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The Vulvar Vestibule, a Small Tissue with a Central Position: Anatomy, Embryology, Pain Mechanisms, and Hormonal Associations

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Abstract
The vulvar vestibule is the tissue located between the vagina and the vulva, comprising the vaginal introitus. It is mainly considered in the context of localized provoked vestibulodynia, a pain condition causing dyspareunia in 8–16% of women.
Purpose of Review To describe the vulvar vestibule's unique properties, which may have a role in the development of vestibular tenderness and dyspareunia.
Recent Findings The vestibule displays different neural features when compared with neighboring organs. It is an immunologically diverse tissue that exhibits highly localized and tissue-specific pro-inflammatory responses. Systemic, as well as local hormonal-associated processes regarding estrogen, androgens, and usage of hormonal contraception apear to influence the pain threshold in the vestibule.
Summary The vestibule, emerging from the embryonic endoderm, exhibits unique features regarding innervation, inflammation, and hormonal stimuli. A better understanding of these mechanisms is required in order to advance our understanding of introital dyspareunia.

Keywords Vulvar vestibule · Provoked vestibulodynia · Dyspareunia

Introduction

The vulvar vestibule, located between the vagina and the vulva, is an embryologically distinct band of tissue. It is mainly discussed in the context of localized provoked vestibulodynia, a common pain condition causing dyspareunia in 8–16% of women. This review describes its anatomy and embryologic origin, and unique features regarding innervation, inflammation, and hormonal stimuli that may have a role in the evolution of vestibular tenderness and dyspareunia.

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Anatomy of the Vulvar Vestibule

The vestibule is the anatomic area that constitutes the vaginal introitus and is demarcated anteriorly by the clitoral prepuce, laterally by the labia minora, and posteriorly by the fourchette, a fold of skin where the labia minora join (Fig. 1). It lies exterior to the hymenal ring, which marks the boundary of the vagina, and interior to Hart's line, forming the medial border of the labia minora [1]. Hart's line comprises the mucocutaneous junction, a zone of transition between hairless skin and squamous mucosa [2], however not uniformly visible on clinical examination.

The vestibule contains the following structures [1]:

- Paired major vestibular glands or Bartholin's glands—analogous to the bulbourethral glands in the male—are paired tubuloalveolar glands located within the subcutaneous tissue deep to the labia minora, vestibule, and hymen. The Bartholin's glands consist of mucus-secreting columnar epithelial cells that secrete into a pair of ducts that open at the vestibule at the 5 and 7 o'clock locations.
- Paired erectile vestibulovaginal bulbs—located laterally within the subcutaneous tissue—are paired bulbs covered

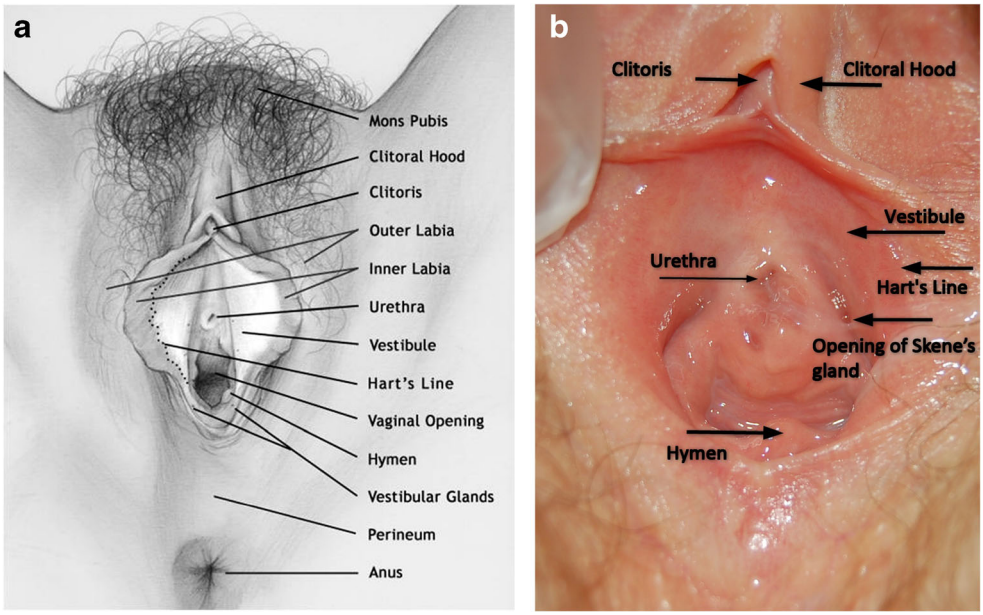

Fig. 1 Vulva anatomy. **a** Image courtesy of Dawn Danby and Paul Waggoner. **b** Vulvar vestibule

by the bulbospongiosus muscles containing vascular erectile tissue.

- The urethral meatus—lined by a transitional epithelium that merges with the stratified squamous epithelium of the vestibule. Throughout the urethra are multiple minor periurethral glands (of Huffman) composed of columnar, mucinous epithelium.
- Paired periurethral (Skene's) glands—paired structures analogous to the male prostate. The glands are composed of mucinous pseudostratified columnar epithelial cells that secrete into ducts lined by a transitional epithelium, which merges with the vestibular squamous epithelium. The bilateral ducts' openings are found immediately posterolateral to the urethral meatus.
- Minor vestibular glands, analogous to the penile glands of Littre in the male, are simple, shallow (no deeper than 3 mm) tubular glands lined by a single layer of mucus-secreting columnar epithelium. Located concentrically within the vestibule, these glands open directly onto the vestibular surface.

Embryology

The urinary and reproductive systems develop from a urogenital ridge of intermediate mesoderm which is located along the posterior body wall in the evolving abdominal cavity [1]. Both systems open into an endoderm-lined cloaca at the caudal end of the embryo [3]. A portion of the cloaca will then invaginate to form the urogenital sinus, which contributes to the bladder, urethra, and the reproductive tracts in both males (prostate and prostatic urethra) and females (vagina and vestibule).

The external and the internal genital organs are undifferentiated for the first few weeks of development. From the 7th week of gestation and on, the male and female systems diverge greatly as the process of sexual differentiation occurs. In the female, the sex-determining region of the Y-chromosome gene (SRY) expression is absent so the dominant genetic program is directed by WNT4, which initiates differentiation of the female phenotype [4]. Estrogen is secreted by the gonads, promoting the growth and development of the paramesonephric ducts and female external genitalia. The paramesonephric ducts develop into the uterus, fallopian tubes, and cervix, whereas the vagina develops from both the paramesonephric ducts and the urogenital sinus.

Vaginal development involves a process of canalization such that the mesenchymal walls of the vaginal fornices are derived from the paramesonephric tubercle and the squamous epithelial surfaces. The initial site where the growth of the vaginal plate from the wall of the urogenital sinus originated usually does not fully canalize, thus leaving a membranous hymen that separates the vaginal canal from the urogenital sinus.

Under the influence of estrogens, the genital tubercle develops into clitoral glans, while the urogenital and labioscrotal folds give rise to the labia minora and majora, respectively [5] (Fig. 2). The labia majora merge anteriorly to form the mons pubis, and posteriorly, they fuse with the perineal body, anterior to the anus. The remaining opening of the urogenital sinus exterior to the vaginal introitus expands to form the vestibule. This structure is homologous to the external urethra (penile) in the male [6]. The lining of the vestibule is endodermally derived, as opposed to the other structures of the vulva and vagina, which are of ectodermal and mesodermal origin, respectively [1].

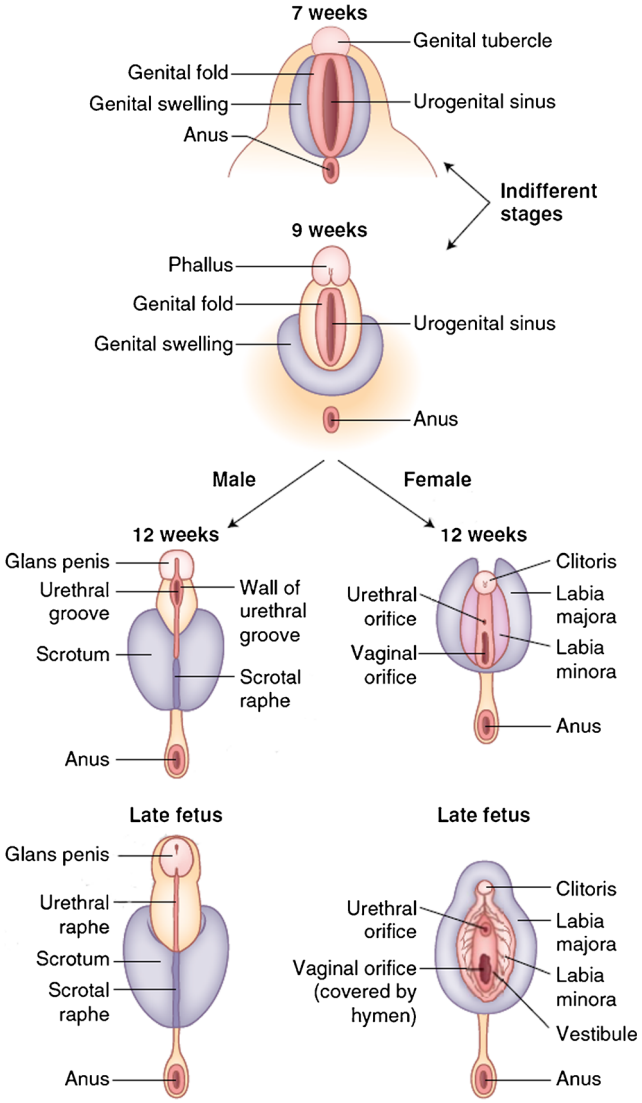

Fig. 2 Embryological development of the female and male external genitalia (From Velkey JM et al. Normal Vulva: Embryology, Anatomy, and Histology, in: Vulvar Pathology, editted by Hoang MP, Selim MA. 2015 Springer-Verlag New York)

Participation in Pain Mechanism

Due to its location between the vulva and the vagina, any vulvovaginal inflammation or infection can result in severe irritation of the vestibule [7]. Therefore, any inflammatory process may cause introital dyspareunia and vestibular tenderness on examination. This is evident in vulvovaginal candidiasis, vaginal atrophy, and purulent vaginitis, as well as with vulvar dermatoses (lichen sclerosus and lichen planus) and with external irritation, i.e., contact dermatitis. In the presence of vestibular inflammation, pain and dyspareunia may result from friction and damage of irritated tissues, tears, fissures, and irritation of sensitized and inflamed nerve fibers.

In addition to these conditions, a vulvar pain syndrome, currently known as provoked, localized vestibulodynia (PVD), is described as superficial pain confined to the vulvar vestibule, provoked by touch, without clear identifiable cause [8]. In some women, the pain can be caused by minimal touch, such as sitting or tight-fitting clothing, whereas in others, it is provoked with vaginal penetration during sexual intercourse, tampon insertion, or gynecological examination, resulting in dyspareunia or complete inability to have intercourse. The clinical diagnosis of PVD is defined by subjective signs and symptoms of entry dyspareunia and vestibular tenderness to gentle touch, after exclusion of other defined disorders. The pain is often described as a burning or a cutting sensation and may be located throughout the vestibule or confined to the posterior (lower) vestibule.

No single causative factor of PVD has yet to be identified, and it has a multifactorial etiology [9•]. The accepted theory is that PVD represents a diverse group of disorders, causing similar symptoms. Some women have primary PVD, experiencing pain at first introital touch, while others describe a period of pain-free vaginal penetration before the onset of symptoms, and thus are diagnosed with secondary PVD.

Much effort has been made to find pathophysiological changes characteristic of PVD. Research suggests abnormalities in different systems, including the vestibular mucosa, pelvic floor musculature, peripheral and central pain regulation, as well as diverse pathways (inflammatory, hormonal, genetic, psychosocial etc.) [9•]. These factors can be the primary cause of the disorder, or develop secondarily to the recurrent provoked pain.

Given the localization of pain in patients with PVD to the vestibule only, sparing the vulva and the vagina, it is accepted that the vestibule is a distinct tissue, characterized by different pathophysiological properties. This distinction is attributed to the separate embryological origin, causing unique changes in the vestibule, in response to various stimuli.

Hyperinnervation of the Vestibular Mucosa

Hypersensitivity of the vulvar vestibule is one of the defining characteristics of PVD. A suggested mechanism explaining this feature is hyperinnervation of the vestibular epithelium in affected individuals. Increased vestibular nerve fiber density was first described by Westrom and Willen [10], and later confirmed by others [11–16]. Immunohistochemical methods define these nerve endings as nociceptors [17]. This hyperinnervation corresponds to heightened mechanical allodynia (pain from light touch) and hyperalgesia (enhanced pain perception), it was termed “neuroproliferation” and is hypothesized to be either congenital [18, 19] or acquired. Since embryologically, the vestibule is of endodermal origin, neuroproliferation may represent a congenital anomaly. In such cases, the neural hypersensitivity is primary and may be present in other tissues derived from the urogenital sinus as well. This may explain the coexistence of PVD and interstitial cystitis in some women

[20], as well as the significantly higher level of umbilical sensitivity in women with primary neuroproliferative PVD [21]. Acquired neuroproliferative PVD has been attributed to neural proliferation in response to an inflammatory process (see below) or endocrine factors [22, 23].

An additional observation supporting the contribution of localized hyperinnervation to pain mechanisms in PVD is that 80% of patients undergoing a vestibulectomy, surgical excision of the vestibule, experience symptomatic relief.

The significance of nociceptor hypertrophy is controversial; some investigators consider neuroproliferation as a nonspecific reaction to previous mucosal trauma or inflammation [24] and attribute the heightened pain perception to neurogenic inflammation [24].

In summary, in PVD, the vestibule exhibits mechanical allodynia and hyperalgesia when compared with adjacent tissues. These characteristics, defining this clinical entity, can result from nociceptor hypertrophy as well as from neurogenic inflammation or simply thinning of vestibular tissue (see below). However, they emphasize that the vestibule displays different neural features when compared with neighboring organs.

Inflammatory Mechanisms in the Vulvar Vestibule

Multiple studies suggest that inflammation may play a role in the development of PVD. The suggested mechanism, "neurogenic inflammation", hypothesizes that persistent inflammation in the vestibular mucosa promotes both hyperplasia of nociceptive c-fibers, secondary to production of nerve growth factor, altered receptor expression [25], and a constant elevation of pro-inflammatory substances [11, 17, 26, 27].

Vulvovaginal infections are frequently cited as an inciting inflammatory event triggering the development of PVD. Often, PVD patients report a history of recurrent vulvovaginal candidiasis and relate the onset of their symptoms to a symptomatic vaginal candidiasis [9•]. Foster et al. reported that in both women with PVD and controls, vestibular fibroblasts release elevated levels of inflammatory cytokines compared with fibroblasts isolated from non-painful vulvar sites following stimulation by irritants [28, 29••]. In women with PVD, an exaggerated response was observed as compared with women without PVD. The authors suggested that the vestibule of PVD patients is inherently sensitive to yeast and that even a subclinical infection may trigger a maladaptive immune response in these fibroblasts.

These observations strongly support the hypothesis that the vulvar vestibule is immunologically unique tissue that exhibits a highly localized and tissue-specific pro-inflammatory response. The degree of this inflammatory response varies between women and may contribute to the development of PVD.

Hormonal Considerations

The vestibular lining is of endodermal origin and is predominantly nonkeratinized, stratified squamous epithelium. Like other urogenital tissues, the vestibular epithelium is estrogen-dependent. Other hormones found to affect the vestibule are progestins and androgens.

The steroid hormones exert their effects through the nuclear receptors of estrogen (ER), progesterone (PR), and androgen (AR). The receptor expression is a dynamic process, with up- and down-regulation. Studies have confirmed the localization of these receptors in the vestibule [30–32].

Vestibular pain and tenderness are reported with estrogen depletion occurring during menopause [33] and breastfeeding [34] and is also associated with hormonal contraception (HCs) usage [35–39].

Estrogen Deficiency

Estrogen is a dominant regulator of physiology in urogenital tissues, maintaining the normal urogenital environment [40]. With estrogen withdrawal, such as during menopause, significant anatomic and physiologic changes occur in these tissues. The epithelium becomes pale, thin, less elastic, and progressively smoother as rugal folds decrease. Other changes include reduced collagen content and hyalinization, decreased elastin, increased density of connective tissue, and fewer blood vessels. Blood flow and secretions diminish, flexibility and elasticity decrease, and thus, the tissues become more friable.

In clinical practice, it is common that patients whose symptoms are mainly vestibular, such as painful penetration or vestibular dysuria, fail to improve when treated with systemic or intravaginal estrogen [41], and on examination, it is not uncommon to find well-estrogenized vaginal epithelium with an atrophied vestibule. In addition, the vagina can also show marked atrophy but does not become tender to the same degree as the vestibule [42].

In such cases, it is recommended that the patient apply estrogen directly to the vestibule rather than the vagina [43]. In a study by Murina et al. [42], postmenopausal women with vestibular dyspareunia were instructed to apply estriol gel to the vestibule daily for 3 weeks and then twice weekly for up to 12 weeks. Dyspareunia was improved or was cured in over 80% of the patients. Cotton swab test scores were reduced and vestibular atrophy was improved at the end of treatment. The authors attributed this effect to a reduction in sensory vestibular innervation sensitivity [41]. However, it is possible that local effects of estrogen may vary, based on the anatomical absorption location. Doppler flow studies showed preferential delivery toward the uterus when a 17beta-estradiol vaginal tablet was placed in the inner third of the vagina, and

preferential delivery toward periurethral areas when placed in the outer one third of the vagina [44].

Androgen and the Vestibule

Androgens were suggested to play a role in vestibular pain in both HC users and menopausal women. The role of androgens in female genitourinary tissues is based largely on data from animal studies [45•] as well as from analogy with the male genitourinary tissues [46]. However, there is evidence that androgens participate in female human genitourinary physiology as well. First, androgens are necessary precursors for the synthesis of estrogens. In postmenopausal women, while estrogen production declines, circulating androgens are significant precursors for the local synthesis of testosterone and estradiol in extragonadal tissues [45•]. In addition, the detection of androgen receptors (ARs) in genitourinary tissues, including the vestibule [31, 41, 47], proposes that androgens have direct effects on these tissues. One possible mechanism is the direct effect on vaginal epithelium. Studies suggested that vaginal atrophy was less prevalent in postmenopausal women with higher levels of androgens [45•]. In a small study in women with breast cancer using aromatase inhibitor therapy (blocking possible conversion of testosterone to estrogen), intravaginal application of testosterone for 4 weeks was shown to improve signs and symptoms of atrophy, including dyspareunia and dryness [48], suggesting that androgens could have a direct effect on vaginal epithelial growth and differentiation. Animal studies have also shown that androgens and estrogens regulate vaginal mucin production in epithelial cells, and AR have been localized to the mucin-secreting vestibular glands in humans [45•]; therefore, it is suggested that androgens contribute to mucin secretion and lubrication [49].

As with estrogen, the effect of androgens on vestibular pain may result from alteration of serum hormone levels, interaction with ARs or vestibular expression of ARs.

Another possible mechanism involves AR polymorphism. The AR gene contains a highly polymorphic cytosine–adenine–guanine (CAG) repeat sequence which ranges in size. Fewer CAG repeats are associated with a more efficient receptor activity, while more repeats are associated with a weaker receptor activity. Goldstein et al. [50] found a larger number of CAG repeats in patients who developed PVD while using HCs as compared to women who took the similar HC but did not develop vestibulodynia and suggested that an inefficient AR predisposes women to develop vestibular pain when the hormonal milieu changes, as with HC usage.

In the practical aspect, there is no enough data to draw conclusions whether the addition of testosterone to topical estrogen preparations improve clinical results as compared with estrogen alone. A study comparing the effects of local estrogen with or without local testosterone on urogenital and sexual health in postmenopausal women found similar improvement in urogenital atrophy symptoms, vaginal health, and maturation index among both study groups as compared with the control group [51]. However, the combination therapy also produced significant improvement in sexual function as compared with local estrogen alone. The authors sugested that combined local estrogen–androgen therapy may be considered in women having inadequate control of symptoms with local estrogen therapy.

Vestibular Changes with Hormonal Contraception

A possible correlation between HCs and PVD has been investigated primarily in epidemiological studies [37, 52–54]. Results showed a 6.6 relative risk of PVD for ever-users of HCs compared to non-users, rising with increased duration of use [37]. Burrows and Goldstein [55] described a case series of 50 women who developed PVD while on HCs and who were successfully treated with topical estradiol and testosterone.

It has been reported that many women with superficial dyspareunia while taking HCs displayed an erythematous and hypersensitive vestibular mucosa which disappeared 4–6 months after cessation of HCs [35]. This effect is probably multifactorial, involving morphological changes, interaction with hormone receptors or alteration of receptor expression. HC cause a suppression of ovarian estradiol and testosterone production as well as an increase in sex hormone binding globulin (SHBG) synthesis. This combination leads to low estradiol and low calculated free testosterone. Decreased estradiol levels may further contribute to vestibular atrophy found in patients with PVD [36], causing introital pain. Additionaly, some HCs contain synthetic progestins that act as testosterone antagonists at the AR [39].

Johannesson et al. evaluated the morphology of the vestibular mucosa in 20 women using HCs and in 25 non-users [35]. They did not find any changes in the number of cell layers in HC users and between the follicular and luteal phases in non-users. However, they described a larger distance between the dermal papillae and a larger space from the dermal papillae to the epithelial surface, appearing as shallow and sparse dermal papillae in the HC group, despite a similar number of cell layers. This phenomenon may be attributed to larger keratinocytes. Nevertheless, the superficial cells in HC users also appeared distended. Non-users displayed similar findings in the luteal phase compared with the follicular phase. The authors concluded that these morphological alterations indicate a gestagenic effect on the mucosa, making the vestibular mucosa more sensitive to mechanical strain. These morphological alterations may influence mechanical properties by thinning the epithelium and causing nerve endings to become more superficially located, thus altering the transduction of mechanical pressure to the receptors, without affecting nerve fibers.

The same authors evaluated the influence of HCs on the steroid receptor expression in the vestibular mucosa as compared with non-users in the same group of patients [30].

They found a higher expression of ER-β in vestibular stromal tissue of women using HCs as compared with non-users. PR-B was more abundant in the stromal tissue in the follicular phase than in the luteal phase. They did not find differences in the expression of ER-α, PR-A, and AR between the groups or with menstrual cycle.

Results from studies investigating the expression of ER-α in PVD patients are contradictory. Eva et al. [56] reported a decrease in vestibular ER-α in women with PVD, while Johannesson et al. [47] reported an increased amount of vestibular ER-α in patients who were past HC users as compared with controls.

Localization of AR in vestibular tissues in patients with PVD has also shown contradictory results. Goetsch et al. [31] found that AR was present at increased frequency in tender vestibular biopsies compared with nontender biopsies, while Johannesson et al. [47] reported no differences when compared to the controls.

Goldstein et al. [50] identified a genetic polymorphism in the AR in PVD patients and concluded that inefficient AR combined with a lowered free testosterone predisposes women to PVD.

In summary, hormonal fluctuations as well as local hormonal-associated processes appear to influence the pain threshold in the vestibule. The association between HC use and the development of PVD is probable, but the actual prevalence and susceptibility factors remain incompletely elucidated.

Conclusion

The vulvar vestibule expresses unique features regarding innervation, inflammation, and hormonal stimuli, presenting clinically as vestibular pain and dyspareunia. The common explanation to this phenomena is its specific embryologic origin, explaining these differences as well as the possible association to other pain syndromes involving endodermal tissues. However, given the variability among affected individuals and women without vestibular pathology, a better understanding of these mechanisms with regards to pathophysiology, genetic suseptability, and congenital factors are required in order to advance our understanding of PVD.

Compliance with Ethical Standards

Conflict of Interest Ahinoam Lev-Sagie, Ronit Gilad, and Diana Prus each declares that they have no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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