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# Contractile Properties of Vaginal Tissue

*The vagina is an important organ of the female reproductive system that has been largely understudied in the field of biomechanics. In recent years, some research has been conducted to evaluate the mechanical properties of the vagina, but much has focused on characterizing the passive mechanical properties. Because vaginal contractions play a central role in sexual function, childbirth, and development and treatment of pelvic floor disorders, the active mechanical properties of the vagina must be also quantified. This review surveys and summarizes published experimental studies on the active properties of the vagina including the differences in such properties determined by anatomic regions and orientations, neural pathways, life events such as pregnancy and menopause, pelvic floor disorders such as prolapse, and surgical mesh treatment. Conflicting experimental findings are presented, illustrating the need for further research on the active properties of the vagina. However, consensus currently exists regarding the negative impact of surgical mesh on vaginal contractility. This review also identifies knowledge gaps and future research opportunities, thus proving a firm foundation for novice and experienced researchers in this emerging area of biomechanics and encouraging more activity on women's sexual and reproductive health research.* [DOI: 10.1115/1.4046712]

## Introduction

"I bet you're worried. We were worried. We were worried about vaginas [1]." The opening lines to the influential *Vagina Monologues* address the audience's likely anxiety about viewing a performance about vaginas and convey the work's intentions to diffuse some of that discomfort and promote awareness about vaginas. Though the play is over two decades old, the relevance of these lines persists since references to this sexual organ still generate discomfort and embarrassment for many. As mechanicians, we should be worried that the mechanical properties of the vagina remain understudied in our field.

The vagina is a fibromuscular tubular organ that extends from the vulva to the cervix (Fig. 1). The organ is comprised of three main layers: the internal mucosa, the intermediate muscularis, and the external adventitia. The mucosa layer contains a stratified squamous nonkeratinized epithelium and the lamina propria made of a dense irregular connective tissue. The muscularis layer is composed of two layers of smooth muscle cells (SMCs): an inner layer with circumferentially oriented SMCs and an outer layer with longitudinally oriented SMCs. The adventitia is a thick layer of loose connective tissue that contains vasculature, lymphatics, and nerves [2].

The proximal and distal regions of the vagina have been found to have compositional and structural differences in the mouse, rat, and rabbit [3–5]. However, there is a disagreement as to whether the proximal vagina has equal [5] or greater [3,6] relative smooth muscle content than the distal vagina. There is a similar disagreement about whether the wall of the proximal vagina is thicker [3] or thinner [4,5] than the wall of the distal vagina. The distal portion of the vagina has a richly innervated sphincter structure of circumferentially aligned SMCs which is thought to offer mechanical support to the vaginal opening [4,7]. These regional variations are likely due to the difference in embryological origin between the proximal and distal vagina and may reflect unique functional purposes [8].

Vaginal innervation is both extensive and diverse, arising from the hypogastric nerves, the pelvic splanchnic nerves, and the pudendal nerves and being comprised of adrenergic, cholinergic, and nonadrenergic noncholinergic nerves in both sympathetic and parasympathetic pathways [9,10]. Researchers have observed regional variation in the distribution of these nerves. Rat studies have shown the proximal vagina to have a higher proportion of cholinergic nerves compared to adrenergic nerves [4]. Conversely, the contractions of the distal vagina have been found to be preferentially mediated by adrenergic nerves [11]. The distal vagina also contains substantial nitric oxide-mediated nerves, which cause a relaxation response in rats [3,7]. It is believed that relaxation of the smooth muscle allows for vaginal distention during labor and arousal. Nitric oxide synthase and several other nonadrenergic noncholinergic mediators have been observed in the human vagina [12,13]. Finally, vaginal contractions can be also induced by hormones, such as oxytocin, which is released during

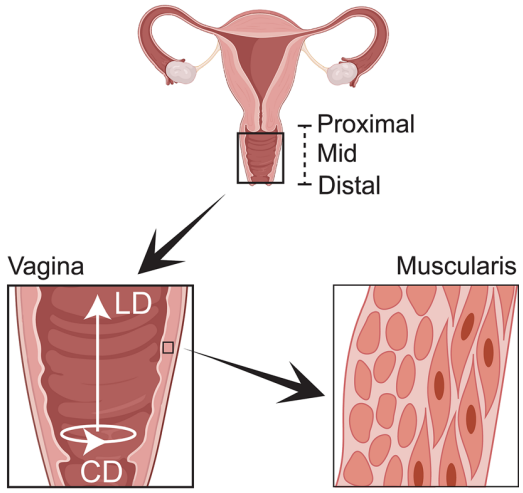


**Fig. 1 Schematic of the vagina denoting proximal, mid, and distal regions, LD and CD, and smooth muscle cell orientation within the muscularis**

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sexual stimulation [14]. The combination of this neurologic activity regulates vaginal blood flow, excretions, and arousal [13].

Although some progress has been made in characterizing the passive mechanical properties of the vagina [15], little is known about the active mechanical properties. This is especially problematic due to the critical role of the contractile vaginal smooth muscle (VaSM) in a woman's reproductive life. For example, alterations to the contractile properties of the vagina that occur during and after pregnancy have been overlooked, despite the fact that more than 80% of women in the U.S. over the age of 30 give birth [16]. In one study on the pregnant rat vagina [17], many of the SMCs transform from quiescent and contractile to proliferative and synthetic phenotype. The vaginal SMCs become diffusely spaced and surrounded by increased extracellular matrix. These changes may result in increased vaginal compliance and likely ease parturition. Immediately following parturition, the SMCs experience a reduction in synthetic characteristics and, 3 weeks after parturition, they are highly similar to vaginal SMCs in virginal rats in terms of organization, extracellular matrix proliferation, and phenotype [17]. However, whether the mechanical function of the vagina completely recovers after parturition remains unclear.

During menopause, when a woman ceases to be fertile, there is a decline in reproductive hormones. The absence of such hormones, especially estrogen, contributes to vaginal atrophy where the vaginal tissue thins and becomes dry causing discomfort and dyspareunia [18]. Decrease in VaSM occurs abruptly at the age of menopause [19]. Estrogen replacement therapy can be used to alleviate these symptoms, but objective outcomes of these treatments are not well evidenced and do not include measures of VaSM [20,21]. In rats, estrogen replacement therapy has resulted in restoration of the VaSM layer thickness as measured through histology [22]. Whether this is true for humans and whether the mechanics of the vagina are restored with such therapies are unknown.

Pelvic organ prolapse, a highly prevalent disorder in women, has also been found to alter VaSM. Prolapsed vaginal tissue shares several architectural hallmarks of pregnancy: reduced SMC content [23–25], SMC disorganization [23,24], and increased extracellular matrix [26]. Still, the consequences of these alterations on the contractile properties of the vagina are unknown. Surgical mesh implantation is a common intervention for correcting pelvic organ prolapse but it is associated with high failure rates and serious complications [27,28]. In primates, mesh implantation has resulted in decreased VaSM and SMC disruption and disorganization [29]. The lack of knowledge about the contractile properties of the vagina likely contributes to the poor outcome of surgical procedures for treating pelvic organ prolapse.

In this paper, an overview of current experimental studies that focus on characterizing the stresses and forces due to contraction of the vaginal tissue will be provided. First, the most commonly used experimental protocols and methods are presented. Then, the effect of anatomic (distal, mid, and proximal) regions and (longitudinal and circumferential) directions of the vagina on the active mechanical properties are reviewed. Studies on nerve-mediated contractions of the vaginal tissue are also summarized. Moreover, the alterations in vaginal contractility caused by pregnancy, menopause, prolapse, and mesh implantation are reviewed. In the Conclusion section, findings of published studies, potential limitations, and current gaps will be discussed while offering suggestions for future investigations that would allow progress in women's sexual and reproductive health.

## Testing Methods

Contractions of the vaginal tissue have been quantified primarily through uniaxial tests of rectangular strips of tissue. These strips are isolated either along the longitudinal direction (LD) [3,6,7,22,25,30–32] or along the circumferential direction (CD) [3,4,7,11,14,33–40] of the vagina as defined in Fig. 1. These types of tests are useful for revealing the effect of anatomic differences on the contractile properties of the vagina. Multiple strips of tissue can be isolated from various locations (e.g., distal versus proximal, anterior versus posterior) within the same organ/animal thus reducing potential animal-to-animal variability. However, uniaxial tests do not simulate the in vivo loading condition of the vagina. Very recently, the active properties of the vagina have been characterized by loading the whole vagina simultaneously in the LD and CD via planar biaxial tests [41] and inflation–extension tests [5]. All studies have been conducted ex vivo, with one exception [42]. Tissue specimens from several animal models have been used including mice [5,14,37], rats [4,6,7,11,22,31–33,41,42], rabbits [3,30,40], sheep [35,38], and nonhuman primates [34,36,39]. In a few cases, contractions of the human vaginal tissue were measured [25,31,43]. Figure 2 presents a summary of the types of mechanical tests and animal models used in all the published studies.

Contraction of the vaginal tissue has been experimentally induced through direct membrane depolarization using high concentrations of KCl or $K^+$ [3–7,14,22,25,30–36,38–41], nonspecific nerve stimulation using electric field stimulation (EFS) [3,4,7,11,30,33,36,37,39–42], and receptor-mediated pathways using receptor-specific agonists [7,11,14,25,30,31,33,36,37,40]. The contraction response of the vaginal tissue to potassium is independent of innervation or receptor density and proportional to the amount of smooth muscle in the tissue. For this reason, the contractile forces that are generated in response to potassium are often used as standard values of the contractility of the tissue, and contractile forces that are generated using other stimulation methods are often normalized using these standard values. Studies in this review typically used solutions with concentrations of potassium ranging between 40 mM [5,14] and 125 mM [25], though studies in which sensitivity analyses were performed tested the effect of lower concentrations [4,32]. Figure 3 presents potassium-induced contractile forces per unit volume of control, healthy, and untreated specimens from several studies [3,6,22,25,31,32,34,36,38,40,41]. It should be noted that some data in Fig. 3 are from representative specimens rather than averages of several specimens. When numerical values of the contractile forces were not provided explicitly, data in Fig. 3, and throughout this review, were retrieved from plots and curves in published papers using ImageJ (National Institutes of Health, Bethesda, MD). Additionally, when either force data or force data normalized by cross-sectional areas were reported, the reported values were divided by the appropriate specimen dimensions in order to estimate contractile forces per unit volume. This normalization was performed to make meaningful comparisons across published studies. Multiple data points were included for studies that presented results for multiple regions of control specimens. The contractile forces normalized by specimen volume ranged between 0.04 $mN/mm^3$ and 3.48 $mN/mm^3$.

Inducing contractions using EFS involves positioning the specimen between two electrodes in a physiologic bath and applying an electric field using a pulse stimulator. EFS typically produces frequency-dependent contractions; the studies presented in this review used frequencies ranging from 0.5 Hz to 70 Hz [33,41]. Unfortunately, the size and specific positioning of electrodes have not been reported for the most part, which makes estimating the strength of electric field applied to the specimen difficult. That being said, most studies in this review applied voltages between 6 V [42] and 30 V [37].

In many studies, possible effects of the estrous cycle on the contractile properties of the vagina were not considered. In the few studies in which the estrous cycle was taken into account, vaginal specimens were isolated from animals at the same phase of estrous [6,31,38] or the estrous phase of the animals was used as an experimental variable when comparing the results from vaginal specimens [7,14,33]. No differences in vaginal contractility were found with respect to estrous cycles [7,33], though spontaneous contractions of uterine and cervical tissue were affected [14].

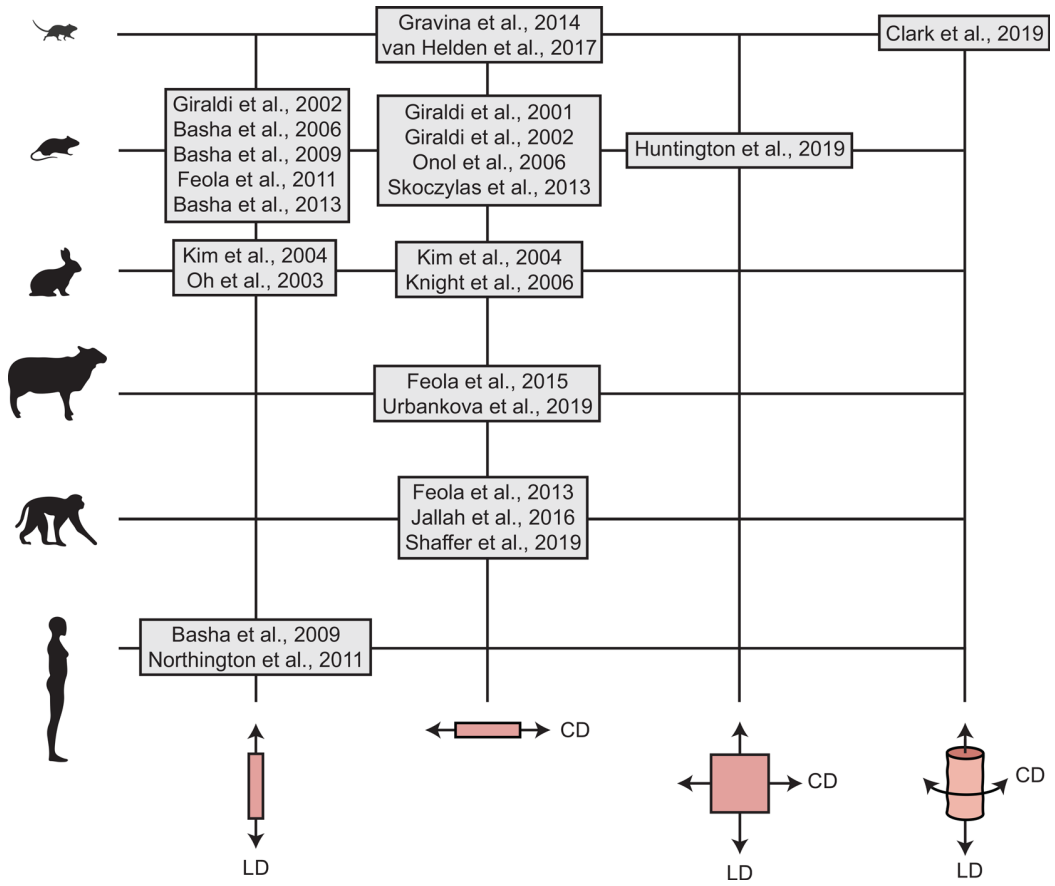


**Fig. 2 Ex vivo mechanical tests (left to right: uniaxial tests in the LD, uniaxial tests in the CD, biaxial tests, and inflation tests) and animal models (top to bottom: mice, rats, rabbits, sheep, nonhuman primates, and humans) used to measure the contractility of the vaginal tissue in the cited studies. CD: circumferential direction, LD: longitudinal direction.**

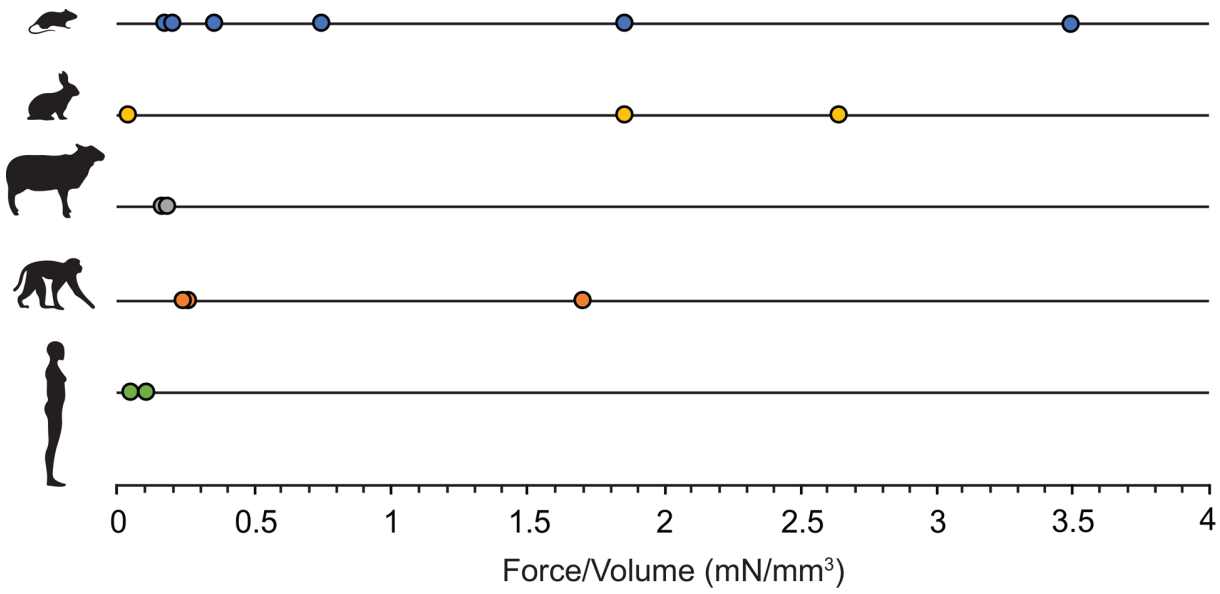


**Fig. 3 Contractile forces of the vaginal tissue in various animal models (top to bottom: rats, rabbits, sheep, nonhuman primates, and humans) in response to potassium normalized by specimen volume. These forces are used as standard/control values [3,6,22,25,31,32,34,36,38,40,41].**

### Distal Versus Proximal Vagina

Variations in the active mechanical properties of the vagina along the LD have been investigated. Those who studied regional differences typically considered the proximal region, which is the upper third or upper half of the vagina that is closer to the uterus, and the distal region, the lower third, or lower half of the vagina that is closer to the introitus. Some studies further divided the vagina into three regions by including a midregion (Fig. 1). While the vagina is generally accepted to be in-homogenous, both in structure and function, along the LD, there are conflicting reports on which anatomic region has higher smooth muscle content, contractility, or innervation density.

As mentioned earlier, the composition of the vagina has been reported to vary along the LD. In the distal region, smooth muscle cells appear to be scattered and unorganized while they are more compact and organized in the proximal region [4,38]. A clearly defined sphincter-like structure of muscle has been identified in the distal vagina [7], and the vagina has the highest overall thickness in this region [3,4]. In terms of smooth muscle quantity and functionality, there are conflicting accounts as to which, if any, region contains more VaSM and generates higher contractile forces.

Figure 4 presents a summary of published studies on the variation in contractile forces with respect to anatomic region. Data that report the contractility of the mid and proximal regions in this figure were normalized by the corresponding data of the distal region. As such, data points with a fold-change value greater than 1 denote studies in which the mid or proximal regions contracted more than the distal region. According to some reports, the proximal vagina had a larger content of smooth muscle and exhibited stronger contractile force than the distal vagina [3,6]. For example, stronger contractions were observed in the proximal and midregions compared to the distal region in response to both 60 mM KCl [3] and 50 Hz EFS [3,37] (proximal: 36.6 force (g)/wet tissue weight (g), mid: 13.5 g/g, distal: 4.1 g/g) [3], and 50 Hz EFS (proximal: 1.1 mN, distal: 0.18 mN) [37]. Likewise, Basha et al. [6] compared the contractile response of strips of rat vaginal tissue isolated from the proximal and distal regions to 110 mM KCl. When normalized by the overall cross-sectional area of the tissue strips, stronger peak contractile forces were observed in the proximal region as compared to the distal region (proximal: $11.13 \pm 1.57\ mN/mm^2$, distal: $4.47 \pm 0.92\ mN/mm^2$). However, there are also reports according to which the proximal rat vagina had a lower smooth muscle content and contracted less than the distal rat vagina [7,11]. Önol et al. [11] found that strips of rat vaginal tissue generated stronger peak contractile forces in response to both 120 mM KCl and 1–40 Hz EFS in the distal region than in the proximal region. The peak force in response to 40 Hz EFS was reported to be 3.6 force (g)/wet tissue weight (g) in the distal vagina and 0.9 g/g in the proximal vagina. Giraldi et al. [7] observed little to no contractions in strips of rat vaginal tissue from the proximal region, and thus used strips of vaginal tissue from the distal region, which contracted consistently, for the majority of testing. Urbankova et al. [38], on the other hand, reported that there were no significant differences in the contractile forces of ovine vaginal tissue with respect to region (distal: $0.17\ mN/mm^3$, mid: $0.19\ mN/mm^3$).

### Longitudinal Versus Circumferential Direction

The muscularis of the vagina contains an inner layer of circumferentially oriented smooth muscle cells and an outer layer of longitudinally oriented smooth muscle cells [30] (Fig. 1). The relative content of smooth muscle cells aligned in the LD and CD has not been determined, although it likely dictates the overall contraction of the organ in each of these directions. Some research has been done to quantify the vaginal contractions in the LD and CD using uniaxial tests [3,7], planar biaxial tests [41], and extension–inflation tests [5] (Fig. 2). By comparing results of uniaxial tests of strips of rat vaginal tissue oriented along the LD and CD, Giraldi et al. [7] and Önol et al. [11] found that, while circumferentially oriented strips of tissue contracted, longitudinally oriented strips of tissue did not reliably produce contractions. Using comparable testing methods, Oh et al. [3], on the other

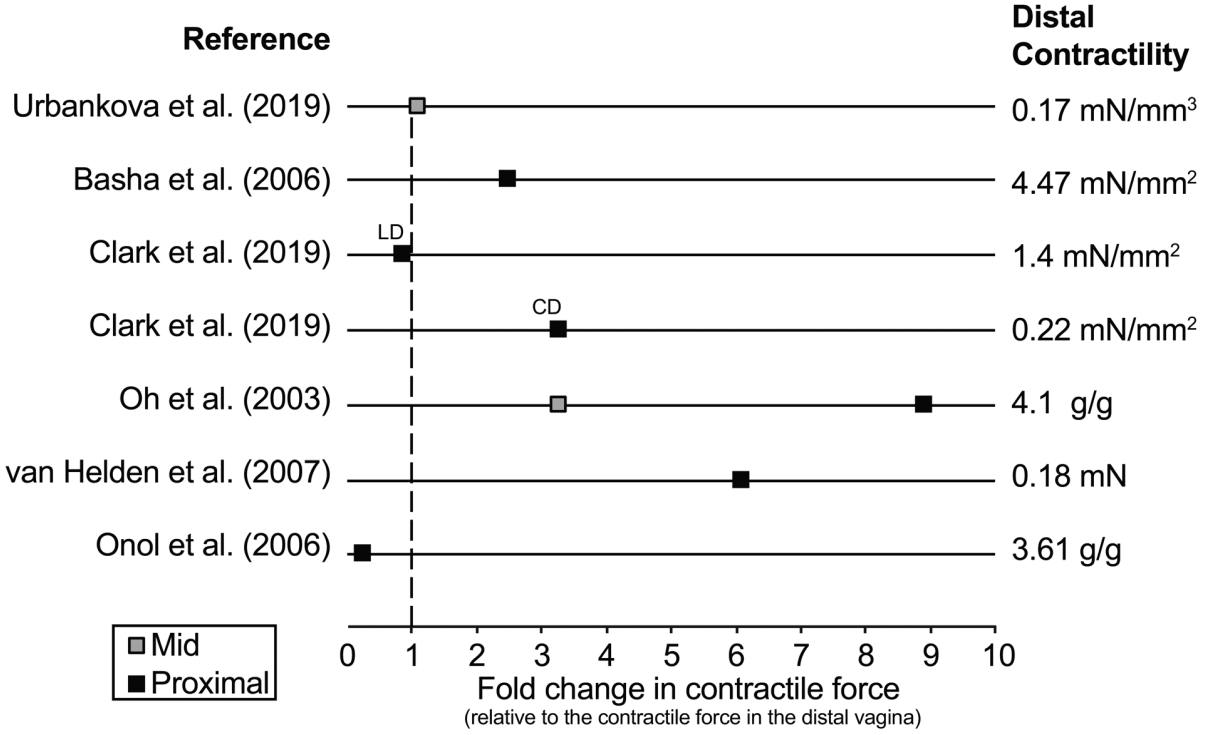


**Fig. 4 Fold change in contractile forces with respect the contractile forces in the distal region. The contractility in the distal region is reported for each study [3,5,6,11,37,38]. LD: longitudinal direction, CD: circumferential direction.**

hand, observed no difference in contractions between strips of rabbit vaginal tissue isolated along the two directions.

Recently, we investigated the anisotropy of the rat vagina by performing planar biaxial tests. Contractions were induced by EFS at various stretch lengths and by KCl, and the contractile forces were measured along the LD and CD simultaneously [41]. While EFS caused higher contractile stresses in the CD than in the LD (CD: 1.88 mN/mm$^2$, LD: 1.17 mN/mm$^2$), KCl caused higher contractile stress in the LD than in the CD (LD: 3.46 mN/mm$^2$, CD: 2.15 mN/mm$^2$). Clark et al. [5] conducted extension–inflation testing of the murine vagina. In this study, potassium-induced contractions also resulted in higher contractile stresses in the LD than in the CD in both the proximal region (LD: 1.23 mN/mm$^2$, CD: 0.72 mN/mm$^2$) and distal region (LD: 1.4 mN/mm$^2$, CD: 0.22 mN/mm$^2$).

## Innervation and Contraction Pathways

Since the contractions of the vagina are mediated by its innervation, there have been several studies that sought to identify the dominant neural pathways within the vagina. These studies typically involved measuring the vaginal contractions that are either caused by the addition of agonists or inhibited by the addition of antagonists during EFS. Phenylephrine, epinephrine, and norepinephrine are the most commonly used adrenergic agonists, and carbachol is the most commonly used cholinergic agonist. There is evidence suggesting that the contractile activity of VaSM is controlled by adrenergic, cholinergic, and nonadrenergic noncholinergic pathways [7,33]. However, the exact mechanisms by which the different anatomic regions of the vagina are controlled are still unknown. Research thus far indicates that the proximal region responds primarily to cholinergic stimulation, and the distal region responds primarily to adrenergic stimulation, though neither exclusively so.

Giraldi et al. [7] observed that the distal vagina in the rat responded to both adrenergic and cholinergic stimulation as both norepinephrine and carbachol elicited contractions. Oh et al. [3] did not observe evidence supporting cholinergic control of the rabbit vagina and found that the distal vagina contracted more in response to adrenergic stimulation than the proximal vagina. Phenylephrine, epinephrine, norepinephrine, and isoproterenol all induced contractions in all regions of the vagina, and all induced stronger contractions than those induced by KCl in the proximal vagina. Carbachol, on the other hand did not induce any significant contractions. Along these lines, metoprolol, an adrenergic antagonist, and prazosin, an adrenergic inverse agonist blocked EFS-induced contractions, while atropine, a cholinergic antagonist, did not [3]. Adrenergic control of the distal vagina was supported by the results of Önol et al. [11]. In the distal region of the rat vagina, carbachol produced only weak contractions, while phenylephrine produced dose-dependent contractions. EFS-induced contractions were blocked in the distal vagina by the addition of alpha-1 and alpha-2 antagonists, indicating that the EFS had been working through adrenergic pathways.

Basha et al. [31] stimulated strips of rat vaginal tissue using carbachol to assess the role of cholinergic nerves in vaginal contractions. Tissue strips from both the proximal and distal regions exhibited dose-dependent contractions in response to carbachol. Carbachol-induced contractile forces were normalized by 110 mM KCl-induced forces to account for variation in the quantity of smooth muscle across the two anatomic regions. After normalizing, the proximal vagina was found to contract 1.5 times more than the distal vagina, despite findings indicating a similar receptor affinity across the two regions. In this study, one human specimen was also tested and contracted in response to both potassium and carbachol, indicating that cholinergic nerves may play a role in the contraction of the human vagina as well.

Skoczylas et al. [4] also provided support for the prominence of cholinergic activity in the proximal vagina, with the finding that carbachol induced a higher peak contractile force than phenylephrine in the proximal vagina (carbachol: 2.44 mN/KCl-induced force (mN), phenylephrine: 1.11 mN/mN). Van Helden et al. [37] observed response to cholinergic stimulation in the proximal vagina and response to both cholinergic and adrenergic stimuli in the distal vagina. The addition of atropine, a cholinergic antagonist, significantly reduced the magnitudes of EFS-induced contractions in both proximal and distal regions of the vagina. Neostigmine, which blocks the breakdown of acetylcholine and, hence, promotes cholinergic activity, enhanced the magnitudes of EFS-induced contractions in both anatomic regions. The addition of phentolamine, an adrenergic receptor antagonist, did not have significant effects on contractile response, further indicating that EFS works through primarily cholinergic pathways to cause contractions. In the distal region though, the addition of phenylephrine resulted in contraction, providing evidence for adrenergic activity in the distal region.

## Pregnancy, Menopause, Prolapse, and Mesh Implantation

Mechanical tests of the vagina in the active state are primarily performed to reveal how life events such as pregnancy, menopause, pelvic organ prolapse, and treatment strategies affect vaginal contractility. A summary of the published findings is reported in Fig. 5. The vagina undergoes significant remodeling throughout pregnancy to prepare for delivery and to recover after delivery [17]. Feola et al. [32] measured the contractility of the rat vagina at various stages of pregnancy, including virgin, midpregnant, late-pregnant, immediate postpartum, and 4 weeks postpartum. Contractions were induced in dog-bone shaped vaginal specimens using increasing concentrations of KCl (5.88–124 mM) to measure peak contractile force as well as to determine the KCl concentration that induced contractions with 50% of the magnitude of contractions induced by 124 mM KCl. The peak contractile force was lower for midpregnant (1.9 mN/mm$^2$), late pregnant (3.3 mN/mm$^2$), and immediate postpartum groups (1.89 mN/mm$^2$) compared to the virgin group (3.78 mN$^2$), but this reduction was not present in the 4-week postpartum group (4.2 mN/mm$^2$). There was, however, an increase in KCl sensitivity during pregnancy that remained present at 4-weeks postpartum. Recently, Urbankova et al. [38] reported the long-term effects of the first vaginal delivery on the contractility of the ovine vagina. Active forces in response to KCl significantly decreased within the distal vagina (0.167 mN/mm$^3$ for nulliparous sheep versus 0.065 mN/mm$^3$ for primiparous sheep 1 year after delivery) but were not significantly altered in the midvagina (0.185 mN/mm$^3$ for nulliparous sheep versus 0.209 mN/mm$^3$ for primiparous sheep 1 year after delivery).

The hormonal changes that occur during menopause decrease the functionality of VaSM. Menopause, a process during which the ovaries drastically reduce their hormone production, can be experimentally induced by surgically removing the ovaries. The muscularis of the vagina in ovariectomized animals was reduced compared to control animals [11,30]. Contractile forces in the distal rat vagina were found to be significantly higher in control specimens than in ovariectomized specimens in response to EFS (control: 3.6 force (g)/wet weight (g), ovariectomized: 0.4 g/g), phenylephrine (control: 2.5 g/g, ovariectomized: $< 0.1$ g/g), and KCl (data not reported) [11]. Contractile forces in the proximal rat vagina were also higher in control specimens than in ovariectomized specimens in response to KCl (control: 9.4 mN/mm$^2$, ovariectomized: 6.2 mN/mm$^2$) [22]. However, vaginal muscle thickness and contractility in ovariectomized rabbits was increased with testosterone and estradiol therapies [30].

To date, there has been only one study evaluating the effect of prolapse on vaginal contractility [25]. There have, however, been a few studies on the changes in VaSM content that are associated with prolapse. Namely, premenopausal and postmenopausal women with prolapse have a lower content of VaSM [23,44]. Northington et al. [25] sought to determine whether prolapse

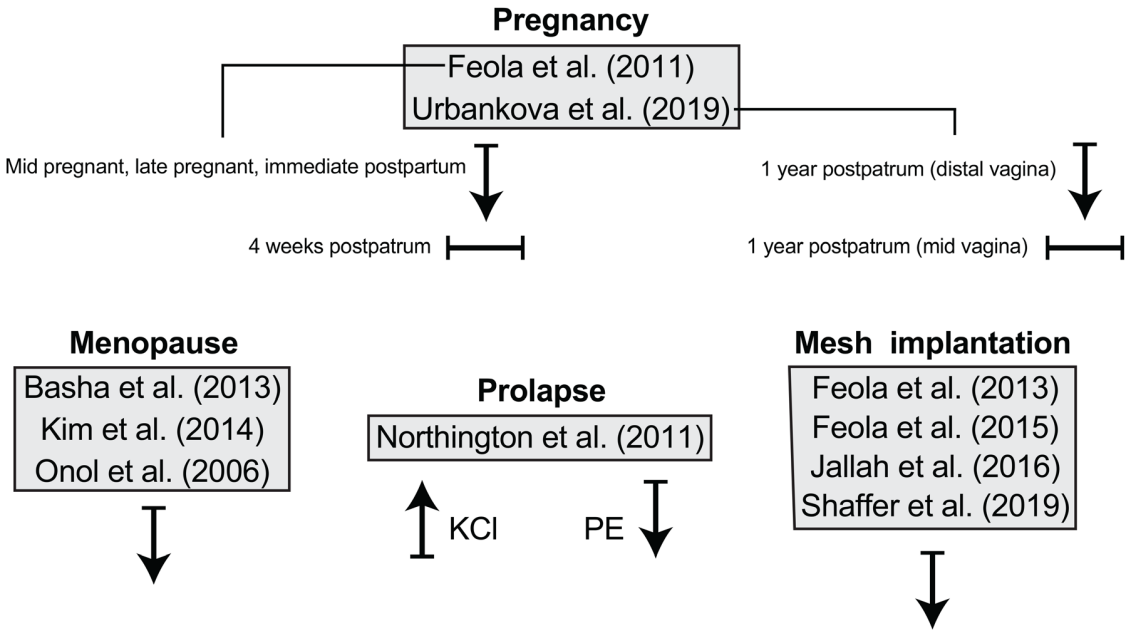


**Fig. 5 Effects of pregnancy, menopause, prolapse, and mesh implantation on vaginal contractility. The down arrow (↓) denotes a decrease in contractility and the horizontal line (—) no change in contractility. KCl: potassium chloride, PE: phenylephrine.**

affected the contractile properties of the human vagina. Longitudinally, oriented strips of tissue were collected from the anterior vagina from premenopausal women with and without prolapse undergoing hysterectomies. Tissue from women with prolapse had a smaller proportion of muscle. Surprisingly though, strips from patients with prolapse generated higher contractile forces than control strips in response to KCl (control: 0.95 mN/mm$^2$, prolapse: 5.33 mN/mm$^2$). However, specimens from women with prolapse did not contract in response to phenylephrine, while control specimens consistently did. In agreement with these findings, immunofluorescence microscopy revealed fewer adrenergic receptors in tissues from women with prolapse. These results indicate that, in addition to muscle loss, prolapse decreased the ability of the vaginal tissue to contract in response to adrenergic stimulation.

The use of mesh devices for the treatment of prolapse has long been surrounded by controversy due insufficient evidence of their safety and effectiveness. Nonhuman primates [34,36,39], sheep [35], and rabbits [40] have been used as animal models for vaginal mesh studies. In these studies, one group of animals was subjected to a surgical procedure in order to implant the mesh, while another group of animals underwent a sham procedure and was used as the control group. The mesh was left in place for several weeks/months to allow for tissue growth and remodeling before tissue–mesh complexes were collected for mechanical testing. Shaffer et al. [39] found that the vaginas from the group of animals who received a mesh had small and scattered muscle bundles and a reduction of nerve density compared to the control group, and Knight et al. [40] observed significant thinning of the muscularis. There is an agreement that mesh implantation also leads to decreased vaginal contractility [34–36,39,40]. The contractility of the vagina was reduced by the implantation of any mesh. Feola et al. [34] tested the effects of four different synthetic surgical meshes implanted and found that the stiffest mesh caused the greatest reduction in contractile force (control: 2.1 mN/mm$^2$, with Gynemesh$^{TM}$ PS: 0.4 mN/mm$^2$). To evaluate the effect of mesh stiffness independently from the differences across various mesh products, the same type of mesh, UltraPro$^{TM}$, which is anisotropic, was implanted in multiple orientations [34,36]. When the stiffer direction was aligned parallel to the LD of the vagina, there was a greater reduction in contractility than when the stiffer direction was perpendicular to the LD (control: 0.24 mN/mm$^3$, UltraPro parallel: 0.07 mN/mm$^3$, UltraPro perpendicular: 0.17 mN/mm$^3$) [36].

## Discussion

Smooth muscle activity within the vagina plays a critical role in women's sexual and reproductive health. As reported in this article, the contractility of the healthy vagina has been, to some extent, quantified via uniaxial and biaxial tests using several animal models (Fig. 2), but conflicting results exist suggesting the need for further research. The contractility of the vagina varies between the LD and the CD, and among the distal, mid, and proximal regions. The differences in the contractile properties in the LD and CD have only been studied by a few investigators. Recently, the anisotropy of the vaginal tissue in the active state has been determined via planar and inflation–extension biaxial tests [5,41]. In these studies, the vagina exhibited stronger contractions in the LD compared to the CD in response to KCl, which stimulates muscle nonselectively. This could indicate that there is a larger quantity of smooth muscle cells oriented longitudinally. However, in response to EFS, which stimulates VaSM via nerves, the vaginal tissue in the CD exhibited stronger contractions [41], possibly indicating a difference in the innervation in the CD and LD. Interestingly, Giraldi et al. [7] and Oh et al. [3] both found that longitudinally oriented strips of vaginal tissue did not produce reliable contractions despite several other studies reporting contractions in the LD [6,22,25,30–32]. This discrepancy may simply be the result of differences in animal models, experimental protocols, and testing methods.

Typically, the vagina was found to have greater contractility toward the proximal end, closer to the cervix. However, it should be noted that this was not consistently observed across published studies (Fig. 4). The presence of larger amounts of smooth muscle and stronger contractions in the proximal region may reflect unique functional purposes. It is believed that uterine contractions facilitate sperm transport [45], so perhaps the proximal region of the vagina, which is closest to the uterus, may have a similar function.

In this review, contractile forces refer to forces that are generated in the tissue in response to an external stimulation. However, the vagina appears to have a basal tone, so smooth muscle cells are contracted to maintain the shape of the organ even when they are not externally stimulated [5]. Therefore, strictly speaking, the contractile forces are the additional forces that are generated by the external stimulation of the vaginal tissue. The basal tone has been measured by comparing the properties of the vagina without any additional stimulants to the properties of the chemically passivated vagina by Clark et al. [5]. With basal tone, the vagina was found to have a decreased length and diameter, an increased distensibility, and significantly lower circumferential and longitudinal tangent moduli in the proximal region than it had without basal tone. For this study, the tangent modulus was defined as the slope of the stress–strain curve within the range of physiological pressure ($7 \pm 2$ mmHg) at the physiological length of the specimen. The inability of smooth muscle cells to maintain an adequate basal tone of the vagina may be implicated in the development of sexual and reproductive disorders and, therefore, must be further investigated.

Potassium concentrations or electric field parameters are often selected so that the maximal contractile response of the vagina is achieved (Table 1). Potassium sensitivity tests on the rat vagina have revealed that the lowest concentration of potassium evoking the maximal contraction varies between 34 mM and 74 mM [32]. In the majority of the published studies, higher concentrations of potassium, between 110 and 125 mM, were used to achieve the maximal contractile response [6,7,11,22,25,30,31,33,34,36,40,41]. The ideal parameters of EFS (e.g., voltage, stimulus frequency, pulse duration, or current) that generate the maximal contractile force in vaginal tissue have not been identified. Usually, investigators perform pilot experiments to select such parameters, incrementally increasing the voltage for the EFS until no change in the contractile force is detected. Most studies reported voltages for the EFS protocols to be between 6 V [42] and 30 V [37]). However, since the same voltage will produce different electric fields depending on the distance between the electrodes, the electric field strength, with units of volts per meter (V/m), should be reported so that research findings across all the studies can be better compared and their differences can be better interpreted. The direction of the electric field relative to the vaginal specimen may also influence the contractile properties of the vagina. For example, in the cat intestine, smooth muscle exhibited stronger contractions when the applied electric field was aligned with the smooth muscle cells, and progressively weaker contractions when the applied electric field was at 45 and 90 deg orientation relative to the smooth muscle cell [46]. Since the VaSM is organized in circumferentially and longitudinally oriented layers within the muscularis, the directionality of the applied electric fields likely has an effect on the relative strengths of the contractions observed in the tissue. Future studies on EFS-induced contractions of the vagina should report the electric field strength (or distance between electrodes) and the orientation of the electric field with respect to the tissue during testing.

The contractile force that the vaginal tissue generates depends on the applied stretch, with the peak contractile force occurring at the so-called optimal length. In the cited studies, vaginal specimens were incrementally stretched and stimulated until the contractile force reached its peak value [3,6,11,22,25,30–32,41] but the optimal length was often not reported. In some studies, vaginal specimens were stimulated starting from a preload state and, for this reason, the peak contractile force values may have been underestimated. In our study, we reported the stretch at which the peak contractile force was recorded, that is the optimal stretch, to be 1.32 in the LD and 1.25 in the CD [41], and in preliminary testing, Oh et al. [3] found the optimal length to be approximately 1.8 of the resting length of the specimens. It remains unclear, however, how the ex vivo estimate of the optimal stretches of the vagina compare to the in vivo ones. Spontaneous contractions of the vagina may also depend on the applied stretch. Indeed, when a 5 mN preload was applied to vaginal strips by Gravina et al. [14], vaginal tissue was almost never spontaneously active, as only 1 of 22 strips tested exhibited spontaneous contractions. On the other hand, when vaginal strips were preloaded to 20–40 mN, Fu et al. reported that 34 of 42 strips tested contracted spontaneously [43].

While all the studies reviewed here reported contractile forces/stresses, there are currently no studies reporting accurate strain measurements of the vaginal tissue during contractions. When reported, strain and stretch are usually estimated from the crosshead displacement of the testing apparatus and, therefore, they are not very accurate. Very recently, the change in the length of the outer diameter of the murine vagina during contractions was

**Table 1 Stimulation methods used to induce contractions in vaginal tissue**

| Reference | KCl molarity | EFS parameters | Chemicals |
|---|---|---|---|
| Basha et al. [6] | 110 mM | — | — |
| Basha et al. [31] | 110 mM | — | CCh |
| Basha et al. [22] | 110 mM | — | — |
| Clark et al. [5] | 40 mM | — | — |
| Feola et al. [32] | 5.88–124 mM | — | — |
| Feola et al. [34] | 120 mM | — | — |
| Feola et al. [35] | 80 mM | — | — |
| Giraldi et al. [33] | 124 mM | 1–40 Hz | NE |
| Giraldi et al. [7] | 124 mM | 1–50 Hz | NE, CCh |
| Giuliano et al. [42] | — | 6 V, 10 Hz | — |
| Gravina et al. [14] | 40 mM | — | — |
| Huntington et al. [41] | 124 mM | 700 mA, 70 Hz | — |
| Jallah et al. [36] | 120 mM | 1 Hz | CCh, PE |
| Kim et al. [30] | 120 mM | 10 V, 0.5–40 Hz | NE |
| Knight et al. [40] | 120 mM | 20 V, 1–64 Hz | PE |
| Northington et al. [25] | 125 mM | — | PE |
| Oh et al. [3] | 60 mM | 70 V/cm, 1–50 Hz | EPI, NE, PE, CCh, IPR |
| Önol et al. [11] | — | 10 V, 1–40 Hz | PE, CCh |
| Shaffer et al. [39] | 120 mM | 20 V, 1–64 Hz | — |
| Skoczylas et al. [4] | 10–120 mM | 20 V, 1–64 Hz | — |
| Urbankova et al. [38] | 80 mM | — | — |
| van Helden et al. [37] | — | 30 V, 50 Hz | PE, CCh, CPA |

KCl—potassium chloride, EFS—electrical field stimulation, CCh—carbachol, NE—norepinephrine, PE—phenylephrine, EPI—epinephrine, IPR—isopreternol, and CPA—cyclopiazonic acid.

measured optically by Clark et al. [5], but this represents the only attempt in which strain was computed more accurately. The deformations of the vagina during ex vivo testing that induce contractions must be quantified in order to fully characterize the contractile behavior of this organ. Along these lines, studies should also be conducted to investigate how much the vagina deforms in vivo during normal activity such as intercourse. This will help to further establish the mechanical requirements of medical devices such as meshes. Meshes used for surgical correction of prolapse become integrated with the vaginal wall, and therefore, should be able to strain as much as the native tissue.

Obtaining vaginal tissue from young and healthy human donors is ideal but nearly impossible, especially considering that tests for measuring contractile properties must be performed quickly after isolation to maintain tissue viability and functionality. Animal models are, therefore, invaluable to characterize the contractions of the vagina. Similarities and differences of the vagina across animal species and between animal species and humans need to be investigated for animal testing to have implications on human health. The inevitable differences need to be taken into account in the design of experiments and interpretation of findings, but these differences are not always detrimental as they can often help in answering specific research questions. For example, the content of VaSM in the rabbit is higher than in the nonhuman primates or humans and, for this reason, the rabbit has been recently selected for studying the effects of mesh implantation on VaSM [40].

While the link between pelvic organ prolapse and VaSM degeneration is well established, it is unclear whether the decrease in smooth muscle quantity contributes to the development of prolapse, or if the development of prolapse leads to the degeneration of smooth muscle [25]. Answering this "chicken and egg" question would have crucial implications for the prevention and treatment of prolapse. On the other end, it is well established that the use of vaginal mesh alters the VaSM significantly, with the mesh leading to lower peak contractile forces. This is the most agreed-upon finding on VaSM contractility and it has been demonstrated in sheep [35], nonhuman primates [34,36,39], and rabbits [40]. Meshes must be developed such that their material properties do not damage host tissue and are compliant enough to withstand the strains experienced by the vagina in both the active and passive states.

## Conclusions

Vaginal contractility has been quantified with respect to anatomic regions and orientations, neural pathways, pregnancy, menopause, prolapse, and mesh implantation. In the vast majority of existing studies, strips of vaginal tissue have been subjected to uniaxial tests, though planar biaxial and inflation–extension testing methods have been utilized in some studies. The most notable findings are that the contractile properties are nonhomogeneous, direction-dependent, and inhibited by the implantation of vaginal mesh. Despite the progress made in measuring the contractility of the vagina, rigorous testing methods are needed to facilitate more research that examines the differences in the active mechanical properties stemming from differences in factors such as pregnancy, menopause, and aging. New efforts on healthy and diseased vaginal tissue are needed to accelerate research advances for the sexual and reproductive health of women.

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