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NEUROVASCULAR ANATOMY OF THE PENIS FOR SEX REASSIGNMENT SURGERY

Mr. Thanakul Wannaprasert

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By	Mr. Thanakul Wannaprasert
Field of Study	Medical Science
Thesis Advisor	Associate Professor Tanvaa Tansatit, M.D.
Thesis Co-advisor	Associate Professor Pichet Sampatanukul, M.D.
	Associate Professor Sirachai Jindarak, M.D.

Accepted by Faculty of Medicine, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…………………………………… Dean of the Faculty of Medicine
(Professor Pirom Kamolratanakul, M.D.)

Thesis Committee

…………………………………… Chairman
(Associate Professor Vilai Chentanez, M.D., Ph.D.)

…………………………………… Thesis Advisor
(Associate Professor Tanvaa Tansatit, M.D.)

…………………………………… Thesis Co-advisor
(Associate Professor Pichet Sampatanukul, M.D.)

…………………………………… Thesis Co-advisor
(Associate Professor Sirachai Jindarak, M.D.)

…………………………………… Member
(Associate Professor Vinida Bundit, M.D., Ph.D.)

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วัตถุประสงค์ เพื่อศึกษากายวิภาคของระบบประสาทและหลอดเลือดบริเวณ shaft และ hilum ขององคชาต รวมทั้งเส้นประสาทที่ไปยัง glans penis สำหรับนำไปประยุกต์ใช้ในการผ่าตัดแปลงเพศ และการผ่าตัดอื่นๆ ที่เกี่ยวข้องกับองคชาตต่อไป

วิธีดำเนินการวิจัย ทำการศึกษาเส้นทางและรูปแบบของ deep dorsal veins, dorsal arteries และ dorsal nerves ตลอดความยาวของ shaft ในอาจารย์ใหญ่ที่เสียชีวิตใหม่ๆ จำนวน 32 ท่าน ในส่วนบริเวณ hilum ทำการ dissect อาจารย์ใหญ่เพศชายที่เก็บรักษาแบบ soft-preserved จำนวน 11 ท่าน สังเกตและบันทึกข้อมูลของเส้นประสาทและหลอดเลือดแดงที่พบ นอกจากนี้ยังใช้การวิเคราะห์ทาง immunohistochemistry ในการศึกษาการกระจายตัวของเส้นประสาทที่ไปยัง glans penis และลักษณะของ genital corpuscles ในชิ้นเนื้อเยื่อของอาจารย์ใหญ่เพศชาย 32 ท่าน และยังทำการศึกษาเปรียบเทียบกับข้อมูลที่พบใน glans clitoris จากชิ้นเนื้อเยื่ออาจารย์ใหญ่เพศหญิงจำนวน 3 ท่าน

ผลการศึกษา จากการศึกษาที่ shaft พบว่าในอาจารย์ใหญ่ 22 ท่าน จากทั้งหมด 32 ท่าน มี dorsal arteries ทั้ง 2 ข้าง (ซ้าย-ขวา) ขณะที่ในอาจารย์ใหญ่ 10 ท่านที่เหลือมี dorsal arteries เส้นเดียว มักเป็นข้างซ้าย ระยะทางเฉลี่ยระหว่าง dorsal arteries ทั้ง 2 ข้างที่คอขององคชาต เท่ากับ 1.77 เซนติเมตร อย่างไรก็ตามในอาจารย์ทุกท่านพบว่ามี dorsal nerves ทั้ง 2 ข้าง จากส่วนฐานถึงส่วนคอขององคชาต dorsal nerves แตกแขนงออกเป็นเส้นย่อยหลายเส้นในทิศทางขนานกัน สามารถแบ่งได้เป็น 2 กลุ่ม คือ กลุ่มที่ 1 เป็นกลุ่มที่ทอดตัวตามผิวทางด้าน dorsolateral ของ shaft และแทงผ่านเข้าไปเลี้ยงในส่วนของ glans penis ส่วนกลุ่มที่ 2 ทอดตัวแผ่ออกไปทางด้านข้างของ shaft ไปเลี้ยงด้านข้างและด้านท้องของ shaft ระยะทางเฉลี่ยระหว่างเส้นประสาทแขนงหลักใกล้แนวกลางทั้ง 2 ข้างที่ไปสู่ glans เท่ากับ 1.18 เซนติเมตร ตรงบริเวณ hilum พบว่าหลอดเลือดที่มาเลี้ยงองคชาตมีการผันแปรของรูปแบบค่อนข้างมาก ตัวอย่างเช่น การพบ accessory pudendal arteries ซึ่งเป็นหลอดเลือดเสริมที่มาเลี้ยงองคชาต การพบว่า cavernous และ bulbourethral arteries มีหลายเส้น และมีจุดกำเนิดมาได้จากหลายที่ เป็นต้น สำหรับผลที่ได้จากการวิเคราะห์ทาง immunohistochemistry แสดงให้เห็นว่า dorsal nerves ที่เข้าสู่ glans จะแตกแขนงย่อยลงเรื่อยๆ และพบอยู่หนาแน่นรอบ urethra ตรงส่วนปลายของ glans นอกจากนี้ยังพบความแตกต่างทั้งในด้านโครงสร้างภายในและลักษณะของ genital corpuscles ระหว่าง glans penis และ glans clitoris

สรุปผล ข้อมูลทางกายวิภาคของระบบประสาทและหลอดเลือดในองคชาต ซึ่งได้จากงานวิจัยในครั้งนี้จะให้แนวทางที่มีประโยชน์สำหรับการนำไปใช้ในการผ่าตัดแปลงเพศ รวมทั้งการผ่าตัดที่เกี่ยวข้องกับองคชาตและการผ่าตัดในเชิงกรานอีกด้วย

สาขาวิชา วิทยาศาสตร์การแพทย์ ลายมือชื่อนิสิต..

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.......................................

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..................................

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..................................

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KEY WORDS: NEUROVASCULAR ANATOMY / PENIS / SEX REASSIGNMENT SURGERY

THANAKUL WANNAPRASERT: NEUROVASCULAR ANATOMY OF THE PENIS FOR SEX REASSIGNMENT SURGERY. THESIS ADVISOR: ASSOC. PROF. TANVAA TANSATIT, M.D. THESIS COADVISOR: ASSOC. PROF. PICHET SAMPATANUKUL, M.D., ASSOC. PROF. SIRACHAI JINDARAK, M.D., 55 pp. ISBN 974-17-3688-6.

Objective: To determine the neurovascular anatomy along the penile shaft and in the penile hilum, including glanular innervation for applying to sex reassignment surgery and other penile surgical procedures.

Materials and methods: The topography of the deep dorsal veins, and the dorsal arteries and nerves along the penile shaft was investigated in 32 fresh adult cadavers. 11 soft-preserved adult male cadavers were dissected to document the neurovascular structures in the penile hilum. Concerning the innervation of the glans penis, immunohistochemical study was employed to observe and document the distribution of the dorsal nerves and the genital corpuscles in 32 adult male cadavers. The clitoral specimens were obtained from 3 cadavers for comparative study of the distribution of nerves and the genital corpuscles.

Results: On the shaft, the dorsal artery was present bilaterally in 22 of 32 cadavers; however, it was present unilaterally in remaining cadavers, predominantly on the left. The mean distance between the left and right dorsal arteries at the neck of the penis was 1.77 cm. The dorsal nerve was identified bilaterally in all cadavers. From the root to the neck of the penis, it fanned out into small branches that divided into two groups. The first group of fibers innervating the glans coursed along the dorsolateral surface of the shaft and pierced the entire area of the corona of the penis posteriorly. The other group diverged to distribute throughout the lateral surface of the shaft to innervate the lateral and ventral portions of the shaft. The mean distance between the left and right medial main branches of the dorsal nerves, which terminated in the glans penis, was 1.18 cm. In the hilum, the penile arterial supply showed several anatomical variations such as the presence of accessory pudendal arteries, multiple cavernous and bulbourethral arteries, and the origin of cavernous and bulbourethral arteries. Immunohistochemical analysis revealed that the main branches of the dorsal nerves after entering the glans divided into terminal branches that concentrated around the urethra. Also, there were differences in the genital corpuscles and the structures within the glans penis and glans clitoris.

Conclusion: This detailed anatomy of the neurovascular structures along the penis can provide a valuable guide for applying to sex reassignment surgery, other penile surgical procedures and pelvic surgeries.

Field of study Medical Science

Academic year 2004

Student's signature..

Advisor's signature...

Co-advisor's signature...

Co-advisor's signature...

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LIST OF ABBREVIATIONS

a.	=	artery
cm	=	centimeter
DAB	=	3, 3’-diaminobenzidine tetrahydrochloride, anhydrous
et al.	=	et alii
g	=	gram
i.e.	=	id est (that is)
inf.	=	inferior
kg	=	kilogram
KNO_3	=	Potassium nitrate
ml	=	milliliter
mm	=	millimeter
NaOH	=	Sodium hydroxide
PBS	=	Phosphate Buffer Saline
S.D.	=	Standard Deviation
µm	=	micrometer

CHAPTER I
INTRODUCTION

1. Background and Rationale

Transsexualism is a unique condition which is a source of major physical difficulties, and creates enormous psychological and emotional conflicts for patients with gender dysphoria [1]. The term transsexual is attributed to Cauldwell [2], and is defined as a permanent and extreme sense of dissatisfaction because of a sense of gender that is opposite to external anatomical presentation. It causes these patients to have extreme psychological pain, societal misunderstanding and personal conflict that surrounds gender identity and desire to become as perfect and consonant as possible in body image and physical presentation [1]. The estimated prevalence of transsexualism varies in different societies and cultures [3-5]. The etiology is unknown but has been proposed to be a result of psychological conditioning in childhood, an unusual paranoiac state or genetic disturbances [6]. Recently, Sex reassignment surgery has proved to be the best way to solve the problem of primary transsexualism [1,7-10]. Many previous reports revealed that there was a marked improvement in the antisocial and self-destructive behaviour, and the quality of life after sex reassignment surgery. Moreover, a decreasing of suicides, criminal activity and drug usage were noted in postoperative patients as well.

The procedures in male to female surgery are based on the understanding of perineal and penile anatomy. The surgical technique is mainly composed of clitoroplasty, vaginoplasty and labioplasty. Clitoroplasty seems to be more important than the others in sex conversion from male to female [7]. Since the glans penis and the clitoris are embryologically corresponding organs, it can be logical to conserve the glans as a clitoris in sex conversion operations [1,11-13]. The anatomy of penile neurovascular structures has been described [14-16], but the detail of neurovascular pattern in the hilum and shaft of the penis and nerve distribution in the glans penis has remained unclear. A precise understanding of this anatomy is important in preserving neurovascular structures for clitoroplasty. A created sensate clitoris enhances the completeness of the external genitalia appearance and the ability to attain orgasm. Furthermore, the new knowledge in this study may be useful in the strategic design of sex reassignment surgery and in other related clinical conditions.

2. Research Questions

Primary research question

To determine how the dorsal arteries and nerves course along the penile shaft.

Secondary research questions

To define how the neurovascular structures relate to anatomical landmarks in the penile hilum and glans penis.

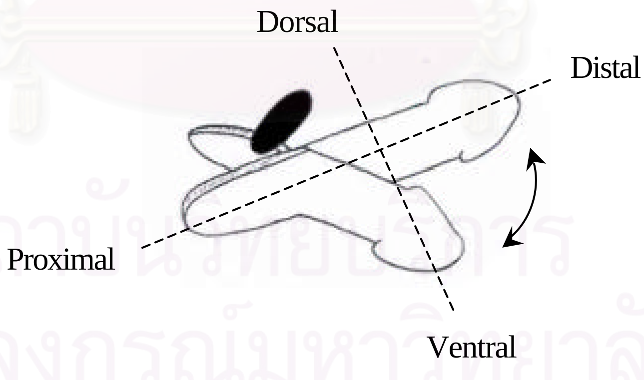
To determine whether there are differences in the structures and the genital corpuscles between glans clitoris and glans penis.

3. Objectives

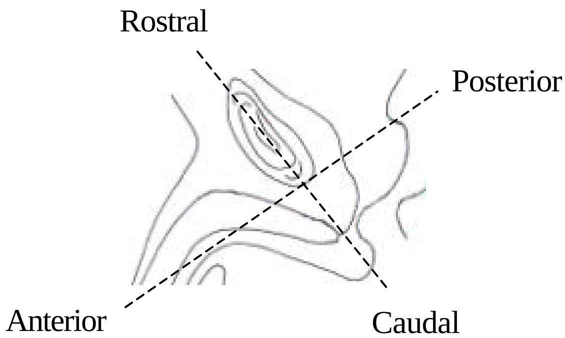
1. To define the neurovascular anatomy along the shaft of the penis.
2. To determine the neurovascular pattern in the penile hilum and glans penis.
3. To investigate the differences in the structures and the genital corpuscles between glans clitoris and glans penis.

4. Assumptions

1. The measurement has the validity and reliability.
2. The cadavers have no impairment of the external genitalia and pelvis.
3. A direction of the penis



4. A direction of pubic symphysis



5. Operational Definitions

Clitoroplasty: the procedure which consists of preservation neurovascular bundle on the dorsum of the penis and trimming the glans to match the size of a normal clitoris

Dysphoria: a state of feeling unwell or unhappy

Genital corpuscles: special encapsulated nerve endings found in the skin of the genitalia and nipple

6. Key Words

Neurovascular anatomy

Penis

Sex reassignment surgery

7. Expected Benefits & Applications

A better understanding of neurovascular anatomy at the hilum of the penis, shaft and glans penis may aid in planning penile surgical procedures including pelvic surgeries. For sex reassignment surgery, this detailed knowledge provides a valuable guide for protection of bleeding too much during surgery, and is useful in the strategic design of clitoroplasty and vaginoplasty for creating the effective neoclitoris and neovagina respectively.

CHAPTER II
REVIEW OF RELATED LITERATURES

Gender surgery

Although Edgerton and Bull [17] observed that a small clitoris-like structure may be formed during the final trimming and fitting of the scrotal flap, they did not detail their technique. However, the glans was not used for this propose, since it was kept attached to the penile skin to simulate a female cervix at the apex of the neovagina, as has been reported by others as well [18-20].

Laub and colleagues [20] described the eversion of urethral mucosa by Z-plasty to provide for a highly sensitive erogenous area in the superior aspect of the vulva normally occupied by the clitoris. Likewise, Wesser [18] devised a technique in which a V-shaped cut is made in the skin at the apex of the entrance of the vaginal skin where the urethral mucosa is sutured to the skin to create a new meatus. The small V-shaped skin flap is sutured in hood fashion at the apex of the meatus to simulate a clitoris. A small triangle of intact spongiosum is deliberately left beneath this to afford some erotic sensation.

Various authors reported suturing both stumps of corpora cavernosa together in the midline to form an erectile and sensitive neoclitoris [19,21,22].

In 1976, Brown [23] described creating a functional clitoris by using the reduced glans, which remained attached to its dorsal neurovascular pedicle. The long pedicle was foled up into the mons pubis. In 1993, Eldh [13] reported the use of the same technique. In addition, Fang and colleagues [7] presented the results of this technique as applied in nine of their patients.

Some authors reported applying a combination of these last two methods in severely masculinized female intersex patients [24,25]. The enlarged corpora cavernosa were reduced and ligated together in the midline. On the stump thus created, the reduced glans was transplanted pedicled on its dorsal neurovascular supply.

In 1980, Rubin [11] presented his method of creating a clitoris in sex conversion operation by using the glans penis, based on the urethra and corpus spongiosum as a vascular pedicle. For this, two midline slits were made in the penile skin flap. The larger, ventral slit which was taken just over the symphysis was sewn to the preputial border of the glans penis, while the smaller, dorsal slit beneath the symphysis was sewn to a urethral stoma, thus forming the neomeatus. Rubin reported that as a result of this technique, drops of urine appeared at the top of the neoclitoris. For this reason, he sealed the urethra by suturing the mucosa just distal to the new urethral orifice during a second operation. Von Szalay [26] slightly modified this technique and reported results in four patients. A comparable technique has been applied in the correction of intersex genitalia [27].

Small [28] presented his results of vaginoplasty in 11 patients in 1987. He reported developing the neoclitoris by using a large silicone chin implant placed in Gore-Tex and sutured to the periosteum of the symphysis pubis. This may be done either as a primary or a secondary procedure.

In 1991, Eicher and colleagues [29] published results of neoclitoroplasty in a series of 50 male-to-female transsexuals. They reported transplanting a free graft of the tip of the glans penis "as large as the seed of a sunflower" to a deepithelialized spot just ventral to the newly created urethral orifice. They did not indicate whether the dorsal nerves were transplanted to the location of the neoclitoris but reported clitoral sensibility in half their patients.

To improve further patient satisfaction as well as appearance, Perovic [30] used the glans to simulate a uterine cervix. The vascularized tube, consisting of penile skin and urethral flap in conjunction with the ventral part of the glans, was employed to create a new vagina with a pseudocervix at its base. The new vagina provided sufficient moisture for intercourse to be carried out. The formation of the pseudo-cervix also contributed to the sensation of orgasm during intercourse. In clitoroplasty, the new clitoris was achieved by its well-preserved neurovascular pedicle. It enabled the patients to attain orgasm.

In 1994, Hage and colleagues [12] made a neoclitoris from the glans and sculptured its final appearance during vaginoplasty using a free composite graft of the tip of the penile glans to cover the shortened dorsal neurovascular bundle. They considered transpositioning of the glans on its long dorsal neurovascular pedicle too risky. Later in 1996, Hage and Karim [31] used Brown pedicled sensate neo-clitoroplasty instead. Six such patients who were followed had satisfying sexual, cosmetic and functional results.

In 1999, Rehman and Melman [1] used an abdominal pedicled inverted penile skin technique to create a vagina and extra folds of skin to create a clitoral hood during 57 male-to-female gender surgeries. Because of general request for the placement of a sensate and erectile clitoris, the procedure was modified in the last 10 patients to create a neoclitoris from the glans penis. Glans volume was reduced to match that of a normal size clitoris and the entire length of the dorsal neurovascular bundle was preserved. They reported the good functional and cosmetic results in the majority of patients.

A few years ago, there were many issues which described the method of clitoroplasty in intersex patients. For example, Papageorgiou and coworkers [32] operated a 22-year-old woman to correct clitoral hypertrophy caused by congenital adrenal hyperplasia (CAH). Clitoral reduction was done through a semicircular incision in the phallus, with preservation of dorsal and ventral neurovascular pedicles. Moreover, Zaparackaite and coworkers [33] described his results of clitoroplasty in 28 CAH patients in 2002. The patients underwent clitoroplasty while the glans and the neurovascular bundle were preserved and clitoral skin was used for plasty of the labia minora. The patients were postoperatively observed for 0.5-5 years, the results showed to be good.

Though many issues reported that most patients were satisfied with the results after operation, some problems always happened during surgery or postoperative follow-up. For example, there were necrosis of neoclitoris caused by lacking in blood supply, bleeding too much during penectomy, the width of neurovascular pedicle on the shaft or the depth of the glans which was used to create neoclitoris for sparing the main nerves. A precise knowledge of penile anatomy could guide to improve surgical techniques to solve these problems.

Penile neurovascular anatomy

Vascular anatomy

In 1989, Breza and colleagues [14] described the dissection of 10 formalin-preserved adult male cadavers. The dissection revealed a surprisingly large variation in penile arterial supply, especially in the cavernous artery. The accessory pudendal artery was found in seven of 10 cadavers, predominantly on the left side. It originated from the superior or inferior vesical arteries, or from the obturator artery. No significant variations were found in the origin and course of the dorsal artery of the penis. There was no extracavernous connection between the dorsal artery and the cavernous arteries, as previously reported [34].

Between 1987 and 1994, Polascik and Walsh [35] identified the accessory pudendal arteries in potent men who underwent radical prostatectomy. The presence of accessory pudendal arteries was found in only 33 of 835 patients (4%). Once the operative technique was developed, they were able to preserve these arteries in 19 of 24 patients, but found that potency rates were similar in men with or without preservation of accessory arteries. Consequently, they concluded that the presence of these arteries was rare and the routine preservation might not be productive.

In 1997, Pineiro et al. [15] reported the dissection of penile arteries in 12 formalin-preserved adult male cadavers. Like Breza, several anatomical variations were observed in penile arterial system and the dorsal artery was almost consistent. However, the incidence of an accessory pudendal artery was not high. Recent immunohistochemical studies by Baskin et al. [36] as well as Pineiro showed that the cavernous artery perforated into the tunica albuginea of corpus cavernosum near the midline.

Subsequently, Benoit et al. [37] reported that accessory pudendal arteries were present in the pelvis in 17 of 20 cadavers (85%). 70% of them anastomosed with the cavernous arteries that originated from the pudendal arteries. The accessory pudendal arteries mostly originated from the inferior vesical and obturator arteries, suggesting that they could be injured during pelvic surgery and especially radical prostatectomy. With respect to function of penile arteries, the authors proposed that these surgeries will result in an acute decrease of penile inflow if the accessory arteries are the main supply for the corpora cavernosa. This attitude was similar to the report of Aboseif et al. [38] in which 15% of the patients were severe vasculogenic impotence after radical prostatectomy.

Based on the findings described by Polascik and Walsh [35], Rogers et al. [39] expanded their works and refined the surgical technique. In 2004, they reported the identification of accessory pudendal arteries in 84 of 2399 (4%) patients underwent radical prostatectomy. The study revealed that the accessory arteries could be preserved, and preservation might improve the potency rates and the recovery of sexual function after surgery.

Neuroanatomy

In the past, there were numerous clinical studies in animals and humans which showed the significance of the dorsal nerve of the penis in erectile and ejaculatory function. For instance, Bors and Comarr [40] observed impairment of erectile capacity in patients with spinal cord injury after pudendal neurectomy. Animal studies suggested that this deficit was caused by the interruption of that portion of the pudendal nerve conveying axons of the dorsal nerve [41,42]. Moreover, the crucial role of the dorsal nerve in human erectile function was supported by Seftel et al. [43], who showed that reflex erections could be abolished by anaesthetizing the dorsal nerve. For ejaculatory function, the contraction of the bulbocavernosus muscle was the predominant force for expelling semen from the bulbous urethra. Reflex contraction of this muscle after stimulation of the dorsal nerve was documented with electrophysiological studies [44]. Vibratory stimuli applied to the glans penis induced ejaculation in men with spinal cord injury [45,46]. Altogether, the dorsal nerve was supposed to be carrier of sensory impulses to the CNS for inducing these sexual reflexes.

Many authors believed that the glans acted as a sensory end-organ for sexual function. Kuhn [47] and others [45,46] revealed that the corona glandis and frenulum were areas of lower threshold for induction of erection and ejaculation than were other areas of the penis. In addition, Halata and Munger [48] reported a higher density of sensory receptors, called genital corpuscles, in the region of the corona and frenulum. However, More studies on genital receptors and neurophysiology were necessary.

Breza et al. [14] demonstrated that the topographical anatomy of the dorsal nerve of the penis was constant in their study. It passed along the dorsolateral surface of the penis, lateral to the dorsal artery, where it gave off multiple branches and terminated in the glans. Later, the data based on immunohistochemical studies [36,49] confirmed that the nerves organized into many bundles running alongside each corpus cavernosum and continued into the glans on the dorsal aspect. These suggested that the glans reduction in feminizing genitoplasties should be performed on the ventral aspect. No nerves were identified at 12 o’clock position in the dorsal midline.

In 1998, Yang and Bradley [16] described the distribution of the dorsal nerve of the penis along the penile shaft and within the glans penis. 22 human cadavers were dissected with an operating microscope. The results showed that the dorsal nerve was composed of two different populations of axons. The first group consisted of fibres travelling along the dorsal midline and terminating in the glans. The other group of fibres radiated from the main trunk over the lateral and ventral aspects of the penile shaft. There was no evidence of axons crossing the dorsal midline. The dorsal trunk divided into 2-3 nerve bundles which entered the glans. The dorsal bundles extended straight into the glans to form medial glanular branches, and ventrolateral branches arose from the lateral bundles.

Later, Benoit et al. [37] used gross and microscopic anatomical analysis to examine the dorsal nerve of the penis in 20 fresh male cadavers. At the penile hilum, the connecting fibres between the dorsal nerves and the cavernous nerves were observed. No anastomoses were found on the free part of the penis (pars pendula). These were the same results as described previously [14], but not as Paick et al. [50] who found many connections along the entire length of the penis. At the penile shaft, Benoit et al. demonstrated that the dorsal nerves coursed longitudinally on the dorsal surface of the corpora cavernosa, partially covering the dorsal artery. About half of the fibres were destined for the penile foreskin, perforating the penile fascia at the level of suspensory ligaments. They gave several fan-shaped branches surrounding the corporal surface towards the glans. These findings revealed the role of the dorsal nerves in sensory input from the glans and penile skin.

In 2001, Akman et al. [51] reported using the immunocytochemical techniques and three-dimentional reconstruction for analysis of nerves and crural bodies in relation to the pubic arch and surrounding structures. Under the pubic arch, the dorsal nerves were located near the ischial bones, slightly lateral to the urethra and proximal to the crural bodies. At the origin of the crural bodies where they touched the ischial bones, there were no well-defined nerve bundles. The dorsal nerves joined the corporeal bodies at the proximal origin where the 2 crural bodies fused together. At this point, perforating branches into the corporeal bodies from the cavernous nerves were documented. The topography of the dorsal nerves on the dorsum of corporeal bodies showed the same result as previous studies [36,49].

Although the detailed anatomy of the penis was described above by many authors, the neurovascular pattern in the hilum and along the shaft, including glanular innervation, has been still controversial. The purpose of this research was focused on the pattern and the distances between two dorsal arteries and between two dorsal nerves on the shaft. In aspect of innervation of the glans, immunohistochemical analysis was employed to prove the previous results in gross and physiological studies. Moreover, we delineated the number and position of genital corpuscles to be a basic knowledge of glanular sensation. A description of these neurovascular structures along the penis provided pertinent information for applying to sex reassignment surgery and other penile surgical procedures. In addition, the knowledge of sensory receptor provided insight into tactile sensation.

CHAPTER III
RESEARCH METHODOLOGY

Target Population and Sample Population

Adult male cadavers

Inclusion Criteria

- Adult male cadavers
- No impairment of the external genitalia and pelvis

Exclusion Criteria

- The cadavers have impairment of the external genitalia and pelvis.
- The cadavers infected AIDS, hepatitis B virus or severe diseases.

Sample Size Determination

In pilot study, the mean distance between the left and right dorsal nerves in 14 cadavers was 1.267 ± 0.114 cm.

<u>Continuous response variables and one-sample problem</u>

$$n = Z^2_{\alpha/2}\,\sigma^2/d^2$$

where; $Z_{\alpha/2} = Z_{0.05/2} = 1.96$ (two tail) at 95% CI

σ^2 = Variance = $(0.114)^2$

d = Acceptable error = 0.03 cm

so; $n = Z^2_{\alpha/2}\,\sigma^2/d^2$

$n = (1.96)^2\,(0.114)^2 / (0.03)^2$

$n = 28.33$

\ The sample size was at least 29 cadavers.

Materials

Cadaveric dissection

- Disposable plastic container
- Acetone, KNO_3 solution
- Feeding tube Fr.5, needles
- 10-cc syringes
- Polyester resin, hardener, cobalt (Co), red oil paint
- Vernier caliper
- Probe, forceps, operative knife
- Adult male cadavers
- Latex examination gloves

Immunohistochemical technique

- Tissue processing apparatus
- Adult penile and clitoral specimens
- Light microscope
- S-100 antibody

Methods

Cadaveric dissection (Penile shaft)

The dorsal penile arteries and nerves in 32 fresh adult cadavers (mean age 39 years) were dissected. The attachment of the prepuce around the neck of the penis including the frenulum was cut and the pliable tubular penile skin was retracted proximally to the root of the penis. Buck's fascia was opened longitudinally, exposing the deep vein and the dorsal arteries and nerves. The bleeding from the superficial vein was wiped carefully. The topography of the veins, arteries and nerves was noted throughout the entire length of the penis. Also, the distances between the left and right dorsal nerves, and between the left and right dorsal arteries were evaluated. A 3-mm thick paramedian longitudinal slice or a proximal transverse slice of specimens from the glans penis was obtained for immunohistochemical analysis.

Cadaveric dissection (Penile hilum)

11 soft-preserved adult male cadavers were dissected to document the penile neurovascular structures. To comfort for studying these structures, each cadaver was sectioned in transverse plane at the level of anterior superior iliac spine and both thighs. Later, pelvic block from transverse section was divided into two parts (left and right) in the midline. The pelvic visceral organs were retracted medially and the internal pudendal artery was exposed to inject coloured resin. Infusion was done with constant pressure and it was continued until the polyester resin filled the main artery and its branches. Once the resin was completely hardened, approximately 24 hours, dissection of the artery cast guided by its colour was done carefully layer by layer to investigate vascular pattern in the hilum of the penis. Moreover, Courses of nerves were followed throughout perineum in the same time.

After dissection, the nerves and arteries in relation to the surrounding structures were analyzed by measurement:

- The distances between the cavernous artery piercing into the penis and the inferior border of pubic symphysis, the junction of both crural bodies and the origin of crural bodies.
- The distance between the bulbourethral artery entering the bulb and perineal body.
- The height from the ischial tuberosity to the pudendal canal.

__Note__: The distance between two structures each was measured three times without bias to be analyzed by descriptive statistics.

Immunohistochemical analysis

All 32 specimens were fixed in formalin, embedded in paraffin and serially sectioned at 3 µm. Sections were stained with haematoxylin and eosin. Selected sections were stained immunohistochemically with antibody raised against the neuronal marker S-100. All immunohistochemical analysis was controlled with non-immune or pre-immune serum, or IgG at equivalent dilutions.

Each specimen was analyzed for a direction and distribution of nerve traveling into the glans, distance between main nerves within the glans and epithelial surface, and vascular structures. In regard to the genital corpuscle, it was scored in the measurable area of skin of the glans penis beneath the epidermal basement membrane. Finally, the distribution of the genital corpuscles per unit area was calculated. The specimens of which the skins were peeled during tissue processing were excluded from the analysis of the genital corpuscles and the distance between main nerves and epithelial skin.

In addition, normal clitoral specimens from 3 female cadavers were made histological sections for comparative study of the distributions of nerves and the genital corpuscles with normal glans penis.

Statistical analysis

Statistical analysis was undertaken with SPSS version 12. The data of measurements were analyzed by descriptive statistics as means, standard deviations, ranges and percentages. The association between the age and the distribution of the genital corpuscles per unit area was assessed by Pearson correlation analysis. The p-value of less than 0.05 was set for the significant difference.

CHAPTER IV
RESULTS

Neurovascular anatomy of the penile shaft

At proximal penile shaft, the dorsal artery coursed on the dorsal surface of the penis medial to the dorsal nerve. It then traveled divergently along the shaft to the lateral surface of the corpus cavernosum toward the glans penis. In ten of 32 fresh cadavers, the dorsal artery was present unilaterally, predominantly on the left, whereas in the others it was present bilaterally. There was only one of 10 cadavers in which it was found on the right. Special patterns were observed in two cadavers: the artery on the left curved immediately to the ventral aspect of the corpus cavernosum at the level of midshaft (Fig. 3A) and the artery on the right terminated as small branches at distal one-third of the shaft (Fig. 3B). The mean distance between the left and right dorsal arteries at the neck of the penis was 1.77 cm. In the same time during dissection, multiple small veins were seen emerging from the glans penis to drain into the deep dorsal vein. The circumflex veins, which were identified around the lateral surface of the penis superficial to the tunica albuginea, also opened into this vein. The deep dorsal vein coursed along the groove between the two corpora cavernosa and entered the pelvis through the suspensory ligament to drain into the periprostatic plexus.

The dorsal nerve of the penis was identified bilaterally in all cadavers dissected, lateral to the dorsal artery. This nerve as well as the dorsal artery has a characteristically crooked shape along its course. From the root to the neck of the penis, the dorsal nerve fanned out into small parallel branches that divided into two groups. The first group was composed of fibers traveling along the dorsolateral surface of the penile shaft and pierced the entire area of the corona of the glans penis posteriorly. The other group of fibers diverged to distribute throughout the lateral aspect of the shaft. A few variations were observed concerning the number and distribution, i.e. the number of dividing branches between sides. The mean distance between the left and right medial main branches of the dorsal nerves, which terminated in the glans, was 1.18 cm.

Table 1 Variations in the presence of the dorsal artery on the penile shaft in 32 cadaveric dissections

	n	%
Unilaterally on the left	9	28.1
Unilaterally on the right	1	3.1
Bilaterally	22	68.8

Table 2 Mean distances (in cm) between two structures at the neck of the penis

	n	Mean	S.D.	Range
The left and right dorsal arteries	20	1.77	0.37	2.50-1.20
The left and right dorsal nerves	32	1.18	0.17	1.50-0.75

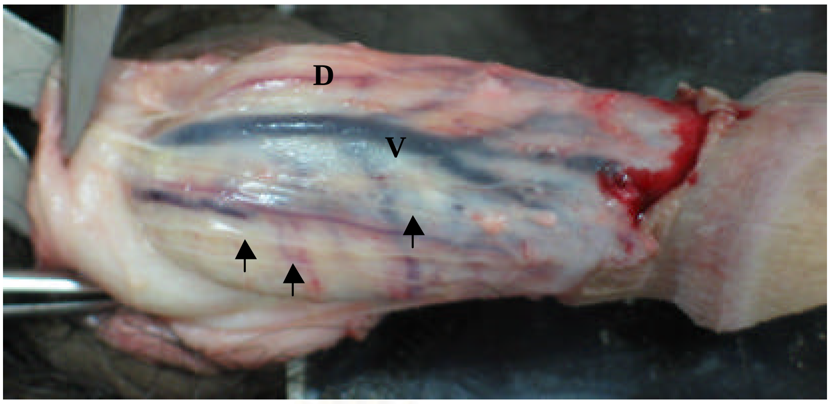


Figure 1. Neurovascular structures along the shaft. Dorsal artery (D) was present bilaterally. V, deep dorsal vein. Dorsal nerves (arrows) fan out into small parallel branches along the shaft.

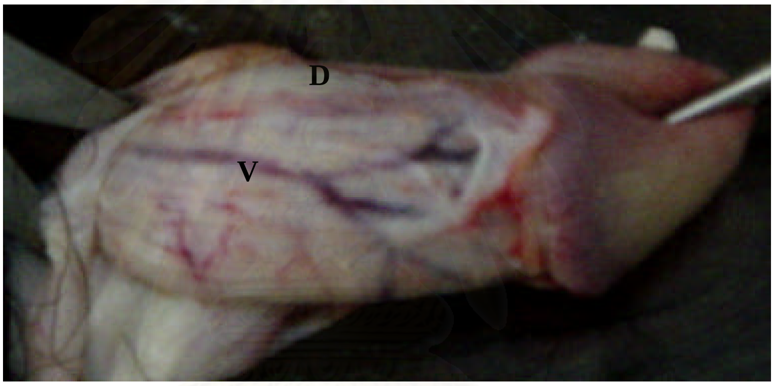


Figure 2. Dorsal artery (D) presenting unilaterally on the penile shaft. V, deep dorsal vein.

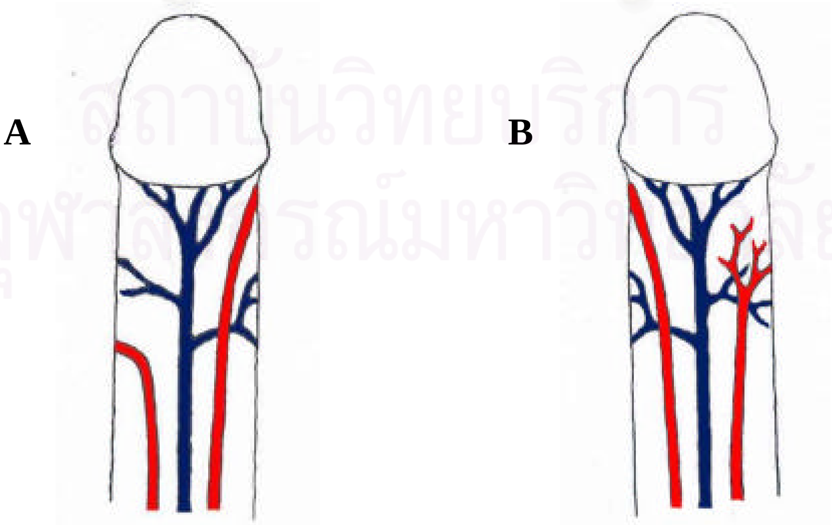


Figure 3. Special patterns of the dorsal artery on the penile shaft.

A, Dorsal artery on the left curves immediately to the ventral aspect at midshaft.

B, Dorsal artery on the right terminates as small branches at distal one-third of the shaft.

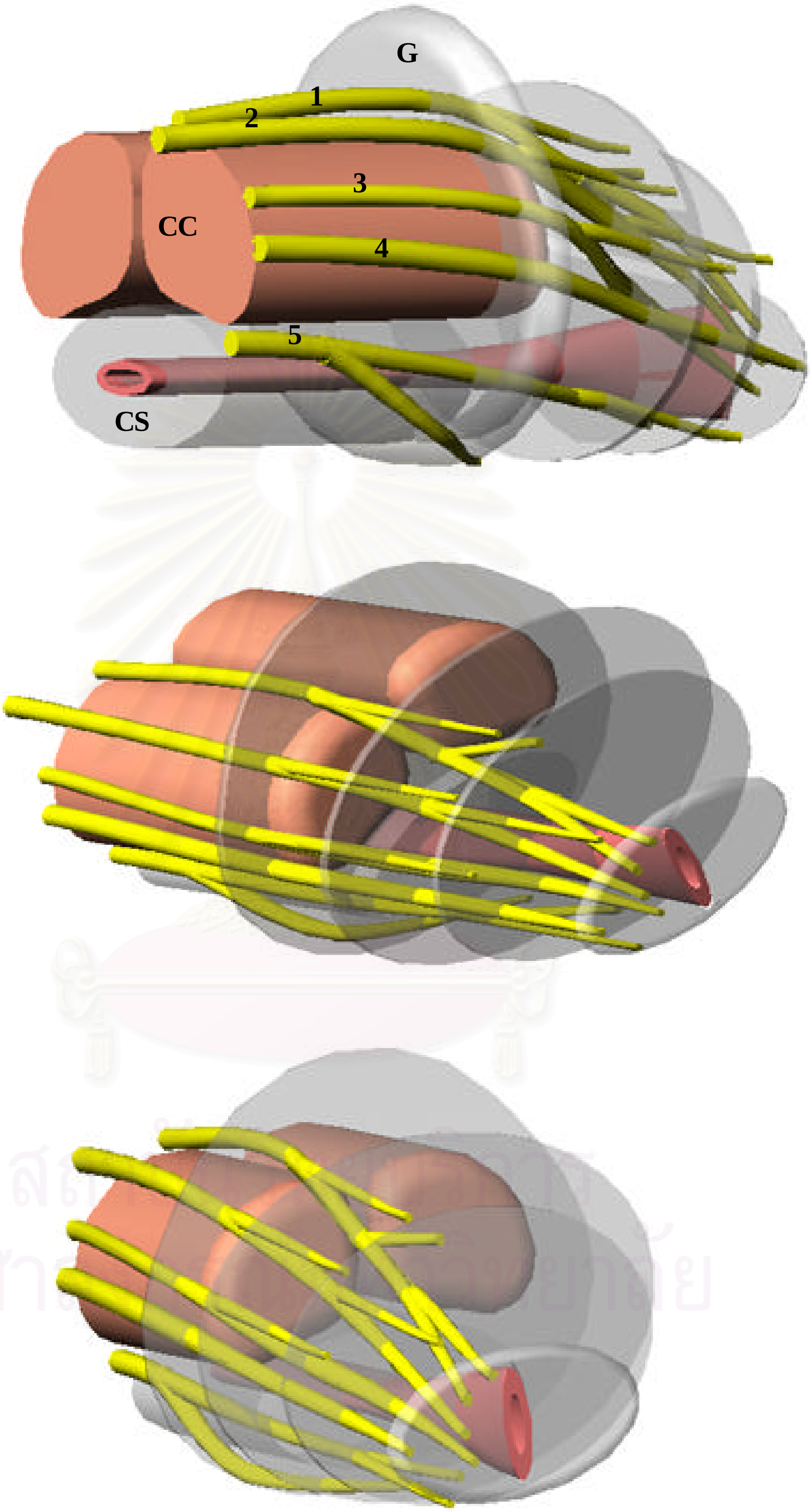


Figure 4. 3-D reconstruction images showing the distribution of nerves at distal penile shaft. Dorsal nerves (yellow, 1-4) surrounding on dorsolateral surface of corpora cavernosa (CC) and piercing the glans penis (G) posteriorly. Perineal nerves (yellow, 5) coursing on the lateral surface of corpus spongiosum (CS). This figure only shows the nerves on one side.

Arterial system in the hilum of the penis

The internal pudendal artery leaves the pudendal canal and gives off its perineal branches in superficial perineal pouch. The perineal branches provide blood supply to the scrotum, muscles and skin of perineum.

After giving off its perineal branches, the internal pudendal artery continues as the penile artery. The latter pierces the urogenital diaphragm and runs along the medial margin of the inferior ramus of the pubis. Near the urethral bulb, it divides into its terminal branches, consisting of the bulbourethral artery, cavernous artery and dorsal artery. This was seen in ten cadavers; however, in one cadaver, the internal pudendal artery terminated as the bulbourethral artery and the cavernous and dorsal artery were found to arise from the accessory pudendal artery.

The accessory pudendal artery was observed in 2 of 11 cadavers, traveling along the lower part of the bladder and the anterolateral surface of the prostate to the root of the penis. In one cadaver, it was present unilaterally on the left and originated from the inferior vesical artery to anastomose with the penile artery proximally to the origin of the cavernous artery. In the other cadaver in which it was present bilaterally, it originated from the inferior vesical artery on the right and the obturator artery on the left.

The first branch of the penile artery is the bulbourethral artery. It is a short artery of large caliber that passes medially to the crus, piercing the inferior fascia of the urogenital diaphragm to enter the dorsolateral aspect of the bulb, at a mean distance of 1.94/2.00 cm (left/right) from perineal body. The origin of bulbourethral artery varied among our cadavers. In five it arose from the main penile artery on both sides (Fig. 5, cadaver 1-5). In three it arose on one side from the main penile artery, but on the other side it originated from a common branch with the cavernous artery in one or with the perineal artery in two (cadaver 6-8). In one cadavers, it arose on one side from the main penile artery, while, on the other side, it arose from both the main penile artery and a common branch with the perineal artery (cadaver 9). In one cadaver, it arose on one side from the main penile artery, but on the other side it originated from both the main penile artery and a common branch with the cavernous artery (cadaver 10). In the last cadaver, it didn't originate from the main penile artery, but it arose on both sides from a common branch with the cavernous artery (cadaver 11). Concerning the variation in number, two bulbourethral arteries were identified on one side in five cadavers and on both sides in one cadaver.

The cavernous artery usually arises distally to the bulbourethral artery. In seven cadavers, it arose on both sides from the main penile artery (Fig. 5, cadaver 1-4, 7-9). In one cadaver, it arose on one side from the main penile artery, while, on the other side, it arose from a common branch with the bulbourethral artery (cadaver 6). In one cadaver, it arose on one side from the main penile artery, but on the other side it arose from both the main penile artery and a common branch with the bulbourethral artery (cadaver 10). In two cadavers, it arose on both sides from the accessory pudendal artery or from a common branch with the bulbourethral artery (cadaver 5, 11). More than one cavernous artery was identified on one side in four cadavers. The cavernous artery travels along the dorsomedial surface of the corpus cavernosum and medially to the dorsal artery. It perforates the albuginea of the corpus cavernosum at a mean distance of 0.25/0.23 cm (left/right) proximal to the junction of both cavernous bodies, 4.48/4.11 cm (left/right) from the proximal ischial attachment of the crus and 0.29/0.90 cm (left/right) caudal to the inferior border of pubic symphysis. The cavernous artery then continues distally in the center of the corpus cavernosum almost to its tip.

In 4 cadavers, small crural arteries were found, arising from the penile artery or accessory pudendal artery and passed laterally to enter the dorsomedial surface of the crus.

The dorsal artery is the terminal branch of the penile artery and has a characteristically tortuous shape that probably adapts to the increased penile length during erection. In ten cadavers, it arose from the penile artery. In the cadaver in which the internal pudendal artery ended on both sides as the bulbourethral artery, the dorsal artery arose from the accessory pudendal artery. The dorsal artery passes anterior to the crus and courses distally along the dorsum of the penis between the deep dorsal vein medially and dorsal nerve of the penis laterally at the proximal shaft of the penis.

Table 3 Mean distances (in cm) between anatomical landmarks in the hilum of the penis

	left				right			
	n	Mean	S.D.	Range	n	Mean	S.D.	Range
Bulbourethral a. to perineal body	10	1.94	0.37	2.56-1.22	11	2.00	0.40	2.54-1.53
CA* to inf. border of symphysis	12	0.29	1.19	1.83-(-1.54)	11	0.90	1.11	2.06-(-2.00)
CA* to corporeal junction	12	0.25	0.86	1.24-(-1.72)	11	0.23	0.85	1.11-(-1.38)
CA* to ischial attachment	12	4.48	1.34	6.59-2.64	11	4.11	1.01	5.66-2.25
Ischial tuberosity to pudendal canal	7	3.49	0.31	3.80-3.06	6	3.56	0.31	4.01-3.18

*CA, cavernous artery, measured from the site on the penis where the cavernous artery perforates the albuginea.

Table 4 Variations of penile arterial systems in 22 cases of 11 cadavers

Variations	No. of cases
Bulbourethral artery and perineal branch arise from a common trunk	3
Bulbourethral artery and cavernous artery arise from a common trunk	4
> 1 bulbourethral artery	7
> 1 cavernous artery	4
Accessory pudendal artery anastomosed with main penile artery	1
Cavernous artery as a branch of accessory pudendal artery	2
Dorsal artery as a branch of accessory pudendal artery	2
Bulbourethral artery as end artery of internal pudendal artery	2
One or more anatomical variations	15/22

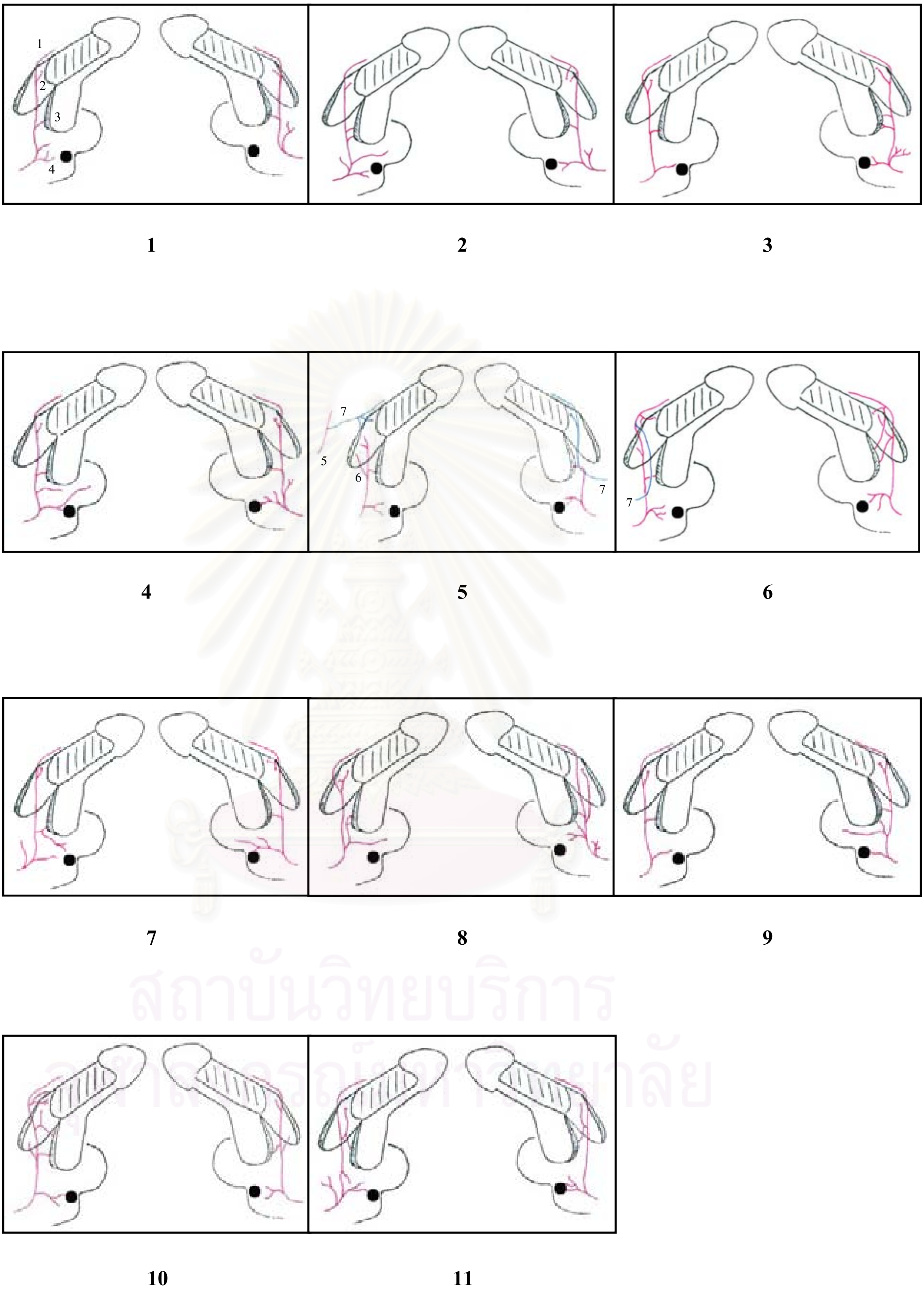


Figure 5. Penile arterial supply of 11 cadavers. It varies among 11 cadavers. 1, dorsal artery; 2, cavernous artery; 3, bulbourethral artery; 4, perineal artery; 5, obturator artery; 6, crural artery; 7, accessory pudendal artery. Black circles represent perineal body.

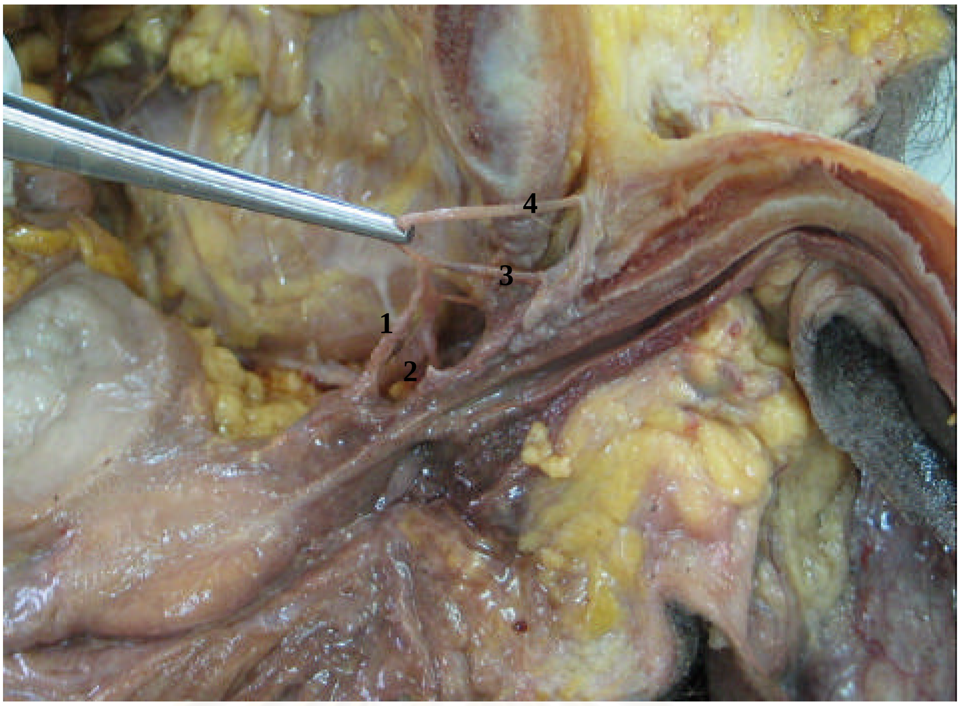


Figure 6. Anastomosis between the accessory pudendal artery and main penile artery. 1, accessory pudendal artery; 2, main penile artery; 3, cavernous artery; 4, dorsal artery.

Figure 7. The accessory pudendal artery (A) arising from the obturator artery (O). It ends as cavernous and dorsal arteries. Bulbourethral artery (B) as end artery of internal pudendal artery.

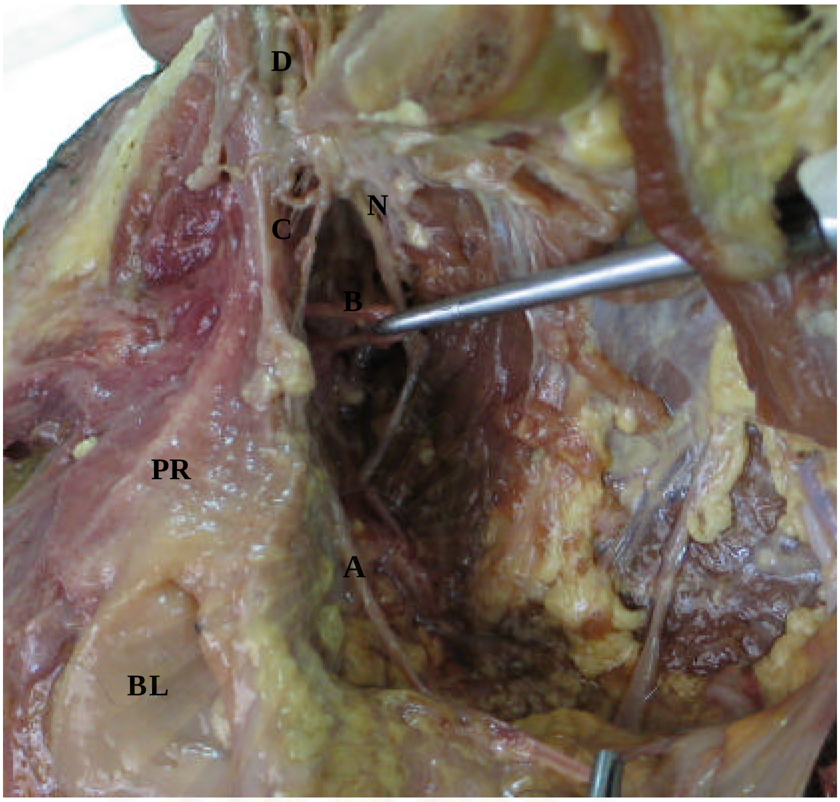


Figure 8. The accessory pudendal artery (A) arising from the inferior vesical artery. It ends as the cavernous (C) and dorsal (D) arteries. Bulbourethral artery (B) as end artery of internal pudendal artery. N, dorsal nerve; BL, bladder; PR, prostate.

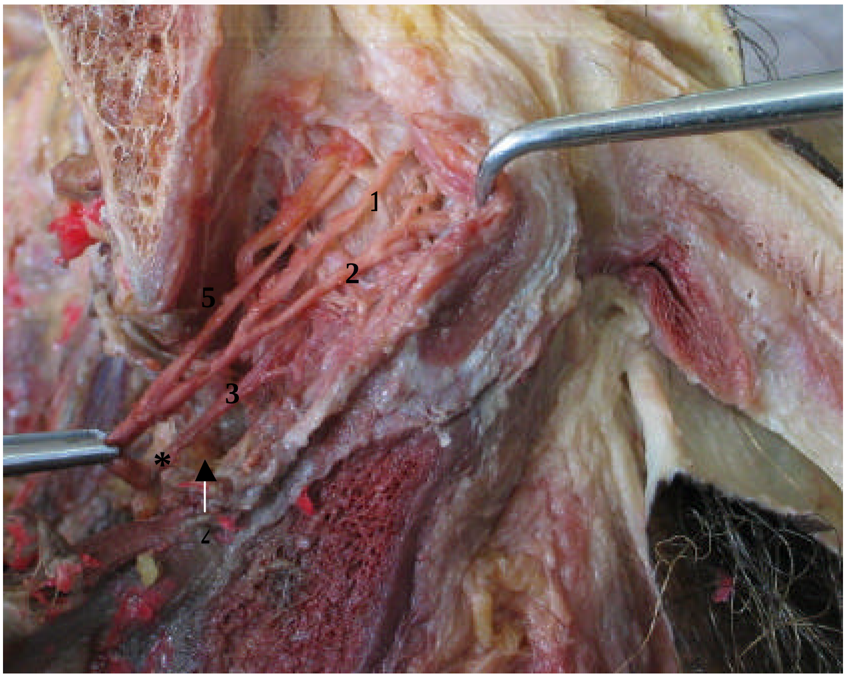


Figure 9. Three cavernous arteries (1, 2 and 3). Bulbourethral artery (4) and cavernous artery (3) arising from a common trunk (*). 5, dorsal artery.

Nervous system in the hilum of the penis

The main trunks of the ventral roots from S-2, S-3 and S-4 combined to form the pudendal nerve, which continued in the covering fascia of the pudendal canal on the lateral pelvic wall. The pudendal canal was 3.49/3.56 cm (left/right) above the ischial tuberosity. The pudendal nerve gave rise to the inferior rectal nerve, which emerged from the medial wall of the pudendal canal, coursing inferomedially in the ischiorectal fossa to divide into multiple branches that innervated the external anal sphincter and perianal skin. After the pudendal nerve left the pudendal canal, it extended its 2 terminal branches of the perineal and dorsal nerve of the penis. The perineal nerve sent the posterior scrotal branches into scrotal septum and the spermatic and scrotal fascia, but also gave several branches that coursed to musculature of urogenital diaphragm, perineal skin and urethral mucosa. The dorsal nerve after branching from the pudendal nerve traveled forward along the margin of the inferior pubic ramus into the urogenital diaphragm. It then coursed through the suspensory ligament of the penis anterior to the pubic symphysis and turned acutely downward to the dorsum of the penis, where it ran distally along the dorsolateral surface of the penis, lateral to the dorsal artery. The diameter of the dorsal nerve was approximately one-third comparing to the perineal nerves.

The cavernous nerves were identified as a thick network of strong fibers accompanied by thin arteries and veins. They passed along the posterolateral aspect of the seminal vesicle and the prostate, and then ran between the prostatic capsule and endopelvic fascia. These nerves were traced back to the inferior hypogastric plexus, which was located at the rectal mesocolon above the ampulla.

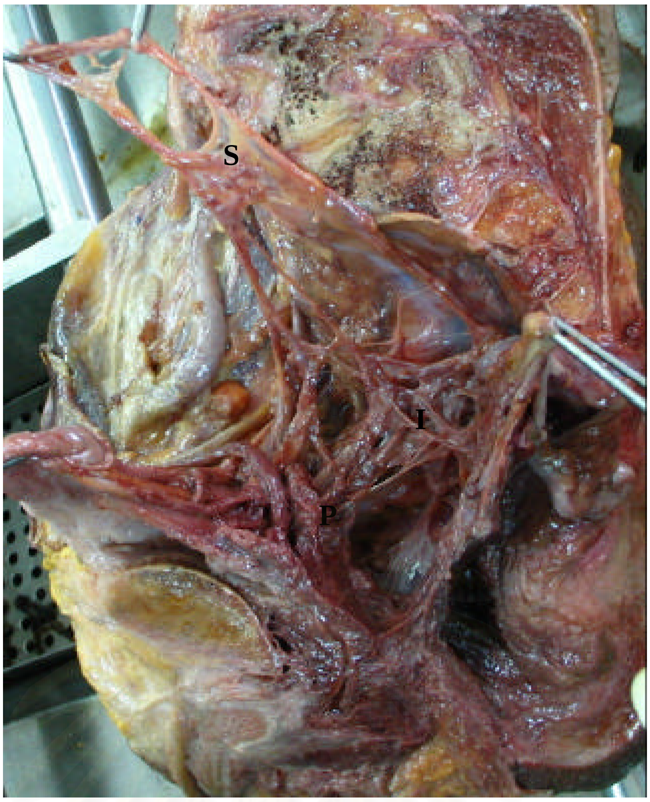


Figure 10. Nerve plexuses. S, superior hypogastric plexus; I, inferior hypogastric plexus; P, prostatic plexus.

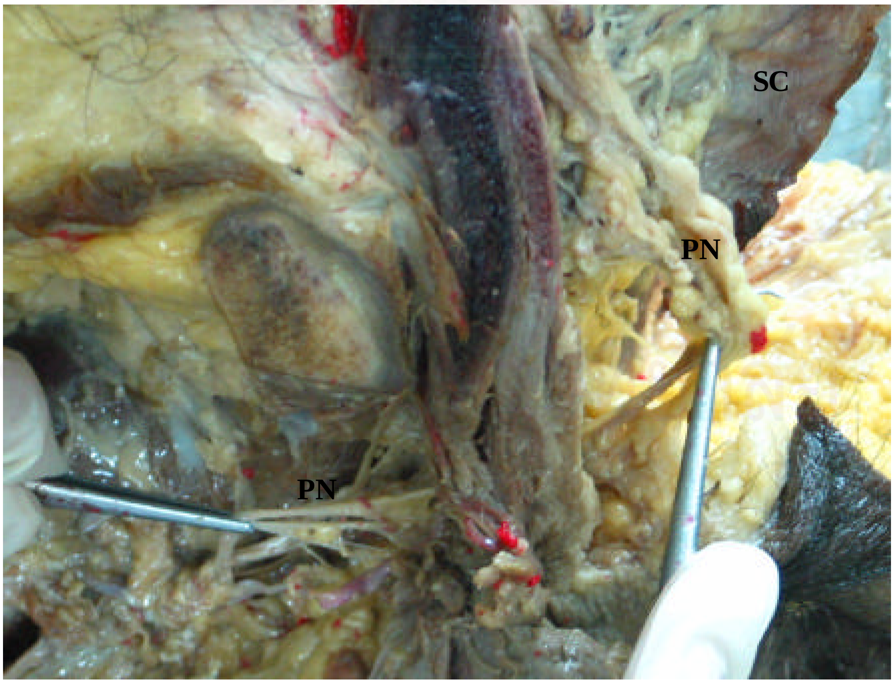


Figure 11. Perineal nerves to the scrotum and perineal skin. PN, perineal nerves; SC, scrotum.

Immunohistochemical analysis

Bell-shaped appearance of the cross sections of the glans clitoris revealed three zones of the structures within. These zones were different from the structural zones in the glans penis. The single core of the glans clitoris was dense mediastinal connective tissue without septum, surrounded with large multiple main branches of the dorsal nerves dorsally and laterally. The dorsal artery and some of its branches were placed laterally in the glans clitoris. Multiple venous channels were observed deep to the dorsal artery and the branches of the dorsal nerves in the border of the core forming the deep dorsal vein. Superficial to the core was spongy tissue zone with empty small venous sinuses. Between this zone and the dermis, the large well-developed densely-stained genital corpuscles lied, varying in size. The female genital corpuscles and the nerve bundles were obviously seen and arranged regularly. The nerve bundles distributed deep to the spongy tissue while the genital corpuscles lied superficially to this layer beneath the dermis. Some genital corpuscles were found in the dermal papillae and the spongy tissue. Four to five large corpuscles were regularly placed in the dorsal aspect of the glans. The thick and smooth dermis with flat dermal papillae was the outermost layer.

Longitudinal sections of the clitoris displayed the dorsal nerves, lying dorsally to the corpora cavernosa and bending down to distribute through the glans clitoris. Sebaceous glands were found in the dermis at the border of the glans. The clitoral hood skin contained Pacinian corpuscles and abundant genital corpuscles.

Cross sections of the glans penis consisted of three zones but were different from the glans clitoris. At proximal end, the inner zone of triple cores was two tips of corpora cavernosa and the single corpus spongiosum with the horizontal star-shaped urethral slit in the center. Some large apocrine glands and small blood-filled venous sinuses lied close to the urethral epithelium. The collagen fibers of tunica albuginea, coverings of the corpora cavernosa, were dense regular-arranged especially at the midline septum. The thin tunica albuginea of the corpus spongiosum blended with the dermis of the glans penis. About three large branches of the dorsal nerves, the superior, middle and inferior branches lied dorsolaterally to the tips of the corpora cavernosa on either side. Small nerve branches were observed among these main branches. Few perpendicular branches to the skin from the main nerves were notified in longitudinal sections. A mean distance from the main nerves to the epithelial surface was 0.71 cm. Large blood-filled thick-walled cavernous tissue layer formed the middle layer between the inner triple core and the outer dermal layer. The genital corpuscles were poorly developed and scantly found in the dermal papillae of the glans penis. In male, most of these thin-capsulated genital corpuscles lied superficially close to the basement membrane of the epidermis. A mean distance between two genital corpuscles was 0.33 cm, implying that the mean distribution of the genital corpuscles was 3 corpuscles per the length of skin of 1 cm. There was no statistically significant correlation between the age and the number of the corpuscles per unit area (r = 0.04, p = 0.86). The dermal and subdermal nerve plexuses were observed. The skin was probably shrunk after the fixing process from some degree of cavernous tissue emptying.

The dorsal artery was identified laterally to the dorsal nerves and could be found variably from 9 o'clock to 10 o'clock position. Multiple dorsal veins lied between the superior branches of the dorsal nerves to form the deep dorsal vein, which was proximal to the neck of the penis. The superior, middle and inferior branches of the dorsal nerves descended to both sides of the urethral slit in the middle of the glans penis, where they divided into small multiple branches distributing anteriorly to the surface of the tip of the glans. All terminal branches were concentrated around the dorsal half of the vertical slit-liked urethral meatus.

Table 5 Mean distances (in cm) between two structures in the glans penis

	n	Mean	S.D.	Range
Main nerve to epithelial surface	21	0.71	0.11	0.94-0.54
Distance between two corpuscles	20	0.33	0.15	0.65-0.07

Table 6 The correlation between the age and the distribution of the genital corpuscles per unit area

		Age	Distribution
Age	Pearson Correlation	1	0.044
	Sig. (2-tailed)	.	0.855
	N	20	20
Distribution	Pearson Correlation	0.044	1
	Sig. (2-tailed)	0.855	.
	N	20	20

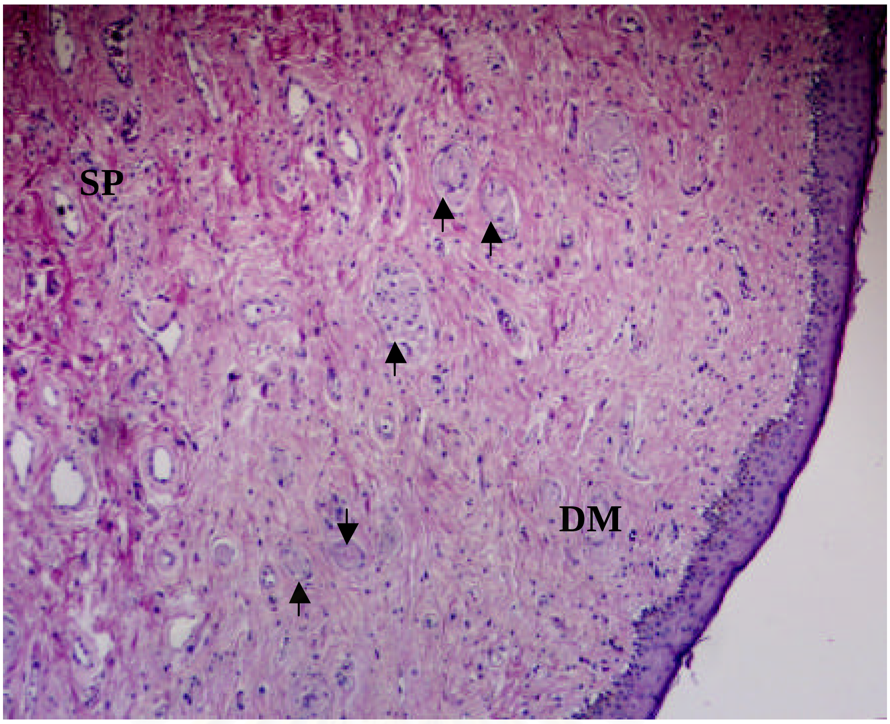


Figure 12. The genital corpuscles in the glans clitoris. Cross section. The genital corpuscles (arrows) lie between the spongy tissue zone (SP) and the dermis (DM).

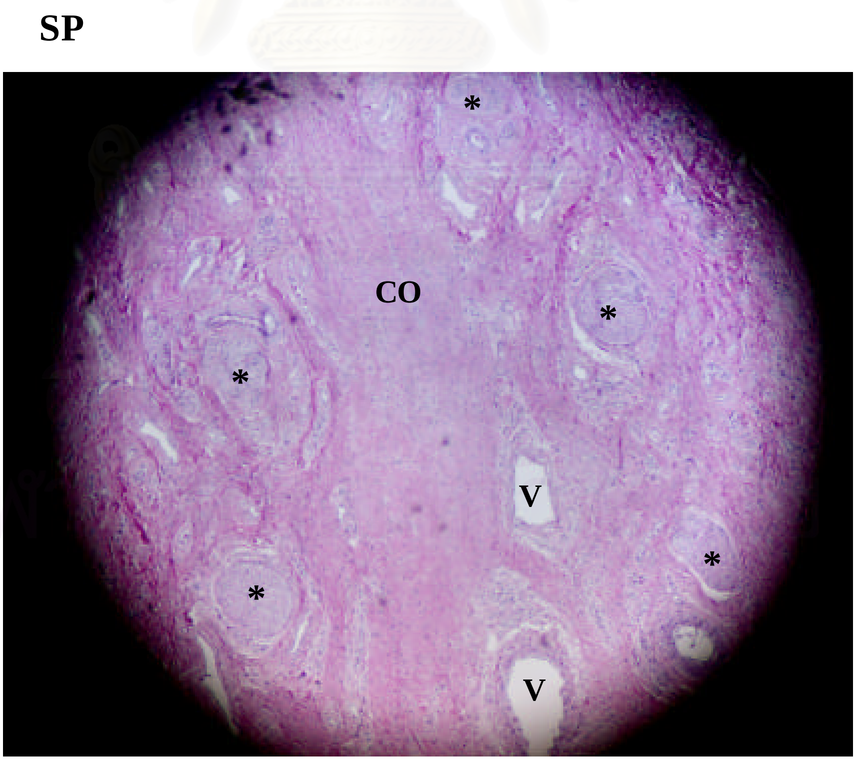


Figure 13. The structures within the glans clitoris. Cross section. Large multiple branches of the dorsal nerves (*) surrounding the core (CO) dorsally and laterally. V, veins.

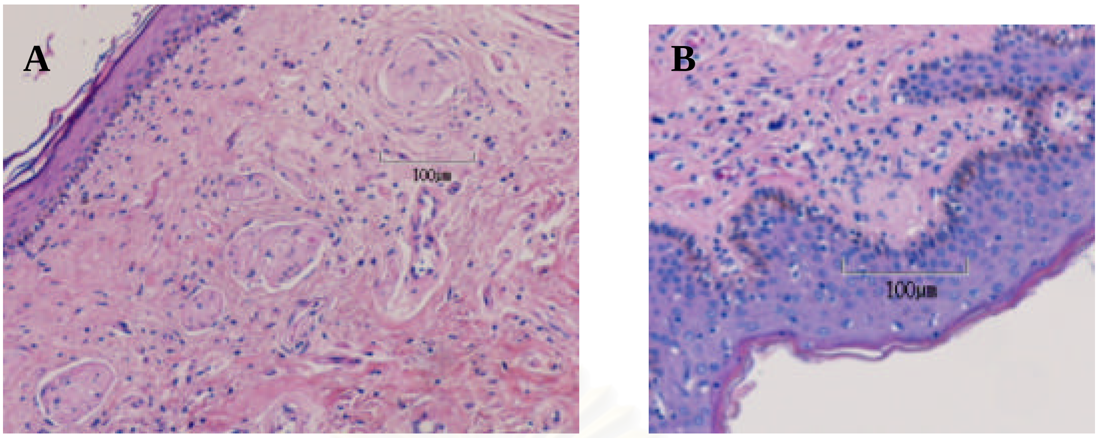


Figure 14. The genital corpuscles. Cross section. A, glans clitoris; B, glans penis. Genital corpuscles in glans clitoris are well developed, but in glans penis are poorly developed.

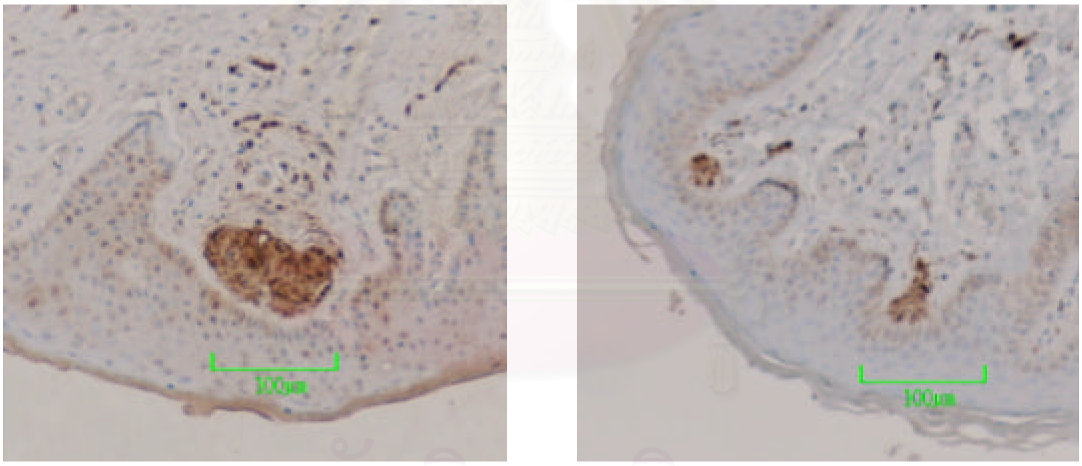


Figure 15. Genital corpuscles stained immunohistochemically with S-100 antibody in the glans penis. The genital corpuscles in glans penis are normally found in the dermal papillae.

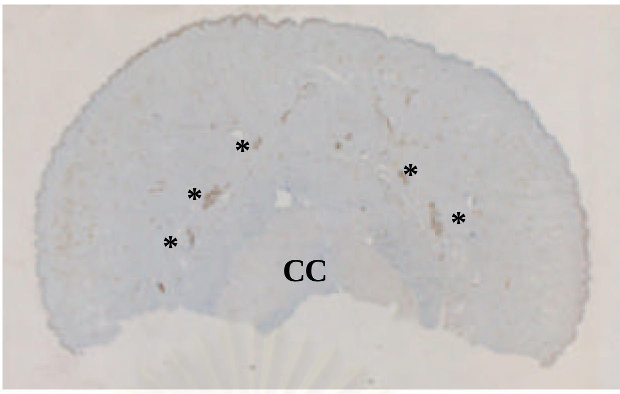


Figure 16. The dorsal nerve of the penis stained with S-100 antibody at the proximal glans penis. Nerves (*) lie dorsolaterally to the tips of corpora cavernosa (CC) on either side.

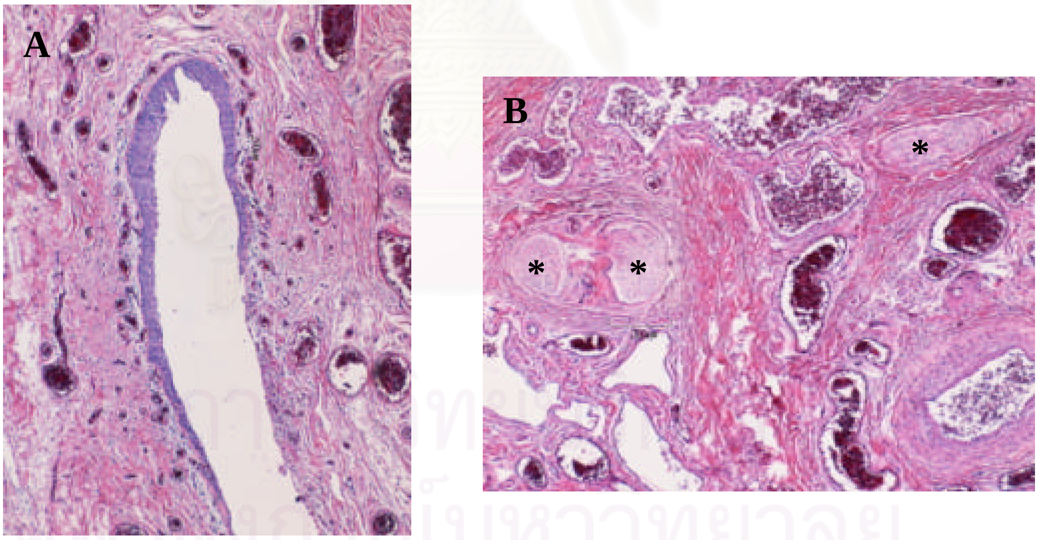


Figure 17. Terminal branches of the dorsal nerves concentrated around the dorsal half of the vertical slit-liked urethral meatus.

A, Small blood-filled venous sinuses lie close to the urethral epithelium.

B, Terminal branches of the dorsal nerves (*) are among venous sinuses.

CHAPTER V
DISCUSSION AND CONCLUSION

The dissections revealed anatomical variations in penile arterial supply. The reported incidences of accessory pudendal artery vary from 4% to 85% in surgical and cadaveric dissections [14,15,37,39]. Some previous reports could not be ensured in results because they did not clearly show the origin and end of artery. Moreover, result may be vary depending on whether small arteries located beneath the prostatic fascia are included. In this study, the accessory pudendal artery was identified in two of 11 cadavers (18%). It was seen originating from the inferior vesical or the obturator artery, as previously described [14,37]. The artery ended as the cavernous artery, dorsal artery or anastomosing with the main penile artery.

In the past, erectile dysfunction was a common complication that occurred after radical pelvic surgery of all types [52-54]. More evidences supported that impotence after pelvic surgery may result from vascular causes [38,55]. Although the corpora cavernosa normally receive their primary arterial supply from the internal pudendal artery, an additional blood supply was perhaps seen providing by an accessory pudendal artery. However, some cases were found that it was the main blood supply to the corpus cavernosum. This finding coincides with many reports [14,15,37], including our study. Because of its closeness to the bladder and prostate, the accessory artery may be inevitably compromised during radical pelvic surgery. This might contribute to vasculogenic impotence especially in patients with atherosclerosis of penile vasculature or with main blood supply existed above pelvic diaphragm. Recent study [39] has demonstrated that preservation of the accessory artery favorably influences potency rates and the time to regain potency after surgery. For this reason, special care should be taken not to injure this artery during surgery.

The results may allow the easier location of the cavernous artery at the penile hilum during surgery. It ran medially to the dorsal artery, nearly close to the inferior border of the pubic symphysis and perforated the tunica albuginea at approximate 4 cm from the proximal ischial attachment of the crus. Thus, after transecting the suspensory ligament, the cavernous artery should be sought at the hilum. Doppler ultrasonography may help delineate the anatomy of cavernous artery before penile vascular surgery to aid in selecting the appropriate procedure for an individual patient [56,57].

The pudendal nerve block technique has long been used in obstetrics. As the pudendal nerve can be easily blocked at the level of the ischial tuberosity, the pudendal nerve block is particularly appropriate to provide analgesia to the caudal and dorsal part of the scrotum which is supplied by perineal branch of this nerve. Watterson [58] described the local anaesthetic injection at a distance of 2.5 cm above the ischial tuberosity. This observation contrasts with our finding demonstrating that the pudendal canal was 3.5 cm above the ischial tuberosity. We suppose that the distance is not sufficient enough to block the pudendal nerve. The patient remains painful during surgery due to ineffective pudendal nerve block.

Constructing a neovagina is a main and original procedure in male-to-female surgery. It is usually done by either inversion of the penile skin [1,12,20] or use of a combination of skin flaps from the penis and scrotum [13,28]. Psychologically, erotic tactile sensation around perineum is crucial role for sexual arousing. From our anatomical point of view, the superficial perineal nerve was the essential sensory nerve of this area including the posterior scrotum. Using posterior-based pedicle scrotal skin flap for the construction of most of the vaginal wall may add some sexual pleasurable sensation compensating the less sensitive neoclitoris. However, Hair on the scrotal flap in the neovagina seemed to be a problem that was found in some of patients [13,28]. This problem can be prevented by using only de-epithelial scrotal flap with the penile skin tube flap for lining within it or using a hair depilating creme. This method may guide formation of an endurable sensate neovagina.

To create the neovaginal space, the rectourethral muscle is divided ventrally to the central tendon of perineum to allow blunt dissection between the urethra and rectum up to the peritoneal reflection. Sharp dissection in lateral space of rectum including using electrocautery for increasing the transverse diameter of the neovaginal space [1] may endanger neurovascular structures on either side in the pelvis, i.e. cavernous and other pelvic autonomic nerves. These pelvic hypogastric plexuses were located on the lateral walls of rectum above the ampulla and continued to the lower half of the prostate, functioning as the sphincteric and erectile control. Injury to these plexuses can cause urinary incontinence but impotence is not the case in male-to-female sex reassignment surgery.

In the step of penectomy, the crura of the corpora cavernosa are separated and amputated from their attachment to the bone. Our study showed that the cavernous artery entered the dorsomedial aspect of the corpus cavernosum adjacent to junction of both cavernous bodies. Consequently, we suggest that cutting the crura should be done close to the pubic rami and cauterized around this area to prevent the problem of perfuse bleeding.

Does the neoclitoris really enable the patient to have the sensation of orgasm during intercourse or the psychogenic stimulation does? Different from the glans clitoris, the glans penis functions as not only a sensory organ but also a penetrating organ. Sizes of the dorsal nerves reaching the glans of both sexes are comparable but the surface area of the glans penis is very large. Abundant nerve plexuses but scanty genital corpuscles in the dermis of the glans penis imply that the glans penis may be less sensitive than the glans clitoris. Because of the deep and wide separated position, both of the main branches of the dorsal nerves on either side were removed from the glans penis after the trimming process during surgery. The lateral position of the dorsal arteries at the neck of the penis suggests its absence in the distal part of the neurovascular pedicle of the clitoroplasty flap in some extent. From this point of view, the sensate neoclitoris of the patient may be less sensitive than the glans penis. A physiologic experiment is required to clarify this question. Most patients still have the sensation of orgasm because this achievable wonderful sensation is combination between psychological and physical stimulation.

To improve the sensate neoclitoris in clitoroplasty, we offer two methods for preserving neurovascular bundle. First, Buck’s fascia on the dorsum of the penis should be incised laterally or ventrolaterally, starting from the base of the penis. Then, the long neurovascular pedicle is raised deep to the tunica albuginea of both corpora cavernosa. This procedure can ensure an adequate blood supply of the flap but the pedicle may be very wide and thick. In the other method, after the penile skin flap is elevated, two longitudinal incisions about 1.2 cm in width are made laterally along Buck’s fascia to free the neurovascular pedicle. An adequate dorsal portion of the glans penis is preserved with a depth of at lease 0.7 cm, incorporating in the pedicle. This method can preserve the main branches of the dorsal nerves but may not preserve the dorsal arteries. Blood supply to the constructed clitoris is obtained from the small branches of dorsal arteries. These methods have their pros and cons, so that selecting to use depend on an opinion of surgeon.

Some surgeons [28,30] suggested closure of the penile skin tube proximally by using the ventral half of the glans penis as a pseudocervix and reported that it contributed to the sensation of orgasm during intercourse. Evidences from our study revealed that the dorsal nerve was the only main sensory innervation of the dorsal half of the glans penis. This nerve distributed three-dimensional branches through the spongy tissue and downed anteriorly forming an extensive network around the urethra. The sensation of the ventral half was innervated by the perineal nerves and dorsal nerves, which communicated each other along the cleft between the corpus cavernosum and the corpus spongiosum [59]. Accordingly, preserving these nerves requires careful dissection of the areolar tissues in this cleft from the excised corpora cavernosa.

In conclusion, the detailed anatomy of the neurovascular structures along the penis in this study can provide a valuable guide for applying to sex reassignment surgery, other penile surgical procedures and pelvic surgeries.

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APPENDICES

APPENDIX A

1. A general knowledge of the penis

The penis is the male organ of copulation and the common outlet for urine and semen. It is composed of three cylindrical bodies of erectile cavernous tissue that are enclosed by a dense white fibrous capsule, the tunica albuginea. Superficial to this layer is the deep (Buck's) fascia of penis which forms a common covering for the two corpora cavernosa and the corpus spongiosum. Two of the three erectile bodies, the corpora cavernosa penis, lie side by side in the dorsum of the penis, whereas the third lies ventrally in the median plane. This is the corpus spongiosum penis, containing the spongy urethra. The corpora cavernosa are fused with each other in the median plane, except posteriorly, where they separate to form two crura. The crura are attached to the medial surface of each side of the conjoint ischiopubic ramus and to the perineal membrane along the bone.

The penis consists of a root, a body and a glans. **The root of the penis** is made up of the bulb of the penis and the right and left crura of the penis. The bulb is situated in the midline and is attached to the inferior fascia of the urogenital diaphragm between the crura. It is traversed by the urethra and is covered on its outer surface by the bulbospongiosus muscles. Each crus is covered on its outer surface by the ischiocavernosus muscle. The bulb is continued forward into the body of the penis and forms the corpus spongiosum. The two crura converge anteriorly and come to lie side by side in the dorsal part of the body of the penis, forming the corpora cavernosa. **The body of the penis** is the free portion of the penis, which is suspended from the sysphysis pubis. It is essentially composed of two dorsally placed corpora cavernosa, which communicate each other and a single corpus spongiosum applied to their ventral surface. At its distal extremity, the corpus spongiosum expands to form the glans penis, which covers the distal ends of the corpora cavernosa. **The glans penis** forms the extremity of the body of the penis. It has a higher concentration of sensory nerve endings than the rest of penile body, and is thus particularly sensitive to physical stimulation. On the tip of the glans penis is the slitlike opening of the urethra, called the external urethral orifice (meatus). Extending from the lower margin of the external orifice is a fold connecting the glans to the prepuce called the frenulum. The edge of the base of the glans is called the corona. The prepuce or foreskin is formed by a fold of skin attached to the neck of the penis. The prepuce covers the glans for a variable extent, and it should be possible to retract it over the glans.

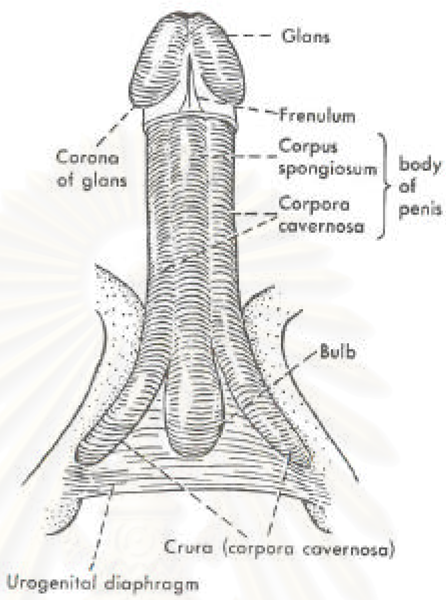

Fig. A. Composition of the penis (ventral view).

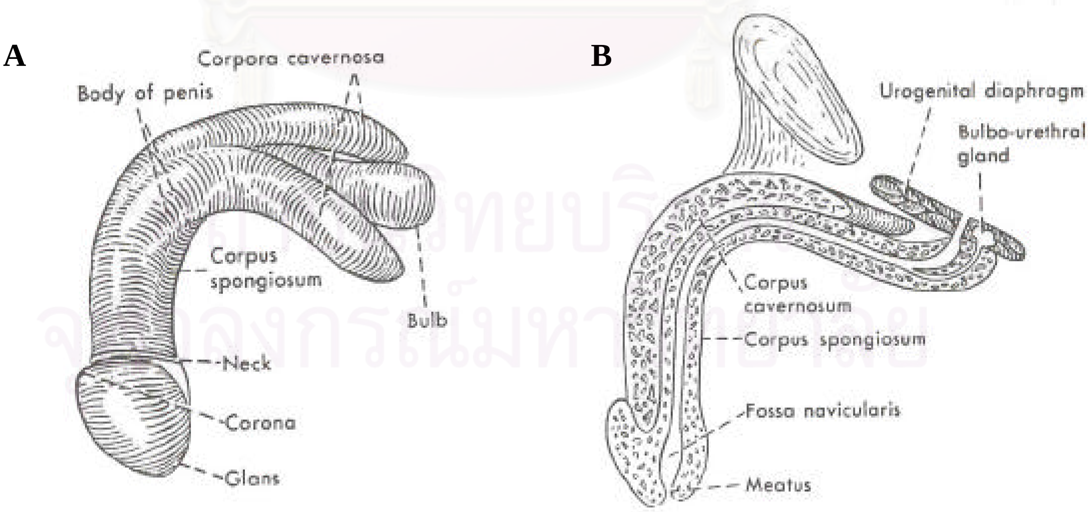

Fig. B. Composition of the penis. *A*, Dorsolateral view (left). *B*, The figure showing the urethra within the corpus spongiosum and the position of the bulbourethral gland.

2. The operative procedure of sex reassignment surgery

The operative technique is mainly composed of clitoroplasty, vaginoplasty and labioplasty.

Vaginoplasty. The patient is placed in the exaggerated lithotomy position. A vertical midline incision is made in the perineum. The transverse perineal muscles are divided laterally, and the central tendon between urethra and rectum is cut. Bilateral orchiectomy is performed, and the vas deferens and spermatic vessels are ligated allowing proximal ends to retract into the inguinal canal. Blunt dissection is performed between urethra and rectum up to the peritoneal reflection to create a neovaginal space. The penile skin flap (or a combination of skin flaps from the penis and scrotum) is sutured to form a skin tube. The skin tube is inverted and inserted into previously created cavity. The tube is held in place by a temporary pack. Two small vertical incisions are made in the penile skin tube to allow for the passage of the urethra and neoclitoris. The urethra and neoclitoris are pulled through the 2 openings.

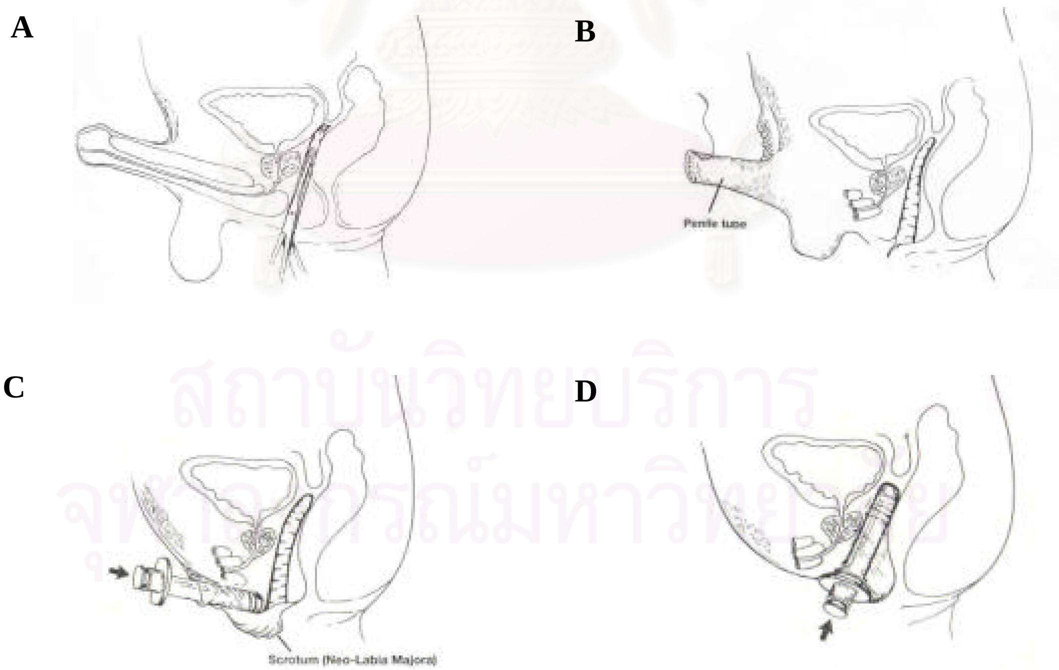

Fig. C. Vaginoplasty. *A*, Blunt dissection to create a new cavity. *B*, Flaps sutured together forming a skin tube. *C* and *D*, Insertion the inverted penile skin into previously created cavity.

Clitoroplasty. A circular incision is made around the coronal sulcus, and tubular dissection of the penile skin superficial to Buck's fascia is performed. The denuded penis is pulled out of the surrounding skin into the perineal incision. On the dorsum of the penis, the dorsal neurovascular bundle is identified above the tunica albuginea consisting of the dorsal vein, and paired dorsal arteries and nerves. Two longitudinal incisions are made lateral along Buck's fascia to free the neurovascular bundle. The dissection is performed distal to incorporate the glans penis and proximal to the symphysis pubis. The glans is trimmed to the size of the normal clitoris.

Labioplasty. The scrotal skin is used to create the labia majora. Two longitudinal incisions are made on each side of the neovagina and the scrotal skin is pulled up to create the labia majora on either side. The medial side of the incised scrotal ends is trimmed to have adequate size labia and the cut ends are sutured.

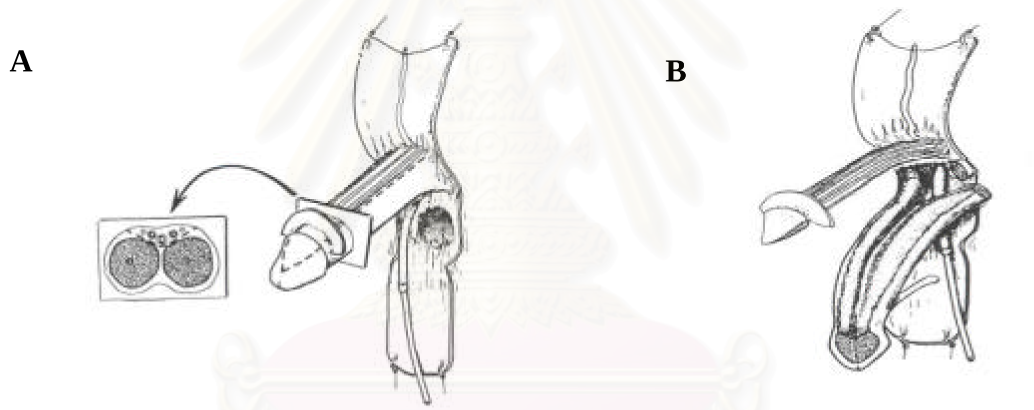

Fig. D. Clitoroplasty. *A*, Incision lines for dissection of the neurovascular bundle marked. *B*, Neurovascular bundle with part of the glans dissected free. Crus of the left cavernous body sectioned.

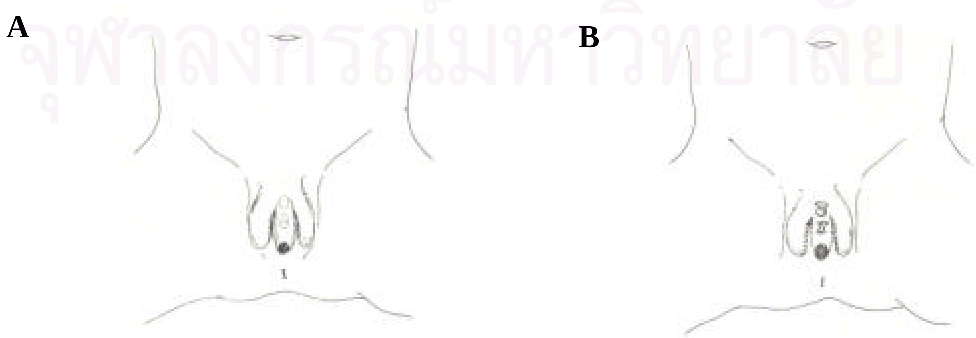

Fig. E. Vaginoplasty and Labioplasty. *A*, Incisions for localization of clitoris and urethra marked. *B*, The appearance of urethra and neoclitoris through 2 openings.

APPENDIX B

1. The procedure of casting the artery

<u>Reagents</u>

6.6 mol/kg KNO_3	800	ml
Polyester resin	100	ml
Acetone	100	ml
Red oil paint	5	ml
Hardener	10-15	ml
Cobalt (Co)	5	ml

<u>Procedure</u>

1) KNO_3 solution is injected into the internal pudendal artery to wash blood clots.
2) A mixture of polyester resin and acetone is added with red oil paint for coloring resin.
3) Before injection into the artery, hardener together with cobalt is added into the mixture in 2). Stirring is done until the mixture blends so well.
4) Finally, infusion is done with constant pressure till the resin fills the main artery and its branches.

2. The procedure of immunohistochemical analysis

<u>Procedure</u>

1) Cut and mount sections on slides coated with 3-aminopropyltriethoxy-silane.
2) Deparaffinize sections and rehydrate by passing tissues through absolute alcohol, 95% alcohol and distilled water.
3) Place in 3% hydrogen peroxide/distilled water for 5 minutes. Wash sections in running tap water.

4) Heat 2,500 ml citrate buffer pH 6.0 (2.1 g citric acid + distilled water 1,000 ml + 13 ml 2M NaOH) until it boils in a stainless steel pressure cooker. Cover but do not lock lid.
5) Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker to ensure that slides are completely immersed in citrate buffer solution. Lock lid.
6) When the pressure cooker reaches operating temperature and pressure, start a timer for 1 minute.
7) When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. Do not open lid until the indicators show that pressure has been released. Open lid, remove slides and place immediately into a bath of tap water.
8) Wash sections in PBS buffer pH 7.4 for 2-3 minutes.
9) Place sections in 3% normal horse serum for 20 minutes.
10) Incubate with S-100 antibody, dilution 1:1,000 for 60 minutes.
11) Wash PBS buffer for 2-3 minutes.
12) Incubate sections with DAKO EnVision peroxidase (KIT) for 30 minutes.
13) Wash PBS buffer for 2-3 minutes.
14) Incubate sections in DAB for 10 minutes.
15) Wash thoroughly in running tap water.
16) Counterstain with haematoxylin and wash in distilled water.
17) Dip slides in acid alcohol and wash in distilled water.
18) Dip in lithium carbonate to improve staining haematoxylin.
19) Dehydrate and mount as soon as possible.

APPENDIX C

1. The variations and mean distances of the dorsal arteries and nerves on the penile shaft

No.	Age	dorsal artery		Distance between two arteries (cm)	Distance between two nerves (cm)
		Left	Right		
1	26	+	+	2.50	1.50
2	47	+	-	-	1.30
3	34	+	-	-	1.30
4	19	+	+	2.21	1.26
5	26	+	-	-	1.23
6	27	+	+	2.13	1.40
7	19	+	-	-	1.20
8	48	+	+	1.67	1.32
9	56	+	+	1.34	1.14
10	67	-	+	-	1.22
11	48	+	-	-	1.25
12	25	+	+	2.42	1.40
13	20	+	+	1.40	1.12
14	35	+	+	1.20	1.10
15	25	+	+	-*	0.75
16	50	+	+	-**	1.04
17	45	+	-	-	1.20
18	42	+	+	1.46	1.00
19	26	+	+	1.49	1.12
20	52	+	+	1.63	1.05
21	35	+	+	1.40	1.00
22	56	+	+	2.05	1.33
23	60	+	-	-	1.30
24	50	+	+	1.72	1.31
25	68	+	-	-	1.07
26	24	+	+	1.58	1.14
27	47	+	+	2.15	1.25
28	23	+	+	1.65	0.84
29	30	+	+	1.70	1.42
30	25	+	+	1.96	1.15
31	64	+	+	1.73	1.24
32	28	+	-	-	0.84

* The artery on the right terminates as small branches at distal one-third of the shaft.

** The artery on the left curves immediately to the ventral aspect of the corpus cavernosum at the level of midshaft.

2. Mean distances between anatomical landmarks in the hilum of the penis (left)

No.	Bulbourethral artery to perineal body (mm)			Mean (mm
	1	2	3	
1	-	-	-	-
2	-	-	-	-
3	22.08	22.80	22.94	22.61
4	18.90	19.22	19.70	19.27
5	19.90	19.16	19.84	19.63
6	11.94	12.26	12.24	12.15
7	19.62	19.10	19.12	19.28
8*	20.16	20.32	20.14	20.21
9	21.10	22.36	22.12	21.86
10	16.06, 25.58	15.56, 25.36	15.82, 25.70	15.81, 25.55
11	17.24	18.10	18.60	17.98

* There are two bulbourethral arteries on one side. Only one bulbourethral a. is measured.

No.	Cavernous artery to inferior border of pubic symphysis (mm)			Mean (mm)
	1	2	3	
1	-4.72	-4.56	-4.84	-4.71
2	8.66	8.58	8.86	8.70
3	-15.56	-15.10	-15.52	-15.39
4	12.44	12.80	12.14	12.46
5	18.34	18.28	18.12	18.25
6	7.96	7.94	8.14	8.01
7	15.36	16.08	15.56	15.67
8	-6.86	-6.84	-6.60	-6.77
9	-	-	-	-
10	5.38, -6.06, -14.98	5.86, -5.94, -14.70	5.58, -5.98, -15.14	5.61, -5.99, -14.94
11	13.88	13.80	14.26	13.98

+ The cavernous artery is caudal to the inferior border of pubic symphysis.

- The cavernous artery is rostral to the inferior border of pubic symphysis.

No.	Cavernous artery to corporeal junction (mm)			Mean
	1	2	3	(mm)
1	6.48	6.72	6.54	6.58
2	4.50	4.62	4.48	4.53
3	5.62	5.80	5.48	5.63
4	12.20	12.04	12.10	12.11
5	2.12	2.14	2.06	2.11
6	-2.32	-2.44	-2.50	-2.42
7	4.46	4.44	4.24	4.38
8	12.46	12.34	12.30	12.37
9	-	-	-	-
10	4.36, -9.32, -17.10	4.36, -9.16, -17.48	4.24, -9.58, -17.14	4.32, -9.35, -17.24
11	7.84	7.50	7.12	7.49

+ The cavernous artery perforates the albuginea proximal to the corporeal junction.

- The cavernous artery perforates the albuginea distal to the corporeal junction.

No.	Cavernous artery to ischial attachment (mm)			Mean
	1	2	3	(mm)
1	26.28	26.76	26.58	26.54
2	28.42	28.16	28.46	28.35
3	60.00	59.60	59.46	59.69
4	42.42	43.54	42.08	42.68
5	26.02	26.56	26.46	26.35
6	46.56	45.24	45.92	45.91
7	41.80	41.84	41.30	41.65
8	66.04	65.78	65.88	65.90
9	-	-	-	-
10	38.78, 49.30, 60.74	38.56, 49.10, 59.62	38.96, 48.66,60.56	38.77, 49.02, 60.31
11	52.04	53.22	53.28	52.85

No.	Ischial tuberosity to pudendal canal (mm)			Mean (mm)
	1	2	3	
1	-	-	-	-
2	-	-	-	-
3	30.42	30.68	30.80	30.63
4	-	-	-	-
5	30.66	30.40	30.86	30.64
6	35.52	36.08	35.82	35.81
7	37.96	38.04	37.98	37.99
8	-	-	-	-
9	35.78	34.36	35.60	35.25
10	36.78	36.92	36.98	36.89
11	37.40	36.96	37.30	37.22

3. Mean distances between anatomical landmarks in the hilum of the penis (right)

No.	Bulbourethral artery to perineal body (mm)			Mean (mm)
	1	2	3	
1	-	-	-	-
2	-	-	-	-
3	17.08 , 22.00	17.46 , 22.86	18.10 , 22.08	17.55 , 22.31
4	24.58	24.72	24.48	24.59
5	15.44	15.46	15.08	15.33
6*	18.48	18.70	18.36	18.51
7	15.82	15.74	15.64	15.73
8	16.76, 24.48	16.80, 24.44	16.60, 24.00	16.72, 24.31
9*	25.54	25.40	25.38	25.44
10	15.92	15.80	16.04	15.92
11	23.02	24.02	23.54	23.53

* There are two bulbourethral arteries on one side. Only one bulbourethral a. is measured.

No.	Cavernous artery to inf. border of sysphysis (mm)			Mean (mm)
	1	2	3	
1	-	-	-	-
2	11.40 , 14.38	11.38 , 14.50	11.54 , 14.56	11.44 , 14.48
3	-19.88	-20.16	-19.84	-19.96
4	18.02	17.74	18.08	17.95
5	15.16	15.06	14.88	15.03
6*	20.32	20.62	20.70	20.55
7*	13.10	13.06	13.22	13.13
8	11.42	11.56	11.64	11.54
9	0	0	0	0
10	6.22	6.16	5.92	6.10
11	8.66	8.54	9.32	8.84

+ The cavernous artery is caudal to the inferior border of pubic symphysis.

- The cavernous artery is rostral to the inferior border of pubic symphysis.

* There are two cavernous arteries on one side. Only one cavernous a. is measured.

No.	Cavernous artery to corporeal junction (mm)			Mean (mm)
	1	2	3	
1	-	-	-	-
2	7.62 , 9.38	7.20 , 9.48	7.82 , 9.20	7.55 , 9.35
3	-2.50	-2.10	-2.18	-2.26
4	8.32	8.20	8.50	8.34
5	1.36	1.58	1.22	1.39
6*	-13.64	-13.96	-13.90	-13.83
7*	4.76	4.32	4.34	4.47
8	11.24	10.96	11.08	11.09
9	-12.60	-12.34	-12.30	-12.41
10	4.04	4.28	4.10	4.14
11	7.50	7.12	6.98	7.20

+ The cavernous artery perforates the albuginea proximal to the corporeal junction.

- The cavernous artery perforates the albuginea distal to the corporeal junction.

* There are two cavernous arteries on one side. Only one cavernous a. is measured.

No.	Cavernous artery to ischial attachment (mm)			Mean
	1	2	3	(mm)
1	-	-	-	-
2	22.32 , 24.30	22.40 , 23.96	22.64 , 24.66	22.45 , 24.31
3	50.48	50.26	50.72	50.49
4	41.30	40.98	42.30	41.53
5	45.90	45.46	45.88	45.75
6*	43.76	43.90	43.80	43.82
7*	41.7	41.16	41.12	41.34
8	46.24	46.70	46.84	46.59
9	56.12	56.78	56.74	56.55
10	40.04	40.92	40.08	40.35
11	38.80	39.44	39.20	39.15

* There are two cavernous arteries on one side. Only one cavernous a. is measured.

No.	Ischial tuberosity to pudendal canal (mm)			Mean
	1	2	3	(mm)
1	-	-	-	-
2	-	-	-	-
3	32.82	31.44	31.14	31.80
4	-	-	-	-
5	34.12	34.18	33.90	34.09
6	37.38	36.80	37.40	37.06
7	36.96	37.18	37.04	37.06
8	-	-	-	-
9	32.88	33.12	33.20	33.07
10	39.52	39.98	40.76	40.09
11	-	-	-	-

4. The distribution of the genital corpuscles per unit area

No.	Age	The number of corpuscles	The length of skin (cm)	The length/The number
1	47	3	1.95	0.65
2	34	16	5.68	0.36
3	19	25	1.79	0.07
4	26	7	2.73	0.39
5	27	17	3.55	0.21
6	19	8	3.30	0.41
7	48	5	2.64	0.53
8	56	17	2.71	0.16
9	25	8	3.19	0.40
10	20	18	3.83	0.21
11	25	14	2.50	0.18
12	50	15	3.86	0.26
13	45	26	5.08	0.20
14	26	5	2.30	0.46
15	35	7	3.15	0.45
16	50	14	3.89	0.28
17	68	32	5.37	0.17
18	24	16	4.13	0.26
19	47	8	4.09	0.51
20	25	13	4.78	0.37

5. Mean distance between main nerve and epithelial surface

No.	The depth (mm)
1	5.8
2	9.4
3	6.4
4	6.9
5	7.8
6	6.3
7	5.4
8	6.6
9	7.4
10	7.1
11	5.9
12	6.3
13	8.9
14	7.0
15	7.1
16	6.8
17	9.2
18	6.0
19	7.6
20	7.0
21	8.8

BIOGRAPHY

Mr. Thanakul Wannaprasert was born on November 15, 1981 in Bangkok, Thailand. He received his Bachelor degree of Science (first class honours) in 2002 from the Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. She has enrolled in graduate programme for Master degree of Medical Science at Chulalongkorn University since 2002.