ESTABLISHMENT OF THE AUTONOMIC NEUROANATOMY TO THE VULVAL ERECTILE TISSUES

by

SHONA MARIE LOUISE PENHALE

B.Sc., The University of British Columbia, 2000

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE

in

FACULTY OF GRADUATE STUDIES

(DEPARTMENT OF ANATOMY)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 2003

© Shona Marie Louise Penhale
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of  Anatomy
The University of British Columbia
Vancouver, Canada
Date  Oct 10, 2003
ABSTRACT

Erectile tissues are the vascular structures within the vulva that fill with blood and are an integral component of the woman’s sexual response. Despite the importance of these structures to her sexual function, the detailed neuroanatomical pathways of the autonomic nerves underlying this mechanism of vascular congestion, have not been elucidated. Therefore, this research had been designed to identify the missing components to answer the question: “What is the pathway of the nerves running from the female pelvic plexus to the external genitalia?”

In the male, the autonomic innervation of the erectile tissue in the penis has been fully described such that damage from surgery can be avoided or minimized whenever possible. The cavernosal nerves arise from the prostatic plexus and pierce the urogenital diaphragm (UGD) to supply the erectile tissue of the cavernosal bodies. In contrast, the description of the innervation of the female erectile tissue is much less complete and there are no descriptions of the nerves between the vaginal plexus and the “anterior parts”. Specifically, their origin from the vaginal plexus and their precise path to the erectile structures of the vulva are unknown and any similarities to male cavernosal nerves are unknown. Additionally, whether or not there are individual branches to the specific erectile structures, which include the right and left clitoral corpora, bisected vestibular bulb and periurethral tissues is also unclear.

Utilizing cadaver tissue, the complete pathway of the cavernous nerve in the female was examined using several methods. Using gross dissection of intact pelvises I identified previously well documented structures of the reproductive organs, major pelvic vasculature, the pelvic promontory, the sacral nerves, the sympathetic chain ganglia, the superior hypogastric plexus (SHP), the inferior hypogastric plexus, and the pelvic splanchnic nerves. The putative cavernous nerve was then identified on the lateral wall of the vagina at the level of the vesicouterine pouch. At this point it coursed inferiorly along with the vagina and
urethra towards the muscular urogenital diaphragm (UGD). During the inferior course it moved anteriorly into the dense connective tissue between the urethra and vagina and ended up 5 mm lateral to the urethra at the level of the UGD. Previously the cavernous nerve was described as a single, large, easily dissected nerve within the erectile tissue that surrounds the periurethral sponge. In contrast, serial sectioning followed by histological analysis allowed me to determine that the cavernous nerve was most commonly a single nerve bundle that branched into 2-5 smaller nerves upon piercing the UGD. Several branches then coursed laterally, came within 1 mm of the pudendal nerve, and innervated the crua; more medial branches carried on in their orientation to feed the vestibular bulbs.

In both serial sectioning and macro dissection I was able to identify differences from previously documented structural anatomy of the bisected vestibular bulbs. We found all our specimens to have much more erectile tissue of the bulbs continue around the anterior wall of the urethra in the shape of an inverted “u”. No evidence of the ‘bisected’ nature of the bulbs was found, as well the bulbs had more fullness of depth than previously documented.

This research has provided a more detailed description of the pathway of the autonomic innervation as it passes through the pelvis to the external genitals than had been previously described in the literature. In particular the course of the cavernous nerve as it passes along the lateral wall of the vagina to pierce the UGD and branch to feed the various erectile tissues. This detailed description should enable the necessary immunohistochemical studies to confirm nerve content. It has also raised questions about the previously established bisected structure of the vestibular bulbs which may have significant implications for engorgement in sexual arousal.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>viii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ix</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>HISTORICAL REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>PELVIC ANATOMY</td>
<td>5</td>
</tr>
<tr>
<td>Bone and muscle structure</td>
<td>5</td>
</tr>
<tr>
<td>Reproductive tract</td>
<td>6</td>
</tr>
<tr>
<td>Perineum</td>
<td>7</td>
</tr>
<tr>
<td>Innervation</td>
<td>9</td>
</tr>
<tr>
<td>FIGURES 1-7</td>
<td>12-24</td>
</tr>
<tr>
<td>THESIS OBJECTIVES AND RATIONALE</td>
<td>25</td>
</tr>
<tr>
<td>METHODS AND MATERIALS</td>
<td>26</td>
</tr>
<tr>
<td>TISSUE PREPARATION</td>
<td>26</td>
</tr>
<tr>
<td>GROSS ANATOMICAL DISSECTION</td>
<td>26</td>
</tr>
<tr>
<td>HISTOLOGICAL PROCESSING</td>
<td>27</td>
</tr>
<tr>
<td>3D MODEL</td>
<td>29</td>
</tr>
<tr>
<td>TABLE 1</td>
<td>32</td>
</tr>
<tr>
<td>FIGURE 8-13</td>
<td>34-44</td>
</tr>
<tr>
<td>RESULTS</td>
<td>45</td>
</tr>
<tr>
<td>MACROSCOPIC</td>
<td>45</td>
</tr>
<tr>
<td>MICROSCOPIC</td>
<td>47</td>
</tr>
<tr>
<td>FIGURES 14-20</td>
<td>51-75</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>76</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>83</td>
</tr>
</tbody>
</table>
APPENDIX A-B...........................................................................................................88-89

LIST OF TABLES

Table 1. Table of variety of specimens and techniques used 32
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure #</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 1</td>
<td>Representative course of the cavernous nerve in the male</td>
<td>16</td>
</tr>
<tr>
<td>Fig. 2</td>
<td>Pelvic bone structure</td>
<td>18</td>
</tr>
<tr>
<td>Fig. 3</td>
<td>Muscles of the pelvic diaphragm</td>
<td>20</td>
</tr>
<tr>
<td>Fig. 4</td>
<td>Suspension of the pelvic organs</td>
<td>22</td>
</tr>
<tr>
<td>Fig. 5</td>
<td>Urogenital and anal triangles</td>
<td>24</td>
</tr>
<tr>
<td>Fig. 6</td>
<td>Urogenital diaphragm</td>
<td>26</td>
</tr>
<tr>
<td>Fig. 7</td>
<td>Innervation of the pelvic viscera</td>
<td>28</td>
</tr>
<tr>
<td>Fig. 8</td>
<td>Illustration of removal/access of abdomen and pelvis</td>
<td>34</td>
</tr>
<tr>
<td>Fig. 9</td>
<td>Mid-sagittal pelvis with rectal branches</td>
<td>36</td>
</tr>
<tr>
<td>Fig. 10</td>
<td>Sample of tissue blocks removed for processing</td>
<td>38</td>
</tr>
<tr>
<td>Fig. 11</td>
<td>Serial section used in 3D reconstruction</td>
<td>40</td>
</tr>
<tr>
<td>Fig. 12</td>
<td>Schematic of 3D image development</td>
<td>42</td>
</tr>
<tr>
<td>Fig. 13</td>
<td>Different views of 3D image</td>
<td>44</td>
</tr>
<tr>
<td>Fig. 14</td>
<td>Gross anatomical dissection of pelvis</td>
<td>51</td>
</tr>
<tr>
<td>Fig. 15</td>
<td>Lateral pelvic wall dissection</td>
<td>53</td>
</tr>
<tr>
<td>Fig. 16</td>
<td>Lateral pelvic wall with IHP visualization</td>
<td>55</td>
</tr>
<tr>
<td>Fig. 17</td>
<td>Lateral view of dissected UGD</td>
<td>57</td>
</tr>
<tr>
<td>Fig. 18</td>
<td>Histological cross-section of cavernous nerve origin</td>
<td>59</td>
</tr>
<tr>
<td>Fig. 19</td>
<td>Histological cross-section UGD</td>
<td>61</td>
</tr>
<tr>
<td>Fig. 20</td>
<td>Histological cross-section of external genitalia</td>
<td>63</td>
</tr>
<tr>
<td>Fig. 21</td>
<td>Histological sections of UGD and area – specimen 7</td>
<td>65</td>
</tr>
<tr>
<td>Fig. 22</td>
<td>Histological sections of UGD and area – specimen 22</td>
<td>67</td>
</tr>
<tr>
<td>Fig. 23</td>
<td>Histological sections of UGD and area – specimen 24</td>
<td>69</td>
</tr>
<tr>
<td>Fig. 24</td>
<td>Histological sections of UGD and area – specimen 9</td>
<td>71</td>
</tr>
<tr>
<td>Fig. 25</td>
<td>Histological whole mount section of UGD</td>
<td>73</td>
</tr>
<tr>
<td>Fig. 26</td>
<td>Illustration of vestibular bulb structure and orientation</td>
<td>75</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>IHP</td>
<td>Inferior Hypogastric Plexus</td>
</tr>
<tr>
<td>L2</td>
<td>Lumbar Nerve 2</td>
</tr>
<tr>
<td>PNS</td>
<td>Parasympathetic Nervous System</td>
</tr>
<tr>
<td>S2</td>
<td>Sacral Nerve 2</td>
</tr>
<tr>
<td>S4</td>
<td>Sacral Nerve 4</td>
</tr>
<tr>
<td>SHP</td>
<td>Superior Hypogastric Plexus</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
</tr>
<tr>
<td>T11</td>
<td>Thoracic Nerve 11</td>
</tr>
<tr>
<td>UGD</td>
<td>Urogenital Diaphragm</td>
</tr>
</tbody>
</table>
LIST OF APPENDICES

Appendix A - Permission from Elsevier for journal figure use..................................................88
Appendix B - Ethics Approval from UBC......................................................................................89
ACKNOWLEDGMENTS

As I write this, the last page of my work, I find myself truly inspired by and grateful for the experiences I have had in this department. Thus thanks must first go to everyone involved with the Roskelley lab. Thanks for your patience and for helping me navigate the grad studies maze.

To the faculty and staff in the Department of Anatomy: Our Friday afternoons together were a great source of enjoyment and enlightenment for me. I won’t miss the smell but I will miss Charlie’s perspective on things, Wayne’s enthusiasm, and the smiles of Betty, Louise and Donna which always greeted me. Bruce, I can never repay you for the countless times you lent me your ear. Thanks to all of you for your never ending support and encouragement....

A very special thank you to Kim Fredriksen and Elizabeth Wellwood. Both of you gave time well beyond what was required of you. You saved me countless hours and always help me stay focussed. Cameron and Karen, your dedication to perfection took my data from ordinary to extraordinary. What started as a research project has culminated in a friendship that will last a lifetime.

To my committee: Drs. Sydney Thompson, Cal Roskelley, Wayne Vogl and Rosemary Basson. Dr. Thompson, thank you not only for participating at my defence but also for your encouragement along the way. Cal, you took a chance on being my supervisor and have given me a second chance to live my dream. “Thank you” seems so lame by comparison. Wayne, your enthusiasm for anatomy is an inspiration to all those around you; thank you for instilling it in me. Rosemary, you are everything one wishes for in a mentor. You have given me such big shoes to fill.

To my husband Bob: thank you for your constant support in every area of our lives together. Despite the difficulties, it is the growing we have both done as individuals that makes us so strong as a couple. To the rest of my two families, thank you. Your unconditional support of Bob and me was a crucial factor in the completion of this work.

Finally, I would like to dedicate this thesis to my beautiful daughters Grace and Katherine. When things got crazy you were always there to bring me back to what is important in life. Some day I hope you will truly understand not only the joy you have brought into my life but that it was the two of you that inspired my belief that anything is possible, you just have to use your imagination. May your desire to learn and grow as individuals throughout your lifetime be as enduring as my love for you.
INTRODUCTION

HISTORICAL REVIEW

The study of functional neuroanatomy has a long history. Claudius Galen of Pergammon (AD 129-199) was a talented anatomic dissector who provided the earliest known description of visceral innervation (the ganglionated sympathetic trunk and its neuronal distributions). He also discovered the anatomic and physiologic basis for the peripheral nervous system after experimental observations that injury to a peripheral nerve produced consequences that included a loss of muscle activity or skin sensitivity. This work resulted in Galen being credited with the discovery of the scientific method. The sophistication of his work becomes all the more remarkable when one recognizes that it was done close to 2,000 years ago.

Vesalius, who wrote *De Humanis Corporis Fabrica*, the first complete anatomy textbook, and many other scientist continued to enunciate the mysteries of the human body and by the 1600's neuroanatomical studies began to accelerate. In 1664 Thomas Willis coined the term “neurology” with the first reasonably accurate drawings of the sympathetic trunk branches, white and grey rami, splanchnic nerves, prevertebral ganglia, and visceral plexuses. His research gave the first physical representation to the theories of innervation of the body (qtd.in Feindel, 1970).

In the late 1700's anatomy continued to evolve into what is commonly considered the “classical” form. In general this was marked by efforts to more precisely portray the general anatomical structures that were already known. For example, Walter, 1804 described spinal nerve outflow and the nerve plexus organization of the thorax, abdomen, and pelvis of the human male in remarkable detail. His illustrations are probably the first to explicitly represent the nerve supply to the visceral structures in the pelvis.

Tiedemann, Beck, and Lee (1822) provided the first modern descriptions of the abdominal and pelvic sympathetic chain ganglia that supply the uterus and adjacent
structures in the female. Of particular note is the work of Frankenhauser, (1865) who went to
great lengths to describe the pelvic origins of the sympathetic nervous system as it related to
uterine anatomy (qtd. in Davis, 1933).

Commensurate with the efforts to describe the physical layout of the human nervous
system were experiments designed to determine function. However, it wasn’t until the mid
1800s that Claude Bernard, whose series of nerve transection experiments in rabbits,
confirmed the existence of efferent and afferent pathways that carry out visceral spinal cord
reflexes (qtd. in Olmsted, 1970).

In 1831 Michael Faraday invented the electrical generator and thus enabled the study
of electrophysiology. Its first known application to pelvic innervation research was by
Budge (1858), who demonstrated that electrical stimulation of the inferior mesenteric
ganglion or hypogastric nerves in rabbits produced contractile responses in the vas deferens
and seminal vesicles. Just five years later Conrad Eckhard established the role of the visceral
rami of the sacral nerves by demonstrating that their stimulation led to penile erection in the
dog (Ekhart, 1863)(qtd in Davis, 1933). John Newport Langley later expanded his studies
into neuropharmacology and his studies of the effects of nicotine enabled him to map out
preganglionic and postganglionic neurons (Langley, 1916); terminology that is still in use
today. Other terms that he coined include “autonomic nervous system” during his study of
the male pelvis (Langley et al., 1895) and “parasympathetic” based on his studies using
nicotine and curare (Langley, 1906).

The groundwork laid by Langley and his contemporaries allowed for rapid
advancement of knowledge in local functions of the nervous system in various parts of the
body, including the pelvis. From this arose the concepts of antagonistic,
parasympathetically-based cholinergic and sympathetically-based adrenergic control of
visceral functions; concepts which were the core of autonomic neurobiologic activity for
greater than half a century.
At the beginning of the last century, the neuroanatomical and neurochemical functioning of the pelvis began to receive great scrutiny. Hashimoto, 1904, Latarjet et al., 1913, Delmas, 1933 and Curtis et al., 1942 all carried out microscopic dissections that revealed the spinal derivations, morphology, anatomic relationships, and neuronal interconnections of the pelvic plexuses. They also documented the anatomic course and regional terminations of peripheral nerves. The net result of this work was one of the first detailed descriptions of the hypogastric plexus. The hypogastric plexus is a considerably sized, widely innervational, three-dimensional structure situated laterally adjacent to the deep pelvic viscera that, is composed of an interlacement of nerve fibers containing numbers of extremely small, almost microscopic, ganglia (Davis, 1933).

Walsh and Donker's work (1982) built on the earlier concepts of autonomic function but focused strictly on male genitourinary system and pelvis. By serial sectioning tissue from male fetuses and newborns, they confirmed that autonomic innervation of the pelvic organs and external genitalia arises from the pelvic plexus. They also demonstrated that the parasympathetic visceral fibers arise from the sacral center (S2-S4) and attach to the pelvic plexus via the pelvic nerve while sympathetic fibers from the thoracolumbar center (T11-L2) flow into the pelvis via the sympathetic trunk to the inferior hypogastric nerve to the plexus. The pelvic plexus forms a fenestrated plate retroperitoneally beside the rectum and extends from the sacrum ventrally as high as the rectouterine pouch in the male newborn. The plexus provides visceral branches that innervate the bladder, ureter, seminal vesicles, prostate, rectum, urethra and corpora cavernosa. These autonomic fibers also innervate pelvic vasculature and contain motor sensory fibers that extend to the levator ani, coccygeus and striated urethral musculature.

Despite the detail in Walsh's work, the precise pathway of the autonomic innervation distal of the prostate was not delineated due to the rich interconnections among fetal nerve bundles making identification difficult. The importance of the innervation of this erectile tissue is paramount to understanding not just penile and clitoral erection but also sexual
arousal in general. In 1993, Paick et al. published their study which focused on the cavernous nerves distal to the prostate. Building on Walsh and Donkers work, this study helped to complete the picture of the autonomic nerve pathway from the pelvis to the penis. They found that the cavernous nerve divides into several branches approximately 10 mm superior to the urogenital diaphragm. These branches then course anterolaterally, remain lateral to the urethra as they pierce the urogenital diaphragm and turn medially to branch to the corpora cavernosa. This branching and lateral course may help explain why some men do not lose sexual functioning despite prostectomy’s or traumatic injury to the UG diaphragm (Paick et al., 1993)(Fig. 1).

Despite such findings, the knowledge of human pelvic anatomy is still not complete. While work in male pelvic anatomy has lead to surgical advances that include nerve sparing prostectomy’s and colo-rectal surgeries, we have not made the same anatomical advances to apply to female pelvic surgery. In 1956 Krantz sectioned vagina and external genitalia of tissue varying from a 8 month fetus to a 55 year old. This extensive serial section study focused on categorizing the type of nerve endings in the female genitals. Quantitative analysis showed the presence of touch, pressure and pain nerve endings in the mons, labia, clitoris and hymeneal ring with only the occasional pain nerve ending within the vagina. Unfortunately Krantz did not describe any of the pathways of these nerves. Smith and Ballantyne (1968) investigated the innervation to the pelvic contents after Lewington, 1956, and Bowers, Moeckel, Yates and Wesson (1957) both reported functional changes to the bladder after total and radical hysterectomy’s. They found the autonomic nerves associated with the bladder course through the pelvis via the inferior hypogastric plexus and move medial just below the uterine artery, to branch to the vagina and bladder.

While there was a lack of published research regarding the anatomical basis of sexual arousal in the 70's and 80's it once again gained prominence in the mid 90's. In 1998 O'Connell et al. did extensive work on the 3-D anatomical relationships of the structures of the external genitals. The work highlighted evidence of significant post-menopausal atrophy
of erectile tissue as well as defining relationship between erectile structures. The relationship of the cavernous nerve to surrounding tissue is described as lying lateral to the urethra within the deep perineal membrane. Baskin et al., 1999 serially sectioned fetal material focusing on the innervation of the human clitoris. In particular, he demonstrated detailed branching of the dorsal nerve and correlated it to nerve sparing technique for feminizing genitoplasty with plastic surgery. Butler-Manuel et al., 2000 detailed the nerve content differences in the uterosacral and cardinal ligaments at the level of division used in radical and simple hysterectomies. Their work, re-confirmed by Kato et al., 2002 demonstrated that both ligaments are more than just supports for the uterus they are also conduits for autonomic nerves to reach the pelvic organs. Specifically, both found that nerve contents varied along the length with and increase in the middle to lateral thirds toward their origin to the pelvic side wall. Both sets of researchers also confirmed that the autonomic nerve content in the uterosacral ligaments were much higher than that in the cardinal ligaments.

As the knowledge of neuroanatomy is necessary to guide surgeons operating for benign and malignant disease this study was designed to document the neural pathways through the urogenital diaphragm (UGD) supplying the female erectile tissue. It completes the missing component of the path of the cavernous nerve. Specifically: the direction and course of the cavernous nerve as it branches off the pelvic plexus, orientates to the vagina and urethra and pierces the UG diaphragm to innervate the external genitals.

FEMALE PELVIC ANATOMY

The female pelvis plays important roles in variety of bodily functions. The significant interactions between organs and systems within the bony pelvis make understanding basic anatomy and anatomical relationships important. Specifically for this research, the basic anatomy and positional relationships of organs will be employed to describe the pathway of nerves as they enter the pelvis until their terminal branches within the external genitals.

Bone Anatomy
A pair of curved bones (os coxae) commonly known as the pelvic bones form the pelvic girdle. Each os coxa develops from ossification centers of three embryonic bones that fuse in the adult. The names of the three bones are retained for the corresponding regions in the adult pelvic girdle: the ilium, the ischium and the pubis. This girdle articulates solidly with the sacrum. It supports the mass of the upper body and distributes it to the legs. Several anatomic regions are recognized in association with the pelvic girdle. The os coxae and the sacrum enclose a space called the pelvic cavity. The brim of the pelvis is formed by a circular line passing along the upper edge of the pubic bones and sacral promontory. The pelvic brim encircles the superior entrance into the true pelvis and is called the pelvic inlet. More importantly for this research is the fact that the sympathetic innervation to the pelvis passes directly over the promontory to enter the pelvis. The pelvic outlet is the inferior opening of the pelvis, and is bounded by the coccyx posteriorly and the ischial and pubic bones anterolaterally. (Fig. 2).

Muscles of the pelvic floor form in an upright funnel shape and support the organs that project into the pelvis from the abdominal cavity. Two main muscles are involved in this task: the levator ani and the coccygeus. The levator ani, the larger and more important of the two, is itself comprised of three smaller muscles: the pubococcygeus, puborectalis, and iliococcygeus. The pubococcygeus forms a "u" shaped sling when attached to its partner. The puborectalis forms the main portion of the levator ani and the iliococcygeus forms the posterior portion. These three muscles form a muscular sling that supports abdominopelvic viscera as well as helping to resist increases in intra-abdominal pressure. The posterolateral portion of the pelvic floor is supported by the coccygeus muscle. In addition to support, these muscles contract voluntarily during defecation, urination and sexual intercourse as well as involuntarily during orgasm (Moore et al., 1999)(Fig. 3).

Reproductive tract

The adult female reproductive tract consists of the ovaries, the oviducts, the uterus, the vagina and the external genitalia. The ovaries are almond shaped, paired organs located
on either side of the pelvic wall. The oviducts, also known as uterine tubes, extend from the horns of the body of the uterus and extend laterally to open adjacent to the ovaries. The lateral ends of the oviducts consist of finger like projections called fimbriae which are involved in 'catching' ova once released (Moore et al., 1999)(Fig 4).

The uterus is a hollow, pear-shaped, thick-walled sac that rests on the floor of the abdominopelvic cavity between the bladder and the rectum. Anatomically it is typically divided into two parts: the body and the cervix. The body of the uterus is held loosely in place by broad, round and ovarian (suspensory) ligaments. The broad ligament, which attaches the uterus and oviducts to the lateral pelvic walls and floor is derived from the double layered peritoneum and is also a conduit for vessels and nerves to pass from the lateral wall to the reproductive organs. The round ligament attaches near the uterine horns and runs lateral to the inguinal canal and eventually the labia majora. The ligament of the ovary is the superiolateral extension of the broad ligament which also attaches to the lateral pelvic wall (Maas et al, 1999)(Fig 4). The hollow uterus connects to the outside through the cervical and vaginal canals. The cervix is a narrow neck-like extension of the uterus that protrudes into the vagina. Although the broad ligament does not cover the cervical portion of the uterus, the cervix is well supported by two other ligaments: the cardinal and the uterosacral (Moore et al, 1999). The cardinal ligaments extend from the cervix to the lateral pelvic wall. The uterosacral ligaments also start at the cervix however they then course posterior to attach to the sacrum. Both of these ligaments provide a pathway for nerves to reach the pelvic organs (Maas et al, 1999; Butler-Manuel et al., 2000; Kato et al, 2002). Given the contents of these ligaments particular attention will be to dissection of them and in the surrounding area. The vagina provides a passage from the uterus to the outside of the body. It is largely a fibromuscular tube that collapses on itself except at its superior end where it attaches to the cervix. This superior portion of the vagina is called the vagina vault and it is slightly widened and allows for sperm accumulation during sexual intercourse. The external genitalia are discussed below with the perineum.
Perineum

The inferior end of the vagina passes through a muscular diaphragm and opens to the outside of the body where it is surrounded by the external genitalia. The area collectively called the perineum includes both the external genitals and the muscular diaphragm by which it is attached to the body. It is a diamond shaped area extending laterally to the ischial tuberosities, anteriorly to the pubic symphysis and posteriorly to the coccyx. The diamond shaped area is typically divided into two by drawing a line between the tuberosities. The anterior portion is known as the urogenital triangle and the posterior portion is the anal triangle (Moore et al, 1999)(Fig. 5). The anal triangle contains skeletal muscle, the terminal portion of the large intestine and shares innervation with the urogenital triangle. The urogenital triangle consists of both a musculofacial diaphragm called the urogenital diaphragm (UGD) and the structures of the external genitalia (Moore et al, 1999). Both the UGD and the external genitals contain organs/tissues of interest for this study.

The UGD, which is a sandwich of fascia over muscles with orifices for both the urethra and vagina, is known as the deep perineal space. The muscles involved are the deep transverse perineal and the urethral sphincter. Along with the muscles of the pelvic diaphragm, the UGD provides both support for the internal structures and attachment for structures which sit inferior to it (Moore et al, 1999)(Fig. 6). The UGD is a key focus area for this research. Specifically, the path of the autonomic cavernous nerve, through the UGD and its subsequent innervation of the tissues of the genitals involved in sexual arousal.

The inferior fascia of the UGD, also known as the deep perineal fascia, consists of a fatty layer and subcutaneous connective tissue (Moore et al, 1999). It is continuous over the labia majora and it attaches medially to the pubic symphysis and laterally to the pubic body. The UGD inferior fascia is also the attachment point for the superficial perineal muscles; the superficial transverse perineal, the ischiocavernosus and the bulbospongiosus. The female external genitalia structures, collectively called the vulva, consists of the mons pubis, labia
majora and minora, the clitoris, the bulb of the vestibule and the greater and lesser vestibular glands (Moore et al, 1999).

**Innervation**

The human nervous system consists of complex networks of neurons that carry information to and from the central nervous system (CNS). The central nervous system develops from the neural plate, a thickened slipper shaped area of embryonic ectoderm. It is the notochord and paraxial mesoderm that induce the overlying ectoderm to differentiate into the neural plate (Larsen, 1993). The neural plate eventually differentiates into the neural tube and crest. The neural tube differentiates into the CNS, consisting of brain and spinal cord while the neural crest cells gives rise to form the majority of the peripheral nervous system (Larsen, 1993). The peripheral nervous system and its central pathways are traditionally divided into two systems. The somatic nervous system is responsible for carrying conscious sensations and for innervating the voluntary muscles of the body. The autonomic nervous system (ANS) is primarily concerned with involuntary processes and is further subdivided into two divisions: parasympathetic and sympathetic. The parasympathetic nervous system (PNS) is considered very generally to be responsible for the visceral activities characteristic of periods of peace and relaxation. The sympathetic nervous system (SNS) controls the involuntary activities that occur under stressful "flight or fight" conditions. Of course, like all parts of the body there are always exceptions. This is particularly true in the case of sexual arousal. The parasympathetic system is responsible for the arousal phase, which is anything but peaceful and relaxing and the sympathetics are responsible for the post-orgasmic detumescence.

The pelvis is innervated by the autonomic sympathetic nerves of the thoracolumbar sympathetic trunk via the superior hypogastric plexus, and the parasympathetics of the sacral trunk. The superior hypogastric plexus (SHP) is a network of sympathetic autonomic nerves fed by the thoracolumbar trunk, which lie posterior to the endopelvic fascia and just inferior to the bifurcation of the abdominal aorta. The SPH splits as it passes over the pelvic
promontory into the left and right inferior hypogastric nerves where they descend into the pelvis under the endopelvic fascia, and expand into a fenestrated fan-like projection called the inferior hypogastric plexus (IHP) (Fig. 7) (Maggi, 1993; Butler-Manuel et al, 2000; Maas et al, 1999).

The sacral trunk is located on the posterior wall of the pelvis and is largely derived from parasympathetic fibers originating from L5-S4. While a significant portion of these nerves leave the pelvis to innervate the lower limb, our focus is on those branches which form the pudendal and pelvic splanchnic nerves. The pudendal nerve is typically derived from parasympathetic fibers of the anterior divisions of S3-4 with occasional input from S2 (Jang et al., 1987). Sympathetic fibers from the lumbar trunk portion of the sympathetic chain ganglia attach to the sacral roots prior to the branching of the pudendal nerve (deGroat et al., 1993). The pathway of the pudendal leaves the pelvis via the greater sciatic foramen and after hooking past the ischial spine, re-enters via the lesser sciatic foramen to innervate the perineum. The pudendal is largely the somatosensory nerve of the external genitalia, terminating as the dorsal nerve of the penis and clitoris. As well, branches of the pudendal feed motor to the muscles of the perineum (Moore and Dalley, 1999). The parasympathetic pelvic splanchnic nerves, originate from sacral roots of S2-4 and course laterally under the endopelvic fascia to join the IHP (Butler-Manuel et al, 2000)(Maas et al, 1999). At this point the IHP contains afferent and efferent sympathetic and parasympathetic autonomic nerves as well as some sensory nerves supplying the rectum, uterus, vagina, vestibular bulbs, clitoris, bladder and urethra (Maas et al, 1999).
Fig. 1  Representative course of the cavernous nerve from the pelvic plexus to the penile shaft. This is one of the clearest documentations of the pathway and branching of the cavernous nerves. Note the pathway and branching as it comes from the posterolateral position on the prostate to the lateral walls of the urethra as it passes through the levator ani muscle. (Modified from Paick et al., 1993).
Fig 2. Bones of the pelvis with focus on general terminology of pelvic areas. Note the pelvic promontory which is a landmark for
Os coxa
Symphysis
False pelvis
• True pelvis

Iliac crest
Sacral Promontory
Sacroiliac Joint
Sacrum
ischial spine

Os coxa
Ilium
Pubis
Ischium

Pubic Symphysis

False pelvis
True pelvis
Fig 3. Muscles of the pelvic diaphragm viewed from above. It is important to understand that these muscles work as a support system for both the internal organs above them and the external genitals below. They also play an important involuntary contraction role during orgasm.
Fig 4: Orientation of the uterus, ovaries and associated structures within the broad ligament. Note that the cardinal and uterosacral ligaments are not wrapped within the broad ligament. They do however play as equally important role as the broad ligament as a conduit for nerves and vessels from the lateral pelvic wall to the pelvic organs. The dotted line is the course of the autonomic nerves traced on gross macrodissection during this study.
Fig 5: Illustration of an inferior view of the female perineum with the musculature of the pelvic diaphragm. The inferior pelvic diaphragm is anatomically divided into two triangle which are named by the more superficial structures located within each triangle. The urogenital triangle contains many of the organs/tissues that are important for this study.
**Fig 6.** Female Urogenital Diaphragm showing the orifices of the vagina and urethra. Along with the muscles of the pelvic diaphragm, the urogenital diaphragm provides both support for the internal structures and attachment for the perineal structures which sit inferior to it. These structures have been added to show their orientation.
Fig 7: Innervation of female pelvic viscera. A generalized schematic overview of the autonomic innervation to the pelvis. Note that the parasympathetics as the pelvic splanchnic nerves to form the pelvic plexus (also known as the inferior hypogastric plexus).
Sympathetic trunk

Superior hypogastric plexus

Inferior hypogastric nerve

Inferior hypogastric plexus

Sacrum

S2

S3

S4

Pudendal Nerve

Nerves

Pelvic splanchnic nerves

Internal pelvic organs
- rectum
- vagina
- uterus
- bladder

Muscles of the pelvic diaphragm
Muscles of the urogenital diaphragm
Somatic sensory to the external genitals
Somatic afferents

External Genitalia
THESIS RATIONALE AND OBJECTIVE

The autonomic innervation of the erectile tissue in the human male has long been identified. In men the cavernosal nerves arise from the prostatic plexus then pierce the urogenital diaphragm to supply the cavernosal bodies and thence the penis. In contrast, the autonomic innervation of the female (via the vaginal plexus) has been described as “supplying its mucosa and erectile tissues at its anterior parts”. Unfortunately, the description is not fully supported by published data as there has been no description of the actual neural pathways that supply the erectile structures of the vulva, nor have their site(s) of origin in the vaginal plexus been defined. Specifically, it has always been supposed, but never definitively demonstrated, that the female innervation generally parallels that of the male (i.e. that there are two major nerve bundles that are equivalent in form and function to the male cavernosal nerves). It is also unclear whether or not there are individual branches to the individual erectile structures, which include the right and left clitoral corpora, vestibular bulb, and periurethral tissue.

Based on similarities of anatomy, I hypothesize that the female innervation of the erectile tissue generally parallels that of the male. Specifically, I predict that the cavernous nerve travels from the pelvic plexus as two major bundles, then branches inferior to the urogenital diaphragm to innervate the left and right clitoral corpora, the vestibular bulb and the periurethral tissue.

To test my hypothesis I macro-dissected preserved female cadavers to determine the pattern of travel through the pelvic and vaginal plexuses. Due to the interwoven nature of the plexuses and the density of the connective tissue between the vagina and urethra, I used histological processing, serial sectioning and 3D reconstruction of both fresh and fixed female cadaver tissue to better visualize the path through the urogenital diaphragm to the external structures of the vulva.
MATERIALS AND METHODS

This study involved cadaver tissue obtained from the Faculty of Medicine at the University of British Columbia. A total of ten specimens were used to fully document the morphology of the neural pathways supplying the female erectile tissue (Table 1). Techniques used ranged from an intact pelvic macro-dissection to specific histological processing. Digital reconstruction a 3D image of the external genitalia was also employed using serial sections.

TISSUE PRESERVATION

Of the ten cadavers, eight were preserved by morgue staff via standard embalming procedures upon arrival at the Department of Anatomy and Cell Biology's morgue at the University of British Columbia. The cadavers were embalmed using the following mixture: 20% isopropanol, 20% propylene glycol, 4% phenol and 5% formalin. This solution was infused under positive pressure (20 psi) via the left and right brachial arteries. To allow infusion of the embalming fluid to the cellular level, the cadavers are left in a cooler at 7°C for two months.

To ensure that the embalming process did not affect data integrity through cell damage, two cadaver specimens had their reproductive organs removed directly upon their arrival at the morgue, both within 24 hours of death. This tissue was immediately processed in tissue prep cassettes in a Leica Automatic 12 Bath Tissue Processor using standard fixation method of 30% xylene/70% alcohol mix for 5 baths, five baths of 100% xylene and 2 baths of liquid paraffin. The samples were then embedded on the cassettes in paraffin and sectioned on a Leica model 1212 microtome.

GROSS ANATOMICAL DISSECTION

The first portion of this study involved the dissection of an intact pelvis. The abdominal cavity was exposed via a mid-sagittal incision. The abdominal organs were removed to aid in the exposure of the posterior abdominal wall and the pelvic organs. The pelvic bones were then cut approximately 3 inches lateral to the pubic symphysis, the bone
then being removed leaving the periosteum intact (Fig 8). The area under the pubic symphysis was then dissected aided by Surgitel 2.75X binocular loupes. The intention of the first dissection was primarily to establish methods of approach to the area in question. However, due to the limited number of female cadavers with intact reproductive organs the first cadaver, along with one other were then further dissected as described below. The two pelvis were removed by transecting the body at L4 and removing the legs at the acetabulum. Like the previous pelvis, the pelvic organs remained intact however the rectum was removed just above the level of the coccyx to aid with visualization of the posterior pelvis (Fig. 9). The entire pelvic block was then cut mid-sagittally. Dissection, aided by Surgitel binocular loupes, began at the superior hypogastric plexus, which was identified prior to transection at L4, and moved caudally following the pelvic plexus to the external genitalia. The results were documented using still photos on a Fuji Finepix A201 digital camera.

**HISTOLOGICAL PROCESSING**

Eight cadavers were prepared for histological processing. The first processing done was with an intact pelvis which had been transected at the body at L4 and had both legs removed at the acetabulum. The entire block was then placed in 10% Formic acid to decalcify the bone thereby enabling sectioning. The pelvis was then cut transversely into 10 blocks, approximately 1 to 3 cm in width. Due to the density of the pelvic bones only one or two cuts could be made prior to the block being returned to the acid for the newly exposed bone to decalcify. The tissue sections were then further processed to ensure cellular fixation for embedding in a large glass jar with an internal mixer in the following manner: 10% Formalin for six hours, 70% Xylene/30% alcohol for 6 hours with 5 bath changes, 100% Xylene for 6 hours with 5 bath changes and 100% Paraffin for 2 hours with 2 bath changes. The sections were then placed in containers, covered in paraffin and allowed to cool in a vacuum oven overnight.

The tissue was then cut serially at thickness of 10 to 25 microns using a Jung K motorized sliding microtome and a carbon tip blade. They were placed on specially cut
8x13cm glass slides (due to the large section size) that was treated with five percent poly-lysine (Sigma) for tissue adherence. The slides were then stained using standard preparation of hematoxylin and eosin (H&E) which was chosen because of its easy adjustability ‘mid-staining’ with the varying thickness of tissue, and cover slipped using Permount and second glass slide. We were unable to locate commercially coverslips that were large enough. This portion was done to obtain a preliminary understanding of cross-sectional relationships of not only bones and organs but also vasculature, muscles, innervation and fascia.

Specimens of five embalmed and two fresh cadavers were used to further the histological component of this study. Each of the seven had the reproductive organs removed enbloc from the pelvis. This was accomplished by cutting the pubic rami 8 cm lateral to the pubic symphysis maintaining the pubic arch. Laterally the ovaries were removed by cutting mid fallopian tube following the broad ligament caudally approximately 2.5cm lateral to the uterine edges. Once removed from the cadaver, each block was then further dissected to remove the compact bone and unneeded tissue. Five of the blocks (two fresh and three embalmed) were then cut mid-sagittally, keeping one side for future reference while the other side was further cut to fit standard tissue prep cassettes. This material was then processed in a Leica Automatic Tissue Processor using 30%Xylene/70%alcohol mix and then a 100%Xylene bath. The samples were then embedded in paraffin and sectioned on a Leica model 1212 microtome.

Two of the embalmed blocks were also processed using the above procedure however these blocks were cut transversely into sections of approximately 5mm keeping right and left together (Fig 10). These ‘intact’ blocks were used to ensure that we did not lose important structure that lay midline. These sections were embedded in paraffin and cut on a Jung K motorized microtome. Due to the larger format of these sections they were placed on specially cut 8x13cm microscope slides treated with 5% poly-lysine (Sigma) for tissue adherence. All of the sections were subsequently stained with H&E for morphometric analysis of nerve size, orientation and general histological organization.
3D MODEL

One of the mid-sagitally cut reproductive blocks was used to develop a 3D model of the UGD and erectile tissue. To obtain a representative specimen from each section the embedded tissue was trimmed on the microtome to get a cross-section of the entire section. Several 5 micron samples were then obtained, placed on slides and stained using H&E. This procedure was repeated for the twenty eight sections of the block. The resulting slides were then scanned in a Kodak slide scanner, processed in Adobe Photoshop® 6.0 to grayscale to aid in visualization (Fig 11). Each of the following structures was identified within each scan: the pudendal nerve, the dorsal vein, the clitoral crura, the urogenital diaphragm and a secondary nerve bundle. Each structure was color coded and the orientation was maintained by orientation points on the scans which were obtained by using a cardiac syringe on the original section prior to cutting. This left a small spherical hole that was later located using the microscope.

Knowledge of anatomy was essential when generating these structures into 3D as their inherent structure had to be added. For example a nerve is flat but tubular where as muscle is solid. The scans with their labeled structures were then converted using the imaging software Cinema 4DXL®, by Carbon Digital Media of Vancouver, B.C., Canada to generate a three dimensional image (Fig 12). When used in conjunction with a computer video player the image can be rotated and viewed on all sides (Fig13).
Table 1. Table showing the variety of processing and techniques employed to determine nerve pathway.
<table>
<thead>
<tr>
<th>Technique or process used</th>
<th>Number of specimens (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gross Anatomical Dissection</strong></td>
<td></td>
</tr>
<tr>
<td>Macro dissection – intact pelvis</td>
<td>3</td>
</tr>
<tr>
<td>Macro dissection – pelvis cut mid-sagittal</td>
<td>2 (both previous dissected when intact)</td>
</tr>
<tr>
<td><strong>Total specimens</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Histologic Processing</strong></td>
<td></td>
</tr>
<tr>
<td>Embalmed</td>
<td></td>
</tr>
<tr>
<td>- whole pelvis</td>
<td>1</td>
</tr>
<tr>
<td>- enbloc reproductive organs</td>
<td></td>
</tr>
<tr>
<td>- whole</td>
<td>2</td>
</tr>
<tr>
<td>- cut mid-sagittal</td>
<td>2</td>
</tr>
<tr>
<td>- UGD and gentials only</td>
<td>1 (used for 4DXL)</td>
</tr>
<tr>
<td>Fresh</td>
<td></td>
</tr>
<tr>
<td>- enbloc reprod. organs cut mid-sagittal</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total specimens</strong></td>
<td>8</td>
</tr>
</tbody>
</table>
Fig 8: Schematic of cuts made to cadaver to expose underlying organs

A) Illustration of cuts made to outer abdominal wall to access pelvis. Procedure the same for access to reproductive organs for harvest ‘enbloc’ with removal of pubic symphysis being done as part of the block, not as a separate step as illustrated for the procedure below.

B) Illustration of exposure of the pelvic organs with the abdominal contents removed. Note the removal of the pubic symphysis but retention of underlying structures.
Fig 9: View of transected pelvis that has been cut mid-sagitally prior to nerves to rectal area being removed. Most the dissected material is being lifted up to aid in the visualization of the rectal branches.
Fig 10: Representative block of tissue from the external genitalia. This section was further cut into 7 - 5 mm sections, processed for fixation, embedded in paraffin and sliced into approximately 5 micron sections and plated on glass slides.

A) Inferior and superior views of a tissue block showing the external genitals.

B) A 10 micron slice of tissue from one of the seven sections that has been stained with H and E and mounted on a glass slide.
Fig 11: Serial section scan used in 3D reconstruction. Note each type of structure was identified via microscope and circled accordingly.
Fig 12: Schematic of how Cinema 4DXL develops the 3D image. A representative slice of tissue from each section was scanned into the computer and then the major structures were identified and outlined using 4DXL. The computer then rendered the 3D image maintaining structural orientation. The resulting image can be shown as static images as seen in figure 16 or used in video software to create a movable 3D image.
Fig 13. Static images of external genitalia and urogenital diaphragm from Cinema 4DXL imaging software.

A + B  Medial and lateral view of the generated 3D reconstruction. Both of these views give a great view of the orientation of the different layers of the uro-genital diaphragm and external genitals.

C + D. Views from inferior and superior. Note on D the nerve bundle associated only with the urogenital diaphragm. This reconstruction along with other serial sections helped determine the path of the autonomic nerves through the UGD and was identified as the cavernous nerve.
RESULTS

Gross anatomical dissections of cadaver material has been carried out for centuries. Human neuroanatomy has been heavily studied in male cadavers and much of the information can be carried over to the female. However, in some areas, such as the pelvis, differences in structure and function requires a direct approach. Cadaver studies have traced autonomic nerves throughout the female pelvis in some detail. They have demonstrated that sympathetic nerves flow into the pelvis via the bilateral inferior hypogastric plexus (IHP) and join with the parasympathetic nerves of S2-4 via the pelvic splanchnic nerves at the pelvic plexus (Campbell, 1950; Maas et al., 1999). These plexuses sit on the lateral walls of the pelvis, and move medially to the reproductive organs via ligaments to supply the bowel, bladder, urethra and entire genital system. (Maas et al., 1999; Butler-Manuel, 1999). The missing component to date is how these nerves continue their path inferiorly to the erectile tissue and the external genitalia. In the present study, dissection and histochemical evaluation of fresh and fixed tissue definitively demonstrated the structure and pathway of the autonomic nerves which feed the external genitals and are involved in sexual arousal.

MACROSCOPIC

The initial gross dissection was undertaken to primarily orientate the researcher with the general layout of the female pelvis. We were, however, able to superficially demonstrate previously well documented structures of the reproductive organs, major pelvic vasculature, the pelvic promontory, the sacral nerves, the sympathetic chain ganglia, the superior hypogastric plexus (SHP), the inferior hypogastric plexus, and the pelvic splanchnic nerves (Fig 14). The only discrepancy noted was the variations of the orientation of the superior hypogastric plexus. Netter, 1997 illustrates the SHP as beginning at the bifurcation of the aorta and ending at the promontory. However there is documentation that the SHP can lay as much as 1-2 cm below the promontory (Maas et al., 1999; Butler-Manuel et al., 2000). The specimen shown in figure 14 is somewhat unusual in that the SHP begins below the promontory but has a short course until it bifurcates into the inferior hypogastric plexus.
Dissection of the two hemi pelvises were then undertaken to properly document in detail the above noted structures. In both cases we obtained access to the posterior abdominal wall with no difficulties. The superior hypogastric plexus (SHP), our starting point, was found attached via fascia one cm below the aortic bifurcation on the posterior abdominal wall, posterior to the endopelvic fascia. This finely fenestrated network of neurons receives sympathetics from the left and right sympathetic chain ganglia. As the SHP continued inferiorly it spread laterally to a width of two to three cm. The network clung firmly to the posterior wall while it continued two and a half cm below the pelvic promontory. At the most inferirolateral ends the SHP bifurcated into three millimeter diameter left and right hypogastric nerves (Fig 9). Each of these nerves followed a singular course, one centimeter medial and parallel to the ureters. In both cases the inferior hypogastric nerve began 1 cm above the level of the common iliac artery bifurcation into the internal and external iliac branches and ended one centimeter below the coccyx at the inferior hypogastric plexus (IHP) (Fig 15).

Parasympathetic nerves entered the pelvis via the sacral nerves of S2-4. These sacral nerves left the sacrum via the anterior foramina and joined together approximately two centimeters inferirolaterally and coursed over the muscles of the posterior pelvic wall. In both pelvises, approximately one centimeter after exit from the foramina, branches arose from the roots of S3 and S4. They ran inferirolaterally to independently pierce the endopelvic fascia and join medially with the IHP four to five centimeters later (Fig 9). These nerves were identified as the pelvic splanchnic nerves.

The IHP, like the SHP was a highly fenestrated web encapsulated in thick connective tissue. The plexus was three centimeter wide and 3 centimeter long triangular shaped structure and it lay approximately three centimeters from the mid-sagittal plane at its superior border at the level of the rectouterine fold. It extended laterally to encompass many of the lower branches of the internal iliac artery and medially to the target organs of the uterus, vagina and bladder (Fig. 16). At the level of the rectouterine fold several branches of the
IHP branched off the to rectum. Once seen the branches were cut and the rectum removed to aid in visualization of the distal area of the IHP (Fig 9).

The density of the connective tissue made it very difficult to differentiate nerves particularly as they joined with other connective tissue support systems of the broad, uterosacral and cardinal ligaments. It was noted however that the majority of the plexus appeared to turn more medial at the level of the vaginal vault (Fig 15). Despite the large amount of branching a major part of the plexus turns toward the midline running in an anteroinferior direction moving from the lateral wall of the cervix and vaginal vault to a position anterior and lateral to the urethra (Fig 17). Like Olelrich, 1983, we noted the problem of tissue identification, despite using 2.75 magnification binocular loupes, through the dense fibrous area between the urethra and vagina at the level of the UGD. Several very small branches of nerves appeared to pierce the UGD and carried on in the same orientation to the external genitals. However due to the density and apparent atrophy of the tissue of the UGD we were unable to confirm unequivocally that these branches were in fact the cavernous nerve feeding the erectile tissue. To attempt to confirm the pathway of the cavernous nerve microscopic processing was employed.

MICROSCOPIC

In general, the hematoxylin and eosin (H&E) staining allowed for easy identification of structures such as skeletal muscle, erectile tissue and nerves. Of course, the one disadvantage was that it did not allow us to differentiate nerve types. For this study however our main goal was to determine the pathway of major nerves piercing the UGD. This was done not only to aid in further the understanding of general gynecological anatomy but also to give a focus for further immuno and neurochemical studies.

The histological sections of the eight cadavers were evaluated starting at the external genitals and moving superiorly to the internal structures at the rectouterine pouch. This allowed the nerves to be followed directly from their target organ to their source. We found two large neurovascular bundles running into the external genitals: one which was found
only on the inferior side of the UGD which we identified as the pudendal nerve and the accompanying vasculature (Paick et al., 1993; Benoit, 1999; Lundberg, 2001) the second came from the lateral wall of the vagina (Fig 18), moved anterior as it coursed inferiorly and pierced the UGD anteriolateral to the urethra to its target erectile tissue. This key feature, that is the well documented course of the pudendal nerve running along the inferior wall of the UGD versus the cavernous nerve which pierces the UGD allowed us to identify this second nerve as the cavernous nerve and vasculature (Fig 19). Second to pathway, size difference is significant between the two with the pudendal being much larger (Fig. 20).

The putative cavernous nerve followed a similar course in each of the cadavers. The cavernous nerve appeared on the lateral wall of the vagina at the level of the vesicouterine pouch. At this point it coursed inferiorly along with the vagina and urethra towards the UGD. As the nerve coursed inferior it moved into the dense connective tissue between the urethra and vagina and ended up 5 mm lateral to the urethra at the level of the UGD. O'Connell et al, 1999 identified the cavernous nerve as a single, large, easily dissected nerve within the erectile tissue which surrounds the periurethral sponge. By histological analysis we found that the cavernous nerve was most commonly a single nerve bundle until it pierced the UGD where it branched. The largest of these branches remained medial and continued in it’s orientation to the urethra moving 2-3mm anterior just prior to piercing the vestibular bulbs(Fig 20) After leaving the UGD the cavernous nerve comes within 1mm of the pudendal nerve as it courses anteriorly to become the dorsal nerve of the clitoris . Despite only being separated by deep perineal connective tissue we were unable to confirm any communicating nerves. We were able to easily see a significant size difference with the pudendal being much larger (Fig. 20).

Given the whole mount nature of some of our specimens we compared the results to current anatomical literature. In particular the orientation of the ligaments and pelvic plexus was similar to that of Butler-Manuel et al., 2000. Like O'Connell et al., 1999 we found a significant variation from the description of the vestibular bulbs. We were unable in our ten
specimens to find any evidence of the tear drop shaped, bisected vestibular bulbs. We found all our specimens to have much more erectile tissue of the bulbs continue around the anterior wall of the urethra in the shape of a inverted u (Fig 25). As well the bulbs had significant fullness of depth than previously documented (Fig 17).

3D Model

The 3D model of the UGD and external genitals provided further confirmation for several anatomical points discussed in this thesis. The first of which is that two large bundles of nerves could easily be identified. Their orientation to the UGD allowed us to confirm them as the pudendal and cavernous nerves. Specifically the superior view of the model allowed for visualization of the course of the cavernous nerve from within the pelvic cavity. The inferior view confirms the pathway of the pudendal nerve on the inferior surface of the UGD (Fig 13). These differences as noted before were further confirmation that we did indeed have two separate nerves with two separate pathways.

As well the model confirmed the inverted u-shape of the erectile tissues of the vestibular bulbs. The post-sectioning shape more like a boomerang than a tear drop. A similar shape was also noted on the macrodissected cadavers however the depth of the tissues(inferior to superior) was as much as 1" at the widest area. (Fig.17)
Fig 14: Pictures of a partially dissected pelvis which has not yet been cut mid-sagittal.

A. Lateral view of the pelvis allowing visualization of the uterus and bladder in orientation to other structures of the abdominal area such as the kidney and muscles.

B. View looking inferior to superior showing the superior hypogastric plexus.
Fig 15: Macro-dissection of the lateral pelvic wall. Note the lines added for orientation to the pelvic promontory, internal iliac artery bifurcation and the hypogastric nerve.
Fig 16: Lateral view of pelvic bowl showing orientation of IHP to the pelvic contents
Fig 18. Histological section of area superior to the UGD. The neurovascular bundle is close to the wall of the vagina and is quite large. This bundle moves anterior as it approaches the UGD as seen in Fig 18 and 19.
*note this section is superior to the UGD
Fig 19: Histological sections of the UGD. The urethra and external sphincter are easily identified as well as their orientation to the anterior vaginal wall. The neurovascular bundle of the cavernous nerve can be seen in the inset.
Fig 20: Histological sections of external genitals just inferior to the UGD. The neurovascular bundle of the dorsal nerve and vein are very easily visualized do to the large size of the bundle. A cross-section of the bundle can be seen on the top left hand side were a small piece of the crua can be seen just beginning to enter the sections.
Fig 21: Histological sections of UGD and surrounding area which repeat the work in Fig 18-20. Note that a UGD representative block has been added to aid in visual orientation.

A. Area superior to the UGD
B. Within the UGD
C. Inferior to the UGD
A. Dorsal Vein
   Pudendal Nerve
   Ischiocavernous Muscle
   Crura

B. Levator Ani Muscle
   Cavernous Nerve
   Bulbospongiosus Muscle
   Ischiocavernous Muscle
   Crura

C. Levator Ani Muscle
   Vestibular Bulb
   Cavernous Nerve

5mm
Fig 22: Histological sections of UGD and surrounding area which repeat the work shown in Fig 18-20. Note that a UGD representative block has been added to aid in visual orientation.

A. Area superior to the UGD
B. Within the UGD
C. Inferior to the UGD
Histological sections of UGD and surrounding area which repeat the work shown in Fig 18-20. Note that a UGD representative block has been added to aid in visual orientation.

A. Area superior to the UGD
B. Within the UGD
C. Inferior to the UGD
Fig 24: Histological sections of UGD and surrounding area which repeat the work shown in Fig 18-20. Note that a UGD representative block has been added to aid in visual orientation.

A. Area superior to the UGD  
B. Within the UGD  
C. Inferior to the UGD
**Fig 25:** Histological section of UGD. Note this section has been cut and mounted whole to give a better understanding of the structural relations of the UGD.
Fig 26: Whole mount tissue cross-section of the external genitals showing the structures. Note how the erectile tissue of the vestibular bulbs continues around the anterior wall of the urethra and it is not bifurcated like the crura.
DISCUSSION

To our knowledge this is the first study to trace the complete pathway of the cavernous nerve in the human female pelvis. The autonomic innervation of the erectile tissue in the human male has long been identified. In men the cavernosal nerves arise from the prostatic plexus then pierce the UG diaphragm to supply the cavernosal bodies and thence the penis. There has been no description of the actual neural pathways that supply the erectile structures of the vulva, nor have their site(s) of origin in the vaginal plexus been defined. The morphology of these pathways has therefore also remained unknown. Specifically it has always been supposed, but never proven, that the female innervation generally parallels that of the male (i.e. that there are two major nerve bundles that are equivalent in form and function to the male cavernosal nerves). It has also been unclear whether or not there are individual branches to the individual erectile structures, which include the right and left clitoral corpora, bisected vestibular bulb, and periurethral tissue.

Sympathetic fibres to the pelvis arise from the thoracolumbar spinal cord (Pick and Sheehan, 1946) and then reach the pelvic plexus via two outflows, namely the prevertebral hypogastric nerve (Barber et al, 2002, Latarjet and Bonnet, 1913, Learmonth, 1931, Kuo et al, 1984) and the paravertebral sacral sympathetic chain. In general parasympathetic innervation arises from the sacral spinal cord (Kokotas et al, 1978) and proceeds to the pelvic plexus via the pelvic nerve. However there is some histochemical evidence (Benoit et al, 1991) that the pelvic nerve contains both adrenergic and cholinergic (i.e. sympathetic and parasympathetic, respectively) fibres in the pelvic roots of the male pelvic plexus (Huselboch and Coggeshall, 1982, Downie et al, 1984, Kuo et al, 1984, Lepor et al, 1985).

Research in male humans and animals has demonstrated that efferent and afferent sympathetic and parasympathetic neurotransmission occurs via the cavernous nerves (de Groat et al, 1993, Vernet, 1964) and also terminal branches of the pudendal nerves. We found no evidence to support autonomic branches of the cavernous feeding the pudendal nerve. The role of the sympathetic fibres of the cavernous nerve remains unclear as the
erectile response elicited in animals by stimulation of the sympathetic chain was still present after unilateral or bilateral transections of the cavernous nerves near their origin (Giuliano et al 1995). The pudendal nerves arise from the sacral roots (typically S2, S3, and S4) and contain motor fibres (for the perineal muscles), sensory fibres, and likely more sympathetic fibres (Katagiri et al 1986, McKenna and Nadelhaft 1986, Lavoisier et al 1988).

Much of the research to date in human females has concerned itself with either the internal structures of the pelvis or the external structures of the genitalia. There has been little work on the neuropathways between these two areas. There are several reasons behind our ignorance of female genital neurophysiology and its anatomic basis. First is a tendency for investigators to be preoccupied with vaginal lubrication, which is but one component of arousal. Second, the majority of women do not have an accurate appreciation of erectile tissue engorgement in any direct sense. Third, the often used measurement of genital congestion does not correlate well with subjective arousal. Finally, information about the effects of surgical procedures on vaginal or vulval innervation is incomplete at best.

Consider the issue of vaginal lubrication and the autonomic nerve supply allowing vasodilation of the submucosal capillary plexus with subsequent increase in transudation. The common plexus that supplies both the vaginal mucosa and the proximal vulval erectile structures, will be affected differently by various surgical procedures. Surgeries which potentially damage cavernous nerves and vaginal branches of the utero-autonomic plexus include Birch procedures (controlling of involuntary urination by elevating the urethra and bladder), anterior vaginal repairs, vaginal hysterectomies (primarily in estrogen-deficient women where there is shrinkage of tissues such that the urethra becomes approximated to the anterior vaginal fornix), radical hysterectomies for vulval cancer (where there is deep dissection of the vesico-vaginal plexus) and direct vulva procedures for various benign and malignant conditions (Ziessen, et al., 2002). It is also noteworthy that the neurotransmitters involved are likely to be different in the two areas. It seems that vasoactive intestinal peptide (VIP) (Ottesen et al., 1987) and another as yet unrecognized neurotransmitter primarily
mediate increased transudation whereas nitric oxide (NO) is a major neurotransmitter of the
“erectile” or congestive response of the vulva (Burnett et al., 1997).

It will not come as a surprise to most persons that women generally do not have an
appreciation of their degree of erectile tissue engorgement. It is equally unsurprising that
most men are exquisitely aware of their degree of erectile tissue engorgement. A woman
generally cannot tell you how engorged her vestibular bulb is, assuming she knows that she
even has one. Nevertheless the simple fact is that vasocongestion of her erectile structures is
critical for her sexual enjoyment for several reasons:

- Her source of sexual stimulation and subsequent arousal may well be genital touch. If the
  structures do not engorge, the touch is not sexually effective.
- Her further stimulation to orgasm usually requires direct massaging of the congested
  structures. This may occur through the labia, the mons, direct clitoral stimulation, or
  through the anterior vaginal wall to the erectile tissue around the urethra. Lack of
  engorgement of these structures tends to cause their stimulation to unpleasantly sensitive,
  irritating, and even uncomfortable and painful. Arousal is obviously dampened and
  orgasm is not reached.

The issue of genital congestion raises the issue of its use as an objective measure of
arousal. Typically vaginal plethysmography is employed for these studies, and this
procedure measures increases in vaginal blood volume and/or pulse amplitude. These
measurements are typically taken when the subject is exposed to an erotic stimulus (usually
an erotic video). Unfortunately, the data is really representative only of objective arousal and
Brotto et al., 2002 have shown that there is poor correlation between objective and subjective
arousal.

The last major reason that female genitalia neurophysiology/neuroanatomy is so
poorly understood is that the effects of surgical interference on autonomic innervation of the
vagina and vulva have only been minimally studied. This knowledge gap has opened the
door to conjecture about these effects and there is no shortage of opinions. Not only are there
markedly different views concerning the disruption of the autonomic nerve supply to the vulva and vagina but the possible repercussions on sexual function are rarely delved into. Loss of sexual arousal and pleasure from stimulation of vulval erectile tissue is not considered in detail. Lack of vaginal lubrication is sometimes raised as a concern but the confounding effects of estrogen status are often not clarified. Typically the focus of research has been centred on “intercourse frequency” and “intercourse satisfaction”. In fact most studies of sexual consequences of hysterectomy refer to orgasm.

Using criteria such as intercourse frequency and/or satisfaction to assess the effect of a given surgical procedure on a patient’s sex life is fraught with problems. This is because it may be completely impossible to differentiate between the negative effects of nerve damage and the positive effects of vaginal repair (or whatever). For example, the patient that has had a cyst the size of a grapefruit removed may say that intercourse is more frequent/pleasureable because it doesn’t hurt anymore. However, it also may not be as complete as it could have been had nerve-sparing surgical techniques been employed.

A review of the literature regarding hysterectomy’s, the most common gynecological surgery performed on women in the UK and USA (Farquhar et al., 2002), is anything but clear. In 1983 Kilkku et al. reported that the frequency of orgasm was significantly reduced one year post operatively in women who underwent total hysterectomy versus those patients who kept their cervixes (ie. subtotal hysterectomy). Kilkku also found that dyspareunia decreased from 31% preoperatively to 16% postoperatively with total hysterectomy, while with subtotal hysterectomy a more spectacular decrease occurred, ie. from 29% pre-op to 6% post-op. An important finding of this study was the decrease in frequency of orgasm only with those patients who had undergone total hysterectomy.

Ten years later, Virtanen (1993) (working in the same institution as Kilkku) concluded that the anatomic basis for his observed lack of problems with orgasm after a total hysterectomy is due to the fact that damage to pelvic nerves and the plexus should be minimal since the nerves are posterior and the main plexus lies below the cardinal ligaments,
which are preserved in total hysterectomy. “Because the pelvic plexus remains intact, the vaginal walls and the erectile tissue of the vestibule and clitoris remain sensitive and, consequently, no symptoms occur”. Virtanen did, however, find a decrease in dyspareunia (pain in the pelvic area during or after intercourse) after total hysterectomy.

Similarly, Nathorst-Boost (1992) comparing total abdominal hysterectomy with supravaginal hysterectomy in 576 women less than 55 years of age, found no differences in sexual outcome between the two groups with over 80% in both groups reporting improvement or no change. Thakar et al., 2002, in study involving 279 women determined that there was little no statistical difference in either type of hysterectomy when related to sexual function. Their results however did show a significant decrease in dyspareunia from 46.2% to 6% in subtotals and 39.3% to 14.3% in total hysterectomies. Interestingly 10% of the subtotal group had an increase in what was termed “good sexual relationship” however there was no change in the total hysterectomy group.

In contrast, Hasson wrote in 1993 that the loss of the major portion of the uterovaginal plexus through excision of the cervix “is bound to have an adverse effect on sexual arousal and orgasm in women who previously experienced internal orgasm”. He based this statement on the concept of stimulation to the cervix leading to a different type of orgasm than one arising from stimulation of the vestibule and clitoris or other parts of the external genitalia - forgetting, perhaps, that orgasm is a reflex and the efferent component, ie. the contraction of internal and external genitalia is a constant entity even if its intensity varies with the type and degree of stimulation.

Zekam et al., 2003 studied gynecologists attitudes regarding hysterectomy’s. Interestingly, advocates for total hysterectomies state that there has been no demonstration that the cervix has any role in sexual, urinary or bowel function. Advocates for subtotals focus on surgical times, decreased morbidity and fewer complications. Ultimately the authors concluded that most gynecologists surveyed favor total abdominal hysterectomy over
subtotal. Few counsel women regarding the options of total and subtotal hysterectomy or offer a choice between the procedures.

These studies emphasize the need to discriminate between various dimensions of surgical outcome. Patients may perceive the benefits to be more important than the problems. For example, patients with significant pelvic pathology may be gratified by relief of their symptoms but may become discontented with other results such as loss of sexual function and vault prolapse over time. One possible way to determine patient perceived differences in outcome would be to run longitudinal studies involving both groups. The variations in outcome post total hysterectomy for benign disease is likely based in anatomy not patient psychology. The pelvic organs, including the uterus and cervix are supported by the endopelvic fascia which attaches to the pelvic sidewalls. Sympathetic and parasympathetic nerves travel from the pelvic plexus inferiorly along the posterio-lateral wall of the pelvis, move medial along the uterosacral and cardinal ligaments (Kato et al., 2002) and pierces the UGD lateral and slightly posterior to the urethra to innervate the external genitals. Cervical removal, and cutting of the cardinal and/or utero-sacral ligaments may result in the loss of innervation which relays with the UGD and external genitals. The pudendal nerve, which has a more exterior route, as previously described may explain why sexual function may only be minimally effected. If there is an anastomosis between pudendal and cavernous, this connection may help replace function that could be lost through lack of surgical skill or anatomical anomalies.

Despite our continued lack of knowledge in the area of women's sexual arousal and gynecological anatomy, there have been significant strides made in the last 10 years to finding better answers than the simple application of male anatomy. With the knowledge of the pathway of the autonomic through the UGD, educators can ensure dissemination at all levels of health care from developing more precise surgical techniques to giving women a better understanding of the balance of options with outcomes. As women become more
educated on their options for sexual intimacy they may feel more control and with that comes a great enjoyment and comfort level.

**Future directions**

Ultimately while the information obtained from this study may have clarified some points regarding neuroanatomy of the pelvis, it has also raised many more. Certainly the nerve content of the neurovascular bundles of both the pudendal and cavernous nerves need to further clarified. Does the pudendal receive sympathetics from the pelvic sympathetic trunk or are there communicating branches between the pudendal and cavernous nerves. If these nerves do have communicating branches is there an approximate point that they consistently occur. Along with that comes the need for clarification of the neurotransmitters involved in erectile tissue of the genitals. One of the drawbacks of the cadavers we used was their age with the oldest being over 100. O’Connell, 1998 raised the question of a link between age and atrophy of vasculature and genital tissue. With this in mind it would be prudent to repeat the work, using fresh tissue from a variety of ages. In particular, looking at fetal, pre and post puberty, pre, peri and postmenopausal women. One interesting aspect may be to look at the changes in vasculature and how it correlates to the changes in tissue.
REFERENCES


Langley J. 1906. On the reaction of cells and of nerve endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curare. J. Physio. 27:237-256.

Langley JN. 1916 sketch of the progress of discovery in the eighteenth century as regards the autonomic nervous system. Journal of Physiology. 50:225-258.


Muller J. 1836. Uber die organischen Nerven der erectilen mannlichen Geschlechtsorgane de Menschen und der Saugethiere. F Dummler.


