

REPRODUCTIVE ANATOMY AND HISTOLOGY OF THE MALE FLORIDA MANATEE
(*TRICHECHUS MANATUS LATIROSTIS*)

By

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To Amanda, Mom and Dad: for the support and infinite love.
And to Quique, I miss you.

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LIST OF ABBREVIATIONS

ASG	Accessory Sex Glands
H&E	Hematoxylin and Eosin stain
MMPL	Marine Mammal Pathobiology Lab
NBF	Neutral buffered Formalin
PAS	Periodic acid-Schiff stain
SMA	Smooth muscle Actin Immunohistochemistry stain

Abstract of Thesis Presented to the Graduate School
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Information about genital anatomy of the male Florida manatee (*Trichechus manatus latirostris*) is scarce. Studies have been conducted on the general and histological examination of the testis and epididymis, but a detailed review of the accessory sex glands has not been performed. The objective of this study is to conduct an anatomical and histological description of the entire reproductive tract of male Florida manatee, including the testes, accessory sex glands and penis.

Three reproductive tracts of adult males were collected for histological analysis, and three additional tracts (two adults and one juvenile) for the gross anatomical analysis, from the Marine Mammal Pathobiology Laboratory in St. Petersburg, Tampa. Four archived tracts (two adults, a juvenile and an infant) were included for histological analysis. The general description of the tracts was determined by dissection. Histological analysis used the following stains: hematoxylin and eosin technique, PAS, Gomori's one step trichrome and immunohistochemistry specific for smooth muscle actin.

The accessory sex glands found in the manatee are the vesicular glands and prostate. They lack ampullae and bulbourethral glands. A prominent colliculus seminalis was surrounded by the urethralis muscle. The ductus deferens, vesicular glands and prostate had ducts opening

into the colliculus seminalis. The musculocavernous manatee penis is similar to the stallion (*Equus ferus*), with a large corpus cavernosum dorsally located and smaller corpus spongiosum near the urethra ventro-medially located, all surrounded by a thick tunica albuginea layer. An os penis (baculum) is absent in the Florida manatee.

The male reproductive tract of the Florida manatee has gross and histological similarities with the dugong (*Dugong dugon*), another member of the order Sirenia, and with a close terrestrial relative of the manatee, the elephant (*Loxodonta africana*).

CHAPTER 1 INTRODUCTION

The Order Sirenia includes manatees of the Trichechidae family and the dugong (*Dugong dugon*), present in the Dugongidae family. There are three species of manatees in this Order, the Amazonian manatee (*Trichechus inungis*), the West African manatee (*Trichechus senegalensis*) and the West Indian manatee (*Trichechus manatus*). The West Indian manatee includes two subspecies that are geographically distributed in tropical and subtropical marine habitats and coastal, inland water and promenades along some river systems with connection to the sea (Jefferson et al., 1993; Marsh & Lefebvre, 1994). The Florida manatee (*Trichechus m. latirostris*) is present through the peninsular coast of Florida to southeastern Georgia in the U.S. (Reynolds & Odell, 1991), and the Antillean manatee (*Trichechus m. manatus*) is found from Mexico, the Wider Caribbean, Central America, and northern South America to Brazil (Lefebvre et al., 2001; Marsh et al., 2011)

The earliest known sirenians were both terrestrial and aquatic herbivores. They featured four limbs and a tail, with morphological characteristics suggesting they split time between land and water environments (Domning, 2001). Fossil evidence of this sirenian, the *Pezosiren portelli*, has been found around the Early Eocene, 50 million years ago (mya). Fossils of the three living species of manatees have been aged from the early Miocene (15 mya) to the Recent. Evidence suggests that the first manatee appeared in Florida over one mya (Reinhart, 1971; Berta et al., 2006). Domning (2005) has studied the evolution of sirenians, and stated that the trichechids evolved into the present day manatee species over the last 4 million years. Simpson (1945) related the order Sirenia with the order Proboscidea (elephants) and the order Hyracoidea (hyraxes) as the mammalian clade Paenungulata. More recent publications supporting with

molecular data confirm that elephants and hyraxes are the closest living relatives to the sirenians (De Jong & Zweers, 1980; Kleinschmidt et al., 1986)

The Antillean manatee has been hunted since the 1800's. Manatee hunting has primarily been for food, oil and leather. This practice caused a severe reduction in the population size (Caldwell & Caldwell, 1985; Bonde, 2009). In the case of the Florida manatee, additional anthropogenic causes have been negatively affecting their population. These risks include accidental deaths by watercrafts, increased human population in Florida, tourism, and indirect activities that affect their food availability and distribution (Caldwell & Caldwell, 1985; O'Shea et al., 1997). For these reasons, the West Indian manatee has been considered an endangered species. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since July 1975 classified manatees with the status of "endangered". In 1982 the International Union for the Conservation of Nature (IUCN) considered this species as "vulnerable" (Deutsch et al., 2008). The Endangered Species Act (1973), Marine Mammal Protection Act (1972) and Florida Manatee Sanctuary Act (1978) also protect the Florida manatee.

Assessments of the population and health of sirenians have been conducted by different groups of researchers across various disciplines. Distribution and seasonal habitats have been evaluated by aerial surveys of dugongs (Marsh et al., 1994), Florida manatee (Ackerman, 1995; Garrott et al., 1995) and Antillean manatee in Mexico and Central America (Bengtson & Magor, 1979; Morales-Vela et al., 2000). Distribution in certain environmental conditions where aerial surveys are not optimal can be assessed with side-scan sonar (Gonzalez-Socolozke et al., 2009). Manatee photo identification has been helpful for monitoring manatee population size, documenting habitat use, and estimating adult survival and reproductive success. Aspects of

individual photo identification are facilitated by visible scars, deformities and freeze brands. The most common on Florida manatee are the scars from propeller strikes. (Beck & Reid, 1995; Reep & Bonde, 2006).

Other scientific techniques that complement photo identification include analysis of molecular genetics. Genetic diversity is important to evaluate the health of the population. While a population may be large in number, they could still have low genetic diversity, putting the species at risk of extinction. Genetic diversity can occur by mutation and migration. Manatee genetic diversity varies from species to species. Studies show that the Amazonian manatee and the Antillean manatee present much higher genetic diversity than the Florida manatee (Assis et al., 1988; Gray et al., 2002; Cantanhede et al., 2005). Florida manatee low diversity could result from a possible population bottleneck or a founder effect (Garcia-Rodriguez et al., 1998; Vianna et al., 2006).

Manatees present with little sexual dimorphism to assist with gender identification. Mature males are slightly shorter in length than females, and females tending to be larger with age (Harshaw, 2012). The main external difference is the anogenital distance, which can determine the gender of the specimen. The genital opening in females is close to the anus, while in males the genital opening is closer to the umbilicus (Hartman, 1979; Reynolds & Odell, 1991; Marmontel et al., 1992).

Reproduction in manatees is categorized as promiscuous. The manatee mating system consists of multiple males pursuing an estrous female for as long as two to four weeks. Each male, attempts to grasp the female with its pectoral flippers, trying to position ventrally to initiate mating. The female will mate with multiple males, so determining paternity is difficult without an evaluation of genetics of the calf. The mating encounter is transitory, permanent pair bonds

are not formed (Rathbun et al., 1995; Reynolds et al., 2004; Larkin et al., 2005). Females reach sexual maturity at 3 to 5 years of age (Marmontel, 1995) and males may reach sexual maturity at 2 to 3 years of age (Hernandez et al., 1995). The age at which individuals begin reproducing is an important characteristic in the life history of a species.

The determination of sexual maturity in females involves examining follicular development and identifying the presence of corpora lutea or albicantia in the ovaries, which indicate previous ovulation. Florida manatee are polyovular and produce an average of 36.3 corpora lutea/ovary/pregnancy. The determination of sexual maturation is usually made by three criteria: first ovulation, first conception and first lactation (Marmontel, 1995). It is possible for a female manatee to become pregnant when first ovulates, however, not every ovulation results in a pregnancy (Rathbun et al., 1995).

In males, the determination of sexual maturity involves the increase of testicular activity with the spermatogenic cycle and the diameter of the seminiferous tubules. This occurs when the manatee is approximately 250cm in length. Total length seems to be a better predictor of reproductive status than age (Hernandez et al., 1995). Environmental cycles could affect sperm production in some species of manatees. For example, the Florida manatee may be physiologically stressed in winter due to cold water temperatures and less food availability. Studies confirm that the functionality of seminiferous tubules in male Florida manatee is reduced during the winter (Hernandez et al., 1995; Reynolds et al., 2004). Alternatively, in the Amazonian manatee, the environmental cycle (dry-wet season) suggests a possible stress due to food availability during the dry season. Beyond the presence of these environmental stressor, manatees reproduce throughout most of the year (Marmontel et al., 1992; Hernandez et al., 1995; Reynolds et al., 2004; Reep & Bonde, 2006). In general, Florida manatee calves are born in the

spring or early summer with a single calf born after a gestation period of at least 12 months, twins can occur but are rare (Jefferson et al., 1993; Rathbun et al., 1995).

In female sirenians, the gross anatomy and histology of dugong reproductive tracts were described by Marsh et al. (1984), the anatomical description of the Florida manatee was conducted by Marmontel (1995) and Amazonian manatee (*Trichechus inunguis*) were reviewed by Rodrigues et al. (2008). In males, Marsh et al. (1984) analyzed the reproductive tract of 59 dugongs where testis, epididymis, ductus deferens and vesicular glands were described. Prostate and bulbourethral glands were not distinct macroscopically, but in the histological analysis they identified glandular tissue, of bulbourethral type, surrounding the urethra close to the root of the penis. Hernandez et al. (1995) focused on describing the testes and epididymis of male Florida manatee in order to identify the spermatogenesis cycle. Reynolds et al. (2004) used testicular size as an indicator of sperm competition in the Florida manatee (*Trichechus m. latirostris*). Little effort has been focused on the accessory glands of manatees. Research by Caldwell and Caldwell (1985) revealed the presence of large vesicular glands and a nonglandular prostate, while some studies suggest the presence of only vesicular glands (Bonde et. al., 1984; Reynolds et al., 2004).

Studying the accessory sex glands in the Florida manatee is important to increase the reproductive information of this species. As mentioned above, there is no definitive description of the accessory sex glands in the male Florida manatee. Details of the accessory sex glands could contribute to the hypothesis of sperm competition in this species. This project is focused on the examination of male Florida manatee reproductive anatomy. A chapter is focused on the gross anatomical description of the male reproductive tract. Secondly, a microanatomy chapter (histology studies) provides added detail, complementing the results described on gross anatomy.

Finally, a conclusions chapter is presented at the end of this thesis to provide further context and a broader perspective of results.

CHAPTER 2 GROSS ANATOMY

Male Reproductive tract

Understanding reproductive biology and physiology is important to establish population models and to improve management decisions in captive and wild populations. This knowledge is also important to recognize differences between healthy and unhealthy states (Larkin et al., 2005; Amaral et al., 2009). Structures of male reproductive anatomy in terrestrial mammals are well known. Basic components of the male reproductive system are the testes, epididymides, deferent ducts, accessory sex glands (ampullae, vesicular glands, prostate and bulbourethral glands) and the penis (Senger, 2005).

The reproductive role of the male is to produce sperm and be able to deposit the sperm in the female reproductive tract (Reece, 2009). The production of viable sperm to fertilize oocytes involves the testes, epididymis, and the accessory sex glands. The testes produce and manufacture spermatozoa through the process of spermatogenesis. The testes produce testosterone, inhibin and estradiol essential for normal production and maturation of spermatozoa (Pineda & Faulkner, 1980; Amann & Schanbacher, 1983).

Male mammal reproductive physiology varies between terrestrial and aquatic mammals. In terrestrial mammals, the testes descend from the abdominal cavity into a scrotum. The descent supports the spermatogenesis process which occurs at a lower temperature than the body core (Pineda & Faulkner, 1980; McEntee, 1990; Senger, 2005). Aquatic mammals have testes located in the abdominal cavity, except for eared seals (Family Otariidae) that have scrotal testes (Atkinson, 1997; Robeck, Atkinson, & Brook, 2001; Berta et al., 2006; De Smet, 1977; De Guise et al., 1994; Berta et al., 2006). For adequate function and spermatogenesis process, the testes of aquatic mammals have vascular adaptations to regulate temperatures in the reproductive system

(Rommel et al., 1998). The shape of the testes in cetaceans can be cylindrical, more slender, compressed or rectangular in shape depending on the species (De Smet, 1977). The testes in manatees (*Trichechus manatus latirostris*) and dugongs (*Dugong dugon*) are ovoid in shape, internally situated in the abdominal body cavity and are located laterally and ventral to the kidneys, attached to the peritoneum (Marmontel et al., 1992; Boyd et al., 1999).

Once the spermatozoa have been produced in the testes they enter into the epididymis. The epididymis in mammals is a coiled tubule that allows collection, storage, maturation and the development of motility in the spermatozoa. These structures are located covering each testis on one side (McEntee, 1990; Reece, 2009). There are three main parts of the epididymis; head (proximal part), body (intermediate segment) and tail (most distal part) (McEntee, 1990; Senger, 1997; Reece, 2009). The epididymis can vary in size, shape and attachment to the testes among species. For example, the epididymis of three different rhinoceros species [Asia rhinoceros (*Rhinoceros unicornis*), white rhinoceros (*Ceratotherium simum*) and black rhinoceros (*Diceros bicornis*)] can be tight, loose or more loosely attached to the testes (Schaffer et al., 2001). In another case, between the African elephant (*Loxodonta africana*) and the Asian elephant (*Elephas maximus*) there is no obvious gross differentiation of the three main parts of the epididymis. Moreover, the size of the epididymis of the African elephant is very small compared with the size of the testis of this species (Hildebrandt et al., 1998). In aquatic mammals, the attachment of the epididymis can run the entire length of the testicular border or just a portion depending on the species (De Smet, 1977; De Guise et al., 1994). In sirenians, the epididymial shape and tightness of the attachment to the testis has not been described, as in the terrestrial mammals mentioned above. Only Marsh et al., (1984) showed a picture of the dugong

epididymis, where the epididymis was located along the proximal end of the testis through three quarters of the intermediate segment.

Sperm is stored and nourished within the tail of the epididymis until sexual stimulation occurs. Sperm are transported by the ductus deferens from the tail of the epididymis. The ductus deferens is the continuation of the duct system that extends from the tail of the epididymis to the pelvic urethra. The wall of the ductus deferens is thick and mostly muscular with an irregular and very small lumen (Senger, 1997; Reece, 2009).

The accessory sex glands are the main contributors of seminal fluid production and volume of the ejaculatory secretion. The accessory sex glands empty their products into the lumen of the pelvic urethra (Senger, 1997, Reece, 2009). Reproductive hormones control the function and development of the accessory sex glands. These glands are dependent on testosterone for full development and maintenance of their structure and function (Senger, 1997; Pineda & Faulkner, 1980). Size, shape, presence or absence of the accessory sex glands varies widely among species. Table 2-1 details the accessory sex glands currently characterized from several species.

The ampullae are located within the enlarged distal portions of the deferent ducts. This enlargement is due to dilatation in the mucosal portion of the lumen and the abundant glands embedded in the duct wall, which store and secrete fluid for the sperm (Sorensen, 1979). This accessory sex gland is present in some terrestrial mammals, and absent among the marine mammals on the reference reviewed (Table 2-1).

The vesicular glands are paired accessory sex glands located dorso-cranially to the pelvic urethra. These glands do not store semen; rather they supply the majority of the seminal fluid of the ejaculation, secreting fluid that adds nutriment and volume to the semen. The anatomical

configuration varies among domestic mammals (McEntee, 2012, Reece, 2009). For example, the vesicular glands of the stallion (*Equus ferus*) are elongated pyriform-shaped true vesicles with a large central cavity, and in ruminants the vesicular glands are compact lobulated (Senger, 1997). Rhinoceros have multilobuled and sacculated vesicular glands (Schaffer et al., 2001), whereas vesicular glands are the largest glands in elephants with a cigar-shape characterized by a single cavity (Hildebrandt, et al., 1998; Hildebrandt et al., 2000). Dugongs and Florida manatees have vesicular glands (Harrison, 1969; Marmontel et al., 1992) that are bilaterally located at the dorsal aspect of the urinary bladder. These glands are elongated, ovoid shape, and have a thick wall (Bonde et al., 1983; Marsh et al., 1984; Caldwell & Caldwell, 1985).

The prostate is the most common accessory sex gland found across mammals. It varies in location and shape, but is generally located between the urinary bladder and the pelvic urethra (Reece, 2009). This gland can be found in two structural forms. The first is as a corpus or body structure, located outside the urethralis muscle. The second is the glandular tissue embedded along the urethra near the urethralis muscle, known as the disseminated portion or pars disseminate (Senger, 1997; McEntee, 1990). The prostate secretes a slick fluid that cleanses and lubricates the urethra and adds a small volume to the semen (Sorensen, 1979).

All domestic mammals have a prostate; some species have both structural forms, described above, while others may only have one of them. For example, the bull (*Bos taurus*), boars (*Sus domesticus*) and dogs (*Canis l. familiaris*) have the two structural forms (interestingly, the prostate is the only accessory sex gland present in dogs) (Senger, 1997; Pineda & Faulkner, 1980). In the stallion and cat (*Felis catus*) the disseminated portion of the prostate gland is absent (McEntee, 1990).

The prostate glands are more often present as the body structure rather than the disseminated portion. This gland varies among species in size, shape and number of lobes. For example, rhinoceros (Schaffer et al., 2001) and elephants (Hildebrandt et al., 1998; Hildebrandt et al., 2000) have two lobes of the prostate, whereas in rodents there may be two to three lobes (Çakir & Karataş, 2004; Mollineau et al., 2009; Chaves et al., 2011). Camels (*Camelus dromedarius*) have both structural forms of prostate (Degen & Lee, 1982) like bull, boars and dogs.

In marine mammals, the prostate is the only accessory sex gland described in pinnipeds and cetaceans (Rommel & Lowenstine, 2001). Harrison (1969) defined the prostate as relatively large in most delphinid adults and mentioned that neonatal phocids had a prostate gland that grows with age. de Smet (1977) described the bottlenose dolphins prostate as a “small gland without uterus masculinus”. On the dissection of the beluga whale (*Delphinapterus leucas*) made by De Guise and collaborators (1994) no evidence of the prostate gland around the urethra or in the surrounding area was found. Similarly, dugong dissections did not reveal a discrete structure corresponding to a prostate gland, but prostate was described as disseminated form (Marsh et al., 1984). In the Florida manatee the prostate gland has been described as nonglandular, composed of erectile muscular tissue (Caldwell & Caldwell, 1985).

The most caudal accessory sex gland is the bulbourethral gland. The bulbourethral glands are paired glands generally located dorsally on either side of the pelvic urethra near to the ischiatic arch (Sengel, 1997; Reece, 2009). This gland is characterized as being relatively small, compact and round in shape in most domestic mammals (Pineda & Faulkner, 1980; McEntee, 1990). Their functions are very similar to those of the prostate, cleansing and lubricating the urethra, adding a small volume of fluid to the semen, and aiding in coagulation of the ejaculate

(Sorensen, 1979). These glands vary in shape and size depending on the species, for example, in rhinoceros, they are small and round, or elongated and irregular, (Schaffer et al., 2001). In elephants the bulbourethral glands can be large and ovoid in shape (Hildebrandt et al., 2000), while in camels they are small, almond-shaped (Degen & Lee, 1982).

Bulbourethral glands are absent in most of the aquatic mammals studied. Bulbourethral glands are considered absent in delphinids, including the beluga, phocids and manatees based upon the literature reviewed (Harrison, 1969; Bonde et al., 1984; Caldwell & Caldwell, 1985; De Guise et al., 1994). In dugongs, there are discrepancies in the literature as to the presence of the bulbourethral glands. Harrison (1969) mentioned that the bulbourethral glands appear to be absent in this species. Marsh et al. (1984) remarked that bulbourethral glands were not found macroscopically but histologically they located some bulbourethral glandular-like tissue. Later, Bertha and collaborators (2006) stated that dugongs have bulbourethral glandular tissue within the base of the penis.

This chapter will focus on the evaluation of the gross anatomy of the male Florida manatee reproductive tract. The characteristics of the accessory sex glands will have special attention, since these organs play an important role in reproduction and their presence in the male Florida manatee is unclear. Dissections of adult male Florida manatee tracts will be examined, expecting to at least to find the prostate and bulbourethral glands, as in the elephant and the dugong.

Methods

Sample Collection

Gross anatomical data were collected from three male reproductive tracts, one juvenile (GTM1) and two adults (GTM2 and GMT3). Condition and causes of death of these manatees is show in Table 2-2. In February, 2014, the juvenile was collected; the two adults were collected

in April and May, 2014 (Table 2-3). These male tracts were collected from the Marine Mammal Pathobiology Laboratory (MMPL), in Saint Petersburg, Tampa. The tissue collection consisted of the en block of tissue mass from the caudal area from testes to the penis, including muscles and fat attached. The process of dissection occurred the same day of collection or the next at the University of Florida histopathology laboratory.

Dissection

Using the urinary bladder as a landmark, each tract was dissected carefully (with surgical scissors, to avoid damaging tissues of interest) until the accessory sex glands were visible and identified. In order to keep track of the organs already identified, tags with the name of the organs were used. Morphometric measurements (length, width, depth and diameter) were obtained for the following structures in the adult samples: testes, epididymides (head, body and tail), deferent ducts (thickest and thinnest portions), urinary bladder (empty), vesicular glands (empty), possible prostate and penis (top of crura, middle of the penis, penile tip, and fossa of the penis) and urethra were measured (Figure 2-1). A tape measure (1m), plastic ruler (30cm) and calipers were used for the morphometric measurements. Photographs were obtained with an EOS Rebel T2i Canon camera. Tracts were placed on a flat surface in plastic bags in a -8°C freezer.

Results

Dissection process of GMT1 juvenile reproductive tract showed well-defined structures and the absence of some accessory glands. Testes, epididymides, deferent ducts, urinary bladder and urethralis muscle were identified. Vesicular glands, ampullae and bulbourethral glands were not identified on this specimen. Testes, epididymides, deferent ducts, urinary bladder, ureters, vesicular glands and urethralis muscle were easily recognized and identified in tissues from adults GMT2 and GMT3. Ampullae and bulbourethral glands were not identified, as in the

juvenile. Morphometric data obtain from the adult male reproductive tracts are summarized in Table 2-4 and Table 2-5.

The testes of all manatees examined (n=3) were ovoid in shape covered by a tunica vaginalis. They were located internally in the abdominal body cavity, lateral to the kidneys and dorso-cranially to the urinary bladder. Florida manatee testes (n=2) averaged 15.2cm length, 10.2cm width and 1.5cm depth including both right and left tissues. The left testis was slightly smaller than the right testis. Manatee GTM2 was longer and heavier than manatee GTM3, and the testes in GTM2 were, likewise, larger than GTM3 (Table 2-4).

The epididymides of the adult male manatee (n=2) reproductive tracts showed a tight s-shape curvature at the tail of this structure. The tail of the epididymis was partially covering the caudal border of the testis. In the juvenile, it was difficult to distinguish the exact shape, but both adult males the epididymides had the unusual S-shape configuration (Figure 2-2, Figure 2-3 A). The average measurements (n=2) of the head of the epididymis was 13cm long, 2.8cm width and 0.9cm of depth; the body of the epididymis was 10cm long, 4.9cm width and 1.3cm of depth. Finally, the tail of the epididymis of the Florida manatee measured in average 12.9cm long, 2.9 cm width and 1cm of depth (Table 2-4).

The width of the epididymal tail drastically decreased denoting the beginning of the ductus deferens. The ductus deferens on the male manatee tract was much coiled. No ampullae were found in any of the specimens. The deferent ducts extended to the neck of the urinary bladder, measuring an average of 28.9cm in length. The urinary bladder was not complete in all specimens. A segment of it was removed at the MMPL for analysis of tissues and urine sampling. However, we were able to measure the empty bladders, averaging approximately

10.4cm long, 9 cm width and 2.2 cm in depth (Table 2-5). The urinary bladder was the landmark used to locate and identify the accessory sex glands (Figure 2-2, Figure 2-3).

The most obvious accessory sex glands were the vesicular glands. These were dorsal and caudal to the neck of the urinary bladder, laterally to the deferent ducts junctions to the urinary bladder. They were bilateral, lobulated, large hollow and firm (Figure 2-2, Figure 2-3 B). In length, the right vesicular glands were longer than the left ones. Vesicular glands from GTM2 were longer than those of GTM3 (Table 2-5).

Caudal to the vesicular glands and close to the neck of the urinary bladder were the prostate glands (Figure 2-5). The prostate in the manatee was present as two small ovoid-shaped foamy lobes. They are difficult to locate grossly, but with manual palpations of the specimens, one could feel the lobes on each side. Morphometric data was not obtained for these glands, but they were palpably approximately 15mm in diameter. Grossly identifiable bulbourethral glands were not detected.

The urethralis muscle was located ventral to the urinary bladder and cranial to the root of the penis (Figure 2-3 B). This muscle was large, heart-shaped and a very firm. This structure was initially identified as prostate; upon transecting and later histological analysis, this structure was correctly identified as urethralis muscle. Inside the urethralis muscle, the seminal colliculus was found (Figure 2-3 C). The seminal colliculus was prominent and located ventral to the ureteral openings. Four opening ducts were present in the dorsal side and six were located in the ventral side of the seminal colliculus. It was obvious that the dorsal openings were wider than the ventral ones, though measurements were not taken. A thin probe was used to follow the openings. Of the four dorsal openings, the two inner openings were to the deferent ducts, the two outer openings were to the vesicular glands. Off the six ventral openings, two more were opening to the

vesicular glands and the other four to the prostate lobes. Figure 2-4 shows a schematic diagram of the description of the duct openings in the colliculus seminalis. No other duct openings were found from the neck of the bladder to the penile urethra.

The Florida manatee penis was musculocavernosous in composition. The penis had a bilateral corpus cavernosum and unilateral ventro-medially corpus spongiosum composition. The corpus spongiosum surrounded the penile urethra. A thick cord-like retractor penis muscle was identified, curving dorsally along the penis (Figure 2-6). A glans penis with crura was present in this species. At the dissection, the vestigial pelvic bones were identified on each side of the bulbocavernosum muscle at the base of the penis. The penis was 39.9cm long, 3.8cm width and 3.6cm of depth in average (n=2).

Discussion

The gross anatomy of the two adult Florida manatee male reproductive tracts shows similarities with the typical mammalian reproductive tract. The Florida manatee ovoid-shaped testes are similar in appearance to those of dugongs (Marsh et al., 1984) in contrast to the different shape of testes present in cetaceans (de Smet, 1977). The location of the testes in the abdominal cavity next to the kidneys, attached to the abdominal wall, is more similar to cetaceans (De Smet, 1977; De Guise et al., 1994; Berta et al., 2006) than pinnipeds since walruses and seals have para- abdominal testes located laterally to the penis and eared seals have scrotal testes (Atkinson, 1997; Robeck, Atkinson, & Brook, 2001; Berta et al., 2006). Some studies have found that testes size among mammals is proportional to body mass and body length, mating system and breeding season. In aquatic mammals it has been suggested that the mating system may influence the size of the testes more than the body length (Kenagy & Trombulak, 1986). The size of testes in Florida manatee does not seem to match those patterns seen in other mammals. Florida manatees' testes are larger than those mammals that breed with a

single male, but smaller than species with multi-male breeding. Reynolds et al (2004) indicated that the largest testes found in Florida manatees were present in individuals with body length between 260cm to over 300cm and testes were heavier in non-winter months. In winter months (December to February) Florida manatee appeared to undergo testicular regression, like the beluga and the narwhal (*Monodon monoceros*) (Kelley et al., 2014). The testes from GTM2 and GTM3 were obtained in non-winter months, their lengths were 280cm and 273 cm long, respectively and GTM2, the larger animal, had larger testes.

The epididymis of some marine mammals lies distal to the dorsal aspect of the testis, unlike the epididymis of domestic mammals (McEntee, 1990; Senger, 2005). For example the bottlenose dolphin (*Tursiops truncatus*) has an epididymis that measures 1.5cm from the dorsal part of the testes, but is closer at the caudal end of the testis. The epididymis of the North Atlantic beaked whale (*Mesoplodon bidens*) has the same partial covering at the caudal border of the testis (de Smet, 1977) like was found in this study. Similarly, the beluga has an epididymis loosely attached to the testicular border (De Guise et al., 1994). The s-shaped epididymis found on the Florida manatee, could be a sirenian feature. Marsh et al (1984) described that the dugong epididymal tail as having some caudal migration. Even though the epididymides in the present study were not as completely dissected as did Marsh, they looked very similar in shape to the dugong epididymides shown in that study.

Two accessory sex glands were not identified in the specimens assessed in this study, the ampullae and the bulbourethral glands. Many mammals, especially aquatic ones, do not have a full complement of accessory sex glands. The ampulla is frequently absent in terrestrial mammals, as in the domestic species like the dog, boar, and tomcat (Senger, 1997; Bacha & Bacha, 2000; Reece, 2009). Likewise, rhinoceros (Schaffer et al., 2001), marsupials (Rodger,

1976; De Barros et al., 2013) and rodents (Çakir & Karataş, 2004; Mollineau et al., 2009; Chaves et al., 2011) do not have them. In aquatic mammals, the ampulla has not been found or described in the species looked at thus far, like the dugong, (Hill, 1945; Marsh et al., 1984), pinnipeds and cetaceans (Norris, 1966; Harrison, 1969; Boyd, et al., 1999) such as the beluga (De Guise, et al., 1994).

Bulbourethral glands are present in all domestic mammals, except the dog, and in most of the other terrestrial mammals studied. These glands produce a viscous secretion in domestic animals, important for lubrication of the urethra before ejaculation. The bulbourethral glands are generally located near the ischial arch, along the urethra near the point where it exists from the pelvis. Secretions of the bulbourethral gland are emptied into the urethra by excretory ducts. Stallions, elephants and rhinoceros have several excretory ducts (McKinnon & Voss, 1993; Hildebrandt et al., 1998; Schaffer et al., 2001) from the bulbourethral gland, whereas in bulls, boars, and camels the bulbourethral glands have an excretory duct for each lobe of the gland (Degen & Lee, 1982; McEntee, 1990). In the specimens evaluated in this study, there was no gross macroscopic evidence of these glands. No excretory ducts were found along the urethra from the neck of the bladder to the ischial arch that leads to embedded glands. The absence of these glands may not negatively affect the seminal fluid secretions, as the enlargement of the other accessory glands may compensate for the lack.

The Florida manatee have vesicular glands and a prostate. The vesicular glands of the Florida manatee are similar in shape and structure to the those of the dugong (Hill, 1945; Marsh et al., 1984). However, the vesicular glands of the Florida manatee are about three times longer and wider, and four times deeper, than the vesicular glands of the dugong (Hill, 1945). As in bulls and elephants, the vesicular glands are the largest accessory sex gland in the Florida

manatee (Hildebrandt et al., 1998; Reece, 2009). Vesicular glands may contribute to over half of the total fluid volume of seminal fluid. In the bull, these glands are the sites of fructose synthesis, which provides energy for the sperm, necessary for sperm motility. Lower concentrations of fructose are found in boar and stallion seminal fluid (Sorensen, 1979). Finally, vesicular gland fluid provides an optimum alkaline pH medium that prolongs the life of sperm in the acidic environment in the vagina (Dixson & Bancroft, 1998). These important functions of the vesicular gland secretions may contribute to them being the largest accessory sex glands in some species.

The small bilobed prostate body of the Florida manatee is unusual compared to what is seen in other aquatic mammals. The pinniped prostate surrounds the neck of the bladder, just internal or slightly forward of the pubic arch. It is quite a bulky gland in the elephant seal (*Mirounga sp.*) and Antarctic fur seal (*Arctocephalus gazella*) (Laws & Sinha, 1993) and in the South American fur seal (*Arctocephalus australis*) is described as “a single spherical lobe” (Machado et al., 2012). In cetaceans, like the bridled dolphin (*Stenella frontalis*) and the harbor porpoise (*Phocoena phocoena*), the prostate prominently surrounds the urogenital canal and is the only accessory sex gland present (Harrison, 1969; De Guise et al., 1994). A small prostate gland was described for an immature bottlenose dolphin (*Tursiops truncatus*) male (de Smet, 1977). The development and maintenance of the accessory glands is influenced by testosterone, which increases until maturity. Sexual immaturity may be the reason for the small prostatic size of the bottlenose dolphin mentioned above. Belugas and dugongs seem to have a disseminated part of the prostate in the pelvic urethra (Marsh et al., 1984; De Guise et al., 1994). Even compared with domestic and other terrestrial mammals, the Florida manatee prostate found in this study seems to be unique. The secretions produced by the prostate are a combination of multiple components. For example in the boar, prostate secretions have high concentrations of

inorganic ions (sodium, chlorine, calcium and magnesium)(Bearden & Fuquay, 1992). Proteins are another main component present in the prostate secretion. One of the principal proteins found in prostate secretions is the β -microseminoprotein, present in rodents (Xuan et al., 1999) and boars. In boars this protein helps sperm motility, survival and have immunosuppressive activity (Chao et al., 1996; Manásková et al., 2002). The prostate supplies 4 to 6 percent of the volume of ejaculate in ruminants, 24 to 30 percent in the stallion and 35 to 60 percent in the boar (Sorensen, 1979).

Sperm competition is described as competition between sperm from different males to fertilize the ova of a female (Parker, 1970). Sexual selection by sperm competition has developed different morphological mechanisms. One of those mechanisms is the copulatory plug (Ramm et al., 2005) which may function to prevent leakage of spermatozoa from the female's reproductive tract (Jia et al., 2002; Anderson et al., 2004). This coagulated mass of proteins formed the copulatory plug with the mixture of vesicular gland and prostatic secretions. For example, vesicular glands in rodents secrete proteins like Svs2, that when mixed with a prostate derived transglutaminase at ejaculation obstructs the female reproductive tract (Ramm et al., 2008). Likewise, a comparative study of seminal coagulation rate for different mating systems in primates demonstrates that copulatory plug formation was significantly more frequent in primates with a multi partner mating systems (Dixon & Anderson, 2002). It would be of interest to evaluate if the mechanism of copulatory plug formation is found in male Florida manatee. If so, this may contribute to the hypothesis of sperm competition.

The accessory sex glands found in the two adult Florida manatee specimens were not located in the juvenile specimen. With only one specimen in this study, we are reluctant to conclude that these glands are absent in younger specimens. In many mammals, the inactive

prostate in juveniles develops with age, like in camels (Degen & Lee, 1982), pinnipeds (Laws & Sinha, 1993) and delphinids (Harrison, 1969). There also can be migration of the location, for example in the dog, the prostate moves from a pelvic to abdominal position as the animal matures (McEntee, 1990).

The colliculus seminalis is the area of the urethra where all the openings of the accessory sex glands merge with the urethra. The Florida manatee colliculus seminalis has a total of ten duct openings: 1) ductus deferens (two openings), 2) vesicular glands (four openings) and 3) prostate gland (four openings). Among species, this structure varies in shape and number of openings. Domestic mammals, like the stallion and the dog, have the opening duct of the prostate lateral to the colliculus seminalis (McEntee, 1990). In rhinoceros, the ducts of the ductus deferens, vesicular glands and prostate gland empty at the colliculus seminalis (Schaffer et al., 2001). Similarly, the colliculus seminalis in elephants contains ducts of the ampullae and vesicular glands and at least three from each prostate lobe. Shehata (1980) described the shape of the colliculus seminalis of different species, and mentioned that the colliculus seminalis is considered an active organ (described as a structure with glandular acini present and secretions similar to the prostate). We do not have evidence to state that the colliculus seminalis in the Florida manatee has an active function in the reproductive tract. It is interesting to note that both accessory sex glands found in the male Florida manatee, have the same number of duct openings, but the vesicular glands presented two wider ducts openings than that of the prostate. It might be possible, that this arrangement of the ducts depends on the size of the accessory sex glands, suggesting that bigger accessory sex glands contribute bigger volumes of secretions.

Most mammals reviewed are described as having a fibroblastic penis, like the bull and boar (McEntee, 1990), camels (Degen & Lee, 1982) and also most cetaceans (Norris, 1966), like

the beluga (De Guise et al., 1994) and the Sperm whale (*Physeter catodon*) (Bland & Kitchener, 2001). The Florida manatee penis in this study was classified as musculocavernosous (vascular), which contains a large amount of erectile tissue that upon engorgement will increase in length, diameter and turgidity (Sorensen, 1979) This type of penis is also present in the stallion (McKinnon & Voss, 1993), African elephant (Short et al., 1967; Hildebrandt et al., 2000), the dugong (Marsh et. al., 1984), and in some pinnipeds like the South American fur seal (Machado et al., 2012). The retractor penis of the male Florida manatee located on the dorsal surface of the penis is located opposite from the retractor penis of almost all domestic mammals, on the ventral side of the penis (Senger, 2005). Even though most aquatic mammals have the retractor penis in the ventral side of the penis (Norris, 1966). However, there are variations among species, such as the Southern black right whale (*Balaena g. australis*) which has a relatively long retractor muscle inserted into the dorsal aspect of the penis (Harrison, 1969). The Sperm whale is described with a pair of retractor muscles in a ventro-lateral aspect of the penis (Bland & Kitchener, 2001). The retractor penis of the Florida manatee seemed to be tendon-like than a muscle.

This study was the first approach to fully describe the male reproductive anatomy in the Florida manatee. The gross tissues consist of testes, epididymis and large vesicular glands similar to the dugong, a unique bilobed prostate, a prominent colliculus seminalis with opening ducts of the ductus deferens and the two accessory sex glands present, and a musculocavernosous penis like the dugong and the stallion. In the future, new techniques and methodology like perfusion fixation (Forssmann et al., 1977), electron microscopic studies (Agrawal & Vanha-Perttula, 1988), tridimensional methods (Timms, 2008), and also the study of the male accessory glands-specific proteins found in seminal fluid (Ramm et al., 2008) can

contribute more anatomical and physiological information about the accessory sex glands present in the male Florida manatee.

Table 2-1. Comparative evaluation of the accessory sex glands (ASG) shapes in mammals

	Ampulla	Vesicular gland	Prostate	Bulbourethral gland	Reference
			Body	Disseminated	
Domestic mammals					
Bull (<i>Bos taurus</i>)	Present. Not described	Largest ASG, markedly lobulated hard and compact, elongated irregular form	Bilobed	Enclosed at the urethralis muscle	Small, covered by bulbocavernosus muscle
Stallion (<i>Equus ferus</i>)	Present. Not described	Elongated H-shaped, true vesicles	Lobulated gland, bilobed	-	Ovoid-shape
Boar (<i>Sus domesticus</i>)	-	Heart-shaped, large three sided pyramidal massess	Small, flat mass	Enclosed in the urethralis muscle	Very large and dense, two cylindrical lobes
Dog (<i>Canis l. familiaris</i>)	-	-	Compact, large, globular in form, symmetric	Small number of lobules in wall of urethra	-
Tom (<i>Felis catus</i>)	-	-	Bulb-shape, four spherical lobes,	-	Bilobed glands
Other terrestrial mammals					
Rhinoceros					
Asian (<i>Rhinoceros unicornis</i>)	-			-	Small and round
White (<i>Ceratotherium simum</i>)	-	Paired, multilobulated, sacculated	Two triangular lobes	-	Elongated and irregular
Black (<i>Diceros bicornis</i>)	-			-	Small and round
Elephants	Cone-shaped	Largest ASG, paired cigar-shaped	Lobulated paired structure w/ three lobes*	-	Large, paired glands solid tissue w/ central irregularly shaped cavity
Camel (<i>Camelus dromedarius</i>)	Glandular	-	Rounded or oval disc-shape	Surrounds the pelvic urethra	Almond-shaped pair

Table 2-1. Continued.

	Ampulla	Vesicular gland	Prostate	Bulbourethral gland	Reference	
			Body	Disseminated		
Pampas Deer (<i>Ozotoceros bezoarticus</i>)	Present. Not described	Present. Not described	-	Poorly developed	-	Pérez et al., 2013
Rodents						
Souslik (<i>Spermophilus xanthoprymnus</i>)	-	-	Single lobe	-	Paired lobes	Çakir & Karataş, 2004
Agouti (<i>Dasyprocta leporina</i>)	-	Branched rubber-like, tightly coiled	U-shaped	-	Paired gland bean-shaped	Mollineau et. al., 2009
Viscacha (<i>Lagostomus m. maximus</i>)	-	Paired, elongated and tortuous in shape	Two partially lateral lobules	-	Small and spheroid	Chaves et. al., 2011
Shrew (<i>Tupaia belangeri</i>)	Glandular	Simple, paired glands	Paired lobules	-	**	Collins et. al., 1982
Marine mammals						
Pinnipeds	-	-	Bulky gland	Convoluted tubules	-	Harrison, 1969 Laws & Sinha, 1993
Odontocets			Relatively large			(1)
Beluga (<i>Delphinapterus leucas</i>)	-	-	-	Only very few small remnants were found microscopically	-	De Guise et al., 1994
Sirenians						
Dugong (<i>Dugong dugon</i>)	-	Large, thick-walled		Along the wall of the pelvic urethra	(?)***	Marsh et. al., 1984
Florida Manatee (<i>Trichechus m. latirostris</i>)	-	Bilateral and dorsal to the urinary bladder	Non glandular prostate, composed of erectile muscular tissue		-	Bonde et al., 1984 Caldwell & Caldwell, 1985

(-) Not present in this specie. *Significant differences in size and morphology between African and Asian elephants. ** Pair of spherical structures where located with bulbourethral like histology. ***Not observed macroscopically, but some histological sections present evidence of the glands. (1) Harrison did not specify what species, Odontocetes were generally referring to *Delphinus sp* and *Stenella sp*. Laws & Shia referred to the elephant seal (*Mirounga sp.*) and Antarctic fur seal (*Arctocephalus gazella*) as pinnipeds.

Table 2-2. Condition and cause of death of the male manatees used for gross anatomical analysis.

Study ID	Field ID	Collection date	Necropsy Date	Age at death	Condition	Cause of death
GTM1	MSE14020	02/19/14	02/20/14	J	BD	Flood gate lock
GTM2	MSE14028	04/05/14	04/07/14	A	MD	HR- Other
GTM3	LPZ103167	04/29/14	05/02/14	A	F	Watercraft collision

F- Fresh, MD- Moderately decomposed, BD- Badly decomposed. HR- Human related. Date- mm/dd/yy, A- Adult, J- Juvenile.

Table 2-3. Male manatee carcass data obtained for gross anatomical analysis.

Study ID	Field ID	Samples collected	Age at death	Length (cm)	Weight (Kg)	Necropsy Date
GTM1	MSE14020	WRT	J	246	NT	02/20/14
GTM2	MSE14028	WRT	A	280	396	04/07/14
GTM3	LPZ103167	WRT	A	273	269	05/02/14

WRT- Whole reproductive tract, NT- not taken, Date- mm/dd/yy, A- Adult, J- Juvenile.

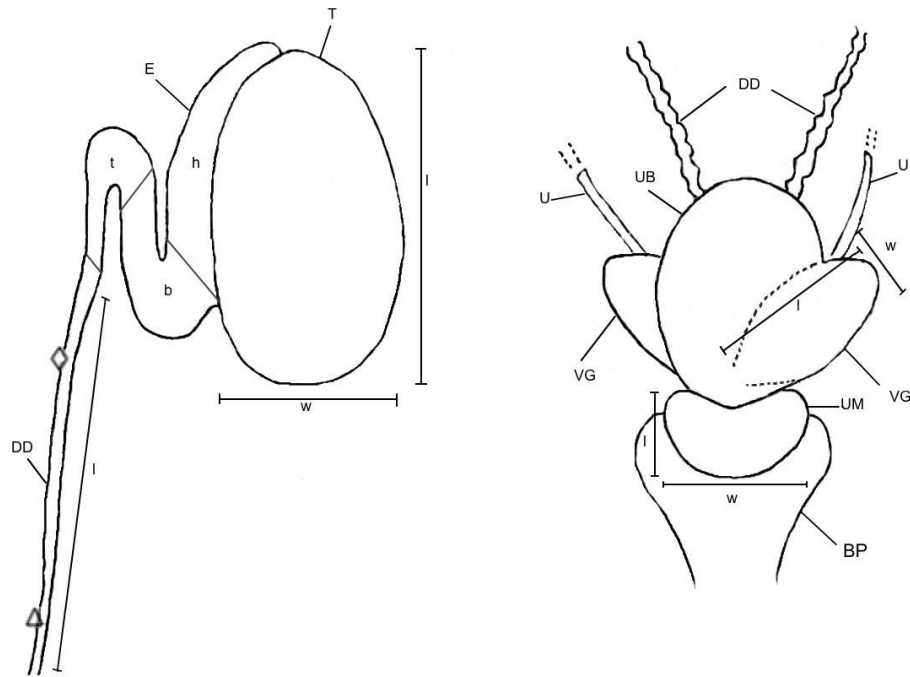


Figure 2-1. Schematic of the morphometric measurements. Left side of the figure: Dorsal view of the left testis (T), epididymis (E) with the division made for the head (h), body (b) and tail (t), and ductus deferens (DD) width and diameter were measured on the thickest (diamond) and thinnest (triangle) portion along the duct. Right side of the figure: Ventral view of the tract shows base of the penis (BP), deferent ducts (DD), ureters (U), urinary bladder (UB), Urethralis muscle (UM) and vesicular glands (VG). Morphometric of the left vesicular gland is showed. Length (l) and width (w) are presented.

Table 2-4. Morphometric data (cm) of testes and epididymides from two adult male Florida manatees.

		Testes			Epididymides				
		R	L	H	R B	T	H	L B	T
GTM2	Length	16.5	16.4	16	7.5	13.2	12.3	8	15.3
	Width	11.6	11.3	3.1	5.1	2.7	2.6	3.9	2.6
	Depth	1.8	1.7	0.7	1.6	1.1	1.1	1.1	0.7
GTM3	Length	15.5	12.4	11.5	14	12	13	10.3	11.2
	Width	10.3	7.5	3.1	5	3.2	2.3	5.4	2.9
	Depth	1.3	1.2	0.8	1.3	1.2	1	1.2	1.04

R= right, L= left, H= head of epididymis, B= body of epididymis, T= tail of epididymis.

Table 2-5. Morphometric data (cm) of ureters, urinary bladder, deferent ducts, vesicular glands, urethralis muscle, and penis from two adult male Florida manatees.

		Ureters		Urinary Bladder	Deferent Ducts		Vesicular glands		Urethralis muscle	Penis
		R	L		R	L	R	L		
GTM2	Length	12	17.5	10	28.5	29.2	10.1	8.9	3.3	42
	Width			7.8	1.4	1.3	4.3	4.9	5	3.6
	Depth			2.1	0.6	0.5	1.2	1.6	2.9	4.5
	Diameter	0.9	0.9							
GTM3	Length	22	27.4	10.8	29.2	25.8	8.2	7.9	3.7	37.8
	Width			10.2	1.4	1.2	4.4	3.9	4.5	3.9
	Depth			2.2	0.5	0.4	2.2	2.7	2.6	2.6
	Diameter	0.9	0.8							

Ductus deferens width and depth were measured at the thickest and thinnest portion along the duct, the table shows the median of the two measurements. Ureters were cut from the kidneys and measure from there to the urinary bladder. Urinary bladder and vesicular glands were measured empty. Penis length was measured from the top of the crura to the tip, width and depth were measured approximately “halfway” from the crura to the tip.

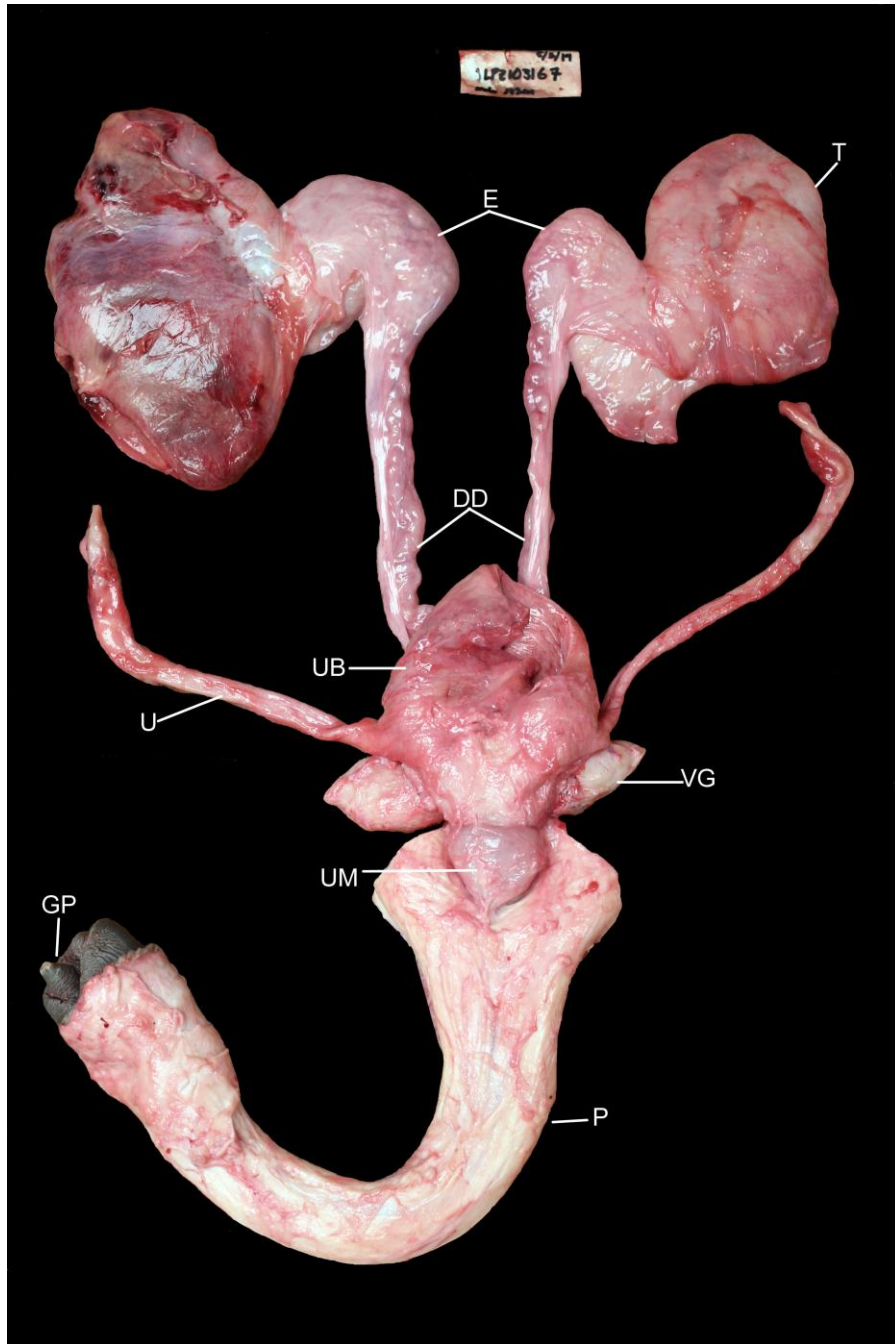


Figure 2-2. Male Florida manatee (GTM3) reproductive tract dissection, ventral view. Deferent ducts (DD), epididymides (E), glans penis (GP), testis (T), ureter (U), urinary bladder (UB), urethralis muscle (UM), vesicular gland (VG) and penis (P). (Photo courtesy of author).

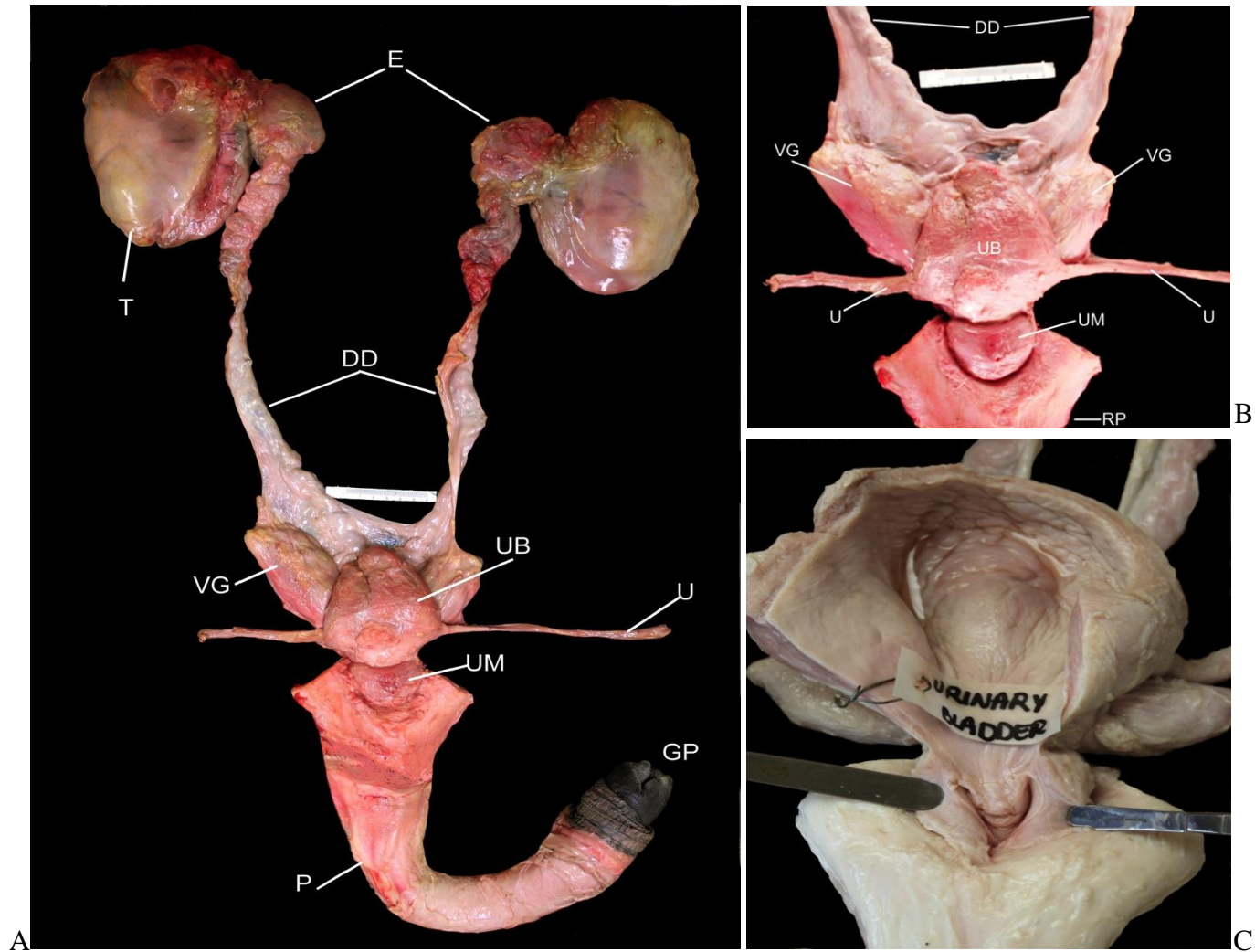


Figure 2-3. Male Florida manatee (GTM2) reproductive tract dissection, ventral view. A) Complete reproductive tract. Deferent ducts (DD), epididymides (E), glans penis (GP), testis (T), ureter (U), urinary bladder (UB), urethralis muscle (UM), vesicular gland (VG) and penis (P). B) Close up of deferent ducts (DD), ureter (U), urinary bladder (UB), urethralis muscle (UM), vesicular gland (VG) and root of the penis (RT). C) The urethralis muscle was transected. Colliculus seminalis was found within the urethra. (Photo courtesy of author).

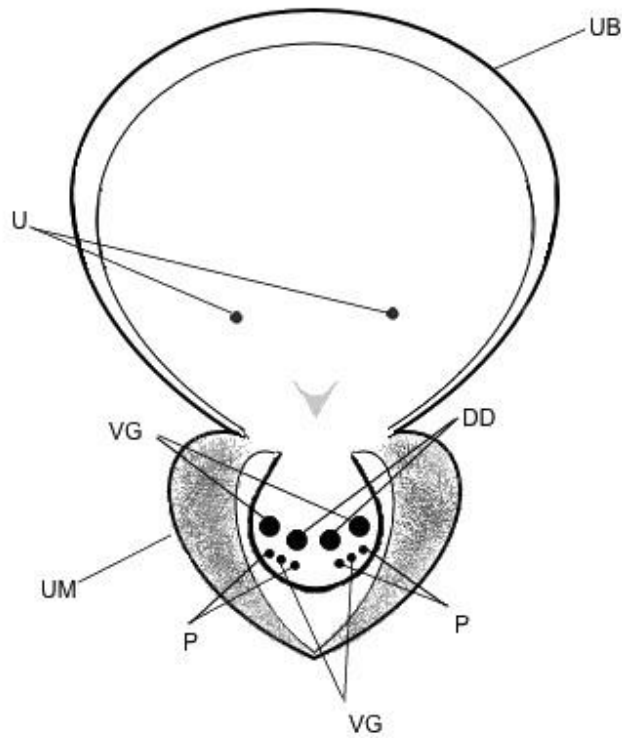


Figure 2-4. Colliculus seminalis of the adult male Florida manatee. Deferent ducts opening (DD), ureters openings (U), urinary bladder (UB), urethralis muscle (UM), vesicular gland opening ducts (VG) and prostate opening ducts (P).

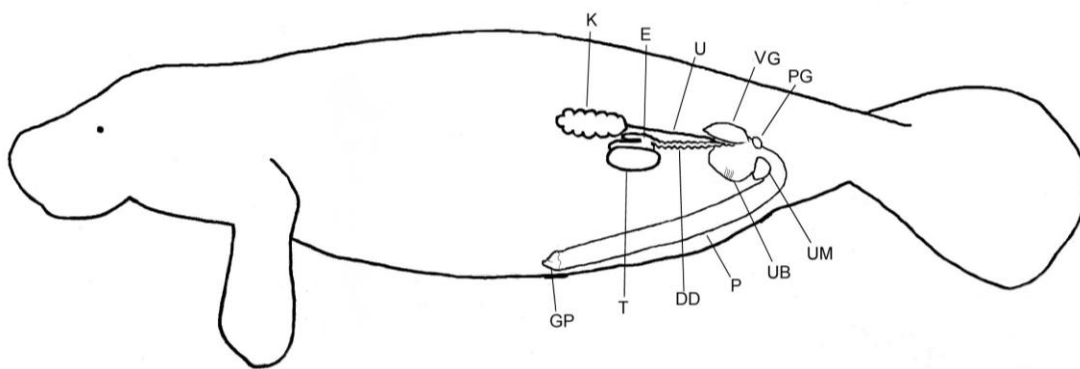


Figure 2-5. Male Florida manatee (*Trichechus m. latirostris*) reproductive tract (left lateral view). Ductus deferent (DD), epididymis (E), glans penis (GP), kidney (K), testis (T), ureter (U), urinary bladder (UB), urethralis muscle (UM), vesicular gland (VG), penis (P) and prostate gland (PG), Modified from Rommel & Reynolds, 2000.



Figure 2-6. Cross-sections of the penis of the Florida manatee. A) Cross-section closest to the base of the penis. B) Cross-section 2 inches from cross-section A. C) Cross-section 2 inches from cross-section B. D) Cross-section 2 inches from cross-section C. E) Cross section 2 inches from cross-section D, most distal part of the penis closest to the tip of the penis. Artery (A), corpus cavernosum (CC), corpus spongiosum (CS), glans (G), retractor penis ligament (RP), septum (S), skin (SK), tunica albuginea (TA), urethra (U), vein (V). (Photo courtesy of author).

CHAPTER 3 HISTOLOGY ANALYSIS

Microanatomy of the male reproductive tract

The male reproductive tract in mammals has many similarities among species, but sexual selection adaptations can be associated to anatomical and functional differences among males. The microanatomical description of the organs and tissues present in the Florida manatee male reproductive tract is essential to: 1) support the gross anatomy description in the previous chapter, 2) compare the histological structures described among species and 3) contribute knowledge of tissue functionality. The histological description of the male reproductive tract has been fully studied for domestic mammals, like the bull (*Bos Taurus*), boar (*Sus demesticus*) (Dellmann & Dellmann, 1976) and stallion (*Equus ferus*) (Bacha & Bacha, 2000). General histology of male reproductive tract in domestic mammals are described as follows: The testes are a complex structure, important for spermatozoa production and hormone secretion (Amann & Schanbacher, 1983). Testes are compound tubular glands surrounded by a thick capsule of dense irregular connective tissue, named the tunica albuginea (Bacha & Bacha, 2000; Reece, 2009). From the tunica albuginea into the testis, connective tissue (septa) divides the testicular lobules. Each testicular lobule contains the seminiferous tubules, which are surrounded by a basal lamina and a layer of collagen fibers. Seminiferous tubules are lined by a stratified epithelium with two different basic cells; supporting and spermatogonic cells (Dellmann & Wrobel, 1976; Reece, 2009).

The microanatomy of the epididymides is commonly studied for reproductive purposes. This single coiled and tortuous tube plays an important role in growth, nourishment and storage of spermatozoa. In order to understand the function of the epididymides, the histology of some terrestrial species has been fully studied. For example, the marsupial (*Didelphis azarae*), cebu

(*Bos indicus*), buffalo (*Bubalus bubalis*), and llamas (*Lama glama*) (Orsi et al., 1980; Orsi et al., 1985; Abdou et al., 1985; Delhon & Lawzewitsch, 1994). Similarly to the testes, the epididymis is surrounded by a thick tunica albuginea composed of dense irregular connective tissue. The coiled duct of the epididymides has a pseudostratified columnar epithelium with stereocilia cells. The epithelium decreases in thickness moving from the head of the epididymis to the tail of this structure (Dellmann & Wrobel, 1976; Bacha & Bacha, 2000).

The epididymides transition into the deferent ducts. The beginning of the deferent ducts is denoted when the epididymidal tail appears the thinnest. The ductus deferens has a thick muscular wall that distinguishes it from the epididymal duct. This thick muscular coat is present as inner circular and an outer longitudinal layers of smooth muscle (Sisson, 1910; Bacha & Bacha, 2000). The folded mucosa of the ductus deferens is lined by a pseudostratified columnar epithelium with some cells with stereocilia that become a simple columnar epithelium distally (Dellmann & Wrobel, 1976).

The accessory sex glands are in charge of producing and discharging secretions into the pelvic urethra to form the seminal fluid (Senger, 2005). The function of the seminal fluid is to transport, providing an energy source for spermatozoa motility (like fructose), lubricate the urethra, and add volume to the seminal fluid, enhancing sperm viability in the female reproductive tract (Gartner & Hiatt, 2001). The accessory sex glands are composed of branched tubular or tubuloacinar secretory units that often have vesicular dilations. The secretory epithelium of these glands is classified as pseudostratified because, although consisting primarily of columnar cells (or sometimes cuboidal cells such as in the prostate), occasional basal cells are present (Bacha & Bacha, 2000). Table 3-1 describes the variation of the microanatomy of the accessory sex glands among some terrestrial and marine mammal species.

In some domestic mammals [stallion, ruminants and dog (*Canis l. familiaris*)] the terminal portion of the deferent ducts is dilated, this segment is known as the ampullae. The lamina propria and submucosa of the ampullae contain simple branched tubuloalveolar glands (Bacha & Bacha, 2000). Ampullary secretion in the bull is rich in electrolytes and citric acid (Ruckebusch et al., 1991). These glandular secretions may facilitate sperm storage (Nissar & Suri, 2012). The ampullae are lined by two forms of epithelium: tall columnar cells with ovoid nuclei or cuboidal cells with spherical nuclei (Dellmann & Wrobel, 1976).

Vesicular glands are paired compound tubular or tubuloalveolar glands, present in most domestic mammals, except in the dog and tomcat. The epithelium of these glands is pseudostratified with tall columnar or cuboidal secretory cells and small, spherical often basal cells (Aughey, 2001). The septa contain connective tissue and smooth muscle strands. The wall of the vesicular glands is thick fibromuscular. The secretion of the vesicular glands is rich in fructose, which serves as an energy source for ejaculated spermatozoa (Sisson, 1910; Dellmann & Wrobel, 1976). Fibrinogen, vitamin C and prostaglandins are also present in the fluid secreted by the vesicular glands (Peckham, 2011), which add the volume to seminal fluid, provide nutritive substance and act as transport medium for spermatozoa (Nissar & Suri, 2012).

The only accessory sex gland present in all domestic mammals is the prostate. The prostate gland is a compound tubular or tubuloalveolar gland (in ruminants), seromucous secretory, except in the dog which is entirely serous (Dellmann & Wrobel, 1976; Bacha & Bacha, 2000). This gland varies in form among species. For example, the disseminated portion of the prostate can be present as a glandular layer in the submucosa of the prostatic urethra (boar and ruminants), or as scattered glands in the stallion and carnivores. The body of the prostate in the stallion and carnivores is a discrete compound tubular gland with a thick fibromuscular

capsule (Aughey, 2001). The simple cuboidal or columnar epithelium with occasional basal cells is found in a fibroelastic lamina propria. The secretion of the prostate gland is rich in citric acid, proteins, and hydrolytic enzymes. These components support sperm in the female genital tract, neutralizing acid vaginal secretions and liquefying coagulated sperm (Dellmann & Wrobel, 1976; Peckham, 2011). The prostate is the only accessory sex gland found in pinnipeds and cetaceans. This gland is described as a tubuloalveolar gland with a cuboidal to low-columnar pseudostratified epithelium with basal nuclei and pale apical cytoplasm (Harrison, 1969).

The most caudal accessory sex gland is the bulbourethral gland. This gland is compound tubular (in boar and tomcat (*Felis catus*) or tubuloalveolar (in stallion and bull) with mostly mucous secretion (Bacha & Bacha, 2000). Cells lining the lumen of this gland are cuboidal to columnar epithelium. These cells are flattened with a basal located nuclei that exhibit a light staining (Dellmann & Wrobel, 1976; Gartner & Hiatt, 2001). This gland secretes galactose and sialic acid into the pelvic urethra prior to ejaculation. These secretion may serve as lubricant for the epithelial surface of the urethra (Peckham, 2011).

The penis is the male organ for copulation. This organ consists of a dense irregular connective tissue capsule known as the tunica albuginea, which is well developed in the fibroelastic type penis of the boar and bull (Dellmann & Dellmann, 1976). The tunica albuginea encloses two sections of erectile tissue. First, the corpus cavernosum, which is surround by a trabeculae consisting of smooth muscle and dense connective tissue (Bacha & Bacha, 2000). Second, the corpus spongiosum, that presents more elastic tissue and less smooth muscle in the trabeculae. This corpus surrounds the urethra (Sisson, 1910). The urethra is a long tube, which extends from the bladder to the glans penis. The urethra is lined by a transitional epithelium, which may become stratified squamous distally (Gartner & Hiatt, 2001).

Methods

Sample Collection

Three adult male reproductive tracts (TM1, TM2 and TM5; Table 3-2) were collected for histological analysis, at the Marine Mammal Pathobiology Laboratory (MMPL), Saint Petersburg, Florida in April 2013. The condition and cause of death of these animals is shown in Table 3-3. Members of the MMPL provided an en bloc mass of tissue that contained the reproductive tract. The tracts were placed in 10% neutral buffered Formalin (NBF) in enough volume to cover all the tissues. Prior to submersion in formalin, superficial slices were made on the tract to aid fixation. These tracts were transported to the Laboratory of the Aquatic Animal Health Program at the University of Florida, Gainesville until processing. At least 24hrs in formalin was required to insure a complete fixation process. The day following tissue collection, additional deep slices in the same pattern in all tracts were made. First, a sagittal plane cut was performed a long the whole tract and then multiple transversal plane cuts were made. Cuts did not follow any protocol, but was a process needed to ensure the fixation process in all the tissues and areas of the tract. Furthermore, fat and muscle were dissected from the tract to facilitate the histological procedures. Morphometric data were not obtained prior to formalin fixation.

Archived Samples

Four archived male reproductive tracts (TM3, TM4, TM6 and TM7; Table 3-2) collected between January and February 2012, were also included in this study. Of the four tracts, two were adult (one complete tract and one with only parts: testes, epididymal duct, vesicular glands and base of the penis), one juvenile and one calf, both with complete tracts. The evaluation of different age stages can give us an idea of the variation at a histological level of the male reproductive tract. These tracts were stored in 10% NBF and data (date of collection size of the manatee and ID) were recorded.

Sample Processing

With these specimens (five adults, one juvenile and one calf) the following organs and tissues of interest were evaluated: testis, epididymis, ductus deferens, urinary bladder, vesicular glands, prostate, and penis. All samples were taken in the same pattern and location of the tissue block in a cranial to caudal order. Photographs were taken at each tissue sample point for the record.

Each tissue sampled were placed in cassettes (Leica Microsystems Inc.) and put in 10% NBF overnight. The cassettes were then placed in an Excelsior™ ES Tissue Processor, where the tissues were dehydrated in alcohol and cleared in xylene. Then the tissues were embedded in paraffin in a Tissue Tek consoles Miles Scientific, cut at 5 µm from the paraffin block on a Leica Reirched 2030 Microtome and dried out in Blue M oven overnight, a standard protocol by Sheehan & Hrapchak, 1980.

Staining

Slides were stained with hematoxylin and eosin (H&E) by the Tissue-Tek DRS 2000 Multiple Slide Strainer Sakura, for examination of general cell structure, as describe by Humason (1979). Periodic acid-Schiff (PAS) was used to stain glycogen and mucus-secretions, and Gomori's one step trichrome was used to demonstrate collagen of connective tissue and muscle fibers. These last two stains were deparaffinized on the Tissue-Tek DRS 2000 Multiple Slide Strainer Sakura, the next steps of the staining procedure were by hand, as described by Sheehan & Hrapchak (1980), and Carson (1997). Dako Smooth Muscle Actin (SMA) Mouse monoclonal IgG_{2A}, kappa kit was used to differentiate and identify the muscle present on different structures of the reproductive tract and localize the myoepithelial cells on glandular tissue. A counter stain of hematoxylin was applied to the SMA slides. All the slides were

analyzed in an American Optical AO Spencer dual Viewing Microscope. Pictures were taken with a Zeiss Axiophot light microscope.

Results

Almost 770 slides were evaluated for male reproductive microanatomy of the Florida manatee. At least two out of the five adults' sections obtained had proper condition (optimal multicellular integrity) for histological description. The majority of the sections presented autolysis or tissue damage that were excluded from the analysis. The juvenile and calf sections were in good histological state. Unfortunately, for these specimens the accessory sex glands were not found, possibly due to late development related to age. This would be interesting for comparison analysis but was beyond the current study.

Testes

Manatee testes were surrounded by the tunica albuginea that was characterized by distinctive layers. These layers were a smooth muscle layer (inner), an adipose and vascular layer in the middle, and a dense irregular connective tissue (outer) (Figure 3-1 A). This tunica extended into the testis as septa (connective tissue) that divides the lobules that contains the seminiferous tubules. The seminiferous tubules were outlined by a thick basement membrane (smooth muscle) with myoid cells. A stratified layer of spermatogenic and Sertoli cells lay within the tubules. Spermatogenic cells in adult male manatees were present at different stages: spermatogonium, spermatocytes and spermatids. Between the tubules, interstitial tissue contained interstitial cells and blood vessels (Figure 3-1 B). In the juvenile and calf, the tunica albuginea showed a thinner layer of smooth muscle and the seminiferous tubules were less abundant than in adults. There were Sertoli and interstitial cells present, but spermatogenic cells were absent.

Epididymis

The coiled epididymal duct had a pseudostratified columnar epithelium with stereocilia, surrounded by a smooth muscle wall embedded in loose connective tissue. The height of the epithelium was similar in the three regions (head, body and tail) of the epididymis (Fig. 3-2 A-C). Interestingly, the smooth muscle wall increased in thickness from the proximal region to the distal region of the epididymis duct (Fig. 3-2 D-F). The juvenile had a pseudostratified columnar epithelium (stereocilia absent). Epididymis tissue in the calf was not fully developed.

Ductus Deferens

Pseudostratified columnar cells with stereocilia and a basal cell layer characterized the epithelium of the ductus deferens (Figure 3-3 A). The thin lamina propria (connective tissue), a thick tunica muscularis of smooth muscle (inner circular and outer layer with no distinct arrangement) (Figure 3-3 B), and adventitia were present as in any other tubular organs. The juvenile and calf ductus deferens had pseudostratified columnar epithelium (with no stereocilia), and a lamina propria. The muscularis smooth muscle layer with no distinct arrangement was found in both the juvenile and calf ductus deferens.

Vesicular Glands

These glands were present as hollow pockets with wide lumens. Microscopically, a cuboidal to columnar epithelium (stereocilia present) with occasional basal cells (Figure 3-4 A) was found. The highly folded mucosa was surrounded by a layer of smooth muscle with some strands of connective tissue. Vesicular glands were not found in the juvenile and calf specimen.

Prostate

The prostate gland in the male Florida manatee was a compound tubuloalveolar gland, seromucous secretory. The edge of the lobes found between the vesicular glands and the urinary bladder, were found to have a foamy cuboidal to low columnar epithelium with basal nuclei in a

connective tissue septa. This region mainly consisted of mucous secretory cells with sporadic serous cells (Figure 3-5 A-B). In the proximal region to the urethra (presumably the disseminated part), scattered seromucous glands were present. A cuboidal epithelium embedded in smooth muscle was observed (Figure 3-5 C-D). Prostate glandular tissue was not found in the juvenile and calf samples.

Penis

The penis of the Florida manatee presented with a thick tunica albuginea of dense irregular connective tissue. A cord of collagen, like suspensory ligament, was found dorso-medially in the tunica albuginea. (Figure 3-6 A). From the tunica albuginea, the trabeculae (abundant strands of smooth muscle in the connective tissue) extend to the central part of the penis. The corpus cavernosum was enclosed by the trabeculae (Figure 3-6 B). The penile urethra, in the ventral part of the penis, has a transitional epithelium in a thick lamina propria. Corpus spongiosum was scarce. Additional smooth muscle was found in the urethral opening (Figure 3-6 C). Penis of the juvenile and calf did not present significant differences in the internal structure, relative to the adults.

Discussion

The microanatomy of the male Florida manatee reproductive tract showed interesting similar features with other species. In the case of the testis, the tunica albuginea in most domestic mammals is composed by dense irregular connective tissue, with the exception of the stallion which is mainly composed of smooth muscle (Bacha & Bacha, 2000). The boar and ram also have muscle cells in the tunica albuginea, but less than the stallion does (Chacon-Arellano & Woolley, 1980). The Florida manatee has an inner layer of smooth muscle in the tunica albuginea, different from the stallion where has this layer located in the outer portion of the tunica (Bacha & Bacha, 2000). This layer was thicker in adults than in the juvenile and the calf.

The samples from the juvenile and calf testes do not allow for generalizations about increased thickness in the tunica albuginea with age. Additional samples may support this hypothesis. The function of the smooth muscle in the stallion tunica albuginea has not been clarified. A study dismissed the possible sperm transportation role of this muscle, but suggests a possible association with the vasculature of the spermatic cord (Chacon-Arellano & Woolley, 1980).

The features of the male Florida manatee epididymis in this study resemble the general mammalian pattern with pseudostratified columnar epithelium and smooth muscle thickening from the head towards the tail of the epididymis. This is similar to domestic mammals (Aughey, 2001), camels (*Camelus dromedarius*) (Degen & Lee, 1982), the lesser anteater (*Tamandua tetradactyla*) (Rossi et al., 2013), babirusa (*Babirusa celebensis*) (Ziehmer et al., 2013), rhinoceros (*Rhinoceros unicornis*, *Ceratotherium simum*, and *Diceros bicornis*) (Schaffer et al., 2001) and African elephants (*Loxodonta africana*) (Short et al., 1967). However, all these species present a decrease in the height of the epithelium from head to tail of the epididymis, a feature not clearly identified in Florida manatee samples. Three epididymis sections were processed from specimen TM4 and five sections were processed from TM1. The most distal section of TM1 showed a different configuration than the description of specimen TM4, as mentioned in the results of this chapter. In TM1, the most distal section was found to have wider lumens, with pseudostratified tall columnar epithelium having “villus” like projections. This configuration is present in the stallion (Bacha & Bacha, 2000), African elephants (Short et al., 1967), and dugongs (*Dugong dugon*) (Marsh et al., 1984). Considering the shape and size of the epididymis of the Florida manatee, it is very probable that the sectioning made in specimen TM4 was improperly. Complete dissection of the ductus epididymis and further histological analysis should be performed.

The vesicular gland microanatomy varies among species. For example, the lining of the epithelium can be columnar, as in rhinoceros (Schaffer et al., 2001) and the Asia minor ground squirrel (*Spermophilus xanthoprymnus*)(Çakir & Karataş, 2004), or pseudostratified columnar, like in stallions, and with basal nuclei and lipid droplets as in the bull and the lesser anteater (Dellmann & Wrobel, 1976; Aughey, 2001; Rossi et al., 2013). The African elephant and the dugong present a pseudostratified cuboidal epithelium, which most closely resembles the vesicular glands of the Florida manatee described in this study, than the other terrestrial mammals. Also, the thick layer of the of smooth muscle is a feature shared by these three species (Short et al., 1967; Marsh et al., 1984), in contrast with the babirusa that has a thin smooth muscle capsule surrounding the vesicular glands (Ziehmer et al., 2013).

Interestingly, the body of the prostate section closely resembles bulbourethral type glandular tissue, which complicated the description of this gland. A columnar epithelium with basal nuclei is present in the body prostate of the African elephant (Short et al., 1967) and tree shrew (*Tupaia belangeri*) (Collins et al., 1982). The histological description in the delphinid male reproductive tract has not been fully described, but a picture of the body prostate from an adolescent long-beaked dolphin (*Stenella longirostris*) is shown by Harrison in 1969. This image resembles the foamy simple cuboidal epithelium with basal nuclei present in the prostate body of the Florida manatee. In the body of the prostate can be found different sections of the gland, the alveoli and the ducts (Degen & Lee, 1982). These different structures when sectioned can present different lining epithelium as in the body prostate in rhinoceros (Schaffer et al., 2001) and camels (Degen & Lee, 1982). The capsule present in the body portion of the Florida manatee prostate is similar to the well-developed connective tissue septa and capsule in the stallion and the dog (Bacha & Bacha, 2000). The smooth muscle fibers seen in the capsule and the septa of

the disseminated portion in the Florida manatee has similar characteristics with the boar disseminated portion (Dellmann & Wrobel, 1976). Differences exist compared with dugong. Marsh et al. (1984) described the dugong prostate as disseminated mucous gland along the wall of the pelvic urethra, while the disseminated portion of the prostate in the Florida manatee showed to be a mix gland, but mostly serous secretory.

The penis of the Florida manatee is similar to the dugong, elephant, stallion and lesser anteater penis, a vascular or musculocavernosous type. The tunica albuginea contains some elastic fibers and smooth muscle cells, and the erectile tissue and smooth muscle in the corpus cavernosum play an important role in the erectile process (Short et al., 1967; Marsh et al., 1984; Bacha & Bacha, 2000; Senger, 2005; Rossi et al., 2013). Otherwise, the Florida manatee penis differs from most of the aquatic mammals' penis type (Harrison, 1969; De Guise et al., 1994; Reynolds & Rommel, 1999; Bland & Kitchener, 2001). The retractor penis muscle found in aquatic mammals suggested that this structure may function to regulate the stretching of the penis during ejaculation (Norris, 1966). The South American fur seal (*Arctophoca australis*) has a retractor penis muscle on the ventral side and also has a suspensory ligament located ventro-caudal (Machado et al., 2012). The retractor penis found in the male Florida manatee in this study contained collagen, with no muscle found in this structure, suggesting that this may function as a suspensory ligament rather than a retractor penis muscle. In other species, like the American alligator (*Alligator mississippiensis*), the penis contains large amount of collagen described for rapid retraction (Kelly, 2013).

The microanatomical study of the male Florida manatee reproductive tract contributes to the characterization of reproduction in the Sirenia. It would be interesting to compare the histology of the reproductive tract among different ages and seasonal months, get more samples

and additional samples of other species of manatees for further comparison. Examining the content of the accessory sex glands would provide descriptive data for the role each gland plays in the reproduction of the Florida manatee.

Table 3-1. Comparative microanatomy of the accessory sex glands (ASG) in mammals.

	Vesicular gland	Prostate	Bulbourethral gland	Reference
	Body	Disseminated		
Domestic mammals				
Bull (<i>Bos taurus</i>)	Tubuloalveolar, columnar basal cells w/ lipid droplets		Tubuloalveolar, simple cuboidal epithelium	Dellmann & Wrobel, 1976 Bacha & Bacha, 2000
Stallion (<i>Equus ferus</i>)	Branched tubuloalveolar	Tubules are expanded with accumulations of secretory material	Compound tubuloalveolar	
Boar (<i>Sus domesticus</i>)	Wide tubular lumina, secretory epithelium is folded		Compound tubuloalveolar simple columnar epithelium	
Dog (<i>Canis l. familiaris</i>)	-	Serous gland in a connective tissue capsule	Few glandular lobules	
Tomcat (<i>Felis catus</i>)	-		Scattered	
Other terrestrial mammals				
Rhinoceros				
Asian (<i>Rhinoceros unicornis</i>)	Dense connective tissue bands, columnar epithelium	Thick connective tissue septa, tall columnar epithelium	Foamy cuboidal epithelium	Schaffer et al., 2001
White (<i>Ceratotherium simum</i>)				
Black (<i>Diceros bicornis</i>)				
Camel (<i>Camelus dromedarius</i>)	-	Compound tubular, two layers pseudostratified epithelium	Fibrous connective tissue surrounding, compound tubuloalveolar, tall columnar epithelium cells w/basal nucleus	Degen & Lee, 1982 Hafez & Hafez, 2001
Pampas Deer (<i>Ozotoceros bezoarticus</i>)	Present. Not described	-	Poorly developed	Pérez et al., 2013

(-) Not present in this specie.

Table 3-1. Continued

	Vesicular gland	Body	Prostate Disseminated	Bulbourethral gland	Reference
Rodents					
Souslik (<i>Spermophilus xanthoprimum</i>)	Branched tubuloalveolar, simple columnar epithelium	Tubuloalveolar, pseudostratified epithelium		Large lumens, tubuloalveolar glands	Çakir & Karataş, 2004
Agouti (<i>Dasyprocta leporina</i>)	Branched epithelium folds.	Tubular - tubuloalveolar, pseudostratified columnar epithelium		Convolutated tubular, mucous, simple columnar epithelial cells w/ oval nuclei	Mollineau et. al., 2009
Viscacha (<i>Lagostomus m. maximus</i>)	Tubular glands, cylindrical pseudostratified epithelium cells w/elongated nucleus	Tubuloalveolar adenomers, prismatic epithelium		Tubuloalveolar glands, acini ovoid, simple cylindrical epithelium cells w/basal nucleus	Chaves et. al., 2011
Shrew (<i>Tupaia belengeri</i>)	Alveolar, lined by columnar or cuboidal epithelium	Alveolar, columnar epithelium cells with basal nuclei		Connective tissue, simple cuboidal epithelium cells w/ round nuclei	Collins et. al., 1982
Marine mammals					
Pinnipeds	-		Tubuloalveolar, cuboidal to low-columnar pseudostratified epithelium	-	Harrison, 1969
Cetaceans					
Beluga (<i>Delphinapterus leucas</i>)	-	-	Small acinin lobules	-	De Guise et al., 1994
Sirenians					
Dugong (<i>Dugong dugon</i>)	Pseudostratified epithelium, cuboidal principal cells		Mucous glands	Columnar cells with basal nuclei	Marsh et. al., 1984

Table 3-2. Male manatee carcass data from the samples obtained for histological analysis. Dates of necropsy and dissected for processing.

Study ID	Field ID	Samples collected	Age at death	Length (cm)	Weight (Kg)	Necropsy Date	Dissection Date
TM1	LPZ103081	WRT	A	283	414	04/08/13	06/06/13
TM2	MSW13276	WRT	A	300	NT	04/08/13	06/21/13
TM3	MNE1201	SRT	A	334	616	01/19/12	07/17/13
TM4	SWFTm1203b	WRT	A	275	339	02/24/12	07/18/13
TM5	MNE1323	WRT	A	266	438.5	04/08/13	02/03/14
TM6	LPZ102961	WRT	J	209	160.5	01/17/12	07/16/13
TM7	MSW1210	WRT	C	180	116	01/19/12	07/17/13

WRT- Whole reproductive tract; SRT- Sections of the reproductive tract; NT- Not taken. Date mm/dd/yy; A- Adult; J- Juvenile; C- Calf.

Table 3-3. Condition and cause of death of the male manatees used for histological analysis.

Study ID	Field ID	Collection date	Necropsy Date	Age at death	Condition	Cause of death
TM1	LPZ103081	01/08/13	04/08/13	A	F	Red Tide
TM2	MSW13276	04/07/13	04/08/13	A	MD	Red Tide
TM3	MNE1201	01/18/12	01/19/12	A	BD	Undetermined
TM4	SWFTm1203b	02/23/12	02/24/12	A	F	Undetermined
TM5	MNE1323	04/07/13	04/08/13	A	MD	Watercraft collision
TM6	LPZ102961	01/17/12	01/17/12	J	F	Undetermined
TM7	MSW1210	01/18/12	01/19/12	C	F	Cold stress

F- Fresh; MD- Moderately decomposed; BD- Badly decomposed; Date- mm/dd/yy; A- Adult; J- Juvenile; C- Calf.

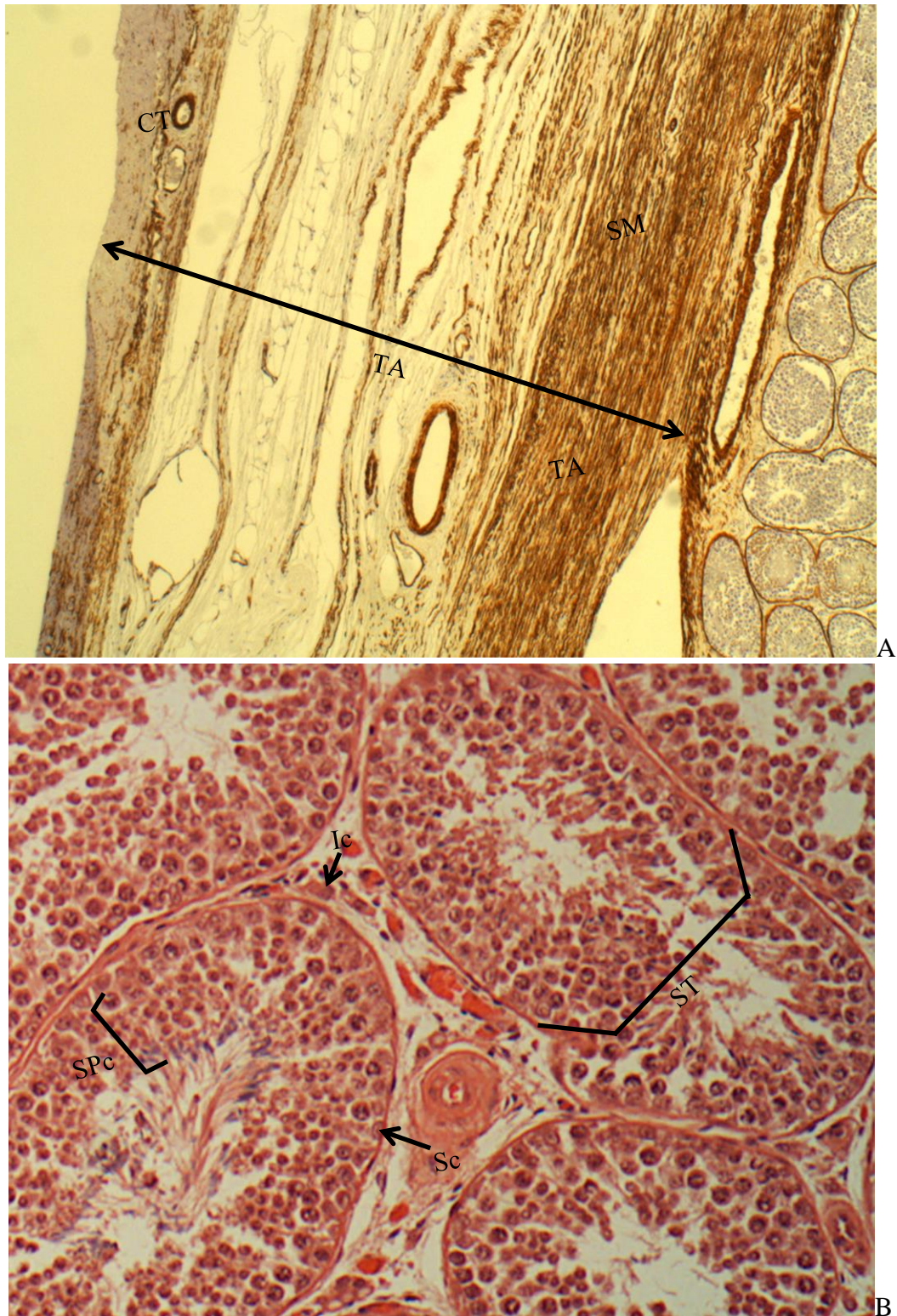


Figure 3-1. Testis of the Florida manatee. A) Layers of tunica albuginea (TA), connective tissue (CT), adipose and smooth muscle (SM) layer (from outer to inner), SMA, 20x. B) Seminiferous tubules (ST), Sertoli cells (Sc), spermatogenic cells (SPc), and Interstitial cells (Ic) are present, H&E, 20x.(Photos courtesy of author.)

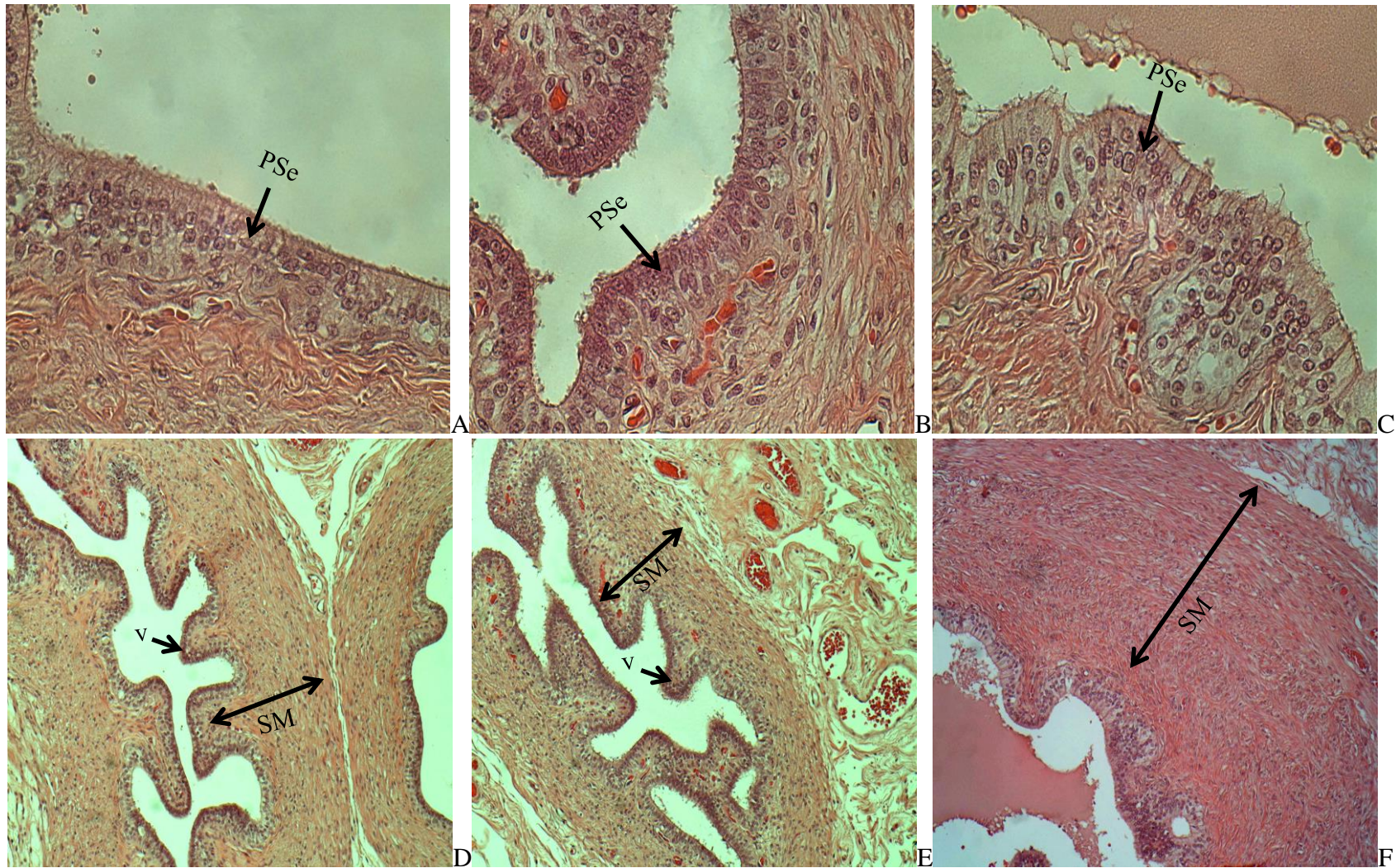


Figure 3-2. Epididymis of the Florida manatee. A) Pseudostratified epithelium (PSe) of the head of the epididymis. B) Pseudostratified epithelium of the body of the epididymis. C) Pseudostratified epithelium of the tail of the epididymis. D) Smooth muscle (SM) wall of the head of the epididymal duct. E) Smooth muscle wall of the body of the epididymal duct. F) Smooth muscle wall of the tail of the epididymal duct. Villus-like projections (v) observed. A-C H&E, 40x. D-F H&E, 10x. (Photos courtesy of author.).

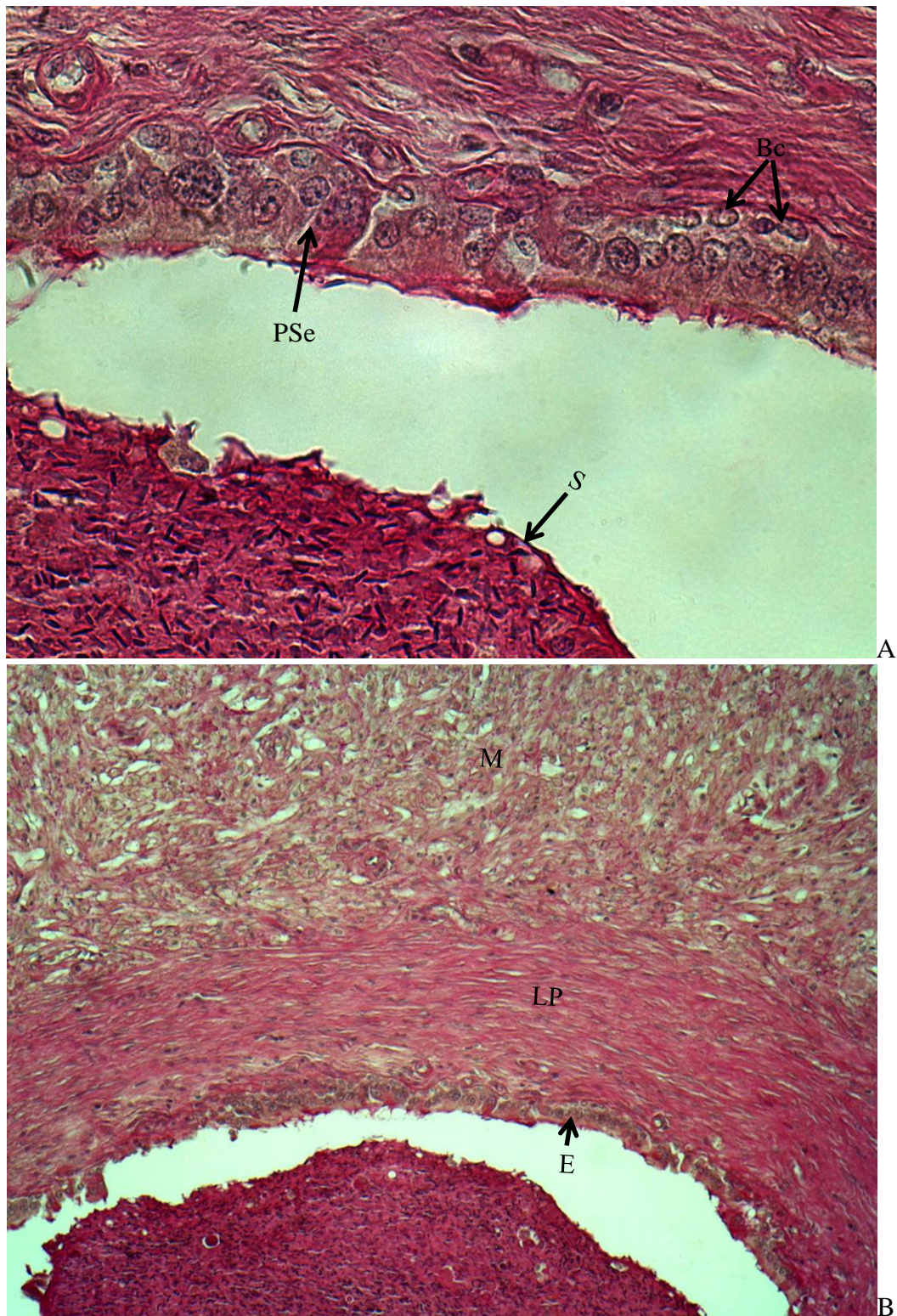


Figure 3-3. Ductus deferens of the Florida manatee. A) Pseudostratified columnar epithelium (PSe) with basal cell (Bc) spermatozoa are present (S), PAS, 40x. B) Epithelium (E), lamina propria (LP) and part of the tunica muscularis (M), PAS, 10x. (Photos courtesy of author).

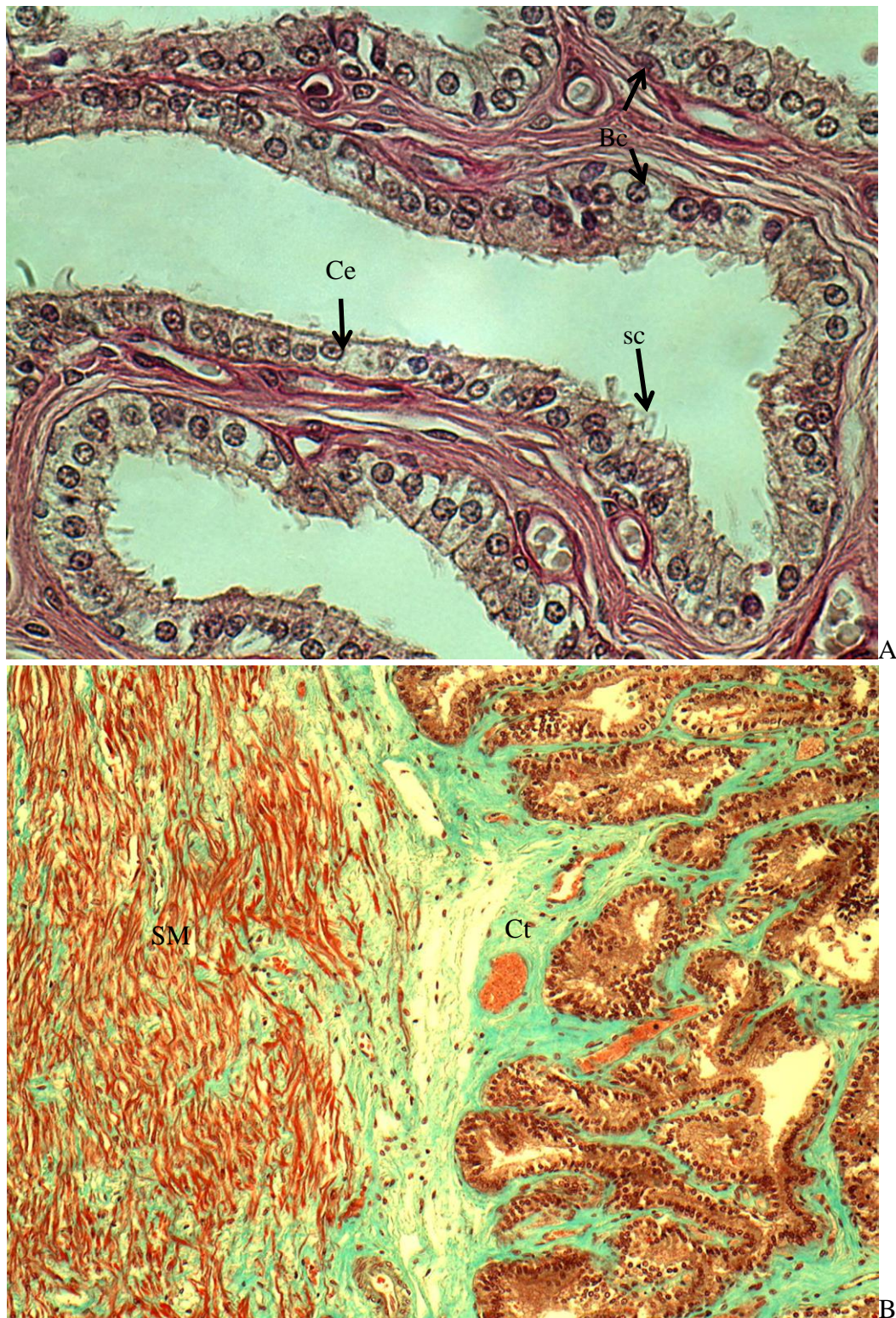


Figure 3-4. Vesicular glands of the Florida manatee. A) Cuboidal epithelium (Ce)(stereocilia (sc) present) with occasional basal cells (Bc) present, PAS, 40x. B) Highly folded connective tissue mucosa (Ct) covered by a layer of smooth muscle (SM), Gomori's Trichrome, 10x. (Photos courtesy of author).

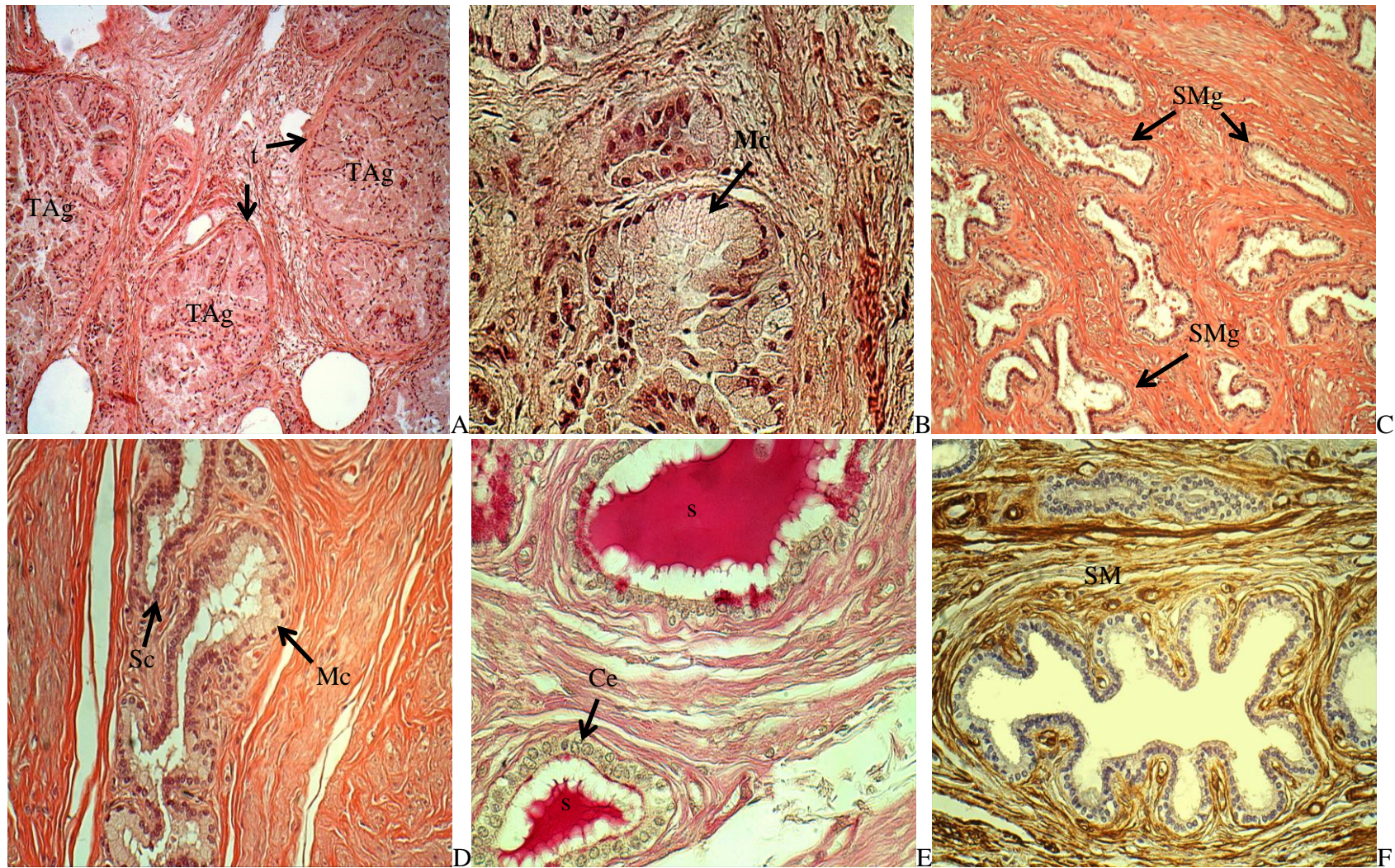


Figure 3-5. Prostate of the Florida manatee. A) Compound tubuloalveolar glands (TAg) in fibromuscular trabeculae (t), body of the prostate, H&E, 10x. B) Cuboidal to low columnar epithelium, foamy cells with basal nuclei (mucous cells-Mc), body of the prostate, H&E, 40x. C) Scattered seromucous glands (SMg), disseminated prostate, H&E, 10x. D) Seromucous secretory cells (serous cells Sc, mucous cell-Mc), disseminated prostate, H&E, 20x. E) Simple cuboidal epithelium (Ce), secretion (s) can be observed, disseminated prostate, PAS, 40x. F) Smooth muscle (SM) layer surrounding the lining of the glands, disseminated prostate, SMA, 20x. (Photos courtesy of author).

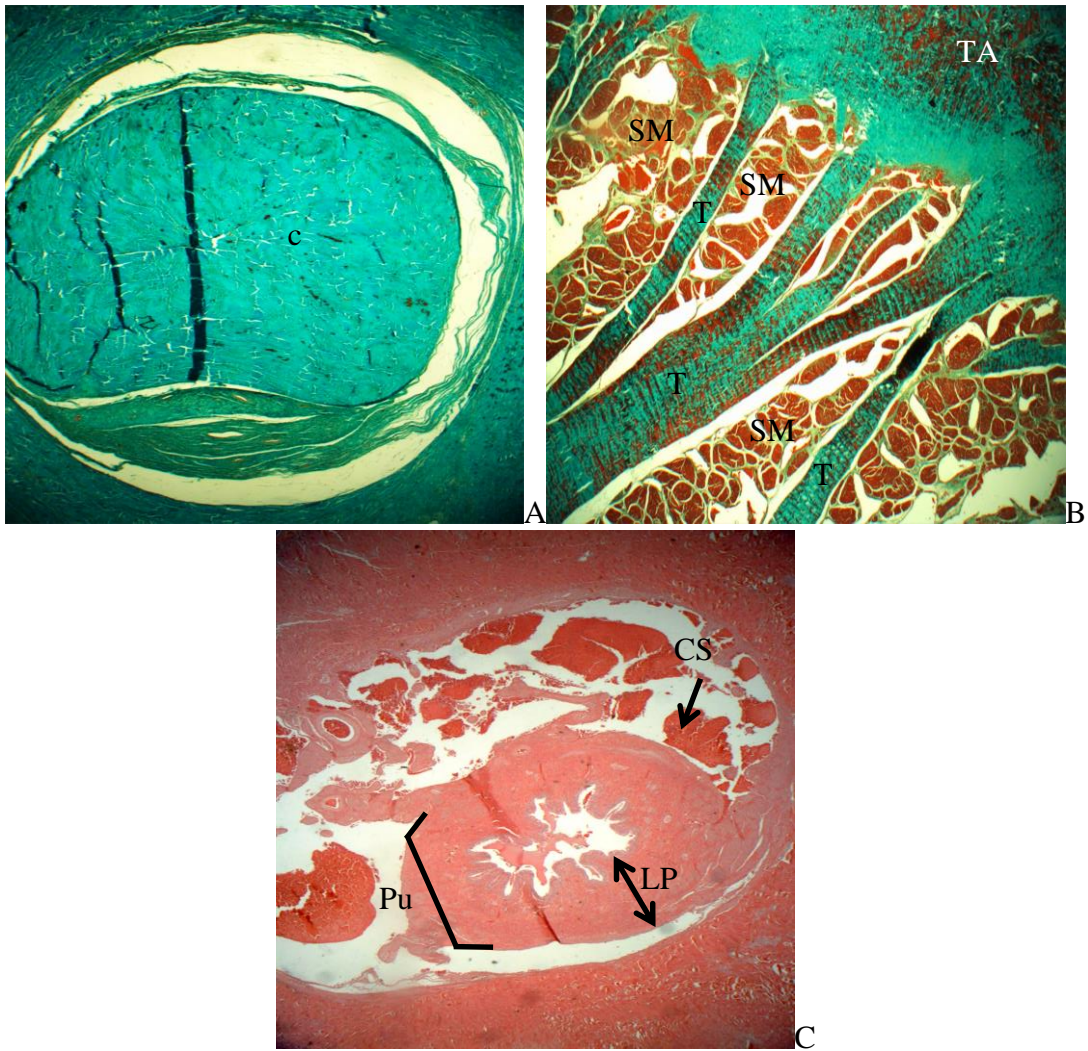


Figure 3-6. Penis of the Florida manatee. A) Collagen suspensory ligament (c), Gomori's Trichrome, 1.25x. B) Tunica albuginea, trabeculae (T) with a dense irregular connective tissue smooth muscle (SM) strand; corpus cavernosum, smooth muscle (SM), Gomori's Trichrome, 1.25x. C) Penile urethra (Pu), transitional epithelium in a thick lamina propria (LP); slight corpus spongiosum (CS), H&E, 1.25x (Photos courtesy of author).

CHAPTER 4 CONCLUSION

The male Florida manatee (*Trichechus manatus latirostris*) reproductive tract was described in a gross and histological manner covering the testes, epididymides, ductus deferens, vesicular glands, prostate and penis. The testes were located internally and contained a smooth muscle layer within the tunica albuginea, surrounding the seminiferous tubules. The function of this smooth muscle maybe contraction to facilitate sperm transport to the epididymis. In this study a large epididymides with an S-shape, which was difficult to distinguish between the head, body and tail of the epididymides, was found. There seems to be a relation between sizes of the epididymis, vesicular glands and prostate of male red squirrels (*Tamiasciurus hudsonicus*) (Bonanno & Schulte-Hostedde, 2009), that has been suggested to increase the ejaculation volume of this species. It may be possible that the shape and size of the manatee epididymides allows for more storage of spermatozoa but it is necessary to obtain more details from this structure. For example, it would be interesting understand which portion of the manatee epididymal duct is head, body or tail. A complete dissection of the whole epididymal duct, several sections of this structure and histological analysis to confirm which part of the epididymal duct is being sectioned, may help. Also, obtaining samples from, at least, five to six adults male to compare would provide better analysis of the function-structure of the epididymis. It may be possible that the manatee epididymal duct can be associated to the largest accessory sex gland found in the Florida manatee, the vesicular glands. The ductus deferens in the male Florida manatee is a convoluted tubule with stereocilia along the lumen epithelium and a thick tunica muscularis, both important characteristics for the transportation of sperm to the urethra. The vesicular glands are the largest accessory sex glands found in the male Florida manatee. These glands are hollow with wide lumens lined with cuboidal to columnar epithelium with stereocillia present. This

configuration suggests that the vesicular glands secrete a large amount of seminal fluid, fructose and prostaglandins, similar to the secretions of other species studied (Sorensen, 1979; Dixon & Anderson, 2002). The Florida manatee appears to have both a prostatic body and a disseminated portion of the prostate. The glandular epithelium in the Florida manatee differs between prostatic portions, but smooth muscle cells surround both parts of the prostate. The smooth muscle cells function through contraction, discharging the prostatic fluid during ejaculation. The Florida manatee has a musculocavernosous penis. A glans penis is present, but without a baculum (os penis), suggesting that the Florida manatee penis increases in size and stiffness during erection.

The Florida manatee have a promiscuous mating system, in which several active adult males pursue and mate with an estrous female for two to four weeks (Rathbun et al., 1995). Males push and fight for access to the ventral side of the female to mate. This can suggest a possible competition between spermatozoa of these males trying to fertilize one egg. This is generally referred to as sperm competition (Parker, 1970). One anatomical feature of sperm competitor species is a large testis relative for producing a large number of sperm. However, Florida manatee does not follow this rule (Reynolds et al., 2004). Also, accessory sex glands are well developed in species with promiscuous mating systems that encounter sperm competitors. Each gland serves a specific purpose, with secretions either nourishing, protecting or improving the quality of sperm viability and in some case producing a vaginal plug (Ramm et al., 2005). Nonetheless, little is known about the specific secretions of each accessory gland and how they support sperm competition.

The presence of accessory sex glands (ampullae, vesicular glands, prostate gland and bulbourethral glands) in male mammals differs among species. All mammals have at least one accessory sex gland, the prostate. It can be present along with two or three other types of

accessory sex glands. Most terrestrial mammals possess at least three of the accessory sex glands. Most aquatic mammals possess only one accessory sex gland, the prostate. Pinnipeds, like the elephant seal (*Mirounga sp*), Antarctic fur seal (*Arctocephalus gazella*) and South America fur seal (*Arctocephalus australis*) all have a prominent prostate (Laws & Sinha, 1993; Machado et al., 2012). In cetaceans, the bridled dolphin (*Stenella frontalis*), bottlenose dolphin (*Tursiops truncatus*), long-beaked dolphin (*Delphinus capensis*) and harbor porpoise (*Phocoena phocoena*) only possess a prostate (Harrison, 1969; de Smet, 1977; De Guise et al., 1994). In terrestrial mammals, the dog (*Cannis l. familiaris*) similarly has only a prostate. Dogs have adapted a mechanism, with extended periods of intromission to ensure reproductive success (Sorensen, 1979). Species with only two accessory sex glands are rare. Tomcats (*Felis catus*) and a rodent, the souslik (*Spermophilus xanthoprymnus*), have only two accessory sex glands, the prostate and the bulbourethral glands (Pineda & Faulkner, 1980; Çakir & Karataş, 2004). One adaptation mechanism of the tomcat is that there are four lobules of their prostate, which may compensate for the seminal fluid volume provided by the vesicular glands in other species (McEntee, 1990).

Florida manatee possess two accessory sex glands: vesicular glands and prostate. Vesicular glands synthesis fructose, an important energy source for sperm. The fluid from this gland also contains prostaglandins, fibrinogen and clotting properties (Dellmann & Wrobel, 1976; Peckham, 2011). Vesicular glands may contribute to over half of the total ejaculate fluid volume in species where it is present. The vesicular glands are the largest of the accessory sex glands in some species, like the bull (*Bos taurus*), African elephant (*Loxodonta africana*), and dugong (*Dugong dugon*). The bull and the elephant have a full complement of the accessory sex glands. The dugong appears to have only the vesicular glands and a disseminated part of the

prostate. The prostate gland is the common denominator in the accessory sex glands across species. This gland secretes multiple components, like inorganic ions and proteins which help sperm motility, survival and immunosuppressive activity of the sperm (Bearden & Fuquay, 1992; Chao et al., 1996).

To achieve reproductive success and perhaps to compensate for the lack of other accessory sex glands beyond the prostate, some aquatic mammals have frequent intromissions into the female reproductive tract. Frequent mating's ensures the deposition of sufficient ejaculate. Also, large testis produce adequate number of sperm, larger diameter of the epididymal tail provides adequate storage and nourish for sperm, and a prominent prostate produce adequate seminal plasma volume. Male Florida manatee has a small prostate relative to their body mass, but larger vesicular glands. Species that minimally have prostate and vesicular glands, and also have a promiscuous mating system like the Florida manatee, often ensure reproductive success by producing a copulatory plug. This copulatory plug can be a physical barrier to decrease loss of sperm or reduce the introduction of another male's sperm, like some rodents (Karn et. al., 2008; Mollineau et al., 2009). If male Florida manatee accessory sex glands were to produce a copulatory plug, this would support the hypothesis that manatee are sperm competitors.

However, there is currently no evidence of the production of a copulatory plug by manatees. It is more likely that male Florida manatees have a combination of features that characterize them as sperm competitors. For example, with large epididymides and vesicular glands, it is possible to both store a large amount of sperm within the epididymis and to produce vesicular secretions to provide energy and protection within the female's reproductive tract. Since the Florida manatee have a large vascular type penis that increases in size, it is postulated that it can deposit sperm

closer to the fertilization point. More analysis needs to be done to support or refute these hypotheses.

Enlargement of certain accessory sex glands is other possible way that sperm competitors compensate to produce more secretions for the seminal fluid in the absences of the other accessory sex glands (Anderson et al., 2004; Ramm et al., 2005). The prostate is the only accessory sex gland in others aquatic mammals, as well as the dog. These species may compensate for the absence of the other accessory sex glands with the enlargement of the prostate. The loss of the other accessory sex glands could be an evolutionary adaptation to avoid physiological cost in maintenance of these structures. In case of the Florida manatee, the larger vesicular glands may fulfill the function of the other accessory sex glands. Further studies of the composition of the secretions of each accessory sex gland would help to understand the specific function and contribution of these glands in male Florida manatee reproduction.

Anatomical variation in the male reproductive tract among species can result from evolutionary aspects associated with the mating system present. The male Florida manatee reproductive tract resembles the dugong and African elephant, which are both closely related to manatees, along with the hyrax (*Procavia capensis*). However, manatees did not appear to have bulbourethral glands as elephants and hyraxes does, and dugongs seem to lack prostatic body. Possibly, the transition to the aquatic environment of the sirenians could be correlated with the loss of the bulbourethral glands. Maybe for the Florida manatee is enough have two of the principal accessory sex glands to achieve reproductive success, considering that this species has a low metabolism that has to focus physical cost for living in the environment.

The basic male reproductive histology of the Florida manatee was similar to other mammals, with a combination of characteristics from several terrestrial mammals. These

similarities are mainly found with the elephant, the stallion and its aquatic relative, the dugong. The characteristics of the prostatic body and disseminated portion of the Florida manatee should be further studied.

The number of specimens in this study was low. Additionally, the tissues obtained were from animals only in winter time and seasonality seems to affect reproduction in this specie. The current study is a first attempt in the description of the male reproductive anatomy. Further studies should include additional specimens from each age class, as puberty obviously affects the reproductive tract configuration. It is recommended to first dissect the reproductive tract, obtain anatomic morphometric data and weight of the organs, and then preserve the tissue for further analysis. Other interesting methodologies can be applied for the reproductive anatomy of the male Florida manatee. For example, ultrasonography in captive animals, direct perfusion fixation for better histological analysis, proteins and enzyme analysis of the secretion of the seminal fluid for description of contents, and behavioral observation combined with genetic analysis.

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BIOGRAPHICAL SKETCH

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