# Functional anatomy of the human vagina

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**ABSTRACT. Anatomy studies normally precede physiology. While the anatomy of the penis and the biochemical and molecular regulation of erection are largely known, the exact anatomical description of the human clitoris was produced in 1998, the taxonomy of female sexual dysfunctions classified in 1999, and 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 and religious considerations have discouraged the scientific study of female sexuality. However, it is now apparent that modern sexology cannot be truly 'medical' if female sexual anatomy and the physiology of female sexual response are unknown.**

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## INTRODUCTION

While anatomy, pathophysiology and possible therapies of male sexual response have been largely explored in the recent years, little and discordant information has been produced for the female counterpart. However, scattered, but interesting data on female functional anatomy are now being published.

### *The molecular bases of sexual excitation*

In the classic Masters and Johnson description of human sexual response, the excitation phase consists of male erection and female lubrication (1). In the male, the mechanism of erection is based on the presence of an integrated biochemical system within the *corpus cavernosum*. The system NO-cyclic guanosine monophosphate cGMP-binding cGMP-specific phosphodiesterase (PDE5) is actually regarded as the principal mediator of male sexual excitation. Nitric oxide (NO), a small lipophilic molecule enzymatically generated from L-arginine by a family of NO synthases (NOS), takes part in several biological events. Constitutive and inducible NOS isoforms exist, which differ in structure and regulation (2). All the isoforms convert arginine to NO and citrulline, and require NADPH as a cofactor. Constitutive NOS isoforms (neuronal NOS, nNOS or $NOS_1$, and endothelial NOS, eNOS or $NOS_3$) produce small amounts of NO over several minutes in response to agonists that elevate intracellular $Ca^{2+}$. The cytokine-inducible, or inflammatory, NOS (iNOS, $NOS_2$) produces NO independently of intracellular calcium elevations and is expressed mainly in inflammatory conditions. It is considered as an enzyme induced during immune response, even if under certain circumstances it may be expressed constitutively. In the penis, NO is released from nerves as well as from the endothelium, where it elicits the production of cGMP (3). This nucleotide is able to relax smooth muscle, inducing erection (4), and is specifically hydrolyzed by PDEs. The main PDE activity is in the corpora cavernosa (5). Sildenafil (6), tadalafil (7), and vardenafil (8), specific PDE5 inhibitors, therefore enhance the action of NO/cGMP on penile erectile activity during sexual stimulation.

## CLITORAL ANATOMY

It has been demonstrated only recently, using gross anatomy techniques, that the clitoris consists of an erectile tissue complex surrounding the

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urethra and embedding the anterior vaginal wall (9). This last is thus of particular physiological interest, considering its role in sexual response (10). The microscopic anatomy of the clitoris is homogeneous among different subjects. It consists of a cavernous tissue, encircled by a thin fibrous capsule that is surrounded by large nerve trunks. Recent data from Burnett *et al*. showed the presence of nitric oxide (NO)-synthase isoforms in the human clitoris, suggesting that NO may be involved in its erectile physiology as a modulator of smooth muscle activity (11).

*Vaginal anatomy*

We previously demonstrated that the microscopic anatomy of the human vagina's anterosuperior wall differed among subjects (12). On histologic examination, the anterior vaginal wall appears as a complex structure formed by: a) the vaginal mucosa; b) a pseudocavernous tissue located in the vaginal chorion; c) a periurethral glandular tissue (13), recently named the 'female prostate' (14). The last is formed by numerous ducts, opening independently in the urethra, and by scarce glandular acini with both exocrinal and endocrinal activity. The immunohistochemical profile of the female prostate is similar to its male counterpart (15, 16). Its glands and ducts are embedded in a fibrous muscular stroma, which is more abundant than in the male prostate and rich in nerves and blood vessels. (Fig. 1). The ultrastructure of the normal adult human female prostate has been studied by Zaviacic *et al*. (17). Skene's gland cells are tall, cylindrical, and secretory, with short stubby microvilli, a protuberance of the apical cytoplasm, and bleb formation. As in the male prostate, the glands displays mature secretory and basal cells.

Interestingly, the microscopic anatomy of the human anterior vaginal wall shows considerable variability among examined subjects. In our experience, the female prostate is observed at autopsy in two thirds of women of reproductive age. The presence of pseudocavernous tissue in the anterior vaginal mucosa is a more frequent finding (86%). This anatomical variability should be taken into account when evaluating the physiology, pathology, and possible medical treatments of female sexual response.

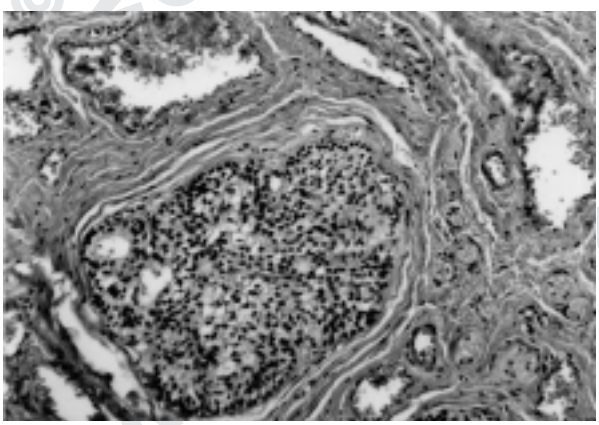

Fig. 1 - *Histological section of the "female prostate". A gland acinus is surrounded by fibromuscular stroma, rich in vessels and nerves fibers. Ematoxilin-eosin stain of autoptic human vagina specimens from a representative subject. Specimen was obtained at autopsy from a phenotypically normal woman aged 23 years with an intact pelvic anatomy and unaffected by the medical history or cause of death. The urethra was removed intact by sharp dissection from the level of the external meatus to the bladder neck. Specimens included periurethral tissue attached to the anterior vaginal mucosa. The vagina was then excised and opened along the posterior wall. The specimens were fixed in 4% buffered paraformaldehyde. After fixing, serial sections were cut perpendicular to the long axis of each specimen. Representative samples were obtained from each level and embedded in paraffin. Four 1nm-thick sections mounted on glass slides were stained with hematoxylin-eosin for examination by light microscope. After preliminary observations of the slides we focused our investigation on the first third of the anterior wall of the vagina. Original magnification: 10X.*

## NOS IN FEMALE GENITAL TISSUES

Our preliminary observations demonstrated a distinct distribution of immunoreactivity for NOS isoforms in the human vagina (18). In all subjects, the nerve bundles and fibers coursing within the organ were positive for the constitutive isoforms of NOS (neuronal and endothelial). These isoforms were also present in the endothelial lining of sinusoids and blood vessels and in the vaginal epithelium. Furthermore, neuronal and endothelial NOS isoforms mapped in the smooth muscle of the cavernous erectile tissue of the anterior wall of vagina were positive, as were most of the cells in the exocrine *acinum* in subjects with Skene glands. Surprisingly, the inducible NOS isoform was expressed in the human vagina, mostly in the epithelium and focally in smooth muscle. To confirm this finding, we tested the inducible NOS antibody in cells harvested *in vivo* from the anterior wall of a volunteer's vagina. These cells were found highly positive. Nitric oxide synthase isoforms are largely distributed in the human vagina (19) and in the vagina of experimental animals (20). The presence of neuronal NOS in nerves suggests that this isoform produces NO as a postganglionic neurotransmitter. Endothelial NOS was mostly localized in the endothelial lining of sinusoids and blood ves-

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sels throughout the erectile tissue of the vaginal anterior wall. Both those findings mirror the anatomical scenario recently found in the human clitoris (11) and penis (21), with some exceptions. While specific immunoreactivity for inducible NOS was not demonstrated within clitoridal specimens (11), we found this isoform mostly in the vaginal epithelium and, as in the penis of experimental animals (22), the smooth muscle cells. Since inducible NOS is stimulated by bacterial products (23), the presence of this inflammatory isoform may be due to normal bacterial flora in the human vagina. Vaginal smooth muscle, as well as smooth muscle fibers of other tissues (24) were stained by constitutive NOS, an isoform that was not identified in the smooth muscle of the clitoris (11). The distribution of NOS isoforms partially supports the recently found relationship between the urethra and clitoris (9). The region we studied can thus be identified as the clitoral bulbs, but it appears much richer in NOS than the clitoris itself.

## PDE5 IN THE FEMALE GENITAL TISSUES

Immunoreactivity for PDE5 partially followed the distribution of NOS isoforms (12). In fact, it was observed focally in the endothelial lining and smooth muscle of the anterior vaginal wall cavernous erectile tissue, and was widespread in Skene glands and the vaginal epithelium. The immunocytochemistry of vaginal cells harvested *in vivo* confirmed the latter finding. No staining of nerve bundles was observed. The anatomical distribution of PDE5 within the endothelial lining and in the smooth muscle of the anterior vaginal wall cavernous erectile tissue, and the acini of Skene glands correlates with that of NOS. This is consistent with the presence of PDE5 activity in the human clitoral corpus cavernosum smooth muscle (25).

The well-defined distribution of the integrated NOS-PDE5 system in the anterior wall of human vagina, here demonstrated in autoptic specimens as well as in epithelial cells harvested *in vivo*, allows speculation regarding its relative roles. The predominance of neuronal NOS in nerves implies that this isoform mediates smooth muscle relaxation in the NOS-rich corpora cavernosa. The abundance of endothelial NOS suggests its prominent role in female sexual response. The presence of inducible NOS may imply that the human vagina can produce NO under certain conditions. The co-localization of PDE5 in the tissues considered as *bona fide* targets for NO (epithelia, exocrine glands, and muscle) may warrant the breakdown of cGMP locally produced upon NO stimulation.

## TYPE-5 INHIBITOR USE IN FEMALES

Female sexual arousal disorders have been defined by the Consensus Panel of the American Foundation of Urological Diseases as the persistent inability to attain or maintain sexual excitement (lubrication/swelling) (26). As this condition is highly prevalent, clinical studies evaluating the safety and efficacy of PDE5 inhibitors in women with sexual arousal disorders are currently in progress. However, clinical data so far produced are discordant. For non-scientific reasons, the first study on the use of sildenafil in women was conducted on postmenopausal women with self-described sexual dysfunction, who were treated with sildenafil for 3 months (27). While overall sexual function did not significantly improve, there were changes in vaginal lubrication and clitoral sensitivity. However, sildenafil has been found capable of antagonizing the sexual discomforts induced by antidepressant drugs (28), as well as of partially reversing sexual dysfunction in women with spinal cord injuries (29). This suggests that the clitoris and vagina are a target for PDE5 inhibitors. Finally, in the first double-blind, cross-over placebo-controlled study performed in young women affected by arousal disorders, sildenafil was recently demonstrated to significantly improve sexual performance (30). Our results may provide an anatomical substrate for the study of specific PDE5 inhibitors in disturbances of female sexual arousal.

## ANATOMY OF G-SPOT AND FEMALE EJACULATION

This area has been very controversial for more than half a century. The term "G-spot" was first introduced 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. They referred to a 1950 scientific paper describing this area written by a gynecologist, Ernst Gräfemberg (31). Indeed, modern sexologists describe realities which were widely known in the medicine of past centuries (32, 33) and since forgotten for political and religious reasons. 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, then producing an intense orgasm, together or not with a semen-like (although less viscous) fluid emission, the so-called "female ejaculation" (34). On the basis of evidence from prostatic components, especially PSA, in the fluid of female ejaculate, the female prostate (Skene gland) is its principal source and participates in this phenomenon (35).

Our preliminary evidence for prostatic markers, such as PSA, bombesin and chromogranin in Skene's gland confirms this hypothesis.

Despite the production of more than 250 papers on this issue, only a few have been published in peer reviewed and impacted journals (36). For this reason skepticism is still widespread. The existence of the G-spot was denied by Masters *et al.* ["Female ejaculation is an erroneous but widespread concept"(1)], and this opinion has been repeated in subsequent sexological textbooks (37). Furthermore, in a recent (and poorly researched) review article, Dr. Hines, from the Psychology Department of the Pace University, Pleasantville, described the G-spot as a "a modern gynecologic myth" (38). Another reason for skepticism is that the presence of a functional G-spot is highly variable. Most women do not in fact ejaculate, and this cannot be exclusively due to inexperience or partner incompetence. It may be due to the large differences in vaginal anatomy existing among individuals. In fact, periurethral glands were found in 7 of 10 female urethras harvested at autopsy (39). These findings are in agreement with the results of our study. The anatomical differences we observed mirror the variability in the distribution and intensity of immunohistochemical staining. Considering that both endothelial and neuronal NOS are subject to regulation by sex hormones (40), the immunohistochemical differences between subjects may also be explained by the different cycle phases.



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