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Blood supply and innervation of the prostate:
Improving clinical outcomes after radical prostatectomy

Ghazi Mobarak Alanazi

A thesis submitted for the degree of

Doctor of Philosophy

The University of Edinburgh

2022
Declaration

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Abstract

**Background:** Radical prostatectomy remains the main choice of treatment for prostate cancer. However, despite improvements in surgical techniques and neurovascular sparing procedures, rates of erectile dysfunction and urinary incontinence remain high. This is due, at least in part, to an incomplete understanding of the neurovascular supply surrounding and inside the prostate.

**Objectives:** To provide a comprehensive, detailed external and internal description of the distribution of nerves and blood vessels associated with the prostate, as well as correlate the internal distribution of prostatic neurovascular structures with patients’ clinical outcomes after radical prostatectomy.

**Methods:** For the gross dissection element of the research, cadaveric bodies were obtained from the University of Edinburgh’s Anatomy unit, regulated by the Human Tissue [Scotland] Act 2006. Detailed dissection of 24 embalmed hemipelvises was performed employing a novel approach designed to preserve the entire prostate in situ, facilitating reliable and consistent identification and tracing of neurovascular structures supplying and surrounding the prostate. For the histological element of the research, internal neurovascular structures of the prostate were investigated in a total of 309 slides obtained from the apex, body and base of prostates from 15 patients who underwent non-nerve-sparing radical prostatectomy. Immunohistochemical staining was performed to identify and distinguish between parasympathetic and sympathetic nerves, whereas H&E staining was used to identify blood vessels. The total number, density and relative position of nerves and blood vessels was established using quantitative morphometry. One-way ANOVA tests and unpaired t tests were applied to establish statistically significant differences across the measured variables. Finally, patient-specific outcome data were used to establish whether the internal distribution of nerves
and blood vessels within the prostate influenced the nature and extent of complications (urinary incontinence and erectile dysfunction) after surgery.

**Results:** A total of 48 prostatic arteries were identified by gross dissection, arising either directly from the internal iliac artery or one of its branches, including the inferior vesical artery, the superior vesical artery and the middle rectal artery. The nerves of the prostate were observed to be derived from the pelvic plexus in all preparations investigated. However, the location of their penetration into the gland was variable. Nerves, as well as blood vessels, were present across all prostatic levels and regions examined at the histological level. However, their number and density varied considerably within regions. Assessment of the precise positioning of neurovascular structures revealed that the majority of nerve fibres were located within dorsal and peripheral aspects of the gland. In contrast, the highest density of blood vessels was found predominantly within ventral and dorsal midline regions. All patients included in the study experienced erectile dysfunction and urinary incontinence after radical prostatectomy. None of the patients recovered their erectile function after two years of follow-up, whereas the recovery period of the urinary continence occurred over a variable time course. The number of intraprostatic nerves was found to be significantly lower in patients who recovered their continence within less than 12 months after surgery, compared to those whose recovery took 12 months or longer. No significant correlation was identified between the distribution of neurovascular structures inside the gland and the aggression level of the prostatic tumour.

**Conclusion:** A novel dissection approach has been developed and successfully applied, facilitating a clear lateral view of the prostate in situ and reliable tracing of associated external neurovascular structures, including the location of penetration into the gland. We identified widespread regional differences in the localization of nerves and blood vessels inside the prostate. We report a surprising disconnect between the localization of nerves and blood vessels, showing that they are predominantly localised to different regions of the prostate.
Sparing of neurovascular structures at any anatomical position of the gland, with less traumatic manipulation, during surgery will be required to protect the majority of neuronal structures and decrease complications following radical prostatectomy.
Lay summary

The prostate is a gland located in the male pelvis, underneath the bladder. It is the largest accessory gland in males and represents the main gland of the male reproductive system. Clinically, the prostate can be affected by several diseases, one of which is prostate cancer. Prostate cancer is responsible for approximately 11% of all cancers in men and is the cause of around 8% of all cancer-related deaths in males. Radical prostatectomy (RP) is the main choice of treatment for prostate cancer, the primary purpose of which is the complete surgical removal of the tumour by taking out the whole prostate gland from the body. This procedure frequently leads to two main side effects in patients: erectile dysfunction and urinary incontinence. These common complications are thought to occur due to nerves and/or blood vessels associated with the prostate being damaged and/or removed during surgery. Understanding the detailed arrangement of nerves and blood vessels around the prostate is therefore of critical importance to guide surgeons performing RP in order to minimise potential complications following surgery.

The aim of this study, therefore, was to investigate the external and internal nerves and blood supply of the prostate, as well as correlate the position of nerves and vessels inside the gland with complications in erectile dysfunction and urinary incontinence that patients reported after surgery. A novel dissection approach has been developed to preserve the entire prostate, facilitating a reliable identification of its external nerves and blood vessels from their origin right down to where they enter the prostate. The nerves and blood vessels inside the gland were also examined using histological staining, applying specific staining protocols to selectively identify different types of nerves and blood vessels within the prostate. In addition, data concerning patients’ outcomes after surgery were used to determine whether the distribution
of nerves and vessels inside the gland affected the extent of urinary incontinence and erectile dysfunction after surgery.

Applying our new dissection approach, we successfully identified the precise origin and location of external blood vessels supplying the prostate, with most arising from one of the major arteries of the pelvis (the internal iliac artery) or one of its branches. External nerves supplying the gland originated from a structure known as the pelvic plexus in all cases, although the location where the nerves then entered the gland was found to differ between individuals. Internally, within the prostate, nerves and blood vessels were found across all regions and areas. However, surprisingly, the position of nerves and blood vessels was not precisely matched, having their largest numbers in different regions of the gland. Moreover, we found that patients who recovered their urinary function within a year after surgery had a significantly lower number of nerves within the prostate than those who took longer than a year to recover their urinary function.

In summary, this study provided the first comprehensive, detailed external and internal description of nerves and vessels related to the prostate gland. It also provided new insights into how the internal distribution of nerves and blood vessels influences the extent of complications a patient is likely to experience after radical prostatectomy.
Dedication

This work is dedicated to

My parents; Mobarak Alanazi & Tamam Alanazi, and to my brothers and sisters; Yasmeen, Sami, Hessa, Mohammed and Hajer for their love, prayers and support throughout the course of this journey
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Chapter 1: Background

1.1 The prostate

The prostate is a fibromuscular gland located in the male pelvis, inferior to the urinary bladder, enclosing the urethra and ejaculatory ducts (Figure 1.1). It is the largest accessory gland and main reproductive gland in males, and their fertility is determined by the contents of the prostatic fluid produced by the epithelium of the gland. The prostate is typically described as ‘walnut-shaped,’ and has a normal weight range from 15 to 20g (Aaron et al., 2016; Lee et al., 2011). In the male foetus, the prostate typically starts to develop around week 10, with the creation of prostatic buds from the urogenital sinus (UGS), before reaching full development at sexual maturity (Kellokumpu-Lehtinen et al., 1980; Marker et al., 2003). Prostatic growth, as well as its preservative and secretory function, is stimulated by the presence of several growth factors and hormones, with testosterone considered the foremost among them (Madersbacher et al., 2019).
Figure 1. 1: The location of the prostate. Schematic illustration (generated using Affinity Designer software) revealing the location of the prostate in male pelvis inferior to the urinary bladder. S: superior; A: anterior; I: inferior; P: posterior.
Anatomically, the prostate is comprised of six surfaces (apex, base, posterior, inferior, anterior and lateral). The apex sits on the urogenital diaphragm’s superior surface and attaches to the levator ani muscles (Standring, 2016). The prostatic urethra passes through the base surface of the gland and connects to the neck of the urinary bladder. The posterior surface is smooth, triangular and connected to the anterior wall of the rectum. The anterior surface joins the inferior and lateral surfaces of the gland, and is located superior to the urogenital diaphragm on the levator ani fascia (Lee et al., 2011). In addition, the prostate is comprised of three zones drawn from different embryologic sources (Figure 1.2): the transition zone (TZ), the central zone (CZ) and the peripheral zone. The peripheral and transition zones are derived from the urogenital sinus and comprise 70% and 5% of the prostatic volume respectively. In contrast, the prostatic central zone arises from the Wolffian duct, and represents 25% of the prostatic volume (Lee et al., 2011).
Figure 1. 2: Schematic illustration (generated using Affinity Designer software) showing the prostatic zones. The prostate consists of three zones and an anterior fibromuscular stroma. It can be noted that the urethra and ejaculatory duct pass through the prostate. S: superior; A: anterior; I: inferior; P: posterior.

1.2 Prostatic diseases

Clinically, three major types of disease are associated with the prostate gland: prostate cancer, benign prostatic hyperplasia (BPH) and prostatitis. Prostate cancer is among the most common causes of cancer deaths in men globally (Ferlay et al., 2010; Retel et al., 2014; Wright et al., 2013). In Europe, approximately 2.6 million patients are diagnosed annually with cancer. Prostate cancer comprises approximately 11% of all cancer occurrences in men and is responsible for approximately 8% of all tumour-related deaths in males (Bray et al., 2002; Heidenreich et al., 2008).

BPH is described as a proliferation of the stromal and epithelial parts of the prostate in the periurethral region. It is one of the most common diseases in men, and its prevalence in men
over the age of 40 years old ranges from 30 to 40%. It increases to 50% in men over 60 years and is as high as 90% in men older than 85 years. However, despite the high incidence of BPH in ageing males, its pathophysiology is not fully understood. For instance, it is yet undiscovered why the weight of the prostate varies significantly among men with BPH (i.e. in some cases the weight of the gland is 40 g and in others reaches 200 g) (De Nunzio et al., 2013; Kramer & Marberger, 2006; Madersbacher et al., 2019).

Prostatitis is the third most common disease affecting the male urinary tract. The incidence of prostatitis ranges from approximately 11% to 13% of all adult males (Verze et al., 2016). In contrast to BPH and prostate cancer, which mainly affect older men, prostatitis is found in men of all ages. Prostatitis, of all prostatic disorders, has the highest potential impact on male fertility (Khan et al., 2017; Verze et al., 2016).

1.3 Diagnosis of prostate cancer

Prostate cancer is caused by a heterogeneous tumour, ranging from asymptomatic to a promptly progressive lethal systemic tumour (Hughes et al., 2005). As a consequence of the emphasis on annual digital rectal examination (DRE) and the extensive use of prostate-specific antigen (PSA) analysis, a high number of men are currently diagnosed in the early stages of prostate cancer. Both types of prostate cells (benign and malignant) produce the PSA protein. However, higher levels of PSA, exceeding 4.0 ng/ml, indicate the possible existence of a tumour (Gomez et al., 1993; Hughes et al., 2005). Thus, patients with high levels of PSA or abnormal DRE readings are offered access to more advanced diagnostic techniques, such as transrectal ultrasound (TRUS) guided biopsy and magnetic resonance imaging (MRI) guided biopsy of the prostate (Kasivisvanathan et al., 2018). During a biopsy, 10–12 samples of prostatic tissue
are obtained for examination by a pathologist, who then determines a Gleason grade for the cells based on their microscopic form and architecture (Litwin & Tan, 2017).

1.4 Prostate cancer Gleason score

Between 1966 and 1974, Donald F. Gleason created a unique histopathological grading scheme for prostate cancer, based on the tumour’s architectural shape (Epstein et al., 2005; Gleason et al., 1974). Using this system, a pathologist gives prostatic cell samples a score from 3 to 5, according to the level of ‘aggression’ present. The Gleason score indicates the most common and second-most common cell patterns in the sample. For example, if the most common pattern was 3 and the second most common was 4, then the Gleason score would be 7 (Gleason et al., 1974; Matoso & Epstein, 2016). In addition, the Gleason scoring system includes grade groups from 1 (being the least aggressive tumour) to 5 (very aggressive tumour). Grade group 1 includes a Gleason score of 6 (3 + 3) and denotes a well-differentiated small and packed gland forming a circumscribed tumour mass. The grade group 2 is represented by a Gleason score of 7 (3 + 4) and is identified as similar to grade 1 with slight variation in the glands’ size and shape. Grade group 3 includes a Gleason score of 7 (4 + 3) and indicates a clear variation in shape and size compared to grade 2, with evidence of individual cells or tiny glands invading the stroma. Grade group 4 denotes a Gleason score of 8 (4 + 4) and is describes large, closely packed and clear tumour cells. Grade group 5 contains a Gleason score of 9 or 10 and references undifferentiated tumours and little or no gland formation (Delahunt et al., 2012; Epstein et al., 2015; Matoso & Epstein, 2016).
1.5 Steps of radical prostatectomy:

The treatment options for prostate cancer include radiotherapy, radical prostatectomy (RP), brachytherapy and active surveillance (Izadpanahi et al., 2014). However, RP is the first-choice treatment for prostate adenocarcinoma. According to a recent study by Covas and colleagues (Covas Moschovas et al., 2021), the surgical steps of robotic radical prostatectomy (RARP) are as follows:

- **Preoperative process:**

  The preoperative process for RARP includes a biopsy from the prostate with a pathological diagnosis of cancer, and sufficient imaging assessment, and determination of the stage of the tumour and the PSA value. Preoperative urinary continence and erectile function are evaluated with the American Urological Associations and Sexual Health Inventory for Men questionnaires, respectively.

- **Patient positioning:**

  During surgery, patients are placed under general anaesthesia and positioned in the Trendelenburg position with an angle of 26°.

- **Surgical steps:**

  1. Dropping of the urinary bladder and dissection of the anterior bladder neck are performed by using the vas deferens and pubic bone as landmarks.

  2. After the anterior dissection of the bladder neck, posterior dissection is performed to identify the seminal vesicles, which are then dissected and lifted.

  3. The prostate is elevated by the seminal vesicles, and a posterior dissection within Denonvilliers’ fascial layers is performed until the dorsal region of the prostate is identified. In addition, the neurovascular bundle and prostatic fascia are observed at this stage and can be preserved bilaterally in patients who meet the requirements for a nerve-sparing procedure.
4. Apical dissection is performed to preserve the greatest possible urethra length, periurethral tissue and prostatic anterior apical attachments.

5. Lymph nodes located anteromedially to the external iliac vein, and adjacent to the obturator nerve and vessels, are dissected in patients who meet the criteria for lymphadenectomy.

- **Post-surgical care:**
  Nonopioid drugs are used to control postsurgical pain if required. In addition, appointments are scheduled for patients 6 weeks after surgery and then at 3, 6, 9 and 12 months to evaluate PSA, erectile function and urinary continence.

- **Clinical outcomes of radical prostatectomy:**
  Despite improvements in surgical techniques and neurovascular sparing procedures, postsurgery problems frequently include loss of erectile function and continence with variable rates, due to damage to or resection of the neurovascular bundle (NVB) components proximal to or associated with the prostate (Sopko & Burnett, 2016).

A study by Ploussard and colleagues (Ploussard et al., 2011) has revealed that men who underwent bilateral preservation attained higher potency rates of 34.7% and 64.6% in the first and second years, respectively, than the 29.5% and 59.2% rates observed after the same period of time in men who underwent unilateral preservation. Another study in 77 patients who underwent non-nerve sparing, unilateral and bilateral nerve sparing laparoscopic radical prostatectomies has reported an overall potency rate of 49.3% 1 year post-surgery (Salomon et al., 2002). In addition, the erectile function rates among patients receiving non-nerve sparing, unilateral and bilateral sparing procedures had increased after the first year to 38.4%, 53.8% and 58.8%, as compared with 7.4%, 15.4% and 23.5% after the first month, respectively.
Surprisingly, Choi et al. (2011) reported that patients who underwent a unilateral nerve-sparing technique showed a better urinary continence rate than those who underwent bilateral nerve-sparing technique 4 months post-surgery, however, there were no significant differences in urinary recovery rate between the groups after a two-year follow up (Choi et al., 2011). However, Hinata et al. (2014) reported a significant difference in the urinary continence rates between patients who underwent bilateral, unilateral and non-nerve-sparing techniques one- and three-months post-surgery. Nevertheless, no significant difference was observed between these patients six months post-surgery (Hinata et al., 2014). The same authors reported that patients in the non-nerve-sparing group showed a better recovery rate than those in the unilateral group six months post-surgery.
1.6 Prostatic neurovascular bundle

Since the pioneering description of nerve sparing radical prostatectomy by Walsh and Donker (Walsh & Donker, 1982), it has been widely accepted that the nerves responsible for erectile function are located within the neurovascular bundle on the dorsolateral aspect of the prostate. However, this concept has recently been questioned, as several studies have reported extensive variation in the distribution of the nerves responsible for erectile function around the prostate. Tewari and colleagues reported three different group of nerves in the periprostatic area: the proximal neurovascular plate, located lateral to the seminal vesicle and the urinary bladder extending laterally to the prostatic base; the predominant neurovascular bundle, the classical bundle, located dorsolateral to the prostate containing the cavernous nerves; and the accessory neural pathways, which included additional nerve fibres around the prostate outside the NVB (Tewari et al., 2006). Notably, Takenaka and colleagues (Takenaka et al., 2004) described the NVB as having more hypogastric nerve fibres than pelvic splanchnic nerves at the bladder-prostate junction. The pelvic splanchnic nerves join the NVB distal to the bladder-prostate junction, in a spray-like distribution. In contrast, in a later study, it was reported that the NVB was located dorsolateral to the prostate, with a consistent straight course, proximal to distal, alongside the urethra (Takenaka et al., 2005).

In an investigation of 12 specimens, Tewari and colleagues (Tewari et al., 2003) identified several small nerve fibres, which were not part of the main NVB, located in the prostatic and Denonvillier’s fascia. These nerves ascended to the cavernous tissue, however, their precise function in generating an erection could not be identified. Moreover, two different further studies reported a significant number of nerves (20-30%) located at the ventral aspect of prostate (Eichelberg et al., 2007; Sievert et al., 2008). Moreover, another study noted the neurovascular bundle dorsolateral to the prostate in only 48% of the cases: in the other 52% of
the cases, the nerves had a spray-like distribution over the lateral and anterior aspects of the prostate, without forming a definite bundle (Kiyoshima et al., 2004)

Taken together, the location and shape of the prostatic neurovascular bundle remains widely debated, with extensive variation reported by previous studies. Undeniably, therefore, improved anatomical understanding of its location and distribution around the prostate is vital to minimize complications following a radical prostatectomy.
1.7 Nerve supply of the prostate

The inferior hypogastric plexus (IHP), or pelvic plexus, comprises nerve fibres from the superior hypogastric plexus and sacral plexus: they are the main source of innervation of pelvic structures including the prostate, urinary bladder, rectum, seminal vesicles, urethra, and penis.

The sympathetic and parasympathetic parts of the inferior hypogastric plexus are derived from spinal cord segments T11-L2 and S2-S4, respectively (Baader & Herrmann, 2003; Mauroy et al., 2003; Röthlisberger et al., 2018; Walsh & Donker, 1982) (Figure 1.3).

![Diagram](image)

Figure 1. 3: Schematic illustration (generated using Paint 3D and Wondershare EdrawMax softwares) showing a lateral view of male pelvis. It can be observed that the IHP runs alongside the rectum and gives branches to innervate the prostate from its dorsolateral aspect. Moreover, the cavernous nerves are derived from the most inferior part of IHP. B: bladder; R: rectum; IHP: inferior hypogastric plexus; PNs: prostatic nerves; PN: pudendal nerve; CNs: cavernous nerves. S: superior; A: anterior; I: inferior; P: posterior.
Several studies have investigated nerve distribution in normal and diseased prostates (Powell et al., 2005). A reduction of nerves in benign prostatic hyperplasia was first identified by Dunzendorfer and colleagues in 1976 (Dunzendorfer et al., 1976). A similar conclusion was reported by other researchers during their exploration of the distribution of nitric oxide synthase in the prostate (Bloch et al., 1997). These findings correlated with the observation of Powell and colleagues (Powell et al., 2005) who revealed that the area of innervation within the transitional zone was significantly greater than the one within the context of benign prostatic hyperplasia. Moreover, Ganzer and colleagues observed that the number of nerves in the dorsal and dorsolateral regions of the prostate was greater than in the anterior and anterolateral regions; they also observed that the number of nerves significantly increased in the apex in comparison to the body and base of the prostate (Ganzer et al., 2008). In a later study, it was reported that the majority of parasympathetic nerves were located in the dorsolateral aspect of the prostate, with only 1.5% being in the ventrolateral region, with its nerve density increasing in the apex. In contrast, sympathetic nerves were primarily located in the dorsolateral region, with 20% being present in the anterolateral aspect: its density decreased in the apex (Ganzer et al., 2012). Similarly, Costello and colleagues (Costello et al., 2011) confirmed that the density of the parasympathetic nerves increases in the apex, mainly located on the dorsolateral region, with only 17.8% being associated with the anterolateral aspect. On the other hand, the majority of the sympathetic nerves were identified in the dorsal and dorsolateral regions of the prostate, with 15% being in the ventrolateral region.

### 1.8 Cavernous nerves

Typically, the cavernous nerve originates from the caudal aspect of the pelvic plexus, which lies lateral to the rectum, close to the seminal vesical, running anteriorly on the lateral side of the prostate between its capsule and the lateral pelvic fascia (Figure 1.3). They contain
sympathetic fibres required for ejaculation and parasympathetic fibres for vasodilation and increasing blood flow to the penis (Röthlisberger et al., 2018; Walsh & Donker, 1982).

The cavernous nerves appear to have varied courses: some were observed to run through a narrow area between levator ani and the urethral rhabdosphincter, lateral to the membranous urethra, whereas others coursed anteromedially in the pararectal space to the penile hilum. (Stolzenburg et al., 2010; Takenaka et al., 2005). Throughout these courses, the cavernous nerves and the nerve to the rhabdosphincter were suspected to pass through the rectourethral muscle. Moreover, the cavernous nerves arising from the NVB penetrated the urogenital diaphragm just dorsolateral to the apex of the prostate. At this level, these nerves divided into several branches, which run on the posterolateral surface of the membranous urethra, posterior to the dorsal penile artery, to innervate the corpora cavernosa (Costello et al., 2004; Gillitzer & Thüroff, 2002; Walsh & Donker, 1982). Mauroy and colleagues (Mauroy et al., 2003) observed that the cavernous nerve crossed the pelvic floor anterior to the apex of the prostate and then ran on the superior surface of the cavernous body.

In summary, it is evident that several studies have revealed a wide range of complexity and variability in the peri- and intra-prostatic nerves. The presence of these variations are due to several factors, such as: (i) differences in the location, structure and size of the nerves around the prostate; (ii) inconsistency in the course of the prostatic and cavernous nerves (Takenaka et al., 2005); and (iii) different roles for the nerves around the prostate in the same patient (Tewari et al., 2006). Therefore, obtaining a comprehensive and detailed study of each category of nerves (sympathetic and parasympathetic) around and inside the gland is vital to limit complications arising from a radical prostatectomy.
1.9 Arterial supply of the prostate

According to leading anatomical textbooks (Moore, 2014; Standring, 2016) the prostate is mainly supplied by arteries arising directly from the internal iliac artery, or from its branches such as the inferior vesical, internal pudendal or middle rectal artery (Figure 1.4). However, several cadaveric and imaging-based studies have been undertaken to investigate the prostatic arteries, revealing a wide range of inconsistency in their origin, course and site of penetration.

![Schematic diagram showing the normal anatomy of the internal iliac artery and its branches in male pelvis.](image)

**Figure 1.4:** Schematic diagram (generated using Paint 3D and Wondershare EdrawMax softwares) showing the normal anatomy of the internal iliac artery and its branches in male pelvis. It can be observed that the umbilical artery and superior vesical artery share a common trunk as they originate from the IIA. Moreover, the prostatic artery is derived from the inferior vesical artery which shares a trunk with middle rectal artery. IIA: internal iliac artery; UA: umbilical artery; SVA: superior vesical artery; MRA: middle rectal artery; IVA: inferior vesical artery; PA: prostatic artery; IPA: internal pudendal artery. S: superior; A: anterior; I: inferior; P: posterior.
1.9.1 Origin of prostatic arteries

Several studies investigating prostatic arteries have been undertaken, using an array of different techniques, revealing considerable inconsistencies in the origin of prostatic arteries (Bilhim et al., 2011; Clegg, 1955; Garcia-Monaco et al., 2014; Moya et al., 2017) (Table 1.1). In all included studies, the prostatic arteries were noted to arise from the internal iliac artery or one of its branches; however, the most and least common sources remain controversial between studies. Recently, Anract et al. (2019) observed a single prostatic artery originating mainly from a common trunk supplying the bladder and the prostate in 55% (n=31) of individuals (Anract et al., 2019). In contrast, Bilhim and colleagues noted that the prostatic artery shared a common trunk with the middle rectal artery in 70% (n=56) of cases: this common trunk was named the prostatorectal trunk, which arose mainly from the internal pudendal artery in 58.9% (n=33) of cases (Bilhim et al., 2013). Moreover, in an investigation of 115 hemipelves, another recent study reported that single and double prostatic arteries were identified in 79.1% (n=91) and 20.9% (n=24) of cases, respectively, with the internal pudendal artery being the most common source of origin and the superior gluteal artery being the least (Xu et al., 2020).

One image-based study performing computed tomography angiography by Bilhim and colleagues (Bilhim et al., 2014) revealed an unexpected source of the prostatic artery as it was derived from the accessory obturator artery, which was either a branch of the inferior epigastric artery or the external iliac artery, with an incidence of 1.8% (n=9). Similarly, Xu and colleagues (Xu et al., 2020) reported that two prostatic arteries that were derived from the obturator artery, which itself arose from the external iliac artery. Another rare source had earlier been reported by Clegg (Clegg, 1955), in which 32.1% (n=9) of prostatic arteries were derived from the superior rectal artery.
None of previous cited studies reported genetics as a factor for the variability of prostatic arteries’ origins. They considered authors’ terminology, individual anatomical variability and different methodology designs as the main factors for the differences between studies. However, in a recent study, Fatma and colleagues (Eldem et al., 2021) who reported SVA as the main source of prostatic arteries with incidence of 36.1% (Table 1.1), suggested that the variation in the incidence of prostatic arteries in their study in comparison to previous studies may be a consequence of racial differences as they performed their study on Turkish patients.
<table>
<thead>
<tr>
<th>Study</th>
<th>Investigated cases</th>
<th>Age (years)</th>
<th>PAs No.</th>
<th>Type of investigation</th>
<th>Sources of prostatic artery % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Clegg, 1955)</td>
<td>21 hemipelves</td>
<td>Range: 36-64</td>
<td>28</td>
<td>Cadaveric dissection</td>
<td>Prostatovesical: 67.9 (19), SRA: 32.1(9)</td>
</tr>
<tr>
<td>(Bilhim et al., 2011)</td>
<td>50 hemipelves</td>
<td>Mean: 72.3</td>
<td>50</td>
<td>MACT/DSA</td>
<td>IPA: 56 (28), GPT: 28 (14), OA: 12 (6), IGA: 4 (2)</td>
</tr>
<tr>
<td>(Bilhim et al., 2012)</td>
<td>150 hemipelves</td>
<td>Mean: 66</td>
<td>214</td>
<td>CT/DSA</td>
<td>IPA: 34.1 (73), SVA: 20.1(43), GPT: 17.8 (38), OA: 12.6 (27), PRT: 8.2 (18), IGA: 3.7 (8), APA: 1.9 (4), SGA: 1.4 (3)</td>
</tr>
<tr>
<td>(Bilhim et al., 2013)</td>
<td>334 hemipelves</td>
<td>Mean: 64.7</td>
<td>56</td>
<td>CT angiography/DSA</td>
<td>IPA: 58.9 (33), IGA: 23.2 (13), GPT: 14.3 (8), OA: 3.6 (2)</td>
</tr>
<tr>
<td>(Garcia-Monaco et al., 2014)</td>
<td>36 hemipelves</td>
<td>Range: 35-68</td>
<td>46</td>
<td>Cadaveric dissection</td>
<td>IIA: 56.5 (26), MRA: 17.4 (8), IPA: 17.4 (8), OA: 4.3 (2), APA: 4.3 (2)</td>
</tr>
<tr>
<td>(Guodong et al., 2015)</td>
<td>55 patients</td>
<td>Mean: 65.1</td>
<td>114</td>
<td>Cone-beam CT/DSA</td>
<td>GPT: 39.5 (45), SVA: 32.6 (37), IPA: 27.9 (32)</td>
</tr>
<tr>
<td>(Bilhim et al., 2014)</td>
<td>9 patients</td>
<td>Mean: 62.9</td>
<td>9</td>
<td>CT Angiography</td>
<td>AOA: 100% (9)</td>
</tr>
<tr>
<td>(Moya et al., 2017)</td>
<td>10 hemipelves</td>
<td>Range: 62-87</td>
<td>10</td>
<td>Cadaveric</td>
<td>IIA: 10 (1), GPT: 30 (3), IPA: 40 (4), MRA: 20 (2)</td>
</tr>
<tr>
<td>(Wang et al., 2017)</td>
<td>148 patients</td>
<td>Mean: 70.5</td>
<td>318</td>
<td>Cone-beam CT/DSA</td>
<td>GPT+IVA: 37.1(118), IIA: 31.1(99), IPA:24.2 (77), OA: 4.7 (15), MRA: 2.8 (9)</td>
</tr>
<tr>
<td>(Xu et al., 2020)</td>
<td>115 hemipelves</td>
<td>N/R</td>
<td>139</td>
<td>Angiogram</td>
<td>IPA: 32.4 (45), SVA: 27.3 (38), OA: 20.1 (28), GPT: 15.1 (21), IGA: 2.2 (3), APA: 2.2 (3), SGA: 0.7 (1)</td>
</tr>
<tr>
<td>(Eldem et al., 2021)</td>
<td>119 hemipelves</td>
<td>Mean: 72.1</td>
<td>119</td>
<td>Angiogram</td>
<td>SVA: 36.1 (43), IPA:28.6 (34), OA: 18.5 (22), IIA: 10.9 (13), others: 5.9 (7)</td>
</tr>
</tbody>
</table>

Table 1: Summary of published studies examining the source(s) of prostatic arteries. MACT: multidetector Angio computed tomography scan; DSA: digital subtraction angiography; MA: multidetector angiography; CT: computed tomography scan; PAs: prostatic arteries; SRA: superior rectal artery; IPA: internal pudendal artery; GPT: gluteal-pudendal trunk; OA: obturator artery; IGA: inferior gluteal artery; APA: accessory pudendal artery; SVA: superior vesical artery; PRT: prostatorectal trunk; SGA: superior gluteal artery; IIA: internal iliac artery; MRA: middle rectal artery; AOA: accessory obturator artery; IVA: inferior vesical artery; N/R: not reported.
1.9.2 Course and location of prostatic arteries

The prostatic artery is a large vessel clearly identifiable between the base and mid-prostate, penetrating the gland on its ventrolateral aspect (Patel et al., 2011). Throughout their course, prostatic arteries give several branches which supply adjacent structures, such as the bladder, seminal vesical, rectum and pelvic floor (Bilhim et al., 2011; Clegg, 1955). Normally, the prostatic arteries are associated with a periprostatic venous plexus around the lateral aspect of the prostate (Benz et al., 2018): the arteries frequently anastomose with adjacent arteries such as rectal, corpus cavernous, vesical and internal pudendal arteries (Anract et al., 2019; Maclean et al., 2018; Wang et al., 2017).

In contrast to Clegg (Clegg, 1955), who emphasized that the prostatic arteries penetrated the gland from its ventrolateral surface in all cases, two imaging-based studies have shown that, at variable distances from their sources and before penetrating the prostate, the prostatic arteries divide into two branches (anterolateral and posterolateral) before reaching the gland. The anterolateral branch ascended anterosuperiorly and penetrated the prostate at the 2 o’clock position on the left side and at the 10 o’clock position on the right side and primarily supplied the central part of the prostate: the posterolateral branch mainly supplied peripheral aspects of the prostate, coursing posteroinferiorly to approach the prostate at the 7 o’clock and 5 o’clock positions on the right and left sides, respectively (Bilhim et al., 2011; Bilhim et al., 2012).

In line with anatomical variability of the prostatic arteries, Garcia-Monaco and colleagues identified two (superior and inferior) prostatic pedicles with constant course and distribution in all specimens investigated. As the superior pedicle approaching the prostate, it divided into lateral and medial prostatic arteries, with the medial branch supplying the superior part of the middle lobe and the lateral branch supplying the lateral lobe: the inferior prostatic pedicle
penetrated the prostate through the posteroinferior part of its lateral side and descended inferiorly to anastomose with the lateral branches of the superior prostatic arteries to supply the apex of the prostate (Garcia-Monaco et al., 2014).

### 1.9.3 Distribution and pattern of prostatic arteries

Several investigations have revealed a wide range of inconsistencies in prostatic artery distribution and patterns within pelvic sides (Table 1.2).

<table>
<thead>
<tr>
<th>Study</th>
<th>Investigated cases</th>
<th>Age (Years)</th>
<th>Type of study</th>
<th>PAs patterns in pelvic sides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Single % (n)</td>
</tr>
<tr>
<td>(Bilhim et al., 2012)</td>
<td>150 hemipelves</td>
<td>Mean: 66</td>
<td>CT/DSA</td>
<td>57 (86/150)</td>
</tr>
<tr>
<td>(Guodong et al., 2015)</td>
<td>110 hemipelves</td>
<td>Mean: 65.1</td>
<td>CT angiography/DSA</td>
<td>96.4 (106/110)</td>
</tr>
<tr>
<td>(Amouyal et al., 2018)</td>
<td>199 hemipelves</td>
<td>Mean: 70</td>
<td>Angiogram</td>
<td>72 (143/199)</td>
</tr>
<tr>
<td>(Demeritt et al., 2018)</td>
<td>72 hemipelves</td>
<td>Mean: 70.1</td>
<td>Angiogram</td>
<td>79.2 (57/72)</td>
</tr>
<tr>
<td>(Anract et al., 2019)</td>
<td>80 hemipelves</td>
<td>N/R</td>
<td>Angiogram</td>
<td>70 (56/80)</td>
</tr>
<tr>
<td>(Garcia-Monaco et al., 2019)</td>
<td>36 hemipelves</td>
<td>Range: 35-68</td>
<td>Cadaveric</td>
<td>77.8 (28/36)</td>
</tr>
<tr>
<td>(Wang et al., 2017)</td>
<td>296 hemipelves</td>
<td>Mean:70.5</td>
<td>Cone-beam CT/DSA</td>
<td>92.6 (274/296)</td>
</tr>
<tr>
<td>(Eldem et al., 2021)</td>
<td>119 hemipelves</td>
<td>Mean: 72.1</td>
<td>Angiogram</td>
<td>97.5 (116/119)</td>
</tr>
</tbody>
</table>

Table 1.2: Summary of published studies examining the distribution and patterns of prostatic arteries in pelvic sides. DSA: digital subtraction angiography; CT: computed tomography scan; PAs: prostatic arteries; N/R: not reported.
Taken together, the study of the literature revealed a wide range of complexity and variability in the arterial supply to the prostate, indicating a wide range of sources, courses and locations where they penetrate the prostate. Moreover, the previously cited studies lacked analysis of the distribution of blood vessels inside the gland, as well as any relationship to complications (erectile dysfunction and urinary incontinence) for patients following a radical prostatectomy. Thus, a comprehensive and detailed cadaveric and histological study of the blood vessels around and inside the prostate, as well as their correlation to patients’ outcomes post-surgery is required to minimize radical prostatectomy complications.
1.10 Blood supply of the penile corpora cavernosa

Anatomically, the penile corpora cavernosa are adjacent structure to the prostate, with many anatomical text books suggesting that it shares the same distribution of neurovascular supply as the prostate (Moore, 2014; Standring, 2016). Understanding the detailed blood supply of the penile corpora cavernosa, including the origin and course and relation to the prostate is essential to avoid damage during radical prostatectomy resulting in impairment of penile function.

1.10.1 Classification of arterial supply to the penile corpora cavernosa

The arterial supply of the penis is mainly derived from the internal pudendal artery which, having given its perineal branches, continues as the common penile artery before dividing into three terminal branches: bulbourethral, cavernous and dorsal arteries. These branches may be supplemented or entirely replaced by an accessory pudendal artery to supply the erectile bodies (Awad et al., 2011). Nevertheless, the arterial supply of the penis is variable in terms of the number of branches, their courses and anastomoses. Droupy et al. (1997) reported three patterns of arterial supply of the penis: Type I, the penis receives its blood supply from the internal pudendal artery only; Type II, it receives its blood supply from both the accessory and internal pudendal arteries, this being the most common (70%, n=14) of cases; and in Type III, the accessory pudendal artery is the main supply (Droupy et al., 1997). Following this classification, Thai and colleagues (Thai et al., 2015) were of the opinion the internal pudendal artery (Type I) was the main arterial supply of the penis, with an incidence of 51.4% (n=57). However, Nehra and colleagues (Nehra et al., 2008) contradicted this confirming that the accessory pudendal artery (Type III) was the dominant blood supply of the penis in 54% (n=15) of cases; while Breza et al., (1989) revealed that the accessory pudendal artery provided an
additional blood supply to the penis along with the internal pudendal artery (Type II) and was the most common with an incidence of 83% (n=5) (Breza et al., 1989).

1.10.2 Accessory pudendal artery prevalence, origin, course and types

The accessory pudendal artery is considered as one of the main branches that supply the penis, with several studies revealing a wide range of variability in terms of their prevalence, origin, course and types. The prevalence of the accessory pudendal artery varies among studies, ranging from 4% to 85% depending on the investigation method used (Table 1.3). The accessory pudendal artery is also known as either the apical accessory pudendal artery or the lateral accessory pudendal artery. Moreover, it can occur bilaterally or unilaterally, arising either directly from the internal iliac artery or from one of its branches, such as the inferior vesical, superior vesical or obturator artery (Allan et al., 2012; Box et al., 2010; Breza et al., 1989; Droupy et al., 1997; Droupy et al., 1999; Dubbelman et al., 2006; Gray et al., 1982; Matin, 2006; Mulhall et al., 2008; Nehra et al., 2008; Park et al., 2009; Polascik & Walsh, 1995; Rogers et al., 2004; Rosen et al., 1990; Secin et al., 2005; Secin et al., 2005; Thai et al., 2015; Whang et al., 2012). Anatomically, the accessory pudendal artery courses with the internal pudendal artery, lateral or anterolateral to the prostate, inferior to the pubic bone and superior to levator ani to supply the penis (Allan et al., 2012; Box et al., 2010; Matin, 2006; Park et al., 2009; Secin et al., 2005; Secin et al., 2005; Secin et al., 2007; Whang et al., 2012). However, Droupy and colleagues (Droupy et al., 1997) reported that the course of the accessory pudendal artery depends on its origin: when it arises from the inferior vesical artery, it passes along the ventrolateral surface of the prostate and is covered by the periprostatic venous plexus (Figure 1.5).
Figure 1. 5: Schematic diagram (generated using Paint 3D and Wondershare EdrawMax softwares) illustrating examples of the origin and course of the accessory pudendal artery in male pelvis. It can be observed in figure (A) that the APA is derived from the IVA and passes along the ventrolateral aspect of the prostate and joins the IPA to supply the penis. Moreover, figure (B) shows another pattern of APA as it originates directly for the IIA and courses lateral to the bladder to join the IPA to supply the penis. IIA: internal iliac artery; IVA: inferior vesical artery; APA: accessory pudendal artery; PA: prostatic artery; IPA: internal pudendal artery. S: superior; A: anterior; I: inferior; P: posterior.
<table>
<thead>
<tr>
<th>Study</th>
<th>Cases No.</th>
<th>Mean age (Years)</th>
<th>Type of investigation</th>
<th>Cases with APA in %</th>
<th>Total No. of APAs</th>
<th>Origins of APAs % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rosen et al., 1990)</td>
<td>195</td>
<td>34.5</td>
<td>Arteriography</td>
<td>7% (13)</td>
<td>13</td>
<td>N/R</td>
</tr>
<tr>
<td>(Curet et al., 1987)</td>
<td>9</td>
<td>29</td>
<td>Arteriography</td>
<td>33.3% (3)</td>
<td>3</td>
<td>N/R</td>
</tr>
<tr>
<td>(Polasek &amp; Walsh, 1995)</td>
<td>835</td>
<td>56.5</td>
<td>RRP</td>
<td>4% (33)</td>
<td>46</td>
<td>N/R</td>
</tr>
<tr>
<td>(Droupy et al., 1999)</td>
<td>12</td>
<td>60.6</td>
<td>Transrectal colour Doppler ultrasound</td>
<td>75% (9)</td>
<td>9</td>
<td>IVA: 78 (7), OA: 22 (2)</td>
</tr>
<tr>
<td>(Droupy et al., 1997)</td>
<td>20</td>
<td>72</td>
<td>Cadaveric Dissection</td>
<td>85% (17)</td>
<td>33</td>
<td>IVA: 46(15), OA: 36(12), EPA: 18(6)</td>
</tr>
<tr>
<td>(Rogers et al., 2004)</td>
<td>2399</td>
<td>54.1</td>
<td>RRP</td>
<td>4% (84)</td>
<td>52</td>
<td>N/R</td>
</tr>
<tr>
<td>(Secin et al., 2005)</td>
<td>325</td>
<td>59</td>
<td>Laparoscopic RP</td>
<td>30% (96)</td>
<td>125</td>
<td>N/R</td>
</tr>
<tr>
<td>(Secin et al., 2005)</td>
<td>285</td>
<td></td>
<td>Laparoscopic RP</td>
<td>25% (72)</td>
<td>92</td>
<td>N/R</td>
</tr>
<tr>
<td>(Matin, 2006)</td>
<td>70</td>
<td>54.5</td>
<td>Laparoscopic RP</td>
<td>25.7% (18)</td>
<td>23</td>
<td>IIA: 60.9 (14), OA: 26.1 (6), EIA: 13 (3)</td>
</tr>
<tr>
<td>(Nehra et al., 2008)</td>
<td>79</td>
<td>40</td>
<td>Pharmacacoangiograms</td>
<td>35% (28)</td>
<td>35</td>
<td>OA: 43 (15), IPA: 28 (10), IIA: 6 (2), IGA: 3 (1), not Identified: 20 (7)</td>
</tr>
<tr>
<td>(Park et al., 2009)</td>
<td>121</td>
<td>62</td>
<td>(MDCT) angiography</td>
<td>30% (36)</td>
<td>44</td>
<td>OA: 77 (34), IIA or one of its branches: 23 (10)</td>
</tr>
<tr>
<td>(Box et al., 2010)</td>
<td>200</td>
<td>61.7</td>
<td>RARP</td>
<td>40% (80)</td>
<td>80</td>
<td>N/R</td>
</tr>
<tr>
<td>(Brezza et al., 1989)</td>
<td>10</td>
<td></td>
<td>Cadaveric Dissection</td>
<td>70% (7)</td>
<td>8</td>
<td>OA: 50 (4), IVA: 37.5 (3), Contralateral SVA: 12.5 (1)</td>
</tr>
<tr>
<td>(Gray et al., 1982)</td>
<td>73</td>
<td>50.5</td>
<td>Arteriography</td>
<td>21% (15)</td>
<td>20</td>
<td>N/R</td>
</tr>
<tr>
<td>(Thai et al., 2015)</td>
<td>111</td>
<td>63.6</td>
<td>Contrast-enhanced MR angiography</td>
<td>48.6% (54)</td>
<td>74</td>
<td>OA: 47.3 (35), IVA: 43.2 (32), IEA: 9.5 (7)</td>
</tr>
<tr>
<td>(Allan et al., 2012)</td>
<td>15</td>
<td></td>
<td>Cadaveric Dissection</td>
<td>27% (4)</td>
<td>6</td>
<td>OA: 67 (4), IVA: 33 (2)</td>
</tr>
<tr>
<td>(Whang et al., 2012)</td>
<td>127</td>
<td>median: 64</td>
<td>contrast enhanced MRA (reader 1)</td>
<td>15.7% (20)</td>
<td>24</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>contrast enhanced MRA (reader 2)</td>
<td>18.1% (23)</td>
<td>28</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>contrast enhanced MRA (reader 3)</td>
<td>18.1% (23)</td>
<td>29</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RALP</td>
<td>12.6% (19)</td>
<td>19</td>
<td>N/R</td>
</tr>
</tbody>
</table>

Table 1.3: Summary of published studies examining types of APAs. APA: accessory pudendal artery; RP: radical prostatectomy; RRP: retropubic radical prostatectomy; MDCT: multidetector-row computed tomography scan; RARP: robotic-assisted radical prostatectomy; RALP: robotic-assisted laparoscopic prostatectomy; MRA: magnetic resonance angiography; N/R: not reported.
1.10.3 Arterial supply of the penile corpora cavernosa and its clinical importance

The correlation between the preservation of arterial supply of the corpora cavernosa and impotence after a nerve-sparing radical prostatectomy is clinically debatable (Droupy et al., 1999; Polascik & Walsh, 1995), with a number of studies having been conducted to examine the relationship to complications following radical prostatectomy.

Several studies, performed using a range of different techniques (open radical prostatectomy, Duplex ultrasonography, intracavernous injections), have confirmed that erectile dysfunction in patients following radical prostatectomy with a nerve-sparing technique may be due to injury/trauma of cavernosal arteries, resulting in insufficient blood supply to the corpora cavernosa (Aboseif et al., 1994; Bahnson & Catalona, 1988; Kawanishi et al., 2001; Kim et al., 1994; Oates et al., 1995; Zelefsky & Eid, 1998). Remarkably, based on age, stage, and neurovascular bundle status-matched analysis, patients with accessory pudendal artery preservation during radical prostatectomy were significantly less likely to report subsequent impotence (Kim et al., 1994). Similarly, Rogers and colleagues (Rogers et al., 2004) emphasized that potency recovery times for patients whose accessory pudendal artery was preserved during surgery were significantly faster than those for patients whose accessory pudendal artery was severed.

Several studies, however, have disagreed with the above findings reporting no significant relation between damage to the blood supply of the corpora cavernosa and complications after radical prostatectomy (Blander et al., 1999; Box et al., 2010; Polascik & Walsh, 1995; Williams et al., 2017). Moreover, there were no significant differences in recovery periods for erectile function after surgery between patients with sacrificed cavernosal arteries, patients whose cavernosal arteries were preserved, and those who did not have these arteries prior to the...
procedure (Blander et al., 1999; Box et al., 2010; Polascik & Walsh, 1995; Williams et al., 2017).

In summary, the anatomical description and clinical significance of the blood supply to the corpora cavernosa is controversial; nevertheless, these arteries could play a major role in the aetiology of impotence in patients undergoing radical prostatectomy. Preservation of these arteries during a radical prostatectomy is therefore of clinical importance. To understand these arteries properly, and thereby avoid radical prostatectomy complications, attaining a detailed and consistent overview of the corpora cavernosa and its neurovascular structures is vital.
1.11 Erectile dysfunction

The definition of erectile dysfunction following radical prostatectomy varies between studies. However, postsurgical erectile dysfunction is commonly described in a number of studies as failure to reach an erection sufficient for penetration (Berg et al., 2014; Kübler et al., 2007; Mickhail et al., 2007; Ploussard et al., 2011; Salomon et al., 2002; Stewart et al., 2011). Some studies consider patients as being impotent following surgery if their erection does not return to the baseline (Tewari et al., 2011), while others are of the opinion that patients are unable to achieve an erection (Katz et al., 2002). Several factors are correlated with the erectile function following radical prostatectomy including: patients’ age, prostate size, time elapsed since the operation, blood vessels injury/trauma, type of the surgical technique, and preservation of the cavernosal nerve (Hollenbeck et al., 2003; Mickhail et al., 2007; Mulhall et al., 2002; Van der Aa et al., 2003; Wiygul et al., 2005).

The potency rate in patients one-year post-surgery ranged from 15 – 87%, despite improvement in surgical techniques for radical prostatectomy (Berryhill et al., 2008; Ficarra et al., 2009; Raina et al., 2005). Using medication, such as sildenafil after surgery, can assist in improving erectile function in 43% to 80% of cases (Zippe et al., 2000). Several open and laparoscopic radical prostatectomies including bilateral, unilateral and non-nerve-sparing techniques, have been undertaken and have revealed a wide range of potency recovery rates post-surgery (Tables 1.4 & 1.5).
<table>
<thead>
<tr>
<th>Study</th>
<th>Surgery</th>
<th>Patients No.</th>
<th>Mean/ median age (years)</th>
<th>Type of NS technique (No.)</th>
<th>Follow up in months and potency recovery rates in % (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unspecified</td>
<td>BiNs</td>
</tr>
<tr>
<td>(Tal et al., 2009)</td>
<td>ORP</td>
<td>142</td>
<td>58</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>(Curet et al., 1987)</td>
<td>ORP</td>
<td>453</td>
<td>66</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>(Tewari et al., 2003)</td>
<td>ORP</td>
<td>100</td>
<td>63.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Tsujimura et al., 2004)</td>
<td>ORP</td>
<td>76</td>
<td>67</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>(Stanford et al., 2000)</td>
<td>ORP</td>
<td>1291</td>
<td>62.9</td>
<td>1291</td>
<td>0</td>
</tr>
<tr>
<td>(Rabbani et al., 2000)</td>
<td>ORP</td>
<td>314</td>
<td>60.5</td>
<td>0</td>
<td>181</td>
</tr>
<tr>
<td>(Walsh et al., 2000)</td>
<td>ORP</td>
<td>64</td>
<td>57</td>
<td>7</td>
<td>57</td>
</tr>
<tr>
<td>(Gralnek et al., 2000)</td>
<td>ORP</td>
<td>129</td>
<td>N/R</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Catalona et al., 1999)</td>
<td>ORP</td>
<td>1870</td>
<td>63</td>
<td>0</td>
<td>1610</td>
</tr>
<tr>
<td>(Quinlan et al., 1991)</td>
<td>ORP</td>
<td>503</td>
<td>59</td>
<td>0</td>
<td>291</td>
</tr>
</tbody>
</table>

Table 1.4: Summary of published studies examining erectile function in patients who underwent open radical prostatectomy with different types of nerve-sparing techniques. ORP: open radical prostatectomy; NS: nerves-sparing; Unspecified: unspecified nerve-sparing; UniNs: unilateral nerve-sparing; BiNs: bilateral nerve-sparing; Non: non-nerve sparing; N/R: not reported.
Table 1.5: Summary of published studies examining erectile function recovery in patients who underwent laparoscopic and robotic assisted radical prostatectomy with different types of nerve-sparing techniques. EERPE: endoscopic extraperitoneal radical prostatectomy; LRP: laparoscopic radical prostatectomy; RALP: robotic assisted laparoscopic prostatectomy; RARP: robotic assisted radical prostatectomy; NS: nerves sparing; Unspecified: unspecified nerve-sparing; UniNs: unilateral nerve-sparing; BiNs: bilateral nerve-sparing; Non: non-nerve sparing; N/R: not reported.

<table>
<thead>
<tr>
<th>Study</th>
<th>Surgery</th>
<th>Patents</th>
<th>Mean/median age (years)</th>
<th>Type of NS technique (No.)</th>
<th>Follow up in months and potency recovery rate in % (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unspecified BiNs UniNs Non</td>
<td>≤ 3 Months</td>
</tr>
<tr>
<td>(Stewart et al., 2011)</td>
<td>EERPE</td>
<td>228</td>
<td>63</td>
<td>102 0 0 126 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Shikanov et al., 2010)</td>
<td>RARP</td>
<td>1436</td>
<td>60</td>
<td>0 1021 322 93 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ploussard et al., 2011)</td>
<td>LRP</td>
<td>740</td>
<td>62.1</td>
<td>0 563 177 0 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Levinson et al., 2008)</td>
<td>LRP</td>
<td>313</td>
<td>56.1</td>
<td>0 226 17 10 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(Fode et al., 2014)</td>
<td>RALP</td>
<td>585</td>
<td>65</td>
<td>0 185 234 166 BiNs: 78 (156/200) Non: 21 (8/38)</td>
<td>N/R</td>
</tr>
<tr>
<td>(Tewari et al., 2003)</td>
<td>RARP</td>
<td>200</td>
<td>59.9</td>
<td>200 0 0 0 N/R</td>
<td>50 (100/200)</td>
</tr>
<tr>
<td>(Madeb et al., 2007)</td>
<td>RARP</td>
<td>55</td>
<td>60</td>
<td>0 45 6 4 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(Chien et al., 2005)</td>
<td>RALP</td>
<td>56</td>
<td>58.9</td>
<td>0 28 20 8 54 (30/56) 66 (37/56) 69 (39/56)</td>
<td>N/R</td>
</tr>
<tr>
<td>(Patel et al., 2007)</td>
<td>RARP</td>
<td>500</td>
<td>63.2</td>
<td>200 0 0 300 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(Zorn et al., 2007)</td>
<td>RALP</td>
<td>291</td>
<td>59.4</td>
<td>0 179 79 33 BiNs: 88 (161/213) UniNs: 59 (52/66)</td>
<td>N/R</td>
</tr>
<tr>
<td>(Bentas et al., 2003)</td>
<td>RARP</td>
<td>40</td>
<td>61.3</td>
<td>40 0 0 0 N/R</td>
<td>21 (8/38)</td>
</tr>
<tr>
<td>(Lavery et al., 2013)</td>
<td>RALP</td>
<td>352</td>
<td>58.5</td>
<td>14 338 0 0 N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(Joseph et al., 2005)</td>
<td>LRP</td>
<td>50</td>
<td>61.8</td>
<td>0 24 10 16 22 (11/50) N/R</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

47
1.12 Urine incontinence

The definition of urinary incontinence varies between studies; however, using at least one pad per day, which is a diaper prescribed for patients who suffer from urinary incontinence and defined as (absorbing incontinence aid) (Mun et al., 2021), following surgery is the most common description (Borchers et al., 2004; Gacci et al., 2011; Nandipati et al., 2007). The incidence of urinary incontinence differs widely following surgery, depending initially on the surgical technique used and secondly on how urinary incontinence is considered (Choi et al., 2011). Immediately post radical prostatectomy, almost all patients experience urinary incontinence, regardless of the surgical technique used; however, this is commonly resolved one-year after surgery (Ko et al., 2012). Several factors, such as body weight, patient age, dysfunction of the pelvic floor muscles, volume of the prostate, length of the membranous urethra and surgical procedure, may crucially affect the urinary continence after surgery, and could question that nerve preservation for retaining urinary continence being the only factor (Cambio & Evans, 2006; Coakley et al., 2002; Lee et al., 2010; Lee et al., 2013).

A wide range of open and laparoscopic radical prostatectomies have been undertaken performing of bilateral, unilateral and non-nerve sparing techniques and revealed a wide range of urinary continence recovery rates after surgery (Tables 1.6& 1.7).
<table>
<thead>
<tr>
<th>Study</th>
<th>Surgery</th>
<th>Patients No.</th>
<th>Mean/ Median of age (years)</th>
<th>Type of NS technique in No.</th>
<th>follow up in months and continence recovery rate in % (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unspecified  BiNs  UniNs  Non  ≤ 3 Months  ≤6 Months  ≤12 Months  ≤18 Months  ≥ 24 Months</td>
<td></td>
</tr>
<tr>
<td>(Gacci et al., 2011)</td>
<td>ORP</td>
<td>1972</td>
<td>65.2</td>
<td>0  689  266  1017  NS: 40 (382/955) Non: 26 (264/1017)</td>
<td>N/R  N/R  N/R  N/R</td>
</tr>
<tr>
<td>(Pick et al., 2011)</td>
<td>ORP</td>
<td>537</td>
<td>63</td>
<td>0  357  143  37  BiNs: 65.6 (233/355) UniNS: 64.5 (91/141) Non: 62.25 (23/37)</td>
<td>N/R  BiNs: 89.2 (296/332) UniNS: 88.9 (120/135) Non: 84.8 (28/33)</td>
</tr>
<tr>
<td>(Shikanov et al., 2010)</td>
<td>ORP</td>
<td>1436</td>
<td>60</td>
<td>0  1021  322  93  N/R  N/R  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Tzou et al., 2011)</td>
<td>ORP</td>
<td>235</td>
<td>63.4</td>
<td>0  73  112  50  BiNs: 65.6 (233/355) UniNS: 64.5 (91/141) Non: 62.25 (23/37)</td>
<td>N/R  BiNs: 855 (62/73) UniNS: 77 (86/112) Non: 84 (42/50)</td>
</tr>
<tr>
<td>(Tewari et al., 2009)</td>
<td>ORP</td>
<td>182</td>
<td>64.1</td>
<td>0  59  82  41  N/R  N/R  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Krambeck et al., 2009)</td>
<td>ORP</td>
<td>588</td>
<td>61</td>
<td>0  509  26  53  N/R  N/R  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Rocco et al., 2009)</td>
<td>ORP</td>
<td>240</td>
<td>63</td>
<td>240  0  0  0  63 (146/233) 83 (189/229) 88 (191/217)  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Tewari et al., 2009)</td>
<td>ORP</td>
<td>182</td>
<td>61.21</td>
<td>182  0  0  0  91.3 (166/182) 97.14 (177/182)  N/R  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Oefelein, 2004)</td>
<td>ORP</td>
<td>60</td>
<td>61</td>
<td>41  0  0  19  N/R  N/R  N/R  95 (57/60)  N/R</td>
<td></td>
</tr>
<tr>
<td>(Borchers et al., 2004)</td>
<td>ORP</td>
<td>80</td>
<td>62.2</td>
<td>38  0  0  42  N/R  N/R  NS: 76 (29/38) Non: 53 (22/42)  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Menon &amp; Tewari, 2003)</td>
<td>ORP</td>
<td>200</td>
<td>59.9</td>
<td>200  0  0  0  N/R  96% (196/200)  N/R  N/R  N/R  N/R</td>
<td></td>
</tr>
<tr>
<td>(Sebesta et al., 2002)</td>
<td>ORP</td>
<td>674</td>
<td>&lt; 65</td>
<td>674  0  0  0  N/R  N/R  N/R  68.1 (459/674)  N/R</td>
<td></td>
</tr>
<tr>
<td>(Catalona et al., 1999)</td>
<td>ORP</td>
<td>1870</td>
<td>63</td>
<td>0  1610  134  126  N/R  N/R  N/R  92 (1223/1325)  N/R</td>
<td></td>
</tr>
<tr>
<td>(Kleinhaus et al., 1999)</td>
<td>ORP</td>
<td>44</td>
<td>68</td>
<td>44  0  0  0  N/R  84.1 (37/44) 97.7 (43/44)  N/R  N/R</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. 6: Summary of published studies examining urinary continence recovery in patients who underwent open radical prostatectomy with different types of nerve-sparing techniques. ORP: open radical prostatectomy; NS: nerves-sparing; Unspecified: unspecified nerve-sparing; UniNs: unilateral nerve-sparing; BiNs: bilateral nerve-sparing; Non: non-nerve-sparing; N/R: not reported.
<table>
<thead>
<tr>
<th>Study</th>
<th>Surgery</th>
<th>Patients No.</th>
<th>Mean/media n age (years)</th>
<th>Type of NS technique in No.</th>
<th>Follow up in months and continence recovery rate in % (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≤ 3 Months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unspecified</td>
<td>BiNs</td>
</tr>
<tr>
<td>(Choi et al., 2011)</td>
<td>RARP</td>
<td>602</td>
<td>59.1</td>
<td>0</td>
<td>469</td>
</tr>
<tr>
<td>(Stewart et al., 2011)</td>
<td>EERPE</td>
<td>228</td>
<td>62.5</td>
<td>102</td>
<td>0</td>
</tr>
<tr>
<td>(Ko et al., 2012)</td>
<td>RARP</td>
<td>1,299</td>
<td>60.1</td>
<td>1299</td>
<td>0</td>
</tr>
<tr>
<td>(Lee et al., 2010)</td>
<td>RARP</td>
<td>88</td>
<td>59.2</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>(Rigatti et al., 2012)</td>
<td>RALP</td>
<td>48</td>
<td>65.5</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>(Hinata et al., 2014)</td>
<td>RARP</td>
<td>211</td>
<td>63.8</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>(Srivastava et al., 2013)</td>
<td>RALP</td>
<td>1417</td>
<td>60</td>
<td>1380</td>
<td>0</td>
</tr>
<tr>
<td>(Joseph et al., 2006)</td>
<td>RARP</td>
<td>325</td>
<td>60</td>
<td>325</td>
<td>0</td>
</tr>
<tr>
<td>(Mottrie et al., 2007)</td>
<td>RALP</td>
<td>184</td>
<td>62</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>(Krambeck et al., 2009)</td>
<td>RARP</td>
<td>294</td>
<td>61</td>
<td>0</td>
<td>221</td>
</tr>
<tr>
<td>(Murphy et al., 2009)</td>
<td>RALP</td>
<td>400</td>
<td>60.2</td>
<td>0</td>
<td>162</td>
</tr>
<tr>
<td>(Rocco et al., 2009)</td>
<td>RARP</td>
<td>120</td>
<td>63</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>(Patel et al., 2007)</td>
<td>RARP</td>
<td>500</td>
<td>63.2</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>(Zorn et al., 2007)</td>
<td>RALP</td>
<td>291</td>
<td>59.4</td>
<td>0</td>
<td>179</td>
</tr>
<tr>
<td>(van der Pool et al., 2009)</td>
<td>RARP</td>
<td>151</td>
<td>60</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>(Rozet et al., 2005)</td>
<td>LRP</td>
<td>599</td>
<td>62</td>
<td>599</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. 7: Summary of published studies examining urinary continence recovery in patients who underwent laparoscopic and robotic assisted radical prostatectomy with different types of nerve-sparing techniques. RARP: robotic assisted radical prostatectomy; LRP: laparoscopic radical prostatectomy; RALP, robotic assisted laparoscopic prostatectomy; EERPE: endoscopic extraperitoneal radical prostatectomy NS: nerves-sparing; UniNs: unilateral nerve-sparing; BiNs: bilateral nerve-sparing; Non: non-nerve-sparing; N/R: not reported.
In a comparison between patients who underwent open radical prostatectomy and robot-assisted radical prostatectomy, no significant differences were observed in urinary continence recovery rates one-year following surgery (Krambeck et al., 2009). Similarly, Stewart and colleagues (Stewart et al., 2011) revealed no significant difference in urinary continence recovery rates between patients who underwent nerve-sparing and non-nerve-sparing endoscopic extraperitoneal radical prostatectomy; however, those who underwent nerve-sparing endoscopic extraperitoneal radical prostatectomy appeared to achieve full continence recovery faster than the non-nerve-sparing group.

Tzou and colleagues (Tzou et al., 2009) reported that the rate of urinary control at one and two years follow-up post-surgery, irrespective of whether they had undergone bilateral, unilateral and non-nerve sparing procedures, was insignificant. Similarly, Pick and colleagues (Pick et al., 2011) revealed no significant differences in urinary control recovery rates at 1, 3 and 12 months follow-up between all patient groups. Talcott et al., (1997) reported that at three months follow-up, patients who had non-nerve-sparing procedure were significantly using fewer diapers than those who underwent nerve-sparing surgery: this difference was not present one-year after surgery (Talcott et al., 1997).
1.13 Aims of the project

Although approximately 40 years have passed since the pioneering description of nerve-sparing radical prostatectomy study by Walsh and Donker (Walsh & Donker, 1982), the precise anatomy of neurovascular structures supplying and surrounding the prostate remain uncertain, with urologists needing to take into account patient variability during prostatectomy in order to alter their dissection strategy.

Given the considerable limitations in previously cited studies regarding methods for investigating external and internal neurovascular structures, new comprehensive approaches were developed to examine these structures.

Thus, this project seeks to achieve the following aims:

1- Develop a new anatomical dissection approach to preserve the entire prostate in situ, facilitating reliable and consistent identification and tracing of the neurovascular structures supplying the prostate and corpora cavernosa

2- Provide a comprehensive and detailed histological overview of the location of the nerve and blood supply within the prostate

3- Examine the relationship between prostatic neurovascular structures and clinical outcomes following a radical prostatectomy
Chapter 2: Gross Dissection

2.1. Introduction

The main treatment option for adenocarcinoma of the prostate is surgical Radical Prostatectomy (RP) (Barazani et al., 2015; Kiyoshima et al., 2004), the aim of which is to achieve complete removal of the tumour. Decreased morbidity rates, as well as complications arising from surgery, have led to greater importance being placed on fulfilling the expectations of patients following surgery (Lunacek et al., 2005), particularly in relation to erectile dysfunction and urinary incontinence resulting from damage or resection of neurovascular bundle components proximal to, and/or associated with the prostate. A review of the existing literature in Chapter 1 has revealed a significant degree of uncertainty with regards to the detailed anatomy of nerve innervation and blood supply to the prostate and the corpora cavernosa, making it difficult for urologists to take patient variability into account during radical prostatectomy. The key to improving post-surgical complications is therefore identified as improved anatomical identification and understanding of the distribution, course and location penetration of periprostatic neurovascular structures. Thus, this section of the thesis addresses the following specific aim:

- Ensure reliable and consistent identification and tracing of the external neurovascular structures supplying the prostate and corpora cavernosa

In order to achieve this, an accurate and repeatable lateral dissection approach for the pelvis has been developed that leaves the prostate and its neurovascular structures intact. A considerable number of previous studies have relied on taking median sagittal sections of the pelvis to investigate the neurovascular supply to the prostate, thereby limiting their ability to accurately map the distribution of the neurovascular bundle around the prostate (Clarebrough et al., 2011; Lepor et al., 1985; Moya et al., 2017). The method used in this current study seeks
to overcome this issue, resulting in the ability to reliably trace these neurovascular structures. Moreover, our improved anatomical dissection approach provides a reliable description of the exact location of the external nerves and vessels of the prostate. Therefore, this description is beneficial for surgeons as it provides them with the opportunity to anticipate the location of these structures around the prostate to preserve them during RP as they perform some of the stages of the operation blindly as reported by Hoznek and colleagues (Hoznek et al., 2001).
2.2 Materials and methods

2.2.1 Specimens

A total of 24 hemipelvises obtained from 12 embalmed cadavers with an average age of 78.75 years (range from 61 to 94 years) were provided by Anatomy, University of Edinburgh, regulated by the Human Tissue (Scotland) Act 2006 guidelines. All donors have donated their body through a bequest program. As part of the process, donors should complete and sign the enclosed bequest form in the presence of a witness. In this form, there is an option to tick a box for taking images. Therefore, in this project, we only use donors who have bequeathed their body knowingly and that have willingly also given approval and consent for imaging of their remains for educational and research purposes. The anatomy act is enforced and regulated by regular inspections from HM Inspector of Anatomy in Scotland.

2.2.2 Gross dissection overview

A novel detailed dissection methodology was developed and successfully performed in 24 pelvic sides to identify the detailed gross anatomy of the external blood and nerve supply of the prostate (Figure 2.1). The purpose of this dissection was to establish a clear lateral view of the pelvic sides and preserve the entire prostate in situ, facilitating reliable and consistent identification and tracing of the external neurovascular structures supplying and surrounding the prostate. The following section of this thesis will provide a detailed overview of this dissection approach.
Figure 2. 1: Schematic diagrams (generated using Paint 3D and Wondershare EdrawMax softwares) illustrating the normal anatomy of the external nerves and blood supply of the prostate. In figure (A), it can be observed that prostatic nerves are derived from the IHP and reach the gland from its dorsolateral aspect. Figure (B) illustrates the normal anatomy of prostatic artery as it is derived from the inferior vesical artery which is a branch of the internal iliac artery. In normal anatomy, prostatic nerves and arteries join each other within the dorsolateral aspect of the prostate to form the neurovascular bundle. IIA: internal iliac artery; IVA: inferior vesical artery; UA: umbilical artery; PA: prostatic artery; IPA: internal pudendal artery; MRA: middle rectal artery; SVA: superior vesical artery; R: rectum; B: bladder; IHP: inferior hypogastric plexus; PNs: prostatic nerves; PN: pudendal nerve. S: superior; A: anterior; I: inferior; P: posterior.
2.2.3 Gross dissection stages

Given its relative size, compactness and complexity, any dissection of the pelvis needs to be performed cautiously to preserve important structures. To accomplish that, each side of the pelvis was dissected and analysed separately. Therefore, this new dissection approach required a considerable amount of effort and time compared to more standard, midline sagittal section approaches. The dissection procedure commenced by removing the skin, superficial and deep fascia of thighs and gluteal regions to expose the muscles of these areas (Figures 2.2 & 2.3). Anteriorly, at the region of the thigh, all adductor muscles located in the medial of the thigh were dissected away (i.e., the adductor magnus, adductor longus, adductor brevis and pectineus muscles) to expose the obturator externus muscle. Then, the obturator externus muscle was cut and the pubic bone completely cleared from all attached structures. The iliopsoas muscle was cut from its insertion, the lesser trochanter of the femur, and reflected superiorly and then cut to clear the iliac fossa (Figures 2.4, 2.5 & 2.6).

Figure 2.2: Image of one male pelvis used in the study. The first step involved removing the skin from the thigh and gluteal region to expose the muscles. S: superior; I: inferior; A: anterior; P: posterior.
Figure 2.3: An illustration of the thigh region following the removal of the skin and fasciae. The second step in the dissection involved removing these muscles to expose the bones. S: superior; I: inferior; A: anterior; P: posterior.
Figure 2. 4: Following the dissection of all the adductor muscles, the obturator externus (OE) was exposed. As shown in the image, the superior pubic ramus (SPR) was clear of all attachments and ready to be cut. In addition, the obturator nerve, artery and vein (2) could be identified as they course through the obturator canal (1). The schematic overview illustrates the superior pubic ramus and obturator canal in a skeletal pelvis. S: superior; I: inferior; L: lateral; M medial.
Figure 2. 5: Representative of the obturator canal, the superior pubic ramus (SPR) and the ischiopubic ramus (IPR) after the obturator externus muscle had been cut. The schematic image illustrates the superior pubic ramus and ischiopubic ramus in a skeletal pelvis. S: superior; I: inferior; L: lateral; M medial.
Figure 2. 6: A lateral view of the iliac fossa (IF). The iliopsoas muscle was cut to clear the way for the IF to be cut. It is possible to clearly observe the superior pubic ramus (SPR), obturator nerve (4), the internal and external iliac arteries (2,3) as they branch from the common iliac artery (1). The schematic overview presents the iliac fossa in a skeletal pelvis. S: superior; I: inferior; A: anterior; P: posterior.
Posteriorly, the hamstring muscles (biceps femoris, semitendinosus and semimembranosus) were dissected from their origin at the ischial tuberosity. The gluteus maximums, gluteus medius and gluteus minimus muscles were observed and removed to clear the gluteal surface of the ilium. The piriformis muscle was observed and dissected from its insertion and reflected medially to observe the greater sciatic foramen and its contents. The quadratus femoris and superior and inferior gemelli muscles were dissected to clear the ischial tuberosity and ischial spine (Figures 2.7 & 2.8).

Figure 2. 7: A posterior view of the gluteal region. The muscles in the gluteal region were removed to allow for the gluteal surface of the ilium (1) to be cut. At this stage of the dissection, it was possible to observe the gluteal region vessels and nerves (2) and the sciatic nerve (SN), which was reflected medially. The schematic overview presents a posterior view of the gluteal region in a skeletal pelvis. S: superior; I: inferior; A: anterior; P: posterior.
Figure 2.8: A posterior view of the gluteal surface of the ilium. This posterior view reveals that the gluteal surface of the ilium (GS) was cleared of all the muscles, nerves and vessels needing to be cut from the middle of the iliac crest downwards to reach the greater sciatic foramen (GSF). The schematic overview presents a posterior view of the gluteal region in a skeletal pelvis. S: superior; I: inferior; L: lateral; M: medial.
The relatively small size of the pelvis can lead to difficulties in identifying blood vessels and nerves reaching the prostate; therefore, after clearing the thigh and gluteal regions from all muscles, the lateral part of the pelvic bone was cut to establish a lateral exposure to the pelvic structures. Hence, the saw was employed to cut the superior pubic ramus and ischiopubic ramus until reaching the obturator foramen (Figures 2.9 & 2.10). After that, the saw was used to make a cut all the way through the ilium, starting from the middle of the lilac crest and cutting inferiorly through the iliac fossa until reaching the greater sciatic foramen (Figure 2.11). Finally, to detach the lateral side of the pelvis, the sacrospinous and sacrotuberous ligaments were cut (Figure 2.12).

Figure 2.9: An illustration of the superior pubic ramus. At this stage of the dissection, after the superior pubic ramus (SPR) had been cleared of all attachments, a saw was used to cut through it (as shown by the black dots) until the obturator foramen (OF) was reached. The schematic image illustrates the superior pubic ramus in a skeletal pelvis. S: superior; I: inferior; L: lateral; M medial.
Figure 2.10: Representative of the ischiopubic ramus. After the superior pubic ramus was cut according to the method shown in Figure 2.8, a saw was used to cut through the ischiopubic ramus (IPR) until the obturator foramen was reached (as demonstrated by the white dots). The schematic image shows the location of the ischiopubic ramus in a skeletal pelvis. S: superior; I: inferior; L: lateral; M medial.
Figure 2. 11: A view of the iliac fossa. After the superior pubic ramus and ischiopubic ramus had been cut, a saw was used to cut through the ilium from the middle of the iliac crest (IC) all the way down through the iliac fossa (IF) until the greater sciatic foramen (GSF) was reached (as shown by the white dots). The schematic image shows the location of the iliac fossa in a skeletal pelvis. S: superior; I: inferior; L: lateral; M medial.
Figure 2. 12: A posterior view shows the sacrotuberous ligament (STL), which was cut (as shown by the black dots) to help with detaching the lateral part of the pelvis. The schematic image illustrates the location of the sacrotuberous ligament in a skeletal pelvis. IT: ischial tuberosity. S: superior; I: inferior; L: lateral; M medial.
After removing the lateral side of the pelvic bone, the obturator internus muscle was exposed and reflected inferiorly to view the internal structures of the pelvis. This step provided a clear exposure of the pudendal canal as well as the fat that covers the prostatic neurovascular bundle (Figure 2.13). The fat and veins were removed to establish a clear view of the arterial and nerve supply to the prostate. Part of the rectum was then dissected to establish an improved exposure to the sacral roots to identify the origin of the pelvic plexus (Figures 2.14, 2.15 & 2.16).

Figure 2.13: A lateral view of a pelvis following the removal of its lateral bone. The obturator internus muscle (OI) was reflected inferiorly. In addition, it is clear from this view that fat covered the nerves and vessels supplying the prostate (P). From this view of the pelvic side, it is possible to identify the pudendal canal (PC), puboprostatic ligament (PPL) and sciatic nerve (SN). S: superior; I: inferior; A: anterior; P: posterior.
Figure 2. 14: A lateral view of a pelvis following the removal of its fat, which revealed the neurovascular supply of the prostate (P). From this view, it is possible to observe the urinary bladder (B) located superior to the prostate. The pudendal canal (PC) and the pubic bone (PB) are visible as well. IL: Ilium bone. S: superior; I: inferior; A: anterior; P: posterior.
Figure 2. A lateral view of a pelvis following the removal of the veins, which made it possible to identify the nerves and arteries that supply the prostate. This lateral view clearly shows the prostate (P) located inferior to the urinary bladder (B). In addition, the internal iliac artery (1) can be observed, as can some of its branches, such as the superior vesical artery (2) and middle rectal artery (3). Moreover, the internal pudendal artery and pudendal nerve can be observed (4). S: superior; I: inferior; A: anterior; P: posterior.
Figure 2. 16: A view of the sacral roots (S1, S2, S3 and S4) and sympathetic trunk (ST), which were studied to identify the exact origins of the nerves that form the pelvic plexus. The schematic overview shows the sacral roots in a skeletal pelvis. LS: Lumbosacral trunk; S: superior; I: inferior; A: anterior; P: posterior.
2.2.4 Nomenclature of arteries:

In his pioneering investigation of the prostatic artery, Clegg has reported that the lack of consistency in terminology for arteries leads to difficulties in assessing and comparing outcomes among studies investigating vascular anatomy. Therefore, the differences in nomenclature, rather than major anatomical variations, may explain the variations in these studies’ findings (Clegg, 1955).

Detailed anatomical understanding of the IIA and its branches is particularly crucial in medical practice (Mohammadbaigi et al., 2019). Two studies investigating IIA branches (Adachi, 1928) and (Yamaki et al., 1998) have identified only three main branches of the IIA: the superior gluteal artery, the inferior gluteal artery and the internal pudendal artery (IPA). However, Adahci and colleagues have reported that the umbilical artery is a terminal branch of the IIA in all cases, whereas Yamaki et al. (1998) have indicated that the umbilical artery may arise as a terminal branch of the IIA or a branch of the inferior gluteal artery, superior gluteal artery or IPA. In addition, a recent study (de Treigny et al., 2017) has confirmed the existence of the UA as the first branch of the anterior division of the IIA in 100% of cases.

The inferior vesical artery is commonly derived directly from the IIA, sharing a trunk with the MRA (Mohammadbaigi et al., 2019). However, owing to the different nomenclatures used, several studies have reported differing origins of the IVA. De Treigny and colleagues (de Treigny et al., 2017) have observed the IVA as a direct branch of the IIA in 72.7% of cases and as a branch of the UA artery in the remaining cases (27.3%). In addition, Assis and colleagues (Assis et al., 2015) have described the identification and catheterization of the IVA during the angiographic procedure as the most challenging and time-consuming step. According to their interpretation of the IVA, Assis and colleagues (Assis et al., 2015) have reported five different
patterns of its origin: I (28.7%), sharing a common trunk with the SVA; II (14.7%), a direct branch of the IIA; III (18.9%), a branch of the obturator artery; IV (31.1%), a branch of the IPA; and V (5.6%), less common sources, such as a branch of the inferior epigastric artery, a branch of the posterior division of the IIA or a branch of the APA.

According to several anatomical textbooks (Moore, 2014; Standring, 2016), the SVA is frequently derived from the anterior division of the IIA. In an investigation of the branches of IIA, Parsons and Keith (Parsons & Keith, 1896) have described the hypogastric trunk as a branch of the IIA before it undergoes anterior and posterior division. In addition, according to their description, the SVA originates from the hypogastric trunk in 75.9% of cases and from the anterior division of the IIA in 22.5% of cases, and it shares a common trunk with the MRA in 1.6% of cases. In a recent study, De Treigny and colleagues (de Treigny et al., 2017) have observed the SVA sharing a trunk with the UA in all investigated cases.

Clegg observed that prostatic arteries originated from a trunk named the prostato-vesical artery; however, this artery was derived from different arteries, with the gluteal pudendal trunk being the most common origin (52.9%, n=9) (Clegg, 1955). Two other image-based studies (Guodong et al., 2015; Wang et al., 2017) also reported the gluteal pudendal trunk as being the main origin of prostatic arteries (39.5%, n=45 and 37.1%, n=118, respectively). In contrast, four other studies observed the internal pudendal artery as the most common source of prostatic arteries (Bilhim et al., 2011; Bilhim et al., 2012; Maclean et al., 2018; Moya et al., 2017). Moreover, A cadaveric study by Garcia-Monaco and colleagues (Garcia-Monaco et al., 2014) observed prostatic arteries arising directly from the internal iliac artery with an incidence of 56.5% (n=26).
Taken together, the arterial supply within the pelvis, including prostatic arteries, is variable and complex in terms of their origin and course. In addition, these arteries may be named differently across research groups and publications. Therefore, it is essential to highlight that the description and terminology of arteries in this project are the author’s own interpretation of these structures.
2.3 Results

2.3.1 Blood supply of the prostate

The prostatic blood supply was investigated through gross dissection of 24 hemipelvises taken from 12 cadavers with an average age of 78.75 years. In total, 48 prostatic arteries were identified and observed to be derived either directly from the internal iliac artery or one of its branches, including the inferior vesical artery, the superior vesical artery and the middle rectal artery (Figure 2.17).

Figure 2. 17: Representative example of a prostatic artery revealed using the newly developed dissection approach. A lateral view of a left pelvic side, which provides an example of a prostatic artery (2) derived directly from the anterior division of the internal iliac artery (1). It was observed that 3 cm from its origin, the prostatic trunk divided into two branches to penetrate the gland from its base and dorsolateral aspects. P: prostate; B: urinary bladder; R: rectum. A: anterior; I: inferior; P: posterior; S: superior.
In 87.5% of the cases (21 sides), prostatic arteries were derived from a single source, whereas in the remaining 12.5% (three sides), they arose from two different arteries. No statistically significant variations were noted between the right and left sides of the pelvis regarding the number of prostatic arteries, as 54% (n = 26) were observed on the right side and 46% (n = 22) on the left side (Figure 2.18). The inferior vesical artery was the most common source of the prostatic artery on the right side (n = 11), and the internal iliac artery appeared to be the main source of prostatic arterial branches on the left side (n = 14).

The diameter of arteries was measured manually using an electronic digital vernier caliper. To ensure the consistency, the measurements were taken at the same location from all arteries, at the region of their origin. Regardless of source, no significant differences were observed between prostatic arteries regarding their diameter (P = 0.40; (Figure 2.19)). Prostatic arteries supplied the gland as a single branch in 21% of the hemipelvises (n = 5), two branches in 58% of the sides (n = 14) and three branches in 21% of cases (n = 5). Furthermore, these arteries penetrated the gland from its dorsolateral aspect within the neurovascular bundle in 63% of cases (n = 30), the base of the prostate in 23% (n = 11), the dorsal aspect of the prostate in 8% (n = 4), the ventral region of the prostate in 4% (n = 2) and the apex of the gland in 2% (n = 1) (Table 2.1 and Figures 2.20, 2.21, 2.22, 2.23 & 2.24).

<table>
<thead>
<tr>
<th>PAs sources</th>
<th>Total Sides (PAs No.)</th>
<th>Right sides (PAs No.)</th>
<th>Left sides (PAs No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal iliac artery</td>
<td>11 (20), 42%</td>
<td>4 (6), 23%</td>
<td>7 (14), 64%</td>
</tr>
<tr>
<td>Inferior vesical artery</td>
<td>7 (15), 31%</td>
<td>5 (11), 42%</td>
<td>2 (4), 18%</td>
</tr>
<tr>
<td>Superior vesical artery</td>
<td>5 (9), 19%</td>
<td>3 (5), 19%</td>
<td>2 (4), 18%</td>
</tr>
<tr>
<td>Middle rectal artery</td>
<td>4 (4), 8%</td>
<td>4 (4), 16%</td>
<td>0 (0), 0%</td>
</tr>
</tbody>
</table>

Table 2.1: The prostatic arteries originated from four main sources in specimens examined in the current study. No statistically significant differences were identified between the right and left sides in terms of the numbers of prostatic arteries. As shown in the table, the most common source of the prostatic arteries among all of the investigated sides was the internal iliac artery. The inferior vesical artery was the main source of the prostatic arteries within the right sides. Moreover, the middle rectal arteries were the least common source of the prostatic arteries in both sides. PAs: prostatic arteries; No.: number.
Figure 2.18: Bar chart summarising the number prostatic arteries in the left and right pelvic sides of the examined cases, as no statistically significant differences were observed applying unpaired t-tests. The bars in the chart indicate the mean, while the error bars show the standard deviation. The dots in the charts refer to the number of pelvises (n=24). PAs: prostatic arteries.
Figure 2.19: Bar chart revealing consistent diameter of the prostatic artery. Indeed, regardless of their source, no statistically significant differences were observed between the prostatic arteries in terms of their diameters applying ordinary one-way ANOVA and Tukey’s multiple tests. The bars in the chart indicate the mean, while the error bars show the standard error of the mean. The dots in the charts refer to number of prostatic arteries from each source. PAs: prostatic arteries; IVA: inferior vesical artery; MRA: middle rectal artery; IIA: internal iliac artery; SVA: superior vesical artery.
Figure 2.20: A lateral view of a left pelvic side, which provides an example of two prostatic arteries derived from the superior vesical artery (SVA). It can be observed that the SVA shared a trunk with the umbilical artery (UA) as they both originated from the internal iliac artery. After its origin, the SVA divided into two prostatic branches that penetrated the gland from its base and dorsolateral aspects. The prostate (P); the urinary bladder (B); the rectum (R); the seminal vesicle (S). A: anterior; I: inferior; P: posterior; S: superior.
Figure 2.21: An illustration of an inferior vesical artery. This lateral view of a right pelvic side shows the prostate (P) located inferior to the urinary bladder (B). In this image, it is possible to observe that the inferior vesical artery (IVA) originated from the internal pudendal artery (IPA), which was a branch of the internal iliac artery (IIA). Before reaching the gland, the IVA divided into two branches: one supplied the seminal vesicle (S) and the other penetrated the prostate from its dorsolateral aspect (BLACK STAR). A: anterior; I: inferior; P: posterior; S: superior.
Figure 2.22: Prostatic arteries were observed to be derived from four sources within the investigated cases. The internal iliac artery was the main source of the prostatic arteries (accounting for 42% of the total arteries), followed by the inferior vesical artery, which was the source of 31% of the prostatic arteries.
Figure 2.23: The number of prostatic arteries varied among the sides. In 58% of the examined cases, the prostate received blood from two branches. Yet, three branches of prostatic arteries were observed in 21% of the examined cases. Moreover, the prostate received blood from one branch in 21% of pelvic sides.
Figure 2. 24: The prostatic arteries penetrated the gland from different aspects. The majority of the identified prostatic arteries penetrated the gland from its dorsolateral aspect (DL) within the neurovascular bundle, while the second most common region for the prostatic arteries to reach the gland was at its base inferior to the urinary bladder. Regions such as the dorsal, ventral and apex regions of the gland were penetrated by the prostatic arteries in 8%, 4% and 2% of the examined cases, respectively.

2.3.1.1 The internal iliac artery

Running anterior to the sacroiliac joint, the common iliac artery bifurcates, giving rise to the internal and external iliac arteries. On average, in the specimens examined in this study, the internal iliac artery measured 4.46 cm in length and 7.7 mm in diameter (Table 2.2). Before it reaches the larger sciatic foramen, it splits into anterior and posterior divisions. The internal iliac artery was the most prevalent source of prostatic arteries, accounting for 42% of all the arteries detected (n = 20). Moreover, the internal iliac artery was the main source of prostatic arteries on the left sides, with 64% (n=14) of identified prostatic arteries. In 46% of the sides (n = 11), a direct prostatic artery with an average diameter of 2.47 mm originated from the anterior division of the internal iliac artery supplying the prostate. In one example, the prostatic
artery arose from the internal iliac artery as a trunk, and then 3 cm from its origin it divided into three branches. These branches penetrated the gland from its base and dorsolateral aspect. On another specimen, one prostatic artery originated from the lateral side of the internal iliac artery, passing away from the neurovascular bundle and close to the pelvic wall. It was joined by two other arteries from the lateral side (which had been cut and so could not be identified) and reached the prostate at its base, inferior to the urinary bladder (Figures 2.25 & 2.26).

<table>
<thead>
<tr>
<th></th>
<th>IIA</th>
<th>Direct prostatic trunk</th>
<th>PAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter mean (mm)</td>
<td>7.7</td>
<td>2.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.94</td>
<td>0.34</td>
<td>0.29</td>
</tr>
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Table 2.2: The average diameters of internal iliac arteries (IIA), their direct prostatic trunks and prostatic arteries (PAs) from specimens examined in the current study.

Figure 2.25: Representative lateral view of a right pelvic side revealing a prostatic artery (1) that originates directly from the anterior division of the internal iliac artery. It can be observed that after its origin, the prostatic trunk splits into three branches, which penetrate the prostate (P) from the base, superior and inferior regions of its dorsolateral aspect. B: Urinary bladder. A: anterior; I: inferior; P: posterior; S: superior.
Figure 2. 26: Representative example of a prostatic artery arising directly from the internal iliac artery. This lateral view of a left pelvic side shows the prostate (P) to be located inferior to the urinary bladder (B) and anterior to the rectum (R). From this view, it is possible to observe a direct branch from the internal iliac artery (1) courses all the way through the pelvis until it reaches the prostate from its base aspect inferior to the urinary bladder. A: anterior; I: inferior; P: posterior; S: superior.
2.3.1.2 Inferior vesical artery

On 29% of the examined sides (n = 7), the inferior vesical artery was the source of the prostatic arteries, accounting for 31% (n = 15) of the identified prostatic vessels. In addition, it was the most common source of prostatic arteries on the right side of the pelvis, accounting for 42% (n = 11). The inferior vesical arteries originate directly from the anterior division of the internal iliac artery or share a trunk with one of the internal iliac branches with an average diameter of 2.6 mm (Table 2.3). The inferior vesical arteries were observed to bifurcate into prostatic branches, at an average of 3.5 cm from their source, to supply the gland. In addition to the prostate, branches of the inferior vesical arteries supply the seminal vesicle (Figure 2.27).

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<tr>
<th></th>
<th>IVA</th>
<th>PAs</th>
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<tbody>
<tr>
<td>Diameter mean (mm)</td>
<td>2.6</td>
<td>1.03</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.46</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Table 2.3: The average diameters of inferior vesical arteries (IVA) and their prostatic arteries (PAs) in specimens examined in the current study.
Figure 2.27: Representative lateral view of a right pelvic side shows the inferior vesical artery (2) originating from the internal iliac artery (1). It can be observed that the inferior vesical artery divides into two branches: the prostatic artery (3) and the artery that supplies the seminal vesicle (4). In addition, the prostatic artery splits into two branches before reaching the gland and then penetrate it from its dorsolateral region and its base. P: the prostate; B: the urinary bladder; R: the rectum. PB: Pubic bone A: anterior; I: inferior; P: posterior; S: superior.
2.3.1.3 The superior vesical artery

The superior vesical artery was observed to be a branch of the anterior division of the internal iliac artery, with an average diameter of 3.26 mm (Table 2.4). The superior vesical artery provided 19% of the prostatic arteries identified (n = 9) within 21% of the investigated sides (n = 5). The superior vesical artery shares a common trunk with the umbilical artery in all cases, traversing the wall of the pelvis, before running medially towards the urinary bladder. In one of the cases, at 3.5 cm from its origin, the superior vesical artery split into three prostatic branches that penetrated the gland from its base, middle and inferior portions of the dorsolateral aspect. In another example, the superior vesical artery provided the prostate with two arterial branches, penetrating the gland from its dorsal and anterior aspects (Figures 2.28 & 2.29).

<table>
<thead>
<tr>
<th>SVA</th>
<th>PAs</th>
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<tbody>
<tr>
<td>Diameter mean (mm)</td>
<td>3.26</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 2.4: The average diameters of superior vesical arteries (SVA) and their prostatic arteries (PAs) in specimens examined in the current study.
Figure 2.8: Representative example of prostatic arteries arising from the superior vesical artery. This lateral view of a left pelvic side shows two branches of the internal iliac artery. UA: the umbilical artery coursing toward the urinary bladder; SVA: the superior vesical artery bifurcating into three prostatic arteries; WHITE STARS. The three prostatic arteries (as branches of the superior vesical artery) penetrated the prostate from its base and dorsolateral aspects. A: anterior; I: inferior; P: posterior; S: superior.
Figure 2. 29: Representative example of prostatic arteries originating from the superior vesical artery. This lateral view of a left pelvic side shows the location of the prostate (P) inferior to the urinary bladder (B) and anterior to the rectum (R). It can be observed that the superior vesical artery (SVA) shared a trunk with the umbilical artery (UA) as they both originated from the internal iliac artery. After its origin, the SVA divided into two prostatic branches that penetrated the gland from its dorsal and anterior regions. A: anterior; I: inferior; P: posterior; S: superior.
2.3.1.4 The middle rectal artery

The middle rectal artery was identified in 75% of specimens (n = 18) examined, with an average diameter of 2.2 mm (Table 2.5). In all cases, it originated from the anterior division of the internal iliac artery and coursed inferiorly to supply the rectum by penetrating it with several small branches. In 22% of the sides (n = 4), the middle rectal artery was observed to be the source of prostatic arteries. Moreover, in all cases, the middle rectal artery provided the prostate with only one branch, which penetrated it from its dorsal aspect anterior to the rectum (Figure 2.30).

<table>
<thead>
<tr>
<th></th>
<th>MRA</th>
<th>PAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter mean (mm)</td>
<td>2.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.21</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2.5: The average diameter of the middle rectal artery (MRA) and their prostatic arteries (PAs) in specimens examined in the current study.
Figure 2. Lateral view of a right pelvic side revealing the prostatic artery arising from the middle rectal artery. It can be noted that the middle rectal artery (2) originated from the anterior division of the internal iliac artery (1). Moreover, the middle rectal artery coursed inferiorly toward the rectum (R) and provided a prostatic branch (3) to supply the gland from its dorsal aspect anterior to the rectum. B: urinary bladder; A: anterior; I: inferior; P: posterior; S: superior.
2.3.2 Blood vessels of the corpora cavernosa

Anatomically, the penile corpora cavernosa are adjacent structure to the prostate, and many anatomical textbooks (Moore, 2014; Standring, 2016) suggest it shares the same neurovascular supply with the prostate or at least, the transition of its neurovascular supply passes adjacent to the prostate. Therefore, understanding the blood supply of the penile corpora cavernosa in terms of its origin and course in relation to the prostate is essential, because damage to these vessels during a radical prostatectomy could impair the function of the penis. Using the dissection approach detailed above, the neurovascular supply of the corpora cavernosa remains intact, allowing investigation of its origin, course and location of penetration.

The arterial supply to the corpora cavernosa was mainly derived from the internal pudendal artery and the accessory pudendal artery. The internal pudendal artery, which is a branch of the anterior division of the internal iliac artery, was the only source for the cavernosal artery in 92% of the examined sides (n = 22). The mean diameter of the internal pudendal artery was 4.5 mm. The internal pudendal artery runs inferior to the piriformis muscle, entering the gluteal region within the greater sciatic foramen. After this, it courses around the ischial spine entering the pelvis through the lesser sciatic foramen. After it re-enters the pelvis, the internal pudendal artery joins the pudendal nerve and the internal pudendal vein within the pudendal canal. Along its course, the internal pudendal artery provides branches to the bulbourethral artery, cavernous artery and dorsal penile artery (Figures 2.3.1). However, in 8% (n = 2) of the sides, the internal pudendal artery was joined by an accessory pudendal artery, which also supplies the corpora cavernosa. These accessory pudendal arteries were found on two sides of the same specimen, branched directly from the internal iliac artery. Interestingly, the prostatic arteries in these sides were received from the same source (Figure 2.3.2).
Figure 2. 31: Representative lateral view of a left pelvic side revealing the blood vessels of the corpora cavernosa. The internal pudendal artery (A) originated from the anterior division of the internal iliac artery. From this view, it can be observed that the internal pudendal artery splits into three branches: 1: bulbourethral artery, 2: cavernous artery, and 3: dorsal penile. P: prostate; B urinary bladder; PE: penis; A: anterior; I: inferior; P: posterior; S: superior.
Figure 2.32: Representative example of an accessory pudendal artery. A lateral view of a left pelvic side revealing the prostate (P), the penis (PE) and the rectum (R). A direct branch (2) was noted to be derived from the internal iliac artery (1), which provided a branch to supply the prostate from its dorsolateral aspect (3) and coursed as an accessory pudendal artery with a branch of the bulbourethral artery (5) and a branch to supply the corpora cavernosa (4). A: anterior; I: inferior; P: posterior; S: superior.
2.3.3 Pelvic plexus

In addition to the blood vessels supplying the prostate and corpora cavernosa, the newly developed dissection technique described in this study provided the opportunity to obtain a clear lateral view of the nerves surrounding and supplying the prostate, without destroying them. The nerves of the prostate and corpora cavernosa were therefore traced from their origin to the location at which they penetrated their target structures.

The inferior hypogastric plexus (pelvic plexus) was identified in all the examined hemipelvises. The nerves that formed the pelvic plexus were derived from the pelvic splanchnic nerves of the sacral roots S2, S3 and S4 and the superior hypogastric plexus. In all cases, the pelvic plexus ran for an average of 4.5 cm along the lateral side of the rectum until reaching the prostate. Once it reached the prostate, it formed the prostatic plexus and joined the blood vessels supplying the gland to form the neurovascular bundle. Throughout its course, the pelvic plexus yields multiple branches to innervate both the seminal vesicle and the urinary bladder. In addition, the most inferior part of the pelvic plexus provides branches that run between the prostate and the rectum to the cavernous nerve, and the nerve to the internal urethral sphincter muscle (Figure 2.33).
Figure 2.33: Representative lateral view of a right pelvic side clearly showing the pelvic plexus (1), passing alongside the lateral aspect of the rectum (R) to reach the prostate (P). It can be observed that the most inferior part of the pelvic plexus provided a branch of the cavernous nerve (WHITE STAR) that passed within the dorsolateral aspect of the gland toward its apex in order to leave the pelvis to innervate the cavernous bodies. In addition, the pudendal nerve and internal pudendal artery (2) are clearly visible coursing toward the penis. (B): the urinary bladder; A: anterior; I: inferior; P: posterior; S: superior.
2.3.4 Nerves of the prostate and corpora cavernosa

The nerves of the prostate were observed to be derived from the pelvic plexus in all cases. However, the location of their penetration within the gland differed. It was noted that the dorsolateral aspect of the gland was the most common site of penetration, to receive nerves in all the sides examined (n = 24). In addition, the prostate was found to be innervated within its base inferior to the bladder, with branches to the anterior surface of the gland in 79% (n = 19) of cases, and within its dorsal regions with branches to the apex of the prostate in 87.5% (n = 21) of cases. The prostatic nerves joined the prostatic blood supply to form the neurovascular bundle within the prostatic dorsolateral aspect. It was noted that the cavernous nerve was derived from the inferior part of the neurovascular bundle in all cases. The cavernosal nerves ran along the dorsolateral side of the prostate anterior to the rectum and left the pelvis to supply the cavernous bodies through the urogenital diaphragm adjacent to the prostatic apex (Figure 2.34).
Figure 2. Lateral view of a left pelvic side illustrating the prostatic and cavernous nerves. The pelvic plexus (1) passed within the lateral side of the rectum (R) to reach the prostate (P). It can be noted that the prostatic nerves penetrated the gland from its dorsolateral aspect. In addition, it can also be observed that the most inferior part of the pelvic plexus provided a branch of the cavernous nerve (WHITE STAR) that passed within the dorsolateral aspect of the gland anterior to the rectum toward the prostatic apex in order to leave the pelvis through the urogenital diaphragm and innervate the cavernous bodies. (B): the urinary bladder; (S) the seminal vesicle, (PE) the penis. A: anterior; I: inferior; P: posterior; S: superior.
2.3.5 The pudendal nerve

The pudendal nerve was derived from the anterior division of sacral roots S2, S3 and S4 in all instances. This origin was expected as it agrees with the description of one of the leading anatomical textbooks (Standring, 2016). The pudendal nerve ran to the gluteal region inferior to the piriformis muscle. It then passed through the lesser sciatic foramen, exiting the gluteal region to join the internal pudendal artery and vein in the pudendal canal. In all the investigated cases, the pudendal nerves gave branches off to the inferior rectal nerve and then split into two terminal branches, i.e., to the perineal nerves, which supply the external urethral sphincter muscle, and the dorsal nerve of the penis. The dorsal nerves of the penis course superior to the internal pudendal artery as they penetrate the penis, whereas the perineal nerves run inferior to it (Figure 2.35).
Figure 2. 35: Representative lateral view of a right pelvic side showing the pudendal nerve and its branches. It can be noted that the pudendal nerve splits into two terminal branches: (1) the dorsal nerve of the penis ran toward the penis and (2) the perineal nerve innervated the external urethral sphincter muscle. In addition, the internal pudendal artery (3) was observed coursing between the two branches of the pudendal nerve. (P): the prostate; (B): the urinary bladder; (PE) the penis. A: anterior; I: inferior; P: posterior; S: superior.
2.4 Discussion

This chapter provides a comprehensive and detailed description of the external nerves and blood vessels supplying the prostate utilizing a newly developed dissection approach. Until the 1980s, it was not known whether RP damaged the neural and/or vascular supplies to the corpora cavernosa. In 1982, Walsh and Donker hypothesized that the nerves were being damaged inadvertently (Walsh & Donker, 1982). Originally, it was believed that preservation of the dorsolateral NVBs was essential for erectogenic function, but subsequently an array of modern anatomical studies revealed that periprostatic nerve distribution is highly variable and often erratic, with up to 25% of nerves found anterolaterally to the prostatic capsule (Eichelberg et al., 2007; Ganzer et al., 2008; Ganzer et al., 2009; Lee et al., 2008). Therefore, better anatomical identification and understanding of the distribution and course of periprostatic neurovascular structures is vital to improve post-surgical outcomes.

The study of literature revealed that different assessment approaches as well as considerable anatomical variations in specimens and patients and authors’ terminology have led to extensive complexity and variability in the findings of previous studies regarding the external nerves and blood supply to the prostate. However, in the current study, a new dissection approach was developed (and successfully repeated on 24 pelvic sides), establishing a clear lateral view of the prostate and its external neurovascular structures. Moreover, it is vital to highlight that the description and terminology of arteries in this study are the author’s own interpretation of these structures. Therefore, considering the findings of this study, the nomenclature in relation to pelvic arteries may now require more clarification of their description.
In total, 48 prostatic arteries were identified and found to be derived from four distinct sources. The internal iliac artery was the most common source of prostatic arteries at 42% (n = 20), followed by the inferior and superior vesical arteries at 31% (n = 15) and 21% (n = 5), respectively. The middle rectal artery was the least common source of prostatic arteries, at 8% (n = 4). In his pioneering investigation of the prostatic artery, Clegg (Clegg, 1955) noted that prostatic arteries were derived in all cases from an obvious trunk, the prostatic-vesical artery. However, this prostatic-vesical artery itself was derived from multiple sources. Two imaging-based investigations (Guodong et al., 2015; Wang et al., 2017) reported the gluteal pudendal trunk as the principal origin of prostatic arteries in 39.5% (n = 45/114) and 37.1% (n = 118/318), respectively. Moreover, in our study, the prostatic arteries were not observed to be derived from the internal pudendal artery, which was the most common source of prostatic arteries in four other studies, with incidences of 56% (n = 28/50) (Bilhim et al., 2011), 34.1% (n = 73/214) (Bilhim et al., 2012), 29.3% (n = 17/58) (Moya et al., 2017), and 36.4% (n = 78/214) (Maclean et al., 2018). Moreover, one cadaveric-based investigation observed the prostatic arteries to be mainly derived directly from the internal iliac artery, with an incidence of 56.5% (n = 26/46) (Garcia-Monaco et al., 2014).

No significant differences were noted between the right and left sides of the pelvis regarding the number of prostatic arteries, as 54% (n = 26) were identified on the right side and 46% (n = 22) on the left. We noted that the location of penetration of prostatic arteries within the gland differed by pelvic side. Prostatic arteries supplied the gland from its dorsolateral aspect within the neurovascular bundle in 63% of cases (n = 30). However, in his pioneering study, Clegg highlighted that the prostatic arteries penetrate the gland from its ventrolateral surface in all instances (Clegg, 1955). In our study, we found the base of the prostate to be the second-most common region to be penetrated by prostatic arteries, i.e., in 23% (n = 11) of cases, followed
by the dorsal aspect of the prostate in 8% (n = 4), the ventral region of the prostate in 4% (n = 2), and the apex of the gland in 2% (n = 1). Elsewhere, two image-based studies confirmed the prostatic arteries penetrate the prostate at the 2 and 5 o’clock positions on the left and at the 10 and 7 o’clock positions on the right side (Bilhim et al., 2011; Bilhim et al., 2012).

Moreover, in this study, the prostate was observed to receive its blood supply from two prostatic arteries in 58% of cases. However, the prostatic arteries supplied the gland from a single branch in 21% of the hemipelvises and three branches in 21% of cases. Guodong et al. (2015) identified a total of 114 prostatic arteries, as single or double arteries in 96.4% (n = 106) and 3.6% (n = 4), respectively (Guodong et al., 2015). Similarly, a recent study based on angiograms of 199 hemipelvises revealed additional variability in the distribution of the prostatic arteries. One prostatic artery was observed in 72% (n = 143) of cases, and two or more prostatic arteries in the remaining 28% (n = 56) (Amouyal et al., 2018). Correspondingly, three studies reported incidences of solitary prostatic arteries in 70% (n = 56/80) (Anract et al., 2019), 77.8% (n = 28/36) (Garcia-Monaco et al., 2014), and 57% (n = 86/150) (Bilhim et al., 2012) of examined pelvises.

Arterial supply to the penis is mainly derived from the internal pudendal artery, which yields perineal branches and then continues as the common penile artery, terminating where it splits into three branches: the bulbourethral, cavernous, and dorsal arteries. These branches may be supplemented or entirely replaced by the accessory pudendal arteries that supply the erectile bodies (Awad et al., 2011). However, the arterial supply of the penis remains highly inconsistent in terms of its branches, courses, and anastomoses. In our study, two patterns of arterial vessels were found to supply the corpora cavernosa. In 92% of the investigated sides, the corpora cavernosa received its arterial supply from a single source, the internal pudendal
artery. However, in the remaining 8%, the internal pudendal artery was joined by an accessory pudendal artery to supply the corpora cavernosa. These accessory pudendal arteries were located on two sides of the same specimen and were direct branches from the internal iliac artery. Droupy et al. (1997) and Breza et al.’s (1989) research confirmed that the penis received its blood supply from both the internal pudendal artery and accessory pudendal artery in 70% (n = 14/20) and 83% (n = 5/6) of examined cases, respectively (Breza et al., 1989; Droupy et al., 1997). However, Thai and colleagues (Thai et al., 2015) reported that the internal pudendal artery was the chief arterial supply to the penis in 51.4% (n = 57/111) of investigated cases. On the other hand, Nehra et al. (2008) observed the accessory pudendal artery to be the dominant source of blood supply to the penis in 54% (n = 15/28) of cases (Nehra et al., 2008).

From a clinical perspective, the correlation between the preservation of the arterial supply to the corpora cavernosa and impotence after a nerve-sparing radical prostatectomy is debatable (Droupy et al., 1999; Polascik & Walsh, 1995). Several studies applying different techniques, such as open radical prostatectomy, duplex ultrasonography, and intracavernous injections, have confirmed that the reason for erectile dysfunction in patients after radical prostatectomy employing the nerve-sparing technique may be due to injuries sustained by the cavernosal arteries, resulting in insufficient arterial supply to the corpora cavernosa (Aboseif et al., 1994; Bahnson & Catalona, 1988; Kawanishi et al., 2001; Kim et al., 1994; Oates et al., 1995; Zelefsky & Eid, 1998). Conversely, several investigations reported no significant relationship between damage to the arterial supply of the corpora cavernosa and complications following a radical prostatectomy (Blander et al., 1999; Box et al., 2010; Polascik & Walsh, 1995; Williams et al., 2017).
The inferior hypogastric plexus (IHP) or pelvic plexus is commonly known as the source of nerve innervation for pelvic structures, including the rectum, prostate, urinary bladder, seminal vesicles, urethra, and penis (Mauroy et al., 2003; Röthlisberger et al., 2018; Walsh & Donker, 1982). It is widely agreed that the IHP nerve fibres are responsible for ensuring the mechanisms of erection, ejaculation, and urinary continence (Mauroy et al., 2003; Walsh & Donker, 1982; Walz et al., 2010). For the purpose of our investigation, the nerves of the pelvic plexus were derived from the pelvic splanchnic nerves originating from the sacral roots S2, S3, and S4 and the superior hypogastric plexus. Moreover, it was observed that the dorsolateral aspect of the gland was the most common site of penetration, receiving nerves on all the examined sides. In addition, the prostate was innervated within its base inferior to the bladder, with branches passing to the anterior surface of the gland, and within its dorsal regions, and branching to the apex of the prostate. The neurovascular bundle, comprised of prostatic nerves and blood vessels, was evident in all cases in the dorsolateral aspects of the prostate.

Anatomically, the cavernous nerve contains the sympathetic fibres responsible for ejaculation, and the parasympathetic fibres that control the process of vasodilation providing increased blood flow to the penis (Röthlisberger et al., 2018; Walsh & Donker, 1982). In our study, the cavernous nerves were identified as branches of the most inferior part of the pelvic plexus, and they were found to run between the prostate and rectum, exiting the pelvis through the urogenital diaphragm adjacent to the prostatic apex, to supply the cavernous bodies.

From a surgical vantage point, several studies have reported that the preservation of anterior fibres is beneficial, improving both erectile function and urinary control, with good tumour control (Nielsen et al., 2008; Stolzenburg et al., 2010; van der Poel et al., 2009). Nevertheless, it is essential to note that any further nerve preservation, with less traumatic manipulation of
the NVB in any anatomical position proximal to the prostate, is likely to deliver advantages in terms of improving erectile function and urinary control following surgery. Stewart et al. (2011) reported a significant difference in the erectile function recovery rates of patients who underwent nerve-sparing and non-nerve-sparing endoscopic extraperitoneal radical prostatectomy 1-year post-surgery, namely 71% and 29%, respectively (Stewart et al., 2011). Moreover, Kübler et al. (2007) stated that nerve-sparing radical prostatectomy correlates significantly with a higher recovery rates for erectile dysfunction (Kübler et al., 2007). In addition, men who underwent nerve-sparing endoscopic extraperitoneal radical prostatectomy appeared to achieve a more rapid return to full continence. One year post-follow-up, 97% of patients who had undergone nerve-sparing EERPE had recovered their continence, in contrast to 86% of those who underwent non-nerve-sparing EEPRE (Stewart et al., 2011).

Thus, it can be concluded that the clinical significance of the periprostatic neurovascular structures is debatable. Nonetheless, it is clear that these structures potentially play a major role in the aetiology of impotence and incontinence for patients undergoing a radical prostatectomy. Therefore, the preservation of these nerves and vessels during radical prostatectomy is vital to improving patients’ clinical outcomes.
Chapter 3: Histology

3.1 Introduction

Since Walsh and Donker introduced their pioneering description of nerve-sparing radical prostatectomy (Walsh & Donker, 1982), the nerves responsible for erectile function have been widely accepted to be located in the neurovascular bundle that enters the dorsolateral aspect of the prostate. However, this assertion has been questioned, as several studies have reported extensive variation in the distribution of the nerves responsible for erectile function around the prostate (Kiyoshima et al., 2004; Sievert et al., 2008). Thus, some authors have suggested adapting the standard nerve-sparing procedure to improve the preservation of penile function after surgery (Costello et al., 2004; Graefen et al., 2006; Montorsi et al., 2005). Despite this, the complex neurovascular elements associated with the prostate, and highly variable interindividual anatomy, make functional preservation challenging, especially for high-risk patients in need of aggressive resection. Therefore, improved anatomical identification and understanding of the distribution and course of the neurovascular structures that supply are and located within the prostate may be required to limit complications following a radical prostatectomy.

A study of the literature revealed considerable limitations across studies that previously attempted to investigate neurovascular structures inside the prostate. Several of these studies were conducted without a nerve-staining procedure (Eichelberg et al., 2007; Kiyoshima et al., 2004), whereas others utilized an unspecified nerve-staining process (Ganzer et al., 2008; Ganzer et al., 2009; Sievert et al., 2008; Sievert et al., 2009). Therefore, it was not possible for these researchers to differentiate between autonomic nerve types, thereby limiting their ability to identify the precise location and distribution of each type of nerve (sympathetic or parasympathetic) inside the gland. Although two different studies (Costello et al., 2011; Ganzer et al., 2012) did manage to distinguish between different nerve types within the prostate, their
studies were limited as their counting methodology enabled them to solely include nerves located in the prostatic peripheral region, close to the capsule. Thus, these two studies could not provide a detailed mapping of all the nerves inside the prostate, as they neglected the neural structures in the central region of the prostate around the urethra. Moreover, all previous studies share a common limitation, in that they focused their investigations on the prostatic nerves without considering the blood vessels. This meant the evidence presented did not address the issue of how nerves and vessels relate to one another inside the gland.

Given the considerable limitations within the literature, additional research is essential to characterize the neuronal and vascular structures inside and proximal to the prostate more fully, along with their anatomical relationship to the gland. Investigating the external nerves and blood supply by applying gross anatomy was worthwhile (as shown in the previous chapter). Nevertheless, investigation into the external nerves and vessels of the prostate requires gross dissection of whole pelvises, which is extremely time-consuming to do well. Therefore, additional approaches need to be undertaken to fully investigate and characterise prostatic neurovascular structures, one of which involves internal viewing by applying histological techniques. In contrast to the investigation of external nerves and vessels of the prostate, internal analyses were performed examining prostatic tissues obtained from patients following non-nerve sparing radical prostatectomy. Moreover, at the macroscopic level of investigation, it is challenging to differentiate between autonomic nerve fibres. Thus, a specific staining immunohistochemical technique was performed to distinguish between different types of autonomic nerves supplying the prostate.

This chapter reports on a combined, comprehensive and detailed histological investigation of the sympathetic and parasympathetic nerves, as well as blood vessels, within the prostate.
Importantly, this investigative approach overcame technical and methodological limitations of previous studies by applying an adapted and advanced study design, as well as developing approaches to better visualize the location and density of intra-prostatic nerves and vessels.
3.2 Materials and Methods

3.2.1 Specimens

All tissues were obtained from the Tayside Biorepository at Ninewells Hospital, Dundee, UK under ethical approval code 17/ES/0130. All prostatic tissues were taken from patients with an average age of 67.8 years (ranges from 60 to 75 years) who underwent non-nerve-sparing radical prostatectomy.

3.2.2 Processing Protocol

Tissue processing and staining were performed following standard methodology, as described previously (Costello et al., 2011; Riegger et al., 2016). Tissues were fixed in neutral buffered 10% formalin (NBF) that was supplied by Genta Medical. Fixative volume was 2 ml of formalin per 100 mg of tissue. Tissues were fixed for a minimum of 48 hours at room temperature. Tissues were dehydrated through a series of graded ethanol immersions that was supplied by Genta Medical to displace the water, and then infiltrated with wax. The infiltrated tissues were then embedded into wax blocks. Once fixed, tissues were processed as follows on a Leica Peloris tissue processor as per the standard NHS Tayside Pathology laboratory 8-hour protocol:

1. Formalin x 2 (Genta Medical)
2. 95% ethanol (Industrial Methylated Spirit) x 4 (Genta Medical)
3. 99% ethanol (Absolute Alcohol) x 4 (Genta Medical)
4. Xylene x 4 (Genta Medical)
5. First clearing agent, Xylene (Genta Medical)
6. Second clearing agent, 99% ethanol (Genta Medical)
7. First paraffin wax (Leica Biosystems)
8. Second wax paraffin (Leica Biosystems)
Tissues were sectioned using a Leica microtome, 4 µM thickness. Then dried in a 37°C oven.

### 3.2.3 Staining of slides

Immunohistochemical staining was performed by applying Anti-Nitric Oxide Synthase (Merck AB5380) antibodies to localise parasympathetic nerves and Anti-Tyrosine Hydroxylase EP1533Y (Abcam ab75875) antibodies to localise sympathetic nerves (Costello et al., 2011) in 206 slides. Antigen retrieval and de-paraffinization was performed using DAKO EnVision™ FLEX Target Retrieval solution (high pH) buffer (50x concentration) (K8004) in a DAKO PT Link (serial number PT2794Y1205) for 20 minutes at 97°C. Immunostaining using DAKO EnVision™ FLEX system was performed manually according to the manufacturing guidelines. Sections were initially washed in Flex Wash Buffer (K8006). The following steps were then performed:

1. Flex Peroxidase-Blocking Reagent (SM801) applied for 5 mins
2. Incubation with Anti-Nitric Oxide Synthase (Merck AB5380) and Anti-Tyrosine Hydroxylase EP1533Y (Abcam ab75875) Primary Antibodies (diluted to optimal dilution, 1-5000 for nNOS and 1-1000 For TH, in Flex Antibody Diluent K8006) overnight at 4 degrees centigrade).
3. Flex/HRP labelled polymer (SM802) for 20 minutes
4. Flex DAB+ working solution (SM803) for 2 x 5 minutes
5. Copper Sulphate solution for 5 minutes
6. Flex Haematoxylin for 5 minutes

In between steps, sections were rinsed with Flex Wash Buffer with a final wash of dH2O. Slides were manually washed in tap water before being rinsed in graded concentrations of alcohol, with a final rinse in Xylene. Glass coverslips were applied.
In addition, Haematoxylin and Eosin staining (H&E) standard processing (Eichelberg et al., 2007) was performed on 103 slides in order to identify blood vessels as follows:

1. Oven at 60°C for 15 mins
2. Deparaffinise sections, 3 changes of xylene, 30 seconds each.
3. Re-hydrate in 2 changes of absolute alcohol, 30 seconds each.
4. 99% alcohol for 2 minutes and 95% alcohol for 2 minutes.
5. Wash in water 30 seconds.
6. Stain in Harris haematoxylin solution for 4 minutes.
7. Wash in running tap water for 1 minute.
8. Differentiate in 0.1% acid alcohol for 1 minute.
9. Wash in water for 1 minute.
10. Bluing in saturated lithium carbonate solution for 1 minute.
11. Wash in tap water for 30 seconds.
12. Counterstain in eosin solution for 20 seconds
13. Wash in tap water for 30 seconds.
14. Rinse in 95% alcohol for 30 seconds x 2.
15. Rinse in 99% alcohol for 30 seconds x 2.
16. Rinse in Isopropyl alcohol for 30 seconds
17. Clear in 3 changes of xylene, 30 seconds each.

Sections were cover slipped by a Leica Cover slipper CV5030 using DPX mountant.

3.2.4 Slide total number and orientation

Parasympathetic and sympathetic nerves as well as blood vessels were investigated in a total of 309 slides obtained from the prostates of 15 patients who underwent non-nerve-sparing radical prostatectomy. Prostates were divided into three levels (Apex, Body and Base) which
represent 20%, 60%, and 20% of the gland, respectively (Figure 3.1). The number of slides from each prostate ranged from four to nine depending on overall prostate size. Slides were scanned using an Axio Scan Z1 with Zen 2.3. The slides were oriented by first identifying the urethra and ventral and dorsal aspects of the gland. Green (left) and black (right) inks on each side of slides were used to identify the left and right sides of the prostate. NanoZoomer Digital Pathology (NDP) software was then used to divide each slide into 12 sections: 6 peripheral and 6 central. Firstly, the centre of the slide was identified manually and then three lines were drawn to divide the slides into 6 sections: ventral, right ventrolateral (VLR), right dorsolateral (DLR), dorsal, left dorsolateral (DLL) and left ventrolateral (DLR). Secondly, these six sections were divided into central and peripheral aspects by a central circle with a diameter of 1.5 cm. Therefore, the six triangles of the circle are considered as central regions and the six aspects out of the circle are considered as peripherals. This division of the slide improves the identification of the precise number, location and course of the neurovascular structures within the prostate (Figure 3.2).
Figure 3.1: Overview of experimental design. Schematic illustration (generated using Affinity Designer software); (A) showing the location of the prostate and its relationship to adjacent structures such as the urinary bladder, seminal vesical, and penis. Section B shows a magnification of the prostate to delineate the three levels of the gland (base, body, and apex), which represent 20%, 60%, and 20% of the gland, respectively. S: superior, A: anterior, P: posterior, I: inferior.
Figure 3. 2: An IHC-stained transverse section of the prostate taken from the apex, illustrating how each slide/prostate was divided up for spatial morphometric analysis. The prostate was oriented by first identifying the urethra and ventral and dorsal aspects of the gland. Green (left) and black (right) inks on each side of the gland were used to identify the left and right sides of the prostate. NanoZoomer Digital Pathology (NDP) software was then used to divide each prostate into 12 regions, including 6 peripheral and 6 central sections. First, the centre of the prostate was identified manually, and then three lines were drawn to divide it into 6 sections, namely the ventral, right ventrolateral, right dorsolateral, dorsal, left dorsolateral, and left ventrolateral sections. Second, the 6 sections were divided into the central and peripheral aspects with a central circle of 1.5 cm in diameter. Therefore, the 6 triangles within the circle were considered to represent central regions, and the six aspects outside the circle were considered peripheral regions. S: superior, A: anterior, P: posterior, I: inferior, C: central, P: peripheral.
3.2.5 Nerve and blood vessel quantification

Nerve fibres were identified based on positive immunohistochemical staining (Costello et al., 2011; Ganzer et al., 2012), whereas blood vessels were identified based on their standard morphological appearance within H&E-stained slides (Pearce & Thomsen, 2000). Nerves were identified and quantified by applying the colour threshold tool in ImageJ software, which then performed an automatic detection and quantification of immuno-positive structures within slides, as follows:

1- Each section of prostatic slides was exported as a tiff image to be analysed separately

2- Upload the image of each section into ImageJ software

3- Set the image scale (analyse → set scale)

4- Measure the area of the section

5- Clear the background (edit → selection → make inverse → fill) (Figure 3.3)

Figure 3. 3: A representative micrograph of the dorsal regions of a transverse section of a prostate at the apex level that was stained with antibodies against neuronal nitric oxide synthase (nNOS) to identify the location and distribution of parasympathetic nerves within the gland. During this step of nerve counting, each section of the prostate was examined separately.
6- Initiate the colour threshold option and adjust the colour values to suit the analysed section

7- Run the colour threshold to detect positive stained structures (Figure 3.4)

Figure 3.4: Identification of positively stained structures. After initiating the colour threshold option, the background of the slide section was cleared and positively stained structures were identified. After this stage, the nerves were counted automatically.

8- Start automated counting (analyse particles)

9- Apply the overlay option of the detected structures over the original section to make sure that only positive stained structures were detected (Figure 3.5)
Figure 3. 5: Following the automated counting of nerves, the detected structures were pseudo-imposed over the examined section to ensure that only the positively stained structures were identified (as illustrated by the red points).
Blood vessels were identified and counted manually using ImageJ software, as follows:

1- Each section of prostate was exported as a tiff image to be analysed separately

2- Upload the image of the section into ImageJ software

3- Set the image scale (analyse set scale)

4- Measure the area of the section

5- Clear the background (edit selection make inverse fill)

6- Manually count blood vessels (plugins analyse cell counter)

Positive identification of a blood vessel required that the lumen and/or one of the tunicae of the structure were clearly identifiable, regardless of size or shape (Pearce & Thomsen, 2000) (Figure 3.6).
Figure 3. 6: Representative example of prostatic blood vessels identified using H&E staining. Blood vessels were scattered throughout all the regions of the prostate, although their shapes and sizes differed among the prostatic regions. This figure provides some examples of the different shapes and sizes of the blood vessels (red arrows) identified in this study.
3.2.6 Statistical analysis

Graphpad Prism, Version 9.0.2 (161), was used to perform all statistical analyses. One-way ANOVA tests and unpaired two-tailed t-tests were conducted. Values are reported as mean with standard error of the mean. P values <0.05 were considered statistically significant.

The Pearson correlation coefficients test was applied to examine the reliability of nerve and blood vessel counting procedures. To perform reliability tests, the original count and density of nerves and blood vessels were first compared to repeated analyses by the same observer. Then, the original count and density of nerves and blood vessels were compared to a repeated analysis performed by a different observer (a colleague from the Anatomy department, University of Edinburgh). The comparison for the same observer revealed correlation coefficients (r) of 0.98 and 0.97 for nerve and vessel numbers, respectively (Figure 3.7). Moreover, repeated analyses by the second observer showed correlation coefficients (r) of 0.95 and 0.94 for nerve and blood vessel counts, respectively (Figure 3.8). Regarding nerve and vessel density, the repeated analyses by the same observer presented a correlation coefficient (r) of 0.96 for nerve density and 0.97 for vessel density (Figure 3.9). Additionally, reliability tests of density conducted by the second observer revealed correlation coefficients (r) of 0.93 and 0.94 for nerves and vessels, respectively (Figure 3.10). The results suggest that the counting methodology for nerves and blood vessels employed in this study is both reliable and repeatable.
Figure 3. 7: Nerve and blood vessel counting methodology applied in this study was reliable and repeatable. The Pearson correlation coefficient test was performed to examine the reliability of the counting procedure, and it revealed correlation coefficients (r) of 0.98 and 0.97 for the same observer for the nerves (Chart A) and vessels (Chart B), respectively. In addition, it indicated a confidence interval of 0.96–0.99 for the nerves and 0.90–0.99 for the blood vessels.

Figure 3. 8: The investigation methodology used to determine the numbers of nerves and vessels inside the prostate was reliable. The Pearson correlation coefficient test was used to examine the reliability of the counting procedure, and it revealed correlation coefficients (r) of 0.95 and 0.94 for the second observer for the nerves (Chart A) and vessels (Chart B), respectively. Moreover, it indicated a confidence interval of 0.89–0.98 for the nerves and 0.80–0.98 for the blood vessels.
Figure 3. 9: The investigation methodology used to determine the density of the prostatic neurovascular structures inside the prostate was reliable. The Pearson correlation coefficient test was applied to examine the reliability of the neurovascular structure density analysis method, and it revealed correlation coefficients (r) of 0.96 and 0.97 for the same observer for the nerves (Chart A) and vessels (Chart B), respectively. Moreover, it indicates a confidence interval of 0.90–0.98 for the nerves and 0.90–0.99 for the blood vessels.

Figure 3. 10: The methodology used to investigate the density of the prostatic neurovascular structures inside the prostate was reliable. The Pearson correlation coefficient test was used to examine the reliability of the neurovascular structure density analysis method, and it revealed correlation coefficients (r) of 0.93 and 0.94 for the second observer for the nerves (Chart A) and vessels (Chart B), respectively. Moreover, it indicated a confidence interval of 0.84–0.97 for the nerves and 0.80–0.98 for the blood vessels.
3.3 Results

Qualitative investigation of immunohistochemically stained and H&E processed sections revealed that neurovascular structures could be reliably identified throughout all prostatic levels and scattered across all the regions of the prostate. Nevertheless, there was evidence of significant structure-specific clustering within regions and levels of the prostate. Consequently, various neurovascular structures across the different regions of the prostate were compared quantitatively. In general, the quantitative investigations supported the qualitative observations, with the neurovascular structures being identifiable across all prostatic levels and regions (Figures 3.11 & 3.12). In addition, no significant correlations were found linking patients’ age and internal prostatic neurovascular structures (Figure 3.13).
Figure 3.11: Representative micrograph of a transverse section of prostate at the level of the apex, stained with haematoxylin and eosin (H&E) to identify the location and distribution of blood vessels within the gland. (A) shows an example of how blood vessels can be scattered in all regions of the prostate. Magnification boxes (B and C) illustrate some of blood vessels that were identified within each region of the same prostatic section. Blood vessels can be observed in all prostatic sections; however, there are clearly differences in the number, shape, size, and the location of them. Magnification of box (B) represents blood vessels (pointed by yellow arrows) that were located within the peripheral ventral region of the prostate. Magnification of box (C) shows blood vessels (pointed by yellow arrows) that were located within the peripheral dorsal aspect of the prostate. Moreover, in addition to the variety in their sizes and shapes, it can be clearly noted that some of the identified blood vessels were filled with erythrocytes. V: ventral; R: right; L: left; D: dorsal.
Figure 3.12: Representative micrograph of a transverse section through the prostate at the level of the base, labelled with antibodies against TH to identify the location and distribution of sympathetic nerves within the gland. Sympathetic nerves were observed to be scattered throughout all prostatic regions. For example, in magnification boxes (B and C), the dark brown stained structures represent the sympathetic nerves. They can be clearly identified in all prostatic sections; however, there are clearly differences in both the relative number and size of the axon bundles. Magnification of box (B) represents sympathetic nerves that were identified within the central dorsolateral left section (red circles refer to some examples of these nerves). Magnification of box (C) shows sympathetic nerves (red circles) that were located within the peripheral dorsal aspect of the prostate. V: ventral; R: right; L: left; D: dorsal.
Figure 3. 13: Correlation between patients’ age and distribution of intra-prostatic neurovascular structures. As shown in figures (A, B and C) no statistically significant correlation was identified between the age of patients and the number of nerves as well as number of blood vessels inside the prostate. PSNs: parasympathetic nerves; SNs: sympathetic nerves.
3.3.1 Distribution of neurovascular structures across prostatic levels

As figures 3.11 and 3.12 demonstrate, the nerves and blood vessels were found to be located throughout the prostate. However, variations in their clustering occurred within the regions and levels of the prostate, which required further examination. Neurovascular structures across the different regions of the prostate were quantitatively compared. The parasympathetic and sympathetic nerves across the prostatic levels (apex, body and base) were compared performing three approaches: total nerve number, nerve surface area and nerve density (nerves per 1mm²). These analyses revealed no significant differences in the number, surface area or density of the parasympathetic nerves across any of the three levels; indeed, parasympathetic nerve fibres were equally distributed throughout all the prostatic levels. In contrast, the distribution of the sympathetic nerves was inconsistent between levels, as the number of nerves and their surface area at the base level were significantly higher than at the apex level. However, similar to the parasympathetic nerves, no significant differences were found between the prostatic levels in terms of sympathetic nerve density. Meanwhile, blood vessels across the prostatic levels were compared by employing two approaches: number and density (number of vessels per 1mm²) of total vessels. These analyses showed no significant differences between the number or density of the blood vessels across the three levels. Thus, the vessels were reported to be equally distributed throughout all the prostatic levels (Figures 3.14, 3.15 & 3.16).
Figure 3. Parasympathetic nerve fibres were found to be equally distributed throughout the apex, body and base levels of the prostate. These nerves were identified and counted automatically by investigating the prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). Three different comparison analyses involving the ordinary one-way ANOVA and Tukey’s multiple comparison tests were conducted between the prostatic levels to investigate the parasympathetic nerves count, surface area and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison analyses of the total number of parasympathetic nerves (PSNs number) across all three levels, and it clearly shows that the numbers of parasympathetic nerves were consistent across the levels. Chart (B) shows the results of the comparison analyses across the three levels in terms of the parasympathetic nerves surface area, and it reveals no significant differences between the prostatic levels. Chart (C) shows the results of the comparison analyses of the parasympathetic nerves density (number of nerves within a 1 mm\(^2\) area) across the three levels of the prostate. This analysis revealed that the parasympathetic nerves density was consistent across the prostatic levels. It can be concluded that none of the analyses revealed a significant difference between the prostatic levels. PSNs: Parasympathetic nerves.
Figure 3.15: Sympathetic nerve fibres showed an inconsistent distribution throughout different levels of the prostate. The sympathetic nerves were identified and counted automatically by investigating prostatic slides labelled with antibodies against tyrosine hydroxylase (TH). Three different comparison analyses applying the ordinary one-way ANOVA and Tukey’s multiple comparison tests were conducted among the prostatic levels to investigate sympathetic nerves count, surface area and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison test of the total number of sympathetic nerves (SNs number) across all three levels, and it clearly shows that the base level contained significantly higher number of sympathetic nerves than apex. Chart (B) shows the results of the comparison analyses across the three levels in terms of the sympathetic nerves surface area. This analysis revealed that the sympathetic nerves located within the base level had significantly larger surface area than the nerves located within the apex of the prostate. Chart (C) presents the results of the comparison analyses of the sympathetic nerves density (number of nerves within a 1 mm² area) across the three levels of the prostate. This analysis revealed that the sympathetic nerves density was equal across the three prostatic levels. SNs: sympathetic nerves.
Figure 3.16: Blood vessels were equally distributed throughout the apex, body and base levels of the prostate. Blood vessel counting involved the manual investigation of haematoxylin and eosin (H&E)-stained prostatic slides (as shown in Figure 3.11 above). Two different comparison tests applying ordinary one-way ANOVA and Tukey’s multiple comparison tests were conducted among the prostatic levels to investigate the vessels number and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison test of the total number of blood vessels across all three levels, and it clearly shows a consistent number of blood vessels across all the prostatic levels. Chart (B) shows the results of the comparison of the blood vessels density (number of vessels within a 1 mm² area) across the three levels of the prostate. It can be concluded that all the analyses revealed the blood vessels to have a consistent distribution across the prostatic levels, and no significant differences were identified among the prostatic levels in either investigation.

In conclusion, it was evident that parasympathetic nerves and blood vessels had a consistent distribution across all prostatic levels, but this did not correlate with the distribution of sympathetic nerves which were unequally distributed, being located mainly within the base of the prostate.
3.3.2 Distribution of neurovascular structures in ventral and dorsal regions of the prostate

Next, the distribution of neurovascular structures within ventral and dorsal halves of the prostate was examined. The resulting data were divided into two groups (ventral and dorsal): the ventral group included the ventral, VLR and VLL regions; whereas the dorsal group included the dorsal, DLR and DLL regions. Similar to previous analyses, comparisons between the nerves were made using three approaches: total nerve number, nerve surface area and nerve density per 1 mm$^2$. Likewise, comparisons in the blood vessels were performed by applying two approaches: total number of vessels and density per 1 mm$^2$. These analyses revealed that 63% of parasympathetic nerves were located in the dorsal half of the gland, whereas the remaining 37% were located within the ventral half of the prostate. Moreover, 67% of the sympathetic nerves were observed within the dorsal regions, while 33% were identified within the ventral half of the prostate. Significant differences were identified between the ventral and dorsal halves of the prostate in terms of nerve number, surface area and density for both the parasympathetic and sympathetic nerves. In contrast, the analysis of blood vessels revealed that they were consistently distributed across both prostatic halves, with 54% in the dorsal half and 46% within the ventral half, with no significant differences arising in terms of total number and density (Figures 3.17, 3.18, 3.19 & 3.20).
Figure 3.17: Parasympathetic nerve fibres showed an inconsistent distribution within the dorsal and ventral halves of the prostate. The nerves were identified and counted automatically by investigating prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). Three different comparison tests applying unpaired t-tests were conducted between the dorsal and ventral halves of the gland to investigate the parasympathetic nerves count, surface area and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison analyses of the total number of parasympathetic nerves (PSNs number) across the ventral region when compared with the dorsal region of the prostate, and it shows that the dorsal half of the gland contained significantly higher number of parasympathetic nerves than the ventral half. Chart (B) shows the results of the comparison across the prostatic ventral and dorsal halves in terms of the parasympathetic nerve surface area. This analysis revealed that the parasympathetic nerves located within the dorsal region of the prostate had significantly larger surface area than the nerves located within the ventral region. Chart (C) presents the results of the comparison of the parasympathetic nerves density (number of nerves within a 1 mm² area) across the ventral and dorsal aspects of the prostate. This analysis showed that the dorsal half of the prostate had significantly higher nerves density than the ventral half. PSNs: parasympathetic nerves.
Figure 3. 18: Sympathetic nerve fibres exhibited an unequal distribution within the dorsal and ventral halves of the prostate. The sympathetic nerves were identified and counted automatically by investigating prostatic slides labelled with antibodies against tyrosine hydroxylase (TH). Three different comparison investigations applying unpaired t-tests were applied between the dorsal and ventral halves of the gland to examine the sympathetic nerves count, surface area and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison analyses of the total number of sympathetic nerves (SNs number) across the ventral region when compared with the dorsal region of the prostate, and it shows that the dorsal half of the gland contained significantly higher number of sympathetic nerves than the ventral half. Chart (B) shows the results of the comparison across the prostatic ventral and dorsal halves in terms of the sympathetic nerves surface area. This analysis revealed that the sympathetic nerves located within the dorsal region of the prostate had significantly larger surface area than the nerves identified within the ventral region. Chart (C) presents the results of the comparison of the sympathetic nerves density (number of nerves within a 1 mm$^2$ area) across the ventral and dorsal aspects of the prostate. This analysis revealed that the dorsal half of the prostate had significantly higher density of sympathetic nerves than the ventral half. SNs: sympathetic nerves.
Figure 3. 19: Differential location of nerves within ventral and dorsal aspects of the gland. A representative heatmap (generated using MATLAB software) of the relative location of both parasympathetic and sympathetic nerve fibres within the prostate. The dorsal half of the prostate contained significantly higher numbers of both types of nerves than the ventral region of the gland.
Figure 3.20: Unlike the neuronal structures, blood vessels showed a consistent distribution within the dorsal and ventral halves of the prostate. The blood vessel counting was based on the manual investigation of haematoxylin and eosin (H&E)-stained prostatic slides (as shown in Figure 3.11 above). Two different comparison tests applying unpaired t-tests were conducted between the dorsal and ventral halves of the gland to investigate the vessels count and density. The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) presents the results of the comparison test of the total number of blood vessels across the ventral region when compared with the dorsal region of the prostate, and it shows no significant difference between the two halves in terms of the amount of blood vessels. Chart (B) shows the results of the comparison of the blood vessel density (number of vessels within a 1 mm$^2$ area) across the ventral and dorsal aspects of the prostate. Similar to the blood vessels number, the blood vessels density was consistent between the prostatic halves, with no significant difference being identified.

Taken together, in contrast to parasympathetic and sympathetic nerves, which have inconsistent distribution within prostatic halves and are located mainly within dorsal regions of the gland, the blood vessels revealed an equal distribution within the ventral and dorsal halves of the prostate.
3.3.3 Distribution of neurovascular structures in central and peripheral regions of the prostate

In keeping with previous investigations, a further experiment was conducted to compare neurovascular distribution within peripheral aspects and central aspects of the prostate. Again, measurements of nerves were completed using three approaches: total nerve number, nerve surface area and nerve density per 1 mm$^2$. Similarly, measurements of blood vessels were performed applying two approaches: total vessel number and density per 1 mm$^2$. The comparison analyses of the neuronal structures revealed that 90% of parasympathetic nerves were located in the peripheral regions, and only 10% in the central aspects of the prostate. Furthermore, 85% of the sympathetic nerves identified were located within peripheral aspects, and 15% were observed within the central regions of the prostate. A similar comparison of blood vessels revealed that 78% of the identified vessels were located in the peripheral regions, with only 22% in the central aspects of the prostate. Furthermore, significant differences were identified between the peripheral and central aspects of the gland in terms of the number, surface area and the density of the parasympathetic nerves. Likewise, significant differences were observed between the peripheral and central regions of the prostates in terms of sympathetic nerve numbers and surface area. However, surprisingly, no significant difference was identified between the peripheral and central regions in terms of sympathetic nerve density. Moreover, regarding the distribution of vessels, a significant difference was identified between the peripheral and central aspects of the gland in terms of number of vessels. On the other hand, no significant differences in term of vessel density were apparent between the two regions (Figures 3.21, 3.22 & 3.23).
Figure 3. 21: 90% of parasympathetic nerves were located within peripheral aspects of the gland whereas only 10% were located within central regions of the prostate. These nerves were identified and counted automatically by investigating prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). Three different comparison tests applying unpaired t-tests were conducted between peripheral and central regions of the gland to investigate: (A) parasympathetic nerves count, (B) parasympathetic nerves surface area and (C) parasympathetic nerves density (number of nerves within an area of 1 mm²). The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) represents the comparison analyses of the total number of parasympathetic nerves (PSNs number) across central regions compared to peripheral regions of the prostates and shows that peripheral regions of the gland contained significantly higher number of parasympathetic nerves than central regions. Chart (B) shows the comparison across central regions and peripheral regions in terms of the parasympathetic nerves surface area. This analysis revealed that parasympathetic nerves that were located within the peripheral regions of the prostate had significantly larger surface area than nerves that were located within central regions of the prostate. Chart (C) shows the comparison of parasympathetic nerves density across the central and peripheral aspects of the prostates. This analysis showed that peripheral regions of the prostates had significantly higher nerves density than the central regions. PSNs: Parasympathetic nerves.
Figure 3. 85% of sympathetic nerves were located within peripheral aspects of the gland whereas 15% were observed within central regions of the prostate. Sympathetic nerves were identified and counted automatically by investigating prostatic slides that were labelled with antibodies against tyrosine hydroxylase (TH). Three different comparison investigations applying unpaired t test were conducted between peripheral and central regions of the gland to examine: (A) sympathetic nerves count, (B) sympathetic nerves surface area and (C) sympathetic nerves density (number of nerves within an area of 1 mm²). The bars of all the charts show the mean and the standard error of the mean, and the dots in the charts refer to each prostate (n=15). Chart (A) represents the comparison analyses of the total number of sympathetic nerves (SNs number) across central regions compared to peripheral regions of the prostates and shows that peripheral regions of the gland contained significantly higher number of sympathetic nerves than central regions. Chart (B) shows the comparison across central regions and peripheral regions in terms of the sympathetic nerves surface area. This analysis revealed that sympathetic nerves located within the peripheral regions of the prostate had significantly larger surface area than nerves that were identified within central regions of the prostate. Chart (C) shows the comparison of sympathetic nerves density across the central and peripheral aspects of the prostate. This investigation interestingly showed no significant difference between peripheral and central regions in terms of sympathetic nerves density. SNs: sympathetic nerves.
Figure 3. 23: 78% of blood vessels were located within peripheral aspects of the gland whereas 22% were located within central regions of the prostate. Blood vessel counting was based on manual investigation of H&E-stained prostatic slides, such as those shown in figure 3.11 above. Two different comparison tests applying unpaired t test were conducted between peripheral and central regions of the gland to investigate vessels count and density. The bars of all charts are showing the mean and the standard error of the mean and the dots in these charts refer to each prostate (n=15). Chart (A) represents the comparison test of the total number of blood vessels across central regions compared to peripheral regions of the prostates and shows that peripheral regions the gland contained significantly higher amount of blood vessels than central regions. Chart (B) shows the comparison across central regions and peripheral regions in terms of the blood vessels density and remarkably revealed no significant differences between the two regions in terms of the density.

In summary, this part of investigation revealed that the total number of neurovascular structures within peripheral regions of the prostate was significantly higher than in the central regions. Furthermore, sympathetic nerves and blood vessels revealed a consistent density distribution between the two regions which did not correlate with the parasympathetic nerves, which showed a significantly higher nerve density within the peripheral regions of the gland.
Following the three general investigations of the neurovascular supply distribution within the prostate, further observations were conducted to develop a more detailed insight into their distribution in each region and level of the gland. Firstly, the peripheral and central regions of the prostatic levels were examined separately. A quantitative comparison across the peripheral and central regions of the apex, body and base levels of the prostate was performed to determine the parasympathetic and sympathetic nerve count, surface area and density. These analyses revealed the parasympathetic nerves had a consistent distribution within the peripheral and central regions at all levels, and no significant differences were observed between the peripheral regions of these levels, or between the central regions of the levels (Figures 3.24 & 3.25).

Figure 3.24: Parasympathetic nerve fibres are equally distributed throughout peripheral regions at all levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, the bars charts show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the test comparing the total number of parasympathetic nerves (the PSNs number) across all three levels’ peripheral regions, showing their consistent distribution. Chart B depicts the comparison of parasympathetic nerves’ surface area across all three levels’ peripheral regions. No significant differences were observed in this regard. Chart C represents parasympathetic nerve density (the number of nerves within an area of 1 mm$^2$) across the three prostatic levels’ peripheral aspects and reveals no significant differences. PSNs: Parasympathetic nerves.
Figure 3. 25: Parasympathetic nerve fibres were consistently distributed throughout central regions at all levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, the bars charts show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A depicts the test comparing the total number of parasympathetic nerves (the PSNs number) across all three levels’ central regions, showing their consistent distribution. Chart B shows the comparison of parasympathetic nerves’ surface area across the three levels’ central regions with no significant differences were observed. Chart C depicts the comparison of parasympathetic nerves’ density (the number of nerves within an area of 1 mm²) across central aspects of prostatic levels. This analysis revealed an equal parasympathetic nerves density across all central regions of prostatic levels. PSNs: Parasympathetic nerves.
Conversely, the analyses of sympathetic nerves revealed that these nerves had an unequal distribution within the peripheral regions of the prostatic levels, and a significant difference was identified between the base and apex in terms of the total number of sympathetic nerves and the total surface area of the nerves. However, the sympathetic nerves revealed a consistent distribution in terms of their density across all peripheral regions in all levels of the prostate. Furthermore, with regard to the central regions, the sympathetic nerves were equally distributed across all levels and no significant differences were observed in terms of nerve count, surface area or density (Figures 3.26, 3.27).

Figure 3. 26: In contrast to parasympathetic nerve fibres, sympathetic nerves were distributed inconsistently throughout peripheral regions at different levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, The charts’ bars show the means and standard error of the means, while the dots represent each prostate (n = 15). Chart A represents the comparison test of the total number of sympathetic nerves (the SNs number) across all three levels’ peripheral regions, showing that peripheral regions of the base contained significantly more sympathetic nerves than peripheral aspects of the apex. Chart B shows the comparison of the sympathetic nerves’ surface area across peripheral regions of prostatic levels. This analysis revealed that sympathetic nerves in the peripheral regions of the base had significantly larger surface areas than nerves in peripheral regions of the apex. Chart C depicts the comparison of sympathetic nerves density (the number of nerves within an area of 1 mm$^2$) across peripheral aspects of prostatic levels. This analysis showed that sympathetic nerves density was consistent across all peripheral regions of prostatic levels. SNs: sympathetic nerves.
Figure 3. 27: Sympathetic nerve fibres were equally distributed throughout central regions at all levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, the bars charts show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparison test of the total number of sympathetic nerves (the SNs number) across central regions of prostatic levels, showing that they were distributed consistently. Chart B shows the comparison of the sympathetic nerves’ surface area across central regions of prostatic levels and revealed no significant differences in this regard. Chart C represents the analyses of sympathetic nerves density (the number of nerves within an area of 1 mm$^2$) across central aspects of prostatic levels and no significant differences were identified in this comparison. SNs: Sympathetic nerves.
In addition, the analysis of blood vessels was conducted using two approaches: determining number and density of blood vessels. It was observed that the peripheral aspects of the prostatic base clearly contained a higher number of blood vessels, with significant differences from the peripheral apex. On the other hand, the density distribution of vessels within the peripheral regions of the prostate was consistent across all levels. Furthermore, the number and density of blood vessels were equally distributed within the central regions of the prostate across all levels (Figures 3.28 & 3.29).

![Figure 3.28](image-url)

Figure 3. 28: Blood vessels were unequally distributed throughout peripheral regions at different levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, the bars charts show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A depicts the comparison test of the total number of blood vessels across all three levels’ peripheral regions, showing that the peripheral regions of the base contained significantly more blood vessels than peripheral aspects of the apex. Chart B shows the comparison of blood vessels density (the number of vessels within an area of 1 mm²) across peripheral aspects of prostatic levels. This analysis identified no significant difference in blood vessels density between all levels’ peripheral regions.
Figure 3. 29: Blood vessels were distributed consistently throughout central regions at all levels of the prostate. Applying ordinary one-way ANOVA and Tukey’s multiple comparison tests, the bars charts show the means and the standard errors of the means, while the dots in these charts represent each prostate (n = 15). Chart A depicts the comparison test of the total number of blood vessels across all three levels’ central regions, showing their consistent distribution. Chart B shows the comparison of blood vessels density (the number of vessels within an area of 1 mm$^2$) across the three prostatic levels’ central aspects, which were consistent. Therefore, no significant differences between prostatic levels were identified in any of these investigations.

In conclusion, this part of the study showed that blood vessels and sympathetic nerves correlate with each other with regards to their number and distribution within peripheral regions of all prostatic levels, in contrast to parasympathetic nerves. On the other hand, all the neurovascular structures corresponded well to each other in terms of density within peripheral regions across all prostatic levels. Furthermore, with regards to central regions of the gland, both types of nerves and blood vessels revealed a corresponding distribution in terms of total number and density.
Qualitative observations and quantitative analyses of the distribution of neurovascular structures have revealed significantly more nerve fibres and vessels within the peripheral regions than the central regions across all three prostatic levels. Typically, the total number of nerves and vessels decreases from the prostate base to the apex. At the base of the prostate, 90% of the parasympathetic nerves were located in peripheral regions, and 10% were found within the central regions. Moreover, 84% of the sympathetic nerves were observed within peripheral regions, and 16% were found within central regions; 83% of the blood vessels were located in the peripheral regions of the gland, whereas 17% were identified within the central regions. In the body of the prostate, 92% of the parasympathetic nerves were identified in the peripheral regions, and only 8% were found within the central regions. However, 88% of sympathetic nerves were located within the peripheral regions, and 15% were located within central regions. In addition, at the body level, 81% of the prostate’s blood vessels were within peripheral regions, and the remaining 19% were within the central regions. The apex level appeared to have more parasympathetic nerves present within its central regions (13%); however, its peripheral regions continued to show the greatest number of parasympathetic nerves (87%). Similarly, sympathetic nerves were located mainly within the peripheral regions at the apex level (84%) and within the central regions (16%). Similarly, the apex level appeared to have more blood vessels within its central regions at 32%. Nevertheless, its peripheral regions continued to show the largest number of blood vessels (68%).
Among the peripheral aspects of the sections at the base, the DLR contained the largest number of parasympathetic nerves; meanwhile, the ventral region had the fewest parasympathetic nerves. The DLR at the base contained parasympathetic nerves with greater surface areas than those of the nerves in the other regions. In contrast, the DLL had the most parasympathetic nerves, including parasympathetic nerves with greater surface areas at the body and apex levels. The dorsal aspects had higher parasympathetic nerve density among the peripheral regions of all sections across all levels. Similarly, the peripheral regions of DLR appeared to have the greatest number of sympathetic nerves at the base and body levels. However, the peripheral aspects of DLL were the areas richest in sympathetic nerves at the apex. Furthermore, these two areas, compared with the other peripheral regions, contained sympathetic nerves with greater surface areas across all levels. Similarly to the parasympathetic nerves, the peripheral regions of the dorsal sections contained higher sympathetic nerve density across all prostatic levels. With regard to the distribution of blood vessels among the peripheral aspects of the sections at the base, the DLL contained the largest number of blood vessels within all peripheral sections of the prostatic levels. In contrast, the fewest blood vessels within the peripheral regions of prostatic levels were located in the ventral region within the base, and the VLR aspect within the body and dorsal regions within the apex. The ventral aspect had higher blood vessel density among the peripheral regions of the apex, whereas the peripheral dorsal section had greater blood vessel density within the body and at the base of the gland (Tables 3.1, 3.2 & 3.3).
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<td>DLL</td>
<td>29500.45 ± 21490.68 (29.50)</td>
<td>187.05 ± 115.45</td>
<td>33628.9 ± 25651.93 (26.60)</td>
<td>135.49 ± 99.07</td>
</tr>
<tr>
<td>DLR</td>
<td>18773.36 ± 9305.54 (18.80)</td>
<td>147.83 ± 118.66</td>
<td>27026.75 ± 19284.13 (21.30)</td>
<td>116.99 ± 75.11</td>
</tr>
<tr>
<td>Dorsal</td>
<td>13054.32 ± 10010.18 (13.10)</td>
<td>205.28 ± 217.19</td>
<td>19111 ± 15825.38 (15.10)</td>
<td>142.88 ± 96.62</td>
</tr>
</tbody>
</table>

Table 3. 1: Summary of the average number of parasympathetic nerves and density across peripheral aspects of each level of the prostate. Note that the DLR was the richest peripheral aspect of parasympathetic nerves at the base and contained 27% of the nerves. At the apex and body levels, the peripheral DLL included the most parasympathetic nerves, at 29.5% and 26.6% respectively. Moreover, the peripheral ventral regions contained the fewest parasympathetic nerves at all levels, with 7.8% at the apex, 9.2% at the body level and 9.9% at the base. Moreover, as this table shows, the peripheral dorsal regions had the highest nerve densities of all prostatic levels. Therefore, parasympathetic nerves’ average densities (the number of nerves per area of 1 mm²) in the peripheral dorsal regions were 216.85 at the base, 142.48 at the body level and 205.28 at the apex. P values <0.05 were considered statistically significant. VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.
<table>
<thead>
<tr>
<th>Peripheral regions</th>
<th>Apex</th>
<th>Body</th>
<th>Base</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD</td>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD</td>
<td>Mean Number ± SD (%)</td>
</tr>
<tr>
<td>Ventral (4762.41 ± 4286.93 (6.5%))</td>
<td>66.66±44.84</td>
<td>6801.39 ± 5322.82 (6%)</td>
<td>59.86 ± 50.90</td>
<td>8609.82 ± 6817.88 (6%)</td>
</tr>
<tr>
<td>VLL (9846.09 ± 6362.99 (13.5%))</td>
<td>92.70±52.66</td>
<td>13040.17 ± 10588.25 (12%)</td>
<td>78.77 ± 74.31</td>
<td>15241.36 ± 10617.64 (11%)</td>
</tr>
<tr>
<td>VLR (11316.09±11248.38 (16%))</td>
<td>99.90±82.82</td>
<td>13572.75 ± 10644.87 (12%)</td>
<td>77.02 ± 62.79</td>
<td>15773 ± 10428.45</td>
</tr>
<tr>
<td>DLL (19098 ± 9838.25 (26%))</td>
<td>145.18±89.51</td>
<td>24791.92 ± 18008.66 (23%)</td>
<td>108.91 ± 84.39</td>
<td>32335.77 ± 22407.84 (22%)</td>
</tr>
<tr>
<td>DLR (17193.23±10169.72 (24%))</td>
<td>133.36±102.33</td>
<td>30060.27 ± 22457.84 (28%)</td>
<td>130.49 ± 97.75</td>
<td>39376.18 ± 32082.44 (27%)</td>
</tr>
<tr>
<td>Dorsal (10242.27± 5081.70 (14%))</td>
<td>170.29±139.06</td>
<td>20903.24 ± 17374.90 (19%)</td>
<td>169.05 ± 143.53</td>
<td>33184.41 ± 29024.68 (23%)</td>
</tr>
</tbody>
</table>

Table 3. 2: Summary of the average number of sympathetic nerves and density across peripheral aspects of each level of the prostate. Note that the DLR was the richest peripheral aspect of sympathetic nerves at the base and body levels and contained 27% and 28% of the nerves, respectively. However, at the apex, the peripheral DLL included the most sympathetic nerves at 26%. Moreover, similar to the corresponding parasympathetic nerve findings, the peripheral ventral regions contained the fewest sympathetic nerves across all levels, with 6.5% at the apex, 6% at the body level and 6% at the base. Furthermore, the peripheral dorsal regions had the highest sympathetic nerves densities across all prostatic levels. Therefore, the average sympathetic nerve densities (the number of nerves per area of 1 mm²) of the peripheral dorsal regions were 295.88 at the base, 169.05 at the body level and 170.29 at the apex. P values < 0.05 were considered statistically significant. VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.
### Table 3.3: Blood vessel distributions varied across the peripheral aspects of each prostatic level. This table depicts the average number and density of blood vessels. Note that the DLL was the richest peripheral aspect of blood vessels across all prostatic levels as it contained 21% of the vessels at the base, 21% at the body level and 22% at the apex. Moreover, the peripheral ventral regions contained the fewest blood vessels within the prostatic base at 11% of the total number of vessels. On the other hand, the fewest blood vessels at the body and apex levels were in the peripheral VLR regions, with 13% of the total number of vessels at both levels. Moreover, note that the peripheral dorsal regions had the highest vessel densities at the body and base levels, with averages of 2.54 and 2.24 vessels per 1 mm², respectively. However, the peripheral ventral regions contained the highest vessel densities at the prostatic apex, with an average of 3.17 vessels per 1 mm². Additionally, the peripheral VLR regions contained the lowest vessel densities across all prostatic levels, with averages of 1.61, 1.20 and 1.39 vessels per 1 mm² at the base, body level and apex, respectively. P values <0.05 were considered statistically significant. VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.

<table>
<thead>
<tr>
<th>Peripheral regions</th>
<th>Apex</th>
<th>Body</th>
<th>Base</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD (%)</td>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD (%)</td>
</tr>
<tr>
<td>Ventral</td>
<td>189.77 ± 181.77 (17%)</td>
<td>3.17 ± 2.75</td>
<td>217.07 ± 110.52 (14%)</td>
<td>2.07 ± 1.49</td>
</tr>
<tr>
<td>VLL</td>
<td>181.09 ± 105.27 (16%)</td>
<td>1.93 ± 1.57</td>
<td>236.85 ± 109.24 (15%)</td>
<td>1.37 ± 0.58</td>
</tr>
<tr>
<td>VLR</td>
<td>151.23 ± 82.51 (13%)</td>
<td>1.39 ± 0.86</td>
<td>209.08 ± 104.08 (13%)</td>
<td>1.20 ± 0.63</td>
</tr>
<tr>
<td>DLL</td>
<td>254.68 ± 107.70 (22%)</td>
<td>1.64 ± 0.61</td>
<td>332.63 ± 161.67 (21%)</td>
<td>1.39 ± 0.60</td>
</tr>
<tr>
<td>DLR</td>
<td>203.59 ± 101.01 (18%)</td>
<td>1.45 ± 0.97</td>
<td>283.44 ± 144.31 (18%)</td>
<td>1.25 ± 0.67</td>
</tr>
<tr>
<td>Dorsal</td>
<td>155 ± 104.59 (14%)</td>
<td>2.15 ± 1.61</td>
<td>299.71 ± 140.88 (19%)</td>
<td>2.24 ± 0.92</td>
</tr>
</tbody>
</table>
Analysis of the nerve and vessel distribution within the central regions of the six sections of the prostate across all levels indicated that the dorsal aspect was the central aspect richest in parasympathetic nerves, from among the six central regions across all levels. In terms of the parasympathetic nerve surface area, at the base and body levels, the central dorsal aspect included nerves with greater surface areas, whereas the central DLL contained larger nerves at the apex level. Similarly to the peripheral regions, the central dorsal aspect had higher nerve densities across all levels. In agreement with previous findings, the central dorsal aspect of the six sections across all levels contained the most sympathetic nerves, which had greater surface areas and higher nerve density. Notably, the ventral aspect was the central aspect richest in blood vessels among the six central regions across all levels. In contrast, central DLR appeared to contain the fewest blood vessels across the apex and base levels, whereas the central VLL contained the fewest vessels at the body level of the prostate. In contrast to the peripheral regions, the central ventral aspects had higher vessel density across all levels (Tables 3.4, 3.5 & 3.6).
### Table 3.4: Summary of the average number of parasympathetic nerves and density across central aspects of each level of the prostate.

Unlike the peripheral aspects, the central ventral regions across all prostatic levels contained significant number of parasympathetic nerves. Note that the dorsal central aspect was the richest in parasympathetic nerves at the base and body levels as it contained 20% and 22.59% of the nerves, respectively – followed by the ventral aspect, which contained 19.40% and 17.51%, respectively. At the apex, similar to the other levels, the central dorsal aspect included the most parasympathetic nerves at 20.14%; however, the central region second-richest in parasympathetic nerves was the VLL with 17.17%. Note that central the dorsal regions had the highest nerve densities (the number of nerves within an area of 1 mm²) across all levels, with averages of 108.89 at the base, 83.66 at the body level and 98.91 at the apex. P values <0.05 were considered statistically significant.

<table>
<thead>
<tr>
<th>Central regions</th>
<th>Apex</th>
<th>Body</th>
<th>Base</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD</td>
<td>Mean Number ± SD (%)</td>
<td>Mean density in 1 mm² ± SD</td>
</tr>
<tr>
<td>Ventral</td>
<td>2383.59± 1988.53 (16.48)</td>
<td>80.94 ± 67.52</td>
<td>1909.80 ± 2087.09 (17.51)</td>
<td>64.85 ± 70.87</td>
</tr>
<tr>
<td>VLL</td>
<td>2482.23 ± 2133.15 (17.17)</td>
<td>84.29 ± 72.43</td>
<td>1470.97 ± 1560.94 (13.49)</td>
<td>49.95 ± 53.00</td>
</tr>
<tr>
<td>VLR</td>
<td>2168.73± 1893.69 (15)</td>
<td>73.64 ± 64.30</td>
<td>1455.46 ± 1325.55 (13.35)</td>
<td>49.42 ± 45.01</td>
</tr>
<tr>
<td>DLL</td>
<td>2476.95± 2331.18 (17.13)</td>
<td>84.11 ± 79.16</td>
<td>1934.29 ± 2163.63 (17.74)</td>
<td>65.68 ± 73.47</td>
</tr>
<tr>
<td>DLR</td>
<td>2036.32± 1809.58 (14.08)</td>
<td>69.14 ± 61.45</td>
<td>1670.46 ± 1616.60 (15.32)</td>
<td>56.72 ± 54.89</td>
</tr>
<tr>
<td>Dorsal</td>
<td>2912.91± 2850.87 (20.14)</td>
<td>98.91 ± 96.80</td>
<td>2463.69 ± 2972.89 (22.59)</td>
<td>83.66 ± 100.95</td>
</tr>
</tbody>
</table>

VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.
### Table 3.5: Summary of the average number of sympathetic nerves and density across central aspects of each level of the prostate. The central ventral regions appeared to contain significant number of sympathetic nerves across all levels. Note that the central dorsal aspect was the richest in sympathetic nerves across all levels. Moreover, the central dorsal region had the highest sympathetic nerve densities (the number of nerves within an area of 1 mm²) across all levels, with averages of 190 at the base, 107.57 at the body level and 97.12 at the apex. P values <0.05 were considered statistically significant. VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.

<table>
<thead>
<tr>
<th>Central regions</th>
<th>Apex</th>
<th>Body</th>
<th>Base</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Mean Number ± SD (%)</strong></td>
<td><strong>Mean density in 1 mm² ± SD</strong></td>
<td><strong>Mean Number ± SD (%)</strong></td>
<td><strong>Mean density in 1 mm² ± SD</strong></td>
</tr>
<tr>
<td>Ventr</td>
<td>2079.82 ± 1857.55 (16.48)</td>
<td>70.62 ± 63.07 (17.51)</td>
<td>75.27 ± 77.94 (17.51)</td>
<td>3840.86 ± 3777.35 (19.40)</td>
</tr>
<tr>
<td>VLL</td>
<td>1940.82 ± 1804.83 (17.17)</td>
<td>65.90 ± 61.28 (13.49)</td>
<td>74.62 ± 79.42 (12.70)</td>
<td>4893.05 ± 6244.51 (14.40)</td>
</tr>
<tr>
<td>VLR</td>
<td>2030.73 ± 2033.57 (15)</td>
<td>68.96 ± 69.05 (13.35)</td>
<td>82.83 ± 86.46 (14.40)</td>
<td>3853.77 ± 4616.99 (14.40)</td>
</tr>
<tr>
<td>DLL</td>
<td>2217.23 ± 2096.46 (17.13)</td>
<td>75.29 ± 71.19 (17.74)</td>
<td>90.27 ± 92.01 (16.40)</td>
<td>4623.41 ± 5421.91 (16.40)</td>
</tr>
<tr>
<td>DLR</td>
<td>2515.41 ± 2577.13 (14.08)</td>
<td>85.41 ± 87.51 (15.32)</td>
<td>93.94 ± 95.79 (17.10)</td>
<td>3997.45 ± 5079.84 (17.10)</td>
</tr>
<tr>
<td>Dorsal</td>
<td>2860.09 ± 2956.13 (20.14)</td>
<td>97.12 ± 100.38 (22.59)</td>
<td>107.57 ± 122.02 (20)</td>
<td>5595.45 ± 7438.10 (20)</td>
</tr>
</tbody>
</table>
Table 3.6: Summary of the average number of blood vessels and density across central aspects of each level of the prostate. As the table shows, the richest central aspect of blood vessels across all prostatic levels was the ventral region, with 22%, 21.79% and 22.29% of the total number of vessels at the apex, body and base levels, respectively. On the other hand, the central DLR contained the fewest blood vessels at the apex and base levels, at 11% and 13.91% of the total vessels, respectively. At the prostatic body level, the central VLL regions included the fewest blood vessels at 13.83%. Moreover, interestingly, the central ventral region contained the highest vessels densities (the number of vessels within an area of 1 mm²) across all levels, with averages of 2.83 at the base, 2.70 at the body level and 4.01 at the apex. P values <0.05 were considered statistically significant. VLL: left ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; VLR: right ventrolateral; SD: standard deviation.
Peripheral and central regions were examined separately to gain further insights regarding the variation in neurovascular structures clustered between the ventral and dorsal halves of the prostate. Analyses of the peripheral regions revealed that 64% of the parasympathetic nerves were present in the dorsal half, and 36% were located in the ventral half. Furthermore, 70% of the sympathetic nerves were identified within the dorsal half of the peripheral regions, and the remaining 30% were observed within the ventral half of the prostate. Significant differences were identified between the peripheral ventral and peripheral dorsal regions of the gland in terms of nerve number, surface area and density for both types of nerves. Therefore, both sympathetic and parasympathetic nerves showed an unequal distribution within the peripheral aspects of the prostatic halves. Moreover, analyses of the peripheral regions of the prostate revealed that 56% of the blood vessels were in the dorsal half, whereas 44% were in the ventral half, and a significant difference was observed between regions. Nevertheless, the blood vessel density showed a consistent distribution within the peripheral regions of the prostatic halves, and no significant differences were identified (Figures 3.30, 3.31 & 3.32).
Figure 3. 30: Parasympathetic nerve fibres were found to be inconsistently distributed within peripheral aspects of prostatic halves. These nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). After each section of prostatic slides was divided into peripheral and central regions, three different analyses applying unpaired t-tests to compare the peripheral dorsal and peripheral ventral halves of the gland were conducted. These analyses determined (A) parasympathetic nerve counts, (B) parasympathetic nerves surface area and (C) parasympathetic nerves density. The bars charts show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A depicts the comparisons of the total number of parasympathetic nerves (the PSNs number) across the prostate’s peripheral ventral regions compared to the peripheral dorsal regions. It shows that the gland’s peripheral dorsal half contained significantly more parasympathetic nerves than its peripheral ventral half. Chart B shows the comparison of parasympathetic nerves surface area across the peripheral ventral and dorsal halves. This analysis revealed that parasympathetic nerves in the prostate’s peripheral dorsal had significantly larger surface areas than nerves in the peripheral ventral regions. Chart C shows the comparison of parasympathetic nerves density (the number of nerves within an area of 1 mm²) across the peripheral ventral and dorsal aspects. This analysis showed that the peripheral dorsal half had significantly higher nerves density than the peripheral ventral half. PSNs: Parasympathetic nerves.
Figure 3. 31: Sympathetic nerve fibres were unequally distributed in the prostate’s peripheral dorsal half compared to the peripheral ventral half. Sympathetic nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against tyrosine hydroxylase (TH). After each section of the prostatic slides was divided into peripheral and central regions, three different analyses applying unpaired t-tests to compare the peripheral dorsal and peripheral ventral halves were performed. These analyses determined (A) sympathetic nerve counts, (B) sympathetic nerves surface area and (C) sympathetic nerves density within each half of the gland. The charts’ bars show the means and standard error of the means, while the dots in these charts represent each prostate (n = 15). Chart A depicts the comparisons of the total number of sympathetic nerves (the SNs number) across the peripheral ventral regions compared to the peripheral dorsal regions. It shows that the gland’s peripheral dorsal half contained significantly more sympathetic nerves than the peripheral ventral half. Chart B shows the comparison of sympathetic nerves surface area across the peripheral ventral and dorsal halves. This analysis revealed that sympathetic nerves in the prostate’s peripheral dorsal regions had significantly larger surface areas than nerves in the peripheral ventral regions. Chart C shows the comparison of sympathetic nerves density (the number of nerves within an area of 1 mm$^2$) across the peripheral ventral and dorsal aspects. This investigation revealed that the peripheral dorsal half had significantly higher nerves density than the peripheral ventral half. SNs: Sympathetic nerves.
Figure 3. 32: Number of blood vessels was inconsistent within the peripheral regions of prostatic halves. Blood vessel counts were based on a manual investigation of the H&E-stained prostatic slides (as shown in Figure 3.11 above). After each section of prostatic slides was divided into peripheral and central regions, two different analyses applying unpaired t-tests were conducted to examine the distribution of blood vessels within peripheral dorsal and peripheral ventral of the gland. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparison test of the total number of blood vessels across the peripheral ventral regions compared to the peripheral dorsal regions. It shows that the gland’s peripheral dorsal half contained significantly more blood vessels than the peripheral ventral half. Chart B shows the comparison of blood vessels density (the number of vessels within an area of 1 mm²) across the prostate’s peripheral ventral and dorsal aspects. This analysis showed that blood vessels density was consistent across peripheral aspects of prostatic halves.
In line with previous findings regarding the peripheral aspects of the prostatic halves, an investigation of the central aspects revealed that 54% of both the parasympathetic and sympathetic nerves were located in the dorsal regions, and 46% of both nerves were located in the ventral regions. However, in contrast to the findings for the peripheral regions, no significant differences were identified between the central dorsal half and the central ventral half of the prostate in terms of nerve number, surface area, or the density of the parasympathetic or sympathetic nerves. Similarly, 56% of blood vessels were found in the ventral regions, and 44% were observed in the dorsal regions; no significant differences in the number and density of vessels were observed between regions. Thus, both types of nerves and blood vessels showed a consistent distribution between the ventral and dorsal halves within the central regions (Figures 3.33, 3.34 & 3.35).
Figure 3. Parasympathetic nerve fibres were consistently distributed within the central dorsal half and central ventral half of the prostate. These nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). After each section of prostatic slides was divided into peripheral and central regions, three different analyses applying unpaired t-tests were performed to investigating (A) parasympathetic nerve counts, (B) parasympathetic nerves surface area and (C) parasympathetic nerves density within central aspects of prostatic halves. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparisons of the total number of parasympathetic nerves (the PSNs number) across the central regions of the prostate’s ventral half compared to the central regions of the dorsal half and revealing no significant difference between these regions. Chart B shows the comparison of parasympathetic nerves surface area within central regions of prostatic halves and revealed no significant differences. Chart C shows the comparison of parasympathetic nerves density (the number of nerves within an area of 1 mm$^2$) across the central ventral and central dorsal aspects and revealed no significant differences. PSNs: Parasympathetic nerves.
Figure 3. 34: Sympathetic nerves were consistently distributed within the prostate’s central dorsal half compared to the central ventral half. Sympathetic nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against tyrosine hydroxylase (TH). After each section of prostatic slides was divided into peripheral and central regions, three different analyses applying unpaired t-tests were performed to investigating (A) sympathetic nerve counts, (B) sympathetic nerves surface area and (C) sympathetic nerves density. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparisons of the total numbers of sympathetic nerves (the SNs number) across the central regions of the prostate’s ventral half versus the central regions of the dorsal half and shows no significant difference between these regions. Chart B shows the comparison of sympathetic nerves surface area within central regions of prostatic halves and revealed no significant differences. Chart C shows the comparison of sympathetic nerve densities (the number of nerves within an area of 1 mm²) across the central ventral and central dorsal aspects and revealed no significant differences. SNs: sympathetic nerves.
Figure 3. 35: Blood vessels were distributed consistently within the prostate’s central dorsal half compared to the central ventral half. Blood vessel counts were based on a manual investigation of the H&E-stained prostatic slides as shown in Figure 3.11. After each section of prostatic slides was divided into peripheral and central regions, two different analyses applying unpaired t-tests were conducted to investigate blood vessels number and density within central dorsal and central ventral halves. The charts’ bars show the means and standard errors of the mean, while the dots represent each prostate (n = 15). Chart A represents the comparison test of the total number of blood vessels across the central regions of ventral half versus the central regions of dorsal half and shows that no significant difference was identified between these regions. Chart B shows the comparison of blood vessel densities (the number of vessels within an area of 1 mm²) across the prostate’s central ventral and central dorsal aspects and reveals no significant differences between these regions.
3.3.4 Refined localisation of neurovascular structures within the prostate

Next, a more detailed investigation of the distribution and course of the nerves and blood vessels within the prostatic halves was performed by investigating nerves and blood vessels within the ventral and dorsal midlines, in comparison to the ventral and dorsal lateral regions. The measurements in this analysis were the same as those previously applied. The total number, surface area and density of nerves, as well as the total number and density of blood vessels, were examined. The analyses revealed that the parasympathetic nerves were consistently distributed across the ventral and dorsal midlines and the lateral regions, and no significant differences in total number or density were evident. Nevertheless, the lateral regions of the dorsal half contained significantly larger parasympathetic nerves than the dorsal midline (Figures 3.36 & 3.39).
Figure 3. 36: Parasympathetic nerve fibres were consistently distributed within the midlines and lateral regions of the prostatic halves. These nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against neuronal nitric oxide synthase (nNOS). Three different analyses applying ordinary one-way ANOVA and Tukey’s multiple tests were conducted to examine nerves count, surface area and density (the number of nerves within an area of 1 mm$^2$) within midlines and lateral regions of the prostatic halves. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparison of the total number of parasympathetic nerves (the PSNs number) across the ventral midline versus the ventral lateral regions and shows equal nerve distribution. Chart B shows the comparison of the ventral midline and ventral lateral regions’ parasympathetic nerves surface area, with no significant differences between these regions. Chart C shows that the parasympathetic nerves density (the number of nerves within an area of 1 mm$^2$) of the ventral midline and ventral lateral regions were consistent. Chart D illustrates the analysis of parasympathetic nerves’ total counts within the midline and lateral regions of the gland’s dorsal half, with no significant differences. Chart E represents the comparison of the dorsal midline and dorsal lateral regions’ parasympathetic nerves surface area, with significant differences between these regions since the DLL and DLR contained significantly more nerves than the dorsal midline. Chart F shows that parasympathetic nerves density in the dorsal midline and dorsal lateral regions was consistent. PSNs: Parasympathetic nerves. V: ventral; D: dorsal; VLL: left ventrolateral, VLR, right ventrolateral, DLL: left dorsolateral, DLR right dorsolateral.
Meanwhile, analysis of the sympathetic nerves revealed that their distribution was consistent among the dorsal half midline and lateral regions in terms of both total number and density. Nonetheless, their distribution within the ventral half was consistent in terms of density, but unequal in total number, because the ventral lateral regions contained significantly more sympathetic nerves than the ventral midlines (Figures 3.37 & 3.39).
Figure 3. 37: Sympathetic nerves were consistently distributed within the midline and lateral regions of the gland’s dorsal half. Sympathetic nerves were identified and counted automatically through an investigation of prostatic slides labelled with antibodies against tyrosine hydroxylase (TH). Three different analyses applying ordinary one-way ANOVA and Tukey’s multiple tests were conducted to examine nerves count, surface area and density (the number of nerves within an area of 1 mm$^2$) within midlines and lateral regions of the prostatic halves. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). Chart A represents the comparison of the total number of sympathetic nerves (the SNs number) across the prostatic ventral midline versus the ventral lateral regions. It shows inconsistent distribution since the lateral regions contained significantly more nerves. Chart B shows the comparison of the ventral midline and ventral lateral regions’ sympathetic nerves surface area, with no significant differences between these regions. Chart C shows that the sympathetic nerves density in the ventral midline and ventral lateral regions was consistent. Chart D represents the comparison of the total number of sympathetic nerves (the SNs number) across the prostatic dorsal midline versus the dorsal lateral regions. It shows consistent distribution between these areas. Chart E shows the comparison of the dorsal midline and dorsal lateral regions’ sympathetic nerves surface area, with no significant differences between these aspects. Chart F shows that the sympathetic nerves density in the dorsal midline and dorsal lateral regions were consistent. SNs: sympathetic nerves. V: ventral; D: dorsal; VLL: left ventrolateral, VLR, right ventrolateral, DLL: left dorsolateral, DLR right dorsolateral.
Finally, the investigation of blood vessels within the midline and lateral regions of the prostatic halves revealed that their density was significantly higher in the midlines than in the lateral regions in both halves. However, a consistent distribution was observed in the total number within the midline and lateral regions of the ventral and dorsal halves of the gland (Figure 3.38 & 3.39).
Figure 3.38: Blood vessels were unequally distributed in terms of their density in the midline and lateral regions of the prostatic halves. Blood vessels count were based on a manual investigation of the H&E-stained prostatic slides as shown in Figure 3.11. Two different analyses applying ordinary one-way ANOVA and Tukey’s multiple tests were conducted to examine blood vessels number and density (the number of vessels within an area of 1 mm²) within midlines and lateral regions of the prostatic halves. The charts’ bars show the means and standard errors of the mean, while the dots represent each prostate (n = 15). Chart A represents the comparison of the total number of blood vessels across the prostatic ventral midline versus the ventral lateral regions. It shows a consistent distribution of vessels in these areas. Chart B shows that blood vessel density in the ventral midline and ventral lateral regions were inconsistent. Chart C represents the comparison of the total number of blood vessels across the prostatic dorsal midline versus the dorsal lateral regions. It shows a consistent distribution between these areas. Chart D shows that the blood vessels density in the dorsal midline and dorsal lateral regions were inconsistent. V: ventral; D: dorsal; VLL: left ventrolateral, VLR, right ventrolateral, DLL: left dorsolateral, DLR right dorsolateral.
Figure 3. Rose diagrams showing the relative total number and density of neurovascular structures in the prostate. Rose diagrams A-C illustrate the number of nerves and blood vessels within prostatic sections. Rose diagrams D-F illustrate the density of neurovascular structures within the gland. The blue circle in each diagram shows the average number/density of nerves and vessels within all regions. PSNs: parasympathetic nerves; SNs: sympathetic nerves; V: ventral; VLL: left ventrolateral; VLR right ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; D: dorsal.
3.3.5 Overall summary of the histological results:

Neurovascular structures could be reliably identified throughout all prostatic levels and scattered across all the regions inside the prostate. However, there was evidence of significant structure-specific clustering within regions and levels of the prostate and can be summarized as follow:

- Parasympathetic nerves and blood vessels had a consistent distribution across all prostatic levels whereas sympathetic nerves were mainly located at the base of the gland.
- Blood vessels revealed an equal distribution within the ventral and dorsal halves of the prostate in contrast to nerves which were predominantly identified within dorsal regions of the gland.
- Total number of nerves and blood vessels within peripheral regions of the prostate was significantly higher than in the central regions.
- Sympathetic nerves and blood vessels revealed a consistent density distribution between peripheral and central regions of the prostate.
- Parasympathetic and sympathetic nerves density were consistently distributed across the ventral and dorsal midlines and the lateral regions of the gland.
- Blood vessels density was significantly higher in the midlines than in the lateral regions of the gland.
3.4 Discussion

Radical prostatectomy is the primary treatment choice for prostate cancer (Menon et al., 2007). A pioneering study by Walsh and Donker (Walsh & Donker, 1982) has suggested that the nerves responsible for erectile function are located within the dorsolateral aspects of the prostate, as part of the neurovascular bundle (NVB). Nevertheless, the belief that the NVB and cavernous nerves are located exclusively within the dorsolateral aspect of the gland has been contradicted by several investigations reporting different locations of these nerves within prostatic regions (Eichelberg et al., 2007; Kiyoshima et al., 2004; Tewari et al., 2006). This project expands on previous studies by providing a combined comprehensive, detailed description of the absolute number, density and precise location of each type of autonomic nerve fibre and blood vessel within all levels and aspects of the prostate.

Our investigation revealed that neurovascular structures were distributed throughout the gland. However, considerable intra-gland variability was observed, with notable differences in the nerves and blood vessels. In contrast to the sympathetic nerves, which were unequally distributed throughout the prostatic levels, the parasympathetic nerves and blood vessels had a consistent distribution across all prostatic levels. In contrast to the blood vessels which revealed a consistent distribution across prostatic halves, both types of nerves were markedly more abundant within the prostatic dorsal regions than the ventral aspects.

Strikingly, the blood vessels and nerve fibres did not correspond in terms of their locations across several regions within the prostate. For example, the DLL and DLR sections (located adjacent to the prostatic neurovascular bundles running along the surface of the gland), contained the most nerve fibres. In contrast, the blood vessels were most dense within the ventral and dorsal midline regions of the gland.
This finding was unexpected because blood vessels and nerves are routinely found in shared locations throughout the body and create neurovascular bundles that supply target tissues and organs (Larrivée et al., 2009). This phenomenon is largely based on shared developmental pathways (e.g., angiogenesis and neurogenesis) that are closely linked from cellular and molecular perspectives (Riquelme et al., 2008; Segura et al., 2009). Therefore, our discovery of the divergent distribution and localisation of blood vessels and nerves within the prostate was unanticipated. Angiogenesis uses pre-existing blood vessels to develop novel networks of vessels (Rajabi & Mousa, 2017). Although a direct assessment of angiogenic processes in response to prostate cancer has not been undertaken, the presence of tumours in all prostates examined has been found to lead to pathological angiogenesis (Melegh & Oltean, 2019) induced by tumour hypoxia (Fong, 2008).

Nerves have several interactions with the epithelium and stroma of the prostate and have essential functions in the development and preservation of the prostate. Therefore, several studies on rats and dogs have reported that the denervation of the pelvic plexus, through either chemical or mechanical means, alters prostate structure and function (Doggweiler et al., 1998; Lujan et al., 1998; Martínez-Piñeiro et al., 1993; Wang et al., 1991; Watanabe et al., 1988). Perineural invasion (PNI), a process in which tumour cells invade the area around nerves, is a common interaction between cancer and nerves in prostatic carcinoma and is considered the main reason for most of the extracapsular spread of prostatic tumours. PNI enables tumour cells to develop and survive within the perineural space (Ayala et al., 2008; Villers et al., 1989).

According to Ayala and colleagues (Ayala et al., 2008), in early stages of preneoplastic lesions and subsequent tumour stages, PNI may be preceded by neurogenesis. Neurogenesis, the formation of new nerve cells, has been reported to occur in prostate cancer, thus suggesting
that this process influences tumour development and progression (Mauffrey et al., 2019). Tumour-induced neurogenesis occurs in prostate adenocarcinoma through neural progenitor cell migration and variation in nerve cells into cancer (Cervantes-Villagrana et al., 2020). Thus, understanding the mechanisms underlying the interactions between tumour cells and nerves in neurogenesis can lead to improvements in cancer treatment.

Through our investigation, we revealed that the numbers of both types of nerves and blood vessels significantly decreased as they coursed from peripheral regions towards the urethra. This finding is consistent with those reported by Powell and colleagues (Powell et al., 2005), who have reported the presence of a different range of innervation within the same prostate. However, Bloch and colleagues (Bloch et al., 1997) have reported contradictory findings indicating no significant differences in nerve density between prostatic regions.

Moreover, our study revealed that the central regions of the prostate, which are close to the urethra, contain abundant nerves and vessels, thus suggesting that preservation of these structures during radical prostatectomy would be extremely difficult. This finding highlights the importance of understanding the prostatic neurovascular structures; such an understanding could contribute to developing new modifications in nerve sparing techniques to preserve these structures in the future—an approach that could have substantial benefits for patients post-surgery.

Although some prior studies have investigated nerve distribution within the prostate, they have considerable technical and methodological limitations. For instance, several of previous studies have performed quantification in specific regions of the gland (Eichelberg et al., 2007; Ganzer et al., 2008; Ganzer et al., 2012). Our approach overcomes this limitation by using an adapted
and advanced study design. The technique of the division of prostatic slides into 12 regions (six peripheral and six central) enhanced our quantification method, thereby enabling us to identify nerves and vessels in every aspect of the gland. Moreover, we performed a combined investigation of both the nerves and vessels within the gland, thus extending the analyses in previous studies (Ganzer et al., 2009; Kiyoshima et al., 2004; Sievert et al., 2008), which have focused on the prostatic nerves but neglected the blood vessels. Moreover, the previously cited studies lacked clear visualisation of the locations of neurovascular structures within the prostate. In contrast, our study used a heat map and rose diagram visualisation approaches (Figures 3.19 & 3.39) to enable clearer visualisation of the density and distribution of the internal neurovascular structures within the prostate.

Following our comprehensive investigation of the nerves and vessels within the prostate, the next chapter addresses the question of whether the distribution of these structures within the gland influences the nature and extent of positive outcomes after surgery.
Chapter 4 The relationship of prostatic neurovascular structures to clinical outcomes after radical prostatectomy

4.1 Introduction

Prostate cancer is among the most common causes of cancer deaths in men worldwide (Ferlay et al., 2010; Retel et al., 2014; Wright et al., 2013). Prostate cancer accounts for approximately 11% of all cancers in men and is responsible for approximately 8% of all tumour-related deaths in men (Bray et al., 2002; Heidenreich et al., 2008). Radical prostatectomy is the main treatment choice for people with prostate cancer. However, this procedure frequently results in two main complications: erectile dysfunction and urinary incontinence (Barazani et al., 2015; Lunacek et al., 2005).

According to previous research (Maas et al., 1998; Rabbani et al., 2000; Schapira et al., 2001; Sopko & Burnett, 2016; Stanford et al., 2000), between 10% and 100% of patients experience erectile dysfunction after radical prostatectomy, depending on factors including: (1) the patient’s age; (2) the severity of the case; (3) the patient’s erectile function before the operation; and (4) the experience of the surgeon.

As described previously, the cause of postoperative erectile dysfunction has been a matter of considerable debate, although it is typically attributed to nerve damage (Lue et al., 1983) and/or damage of the arterial supply (Van der Aa et al., 2003). Potency rates in patients undergoing unilateral preservation of the neurovascular bundle range between 11% and 58%, whereas those for patients undergoing bilateral reservation of the neurovascular bundle range between 68% and 82% (Catalona et al., 1999; Fowler et al., 1993; Geary et al., 1995; Gralnek et al., 2000; Rabbani et al., 2000). Beyond erectile dysfunction, urinary incontinence is a frequent major clinical complication after radical prostatectomy. However, recovery rates pertaining to
continence range from 47% to 97% post-surgery, depending on the type of nerve sparing procedure performed (Choi et al., 2011; Ko et al., 2012; Link et al., 2005; Zorn et al., 2007).

A study of the literature has revealed considerable limitations in studies investigating neurovascular structures within the prostate (Eichelberg et al., 2007; Ganzer et al., 2008; Ganzer et al., 2012; Kiyoshima et al., 2004; Sievert et al., 2008). No previous studies have assessed the correlation between neurovascular structures within the prostate and patients’ clinical outcomes after surgery. Consequently, those studies have been unable to directly investigate the hypothesis that neurovascular distribution within the gland directly contributes to post-surgical complications such as erectile dysfunction and urinary incontinence. Therefore, in this chapter, we describe the approach that we developed to overcome this limitation and to directly answer the question of whether the internal distribution of nerves and blood vessels within the prostate influences the nature and extent of complications (urinary incontinence and erectile dysfunction) after surgery.
4.2 Patients and methodology

Between April 2017 and August 2018, 15 patients underwent non-nerve-sparing laparoscopic radical prostatectomy (LRP) performed by a single surgeon at Ninewells Hospital, Dundee, UK. Patients’ data were collected with an ethical approval from the Tayside Biorepository at Ninewells Hospital, Dundee, UK with a reference number IGTCAL9861. Preoperative characteristics, such as patient age, prostatic specific antigen level (PSA), prostate weight and Gleason score are presented in Table 4.1. The patients were followed up for 2 years after surgery to assess complications such as erectile dysfunction and urinary incontinence. Erectile function recovery was defined as an erection that was satisfactory for intercourse without any additional aids, such as medications. Patients were considered fully continent if they were completely dry and using no pads. On the basis of data from Chapter 4, the distribution of nerves and blood vessels within the prostate correlated with the Gleason score for tumour and patient outcomes after surgery.

4.2.1 Statistical analysis

GraphPad Prism, Version 9.0.2 (161) was used to perform all statistical analyses. Unpaired two-tailed t-tests were performed, and Pearson correlation coefficients were determined. Values are reported as mean and standard error of the mean. P values <0.05 were considered statistically significant.
<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td><strong>Age (Years)</strong></td>
<td>67.8 ± 5.65</td>
<td>From 60 to 75 years</td>
</tr>
<tr>
<td><strong>PSA level ng/ml</strong></td>
<td>9.7 ± 4.35</td>
<td>From 5.1 to 18.7 ng/ml</td>
</tr>
<tr>
<td><strong>Gleason score</strong></td>
<td>7.8 ± 1.01</td>
<td>From 7 to 9</td>
</tr>
<tr>
<td><strong>Prostate weight (g)</strong></td>
<td>48.54 ± 7.84</td>
<td>From 36.3 to 65.6 g</td>
</tr>
</tbody>
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Table 4. 1: This table depicts the preoperative characteristics of patients who underwent non-nerve sparing laparoscopic radical prostatectomy. PSA: prostatic specific antigen; g: gram; SD: standard deviation; ng/ml: nanograms per millilitre.

### 4.3 Results

After surgery, all patients included in the study experienced erectile dysfunction and urinary incontinence. Three patients were excluded from the follow-up because of the presence of erectile dysfunction before surgery. However, none of the remaining 12 patients had recovered erectile function by the end of the 2-year follow-up (Figure 4.1A). However, the recovery period for urinary continence occurred over a variable timeframe (Figure 4.1B). After the first 3 months of follow-up, 73.30% of patients (n = 11) used three or more pads per day (no recovery), whereas 20% (n = 3) used one to two pads (partial recovery), and 6.7% (n = 1) used no pads (full recovery). After 9 months, the percentage of patients who were completely dry had increased to 40% (n = 6), and the percentage of patients who used three or more pads per day had decreased to 6.7% (n = 1); meanwhile, the remaining 53.3% (n = 8) used one to two pads per day. After 18 months, all patients had recovered urinary continence, except for one patient who still used three pads per day. However, 100% of patients (n = 15) were fully continent and using no pads by the 24th month of follow-up.
Figure 4. 1: Summary of patients’ complications following radical prostatectomy. No patients in this study recovered their erectile function. As Chart A shows, 100% of patients were impotent 24 months after their surgery. However, as Chart B shows, patients’ urinary continence recovery time varied. Note that the number of patients who recovered their continence increased over time, and 100% of patients regained normal urinary continence two years after their operation.
After assessing the patients’ complications after surgery, we examined whether the internal distribution of neurovascular structures within the prostate influenced the nature and extent of the complications experienced after surgery. It was observed that the total number of intraprostatic nerves was significantly lower in patients who recovered continence within 12 months after surgery than in patients whose recovery lasted 12 months or longer. However, no significant differences were identified in blood vessel numbers between groups (Figure 4.2).

Figure 4.2: Distribution of intra-prostatic neurovascular structures and urinary continence. Nerves number inside the prostate were found to be significantly lower for patients who recovered their continence less than 12 months after surgery compared to patients whose recovery took 12 months or longer. However, blood vessels distribution was consistent between the two groups. Three different analyses applying unpaired t-tests to compare the two patient groups: Group 1 recovered their urinary continence in less than 12 months, while Group 2 recovered their urinary continence in 12 months or more. The charts’ bars show the means and standard errors of the means, while the dots represent each prostate (n = 15). As charts A, B and C show, parasympathetic and sympathetic nerves were significantly fewer among the first group, whereas blood vessel distributions were the same across the two groups. UC: urinary continence; PSNs: Parasympathetic nerves, SNs: Sympathetic nerves.
Beyond the previous analysis, because we had the patients’ Gleason scores, we were able to examine whether tumour severity influenced the distribution of the neurovascular structures within the gland. Although the patients had different stages of prostate cancer, no significant differences were identified in the distribution of nerves and vessels within the gland and the aggressiveness of the prostatic tumour (Figure 4.3).

Figure 4.3: Regardless of patients’ Gleason scores of the tumour, the distributions of nerves and vessels within their prostate was consistent. Three different analyses applying unpaired *t*-tests to compare intra-prostatic neurovascular structures of patients with two different Gleason scores (7 and 9) were conducted. The charts’ bars show the means and standard errors of the means, while the dots represent the total number of prostates ($n = 15$). Charts A, B and C represent the comparisons of the total number of parasympathetic and sympathetic nerves and blood vessels, respectively, across prostates with Gleason-score-7 tumours compared to prostates with Gleason-score-9 tumours. These analyses revealed no significant differences in total number of nerves and blood vessels inside the prostate between the two groups. PSNs: parasympathetic nerves; SNs: sympathetic nerves.
4.4 Discussion

Although several studies have examined the nerves within the prostate and complications after radical prostatectomy, and their relationship to nerve sparing techniques (Fode et al., 2014; Ganzer et al., 2009; Stewart et al., 2011; Tal et al., 2009), none have indicated a direct connection between the neurovascular distribution within the gland and complications following surgery.

In this study, we overcame the limitations of previous studies by demonstrating that neurovascular structures within the gland directly contribute to the recovery period for urinary continence. Interestingly, we discovered that the number of intraprostatic nerves was significantly lower in glands from patients who recovered continence within 12 months than in those collected from patients whose recovery took 12 months or longer. Therefore, from a prognostic perspective, the total number of nerves within the prostate may determine post-surgery outcomes; i.e., patients with fewer neuronal structures in their prostates will have faster recovery from urinary incontinence after radical prostatectomy. This phenomenon may indicate a faster autonomic reinnervation after surgery in patients who had lower number of nerves inside their prostates. In addition, it may indicate a potential existence of continence nerves that were preserved during RP as they pass away from the prostate, and therefore, they influenced the urinary continence after surgery. Moreover, preservation of the neck of the urinary bladder during radical prostatectomy may explain this finding as it has been suggested to be associated with higher recovery rates and more rapid recovery of urinary control after surgery (Joseph et al., 2006). On the other hand, the number of blood vessels was consistent between groups, thus demonstrating no connection between the distribution of blood vessels within the gland and the recovery rate for urinary incontinence, thus, demonstrating no
connection between the distribution of blood vessels within the gland and the recovery rate for urinary incontinence.

A review of the literature revealed no previous studies directly addressing the influence of prostatic internal neurovascular structures on the extent of complications after radical prostatectomy. Nevertheless, several studies (Cambio & Evans, 2006; Coakley et al., 2002; Lee et al., 2010; Lee et al., 2013) have indicated that factors such as body weight, patient age, pelvic floor muscle dysfunction, prostate volume, length of the membranous urethra and surgical procedure crucially affect urinary continence after surgery.

Regarding the role of factors beyond nerve sparing, a significant correlation has emerged between perineal body tone (PBT) and urinary continence after radical prostatectomy (Rigatti et al., 2012): patients who were continent 1 month after surgery have been reported to have significantly higher perineometric measures before and after surgery than incontinent men. In another study in which all patients were advised to practice pelvic floor muscle exercises (PFME) after surgery, the perineometric measures were significantly higher in patients who recovered urinary control than those who did not by 3 months post-surgery (Sapsford & Hodges, 2001).

All patients included in this study underwent non-nerve-sparing laparoscopic radical prostatectomy and experienced erectile dysfunction as well as urinary incontinence after surgery. None of the patients had recovered erectile function by the 2 year follow-up, in contrast to the findings from two previous studies reporting potency rates of 10% and 38% 12 months after non-nerve-sparing LPR (Levinson et al., 2008; Salomon et al., 2002). Moreover, in this study, the recovery period for urinary continence varied. Full urinary continence was
recovered in one patient 3 months after surgery, five patients 6 months after surgery, six patients 9 months after surgery, 14 patients 12 or 18 months after surgery, and all patients (n = 15) by the 2-year follow-up. Applying the same procedure, Rigatti and colleagues have reported a urinary continence recovery rate of 53.3% (n = 8) within 3 months after surgery (Rigatti et al., 2012), whereas another study has reported a continence recovery rate of 43.5% (n = 20) in the same period after the same procedure (Srivastava et al., 2013).
Chapter 5 General Discussion

The previously reported data from studies investigating external and internal nerves, and blood vessels that supply the prostate have considerable limitations (Costello et al., 2011; Ganzer et al., 2008; Lepor et al., 1985; Moya et al., 2017). In this study, we overcame these limitations by applying a new dissection approach to the pelvis, which allowed us to preserve the entire prostate and reliably trace the external neurovascular structures of the prostate and corpora cavernosa in the same specimens from their origin to the location of penetration. In addition, we performed an adapted and advanced study design that both enhanced our quantification method and permitted us to identify nerves and vessels in every aspect of the gland. Moreover, we provided a better visual overview of the density and distribution of the internal neurovascular structures within the prostate through more sophisticated visualisation approaches.

In our investigation of the external and arterial supply to the prostate, the dorsolateral regions of the gland were the most common site of penetration. However, this finding contradicted our analysis of the internal blood vessels because their higher density within the gland fell within the prostatic midlines. In addition, the internal distribution of the blood vessels was comparable between the ventral and dorsal halves of the gland. However, we discovered that the blood vessels supplying the gland from its dorsal half were significantly more abundant than those penetrating its ventral surface. These two findings may suggest that after entering the gland, the blood vessels yield several internal branches that course towards the ventral and midline regions. In contrast, the distributions of external and internal nerves around and within the gland were correlated. We identified the dorsolateral region as the most common locus of penetration by the external nerves. Likewise, the dorsolateral regions notably contained the
most nerves within the gland. Moreover, the dorsal half of the gland was significantly richer in external and internal nerves than the ventral half.

In our study, the middle rectal artery was identified as the least common source for prostatic arteries. However, an image-based study by Bilhim et al. (2013), reported that the prostatic artery shared a common trunk with the middle rectal artery in 70% (n = 56/80) of investigated cases (Bilhim et al., 2013). Surprisingly, in a later study, Bilhim and colleagues (Bilhim et al., 2014) reported an unexpected source of the prostatic artery derived from the accessory obturator artery, a branch of the inferior epigastric artery or the external iliac artery, with an incidence of 1.8% (n = 9/491). Another rare source of prostatic arteries was reported by Clegg (Clegg, 1955), who identified 32.1% (9/28) of prostatic arteries as being derived from the superior rectal artery.

The present study revealed that the parasympathetic nerves were consistently distributed across the prostatic levels, in contrast to the sympathetic nerves, which were significantly more abundant in the base region. The nerve distribution findings from this study are supported by previous work by Ganzer and colleagues (Ganzer et al., 2012), who have also found that the number of sympathetic nerves decreases from the base to the apex. In contrast, Costello and colleagues (Costello et al., 2011) have reported a consistent distribution of both types of nerves across all prostatic levels. Moreover, Eichelberg et al. (2007) have investigated the total number of nerve fibres within the prostate, without specifically identifying their subtypes, and have reported a significantly lower median number at the apex than in the body and the base (Eichelberg et al., 2007).
We observed that the DLL and DLR regions contained the most parasympathetic and sympathetic nerves, with 45.85% and 47% at the base, 46.72% and 48% at the body, and 46.14% and 48% at the apex. These findings correspond to the results from two other studies (Costello et al., 2011; Ganzer et al., 2012) reporting the identification of both types of nerves across all levels within the dorsolateral regions of the gland. Two additional studies have reported that a substantial number of nerves; i.e., 20%–30%, are located in the ventral half of the prostate (Eichelberg et al., 2007; Sievert et al., 2008). We confirmed that most nerves were located within the dorsal half of the prostate, and only 37% of the parasympathetic nerves and 33% of the sympathetic nerves were detected in the ventral aspect. Kiyoshima and colleagues (Kiyoshima et al., 2004) have observed a neurovascular bundle dorsolateral to the prostate in 48% of cases; in the remaining 52% of cases, the nerves had a spray-like distribution over the lateral and anterior aspects of the prostate, without definite bundle formation. Kaiho and colleagues (Kaiho et al., 2009) have performed electrical stimulation of the periprostatic nerve fibres in patients during radical prostatectomy and confirmed prior findings indicating that, in addition to the nerves present within the neurovascular bundle dorsolateral to the prostate, nerve fibres are present along the ventral and lateral aspects of the prostate.

The peripheral regions were significantly larger than central regions of the prostate, according to our division of the prostate. Thus, to test whether any differences existed in the relative number of nerves and vessels, we examined the density of vessels and nerves. These analyses revealed that the density of the sympathetic nerves and blood vessels was consistent between the peripheral and central regions; in contrast, the parasympathetic nerves had significantly higher density within the peripheral regions. Thus, we concluded that, given its small size, the central aspect of the gland contains abundant nerves and blood vessels. Hence, this finding highlights the importance of understanding the prostatic neurovascular structures; such an
understanding could contribute to developing new modifications in nerve sparing techniques to preserve these structures in the future—an approach that could have substantial benefits for patients’ post-surgery.

Regardless of age, no patients included in our study recovered erectile function after radical prostatectomy. This finding correlates with those of Kübler and colleagues (Kübler et al., 2007), who have stated that there no relationship exists between the time to regain erectile function and the prostate size or patient age. Similarly, another investigation has confirmed those findings, reporting that neither prostate size nor patient age is associated with impotence recovery time (Wiygul et al., 2005). However, these findings contradict those of Hollenbeck and colleagues (Hollenbeck et al., 2003), who have reported that smaller prostate size and younger patient age are associated with significantly better erectile function recovery rates after surgery. Supporting these findings, Ploussard et al. (2011) have observed that of 740 patients, 563 of whom underwent a bilateral nerve-sparing procedure and 177 of whom underwent a unilateral nerve-sparing procedure, the erectile function recovery rate significantly correlated with age, and patients younger than 60 years had better recovery rates (Ploussard et al., 2011).

Regarding the roles of factors not associated with nerve sparing during the procedure, Lee and colleagues (Lee et al., 2013) have noted that different types of nerve-sparing procedures (non-nerve-sparing, unilateral nerve-sparing and bilateral nerve-sparing) are not correlated with the duration over which patients regain continence; however, the type of surgical procedure (extrafascial or intrafascial) significantly influences urinary continence recovery times. Patients who underwent an intrafascial technique were found to have shorter recovery times than those who underwent an extrafascial technique. Interestingly, Coakley et al. (2002) have reported that the stabilisation of urinary continence in patients after radical prostatectomy is
significantly correlated with the length of the membranous urethra: a shorter time to regain urinary control is associated with a longer membranous urethra (Coakley et al., 2002). This finding had been observed several years earlier by Kleinhans and colleagues (Kleinhans et al., 1999), who proposed a correlation between urinary incontinence post-surgery and the length of the membranous urethra.

On the other hand, another investigation reported no correlation between membranous urethral length and the time to regain urinary control after radical prostatectomy (Borin et al., 2007). Confirming the role of patient age in regaining urinary continence post-surgery, Catalona and colleagues (Catalona et al., 1999) have reported that in 92% of patients (n=1223), regaining control is correlated with age and not nerve-sparing techniques or tumour stage. However, another study has reported that age is not a factor, but that the nerve-sparing technique, the prostate volume and the length of the prostatic urethra are significant variables in continence recovery (Oefelein, 2004).
Chapter 6 Conclusion and future research

6.1 Conclusion

Chapter two of this thesis aimed to precisely identify the external nerves and blood supply to the prostate and the corpora cavernosa. Therefore, this chapter can be concluded as follow:

- A novel anatomical dissection approach with preservation of the prostate in situ was developed and successfully repeated in 24 hemipelvises.
- A total of 48 prostatic arteries were identified and found to be derived either directly from the internal iliac artery or from one of its branches.
- No statistically significant variations were noted between the right and left sides of the pelvis regarding the number of prostatic arteries.
- Regardless of their source, no significant differences were observed in prostatic artery diameter.
- The prostatic arteries were identified to supply the gland from different regions, most commonly the dorsolateral aspect.
- The most inferior part of the pelvic plexus provides branches that run between the prostate and the rectum as the cavernous nerves.
Chapter three of this thesis aimed to provide a combined comprehensive, detailed histological investigation of sympathetic and parasympathetic nerves as well as blood vessels within the prostate. Thus, the take home message of this chapter was:

- Both nerves and blood vessels were present across all prostatic levels and regions.
- Widespread regional differences in the localization of nerves and blood vessels within the prostate.
- A surprising disconnect between the localization of nerves and blood vessels, showing that they are predominantly localised to different regions of the prostate.
- Both types of nerves were found predominantly in the dorsal half of the gland, thus demonstrating a significant difference from the ventral half.
- Sparing of dorsal and peripheral aspects of the gland during surgery will be required to protect the majority of neuronal structures and decrease complications following radical prostatectomy.

Chapter four of this thesis aimed to investigate the relationship between the prostatic internal nerves and vessels and the clinical outcomes after radical prostatectomy and it can be summarised as follow:

- No patients in this study recovered erectile function during the duration of the follow-up period: 100% of the patients were impotent 24 months after the surgery.
- All patients recovered urinary continence; however, the recovery period occurred over a variable time course.
- The number of intraprostatic nerves was significantly lower in patients who recovered continence within 12 months after the surgery than in patients whose recovery lasted 12 months or longer.
No significant correlation was identified between the distribution of the neurovascular structures within the gland and the level of aggression of the prostatic tumour.
6.2 Future research

Although approximately 40 years have passed since the pioneering study of nerve sparing radical prostatectomy by Walsh and Donker (Walsh & Donker, 1982), the neuroanatomical details of the prostate remain unclear, and urologists must consider patient variability during prostatectomy and alter their dissection strategies accordingly. The pelvic plexus lies within the fibrofatty connective tissue between the bladder and the rectum, and lymph nodes can cover this area; thus, considering the location of the pelvic lymph nodes during radical prostatectomy is essential (Heidenreich et al., 2002; Touijer et al., 2007). Given the anatomy of the pelvis and the pelvic plexus, the erectile nerves are at risk during lymphadenectomy and medial dissection of the area of the internal iliac arteries and towards the bladder wall. The nerves might also be affected during node removal in the presacral area or any location medial to the common iliac vessels. Accordingly, several studies have reported that patients with extensive node removal have poorer erectile function than patients with conservative or no lymphadenectomy (Sagalovich et al., 2013; van der Poel et al., 2012).

Moreover, in light of knowledge that was generated and discussed in this project, evidently, more research is needed to ensure better characterisation of the neuronal, vascular and lymphatic structures proximal to the prostate and their anatomical relationships with the gland to improve the anatomical and functional understanding of the components of periprostatic neurovascular bundles. The next steps of research should focus on:

- Clinical anatomy studies based on many cadavers from well-characterised population cohorts to ensure the identification of more anatomical variations in the distribution of the NVB and the course of cavernous nerve fibres.
- Immunohistochemical analyses using up-to-date phenotyping markers in tissue resected from patients undergoing nerve-sparing versus non-nerve-sparing
prostatectomy, to accurately identify the locations of nerve fibres, blood vessels and lymphatics.

- Traditional immunohistochemical analysis should be combined with modern techniques, such as 3D-reconstruction and computerised planimetry, to carefully map the association of neurovascular structures with the prostatic fasciae and capsule. In addition, use of state-of-the-art, high-resolution visualisation and image analysis tools is also recommended.

- More work is also required to characterise the functional properties (sympathetic, parasympathetic and sensory) of the periprostatic nerves through immunohistochemistry and specific markers able to differentiate neuronal subtypes.

- Investigate the distribution of internal prostatic neurovascular structures in prostates with low-grade cancer vs. medium-grade cancer vs. high-grade cancer to analyse whether the cancer grade affected the distribution of nerves and blood vessels inside the gland.

- Investigate the density of prostatic neurovascular structures within the region of tumour in comparison to other regions in the same prostate to examine whether the tumour influenced the nature and extent of internal distribution of nerves and blood vessels within the prostate.
Appendix

Appendix 1. 1: Relationship of prostatic weight to the distribution of neurovascular structures inside the gland ................................................................. 198
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Appendix 1. 1: Relationship of prostatic weight to the distribution of neurovascular structures inside the gland. As shown in figures (A, B and C), no significant correlation was identified between the weight of the prostate and number of intra-prostatic nerves and vessels. PSNs: parasympathetic nerves; SNs: sympathetic nerves.

Appendix 1. 2: Relationship of the PSA level to the total number of neurovascular structures inside the prostate. As revealed in figures (A, B and C), no significant correlation was identified between the level of PSA and number of intra-prostatic nerves and vessels. PSA: prostate-specific antigen; PSNs: parasympathetic nerves; SNs: sympathetic nerves.
Appendix 1.3: Total number of prostatic neurovascular structures within defined anatomical regions across all levels of the gland. The table shows the mean number of nerves and vessels, and standard deviation. The number of nerves and vessels within each section was compared between levels. P values <0.05 were considered statistically significant. VLL: left ventrolateral, VLR: right ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; SD: standard deviation.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Neurovascular structures</th>
<th>Apex</th>
<th>Body</th>
<th>Base</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean number ± SD (%)</td>
<td>Mean number ± SD (%)</td>
<td>Mean number ± SD (%)</td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>Parasympathetic nerves</td>
<td>10140 ± 7989 (9.0)</td>
<td>13555 ± 13312 (9.8)</td>
<td>17254 ± 25320 (10.8)</td>
<td>0.33 ns</td>
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<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>6842 ± 5581 (7.9)</td>
<td>9018 ± 6891 (7.2)</td>
<td>12451 ± 9384 (7.0)</td>
<td>0.037*</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>308 ± 257 (18.5)</td>
<td>297 ± 158 (15.2)</td>
<td>283 ± 134 (12.8)</td>
<td>0.89 ns</td>
</tr>
<tr>
<td>VLL</td>
<td>Parasympathetic nerves</td>
<td>19275 ± 13764 (17.0)</td>
<td>18714 ± 13442 (13.6)</td>
<td>16412 ± 9503 (10.2)</td>
<td>0.71 ns</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>11787 ± 7202 (13.6)</td>
<td>15238 ± 12171 (12.2)</td>
<td>20134 ± 14740 (12.0)</td>
<td>0.06 ns</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>273 ± 176 (16.4)</td>
<td>287 ± 119 (14.7)</td>
<td>387 ± 164 (17.6)</td>
<td>0.01*</td>
</tr>
<tr>
<td>VLR</td>
<td>Parasympathetic nerves</td>
<td>16234 ± 11004 (14.0)</td>
<td>19440 ± 19730 (14.1)</td>
<td>20490 ± 15902 (12.8)</td>
<td>0.68 ns</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>13347 ± 12619 (15.5)</td>
<td>16012 ± 12576 (13.0)</td>
<td>19627 ± 13562 (11.0)</td>
<td>0.26 ns</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>253 ± 146 (15.2)</td>
<td>271 ± 122 (13.9)</td>
<td>330 ± 170 (15.0)</td>
<td>0.14 ns</td>
</tr>
<tr>
<td>DLL</td>
<td>Parasympathetic nerves</td>
<td>31977 ± 22486 (28.0)</td>
<td>35563 ± 26975 (25.8)</td>
<td>31623 ± 21691 (19.8)</td>
<td>0.75 ns</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>21315 ± 10130 (24.7)</td>
<td>27450 ± 19460 (22.0)</td>
<td>36959 ± 24589 (22.0)</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>341 ± 159 (20.5)</td>
<td>388 ± 202 (19.9)</td>
<td>439 ± 220 (20.0)</td>
<td>0.25 ns</td>
</tr>
<tr>
<td>DLR</td>
<td>Parasympathetic nerves</td>
<td>20810 ± 10127 (18.0)</td>
<td>28697 ± 20275 (20.8)</td>
<td>41507 ± 30204 (26.0)</td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>19709 ± 12295 (22.8)</td>
<td>32827 ± 23879 (26.3)</td>
<td>43374 ± 35164 (25.0)</td>
<td>0.008*</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>260 ± 107 (15.6)</td>
<td>339 ± 163 (17.4)</td>
<td>408 ± 193 (18.5)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Dorsal</td>
<td>Parasympathetic nerves</td>
<td>15967 ± 12180 (14.0)</td>
<td>21575 ± 18116 (15.6)</td>
<td>32229 ± 29700 (20.2)</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>13102 ± 7147 (15.2)</td>
<td>24071 ± 19383 (19.3)</td>
<td>38780 ± 31866 (23.0)</td>
<td>0.0005*</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>226 ± 128 (13.6)</td>
<td>362 ± 147 (18.6)</td>
<td>350 ± 140 (15.9)</td>
<td>0.0008*</td>
</tr>
<tr>
<td>Regions</td>
<td>Neurovascular structures</td>
<td>Apex</td>
<td>Body</td>
<td>Base</td>
<td>P value</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>Mean density in 1 mm² ± SD</td>
<td>Mean density in 1 mm² ± SD</td>
<td>Mean density in 1 mm² ± SD</td>
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<tr>
<td>Ventral</td>
<td>Parasympathetic nerves</td>
<td>104 ± 83</td>
<td>88 ± 80</td>
<td>122 ± 177</td>
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<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>69 ± 46</td>
<td>63 ± 52</td>
<td>100 ± 87</td>
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</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>3.62 ± 3.07</td>
<td>2.17 ± 1.41</td>
<td>2.14 ± 1.10</td>
<td>0.006 *</td>
</tr>
<tr>
<td>VLL</td>
<td>Parasympathetic nerves</td>
<td>132 ± 80</td>
<td>90 ± 63</td>
<td>81 ± 51</td>
<td>0.01 *</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>86 ± 52</td>
<td>78 ± 71</td>
<td>106 ± 89</td>
<td>0.28 ns</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>2.20 ± 1.90</td>
<td>1.42 ± 0.57</td>
<td>1.91 ± 0.86</td>
<td>0.008</td>
</tr>
<tr>
<td>VLR</td>
<td>Parasympathetic nerves</td>
<td>109 ± 69</td>
<td>86 ± 66</td>
<td>112 ± 109</td>
<td>0.29 ns</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>93 ± 76</td>
<td>78 ± 65</td>
<td>109 ± 86</td>
<td>0.22 ns</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>1.94 ± 1.43</td>
<td>1.34 ± 0.69</td>
<td>1.72 ± 0.94</td>
<td>0.03 *</td>
</tr>
<tr>
<td>DLL</td>
<td>Parasympathetic nerves</td>
<td>167 ± 101</td>
<td>127 ± 94</td>
<td>124 ± 79</td>
<td>0.19 ns</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>130 ± 73</td>
<td>107 ± 81</td>
<td>157 ± 116</td>
<td>0.07 ns</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>1.80 ± 0.66</td>
<td>1.43 ± 0.65</td>
<td>1.71 ± 0.84</td>
<td>0.06 ns</td>
</tr>
<tr>
<td>DLR</td>
<td>Parasympathetic nerves</td>
<td>128 ± 88</td>
<td>109 ± 70</td>
<td>175 ± 146</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>120 ± 88</td>
<td>126 ± 93</td>
<td>197 ± 184</td>
<td>0.04 *</td>
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<td>Blood vessels</td>
<td>1.54 ± 0.88</td>
<td>1.33 ± 0.71</td>
<td>1.67 ± 0.80</td>
<td>0.16 ns</td>
</tr>
<tr>
<td>Dorsal</td>
<td>Parasympathetic nerves</td>
<td>163 ± 142</td>
<td>131 ± 92</td>
<td>195 ± 142</td>
<td>0.07 *</td>
</tr>
<tr>
<td></td>
<td>Sympathetic nerves</td>
<td>140 ± 100</td>
<td>157 ± 132</td>
<td>278 ± 270</td>
<td>0.008 *</td>
</tr>
<tr>
<td></td>
<td>Blood vessels</td>
<td>2.09 ± 0.97</td>
<td>2.23 ± 0.88</td>
<td>2.33 ± 1.15</td>
<td>0.71 ns</td>
</tr>
</tbody>
</table>

Appendix 1.4: Density of prostatic nerves and blood vessels within defined anatomical regions across all levels of the gland. The table shows the mean density of nerves and vessels, and standard deviation. The density of nerves and vessels within each section was compared between levels. P values <0.05 were considered statistically significant. VLL: left ventrolateral, VLR: right ventrolateral; DLL: left dorsolateral; DLR: right dorsolateral; SD: standard deviation.
References:


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