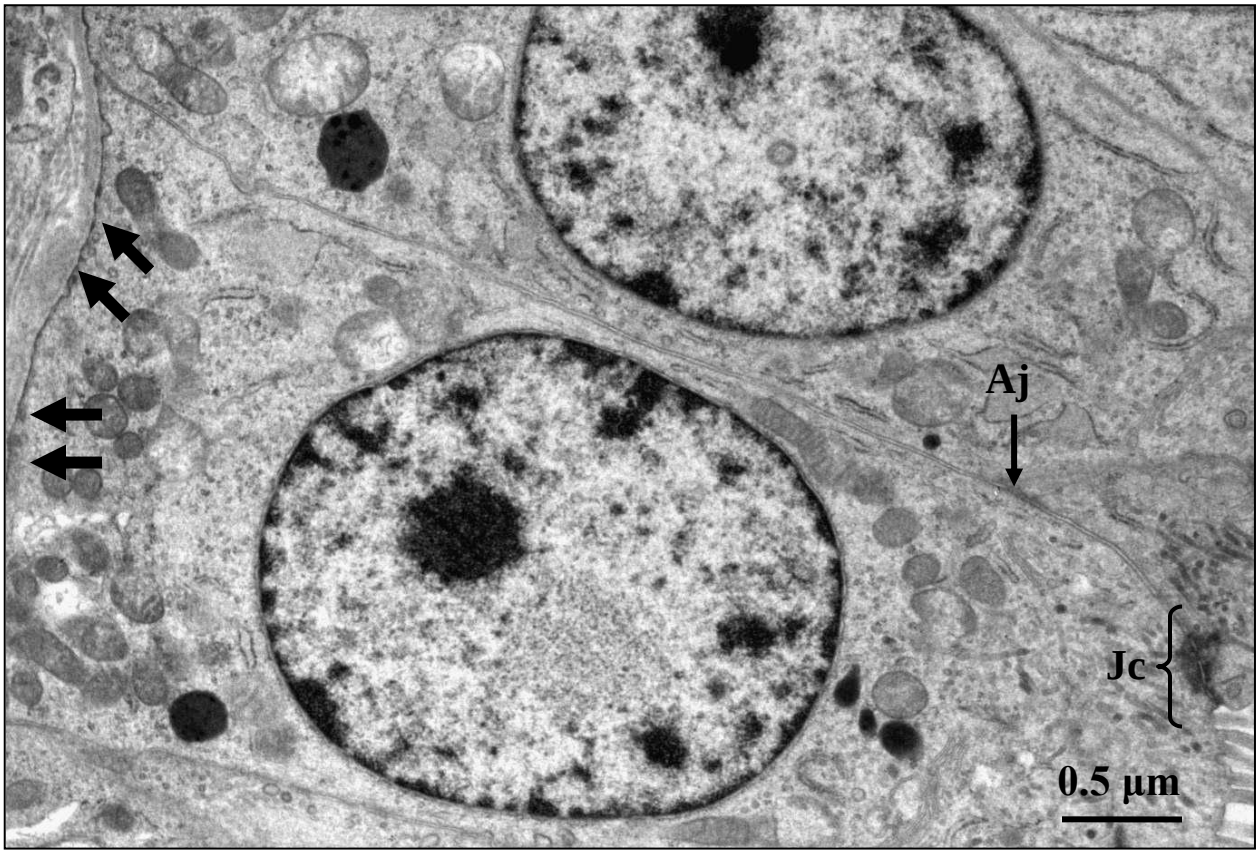

Figure 6.11. A low magnification transmission electron micrograph of the distal efferent duct showing the distribution of the various inter-cellular junctions. The apico-lateral junctional complex (Jc) is situated at the luminal aspect of the cells while the cell base is attached by hemi-desmosomes (thick arrows). Scattered adhering junctions (Aj) link the lateral cell membranes.

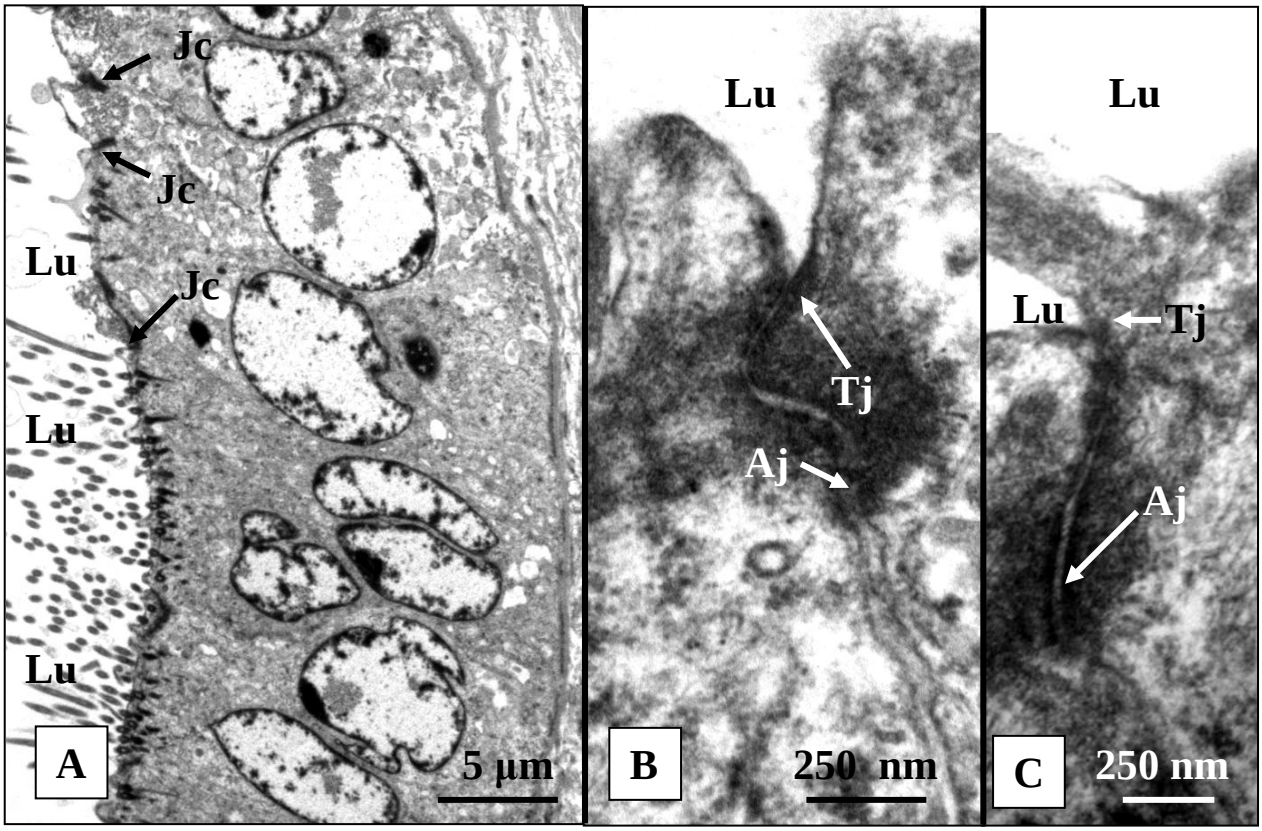

Figure 6.12. A. A low power electron micrograph of a connecting duct, showing the lumen (Lu) of the epithelium and junctional complexes (Jc). **B.** A higher power view of the apical part of the epithelium abeneath the lumen (Lu) showing a tight junction (Tj), and adhering junctions (Aj). **C**. A higher magnification of the apicolateral junctional complex in "B" displaying a tight junction (Tj), below the lumen (Lu), and an adhering junction (Aj) between two epithelial cells.

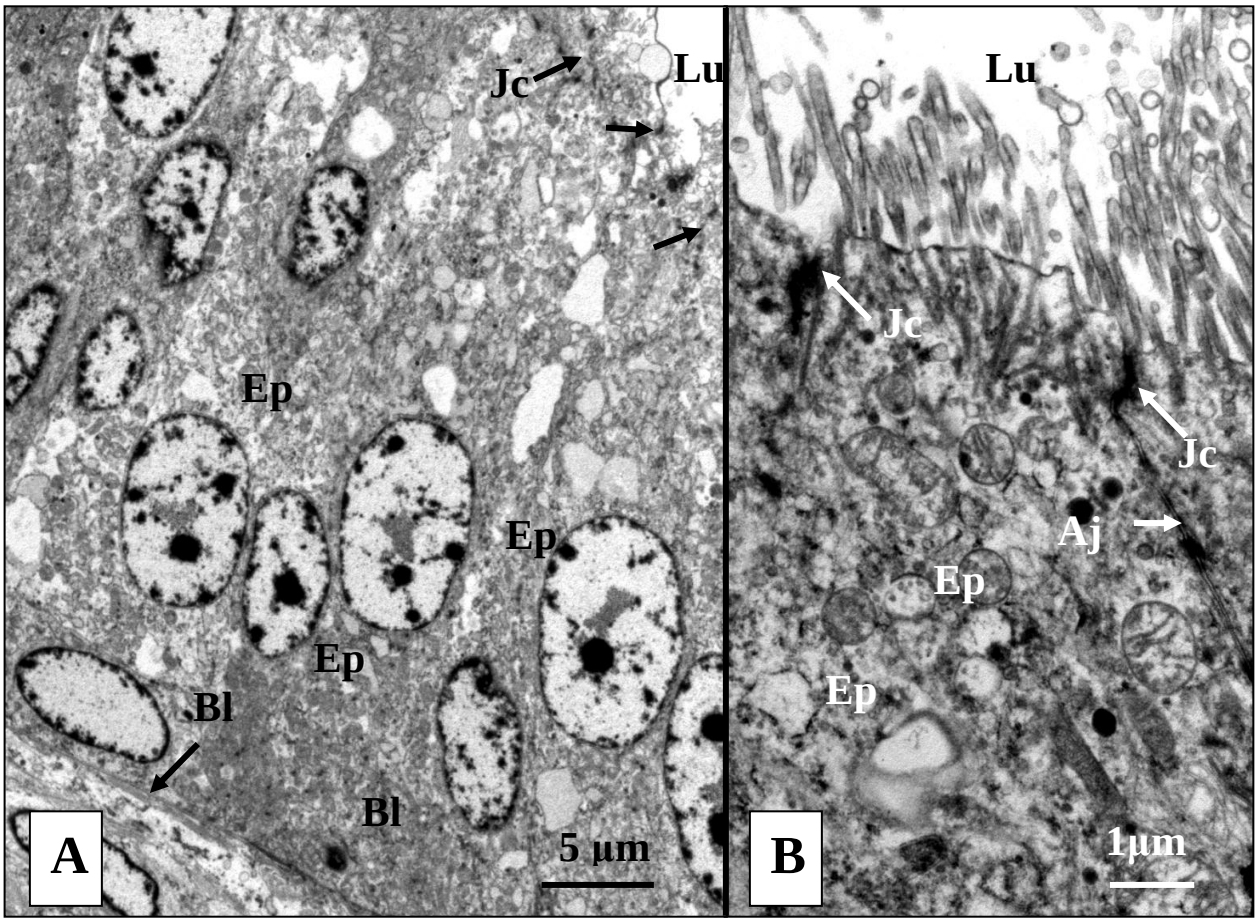

Figure 6.13. A. An electron micrograph of the epididymal duct epithelium (Ep) showing the lumen (Lu) and ad-luminal junctional complexes (Jc). Basal lamina (Bl). **B.** A higher magnification of the ad-luminal epithelium (Ep) displaying tight junctions in the junctional complex (Jc). Adhering junctions (Aj) are visible distally. Lumen (Lu).

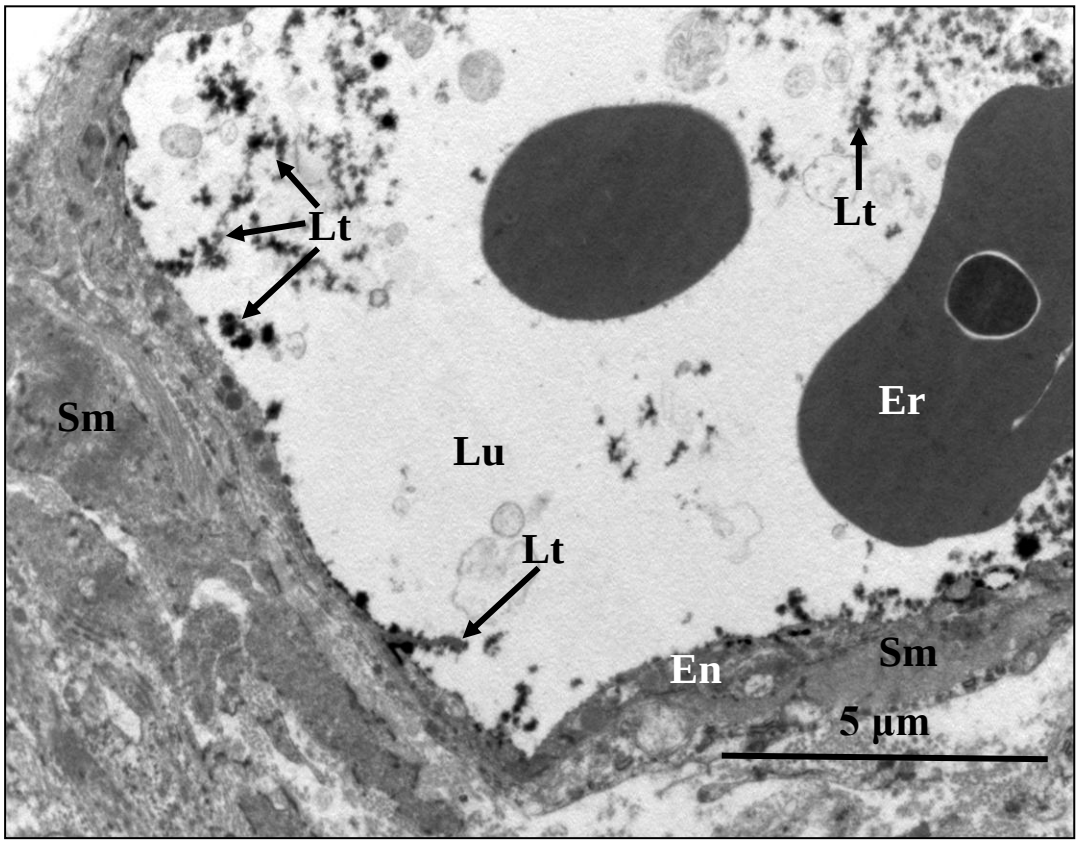

Figure 6.14. Transmission electron micrograph of an arteriole beneath a connecting duct in the epididymis. Lanthanum nitrate (Lt) is seen concentrated at the periphery of the lumen (Lu) and on the endothelial (En) surface. Erythrocyte (Er), smooth muscle cells (Sm).

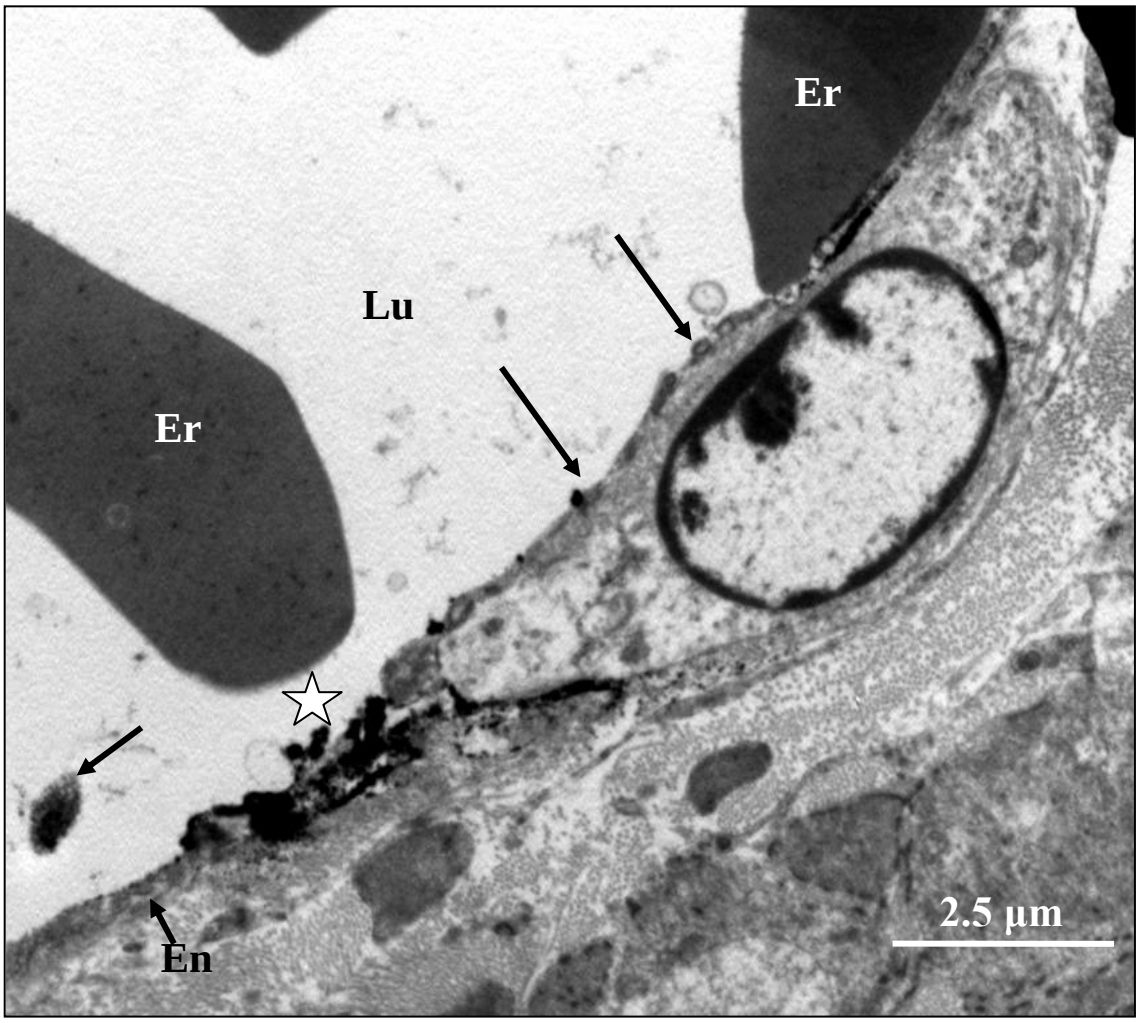

Figure 6.15. An arteriole in the vicinity of a connecting duct showing limited clumping of lanthanum nitrate on the luminal surface (arrows). The tracer is concentrated at a region of endothelial cell overlapping (star). Vascular lumen (Lu), endothelium (En), erythrocytes (Er).

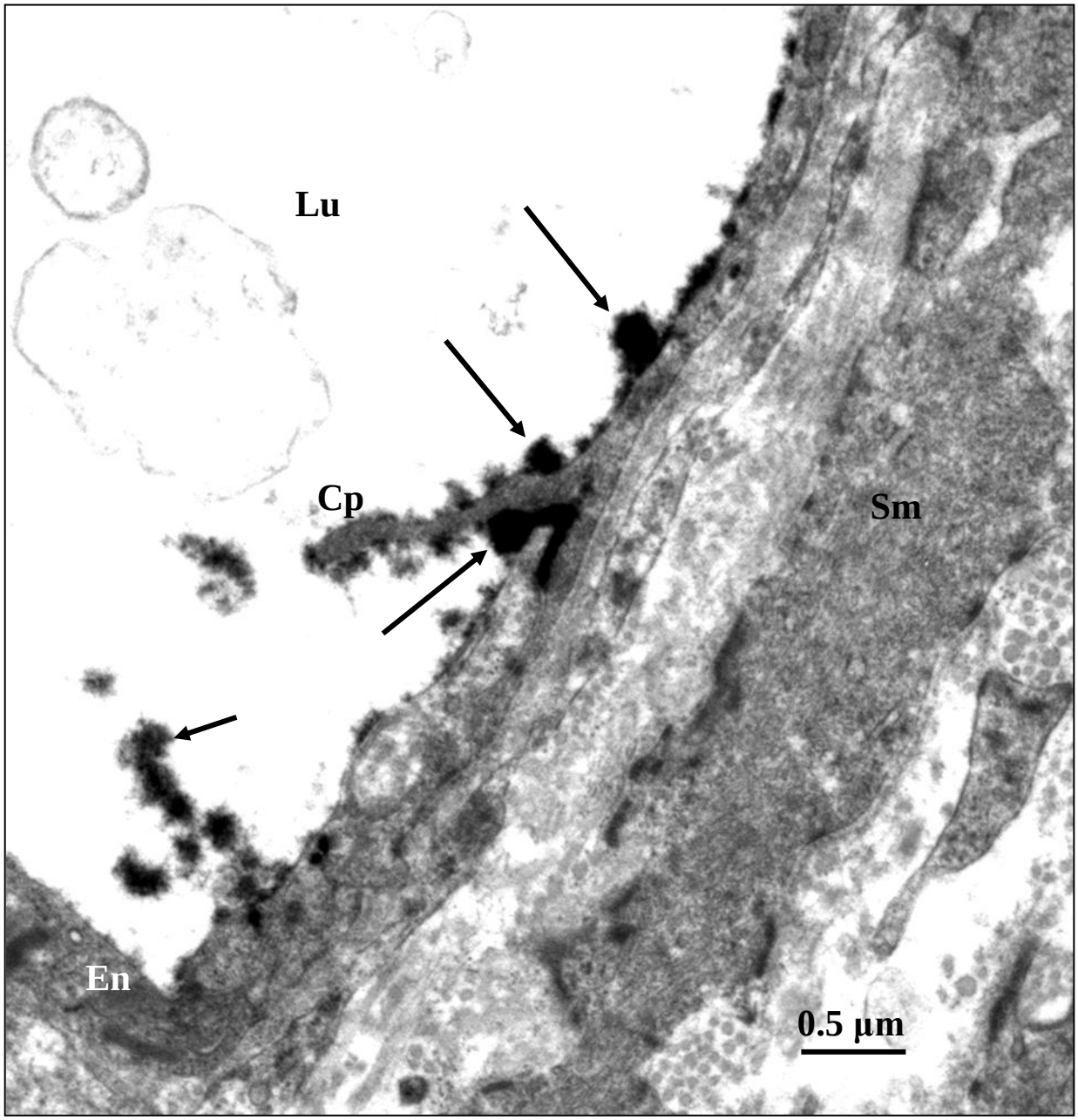

Figure 6.16. Enlargement of the arteriolar wall to show clumping of the tracer (arrows) on the luminal surface of the endothelium (En). A cytoplasmic process (Cp) of the endothelial cell marks an area of intercellular overlapping marked by an accumulation of the tracer. A smooth muscle cell (Sm) lies in close attendance. Lumen (Lu).

CHAPTER 7

GENERAL CONCLUSIONS

The following synopsis presents the main findings of this investigation into the characteristics of the blood vascular system of the reproductive organs of the sexually mature and active male ostrich, *Struthio camelus*.

1. Macroscopic features of the vascular system of the reproductive tract

1.1 The testis, epididymis and cranial segment of the deferent ducts were supplied by left and right cranial renal arteries that arose from the aorta, between the cranial divisions of the kidneys. The testes were additionally supplied by a variable number of accessory testicular arteries that branched directly from the aorta. The cranial portion of the *Ductus deferens* and ureter were supplied by the cranial ureterodeferential branch of the cranial renal artery, which ran caudally to anastomose with the middle renal artery.

1.2. The middle portion of the deferent duct was supplied by the middle ureterodeferential branch of the middle renal artery. The middle renal artery was a branch of the common renal artery which originated from the sciatic artery. A number of variations in this general pattern were, however, observed.

1.3. The caudal segment of the deferent duct was supplied by caudal ureterodeferential branches of the pudendal artery. Additionally, branches from the pudendal artery supplied the cloaca and base of the phallus where they formed an arterial network. This represented an important distinctive feature of the ostrich compared to other birds.

1.4. Each testis was drained by 2 to 4 testicular veins. The right testicular veins drained the right testis, epididymis and its appendix to the caudal vena cava and to the right common iliac vein. In contrast, the left testicular veins drained the left testis, epididymis and its appendix, exclusively into the left common iliac vein. The most caudal testicular vein drained the cranial aspect of the ureter and deferent duct through the cranial ureterodeferential vein. Additionally, there were some smaller veins that drained the testis into the adrenal vein. Based on the relationship of the testicular veins to the caudal vena cava and the common iliac veins, a number of variations were observed.

1.5. The middle portions of the deferent duct and ureter were drained by the middle ureterodeferential veins. These vessels were variable in number, size and point of entry along the caudal renal veins into which they emptied. Depending on individual variation, these vessels terminated in both the fused and separate segments of the caudal renal veins.

1.6. The caudal segment of the deferent duct was drained by tributaries into the pudendal and caudal mesenteric veins. The caudal mesenteric vein was a tributary of a caudal median vein that emptied into a transverse vessel, the inter-iliac anastomosis. The inter-iliac anastomosis interconnected the two internal iliac veins. The surface of the phallus was also drained by tributaries of the pudendal vein. These features are unique to the ostrich.

The vascular pattern outlined above is generally similar to that of the domestic rooster. The few specific differences observed are listed in the final remarks below:

2. Microvasculature of the reproductive organs

2.1. Despite some small differences, the distribution and morphology of the microvasculature of the reproductive tract was generally similar to that of the domestic fowl.

2.2. In the ostrich, the blood capillaries in the testis, deferent duct (cranial and caudal) and receptacle of the deferent duct lack endothelial fenestrations.

2.3. The proximal efferent duct displays fenestrated blood capillaries. This feature is consistent with fluid absorption. Numerous lymphatic vessels are located in the stroma of the epididymis, further indicating active fluid absorption in this region.

2.4. The presence of fenestrations in both capillaries and venules of the spongy tissue at the base of the phallus and erectile tissue of the phallic sulcus, is consistent with the involvement of these structures in the erection mechanism of the phallus in the ostrich.

3. Blood-testis and blood-epididymis barriers

3.1. The blood-testis barrier and blood-epididymal barrier in the ostrich are similar in structure and location to those described in other birds.

3.2. The blood-testis barrier in the ostrich is composed of a number of components. These include the endothelial lining and basal lamina of the blood vessels; the cellular and acellularlayers of peritubularconnective tissue; the basal lamina of the seminiferous epithelium and, most prominently, the series of tight junctions located above the layer of spermatogonia.

3.3. The blood-epididymis barrrier of the ostrich comprises the subepithelial blood capillaries and venules, the peritubular myoid cells and the adluminal apico-lateral

junctional complexes in the epithelium of the rete testis, efferent ducts, connecting ducts and epididymal duct.

4. Final remarks

Generally, the distribution and structure of the vascular network in the reproductive system, and the morphological features of the blood-testis and blood epididymis barriers of the ostrich, are similar to those of the domestic rooster. However, the ostrich displayed some distinct peculiarities:

- An anastomosis was observed between the caudal continuation of the cranial ureterodeferential branch and the middle renal artery, forming a collateral circulation.
- A 2-4 cm long fusion of the left and right caudal renal veins occurred and which received some of the middle ureterodiferential veins.
- A caudal median vein was present that had the caudal mesenteric vein as tributary.
- A number of veins on the surface of the root of phallus drained only the mucosa.
- The capillaries of the testis showed no fenestrations.
- Stromal capillaries were randomly distributed in the epididymis.
- Three vascular networks surrounded the various segments of the deferent duct and receptaculum.
- Fenestrations were obvious in both capillaries and venules in the spongy tissue of the phallus and in the erectile tissue at the phallic groove to allow circulation of lymph for erection.

APPENDICES

APPENDIX I

Papers published

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2007. Macroscopic features of the arterial supply to the reproductive system of the male ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia,* 36: 255-262.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2008. Macroscopic features of the venous drainage of the reproductive system of the male ostrich (*Struthio camelus*).*Onderstepoort Journal of Veterinary Research, 75: 289-298.*

APPENDIX II

1. Papers / Posters presented at local conferences

ELIAS, M. Z. J., SOLEY, J. T. & AIRE, T. A. 2011.Distribuição e ultrastructura da microvasculatura do testículo da avestruz (*Struthio camelus*). Associação de Veterinários de Moçambique. V Jornadas de Medicina Veterinária e Produção Animal.Setembro. Maputo, Moçambique.

ELIAS, M. Z. J., SOLEY, J. T. & AIRE, T. A. 2010. The blood-testis barrier in the ostrich (*Struthio camelus*). 48th Annual Conference of the Microscopy Society of Southern Africa. Warmbaths, South Africa.

ELIAS, M. Z. J., SOLEY, J. T. & AIRE, T. A. 2009. Ultrastructure of microvasculature of the *ostrich Ductus deferens, Receptaculum ductus deferens* and *Phallus).*47thAnnual Conference of the Microscopy Society of Southern Africa. Durban, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2008. Microvasculatura do testículo e do epididimo da avestruz. Proceedings do XI Congresso Internacional de Medicina Veterinária em Língua Portuguesa & I Congresso de Medicina Veterinária de Moçambique. Outubro. Maputo, Moçambique.

ELIAS, M. Z. J., SOLEY, J. T.,AIRE, T. A. & DU PLESSIS, L. 2008. Membrane specializations in the efferent ducts of the ostrich epididymis. 46th Annual Conference of the Microscopy Society of Southern Africa. Gaborone, Botswana.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2008. Intercellular junctions in the ostrich *(Struthio camelus)* testis. 38thAnnual Conference of the Anatomical Society of Southern Africa (ASSA), Kruger Park, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2007. Ultrastructure of the interstitial vasculature of the ostrich testis. 36thAnnual Conference of the Anatomical Society of Southern Africa (ASSA), Magaliesburg, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2006a. The arterial microvasculature of the *distal ductus deferens, receptaculum ductus deferens* and phallus of the ostrich, as revealed by India ink injection. 45thAnnual Conference of the Microscopy Society of Southern Africa. Port Elizabeth, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2006b. Basic macroscopic features of the arterial supply to the reproductive system of the male ostrich (S*truthio camelus*). 36th Annual Conference of the Anatomical Society of Southern Africa (ASSA), Golden Gate, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2005. The microvasculature of the testis, epididymis and proximal ductus deferens of the ostrich (*Struthio camelus*) as revealed by India ink injection. 44th Annual Conference of the Microscopy Society of Southern Africa (MSSA). Pietermaritzburg, South Africa.

ELIAS, M. Z. J., AIRE, T. A. & SOLEY, J. T. 2005. Caracteristicas morfológicas basicas da drenagem venosa do sistema reprodutor do macho de avestruz (*Struthio camelus*). Procedings do IV◦ Seminario de Investigação da Universidade Eduardo Mondlane. Maputo,Moçambique.

2. Papers / Posters presented at international conferences

ELIAS, M. Z. J. 2010. Microvasculature of phallus of the ostrich (*Struthio camelus*) as revealed by transmission electron microscopy. 17th International Microscopy Congress. Rio de Janeiro, Brazil.

ELIAS, M. Z. J., SOLEY, J. T. & AIRE, T. A. 2009.Ultrastructural features of the microvasculature and lymphatics of the ostrich (*Struthio camelus*) epididymis.17th Congress of the International Federation of Associations of Anatomists (IFAA).Cape Town, South Africa.

SOLEY, J.T., ELIAS, M. Z. J. & AIRE,T. A. 2007. Variations in the gross pattern of venous drainage of the ostrich male reproductive tract: A comparison with the general avian model. 1st Conjoint International Conference on Fertility, Anatomy and Morphological Sciences. Lagos, Nigeria.

APPENDIX III

Designation of various arterial and venous vessels according to vessel calibre. The diameter of vessels is expressed in µm unless otherwise indicated.

	Rhodin, 1974	**Leeson & Leeson, 1976**	**Fawcett, 1986**	**Dellman, 1998**	**Dellman & Venable, 1993**	**Plendl, 2006**
Capillaries	3-10	7-9	8-12	5-10	5-10	5-10
Arterioles	> 300	Up to 100	50-300			
Elastic arteries	Large diameter					
Muscular arteries	10–300					
Venules	10-30	< 200	15-20	10-30	20-30	10-30
Collecting veins	30-50			30-50*		
Muscular veins	50-1000					
Small & medium sized veins	1-10 mm	1-9 mm	2-9 mm			
Large veins	< 10 mm					

*Also known as pericytic veins.

REFERENCES

DELLMAN, H. D. 1998**.** Cardiovascular system, in *Textbook of Veterinary Histology*, Vth edition. Edited by H. D. Dellman &J. A.Eurell. Philadelphia: Lippincott Williams & Wilkins. pp. 114-127.

DELLMAN, H. D. & VENABLE, J. H. 1993. Cardiovascular system, in *Textbook of Veterinary Histology,* IVth edition. Edited by H. D. Dellman. Philadelphia:Lea Febiger. pp. 108-119.

LEESON, C. R. & LEESON, T. S. 1976. Circulatory System, in *Histology.* Philadelphia: W. B. Saunders Company. pp. 251-272.

FAWCETT, D. W. 1986. Blood and lymph vascular systems, in *Textbook of Histology.* XIth edition. Hong Kong: W. B. Saunders Company. pp. 337-391.

PLENDL, J. 2006. Cardiovascular system, in *Dellman'sTextbook of Veterinary Histology*, VIthEdition. Edited by J. A. Eurell & B. L. Frappier. Oxford: Blackwell Publishing. pp. 117-133.

RHODIN, J. A.G. 1974. Cardiovascular system, in *Histology. A text book and atlas.* New York: Oxford University Press. pp. 331-370.