SEXUAL MEDICINE REVIEWS REVIEWS

Testosterone and Vaginal Function

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ABSTRACT

Introduction: Androgens have been shown to exert beneficial effects on vaginal physiology, at least partially independent of their aromatization to estrogens. Androgen deficiency in the vagina and in the other genitourinary tissues contributes to the development of vulvovaginal atrophy and genitourinary syndrome of menopause, resulting in impaired arousal and lubrication and dyspareunia.

Objectives: To summarize the role of testosterone in modulating vaginal structure and function.

Methods: A qualitative review of the relevant literature on the topic was performed using the PubMed database. We present a summary of preclinical and clinical evidence supporting the involvement of testosterone (T) in vaginal physiopathology and discuss it in terms of the role of the vagina in female sexual response.

Results: Androgens are important in the differentiation of the vagina and in maintaining trophic and functional actions in postnatal life, as suggested by the detection of the androgen receptor and of the key enzymes involved in androgen synthesis. T is essential for the integrity of vaginal tissue structure (including non-vascular smooth muscle thickness and contractility and collagen fiber compactness) and for the complex neurovascular processes that regulate arousal and lubrication (vascular smooth muscle relaxation via the NO/cGMP/PDE5 pathway, nerve fiber density and neurotransmission). T has also been reported to modulate nociception, inflammation, and mucin secretion within the vagina. Available and potential androgen-based treatments for vulvovaginal atrophy/genitourinary syndrome of menopause and for other conditions leading to female genital arousal disorder and dyspareunia are presented.

Conclusions: The vagina is both an androgen-target and synthesis organ. Preclinical and clinical data consistently suggest that T plays an important role in maintaining vaginal health and genital sexual function. **Maseroli E, Vignozzi L. Testosterone and Vaginal Function. Sex Med 2020;XX:XXX–XXX.**

Key Words: Testosterone; Androgens; Lubrication; Arousal; Female Sexual Dysfunction; Genitourinary Syndrome of Menopause

INTRODUCTION

The vagina is one of the key organs of the female sexual response, and androgens are the most abundant sex hormones in women; in fact, while testosterone (T) serum levels in women during the reproductive age have an average level of 0.7–1 nM, 17 beta-estradiol is about 0.1–0.5 nM.[1] Although the effect of T on vaginal structure and function and the consequences of changes in T intracellular availability have not been fully elucidated, in recent years, evidence from animal and human studies has been accumulating, suggesting that the vagina should in fact be considered as an androgen-dependent organ. Indeed, androgens have been consistently shown to exert potential beneficial effects on vaginal physiology, at least partially independent of their aromatization to estrogens.[2] Meta-analytic data of randomized clinical trials (RCTs) demonstrate that in menopausal women with hypoactive sexual desire disorder (HSDD), systemic T treatment improves not only desire but also arousal, orgasmic function, pleasure, and sexual responsiveness.[3] Nevertheless, it remains unclear whether and in which proportion these effects are mediated by direct mechanisms occurring in the genital organs. To further complicate matters, T and its precursors, beyond their secretion by the adrenal gland and the ovary, are hypothesized to be also formed peripherally, with a complex regulation by steroidogenic enzymes.[4] However, this peripheral production in vagina cells was previously described in rats and only recently in the human vaginal tissue.[5] Therefore, the physiological role of androgens in the regulation of vaginal function is far from being fully elucidated. In addition, tissue sensitivity to T varies in accordance with the amount and activity

Received February 5, 2020. Accepted March 10, 2020.

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https://doi.org/10.1016/j.sxmr.2020.03.003

of the enzymes that synthesize active androgens and estrogens and of the androgen receptors (ARs), which may vary considerably between individuals.[6]

A deeper knowledge of the effects of androgen signaling in the vagina is crucial not only to the understanding of the female peripheral sexual response but also to the development of efficient and safe strategies to treat sexual dysfunction and other genitourinary symptoms related to sex steroid deficiency in women. Anatomo-morphological changes caused not only by estrogen but also by androgen deficiency in genital tissues are typical of the menopausal years, and the resulting signs and symptoms, including vulvovaginal atrophy (VVA), are encompassed in the genitourinary syndrome of menopause (GSM).[7] Nevertheless, such alterations are common also during reproductive years, when they may be even more bothersome. Risk factors for androgen deficiency in young women include systemic hormonal contraception, systemic glucocorticosteroid therapy, hypothalamic amenorrhea, hyperprolactinemia, adrenal insufficiency, hypogonadotropic hypogonadism (eg, in the case of anorexia nervosa, abnormal weight loss, and extreme exercise), and chronic medical conditions (eg, chronic liver disease and HIV infection), in addition to surgical menopause at any age and ovarian failure owing to chemotherapy, radiotherapy, or primary ovarian insufficiency.[8]

THE ROLE OF ANDROGENS IN THE DIFFERENTIATION OF INTERNAL FEMALE GENITALIA

From their organogenesis, female genital tissues are hormone-dependent in their anatomy, histology, and functionality. The first step in sex development is sex determination, governed by the establishment of genetic sex at conception, which directs the development of the bipotential gonadal into either testes or ovaries.[9] Basically, the presence of the Y chromosome containing the *SRY* gene (and the absence of other factors promoting ovary development) will induce the formation of testes in males, whereas the absence of *SRY* will induce the formation of ovaries in females.[9] Subsequent sex differentiation into the male or female phenotype represents the sex-specific response of tissues to androgens; specifically, internal and external genitalia develop after the male pathway in the presence of testicular hormones (T, metabolized into dihydrotestosterone [DHT], and the anti-Müllerian hormone [AMH]), or alternatively, the female pathway in their absence.[10]

The original hypothesis of uterovaginal embryogenesis developed in 1830 by Müller led to the historical notion that the vagina has a dual origin, with the cranial part derived from the mesodermal Müllerian ducts (MD) and the caudal part derived from the endodermal urogenital sinus.[11] In both sexes, 2 pairs of genital ducts form, conjoined in the midline — except for an intermediate septum — in the caudal direction: the Wolffian ducts (mesonephral), which grow caudally below the gonadal ridges in the medial part, and the MD (paramesonephral), parallel to the paramedial side of the Wolffian ducts.[11] Around the 8th week of gestation, in the presence of a male gonad, Sertoli cells produce AMH, which acts in the mesenchyme ipsilaterally, leading to contraction of the surrounding wall layers and preventing the development of the MD.[12] At the same time, Wolffian ducts are maintained by the trophic effect of T, elicited via the AR present in the Wolffian epithelium.[13] Specifically, T acts via a mesenchymal factor, which mediates primary morphogenesis.[13] In the absence of AMH, the 2 paired MD develop and undergo extensive fusion to form the uterovaginal canal: the caudal end gives origin to the upper two-thirds of the vagina,[14] whereas the cranial end gives origin to the uterus, containing mesoderm that will form the endometrium and myometrium. The unfused cranial ends of the MD will differentiate in the fimbrial portions of the fallopian tubes. Conversely, the endodermal urogenital sinus leads to the formation of the inferior third of the vagina, the urethra, the vaginal vestibule, and the urethral, paraurethral, and vestibular glands.[14] During normal female development, lack of T results in the degeneration of the Wolffian ducts.

Although the Müller theory has been confirmed by recent investigations conducted on histological and topographic-anatomical sections of human prefetuses,[15] it has been criticized by some authors, claiming that the whole vagina derives from the mesonephral ducts[16] or that the inferior third of the vagina originates from a caudal extension of the MD itself.[17,18] To demonstrate the latter theory, Drews et al[18] used a murine model of complete androgen insensitivity, observing vaginal development in T-treated female embryos and androgen-insensitive littermates. They were able to show that the evaginations of the urogenital sinus — classically claimed to form the lower end of the vagina — are in fact the caudal ends of the Wolffian ducts and that the vagina forms by downward growth of Müllerian and Wolffian ducts themselves.[18] Furthermore, by immunohistochemical studies in normal mouse embryos, they showed that the AR is expressed in the ends of the Wolffian ducts, whereas it is absent in Müllerian-derived structures, thereby suggesting that the Wolffian ducts are the basis of the negative control exerted by androgens on vaginal development.[18]

The embryogenic origin of the vaginal epithelium has also been a debated issue. In the last century, 2 theories on the topic have been developed, both based on hematoxylin and eosin staining: Koff indicated that the vaginal epithelium derived from the Müllerian epithelium for the upper four-fifth of the vagina (1933), whereas Bulmer indicted the urogenital sinus epithelium (UGE) as the main contributor (1957).[19] Recently, Robboy et al[19] conducted immunohistochemical studies of PAX2 and FOXA1 expression in 12- to 21-week-old human fetuses and observed a progressive cranial displacement of Müllerian epithelium by UGE upgrowth, thus supporting Bulmer's proposal that human vaginal epithelium derives solely from UGE.[19]

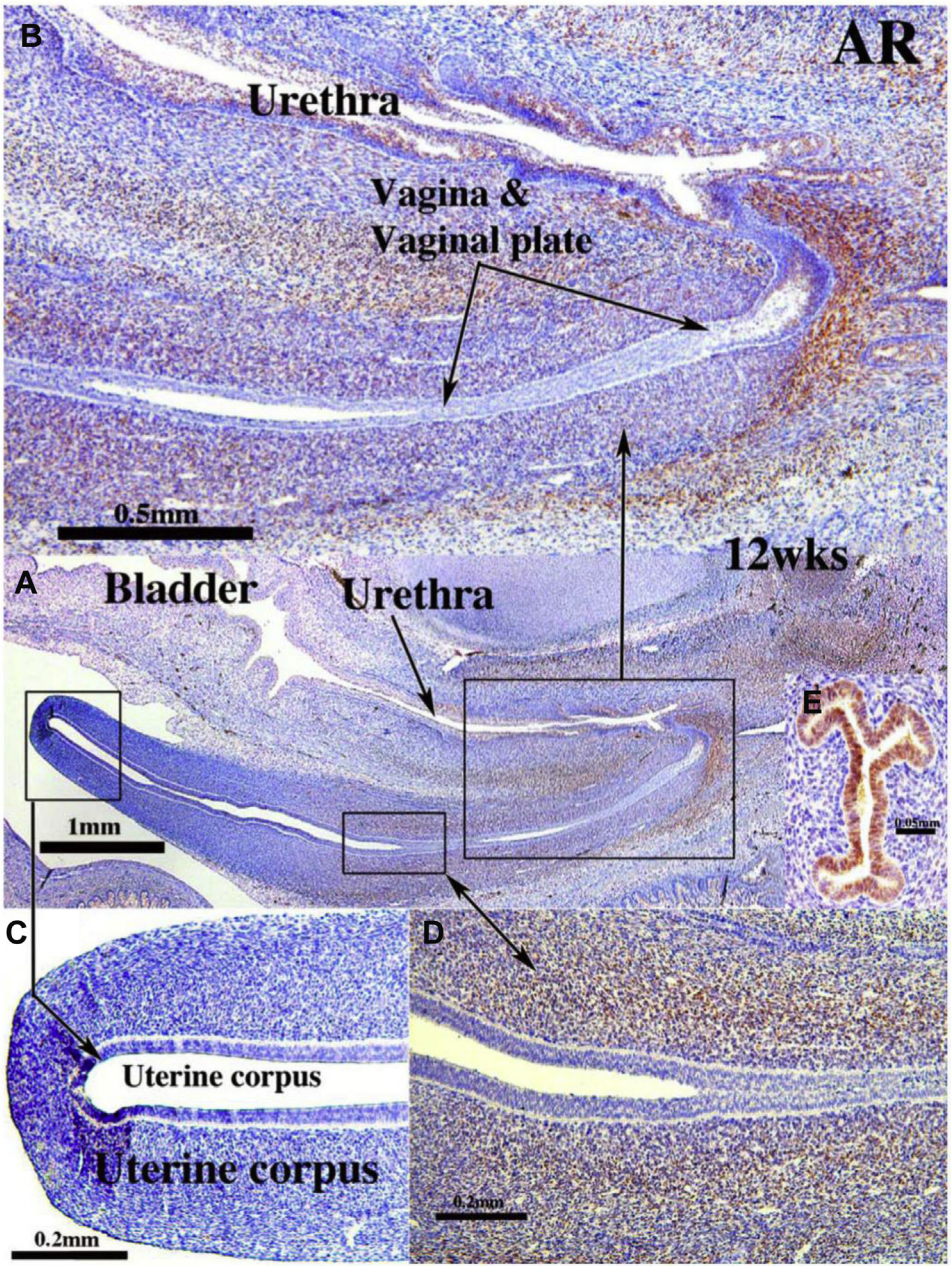

Figure 1. Androgen receptor immunohistochemistry of human fetal female reproductive tracts. Panel A shows low-power overview sagittal section. Note AR staining in mesenchyme associated with the vaginal plate and vagina. Panel B shows higher magnification view of vaginal region showing mesenchymal AR staining. Panel C shows the Absence of AR staining in the uterine corpus ([A] and [C]). Panel D shows intense mesenchymal AR staining associated with the junction of the cervix and vagina. Panel E shows uterine tube of a 16-week fetus showing strong AR immunoreactivity. AR = androgen receptor. Reproduced with permission from the study by Cunha et al.[20] Figure 1 is available in color online at www.jsm.jsexmed.org.

Steroid Receptor Expression During Vagina Organogenesis

Recently, immunocytochemistry studies of steroid receptors revealed that at 14–18 weeks of gestation, estrogen receptor α (ERα) is expressed only in patches in the vaginal plate epithelium and is not detectable in vaginal mesenchymal cells.[20] At 21 weeks, when stimulation by endogenous estrogens occurs, ERα is found in the vaginal epithelium; mesenchymal cells near the vaginal/cervical boundary are also largely ERα positive, whereas near the vaginal introitus, they are mostly ERα negative.[20] Progesterone receptor (PR) positivity is first detected in vaginal stromal cells at 16–18 weeks and becomes uniform in the epithelium at 21 weeks.[20]

With regard to the AR, it has been reported that it is expressed in the mesenchyme associated with the urogenital sinus as early as at 9 weeks (Figure 1). At 21 weeks, it can be widely detected in

vaginal mesenchymal cells; on the other hand, the vaginal epithelium is AR negative, whereas the epithelium of the uterine corpus and cervix exhibits small patches of AR positivity.[20] A particularly strong AR immunostaining has been reported from the 12th week of gestation at the junction of the vaginal plate and the endoderm-derived urethra, and this has been highlighted as an appropriate position to alter the MD-urogenital sinus junction should androgen levels be elevated.[20] Accordingly, manipulations of androgen signaling in utero with AR gene deletions identified a subpopulation of mesenchymal cells that regulate sinus ridge morphogenesis, revealing the mechanisms that lead to vaginal atresia, masculinization of the urethra, and single urogenital sinus in some cases of disorders of sex development with female genital virilization.[21]

FUNCTIONAL ANATOMY OF THE ADULT VAGINA

Gross Anatomy

The human adult vagina is an elastic, fibromuscular canal situated behind the bladder and in front of the rectum. Its walls are ordinarily in contact with an H-shaped transverse cross-section. It extends from the uterine cervix to the vulvar vestibule; its length is 6–7.5 cm along the anterior wall and 9 cm along the posterior wall. It is directed upward and backward, with the axis forming an angle of more than 90° with the uterus, opening forward.[22] The anterior surface is in relation with the base of the bladder and urethra, the sides are enclosed between the levatores ani muscles, and the posterior surface is separated from the rectum by the rectovesical fascia and from the anal canal by the perineal body.[22] Arterial blood is supplied by the vaginal branches of the uterine artery and hypogastric artery for the proximal part and by the middle hemorrhoidal and clitoral arteries for the distal part.[22]

The vagina is not a uniform structure because the proximal and distal parts have distinct properties in accordance with their origin (see "The Role of Androgens in the Differentiation of Internal Female Genitalia"); furthermore, important structural differences have been identified between the anterior and posterior walls. Indeed, in the last 2 decades, research on female orgasmic function has highlighted a surprising lack in our knowledge of the functional anatomy of the vagina and on the relationship between this organ and other structures involved in triggering female pleasure, in particular the clitoris and the urethra. The debated existence and role of the famous Gräfenberg ("G") spot, apparently characterized by a particularly intense sensitivity, led to research on a discrete area within the anterior vaginal wall with an increased nerve density. Although locoregional discrepancies in the innervation of the human vagina are still debated, immunohistochemical studies reported that a greater number of fibers is detectable in distal regions of the vaginal wall compared with the proximal[23] and that the distal anterior vaginal wall is markedly thicker than the proximal.[24] Furthermore, following the studies of O'Connell et al,[25] suggesting that the distal vagina and the urethra display a close spatial, morphological, and functional proximity to the erectile tissue of the bulbs and cavernous bodies of the clitoris and based on dynamic imaging techniques, Jannini et al[26] proposed the existence of a clitourethrovaginal complex, defined as a morphofunctional area stimulated by vaginal penetration and involved in triggering sexual arousal and vaginally activated orgasm.

Overall, these considerations highlight the role of the human vagina not as a passive canal, but rather as a plastic, contractile organ, clinically relevant for women's physical receptivity and sexual responsiveness.[27]

Microscopic Anatomy

In reproductive aged women, the healthy vaginal mucosa comprises a multilayered stratified squamous epithelium that rests on a lamina propria, organized into rugal folds that enable vaginal distensibility. The epithelium contains 2 distinct layers: the stratum basale, mitotically active, and the stratum corneum, a superficial layer of flattened cornified cells.[28] The integrity of the vaginal epithelium promotes the preservation of a eubiotic vaginal microenvironment by stimulating the accumulation of glycogen, the production of lactic acid and a decreased pH, and facilitating the proliferation of *Lactobacilli* with their antimicrobial and anti-inflammatory products.[28] Beneath the epithelium, the lamina propria of the mucosa and the muscularis can be found; the first is rich in vessels that contribute to the diffusion of the vaginal fluid across the epithelium, elastic fibers, lymphatic vessels, and nerves, whereas the second is composed of an outer longitudinal and an inner circular layer of autonomically innervated smooth muscle fibers.[29] Finally, the adventitia is characterized by collagen and elastic fibers and gives structural support to the vaginal wall while allowing expansion during coitus and labor.[29]

The dynamicity of the vagina is reflected in the presence of crucial internal structural differences and in its morphological variability among individuals but also in the remarkable modifications it goes through during a woman's life cycle. The histological analysis on biopsies obtained from the more distal region of the vagina, namely the introitus, has shown dramatic tissue remodeling in postmenopausal subjects with vaginal atrophy symptoms, such as the loss of superficial epithelial layers and epithelial thinning.[30] Moreover, a significant upregulation of genes linked to remodeling of tissues (angiogenesis, wound healing, cell migration, and apoptosis) and inflammation was demonstrated, along with the downregulation of genes related to with epithelial cell differentiation and collagen metabolism.[30]

Increased perfusion in the vagina is critical for its engorgement during sexual arousal. Evidence consistently suggests that the nitric oxide synthase/cyclic guanosine monophosphate/phosphodiesterase-5 (NOS/cGMP/PDE5) system is implicated in regulating vaginal smooth muscle contractility and vascularization.[31,32] In the basal state, norepinephrine from sympathetic, adrenergic nerve terminals determines the constriction of vascular

and non-vascular smooth muscle, thereby maintaining a low perfusion and a high degree of tone in the vaginal wall.[32] In the presence of sexual stimuli, NO is generated in non-adrenergic, non-cholinergic fibers by activation of the neuronal NOS and diffuses into the smooth muscle. In rodent models, diabetes mellitus was found to negatively interfere with the late steps of the NOS/cGMP/PDE5 pathway.[33] In human studies, the constitutive isoforms of NOS (neuronal NOS and endothelial NOS) have been detected in nerve bundles and fibers, the endothelial lining of sinusoids, blood vessels in the epithelium, and the smooth muscle of the cavernous erectile tissue of the anterior wall.[34] In premenopausal female candidates for surgical treatment of stress urinary incontinence, those presenting with poor self-reported sexual arousal had a significantly lower expression of endothelial NOS and PDE5 in the vaginal epithelium of the anterior vaginal wall when compared with asymptomatic controls.[35]

These key structural, neural, and vasomotor characteristics of the adult vagina, essential in maintaining its physiologic function during sexual arousal, seem to be regulated by androgens beyond the prenatal period (see "Vaginal Function in the Female Sexual Response: Potential Mechanisms of Regulation by Testosterone").

THE VAGINA: AN ANDROGEN-TARGET AND SYNTHESIS ORGAN

AR Localization and Modulation

The AR is expressed ubiquitously, and as demonstrated in cell type or tissue-specific AR knockout mouse models, it plays a variety of roles in many extrareproductive processes, including the immune response, insulin sensitivity and metabolism, development and maintenance of bone and skeletal muscle, and in the central nervous system.[36] In the vagina, AR concentration was determined in cytoplasmic and nuclear extracts of tissues derived from intact and ovariectomized (OVX) rabbits treated in vivo with estrogen or androgen replacement therapy.[37] AR expression was evident in both the proximal and distal vagina of intact animals, whereas it was significantly reduced by OVX and differently modulated by androgen and estrogen therapy; in fact, both androgens and estrogens restored AR expression in the proximal vagina, but only estrogens enhanced it in the distal vagina.[37] Similarly, in adult female mice, in vivo estrogen treatment exerted a positive regulation of AR mRNA expression in both epithelial and stromal cells in the vagina.[38] A further characterization of AR localization in monkey vaginas revealed a wide distribution within the epithelium and the lamina propria, with a lower distribution in the muscularis layer and blood vessel walls.[39]

In 1998, one of the first AR localization studies in humans was performed to identify potential target cells of sex steroid action in the female genital skin.[40] Among the investigated receptors, AR appeared particularly abundant in epidermal keratinocytes and dermal fibroblasts of the labia majora, both in premenopausal and postmenopausal women; furthermore, the transition from vaginal epithelium to skin epidermis was marked by an increase in AR and a decrease in ER and PR.[40] In 2013, Baldassarre et al[41] reported AR immunostaining both in the mucosa and stroma of premenopausal and postmenopausal human vagina samples, with no difference between the anterior vs the posterior wall or the proximal vs the distal part, in agreement with previous studies.[42] Notably, AR density in the stroma did not change with aging; conversely, it significantly declined with menopause in the epithelium, suggesting a selective physiological role for T during reproductive years.[41] This reduced AR density could be related to the lower circulating estrogen and androgen levels during menopause and may explain the reduced responsiveness of the vaginal tissue to androgens, potentially leading to the structural and functional alterations typical of GSM.[2,43] Confirming animal data, in the same study, T in vivo treatment in transmen not only increased protein expression of AR in the vaginal stroma[41] but also induced clitoral enlargement.[44]

It is worth noting that a high affinity, specific binding protein has been reported as a specific AR in rat vagina cell nuclei.[45] This protein binds Δ5 androstenediol, a unique steroid causing growth and keratinization of the vaginal epithelium[45] and may be the result of dehydroepiandrosterone (DHEA) transformation, thus reflecting the observed effects of DHEA on vaginal responses.

Local Androgen Synthesis and Its Clinical Implications

Sex steroids are synthesized de novo from cholesterol by the gonads, adrenal glands, and some other tissues, including the kidneys, neurons, glial cells, keratinocytes, adipocytes, and placental trophoblasts, with a limiting step mediated by steroidogenic acute regulatory protein (Figure 2).[46] Cholesterol is converted to pregnenolone by side-chain cleavage enzyme (CYP11A1), and pregnenolone can be transformed into progesterone or into the weak androgen DHEA. DHEA, in turn, can be converted to more active androgens, including T (Figure 2). Subsequently, T can undergo conversion either to the more potent DHT through 5α-reductase activity (SDR5A) or to 17b-estradiol through aromatase (CYP19A1) (Figure 2).[1,46]

Interestingly, these steroidogenic enzymes were first demonstrated by Labrie et al[47] in rat vagina tissue, paving the way for the concept of intracrinology also in the vaginal tissue. In accordance with the principles of intracrinology, in fact, active sex steroids (eg, T and 17b-estradiol) are in large part synthesized locally in peripheral tissues — including the vagina — from DHEA. Indeed, the presence of a sophistically regulated system of steroidogenic enzymes provides every cell with the ability to adjust the intracellular formation and inactivation of sex steroids as per its specific needs, independent of gonadal sex steroid production.[4] This was suggested as having a great importance in

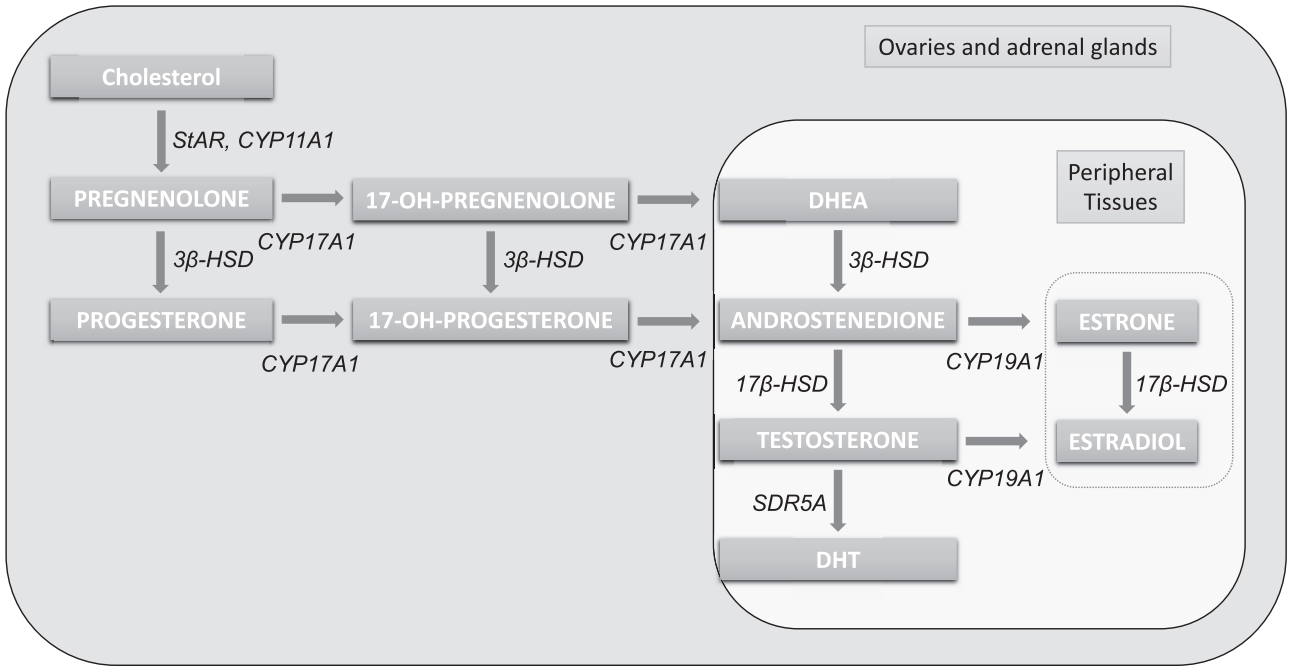

Figure 2. Synthesis of the main sex steroids in women. DHEA is the major androgen secreted by the adrenals. Testosterone is formed from cholesterol in the ovaries and adrenals and from circulating DHEA in the peripheral tissues, including the vagina. Estradiol and estrone derive from precursor testosterone and androstenedione, respectively, and are a minor product of the adrenal glands. 5a-DHT is synthesized from testosterone in target tissues by 5a-reductase (SDR5A). CYP = cytochrome P450; DHEA = dehydroepiandrosterone; DHT = dihydrotestosterone; HSD = hydroxysteroid dehydrogenase; SDR= short-chain dehydrogenase/reductase; StAR = steroidogenic acute regulatory protein.

postmenopausal women, when essentially all estrogens and androgens are synthesized within peripheral tissues,[4] even though human data are still lacking.

In 2003, Berman et al[43] for the first time attempted to analyze aromatase and 5α-reductase type I and II gene expression in vaginal tissue (both proximal and distal) from women aged 31–78 years undergoing vaginal surgery for benign conditions. The study, though innovative, was poorly conducted by using extremely low-sensitive techniques (traditional reverse transcriptase polymerase chain reaction and immunohistochemistry) in a small sample size with a very heterogeneous population of women. Therefore, only 18 of the 20 analyzed specimens showed expression of 5 α -reductase, whereas aromatase expression was only demonstrated in 7 of 10 samples selected.[43] Recently, and only in partial agreement with these findings, our group investigated the mRNA expression of steroidogenic enzymes in distal vaginal tissue biopsies obtained from perimenopausal and postmenopausal women by using real-time reverse transcriptase polymerase chain reaction technique.[5] Expression of steroidogenic acute regulatory protein and of other enzymes involved in the first reactions (CYP11A1 and CYP17A1) were expressed at very low levels compared with the ovary tissue; interestingly, high mRNA levels of genes involved in androgen synthesis, namely HSD17β3 (17β-hydroxysteroid dehydrogenase type 3) and 5 were found, whereas the 3 isoforms of 5α reductase (in particular SRD5A2) showed a dramatic increase compared with the ovarian tissue.[5] Conversely, CYP19A1 mRNA expression was significantly lower, at the limits of detection.[5] These data indicate a physiologic relevance of steroidogenic enzymes, mainly those related to androgen rather than estrogen synthesis, in the human vagina.

T AND VAGINAL FUNCTION IN THE FEMALE SEXUAL RESPONSE

The potential mechanisms of regulation of vaginal function in the context of the female sexual response are summarized in Figure 3.

Epithelium Integrity and Characteristics

It is well known that sex steroid deprivation results in an alteration of vaginal structure toward atrophy, including thinning of the vaginal epithelium, decreased vaginal maturation index (VMI) detectable with a vaginal smear — namely the shift from superficial squamous cells toward intermediate epithelial cells — and decreased smooth muscle, collagen, and elastin content.[2,7] These structural changes contribute in the undermining of the functional properties of the epithelium in terms of fragility, decreased ability to produce glycogen, maintain a low pH and protect from infections, and impairment of intraepithelial nerve terminals.[2,7]

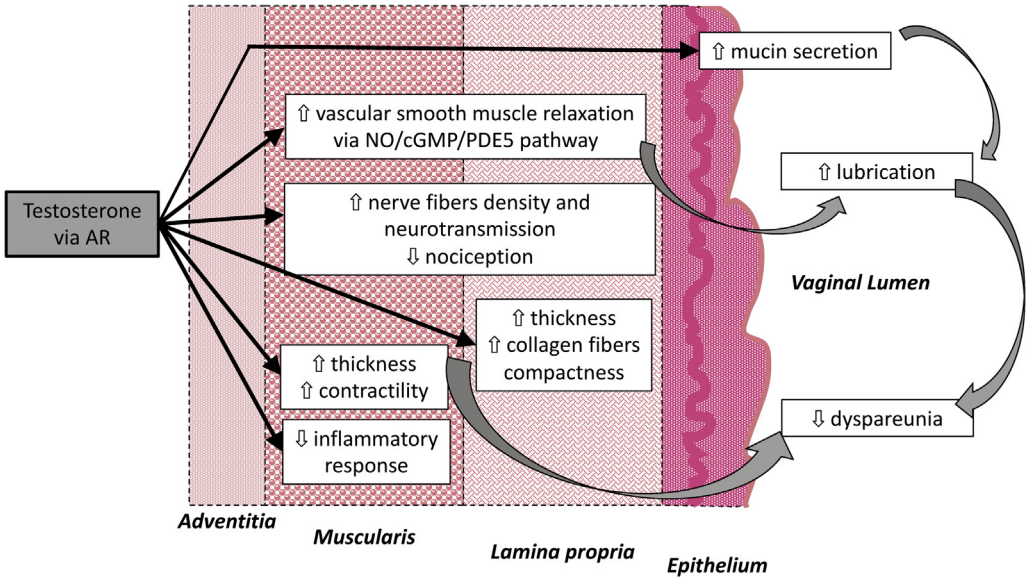

Figure 3. Potential mechanisms of regulation of vaginal function in the context of the female sexual response. AR = androgen receptor. NO/cGMP/PDE5 = nitric oxide synthase/cyclic guanosine monophosphate/phosphodiesterase-5. Figure 3 is available in color online at www.jsm.jsexmed.org.

Vaginal epithelium proliferation appears to be primarily under the control of estrogens. In OVX Sprague-Dawley rats treated with different replacement therapies, only estradiol restored epithelium thickness and morphology up to levels of intact controls, whereas T, similar to progesterone, had little effect on the morphological characteristics of the epithelium in terms of the number of cell layers and cell features, with no significant differences when compared with the vehicle-only treated group.[48] However, it is important to note that coadministration of T with estradiol was able to prevent the notable hyperplasia seen in OVX animals given estradiol alone, thereby suggesting the opportunity of combined replacement regimens to maintain safety and to provide an optimal physiological response.[48]

NO/cGMP Pathway, Blood Flow and Lubrication

Increased pelvic blood flow causing genital vasocongestion and production of plasma transudate within the vagina is the key mechanism underlying lubrication, thus preventing dyspareunia. Administration of inhibitors of NOS and guanylyl cyclase diminished vaginal blood flow in response to pelvic nerve stimulation in the animal model, indicating the NO/cGMP pathway as one of the key mediators of vaginal smooth muscle relaxation.[32] As previously mentioned (see "Microscopic anatomy"), immunohistochemical analysis of the human vaginal wall has also shown the presence of the NO/cGMP/PDE5 biochemical machinery.[34]

Vaginal blood flow measured by laser Doppler flowmetry in response to pelvic nerve stimulation was significantly reduced in OVX rats infused with vehicle when compared with that of controls, and normalized by T treatment.[49] In the clitoris corpora cavernosa, T has been shown to improve the relaxation of vascular smooth muscle cells through the NO/cGMP pathway, maintaining functional relaxant machinery.[50] Given the close interrelation between the distal vagina and the clitoris, which share vasculature and nerve supply,[25] it is likely that the same processes provide the mechanistic basis for the effects of androgens on blood flow in the vagina. In agreement with this, expression and activity of total NOS resulted as being increased by T treatment in the proximal vagina of OVX rabbits and in both the proximal and distal vagina by treatment with DHT.[51] As for PDE5, its androgen-responsiveness is debatable: studies in males have questioned the existence of an androgen responsive element within the promoter region of the PDE5 gene.[52,53] In the bladder of OVX female rats, the activity of PDE5 inhibitor vardenafil in potentiating sodium nitroprusside—induced relaxation was stimulated by the coadministration of T and letrozole and associated with an increased T/estradiol ratio.[54] In the vagina, the responsiveness of PDE5 to androgens in has not been clearly demonstrated.

Mucin secretion from the vestibular glands, which include the Bartholin's, Skene's, and minor vestibular glands, contributes to vaginal lubrification during sexual arousal. Interestingly, these mucin-secreting glands are known to be the embryologic analogs of the Cowper's glands, prostate, and the glands of Littré in males and are androgen-dependent also in females.[55] Consistent with this, vaginal mucin production as assessed by tissue sialic acid content has been historically reported to be androgen-dependent in animal models.[56] Updated studies are needed to consolidate these findings.

Non-Vascular Smooth Muscle Content and Contractility; Lamina Propria Structure

VVA is not limited to changes in the external stratified epithelium, which can be restored by estrogen administration,

but also involves the lamina propria and the muscularis, which appears atrophic[57–59] with a relative increase in connective tissue between the muscle fiber bundles.[48] These 2 layers are essential for vaginal function thanks to their rich innervation and the fact that the relaxation of non-vascular smooth muscle within the vagina is an important event occurring during genital arousal. In OVX Sprague-Dawley rats, Pelletier et al[59] observed a significant reduction in the thickness of the lamina propria area and reported that DHEA was able to restore its thickness to approximately 70% of the intact value. Consistently, T treatment has been shown to restore the muscularis integrity and thickness after OVX, to a lesser extent than estradiol in rats[48] and to a greater extent than estradiol — comparable with that of intact animals — in rabbits.[58] With regard to contractility, in precontracted vaginal strips of OVX rabbits, estradiol diminished the relaxatory response caused by electrical field stimulation or exogenous vasoactive intestinal polypeptide, whereas T and other androgens (including DHT and DHEA) enhanced or normalized these responses.[58] These data suggest that T increases neurogenic input and/or upregulates the number or affinity of neurotransmitter receptors that mediate smooth muscle relaxation in the vagina.

Interesting androgen-dependent effects of DHEA identified, thanks to co-treatment with a blocker of estrogen action (Selective estrogen receptor modulator alcobifene), have also been described on the increase of collagen fiber compactness in the lamina propria in OVX rats.[60]

Innervation, Nerve Fiber Density, and Neurotransmission

Another mechanism by which sex steroid deprivation might induce sexual dysfunction is by deficient vaginal innervation and/or sensitivity; indeed, cholinergic fibers, mostly found in the lamina propria in mammalians, are known to mediate the increase in vaginal blood flow leading to vasocongestion, whereas sympathetic fibers concentrated in the muscularis are responsible for an increased vascular tone in the basal state. In immunostaining studies in which protein gene product 9.5, a general nerve marker, was used in vaginal samples of Sprague-Dawley rats, T was the only replacement treatment able to significantly increase the density and thickness of nerve fibers in the muscularis and lamina propria, compared not only with OVX animals but also with intact controls; this significant effect was lost when T was combined with estradiol.[48] These data are supported by evidence indicating the presence of AR in pelvic ganglions and the neuroprotective role of T in different systems.[61] Other studies in rodents demonstrated that long-term DHEA administration restores pre-OVX vaginal innervation by significantly increasing fiber density, with a more powerful effect than that observed on the area of the 2 layers (muscularis and lamina propria), therefore suggesting a specific positive influence of DHEA on neural tissue.[59] To demonstrate an aromatization-independent effect of DHEA on promoting neural plasticity in the vagina, the same authors subsequently compared DHEA, premarin, and alcolbifene; they found that Premarin had no effect on fiber density, whereas alcobifene did not prevent the positive effect of DHEA, thereby illustrating its predominant non-estrogenic influence.[62]

Finally, T is a well-known pain modulator, with low levels described in chronic pain patients (refer to the study by Maurer et al[63] for a review). It could be speculated that T is also involved in nociception within the female genital tract, acting through a dampened pain signaling within the dorsal horn and other key sites in the central nervous system. Interestingly, a transdermal T gel therapy significantly reduced pain and nociceptive neuronal signaling in women with fibromyalgia in a phase I/II pilot study,[64] whereas higher menstrual phase-related serum T levels have been associated with hypoalgesia in healthy women.[65] Furthermore, AR expression was found to be significantly lowered in the minor vestibular glands of hormonal contraceptive users,[66] and a genetic polymorphism in the AR has been identified in women with provoked vestibulodynia, further suggesting that an inefficient AR combined with low free T may predispose women to genital conditions characterized by chronic pain.[67]

ACTUAL AND POTENTIAL THERAPEUTIC APPLICATIONS FOR T/ANDROGENS IN VVA/GSM AND SEXUAL DYSFUNCTIONS CHARACTERIZED BY IMPAIRED AROUSAL AND LUBRICATION

Low-dose vaginal estrogens represent the most common effective and safe approach to VVA/GSM and their associated sexual symptoms, including poor arousal/lubrication and dyspareunia. In recent years, based on growing evidence supporting a direct role of androgens in vaginal physiology, new therapies targeting androgen activity have been developed. (Table 1). Intravaginal preandrogen DHEA (Prasterone) was approved in 2016 in the United States and in 2018 in Europe for the management of moderate to severe dyspareunia in menopausal women. DHEA is a product of the adrenal zona reticularis (50%) and the ovarian theca (20%) and is derived for 30% from circulating DHEA sulfate[1]; serum concentration of both DHEA sulfate and DHEA decrease with age, and by the age of 70, they are approximately 20–25% of their peak values.[4,68] Intravaginal DHEA is administered as a 6.5 mg ovule, daily at bedtime, and within the vagina, it is transformed not only into estrogens but also into androgens such as androstenedione, T, and DHT.[69] In 3 independent 12-week double-blind, placebo-controlled RCTs summarized in the study by Labrie et al,[70] Prasterone demonstrated significant beneficial effects on dyspareunia and vaginal dryness, restoring VMI and pH, with all serum sex steroids remaining within normal postmenopausal values. An improvement has also been reported in other domains of sexual function, including desire/interest, arousal, and orgasm, evaluated with validated questionnaires.[71] It is worth noting that clinical trials on the possible preventive effect of intravaginal DHEA on recurrent urinary tract infections, which are part of the constellation of the typical symptoms of GSM, have been planned.[72]

Table 1. Available and off-label local and systemic androgen-based therapies for the treatment of VVA/GSM and related sexual dysfunction

Compound	Formulation	Status	Known/proposed benefits on vaginal function	Supposed mechanisms of action in the vagina	Quality of evidence
Local DHEA (Prasterone)	Daily 6.5 mg intravaginal insert 0.50%	Approved in the United States and Europe for moderate-severe dyspareunia in menopausal women	Significant beneficial effects on dyspareunia, vaginal dryness, VMI and pH[71] Improvement in desire/interest, arousal, and orgasm[71] Potential application for GSM-related UTIs[72] (no evidence yet)	Converted into estrogens and androgens within the vagina (intracrine metabolism) Aromatization-independent effects have to be clarified	Placebo-controlled RCTs[70]
Systemic DHEA	Oral 25–100 mg daily	Available over-the-counter in the United States as a food supplement Off-label in Europe	No clear improvement in sexual function in menopausal women with normal adrenal function	Converted peripherally into estrogens and androgens	Meta-analysis of RCTs[74]
Local testosterone	Testosterone vaginal cream	Off-label	Improvement in self-reported vaginal dryness, dyspareunia and sexual function[76–78] Improvement in objective clinical outcomes (vaginal trophism)[76,77]	Increase in local blood flow, density of nerve fibers, mucous secretions, and formation of collagen Studies on women taking aromatase inhibitors support an aromatization-independent effect	Overall low quality (open label designs and lack of control arms)[75]
Systemic testosterone	Different compounds	Off-label	In postmenopausal women T treatment at high physiologic doses, with or w/o HRT, improves not only desire, but also arousal, orgasmic function, pleasure, and sexual responsiveness[83]	Direct vaginal effects of systemic therapy have to be clarified	Meta-analysis of RCTs[3]

DHEA = dehydroepiandrosterone; HRT = hormonal replacement therapy; RCTs = randomized clinical trials; T = testosterone; UTIs = urinary tract infections; VMI = vaginal maturation index.

In accordance with observational data, low circulating DHEA levels may be associated with an increased risk of low sexual function in both premenopausal and postmenopausal women.[73] In the United States, systemic DHEA therapy is readily available over-the-counter as a nutritional supplement aimed at improving aging-related poor libido and well-being, despite the fact that there is scant evidence to support its use in women with normal adrenal function and that safety data are lacking (Table 1). In 2014, a meta-analysis including 23 RCTs (of overall low quality) on oral DHEA vs placebo enrolling more than 1,100 healthy menopausal women found no association with a significant improvement in sexual function.[74]

There are no approved local T products for women diagnosed with VVA/GSM or with sexual dysfunctions characterized by dyspareunia, including the female genital arousal disorder (FGAD) (Table 1). A recent systematic review considered all trials on intravaginal T for the treatment of VVA[75] and concluded that the efficacy and safety of this therapy is uncertain. Indeed, the included studies were only 6, and methodologically flawed by the lack of a T-only or a placebo arm, lack of random allocation and blinding, small sample sizes, lack of objective clinical outcome measures (eg, VMI, pH, vaginal health score), and failure to adjust for baseline values. Moreover, 3 of 6 trials enrolled a specific subpopulation of breast cancer survivors, thus limiting the generalization of the results.[75] Nevertheless, available evidence suggests that intravaginal T in menopause may exert a positive effect on self-reported sexual function domains, including sexual desire, lubrication, pain, and satisfaction and that it is associated with a lower vaginal pH and improved the vaginal health score compared with non-hormonal treatment.[76,77] As for safety, 1 RCT on breast cancer survivors taking aromatase inhibitors showed that 12 weeks of treatment with intravaginal T cream (5,000 μg 3 times/week) resulted in 12% modest sustained serum estradiol elevations, suggesting that lower-dose T may be warranted in these patients.[78] Since Bell's systematic qualitative analysis, only a double-blind, placebo-controlled RCT has been published, enrolling 37 postmenopausal women with VVA taking aromatase inhibitors in which improved self-reported sexual satisfaction and reduced dyspareunia have been reported.[79] Positive outcomes have been also shown with topical estradiol and T on pain scores in young women with hormonal contraception-related vestibulodynia.[80] In the 1990s, topical T was described as exerting some beneficial effects in vulvar lichen sclerosus[81]; however, these data have never been confirmed.[82]

Regarding systemic T treatment, a recent global consensus position statement produced by a task force of representatives from leading societies concluded that in menopausal women diagnosed with HSDD, T at high physiologic doses with or without hormonal replacement therapy improves not only sexual desire but also arousal, orgasm, pleasure, and overall sexual responsiveness (Table 1).[83] Nevertheless, T remains off-label in most countries, both for HSDD and for any other genitourinary or sexual condition. In addition to natural and surgical menopause, exogenous T is supposed to exert a facilitatory action on the genital response in the presence of other alterations that lead to FGAD by interfering with endothelial function and/or neurotransmission.[84] These include diabetes mellitus, different components of metabolic syndrome (eg, obesity),[85] and central and peripheral nervous system disorders (eg, multiple sclerosis).[84] However, direct beneficial effects of T therapy on genital tissues have to be clarified. Unfortunately, in recent decades trials investigating systemic T therapy have not provided data on subjective or objective outcomes relative to urogenital health.

Finally, aging and menopause are associated with chronic systemic and local inflammation and it has been hypothesized that these processes may contribute to the onset of GSM and other disorders characterized by chronic pain in women, including vulvar pain syndrome, genitopelvic pain/penetration disorder and chronic pelvic pain syndrome.[86] In this regard, recent experimental data from our group have shown that human distal vaginal smooth muscle cells derived from perimenopausal and postmenopausal women possess the ability to be involved in the inflammatory (acute and chronic) response, by activating cell-mediated immunity and secreting a number of cytokines.[87] Our experiments have also suggested a significant anti-inflammatory in vitro effect of DHT, which in vagina smooth muscle cells was able to reduce the expression and secretion of inflammatory mediators after stimulation with interferon γ, the key effector cytokine of T helper 1 immunity.[87] These findings are in line with compelling evidence on the pleiotropic immunosuppressive role of androgens on key components of both innate and adaptive immunity, described in recent literature in both preclinical and clinical male models (refer to the study by Trigunaite et al[88] for a review). If these hypotheses are confirmed by further studies, androgen-based treatments may represent a novel therapeutic strategy in genital conditions with an inflammation-based etiology in women.

CONCLUSIONS

The vagina, a crucial organ of the female sexual response, is androgen-responsive from its embryogenesis throughout adult life. In the vagina, T — produced by the ovaries and the adrenals or formed locally — interacts with the AR directly or by reduction to the more potent DHT, positively regulating vascular and non-vascular smooth muscle and collagen growth and function, nerve density and function, and genital hemodynamics, with a facilitating effect on arousal and lubrication. Favorable effects of T in the negative modulation of nociception and inflammation in the vagina have also been hypothesized.

The clinical consequences of androgen deficiency on vaginal health in both reproductive and menopausal years remain understudied. However, local DHEA, which is transformed in the vagina into both active androgens and estrogens, is effective for the management of dyspareunia in menopause, with evidence

pointing toward an aromatization-independent mechanism. Further studies are needed before vaginal or systemic T can be considered for the treatment of VVA/GSM and for other conditions leading to FGAD and dyspareunia.

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Conflict of interest: The authors report no conflicts of interest.

Funding: This research was supported by a PRIN ("Progetti di Rilevante Interesse Nazionale": 2017S9KTNE) and Ministero dell'Istruzione, dell'Università e della Ricerca (Italy) to Linda Vignozzi.

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