

4.3 Histology and immunohistochemical studies of female genital tissue

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Introduction

While the anatomy, pathophysiology, and possible therapies of male sexual response have been extensively explored in recent years, little and discordant information has been produced for the female counterpart. However, scattered but interesting reports of female functional anatomy are now being published, and useful animal models are now available.¹ This chapter considers the three anatomic structures mainly involved in sexual female arousal: the clitoris, vagina, and urethra. Discussion of histologic and immunohistochemical findings may provide important insights into female sexual pathophysiology and therapy.

In the classic Masters and Johnson description of human sexual response, the excitation phase consists of female lubrication.¹

The clitoris

Masters and Johnson found the macroanatomy of the clitoris to be quite variable.² They described women with a long, thin shaft surmounted by a relatively small glans, and those with a short, thick shaft with a rather large glans, with numerous variations and combinations. A study of 200 women revealed that clitoris length varies by more than 25%,¹ demonstrating that individual differences are macroanatomic. It has only recently been clearly demonstrated by gross anatomy techniques that the clitoris consists of an erectile tissue complex surrounding the urethra and embedding the anterior vaginal wall.¹ This is of particular physiologic interest considering the role of this region in the sexual response.

The microscopic anatomy of the clitoris is consistent among different subjects. It consists of cavernous tissue, encircled by a thin fibrous capsule surrounded by large nerve trunks. Its cavernosal tissue consists of trabecular smooth and connective tissues, which encase the cavernosal sinusoidal spaces. The nerve network distribution pattern was studied by S-100 and neuron-specific enolase-immunoreactivity¹ (Fig. 4.3.1). The findings demonstrate that tissue organization in the corpora cavernosa of the clitoris is tunica albuginea and erectile tissue. There is a strong link between an increase in age and a subsequent decrease in clitoral cavernosal smooth muscle fibers with a relative increase of cavernosal connective tissue, as measured by histomorphometry.¹ Vascular risk factors and/or low estrogen levels, important in the pathophysiology of age-associated female sexual arousal disorders, may adversely affect clitoral cavernosal fibrosis.

Table 4.3.1 shows the immunohistochemical substances found in the clitoris and vagina of humans and experimental animals. Recent data from Burnett et al. showed the presence of nitric oxide-synthase isoforms in the human clitoris, suggesting that nitric oxide may be involved in its erectile physiology as a modulator of smooth muscle activity.¹ Findings of phosphodiesterase type 5 activity in human clitoral tissue support this theory¹ (Fig. 4.3.2).

The vagina

The vagina is a fibromuscular virtual tube, designed as the female copulatory organ. It extends from the vestibule, between

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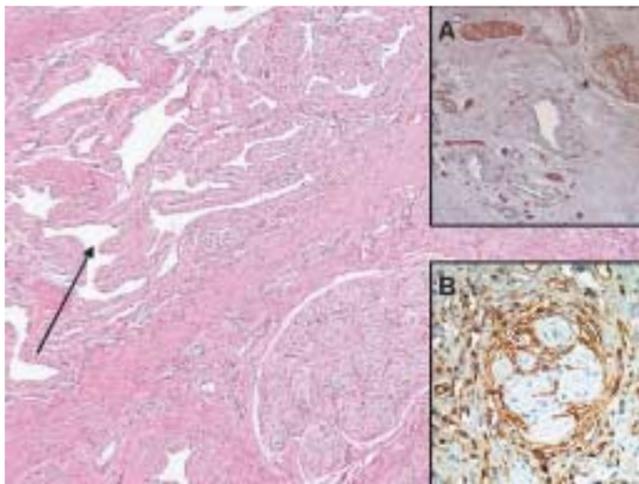


Figure 4.3.1. Human clitoris – specimens in these figures were obtained at autopsy from phenotypically normal premenopausal women aged 23–39 years. The cavernosum tissue (upper left, arrow) is surrounded by nerve fibers. HE staining, $\times 5$. Inset A shows immunostaining with the neuronal specific antibody to S100, demonstrating the abundant presence of nervous fibers ($\times 10$). Inset B is a section stained with the endothelial marker CD31, showing the presence of small vessels ($\times 10$). All the figures in the chapter are original, unpublished pictures obtained in our laboratory.

the labia minora, and the uterus, the anterior urethra and bladder, and the posterior anal canal and rectum.¹ Depending on rectum and bladder content, the vagina describes a 90° angle with respect to the uterus axis, ascending posterosuperiorly. Considered as a parallelepiped, the vagina is composed of a posterior wall, separated from the rectum by the rectouterine

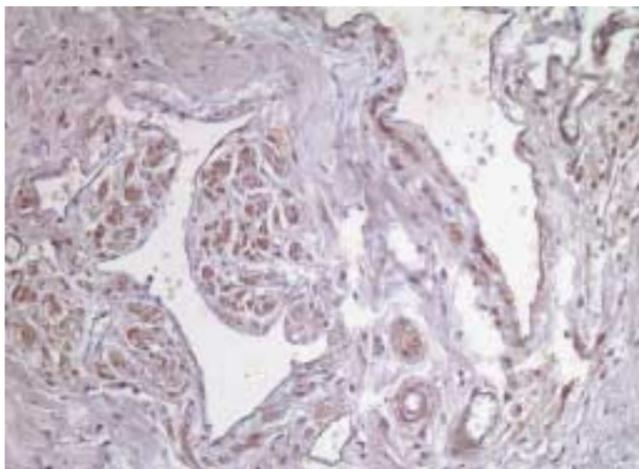


Figure 4.3.2. Human clitoris – expression of PDE5. Smooth muscle cells within the vessels of the cavernous tissue show a diffuse cytoplasmic stain. To our knowledge, this original figure is the first published showing the immunohistochemical distribution of phosphodiesterase within the human clitoris. $\times 20$. For material and methods, see ¹⁴.

pouch and from the anal canal by the fibromuscular structure of the perineal body. The lateral walls are the pelvic fascia and the muscle *levator ani*. From a sexologic point of view, the most important is the anterior vaginal wall, under the clitoris, in which the urethra and clitoral bodies are embedded.

Microscopic examination reveals that the vaginal structure comprises (1) the inner mucosal epithelial stratum, (2) a lamina propria containing thin-walled veins, (3) the intermediate muscularis stratum, and (4) the external adventitial layer. The inner mucosal epithelial stratum adheres strictly to the muscular stratum. Its nonkeratinized squamous epithelium is raised by two median, anterior and posterior, longitudinal ridges, between which are the *rugae* divided by sulci of variable depth, the *vaginal columns*. These conical papillae, most numerous close to the orificium and in the posterior wall, are less developed after natural parturition. Furthermore, the mucosal epithelium is hormone-dependent (estrogen) and changes during the menstrual cycle, having the potential for a basal, nonsexual, moisture. This basal lubrication is usually not sufficient for painless penetration: the vagina needs an enhancement of lubrication for coitus. The epithelium thickens after puberty and is rich in glycogen, mostly during ovulation. Glycogen is fermented by Döderlein's bacillus, lowering the vaginal pH. The lamina propria of the mucosa contains blood vessels contributing to the diffusion of the vaginal fluid across the epithelium, elastic fibers, lymphatic vessels, and nerves. It is a labyrinthine pathway, across which the neurogenically stimulated transudate filters during sexual arousal. An external longitudinal and an internal circular layer of nonstriated muscularis myocells, connected by oblique decussating fasciculi, make up the strong muscular stratum. The muscularis is composed of autonomically innervated smooth muscle fibers, containing a great variety of transmitters. For the majority of these compounds, the function is still unclear. The adventitia is rich in collagen and elastic fibers, giving structural support to the vaginal wall and allowing expansion during coitus and labor. The striated muscle bulbospongiosus surrounds the first third of the vagina. (Fig. 4.3.3).

In evaluating the histology of the human vagina, three concepts should be considered: individual differences (accounting for many discrepancies in the scientific literature), locoregional differences between anterior and posterior vagina, and the fact that the vagina and its histology are hormone-dependent, and thus cyclic.

The microscopic anatomy of the human vagina's antero-superior wall differs among subjects. The presence of pseudo-cavernous tissue (clitoral bulb) in the anterior vaginal mucosa is a frequent, but not universal finding (86%).¹ This anatomic variability should be taken in account when evaluating the physiology, pathology, and possible medical treatments of female sexual response. In the rabbit vagina, there are regional and functional differences. Adrenergic agonists in all regions can induce the contractile response, while prominent nerve-mediated relaxation can be identified only in the lower third.¹ Interestingly, this response is inhibited by the nitric oxide synthase inhibitor *N*- Ω -nitro-*L*-arginine methyl ester (NAME)

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Table 4.3.1. Histologic findings in the clitoris and vagina

Factor	Tissue	Animal	Function/localization	Reference
NOS	Clitoris Vagina	Human Rabbit Mouse Pig Cow	Neurotransmission, blood flow control, and capillary permeability	23, 24, 26, 33, 36, 46
nNOS	Clitoris Vagina	Human Rat Rabbit	Nerve fibers supplying smooth muscle, perivascular nerve plexuses, lamina propria	11, 34, 35, 42, 45, 47
eNOS	Clitoris Vagina	Human Rat Rabbit	Vascular endothelium, perivascular smooth muscle	11, 34, 35, 37, 45, 47
iNOS	Vagina	Human	Production of NO under certain conditions	35
PDE5	Clitoris Vagina Skene's glands	Human Rabbit	Breakdown of cGMP. Decrease of sexual arousal	12, 14, 35, 49
CGRP	Vagina Clitoris	Human Pig	Neurotransmission, blood flow control, and capillary permeability	22, 23, 26
SP	Clitoris Vagina	Human Pig Cow	Neurotransmission, blood flow control, and capillary permeability	22, 24, 26
NPY	Clitoris Vagina	Human Pig	Neurotransmission, blood flow control, and capillary permeability	22, 23, 25, 26
Estrogen receptors	Distal vagina	Rabbit	Downregulation of NOS	44
Androgen receptors	Proximal Vagina	Rabbit	Facilitation of vaginal smooth muscle relaxation	44
TGF β 1	Vagina	Diabetic rat	Fibrosis	29
VIP	Clitoris Vagina	Human Cat Rat Guinea pig Goat Hen Pig	Smooth muscle relaxation, neurotransmission, blood flow control, and capillary permeability	18-20, 22-24, 26, 32
PSA	Skene's glands	Human	Prostatic marker	56, 57, 59, 62
PAP	Skene's glands	Human	Prostatic marker	57, 58, 62
UP1	Skene's glands	Human	Protection of uroepithelium?	64
Chromogranin	Skene's glands	Human Pig	Marker of neurosecretion	35, 63

CGRP: calcitonin gene-related peptide; cGMP: cyclic guanosine monophosphate; NOS: nitric oxide synthase; NPY: neuropeptide Y; PAP: prostatic acid phosphatase; PDE5: phosphodiesterase type 5; PSA: prostate-specific antigen; SP: substance P; TGF: transforming growth factor; UP1: urine protein 1; VIP: vasoactive intestinal protein.

and by D-vasoactive intestinal peptide. In the human fetal vagina, adrenergic innervation is most prominent in the cranial portion and decreases toward the vestibulum.¹ These locoregional differences have been attributed to differences in embryologic origin. In fact, the lower third of the human vagina is derived from the urogenital sinus and the Wolffian duct, while the rest arises from the müllerian structure.¹

Vasoactive intestinal peptide has been previously considered as involved in vaginal vasodilation, the prerequisite for vaginal lubrication.^{1,2,3} It is present in the nerves closely applied to blood vessels in the vaginal wall, and its systemic or local administration increases vaginal blood flow and induces vaginal fluid production (see⁴, and references therein). However, vasoactive intestinal peptide-ergic fibers are distributed in the

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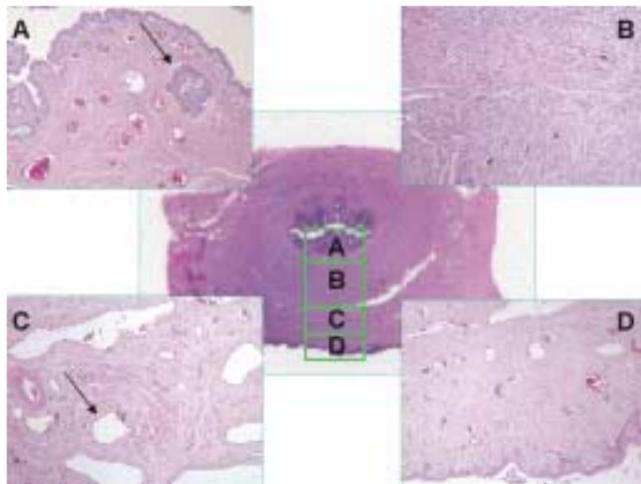


Figure 4.3.3. Human vagina – the central section is obtained by cutting the entire anterior vaginal wall. The urethra (A) was removed intact by sharp dissection. Specimens included periurethral tissue attached to the anterior vaginal mucosa (D). The vagina was then excised and opened along the posterior wall. The inserts are sections obtained at the levels indicated. Insert A: urothelium covering the lamina propria with numerous large vessels and an exocrine (Skene's) gland. Insert B: intermediate muscular stratum. Insert C: lamina propria of vagina with corpora cavernosa (clitoral bulb). Insert D: inner mucosal stratum of vagina covered by the vaginal epithelium. Inserts $\times 5$.

clitoris⁵ and in the animal^{6,7} and human vagina to a lower extent than neuropeptide Y (NPY) (also present in the clitoris⁸), but more extensively than calcitonin gene-related peptide (CGRP) and substance P (SP).⁹ These results imply that nerves that utilize neuropeptide Y, vasoactive intestinal peptide, or calcitonin gene-related peptide as a neurotransmitter may play a role in controlling blood flow and capillary permeability in the vagina. In fact, vasoactive intestinal peptide administration, both intravenously and by subepithelial injection in the vaginal wall, increases blood flow and induces lubrication.¹⁹

A possible alternative avenue for the treatment of erectile dysfunction has recently been found in the calcium-sensitizing ρ -A/Rho-kinase pathway, which, together with norepinephrine and endothelin, plays a synergistic role in cavernosal vasoconstriction to maintain penile flaccidity. In fact, in animal studies, its pharmacologic antagonism with Y-27632 results in increased corpus cavernosum pressure, initiating erection independently of nitric oxide.⁵ The same compound elicits relaxation of the rabbit vaginal wall and clitoral corpus cavernosum.¹⁰

Diabetes may induce lack of vaginal lubrication.¹¹ The smooth muscle profibrotic transforming growth factor (TGF) β_1 is expressed in diabetic rats, but not in normal animals, suggesting its involvement in diabetes-induced female sexual dysfunction.¹² Receptors for TGF β have been detected in rabbit clitoral smooth muscle cell cultures along with androgen and estrogen receptors.¹³

The nitric oxide–cyclic guanosine monophosphate (cGMP)–phosphodiesterase type 5 pathway is an important, if

not the primary, regulator of vaginal hemodynamics.¹⁴ Hen,¹⁵ mouse,¹⁶ and rat¹⁷ vaginal muscular wall contains abundant nitric oxide synthase-reactive nerve fibers running parallel to the smooth muscle bundles and beneath the epithelium. Nitric oxide synthase isoforms are largely distributed in the vagina of humans^{26,18} and experimental animals.¹⁹ A distinct distribution of immunoreactivity for nitric oxide synthase isoforms has been demonstrated in the human vagina.³⁵ In all subjects, the nerve bundles and fibers coursing within the organ were positive for the constitutive isoforms of nitric oxide synthase (neuronal and endothelial). These isoforms were also present in the endothelial lining of sinusoids and blood vessels and in the vaginal epithelium. Furthermore, neuronal (Fig. 4.3.4) and endothelial (Fig. 4.3.5) nitric oxide synthase isoforms have been found in the smooth muscle of the cavernous erectile tissue of the anterior wall of the vagina. In particular, endothelial nitric oxide synthase S has been found in the stratified squamous epithelial lining and in smooth cells, with maximum expression in experimental animals during estrus and proestrus.²⁰ This suggests that nitric oxide may be involved in vaginal secretion. Surprisingly, the inducible nitric oxide synthase isoform was found expressed in the human vagina, mostly in the epithelium and focally in smooth muscle³⁵. To confirm this finding, the inducible nitric oxide synthase antibody was tested for in cells harvested *in vivo* from the anterior wall of a volunteer's vagina. These cells were found to be highly positive. While specific immunoreactivity for inducible nitric oxide synthase was not demonstrated within clitoral specimens, it is present mostly in the vaginal epithelium and in the smooth muscle cells. Since inducible nitric oxide synthase is stimulated by bacterial products,²¹ the presence of this inflammatory isoform may be due to normal bacterial flora in the human vagina. The presence of inducible nitric

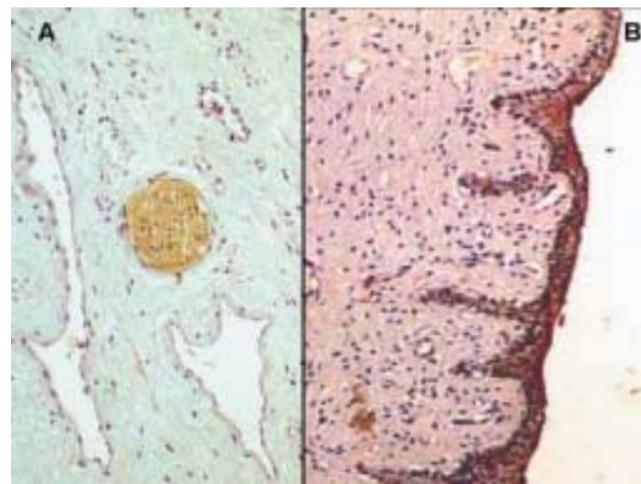


Figure 4.3.4. Human vagina (anterior wall) – staining of neuronal nitric oxide synthase with a specific commercial antibody. In panel A, a single nerve fiber is strongly positive, close to the lacunae of sinusoids. $\times 25$. Panel B shows that the enzyme is also present in the vaginal epithelium $\times 10$.

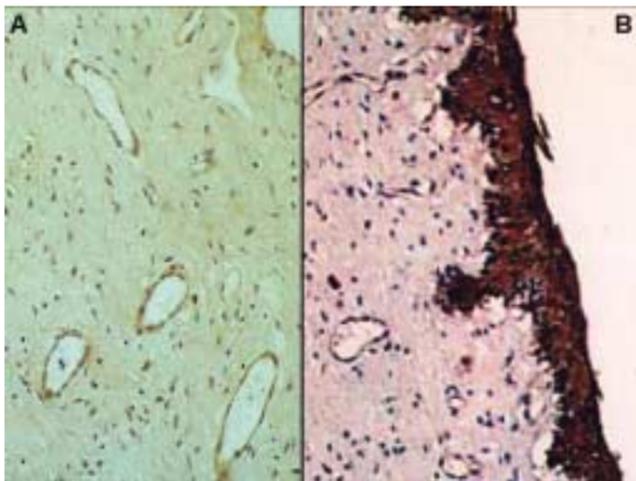


Figure 4.3.5. Human vagina (anterior wall) – staining of endothelial nitric oxide synthase with a specific commercial antibody. Endothelial lumen of sinusoids (panel A) and the vaginal epithelium (panel B) are strongly positive. $\times 20$.

oxide synthase may imply that the human vagina can produce nitric oxide under certain conditions. Vaginal smooth muscle is stained by constitutive nitric oxide synthase, as are smooth muscle fibers of other tissues.²² The presence of neuronal nitric oxide synthase in nerves suggests that this isoform produces nitric oxide as a postganglionic neurotransmitter mediating smooth muscle relaxation in the nitric oxide synthase-rich corpora cavernosa. The abundance of endothelial nitric oxide synthase suggests its prominent role in female sexual response. Endothelial nitric oxide synthase was mostly localized in the endothelial lining of sinusoids and blood vessels throughout the erectile tissue of the vaginal anterior wall. Both those findings mirror the anatomic scenario found in the human clitoris,¹¹ with some exceptions. The distribution of nitric oxide synthase isoforms partially supports the recently found relationship between the urethra and clitoris: gross anatomy techniques have demonstrated that the female perineal urethra is embedded in the anterior vaginal wall and surrounded by erectile tissue forming the bulbs of the clitoris.⁸ The anterior vaginal wall thus contains the clitoral bulbs, but it is probably much richer in nitric oxide synthase than the clitoris itself.

Nitric oxide synthase expression and activity are under steroid hormone regulatory activity. In fact, androgens and female steroids modulate distinct physiologic responses in the vagina. Progesterone upregulates vaginal nitric oxide synthase.²³ Although estrogen treatment reduced total nitric oxide synthase activity in the proximal vagina, estrogens are known to enhance vaginal blood flow.²⁴ Androgens facilitate vaginal smooth muscle relaxation through their own receptor localized in the proximal vagina, while estrogen downregulates nitric oxide synthase activity in the distal vagina.²⁵

This paradoxical observation has been explained by differential, locoregional (distal with respect to proximal vagina)

regulation of estrogen receptors. Both neuronal and endothelial nitric oxide synthase are under estrogen control. In fact, after oophorectomy, levels of both enzymes decline, with a parallel induction of apoptosis, vaginal atrophy, intramural collagen accumulation, and perivascular wall thickening.²⁶ However, these results have not been replicated in other experimental models.^{27,28}

Treatment with sildenafil enhances intracellular cyclic cGMP synthesis and accumulation of cultured human and rabbit vaginal smooth muscle cells.²⁹ This suggests that vaginal smooth muscle expresses phosphodiesterase type 5. Immunoreactivity for phosphodiesterase type 5 partially followed the distribution of nitric oxide synthase isoforms. In fact, it was observed focally in the endothelial lining and smooth muscle of the anterior vaginal wall's cavernous erectile tissue, and was widespread in the vaginal epithelium. The immunocytochemistry of vaginal cells harvested *in vivo* confirmed the latter finding. No staining of nerve bundles was observed and, considering the vagina as a whole, phosphodiesterase type 5 expression was low, confirming the focal expression of this enzyme.³⁰ The colocalization of phosphodiesterase type 5 in the tissues considered as *bona fide* targets for nitric oxide (epithelia, exocrine glands, and muscle) may warrant the breakdown of cGMP produced locally upon nitric oxide stimulation.

The demonstrated presence of the nitric oxide synthase–phosphodiesterase type 5 machinery in the human clitoris and vagina is the anatomic rationale for the use of phosphodiesterase type 5 inhibitors in female sexual arousal disorders, defined by the Consensus Panel of the American Foundation of Urological Diseases as the persistent inability to attain or maintain sexual excitement (lubrication/swelling).³¹ Sildenafil has been found capable of antagonizing the sexual discomforts induced by antidepressant drugs,³² as well as of partially reversing sexual dysfunction in women with spinal cord injuries.³³ In a double-blind, crossover, placebo-controlled study performed in young women affected by arousal disorders, sildenafil was demonstrated to improve sexual performance significantly,³⁴ a result which has been subsequently replicated.³⁵

The urethra

The female urethra from the bladder to the orifice is about 4 cm long, with a diameter of 6 mm. It runs anteroinferiorly behind the symphysis pubis, embedded in the anterior vaginal wall. The mucous membrane lining the lumen is a stratified epithelium under the lamina propria of fibroelastic connective tissue. Its proximal section is a transitional urothelium identical to that of the bladder neck, while the distal section changes into a nonkeratinizing, stratified squamous epithelium. The muscular coat is composed of both the striated, external urethral sphincter and an inner coat of abundant smooth muscles, oriented obliquely or longitudinally.

The female urethra is surrounded by numerous sinusoids (corpus spongiosus of urethra). However, individual differences

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have been found in the presence and extension of these venous/sinuous channels.^{14,35} In the luminal epithelium, serotonin-producing cells have been detected. Mechanical stimulation of the urinary urethra converts it into the "sexual" urethra, possibly by the releasing of 5-HT, which enhances neural afferent input from the organ.

Skene's glands

Classic anatomic textbooks fail in careful and exact description of the macro- and microanatomy of the female genital tract.⁸ Gray's *Anatomy* summarily and inexactly describes the urethral glands (Fig. 4.3.6) and the minute, pit-like recesses (lacunae) opening into the urethral lumen. Some of these glands are grouped together and may open in two symmetric, paraurethral ducts, on the lateral margin of the external urethral orifice. However, numerous ducts open independently in the urethra, coming from glandular acini with both exocrinal and endocrinal activity.³⁶ In 1672, the Dutch anatomist Regnier de Graaf illustrated a set of glands and ducts surrounding the female urethra. Later, in 1880, Alexander Skene redirected attention to this structure, giving his name to these glands and ducts.³⁷

Histomorphologically, Skene's glands (Fig. 4.3.7) strongly resemble the male prostate before puberty.³⁸ In many cases, they remain immature throughout life because of a lack of androgenic stimulus. However, when they appear more mature and with high secretory activity, this could be due to the absolute or relative overpresence of androgens during a certain time of life. They are surrounded by a nonglandular contractile system, composed of smooth muscle cells and/or muscolofibrous

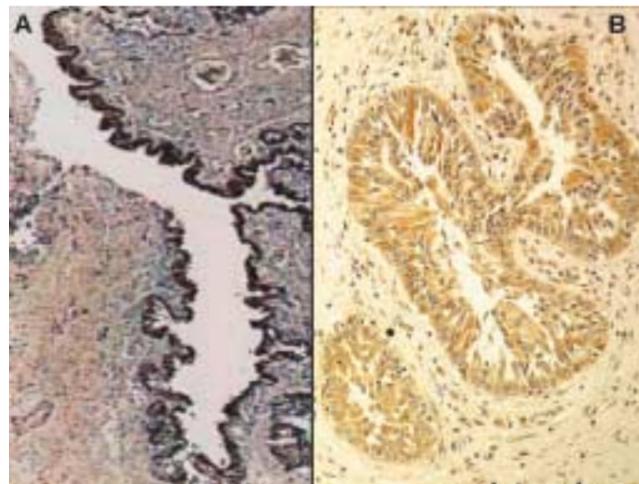


Figure 4.3.7. Skene's glands – panel A: staining with antiendothelial nitric oxide synthase antibody (x25). Panel B: staining with an anti PDE5 antibody, showing a diffuse cytoplasmic positivity (x20).

tissue.³⁹ The complex Skene's glands smooth muscle is strongly stained when nitric oxide synthase and phosphodiesterase type 5 antibodies are used (Fig. 4.3.8),^{14,35} suggesting that upon sexual stimuli the tonus and congestion of the complex may contribute to evacuation of the exocrine glandular product. The immunohistochemical profile of Skene's glands is similar to that of the male prostate.^{40,41} These glands and ducts are embedded in a fibrous muscular stroma that is more abundant than in the male prostate, and rich in nerves and blood vessels. Skene's glands are thought to be the principal source of prostate-specific antigen (PSA) in the fluid of the so-called female ejaculate produced

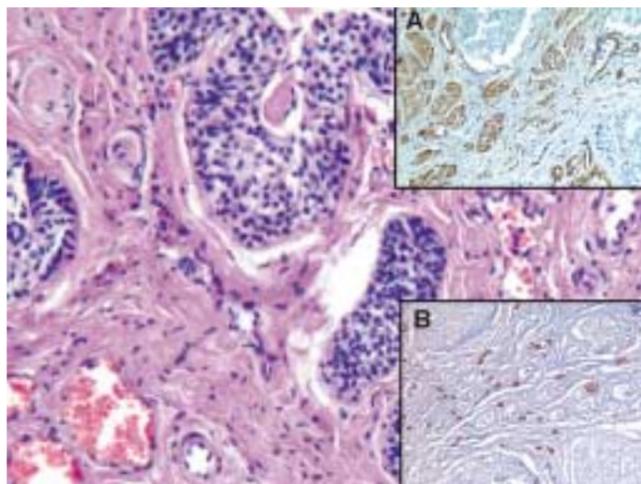


Figure 4.3.6. Skene's glands – the exocrine-endocrine glands are within the anterior vaginal wall, surrounded by fibromuscular stroma (insert A, stained with muscular actin antibody) and vessels (insert B, stained with S100 antibody). HE staining x10.

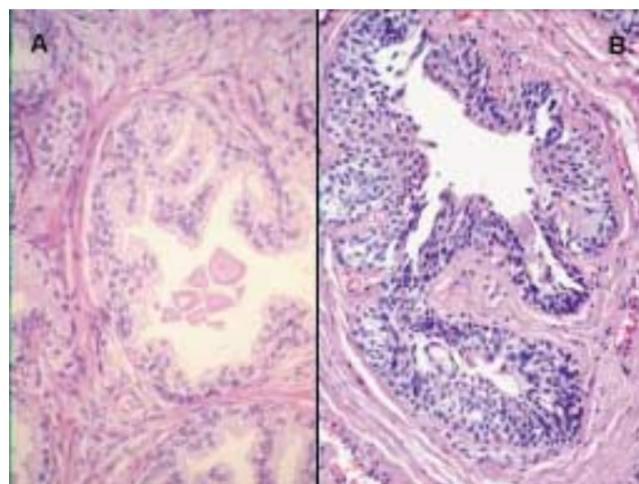


Figure 4.3.8. Prostate – comparison between histology of a male prostatic acinus (panel A) and Skene's glands (panel B). Note the microanatomic identities. HE staining x10.

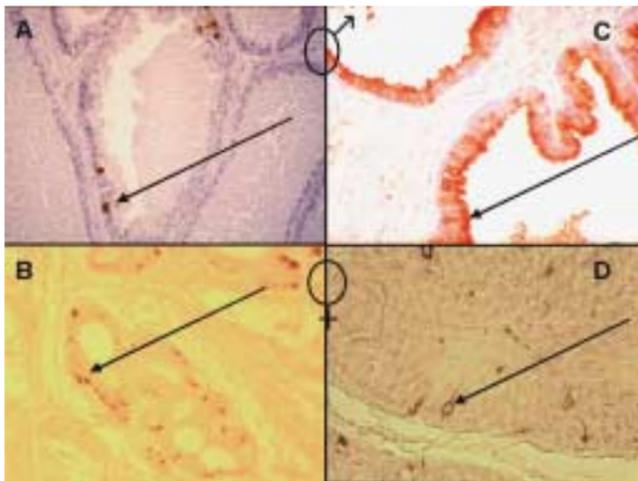


Figure 4.3.9. Prostate – expression of prostatic specific factors in normal male prostate (panels A and C) and in Skene's glands (panels B and D). Panels A and B are stained with the neuroendocrine marker chromogranin A (arrow). Panel B shows the intense staining with PSA (arrow) and panel C the focal distribution of the same antigen in the Skene's glands. $\times 10$.

during direct stimulation of the anterior vaginal wall.⁴² Immunohistochemical evidence for prostatic markers, such as prostate specific antigen,^{57,59,43} prostatic acid phosphatase (PAP),^{57,59} bombesin, and chromogranin,⁴⁴ in Skene's glands supports this hypothesis (Fig. 4.3.9). Urine protein 1 (UP1), found throughout the male prostate, is also present at high levels in human Skene's glands.⁴⁵ Steroid hormones are the putative regulators of Skene's glands differentiation: estrogen receptor-associated protein (ER-D5) was found in the glands,⁵⁷ but the presence of androgen receptors needs to be investigated.

The ultrastructure of normal adult human Skene's glands has been studied by Zaviaciac et al.⁴⁶ Cells are tall, cylindrical, and secretory, with short stubby microvilli, a protuberance of the apical cytoplasm, and bleb formation. The glands display mature secretory and basal cells. The complexity of the biochemical apparatus and enzymatic equipment expressed in Skene's glands makes it unlikely that they are embryonic vestiges,⁴⁷ as previously supposed.

In view of their possibly embryologic origin, expression of prostatic-specific markers, and hypothesized function during female orgasm (see later), it has been proposed to rename Skene's glands as *prostata feminina*, "the female prostate", following de Graff's original definition.⁴⁸ The inference from this definition is that the same diseases of the male prostate may occur in the female, such as carcinoma,⁴⁹ prostatitis,⁵⁰ and, possibly, benign hyperplasia. In fact, the quite frequent female urethral syndrome (retropubic pressure, dyspareunia, urinary frequency, and dysuria) is often diagnosed and treated as a male prostatitis.⁶⁹ Interestingly, the microscopic anatomy of the human anterior vaginal wall, as well the presence of the female prostate, shows considerable variability among examined

subjects.^{35,59} Even immunostaining for PSA and PAP has been demonstrated to vary from 67% to 83%^{57,59,62} of studied cases.

Microanatomy of the "G-Spot"

Within the vaginal wall, generally considered quite insensitive, a position relatively sensitive to electric stimuli can be detected at the 12 o'clock position (the anterior vaginal wall).⁵¹ This area has been very controversial for more than half a century. The term "G-spot" was first used by two researchers, Beverly Whipple and John D Perry, to name the sensitive area felt through the anterior vaginal wall, halfway between the back of the pubic bone and the cervix, along the course of the urethra.⁵² They referred to a 1950 paper describing this area by the gynecologist Ernst Gräfenberg.⁵³ Sexual stimulation of the G-spot can produce a variety of initial feelings: discomfort, sensation of urination, or pleasure. With additional stimulation, the area may begin to swell, and then produce an intense orgasm, possibly together with a semen-like (although less viscous) fluid emission, the so-called female ejaculation.⁷¹ This is thought to be the product of Skene's glands.

More than 250 papers on this issue have been published, but only a few in peer-reviewed journals. This is one reason for skepticism. The existence of the G-spot was denied by Masters and Johnson: "Female ejaculation is an erroneous but widespread concept."⁷² Furthermore, in a recent (and poorly researched) review article, Hines, of the Psychology Department of Pace University, New York, described the G-spot as a "a modern gynecologic myth".⁵⁴ Another reason for skepticism is that the presence of a functional G-spot is highly variable. By studying 48 coitally experienced volunteers, Alzate and Londoño found that the large majority, but not all, reported erotic sensitivity in the upper anterior wall.⁵⁵ Indeed, most women do not ejaculate, and this cannot be exclusively due to inexperience or partner incompetence. It may be due to the large differences in vaginal microanatomy among individuals.

Conclusion

Differences in histology and immunohistochemical findings between the clitoris and vagina are only apparent. Nitric oxide is the key pathway mediating clitoral and vaginal smooth muscle relaxation. However, its role in mediating vaginal muscularis smooth muscle relaxation has been considered controversial.⁵⁶ This apparent discrepancy should be considered in light of the evidence that, while the clitoris is a relatively homogeneous erectile tissue, vagina histology changes in function of its topography, which varies according to the different levels examined. The first third of the vagina should in fact be considered as continuous with the clitoris, as the clitoral bulb, an erogenous zone expressing the same biochemical mediators of arousal, such as nitric oxide synthase, vasoactive intestinal peptide, and phosphodiesterase, is embedded in it. In contrast,

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the remaining two-thirds appears almost devoid of sexually responsive structures. This justifies the traditional assumption of sexologic and anatomic literature that the vagina is almost inert in sexual response. Finally, the large interindividual histologic variability may account for these discrepancies. Given its manifest behavioral and clinical consequences, this evidence needs to be more deeply studied in future.

Anatomy studies normally precede physiology. While the anatomy of the penis and the biochemical and molecular regulation of erection are largely known, the exact anatomic description of the human clitoris was produced in 1998, the taxonomy of female sexual dysfunctions classified in 1999, and the biochemistry of female excitation described only in 2002. There are various reasons for this. Female sexual physiology is much more complex than that of the male, and cultural, political, and religious considerations have discouraged the scientific study of female sexuality. However, it is now evident that modern sexology cannot be truly called sexual medicine if female sexual anatomy and the physiology of female sexual response are unknown.

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