The suspensory ligamentous system of the penis: Macro and micro anatomical examination of the adult male cadaveric specimen

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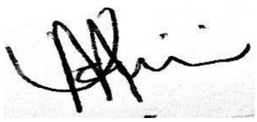
Declaration

I, Ursula Meredith Mariani, student number: 556471, declare that the contents of this dissertation is my own, unaided work. It is being submitted for the degree Master of Science in Medicine at the University of Witwatersrand, Johannesburg. It has not been submitted before for degree or examination at any other University.

______________

Ursula Meredith Mariani

On the 17th of April, 2020, Johannesburg, South Africa.

"You can't improve what you don't measure"

-Michael Hyatt

Abstract

Background: The suspensory ligamentous system of the penis lifts and supports the penis when erect, and plays a key role during coitus. The ligamentous system of the penis is a set of three ligaments that include the fundiform ligament, suspensory penile ligament (SPL) and the arcuate ligament. These ligaments are prone to injury during coitus and also play a key role in specific penile reconstruction procedures. The origin, course and insertion of each ligament remains a highly debated subject. In addition, their dimensions and histological composition are not clearly elucidated. The function of the penis is important, hence it is essential to examine and describe the macro and micro anatomy of the suspensory ligaments of the penis.

Aim: The purpose of the current study was to investigate the macro and micro-anatomy of the suspensory ligamentous system of the penis in order to provide the knowledge base for successful penile reconstruction procedures that involve the suspensory ligamentous system of the penis.

Materials and Methods: The study utilized a total of 49 cadavers from the dissection laboratories housed in the school of anatomical sciences at the University of the Witwatersrand.

Three methods of analysis were employed including gross anatomy dissection to determine the origin, course and insertion of the suspensory ligaments, histological analysis to determine the cellular and fibre composition of each ligament and the MRI to elucidate the precise origin, course and insertion or each ligament complementing the gross anatomy dissection.

Results: The fundiform ligament was found to have two sets of bundle fibres, a superficial and bilaterally placed arising from the Scarpa's fascia and a deep and medially placed arising from the linea alba. Both ligaments split and encircled the penis and inserted into the superficial fascia of the penis. The SPL arose from the perichondrium of the pubic symphysis and inserted into the deep fascia (Buck's fascia) of the penis. The arcuate ligament arose from the inferior aspect of the body of the pubis and pubic symphysis and inserted into the Buck's fascia. The deep bundle of fibres of the fundiform ligament consists of more adipose tissue (43±2.02%) and collagen fibres (43 ±1.6%) than both elastic (13±0.67 %) and reticular fibres (13 ±3.14 %). The superficial bundle of fibres of the fundiform ligament showed more adipose tissue (53±2.55%) and equal amounts of both collagen (21 ±1.79%) and elastic (23±0.35%) fibres but less reticular fibres (14±3.96%). Similarly, the SPL consists of more adipose tissue (53±1.78%), equal amounts of collagen (23±1.35%) and elastic (23±0.82%) fibre and less reticular fibres (12±3.29%).

Conclusion: The present study provided a depth of anatomical knowledge regarding the suspensory ligamentous system of the penis. Presenting clear information on the origin, course and insertion of each suspensory ligament and expounded on their histological composition. This information is valuable to anatomy subject and medical professionals particularly during interventional procedures around the penis and perineal region.

Key words: Suspensory penile ligament; Fundiform ligament; Arcuate Ligament; Penis

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I'd like to acknowledge firstly, the efforts of my supervisors. Secondly, the extensive support of Dr. Pilani Nkomezepi, thank you for the guidance, teaching and encouragement. Thirdly, Dr. Fayman for the conceptualization and support in execution of this project. Lastly the love, support and prayers of my parents, siblings and friends.

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List of abbreviations

ANOVA: Analysis of variance

ARC: Arcuate ligament

BS: Buck's fascia

BV: Blood vessels

DMBF: Deep medial bundle of the fundiform ligament

DV: Deep dorsal vasculature

DF: Dartos fascia of the scrotum

FL: Fundiform ligament

IF: Left and right wings of the deep medial bundle of the fundiform ligament

L.Abd: Lower abdomen

LT: Left thigh

LW: Left wing

PPS: Perichondrium of the pubic symphysis

PSP: Periosteum of the pubic symphysis

PS: Pubic symphysis

P/SP: Shaft of the penis

RT: Right thigh

RW: Right wing

S: Scrotum

SLBF: Superficial lateral bundle of the fundiform ligament

SPL: Suspensory penile ligament

1.0 Introduction

The Suspensory penile ligamentous system of the penis comprises of a set of three ligaments that work together to suspend the penis when it is flaccid or erect. The ligaments, from superficial to deep, are the fundiform ligament, the suspensory penile ligament proper and the arcuate ligament. With the primary observed function of these ligaments being to support and keep penis suspended, the ligaments play a critical role during coitus, aiding penetration, and stabilizing the penis during and after coitus (Hoznek et al., 1998; Li et al., 2006; Li et al., 2007; Chen et al., 2017).

There are certain disorders of the penis, of which the suspensory penile ligamentous system plays a centred role in treatment. Such disorders are penile curvature, erectile dysfunction, micropenis, body dysmorphia, and reconstructive procedures in cases of post-penile trauma treatment or construction of male genitalia in transgender patient (Nyirády et al., 2008; Li et al., 2007). All treatment alternatives that usually fall under the discipline of phalloplasty. Phalloplasty is a specialized branch of plastic surgery that involves the aesthetic of male genitalia (i.e. construction, reconstruction, penile enlargement, etc.).

Considering the significance in function of the suspensory penile ligamentous system and that the ligaments play an indispensable role in the treatment of an array of aforementioned disorders, it comes as a surprise that detailed anatomical descriptions are limited and incoherent. Of the little research conducted on the suspensory ligaments (Hoznek *et al.*; 1998; Chen *et al.;* 2017), settlement of precise anatomical descriptions of origin, course and insertion have not been reached. The origin of the fundiform and SPL are inconsistently defined by different authors (Hoznek *et al.*; 1998; Chen *et al.;* 2017; Standring *et al.,* 2008). The same discrepancy is found with the termination of each ligament. Furthermore, the histological structure of the three ligaments is not well described and there is a dearth of information as to what exactly constitute each ligament. In order for phalloplasty procedures to achieve maximum success the histological composition of the suspensory ligaments is critical, firstly to determine the extent of the function of each suspensory ligament and secondly to best recreate the synthesized replication in case of damage.

The inadequacies in the gross structure and histological composition of the suspensory ligaments system of the penis and the associated risks during surgery of the region, make it imperative to design studies that elucidate the anatomical structure of this system in order to improve the surgical outcomes. Therefore, the current study (using dissection, histological analysis and analysis of magnetic resonance imaging (MRI) of the region containing the penile suspensory ligament) characterized the topographic, macro-anatomy and micro-anatomy of the penile suspensory ligamentous system in order to define their origin, course and attachments and also to understand their histological structure associated with its unique function

2.0 Literature Review

The review below is a literature collection of the anatomy gross, embryological development, histological structure and the clinical correlates (relating disorders and reconstructive procedures) relating to the suspensory ligamentous system of the penis.

2.1 General anatomy of the anterior abdominal wall (Site of ligament origin)

The anterior abdominal wall is made up of four main muscles, each of which is bilateral. The external oblique muscle, its fibres run in an inferiomedial direction. Deep to the external oblique muscle in the internal oblique, its fibres run superiomedially. Deep to the internal oblique muscle lies the transverses abdominis muscle, its fibres run horizontally. These three muscles become aponeurotic towards the midline forming the rectus sheath. Within the rectus sheath the rectus abdominis muscle can be located, a segmented bilateral muscle that runs down the midline of the abdomen (Standring *et al.*, 2008; Moore *et al.*, 2013).

Aponeurotic fibres from the abdominal muscles across the abdomen form sheath like structures called the rectus sheath. The medial termination of the aponeurotic fibres from each of the muscle fibres from the anterolateral abdominal layer forms a dense, firm, fibrous and avascular structure called the linea alba in the midline (Burger, 2006). The orientation of the rectus sheath in relation to the rectus abdominis muscles differs at different anatomical levels.

Above the muscular layers of the anterolateral abdominal wall lies two fascial layers, a superficial fatty layer called Camper's fascia and an inner membranous layer called the Scarpa's fascia (Moore *et al.*, 2013).

2.2 General anatomy of the penis (Site of ligament insertion)

The penis can be compartmentalized into two parts, the root which can be located in the perineum (internal) and the shaft of the penis (external). The suspensory penile ligaments work to suspend the external male genitalia. This external shaft of the penis is comprised of three erectile bodies, a paired (left and right) corpora cavernosa bodies that lie on the body of the corpus spongiosum (median) (Standring *et al.*, 2008; Moore *et al.*, 2013). The bodies are covered by three layers of fascia (Hsieh *et al.*2012). The deepest of the three layers of fascia being the tunica albuginea and lies directly on the erectile bodies. The next fascial layer, is the middle fascial layer of the penis, it is called the deep fascia of the penis or Buck's fascia. The deep dorsal vasculature can be located in the midline of the shaft, between the tunica albuginea and Buck's fascia (Standring *et al.*, 2008; Hsieh *et al.* 2012). The third and most superficial of three penile fascia layers is called Dartos fascia. The superficial dorsal vein lies between Buck's fascia and Dartos fascia (Standring *et al.*, 2008; Hsieh *et al.* 2012).

2.3 Gross Anatomy of the Suspensory ligamentous system of the penis

The suspensory system of the penis consists of three ligaments: the fundiform ligament, the suspensory ligament proper (SLP), and the arcuate ligament (Hoznek et al., 1998). The origin and insertion of these three ligaments have not yet been clearly defined in Anatomy textbooks. These ligaments serve as important structures for the development and maintenance of an erection during coitus (Pryor and Hill, 1979; Li et al., 2007; Chen et al., 2017). Described below is a summarized collection of anatomical descriptions of the suspensory ligamentous system of the penis.

2.3.1 The fundiform ligament

The fundiform ligament is the most superficial of the three ligaments that make up the suspensory ligamentous system of the penis. The ligament is prone to injuries during contact sport and traditional male circumcision because of its superficial location (Standring *et al.*, 2008). It is also frequently involved in phalloplasty procedures (Li et al., 2006; Li et al., 2007). Its susceptibility to injury highlights a necessity to establish fastidious anatomical descriptions.

The fundiform ligament extends from the linea alba superiorly and splits around the penis to unite and blends inferiorly with the dartos fascia which forms the scrotal septum (Moore *et al.* 2013; Standring *et al.*,2008). Hoznek *et al.* (1998) using dissection and magnetic resonance imaging (MRI) technology found that the fundiform ligament arose from the deep fibre attachments to the linea alba, and extends downwards as two lateral bundles that encircled the base of the penis. At the base of the penis the fundiform ligament inserts adjacent to, but not in contact with, the tunica albuginea and forms the origin of the septum of the scrotal sac inferiorly (Hoznek *et al.*,1998). Chen *et al.* (2017) using visible human data sets (CVH-1, CVH-3 and VHM) and MRI technology found that the fundiform ligament arises from the deep layer of the superficial layer of the abdomen, also known as Scarpa's fascia and it had no direct connection with the linea alba. Inferiorly, it was found to have no connection to the tunica albuginea, and below the shaft of the penis it formed the antero-superior end of the scrotal septum (Chen et al., 2017). Liu *et al.* (2013) examined children with congenital complete buried penis disorder, using resection of the fundiform ligament as a surgical technique to straighten the erect penis. The fundiform was found attaching to the distal or middle shaft of the penis and originating from the midline of the pubic symphysis (Liu *et al.*, 2013).

However, most research concurs on the termination of the fundiform ligament below the penis, whereby it tapers inferiorly into the scrotal sac forming the origin of the scrotal septum (Chen et al., 2017; Moore *et al.* 2013; Standring *et al.*,2008; Hoznek *et al.*,1998), but the exact point of origin superiorly and insertion on the shaft of the penis inferiorly remain inconclusive. Defining the anatomical parameters of the fundiform ligament will also assist in assessing the extent of its suspensory function. Essentially, the fundiform ligament will arise from one of the abdominal layers and the insertion on to either the superficial fascia of the penis, deep fascia of the penis or the tunica albuginea.

2.3.2 The suspensory penile ligament

The Suspensory penile ligament (SPL) is the intermediate ligament of the three ligaments that suspend the penis, it lies posteriorly to the fundiform ligament and anteriorly to the arcuate ligament. It is referred to as the suspensory ligament proper (Hoznek *et al.*; 1998) and it carries the bulk of suspensory role of the penis (Hoznek *et al.*; 1998; Chen *et al*.; 2017).

According to Moore *et al.* (2014), the SPL is a condensation of deep fascia that arises from the anterior surface of the pubic symphysis which splits and attaches to the deep fascia of the penis (Buck's fascia). Hoznek *et al.* (1998) using dissection and MRI technology described the SPL as a firm fibrous structure that extends from the pubis. It fuses with the arcuate ligament posteriorly and circumscribes the anterior side of the base of the penis. It then attaches to the tunica albuginea on its outer side. Chen *et al.* (2017) found that it arises from the inferior border of the pubic symphysis and inserts directly on to the tunica albuginea. At its point of insertion on the root of the penis posteriorly, Buck's fascia (deep fascia of the penis) is diminishing (Chen *et al*.; 2017). The fundiform ligament can also be referred to as the triangular suspensory ligament (Standring *et al.*,2008). Furthermore, Standring *et al.* (2008) describes the SPL as arising from the anterior end of the pubic symphysis and the inserts the fascia of the penis. This description is nonspecific and generalized, considering that there are fascial levels (Dartos, Buck's and Tunica albuginea) that surround the three masses of erectile tissue (Standring *et al.*,2008; Hsieh *et al.*2012).

As noted, the origin and insertion of the SPL is a contentious debate among anatomical researchers. The positioning of the origin along the margin of the pubis, most importantly though would be its insertion of the penile shaft, whether it attaches directly on the tunica albuginea or on the deep fascia of the penis (Buck's fascia). It is important to establish the insertion as that would provide clarification as far as the anatomical level depth. This will clarify the extent of which the ligament plays a suspensory role in the suspensory ligamentous system of the penis.

2.3.3 The arcuate ligament

The arcuate ligament is the most posterior of the three suspensory ligaments of the penis. General anatomy textbooks (Moore *et al.*, 2013; Standring *et al.*, 2008) describe the fundiform and SPL briefly, however, make no mention of the arcuate ligament. Hoznek *et al.* (1998) found that the arcuate ligament originates from the inferior border of the pubis and lodged in the subpubic angle. It then attaches to the tunica albuginea of the corpora cavernosa (Chen *et al.*, 2017; Hoznek *et al.*, 1998). The arcuate ligament does not carry the same kind of controversy with that of the two more superficial suspensory ligaments. Although the literature is very limited, an accord has been reached. The arcuate ligament helps in suspension of the penis during erection and coitus (Chen *et al.;* 2017). Due to its deeper location under the pubic arch, it is rarely implicated during penile reconstructive procedures (Shaeer et al., 2006; Li et al., 2007).

2.4 The histology of the Suspensory ligamentous system of the penis

Anatomical literature expanding on the suspensory penile ligamentous system of the penis focuses mostly on its macro anatomical features. Hozek *et al.* (1998) examined the histological structure of the penile ligamentous system and found that the suspensory penile ligament had collagen fibres that are continuous with those of the tunica albuginea. The fundiform ligament was not characterized and its histological composition remains unknown. Standring *et al.* (2008) described the histological composition of the ligaments as largely elastic fibres. Other studies that have examined the penile ligaments, describe their composition only as fibro-fatty without further delineation of the types of fibres and or the specific composition of each ligament (Chen *et al.*, 2017). Hence the current study aims to characterize the histological structure of the fundiform and SPL proper ligaments that are commonly used in reconstructive surgery of the penile disorders.

2.5 Clinical Correlates

2.5.1 Disorders of the penile suspensory ligamentous system

Penile curvature is a condition associated with bent penis either ventrally, dorsally or laterally. Ventral penile curvature is usually associated with hypospadias, whereby the urethral opening is positioned on ventral surface of the shaft of the penis (ref). Dorsal penile curvature is usually associated with epispadias (presence of the urethral opening on the dorsal surface of the penis) (Nyirády et al., 2008). Lateral curvature of the penis usually presents as mono-symptomatic with normal positioning of the urethra and the penis does not present as curved in the flaccid state but rather during an erection (Nyirády et al., 2008). Patients with lateral curvature of the penis experience difficulties with or may not even achieve penetration during coitus (Nyirády et al., 2008). Repair of the SPL is an option for the correction of penile curvature (Li et al., 2007).

Another disorder of the penis is the Peyronie's disease, which is acquired and affects the physical appearance and functionality of the penis. The disease affects the connective tissue of the tunica albuginea of the corpus cavernosum, and presents with excessive fibrosis and plaque formation (Hussein et al., 2015). The disease occurs in two stages, acute and chronic stages. The acute stage is characterized by severe pain. Treatment at this stage is non-surgical and aimed at reducing pain. At the chronic stage, the penis presents with deformities such as shortening, curvature formation, indentation and narrowing (Hussein et al., 2015). Sufferers of the disease may experience pain and erectile dysfunction that could result in an inability to have coitus (Hussein et al., 2015). Surgical repair of the SPL is a treatment option for the penile deformities caused by the chronic stage of Peyronie's disease (Li et al., 2007).

Over the years, people have eulogized penile length extremities, consequently applying social and emotional pressure on men, thus creating insecurities (Shaeer et al., 2006). As a result, penile lengthening cosmetic procedures are commonly requested. Approximately 10 000 men had penile lengthening procedure in the USA between the years 1989-1996 (Shaeer et al., 2006). It has been observed that the insecurities men have regarding penile length stems from psychological issues rather than physical (Li et al., 2006; Li et al., 2007). The majority of patients that request penile lengthening procedures suffer from a perceived psychological disorder referred to as penile dysmorphic disorder, whereby an individual may view their penis as inadequate in length, when it is in actual fact of normal size. The average of a white male penis length is 12.5 cm $\pm$ 2.7 cm (Li et al., 2006). Studies show that most men that request penile enhancement procedures fall within the range of this norm. However, these patients suffered from an altered self-perception, in essence, the length of their penis was not abnormally short but rather the perception they have of what the social standard is, was unrealistic in itself leading to anxiety and/or depression (Li et al., 2006). Such patients are not likely to be satisfied with the penis lengthening procedures available, as their expectations are unrealistic, therefore patients should rather seek psychological treatment (Li et al., 2006; Li et al., 2007).

The same anxiety and depression seen in patients with penile dysmorphic disorder can also be seen in patients who have experienced penile trauma. Penile trauma, such as partial penile amputation, may be a result of traditional male circumcision. Although avenues for mainstream medical circumcision have been widened in South Africa, as the government offers to perform the procedure free of charge (Mayosi et al., 2012), the preference for traditional male circumcision is still a prevalent issue as there is a high complication rate from genital mutilation, partial amputation, genital sepsis, etc. (Kahn et al., 2006; Anike et al., 2013; Lawal and Olapade-Olaopa, 2017).

In large areas of rural South Africa, especially in the Eastern Cape, the holistic rite of passage ceremony associated with the traditional circumcision is an important cultural practice. Studies have shown that traditional male circumcision can result in several extreme complications such as hospital admissions, penile mutilations and even death (Ahmed et al., 1999; Meissner and Buso, 2007). The results emphasized the sad reality of the trauma experienced by many men relating to these traditional practices. Meissner et al. (2007) reports that between the years 2001-2005 there was a total of 107 penile amputation/mutilation cases reported by the Department of Health in the Eastern Cape Province alone.

The statistics of male genital mutilation (Meissner and Buso, 2007) reemphasize the need for extensive research around penile structures for successful reconstructive procedures.

2.5.2 Penile suspensory ligamentous system reconstruction

There are different procedures to rectify various penile disorders. Currently procedures relating to the repair of the suspensory penile ligament are of particular interest. Patients in need of these procedures can be clinically diagnosed, with a presence of a palpable gap between the pubic symphysis and the base of the penis; and by examination of penile stability and medical history (Pryor and Hill, 1979; Li et al., 2007). The procedure entails reinforcement of the suspensory ligament using non-absorbable nylon monofilament sutures. These sutures are placed between the pubic symphysis and the midline of the tunica until the best penile position for coitus is obtained and as seen through an artificial erection test (Li et al., 2007).

Common penile lengthening procedures also revolve around the suspensory penile ligament. The division of the suspensory ligament accompanied with a silicone spacer between the base of the penis and the pubis. Once the surgical wound has completely healed, the next advised step for optimum results is penile stretching, using penile weights, vacuum constriction devices and the use of a penile stretcher device. This procedure can increase the length of the penis by 1.3 ± 0.9 cm (Li et al., 2006). Both the penile lengthening procedure and the suspensory ligament repair procedure are amongst other procedures that involve the suspensory penile ligament (Li et al., 2006; Shaeer et al., 2006; Li et al., 2007). That being said, clearly defined anatomical descriptions (i.e. origins, course, insertions, length, histological structure and magnetic resonance imaging) of the penile suspensory ligaments are necessary to improve the precision, repeatability and outcome of these procedures. This in essence is what this project aims to achieve.

Motivation for the study

The suspensory ligamentous system of the penis is crucial in the integrity of the penile structure holistically. The suspensory ligaments play important roles in erection of the penis and coitus. It withstands risk of injury and reconstruction surgery of the suspensory penile ligaments can be necessary. Information on the anatomical descriptions of the suspensory penile ligaments is insufficient. Therefore, the current study was designed to provide a greater depth of anatomical knowledge of the suspensory ligamentous system of the penis, with the hope of increasing surgical outcomes around this region of study especially with the continuously reported cases of genital mutilation and death from complications during initiation rites in some South African communities.

Aims and Objectives

Aims

The purpose of this study was to investigate the macro- and micro-anatomy of the suspensory ligamentous system of the penis with a view of adding to the anatomical knowledge and with the hope of improving outcomes of surgical procedures relating to the suspensory ligamentous system of the penis.

Objectives

1. Describe the anatomy of the fundiform ligament of the penis by determining its origin, course and attachments.
2. Describe the suspensory ligament (proper) of the penis by determining its origin, course, and attachments.
3. Describe the arcuate ligament of the penis by determining its origin, course, and attachments.
4. Determine the average length and width of each bundle of the fundiform ligament.
5. Determine the average length and height of the suspensory ligament of the penis (proper).
6. Determine the tissue (adipose tissue, collagen, elastic and reticular fibres) composition of the suspensory ligamentous system of the penis.

3.0 Materials and methods

The objectives of the study required different materials and methodology. Three methods were applied to achieve the macro-anatomical dimension quantification, the macro-anatomical descriptions and micro-anatomical composition quantification (i.e. the six stated objectives above). Objective 1, 2, 3, 4 and 5 were achieved by gross metric methodology and MRI analysis whereas objective 6 was achieved by histological analysis methodology.

Gross Morphology of penile suspensory ligaments

Study Sample

The study utilized 49 adult male cadavers from the School of Anatomical Sciences at the University of the Witwatersrand. 26 out of the 49 cadavers had intact (un-dissected) pelvic region, but the remaining 23 had their pelvic region pre-dissected by undergraduate medical students. Ethical clearance pertaining to the use of donated cadavers for teaching and research was obtained (W-CJ-140604-1) from the School of Anatomical Sciences.

Dissection of the suspensory ligamentous system of the penis

Standard dissection procedure of the male perineum was followed according to Grant's Manual of Human Dissection (Tank and Grant, 2012). A T-shaped incision was made in the pubic region as follows: a superior horizontal incision was made along the pubic region similar to the Pfannenstiel- Kerr incision. A vertical incision was made perpendicular to the pubic incision all the way down to the penis. The skin of the pubis was reflected laterally. Dissection of the suspensory ligamentous system was done layer by layer cleaning out the fat and superficial fascia. Caution was exercised not to cut any fibrous materials.

Once the ligaments were exposed, the first step was to thoroughly examine fibrous material to reveal their origin, course and distal attachments. Due to the topographic positioning of the suspensory penile ligamentous system of the penis, the fundiform ligament was examined first followed by the SLP, since the fundiform ligament lies more superficially to the SPL.

The structure of the ligamentous system of the penis was described and interesting features were noted. Images were taken using a NIKON D 3400 camera.

3.1.1 Dimensions of the penile suspensory ligaments

The metric measurements of the fundiform ligament were taken using a sliding Vernier caliper. The different fundiform fibre bundles were measured: a left, right and a middle. The metric measurements of the SPL were taken using both the string and sliding vernier caliper. The various measurements of the penile ligaments were taken as follows:

I. Total Length of the fundiform ligament (TLF): Distance from origin of the Fundiform ligament (abdominal wall) to the first point of insertion on the penile shaft (left) (See Fig. 3.1).
II. Total Length of the fundiform ligament (TLF): Distance from origin of the Fundiform ligament (abdominal wall) to the first point of insertion on the penile shaft (right) (See Fig. 3.1).
III. Total Length of the fundiform ligament (TLF): Distance from origin of the Fundiform ligament (abdominal wall) to the first point of insertion on the penile shaft (middle) (See Fig. 3.1).
IV. Width of fundiform (WF): Distance along the origin of middle bundle of the fundiform ligament, from the extreme left point of the origin to the extreme right point of the origin (See Fig. 3.1)
V. Total Length of the SPL (TLP): Distance of the SPL ligament from the most posterior point of insertion to the free-end (along the shaft of the penis) of the SPL (See Fig. 3.2).
VI. Height of SPL (HP): Distance from the highest point of origin of the SPL along the pubic symphysis to the most anterior point of insertion of the SPL (See Fig. 3.2).

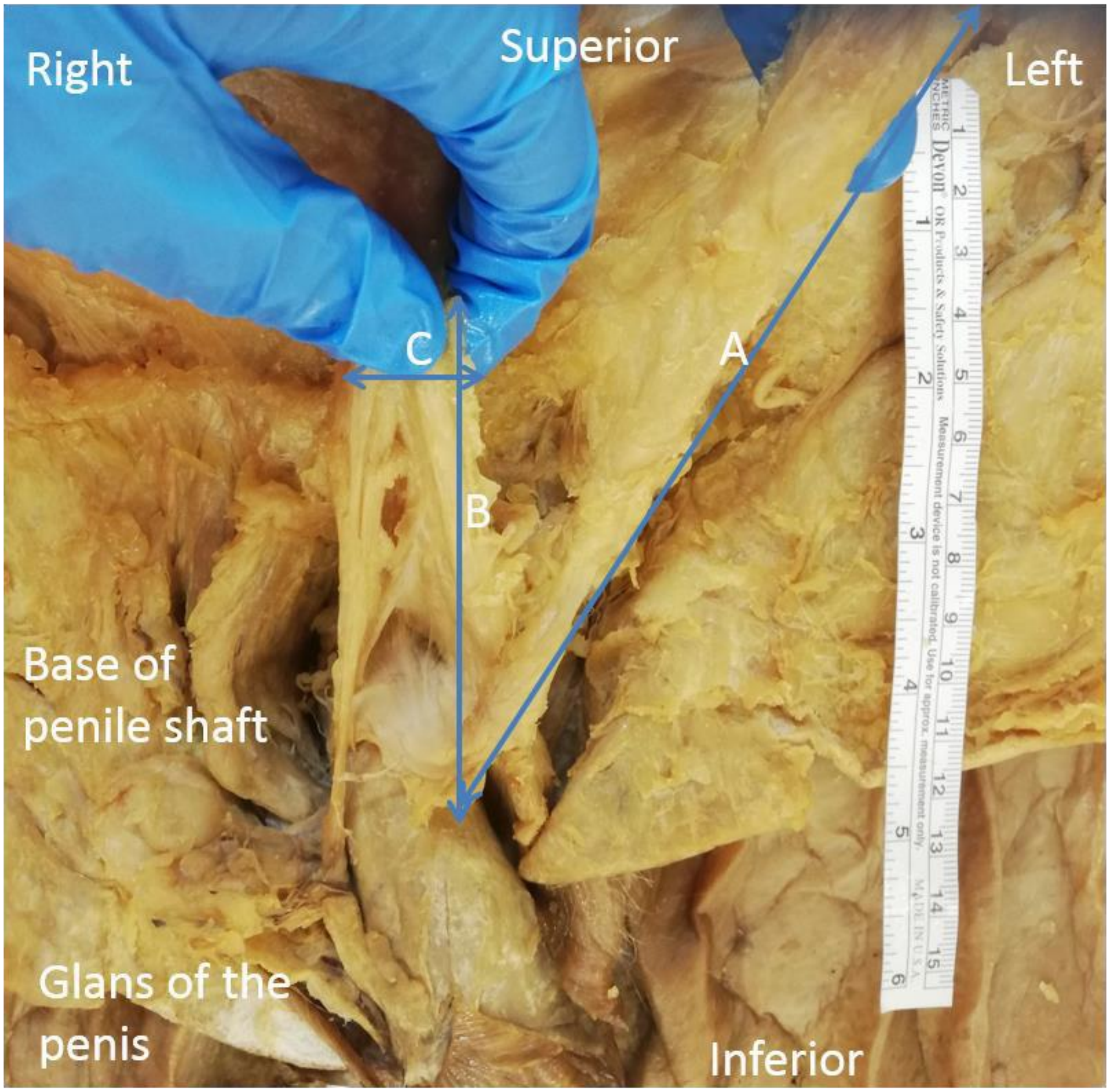

Figure 3.1: The photograph shows dissected penile suspensory ligaments (Anterior view). (A): Length of the left bundle of the fundiform ligament; (B): length of the middle (deep) portion of the fundiform ligament; (C): Width of the middle portion of the fundiform ligament at its origin.

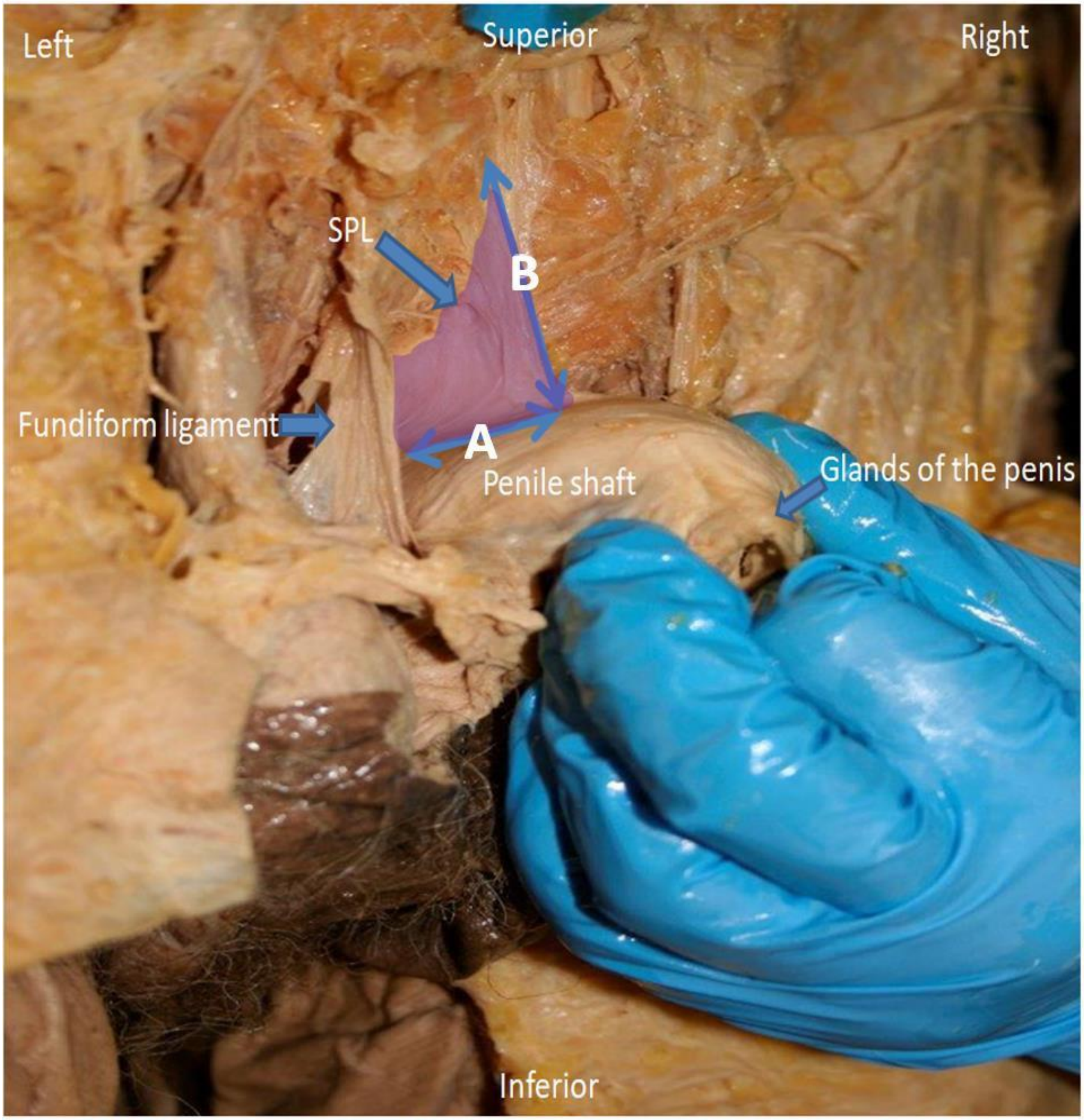

Figure 3.2: The photograph shows a lateral view of a dissected penis. The shaded area: SPL; (A): Length of the SPL; (B): Height of the SPL.

3.2.2 Descriptive collection

During dissection, careful consideration was taken to ensure that each layer was delicately dissected. This allowed for precise descriptions of the origin, course and insertion of the fundiform ligament and SPL to be noted for complete descriptive profiling. Hand notes were made from each dissected cadaver, descriptive pictures were also taken and a comprehensive descriptive depiction of what the anatomy of these two ligaments was then derived.

3.2 Histology of the penile suspensory ligaments

Study Sample

The histological analysis section of the study utilized a preselected 7 adult male cadavers from the School of Anatomical Sciences at the University of the Witwatersrand.

Histological procedures

Tissue samples were harvested from the superficial lateral bundle of the fundiform ligament, deep medial portions of the fundiform ligament and the suspensory ligament proper (SPL). The harvested ligaments were stored in 10% formaldehyde and processed (see Appendix 1) for various histological stains for histological staining. 5µm thick sections were serially sectioned in a 1 in 4 series. Four histological stains as listed below were then performed on the tissue samples.

1. Hematoxylin and eosin staining (H&E) for general anatomical orientation and adipose tissue (see Appendix 2)
2. Masson's trichrome stain for collagen fibres (see Appendix 3)
3. Gordon's sweet silver stain for reticular fibres (see Appendix 4)
4. Verhoeff's modified elastic fibre stain for elastic fibres (see Appendix 5)

There were 4 main tissue types of interest: adipose, collagen, elastic and reticular fibres. In order to select the ideal stain for the best tissue visibility for quantification, some slides were stained with two different stains to highlight the same kind of tissue, the one with the best contrast and definition for this study was then selected.

Image analysis and quantification

From each stained section, ten random camera fields at X10 magnification were acquired using a high-definition video camera (AxioCam HRC) linked to a compound microscope (Zeiss Axioscope). Composite images were prepared with CorelDraw X7 Software (Version 13, Corel Corporation, Ottawa, Canada). No pixilation adjustments of the captured photomicrographs were undertaken except for adjustment of contrast and brightness.

The percentage composition of collagen, elastic, reticular and adipose tissues in each ligament were estimated from the photomicrographs using point-counting plugin of ImageJ software. The area (**A**) and area fraction (**$A_{fraction}$**) occupied by each of the components was calculated as follows:

$$A = ap * \Sigma p$$

where **ap** is area per point (0.007 mm^2) and **Σp** is the sum of points falling over the tissue of component of interest and:

$$A_{fraction} = A \div A_{camera\ field} * 100$$

where **$A_{camera\ filed}$** is the total area of the camera field at X10 (0.60 mm^2).

3.3 Magnetic resonance imaging (MRI)

Study sample

The study utilized 2 adult male cadavers from the School of Anatomical Sciences at the University of the Witwatersrand. Ethical clearance pertaining to the use of donated cadavers for teaching and research was obtained (W-CJ-140604-1) from the School of Anatomical Sciences.

MRI processing

The perineal regions were harvested enbloc including the root and shaft of the penis. The enbloc specimens were scanned using the MRI machine (Siemens AVANTO FIT 1.5T) equipped with Syngo Software version II. Details of the MRI scanning modalities are as follows: the specimens were scanned in a 15 channel Knee Coil with oil marker placed on the left side for specimen A and on the right side for specimen B for orientation purposes. The enbloc ections were scanned at T1, T2 and T3 weighted vibes in both coronary and sagittal place. Section thickness was set at 1mm and FOV at 180mm to the specimens. The MRI method allowed for the visualization of the deeper (middle) fundiform, suspensory ligament proper (SPL) and the arcuate ligaments tracing the points of origin and insertion.

Data analysis

Once the metric measurements were recorded and descriptive statistics, such as mean and range were calculated on excel spreadsheets. A student t-test was used to compare left and right mean. An ANOVA test was used to compare different tissue composition (collagen, elastic, adipose and reticular) of each ligament (SLBF, DMBF and SPL). These tests were performed using R software. The values ($p = 0.05$) was used to infer the level of significance.

4.0 Results

Each method of the study described under materials and methods has its own set of results. The first set of results describes the metric measurements (dimensions) of the fundiform and SPL ligaments and it also describes the observed origin, course, and insertion of the fundiform and SPL ligaments. The second set of results defines the histological findings, i.e. the histological composition of fundiform and SPL ligaments. The last set of results is that of the MRI scan findings, anatomical descriptions of the fundiform ligament, SPL and the arcuate ligament.

4.1 Macro Anatomy

4.1.1 Metric measurements

The data used in this research was collected from 49 cadavers. The dimensions of both the fundiform ligament and the suspensory penile ligament (SPL) were measured. Six metric measurements were recorded: length of the left superficial lateral bundle of the fundiform ligament (SLBF), length of the right SLBF, height of the deep medial bundle of the fundiform ligament (DMBF), width of the DMBF, the height of the SPL and the length of the SPL.

The fundiform could only be analysed on undissected cadavers, as its position is superficial and pre-dissected cadavers presented with damage to the superficial pelvic region. Furthermore, undissected cadavers were dissected above the pelvic area. This pre-dissection of the lower abdomen damaged 6 of the SLBF measurements, decreasing the sample size to 20. The mean length of left SLBF was 14.3±1.3 cm and the mean length of right was 15.0±1.7 cm. A student t-test was performed to compare the left and right SLBF (See Fig. 4.1). There was no statistically significant difference ($p = 0.1422$) between the right and left bundles of the SBLF. The sample size for the examination of the DMBF was 26 undissected cadavers. The mean length of the DMBF was 8.5±1.8 cm and the mean width of the DMBF was 3.1±0.8 cm. The SPL was measured over a sample size of 49 cadavers as the pre-dissected cadavers had no damage to the SPL ligament therefore the sample size could be expanded. The mean height of the SPL was 2.6 ±0.8 cm and the mean length of the SPL was 5.08±1.1 cm as shown in Table 4.1.

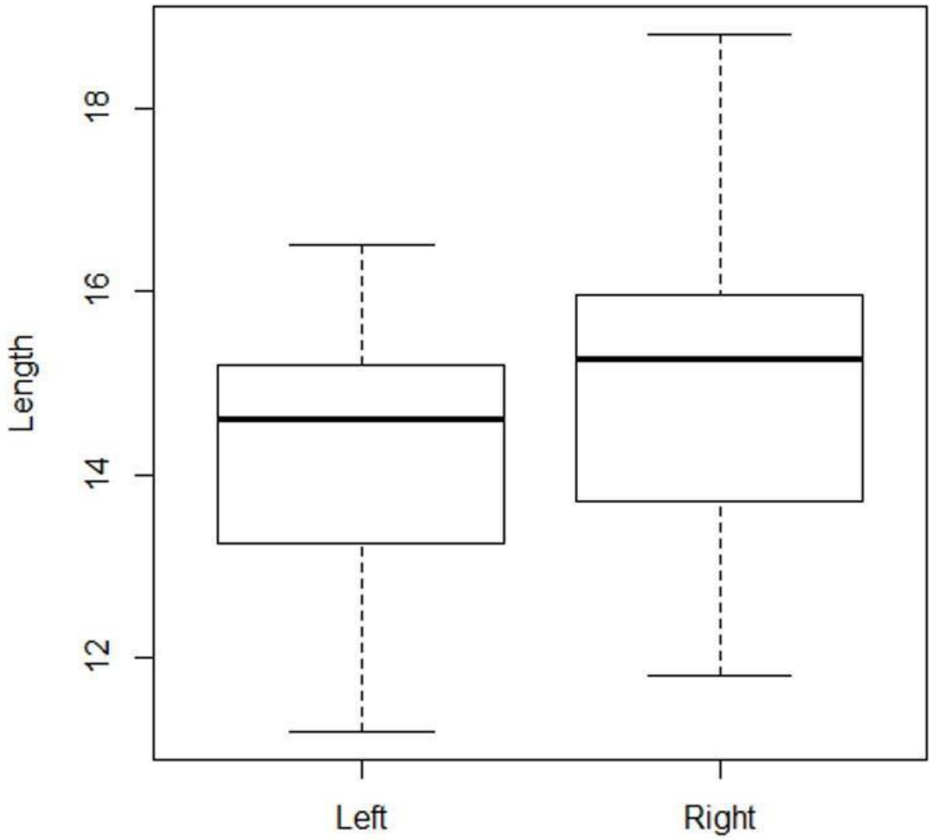

Figure 4.1: The boxplot above depicts the relationship between the mean length of left and right SLBF. Which were found not to have any statistically significant difference.

Descriptive statistics of dimensions

Table 4.1: Metric measurements of the fundiform ligament and the suspensory penile ligament.

	Number of observations	**Mean ± SD***	**Min**	**Max**	**Range**
SLBF Left Length	20	14.3± 1.3**	11.2	16.5	5.3
SLBF Right Length	20	15.0 ± 1.7**	11.8	18.8	7
DMBF Height	26	8.5±1.8	5.6	11.8	6.2
DMBF Width	26	3.1±0.8	1.3	4.8	0.6
SPL Height	49	2.6±0.8	1.1	4.4	3.3
SPL Length	49	5.08±1.1	3.3	8.1	4.8

*SD: Standard deviation

**No significant difference: p-value is 0.1422 (>0.05).

4.1.2 General observed macro anatomy patterns

The two most superficial ligaments were observed and described during the dissection process, the fundiform ligament and SPL. Due to the positioning of the arcuate ligament, it could not be dissected and observed, it is thus thoroughly studied under MRI method.

Fundiform Ligament

The fundiform ligament being the most superficial of the ligaments was the first ligament to be observed. Three fibre bundles can be seen constituting this ligament - two lateral (left and right) and one median. The right and left lateral bundles (SLBF) lie superficially and the unpaired medial bundle (DMBF) that runs in the midline of the anterior abdominal wall lies deep (Fig. 4.2). The fundiform ligament is observed soon after reflection of Camper's fatty fascial layer. A very careful dissection process was followed as the lateral bundles, which lay more superficially than the medial bundle of fibres, tapered off and fused with Scarpa's fascia of the abdomen). At its most lateral extremities, the SLBF appears more fatty than fibrous. As the lateral bundles extend medially and down towards the shaft of the penis, they thicken and become tougher and more fibrous matching in consistency with that of the DMBF. The posterior fibres of the lateral bundles then fuse with the left and right wing of the medial bundle. The medial bundle arises from the linea alba (Fig. 4.3) of the abdomen and extends downwards towards the shaft of the penis. It is fibro-fatty in appearance; shorter than the SLBF and lies deep to the membranous fascial layer of the abdomen. As it extends inferiorly, it splits into left and right wings (LW and RW) before fusing with the corresponding lateral bundles of the SLBF and encircling the base of the penis, inserting into the superficial fascia of the penis directly above Buck's fascia (Fig. 4.2). After it encircles the base of the penis and it terminates inferiorly in the scrotum forming the origin of the scrotal raphe septum (dartos fascia) in the midline (see Fig. 4.4).

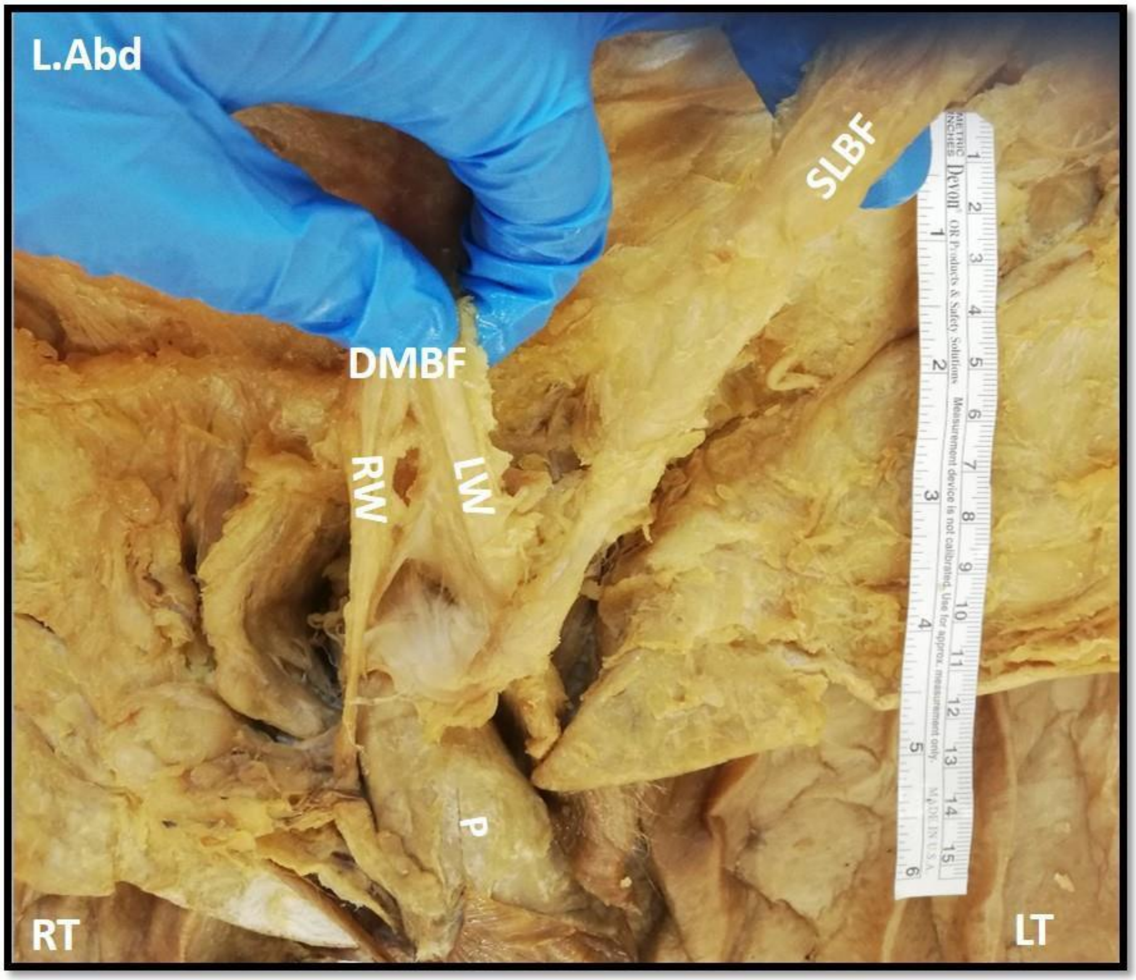

Figure 4.2: The photograph above shows the left superficial lateral bundle of the fundiform ligament (**SLBF**) descending from Scarpa's fascial layer of the abdominal wall fusing with the left wing **(LW)** of the deep medial bundle of the fundiform ligament **(DMBF)**. It also depicts the DMBF splitting into two separate wings, a left and right wing (**LW** and **RW**) which then circles the base of the penile shaft. **LT**: Left Thigh. **RT:** Right Thigh. **L.Abd:** Lower Abdomen.

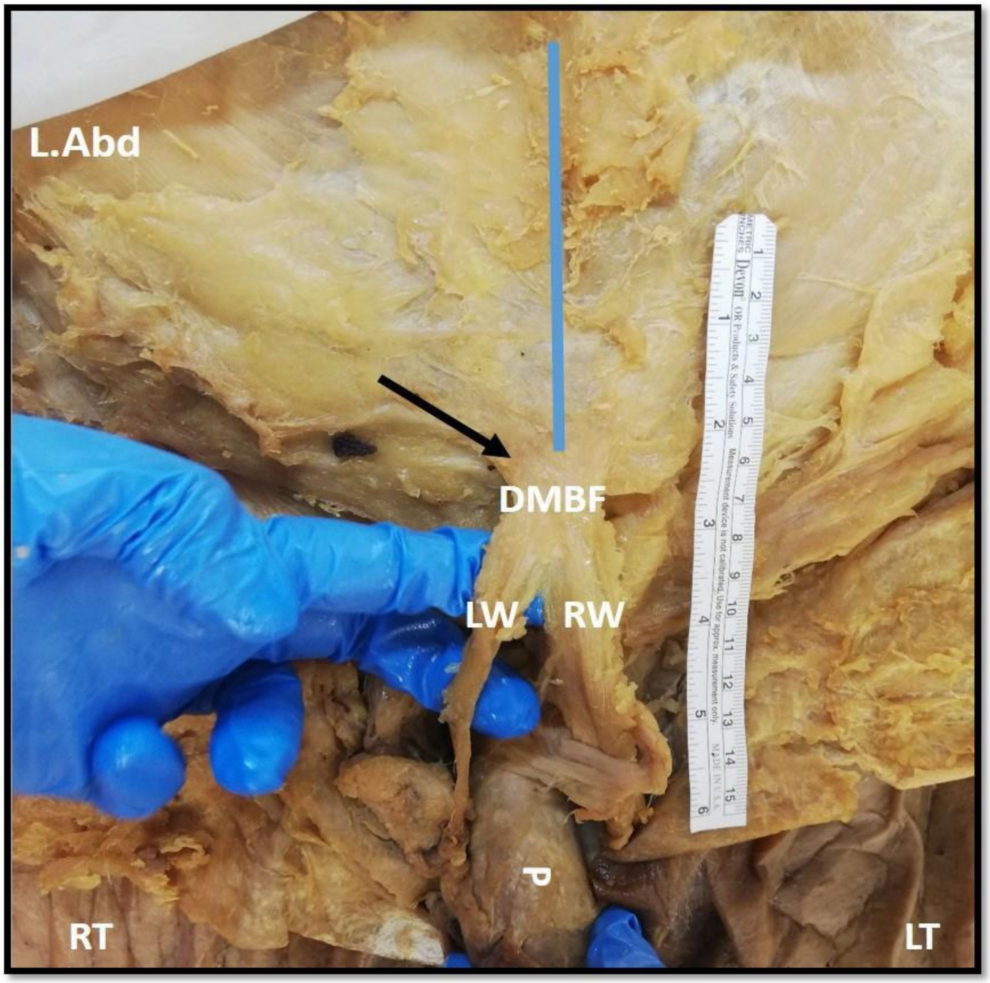

Figure 4.3: The photograph above shows the deep medial bundle of the fundiform ligament arising from the linea alba of the abdomen, indicated by the black arrow. The blue line highlights the linea alba of the abdomen**. L.Abd:** Lower abdomen**. DMBF:** Deep medial bundle of the fundiform ligament. **LW**: Left wing. **RW**: Right wing. **P**: Penile shaft. **LT:** Left thigh. **RT:** Right thigh.

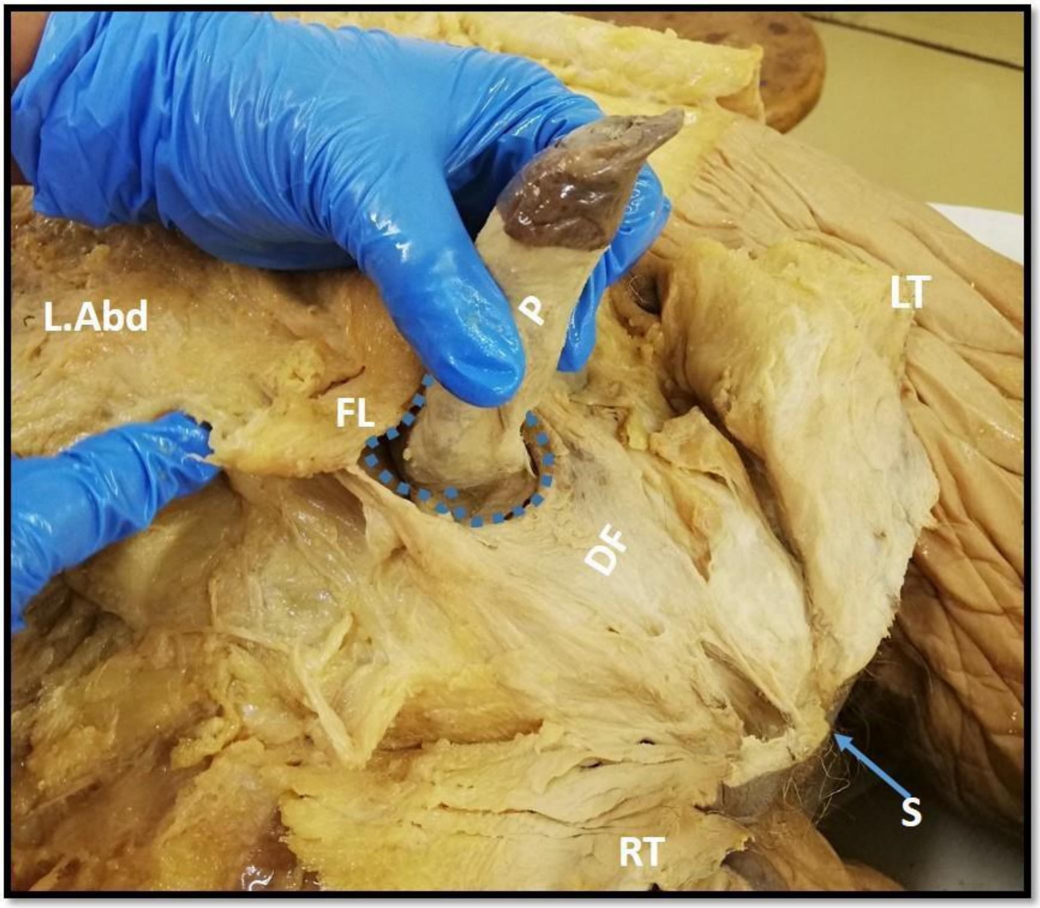

Figure 4.4: The photograph above shows the fundiform ligament (**FL**) as it circles the base of the shaft of the penis, above the deep fascial layer of the penis (Buck's fascia), indicated by the blue line. **L.Abd**: Lower Abdomen. **P:** Penile shaft. **DF**: Dartos fascia of the scrotum. **S**: Scrotum. **RT**: Right thigh. **LT**: Left thigh.

The suspensory ligament

The suspensory ligament proper (SPL) lies deeper to the DMBF and it has a tough fibro-fatty consistency throughout. It arises from the anterior edge of the pubic symphysis, extending inferiorly down the lower border of the pubic symphysis (Fig. 4.5). It travels a short distance to reach the base of the penile shaft. It encircles the base of the penis, however, at a deeper level than that of the fundiform ligament, adhering tightly with the deep facial layer of the penis (Buck's fascia) (see Fig. 4.6).

Figure 4.5: The photograph above shows the suspensory penile ligament (**SPL**) attaching to the anterior and inferior border of the pubic symphysis (**PS**). **RT:** Right thigh. **LT:** Left thigh. **P:** Penile shaft. **L.Abd:** Lower abdomen.

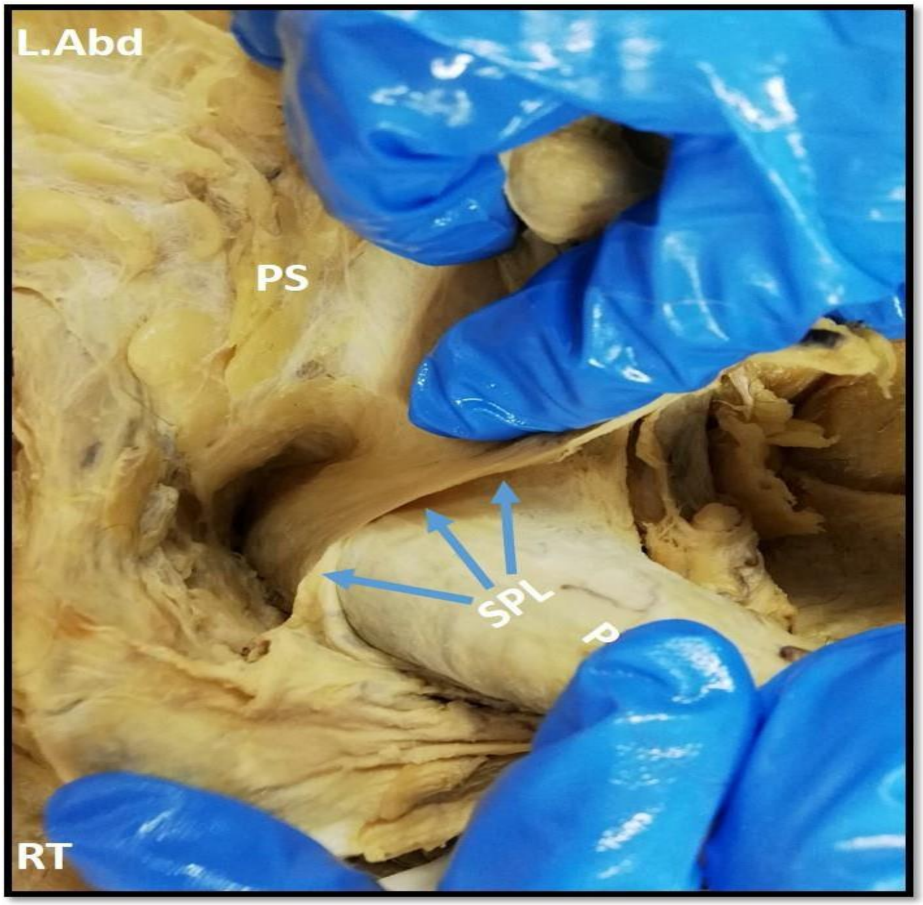

Figure 4.6: The photograph above shows the suspensory penile ligament (**SPL**) attaching to the anterior and inferior border of the pubic symphysis (**PS**). SPL fuses with the deep fascial layer of the penis (Buck's fascia). **RT:** Right thigh. **LT:** Left thigh. **P:** Penile shaft. **L.Abd:** Lower abdomen.

4.2 Microanatomy

4.2.1 General Histology

The ligaments, both bundles of fundiform and SPL are fibro-fatty ligamentous structures. Each ligament had a general composition of mostly adipose tissue, followed by collagen fibres, then elastic fibres, reticular fibres make up a small fraction of the ligaments. The high content of collagen in the ligaments gives the ligaments a very tough fibrous consistency despite high adipose content. The ligaments are also highly vascular, and are riddled with blood vessels.

H&E stained slides were used to examine morphology and calculate an estimation of adipose tissue content. Masson's trichrome stain was used to observe and calculate an estimation of collagen content. Verhoeff's modified elastic stain was used to calculate an estimate of elastic fibre content. Gordon's sweet silver stain was used to calculate an estimate of reticular fibre content.

The estimated average histological composition of each ligament

All the ligaments analyzed are fatty-fibrous structures. The deep bundle of fibres of the fundiform ligament consists of mostly adipose tissue (43±2.02%) and collagen fibres (43 ±1.6 %), there is equal contribution of elastic (13±0.67 %) and reticular fibres (13 ±3.14 %) (see Fig. 4.7). The superficial bundle of fibres of the fundiform ligament is mostly adipose tissue (53±2.55%), equal amounts of both collagen (21 ±1.79%) and elastic (23±0.35 %) fibres and a relatively small amount of reticular fibres (14±3.96%) (see Fig. 4.8). Similarly, to the superficial bundle of fibres, the SPL consists of mostly adipose tissue (53±1.78%), equal amounts of collagen (23±1.35%) and elastic (23±0.82 %) fibre and a relatively small amount of reticular fibres (12±3.29%) (see Fig. 4.9).

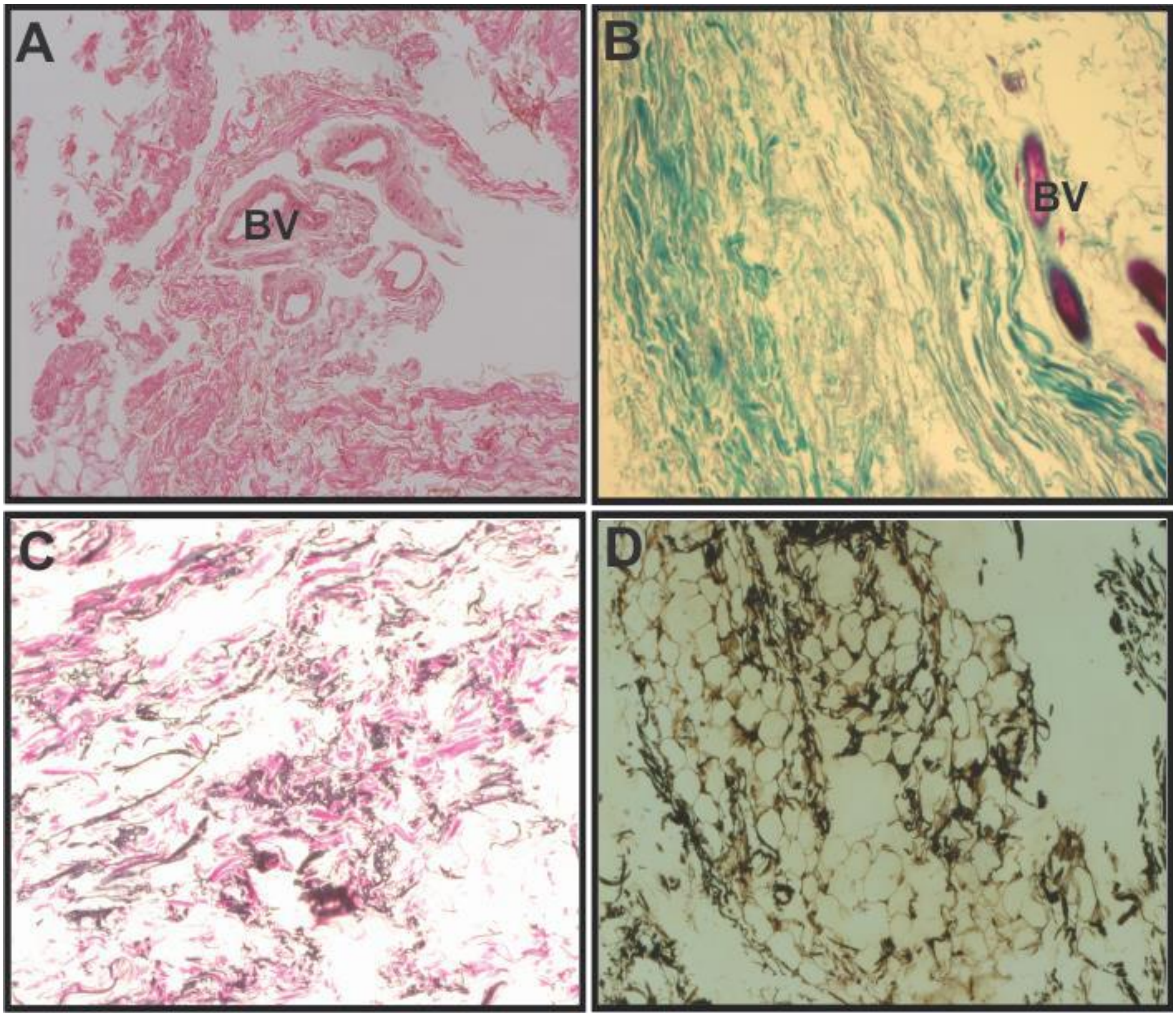

Figure 4.7: The pallet above shows histological composition of the DMBF. **Frame A to Frame D:** From highest to lowest in composition. **A:** H&E stain showing the general morphological structures of the ligament, showing connective tissue and adipose (43±2.02%). **B:** Masson's trichrome stain showing the collagen composition, green (43 ±1.6 %). **C:** Verhoeff's modified elastic stain showing elastic composition, black/brown (13±0.67 %). **D:** Gordon's sweet silver stain showing reticular fibre composition that surround cells, black/brown (13 ±3.14 %) .**BV:** Blood vessels.

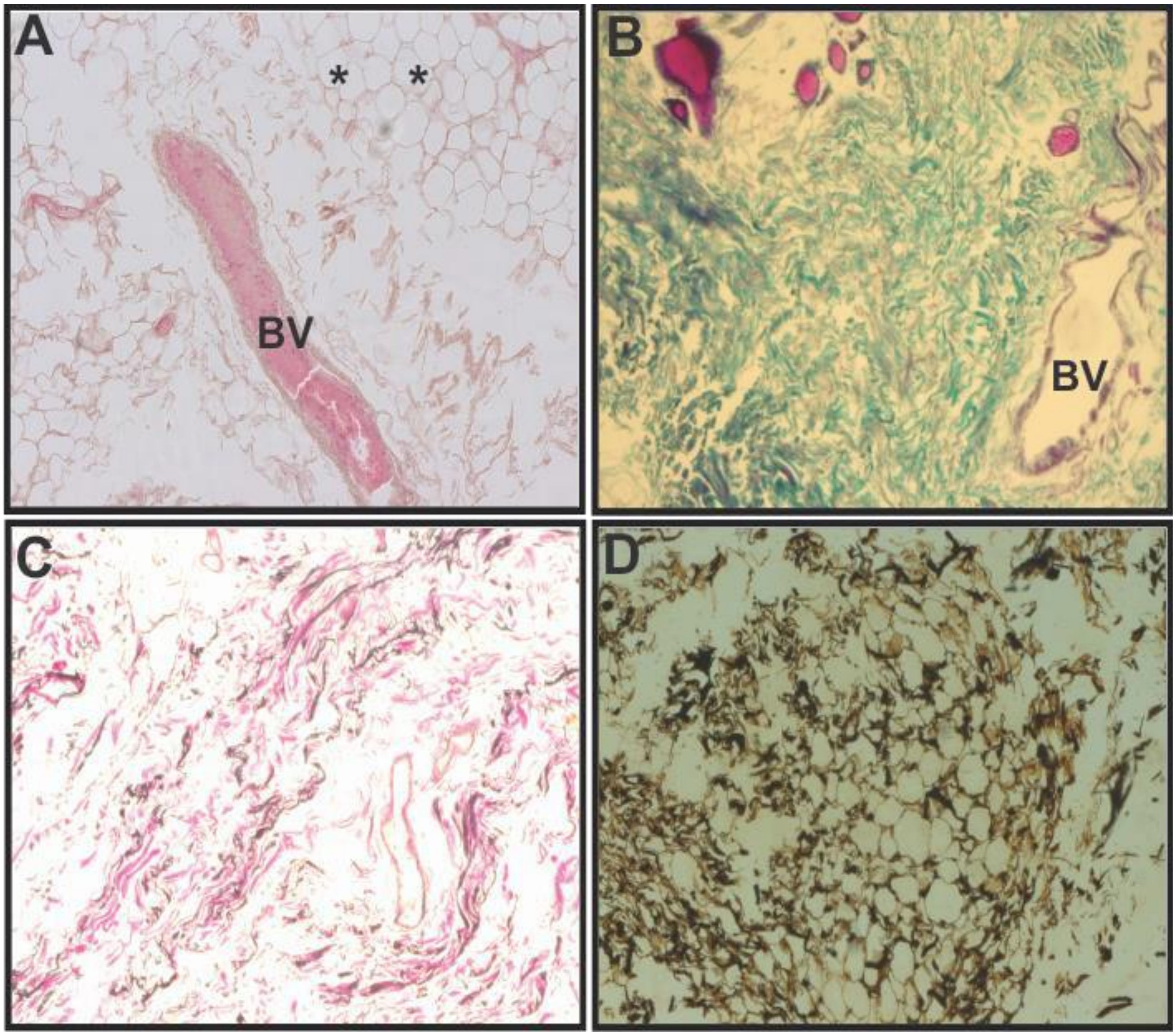

Figure 4.8: The pallet above shows histological composition of the SLBF. **Frame A to Frame D:** From highest to lowest in composition. **A:** H&E stain showing the general morphological structures of the ligament, showing connective tissue and adipose (53±2.55%). **B:** Masson's trichrome stain showing the collagen composition, green (21 ±1.79%). **C:** Verhoeff's modified elastic stain showing elastic composition, black/brown (23±0.35%). **D:** Gordon's sweet silver stain showing reticular fibre composition that surround cells, black/brown (14±3.96%). **BV:** Blood vessels.

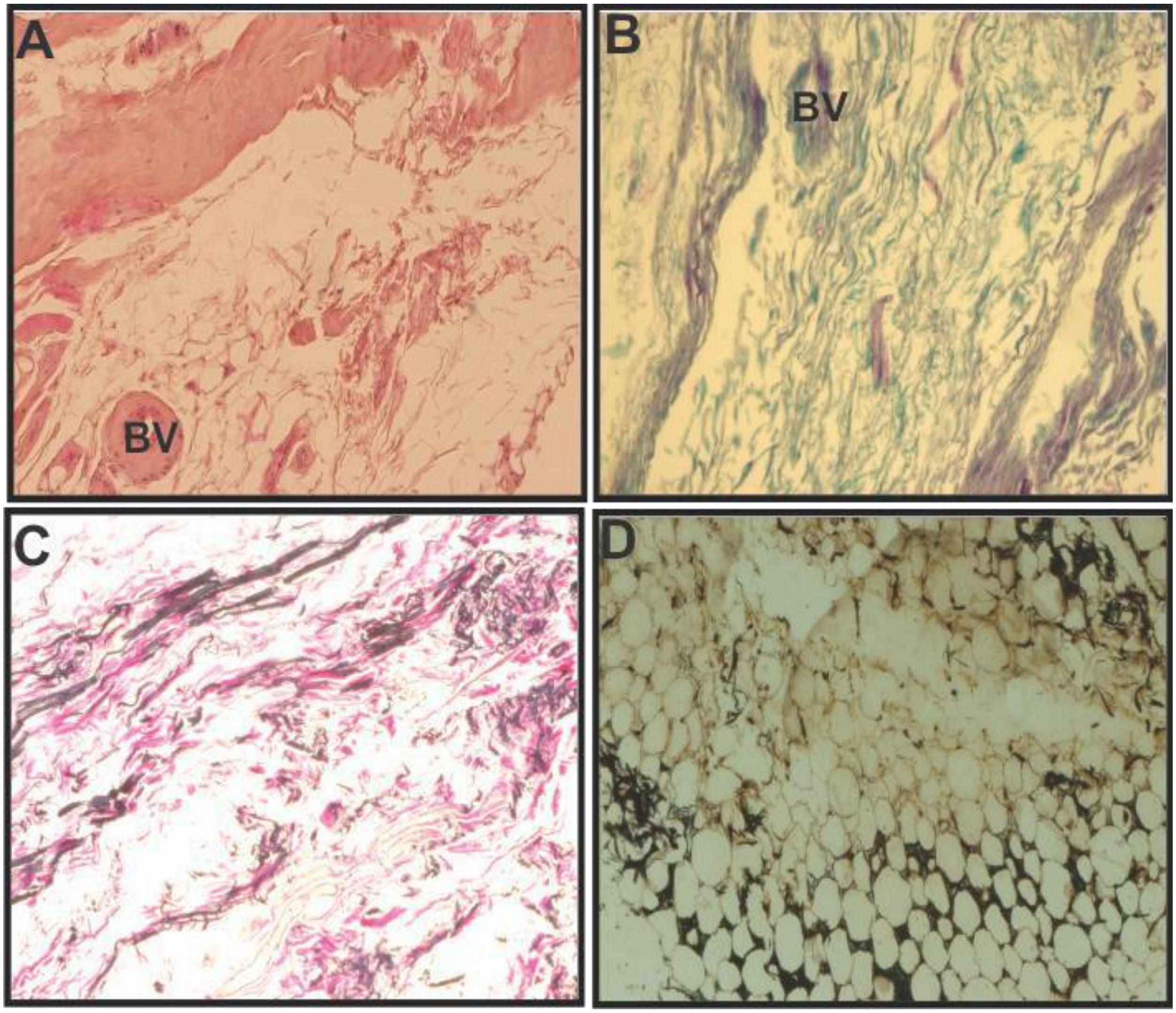

Figure 4.9: The pallet above shows histological composition of the SPL. **Frame A to Frame D:** From highest to lowest in composition. **A:** H&E stain showing the general morphological structures of the ligament, showing connective tissue and adipose (53±1.78%). **B:** Masson's trichrome stain showing the collagen composition, green (23±1.35%). **C:** Verhoeff's modified elastic stain showing elastic composition, black/brown (23±0.82%). **D:** Gordon's sweet silver stain showing reticular fibre composition that surround cells, black/brown (12±3.29%). **BV:** Blood vessels.

4.2.2 Histomorphometry

Adipose tissue

The fat cell content of the ligaments was established using the H&E stain. The percentage composition of adipose tissue was statistically significantly different across all the ligaments studied ($F=45.48$; $P<0.0001$, Fig 4.13D) and the percentage area of adipose tissue was statistically significantly lower in the DMBF compared to SLBF and SPL. There was no statistically significant difference found in the adipose tissue content of the LBF and SPL ($P>0.05$).

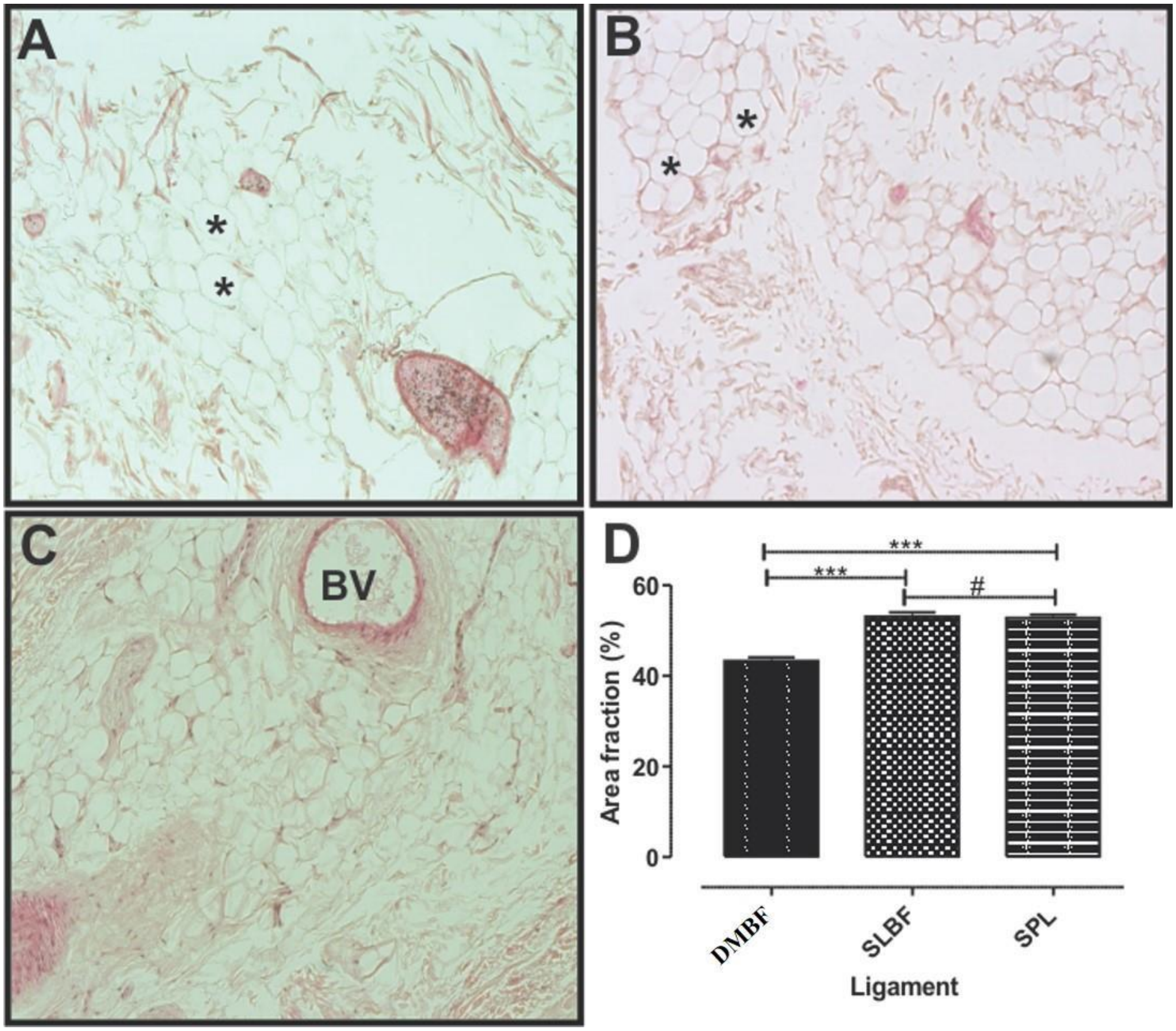

Figure 4.10: The pallet above shows adipose tissue composition of each ligament**. A-C:** H&E stains of each ligament**. Frame A:** Adipose composition of the DMBF. **Frame B:** Adipose composition of the SLBF. **Frame C:** Adipose composition of the SPL. **Frame D:** Graph showing difference of adipose tissue composition of each ligament between each ligament.

#: No significant difference.**BV:** Blood vessels. ***:** Adipose cells.

Collagen fibres

Collagen provides for the tough fibrous consistency of the ligaments. The percentage composition of collagen was significantly different across all the ligaments studied ($F=398.3$; $P<0.0001$, Fig. 4.10 D). More specifically, percentage area of collagen was significantly higher in the DMBF compared to SLBF and SPL ($P<0.0001$). There was no statistically significant difference between the collagen content of the SLBF and SPL ($P>0.05$).

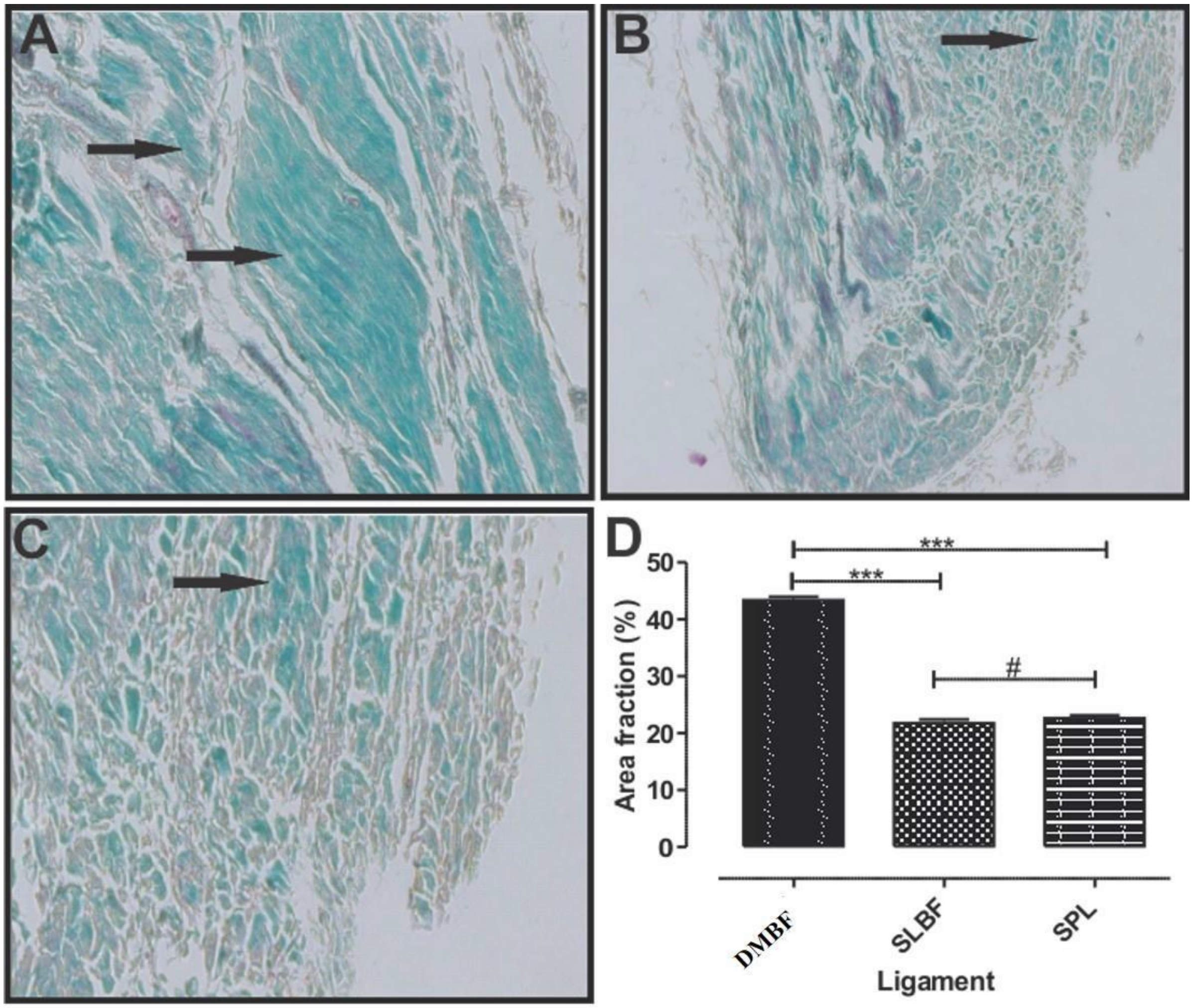

Figure 4.11: The pallet above shows collagen fibre composition of each ligament. **Frame A:** Collagen composition of the DMBF. **Frame B:** Collagen composition of the SLBF. **Frame C:** Collagen composition of the SPL. **Frame D:** Graph showing difference of collagen fibres between each ligament.

*****:** Significant difference. **#:** No significant difference. **Arrows:** highlights collagen fibres (green) of the ligaments.

Reticular fibres

Reticular fibres were found mostly as a thin layer surrounding each adipose tissue cell. The percentage composition of reticular fibres was comparable across the all ligaments DMBF, SLBF and SPL ($F=14.83$; $P>0.05$, Fig. 4.12 D). The reticular fibre content was not statistically different across all ligaments.

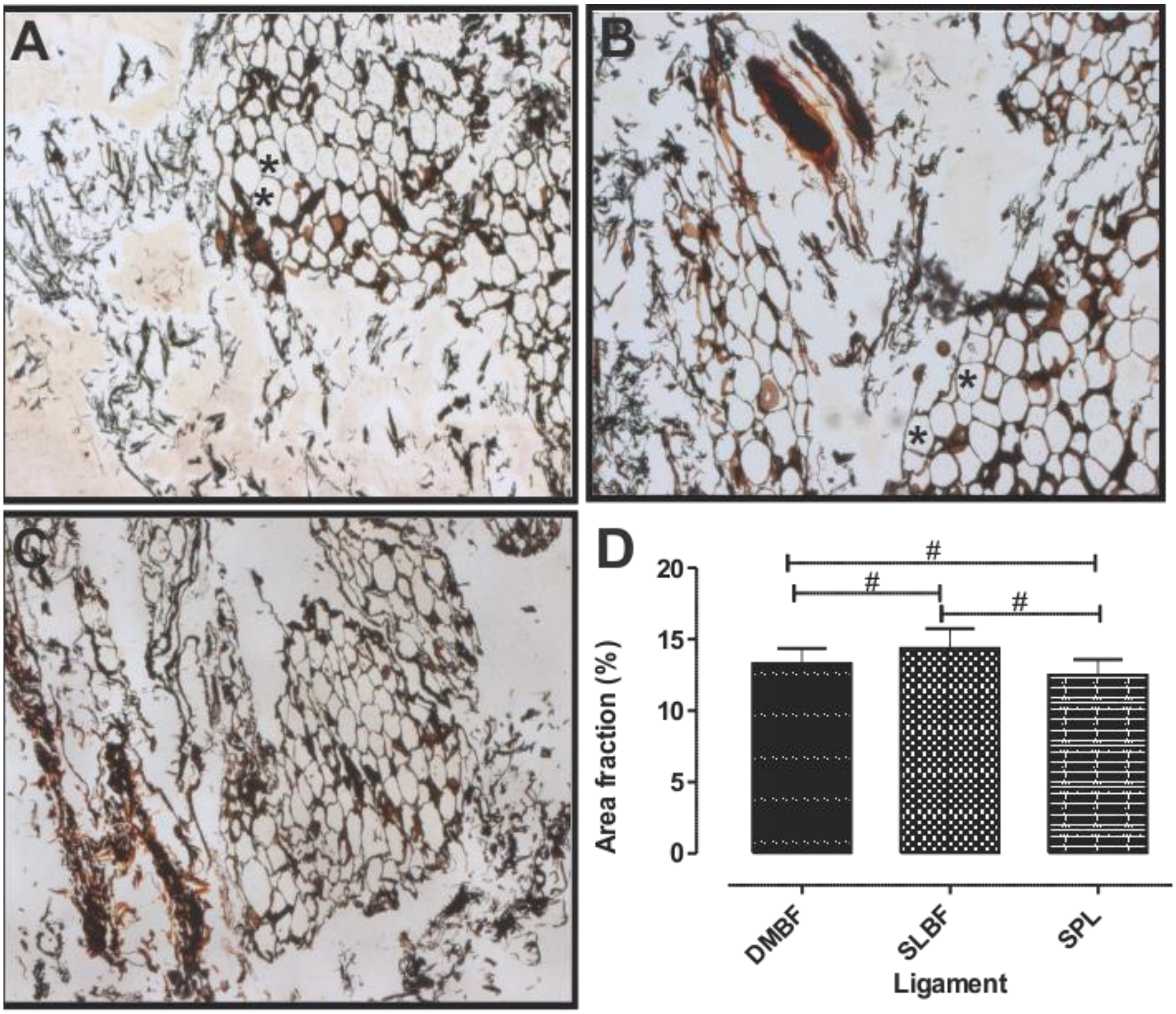

Figure 4.12: The pallet above shows reticular fibre composition of each ligament. **Frame A:** Reticular fibre composition of the DMBF. **Frame B:** Reticular fibre composition of the SLBF. **Frame C:** Reticular fibre composition of the SPL. **Frame D:** Graph showing difference of reticular composition in each ligament between each ligament.

#: No significant difference.

Elastic fibres

The percentage composition of elastic fibres had a statistically significant difference across all the ligaments studied ($F=559.1$; $P<0.0001$, Fig. 4.11 D). The percentage area of elastic fibres was significantly lower in the DMBF compared to SLBF and SPL, but there was no statistically significant difference between the elastic fibre content of the SLBF and SPL ($P>0.05$).

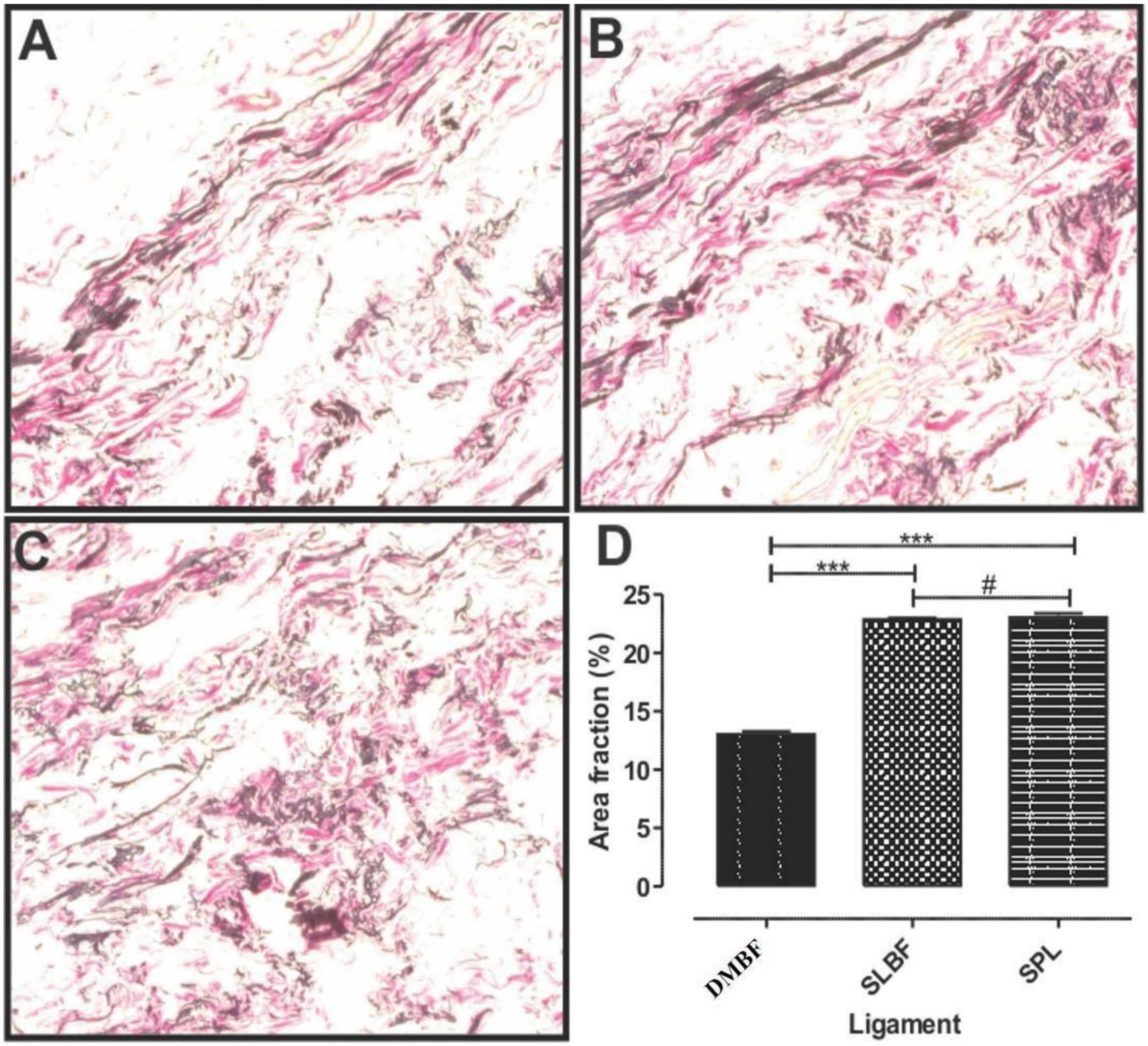

Figure 4.13: The pallet above shows elastic fibre composition of each ligament. **Frame A:** Elastic composition of the DMBF. **Frame B:** Elastic composition of the SLBF. **Frame C:** Elastic composition of the SPL. **Frame D:** Graph showing difference of elastic composition in each ligament between each ligament.

*****:** Significant difference. **#:** No significant difference.

4.3 Magnetic resonance images

Fundiform ligament

The deeper (DMBF) layer of the fundiform ligament was visualized in coronal plane using the MRI system. The superficial part (SLBF) of this ligament was not observed because of the enbloc removal of the pelvic block was closely associated with body of the pubis and hence the proximal and most superficial bundles were omitted. The deeper portion of the fundiform ligament was traced from superficial to deep. In both sections the origin of the deeper layer of the fundiform ligament fell out of the view of the scans but its insertion was clearly visible. In superficial coronal slices, the deeper layer of the fundiform ligament appeared as thick and broad ligament (width) that descended towards the dorsal surface of the shaft of the penis (Fig. 4.14 A-C). Further posteriorly, the deeper layer of the fundiform ligament slit to resemble an inverted **'V'** shape. encircling the three erectile bodies of the penis and the dorsal arteries directly above Buck's fascia (Fig. 4.14 C).

Suspensory ligament proper (SPL)

The origin of the SPL in both specimens was directly associated with the perichondrium of the pubic symphysis as seen in both coronal and sagittal planes (Fig. 4.14 D). In the coronal plane the ligament descended in the median plane and fused with the Buck's fascia on the dorsum of the penis (Fig. 4.14 D). The SPL together with deeper fibres of the fundiform ligament encircles the penile shaft intimately fusing with the Buck's fascia. Insertion into the Buck's fascia can be confirmed by the location of the dorsal arteries and the deep dorsal vein of the penis with lies deep to this fascia (Fig. 4.14 D)

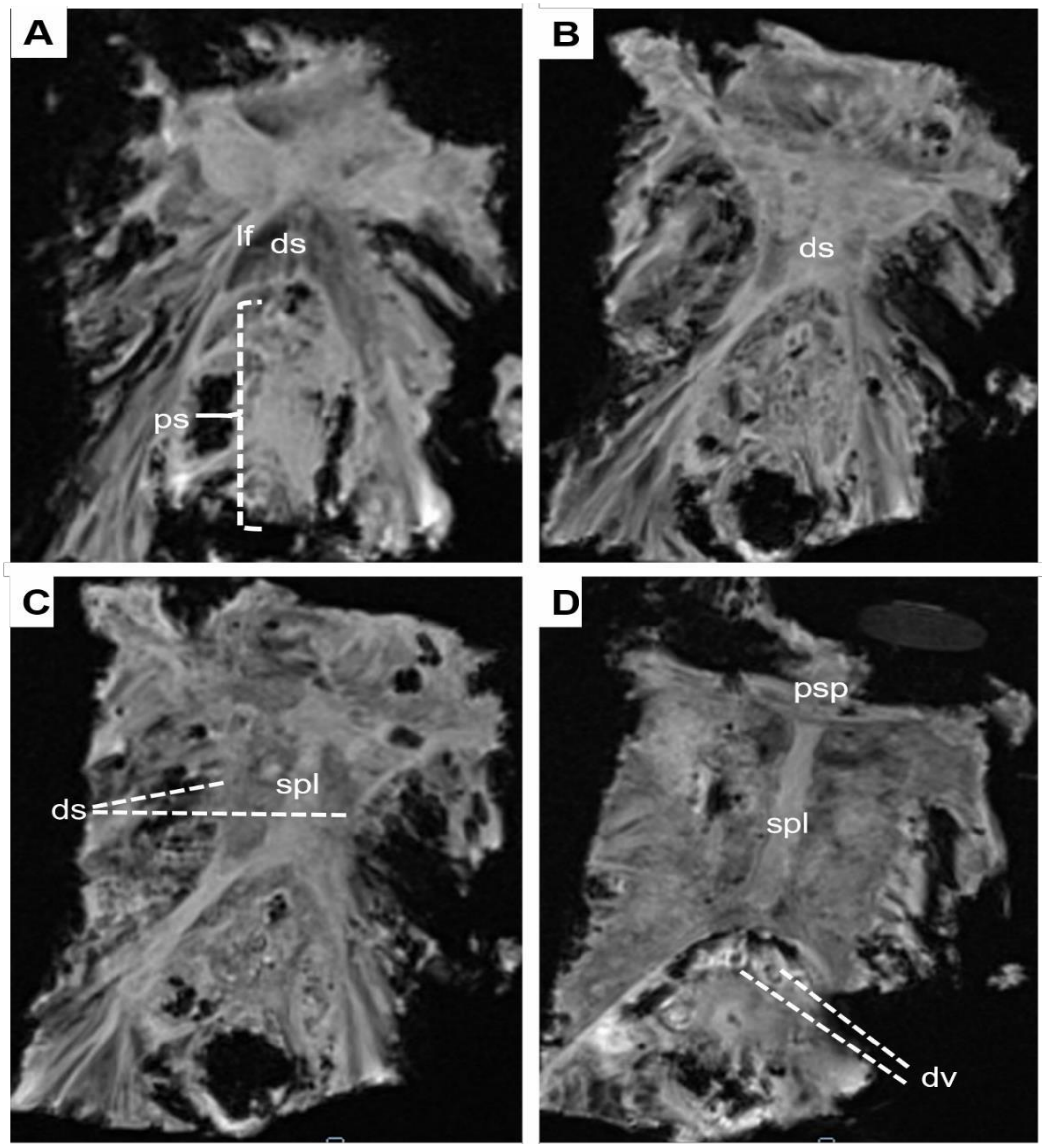

Figure 4.14. The photograph shows a series of coronal plane images of the suspensory ligament of the penis from superficial to deep (**A** through to **D**). (**A**), shows the distal portions of the DMBF split into two wings (**lf**) encircling the penile shaft on each side above the deep fascial layer of the penis (**ds**). (B), shows the deeper fundiform ligament, (**C**), DMBF splitting. Also in view is the suspensory ligament proper (**spl**), (**D**), the suspensory ligament proper (**spl**) inserting above the deep dorsal vasculature (**dv**) and arising from periosteum of the pubic symphysis (**psp**).

Arcuate ligament

In the coronal plane, the arcuate ligament appeared as an arch bridge above the erectile bodies of the root of the penis (Fig. 4.15 A-C). in more superficial sections, the arcuate ligament fuses with the posterior fibres/portion of the SPL making a T-like junction (Fig. 4.15 A). In the sagittal plane, the arcuate ligament appeared to originate from the lower margins of pubic arch including the right and left pubic bodies and the pubic symphysis and insert on Buck's fascia similar to the insertion of the SPL (Fig. 4.15 D-E).

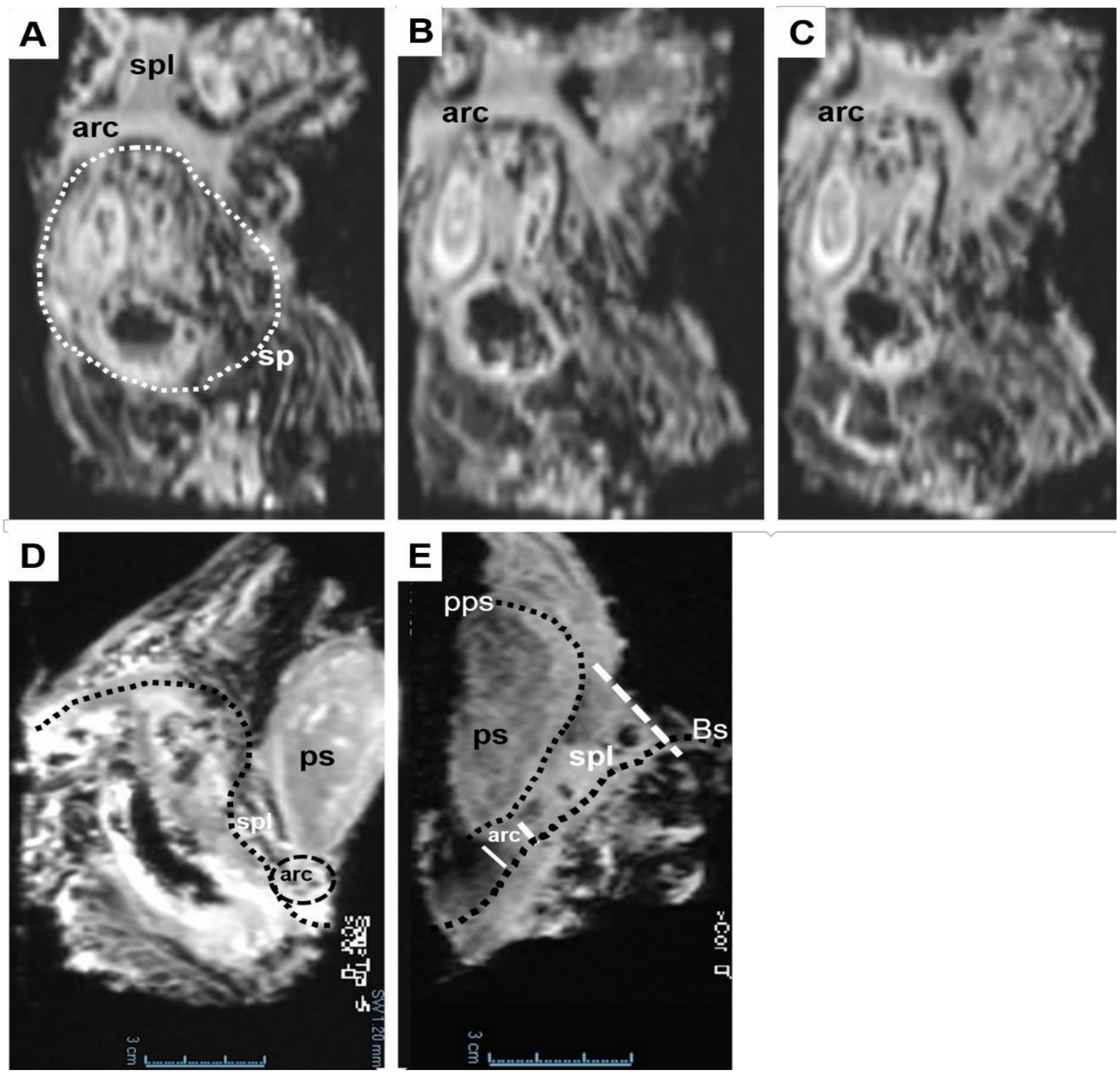

Figure 4.15. The photograph shows a series of coronal plane images of the arcuate ligament from superficial to deep (**A-C**) and the sagittal plane images of both suspensory ligament proper (**spl**) and arcuate ligament (**arc**). (**A**), shows the arcuate (**arc**) and suspensory ligament proper (**spl**) ligaments fusing above the shaft of the penis (**sp**). (**B**) and (**C**) show the arcuate ligament (**arc**) above erectile bodies (root/shaft) of the penis. (**D**) and (**E**), show the suspensory ligament proper (**spl**) and arcuate (**arc**) ligaments in sagittal plane as they arise from the perichondrium (**pps**) of the pubic symphysis (**ps**) inserting on the Buck's fascia (**Bs**) of the penis.

5.0 Discussion

The suspensory ligamentous system of the penis is important for elevating and supporting the penis. The function of the penis and its aesthetic value plays a crucial role in psychological perceptions men have of themselves, i.e. body dysmorphic disorder (Li et al., 2007). The suspensory ligaments of the penis have been described by a few authors (Hoznek *et al.,* 1998; Standring *et al.*, 2008; Moore *et al.*, 2013; Chen *et al.*, 2017) but there is not consensus on the exact origin, course and insertion points of each ligament. In addition, the histological compositions of the penile ligamentous system have not been fully characterized. Hence the current study examined the macro- and micro-anatomy of the suspensory ligamentous system of the penis with a view of adding to the anatomical knowledge. This will improve outcomes for surgical procedures relating to the suspensory ligamentous system of the penis.

5.1 The fundiform ligament

The fundiform ligament is the most superficial of the three suspensory ligaments (Standring *et al.*, 2008). Apart from its topography, the sites of origin from the anterior abdominal wall and insertion on the penile shaft are contentious. In addition, its histological composition is largely unknown. Information about its structure will help towards understanding of the suspensory mechanism of the penis and the associated penile lengthening procedures.

The ligament has been observed to either originate from the linea alba (Moore *et al.,* 2013) or from Scarpa's fascial layer of the abdomen (Chen *et al*., 2017). In the current study, the fundiform ligament presented with two fibre bundles the superficial and deep. The superficial bundle is paired and lies on the lateral edges of the lower part of the anterior abdominal wall while the deep bundle of fibres lies medial along the midline of the lower part of the anterior abdominal wall. The superficial lateral bundle of fundiform ligament (SLBF) was found to be a thickening of fibres within the scarp's fascia as it descends inferomedially towards the penile shaft on each side. The origin and course of the fundiform ligament in the current study is in agreement with Chen *et al*. (2017) who described the superficial bundle of the fundiform ligament as arising from the Scarpa's fascia on the anterolateral abdominal wall. The deep and or medial bundle of the fundiform ligament (DMBF) was found to be arising from the linea alba of the anterolateral abdominal wall.

Similar observations were reported by Hoznek *et al.* (1998), Standring *et al.* (2008) and Moore *et al.* (2013) who found the fundiform ligament to be arising from the inferior end of the linea alba.

The deep fundiform (DMBF) ligament descended medially towards the shaft of the penis and split to give of the left bundle of fibres (left wing, LW) and a right bundle of fibres (right wing, RW) that encircled the shaft of the penis above the layer of the deep fascia of the penis (Buck's fascia). According to the descriptions on the fascial layers of the penis, the superficial fascia (Colles' fascia) of the penis lies directly above the deep fascia and this fascia extends into the scrotum fusing with the scrotal fascia of the scrotum (Dartos fascia) (Standring *et al.*,2008; Moore *et al.*, 2013). Hence, the anterior fibres of the DMBF, those that split into right and left bundles fuse with superficial fascia of penis as they insert on the shaft of the penis. However, as evidenced by the MRI scans, the posterior fibres of the DMBF fuses with the posteriorly located SPL and insert together on the Buck's fascia (deep fascia of the penis). Therefore, the posterior fibres of the DMBF may be classified as an extension of the SPL anteriorly while the superficial portion of the DMBF can be described together with superficial fundiform bundles of fibres (SLBF). This is because the fusion of left and right SLBF fibres to the left and right wings of the DMBF, respectively, happens just before both ligaments encircle the base of the of shaft of the penis. In addition, the fused fibres terminate collectively in the scrotal sac as the origin of the scrotal septum raphe. These findings are in line with Chen *et al.* (2017), who found that the fundiform terminated as fascia in the scrotum sac after encircling the penis. It is therefore, recommended to describe the fundiform ligament according to each different fibre bundle, in order to get a full view of its macro anatomical course and structure. This merges the findings of the different authors who have studied the penile ligamentous system, who described the fundiform as arising from either Scarpa's fascia (Chen *et al*., 2017) or from the linea alba of the abdominal wall (Hoznek *et al.*, 1998; Standring *et al.*, 2008; Moore *et al.*, 2013).

The histological findings of the current study suggest a similar origin and structure to the fibre bundles of the superficial fundiform ligament and this complements the macro anatomical findings. Histological results showed that the SLBF has considerably less (+/-50% less; refer to Fig. 4.9) collagen fibres than that of the DMBF. This echoes the current findings on gross dissection whereby the SLBF arose from the membranous layer of fascia (Scarper's fascia) and the DMBF arose from the linea alba. It is a credible outcome for the DMBF arising from the linea alba which is such a tough fibrous structure to have a significantly higher amount of the collagen fibres than the SLBF. On the other hand, the SLBF showed a higher degree of adipose tissues than the DMBF further indicating its origin from the fatty membranous structure of Scarpa's fascial layer of the abdomen. Hoznek *et al.*, 1998, also found the SPL to have high collagen fibre content.

From these findings, it can be deduced that the DMBF carries more of the suspensory function between the two bundles as it has a significantly tougher structure and that the SLBF plays a supporting role to the DMBF in suspending the penis.

5.2 The suspensory penile ligament

The suspensory penile ligament (SPL) lies between the fundiform ligament anteriorly and the arcuate ligament posteriorly and is often referred to as the suspensory penile ligament proper (Hoznek *et al.*; 1998). The results of the gross dissection study found that the SPL ligament originated from the anteroinferior edge of the pubic symphysis and arched inferiorly downwards towards the shaft of the penis. The origin of the SPL from the pubic symphysis was further confirmed on the MRI scans where the ligament arose from the perichondrium of the pubic symphysis. These findings concurred with observations from previous studies (Moore *et al.* 2014, Hoznek *et al.* 1998 and Chen *et al.* 2017). In females, the superficial suspensory ligament of the clitoris descends from the mons pubis (fatty tissue lying over pubic symphysis) and split to attach to the lateral edges of the glans and body of the clitoris and ultimately extends to the medial aspect of the labia majora (Rees *et al.*, 2000). The dimensions (width= 7-8cm; length= 8-9cm) of the suspensory ligament of the clitoris (Rees *et al.*, 2000) appeared larger than those of the penis (length= 5.08 ± 1.1cm and height = 2.6±0.8 (see Table 4.1; Fig. 3.1 and Fig. 3.2)) observed in the current study. This disparity may be caused by different approach taken to classify the suspensory ligaments of the penis and clitoris. In males the bundles of the fundiform ligament are longer than the suspensory ligament proper (SPL) by a ratio of 1:2. Surprisingly, the dimensions of the SPL of the clitoris are larger than those of penis (Rees *et al.*, 2000) and this may suggest that the authors misconstrued the fundiform ligament to be the SPL of the clitoris. In a similar study by Hoznek *et al.* (1998) the length and width of the SPL were found to be 3.7cm (±0.5) and 2.7cm (±0.8) respectively. Wang *et al.* (2012) vaguely reported on the length of the SPL and found that its length to be 2-4cm. Both Hoznek *et al.* (1998) and Wang *et al.* (2012) reported a smaller range in SPL dimensions, this could be a result of methodology disparity or a difference in overall population demographics.

In the current study, the SPL was found to fuse and insert on the deep layer of the penile fascia (Buck's fascia) (see Fig. 4.6) Similarly, Chen *et al.* (2017) and Moore *et al.* (2013) found that the SPL fuses with Buck's fascia and suggested that it carries the greater part of suspensory function of the penis. However, Hoznek *et al.* (1998) concluded that the SPL had relations with the tunica albuginea of the cavernosa but also noted that the MRI sections were not sufficient to assess the three ligaments. On the other hand, Standring *et al.* (2008) describe the attachment vaguely as attaching to the fascia of the penis, not specifying whether it attaches to Colle's fascia, Buck's fascia or the tunica albuginea.

The SPL is referred as the suspensory ligament proper (Hoznek *et al.*, 1998) which suggests that it carries the greater part of the suspensory function. The SPL is prone to injury, which could be a result of angulation (Standring *et al.*, 2008) or rough coital activity (Pryor *et al.*, 1979). The histological results of the current study show that SPL consist of both fibrous material and adipose tissue. The SPL showed it consists of a higher percentage of elastic fibres, followed by adipose tissue and less collagen fibres. Therefore, the SPL ligament, though short holds firmly to the penis and has more flexible capacity than the fundiform ligament. Affirming that it possesses the bulk of the suspension of the penis (Hoznek *et al.*, 1998).

5.3 The arcuate ligament

Due to the positioning of the arcuate ligament, it could not be examined via dissection nor extracted for histological analysis. Therefore, it was imperative to extend the study, and include an MRI part to the study. MRI technology enabled study of the arcuate ligament in situ.

The arcuate ligament is the least of the three ligaments mentioned in literature. Literature around the suspensory ligamentous system of the penis at times makes no mention of it (Moore *et al.*, 2013; Standring *et al.*, 2008). This could be a result of its intricate positioning. The MRI results of this study have presented motivating findings. The scans found that the arcuate ligament originated from the inferior edges of the pubic symphysis and inserted directly on the deep fascia of the penis (Buck's fascia) on the base of the penis, this was consistent with the results of Hoznek *et al.* (1998) and Chen *et al.* (2017).

Furthermore, however, the MRI scans found that the arcuate ligament and fused with the posterior fibres of the suspensory penile ligament (SPL). This anatomical distinction that has not appeared in available literature. This fusion to the posterior fibres of the SPL puts emphasis on the strength of and surrounding structures that support the suspensory penile ligament proper.

6.0 Limitations of the study

The 26 un-dissected cadavers had other areas not of interest to this study dissected by other students, however, the area of interest for this study (pelvic region) remained intact. 6 of these 26 cadavers had the lower abdominal region dissected, by undergraduate medical students, rendering the SLBF ineligible for quantification. This limited the number of observations of the SLBF ligament to a total of 20. Cadaveric specimens have undergone structural manipulation due to the formaldehyde used to preserve the body tissue, this in turn affects the measurements. The positioning of the arcuate ligament is intricate and it therefore could only be studied solely by MRI technology resulting in the observation of only its origin, course and insertion, but not the fibre composition.

7.0 Conclusion

The aim of this project was to add anatomical knowledge about the suspensory ligamentous system of the penis through macro-anatomical and micro-anatomical examination. The aim and objectives were achieved by quantifying the dimensions of the fundiform and the suspensory penile ligament, providing anatomical descriptions of the fundiform, suspensory penile ligament and the arcuate ligament and providing an in depth histological quantification of the different types of tissue.

8.0 Recommendations

Detailed anatomical descriptions of the suspensory penile ligamentous system were obtained in this study. It is therefore recommended that these anatomical descriptions be reflected on during the conceptualization and design of phalloplasty procedures and any other procedures that implicate the suspensory ligamentous system of the penis. Increase in the depth of contextual knowledge around the penis will in turn increase the quality and accuracy of surgical procedures.

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Appendices

Appendix 1

Tissue Processing

The tissue was processed using an automatic tissue processor (Shandon citadel 1000) as follows:

1. 10% buffered formalin for 4 hours
2. 70% alcohol for 1 hour
3. 95% alcohol for 2 hours
4. 95% alcohol for 2 hours
5. 95% alcohol for 2 hours
6. 95% alcohol for 2 hours
7. 100% alcohol for 2 hours
8. 100% alcohol for 2 hours
9. 100% alcohol for 2 hours
10. Chloroform for 2 hours
11. Chloroform for 2 hours
12. Wax for 2 hours
13. Wax for 2 hours

Paraffin embedding

1. Warm the wax as well as the metal moulds and a pair of forceps making sure that the temperature does not exceed 60^{o}C.
2. Once the wax has melted, fill the metal mould with wax.
3. Cool briefly, and then cover with the plastic cassette accordingly.
4. Quickly top up with more melted wax and cool for approximately 20 minutes.
5. Separate the metal mould from the plastic cassette.

Appendix 2

General tissue structure and adipose tissue staining procedure (Haematoxylin and eosin stain)

Hydration

1. Dewax in xylene for 2x10 minutes.
2. Immerse in 2 changes of 100% ethanol for 3 minutes each
3. Immerse in 95% ethanol for 3 minutes
4. Immerse in 70% ethanol for 3 minutes
5. Wash in distilled water for 5 minutes

Nuclear staining

1. Stain with Mayer's haematoxylin for 10 minutes
2. Wash in running tap water for 2 minutes
3. Differentiate in 1% acid alcohol for 2 dips
4. Wash in running tap water for 2 minutes
5. Blue in Scott's tap water for 2 minutes
6. Wash in running tap water for 2 minutes

Cytoplasm staining

1. Stain in eosin for 2 minutes
2. Wash briefly in running tap water

Dehydration and mounting

1. Immerse in 70% ethanol for 3 minutes
2. Immerse in 90% ethanol for 10 minutes
3. Immerse in 100% ethanol for 10 minutes
4. Clear in 2 changes of xylene
5. Mount in entellen

Appendix 3

Collagen fibre staining procedure (Masson's trichrome stain)

1. *Mordant in Bouin's solution, microwave 1 minute, allow stand for15 minutes.
2. Wash in running tap water to remove the picric acid, 5 minutes.
3. Weigert's working hematoxylin, 10 minutes.
4. Blue in running tap water for 5 minutes, rinse in distilled water.
5. Biebrich scarlet for 5 minutes.
6. Rinse in distilled water.
7. Phosphotungstic/phosphomolybdic acid for 10 minutes, discard solution.
8. Transfer directly into Aniline blue for 5 minutes.
9. Rinse in distilled water.
10. 1% Acetic acid for 1 minute, discard solution, rinse in distilled water.
11. Dehydrate, clear, and coverslip.
12. *Conventional method: Mordant in Bouin's solution, 60°C for 1 hour.

Appendix 4

Reticular fibre staining procedure (Gordan's sweet silver stain)

REAGENTS:

20% Silver Nitrate

Silver nitrate 10.0 gm

Distilled water 50.0 ml

Make fresh.

CAUTION: Skin irritant, avoid contact.

Ammoniacal Silver Solution

20% silver nitrate 50.0 ml

ammonium hydroxide

Add ammonia drop by drop until the

precipitate formed has completely

dissolved. Make right before use and discard after use.

CAUTION: Skin and respiratory system irritant, avoid contact and inhalation.

Developer Stock Solution:

Formaldehyde (37-40%) 20.0 ml

Citric acid 0.5 gm

Nitric acid 2 drops

Distilled water 100.0 ml

Make fresh. Discard after use.

CAUTION: Carcinogenic, corrosive.

Working Solution:

Distilled water 50.0 ml

Ammonium hydroxide 8 drops

Developer, stock 8 drops

Make fresh just before use, discard.

CAUTION: Skin and respiratory system irritant, carcinogenic.

Ammonia Water: 5% Hypo

Distilled water50.0 ml

Ammonium hydroxide8 drops

Make fresh, discard.

CAUTION: Skin and respiratory system irritant, avoid contact and inhalation.

PROCEDURE:

1. Hydrate to distilled water, 3 changes.
2. Preheat a coplin jar of 20% Silver nitrate solution, 35 seconds in the microwave, add slides, place in a 37℃ oven for 15 minutes.
3. Wash in distilled water, 3 changes.
4. Place slides in Ammoniacal silver solution, 10 minutes, in 37℃ oven.
5. Place slides in Working Developer solution, 1 to 5 minutes, check under microscope.
6. Wash in fresh ammonia water.
7. Wash in distilled water, 3 changes.

Appendix 5

Histochemical staining of elastic fibres (Verhoeff's modified stain)

1. Dewax and hydrate sections to water

2. Stain in modified Verhoeff at room temperature for 7 minutes [or in a 60 °Coven for 3 1/2 minutes].

To prepare **modified Verhoeff Working Elastic solution,** mix just before use: • 3 parts Alcoholic Hematoxylin, 3% • 2 parts Ferric Chloride 2%, aqueous • 1 part Lugol's Iodine

3. Wash in running tap water for 1 minute.

4. Differentiate in Ferric Chloride, 0.4% aq. for 75 seconds. Check differentiation microscopically

5. Wash in running tap water for 5 minutes.

6 Counterstain in Van Geisons solution for 60 seconds.

7. Blot dry and clear in 100% alc., 2 x Xylene and mount with Entellen

Lugols Iodine	
Lugols Iodine	1g
Potassium iodide	2g
Distilled water	100ml

Van Gieson	
Sat. aq Picric Acid	100ml
1% aq Acid Fuchsin	18ml
Distilled water	100ml

Appendix 6

Ethical clearance certificate

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
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UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

Ref: W-CJ-140604-1

25/01/2016

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
29 JAN 2016
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Copy - HREC (Medical) Secretariat: Rhulani Mkansi, Zanele Ndlovu.