

Adwoa Baah-Dwomoh

STRETCH Laboratory,
Department of Biomedical Engineering
and Mechanics,
330A Kelly Hall,
Virginia Tech,
Blacksburg, VA 24061

Jeffrey McGuire

STRETCH Laboratory,
Department of Biomedical Engineering
and Mechanics,
330A Kelly Hall,
Virginia Tech,
Blacksburg, VA 24061

Ting Tan

STRETCH Laboratory,
Department of Biomedical Engineering
and Mechanics,
330A Kelly Hall,
Virginia Tech,
Blacksburg, VA 24061

Raffaella De Vita

STRETCH Laboratory,
Department of Biomedical Engineering
and Mechanics,
330A Kelly Hall,
Virginia Tech,
Blacksburg, VA 24061
e-mail: devita@vt.edu

Mechanical Properties of Female Reproductive Organs and Supporting Connective Tissues: A Review of the Current State of Knowledge

Although there has been an upsurge of interest in research on women's sexual and reproductive health, most of the research has remained confined to the obstetrics and gynecology disciplines, without knowledge flow to the biomechanics community. Thus, the mechanics of the female reproductive system and the changes determined by pregnancy, age, obesity, and various medical conditions have not been thoroughly studied. In recent years, more investigators have been focusing their efforts on evaluating the mechanical properties of the reproductive organs and supportive connective tissues, but, despite the many advances, there is still a lot that remains to be done. This paper provides an overview of the research published over the past few decades on the mechanical characterization of the primary female reproductive organs and supporting connective tissues. For each organ and tissue, after a brief description of the function and structure, the testing methods and main mechanical results are presented. Constitutive equations are then reviewed for all organs/tissues together. The goal is to spark the interest of new investigators to this largely untapped but fast-evolving branch of soft tissue mechanics that will impact women's gynecologic, reproductive, and sexual health care.

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1 Introduction

The female reproductive system consists of the vagina, cervix, uterus, and ovaries supported by ligaments, fasciae, and muscles. The elements of this system exhibit an astonishing mechanical performance: they undergo incredibly large deformations and are remarkably strong. For example, the uterus increases in size severalfold during pregnancy, going from the size of a clenched fist to the size of a full term baby. During labor, the uterus generates contractile forces that are high enough to propel a full term baby out of the pelvis. The cervix also dilates significantly; the diameter goes from 1 cm to 10 cm during labor, over only a few hours. During pregnancy, the ligaments supporting the reproductive organs remodel as well. The round ligaments, for example, triple their length due to the increased weight of the organs [1].

The elements of the reproductive system work in unison to perform sexual and reproductive functions while maintaining their positions within the pelvis. The connectivity of the organs and tissues can, however, also give rise to serious health issues. In fact, malfunction of one organ or tissue can compromise the function of the connected organs, setting off a cascade of health problems. For example, when one organ of the reproductive system falls out of place, it presses against surrounding structures. This typically leads to pelvic floor disorders (PFDs) such as urinary incontinence, fecal incontinence, and prolapse. While the exact etiology of PFDs is unknown, mechanical alterations to pelvic organs, supportive ligaments, fasciae, and muscles due to pregnancy, age, and weight gain contribute to the development of these disorders [2–4]. Currently, PFDs affect nearly 30 million women in the

U.S., and this number is expected to rise to an alarming 44 million by 2050 [5]. Treatment for PFDs often involves surgery, but complications or recurrence after surgical interventions are very common and no standardized surgical protocols exist [6]. Annual economic costs associated with PFD surgeries alone are significant and are expected to increase over the next two decades [7,8].

Mechanical alterations of the female reproductive organs and structures such as the uterus and cervix during pregnancy can also result in preterm birth. For example, weakening of the cervical tissue can lead to cervical insufficiency [9]. Preterm births increase the chances of neonatal morbidity, cerebral palsy, sensory deficits, learning disabilities, and respiratory illnesses [10,11]. In 2005, approximately 9.6% of all births worldwide were preterm, and 10.6% of births in the U.S. and Canada were preterm [12]. Preventing preterm birth is challenging since the causes of preterm births are numerous and not well understood [13]. While drug treatments have been shown to be promising, their effectiveness and safety are still being studied [14]. Costs associated with preterm birth for the U.S. were recently estimated at \$26.2 billion [15].

There is an unmet clinical need to characterize the mechanical properties of the female reproductive organs and supporting tissues. Knowledge about these properties can help unravel risk factors, establish preventative methods, standardize surgical protocols, and engineer surgical materials for PFDs and preterm birth. Advances on the mechanical characterization can also lead to new detection, prevention, and treatment strategies for other common diseases that affect women's health such as uterine, endometrial, and ovarian cancers. However, because the material characterization of these tissues in many cases remains fairly superficial, more focused research efforts are necessary to ensure women's reproductive and sexual health at all stages of life.

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The mechanical properties of the female reproductive organs and surrounding tissues can be computed by means of several *ex vivo* and *in vivo* testing methods. Commonly used *ex vivo* methods include uniaxial compression and extension tests, planar biaxial tests, inflation tests, aspiration tests, puncture tests, and indentation tests. *In vivo* techniques include tensile tests, suction tests, ultrasound-based tests, and balloon inflation tests. By using these testing methods, structural (e.g., stiffness) and material properties (e.g., elastic modulus) can be computed. Together with histological and microscopy data, the mechanical data can guide the development of constitutive equations for the reproductive organs and supporting connective tissues. These equations can then be implemented into finite element models, and other numerical models, to predict the behavior of the pelvic floor under a variety of mechanical stimuli that emulate both normal and pathological conditions [16].

With the recent advances in soft tissue mechanics, different methods and testing protocols have been used to quantify the mechanical behavior of the female reproductive organs and supporting connective tissues. In this paper, the authors offer a brief overview of the *in vivo* and *ex vivo* testing methods and mechanical properties of the vagina, uterus, cervix, and pelvic ligaments (Fig. 1). More specifically, in Secs. 2–5, the testing methods and material properties are presented after describing the main function and structural components of each organ and tissue. In Sec. 6, due to the lack of constitutive models for several organs and tissues, the very few existing modeling efforts for all the reproductive organs and pelvic ligaments are presented together. A discussion about the mechanical studies that are reviewed is presented in Sec. 7, and some recommendations for future directions are also offered in Sec. 8. The authors hope that this review will serve as a springboard for new investigators to dive into this fast-evolving branch of biomechanics with immediate impact on women's health.

2 Uterus

2.1 Function and Structure. The uterus is a major hormone-responsive sex organ in the female body. It is connected to the vagina, via the cervix, and to the fallopian tubes (Fig. 1). Also known as the womb, the uterus is held into place by endopelvic fascia or ligaments, such as the pubocervical, cardinal, and uterosacral ligaments. It is typically pear-shaped and approximately 7.6 cm long, 4.5 cm wide, and 3.0 cm thick [17]. During sexual activities, the uterus provides increased blood flow to the pelvis, ovaries, and vagina. The main function of the uterus is, however, to house the fetus during gestational development. The uterus

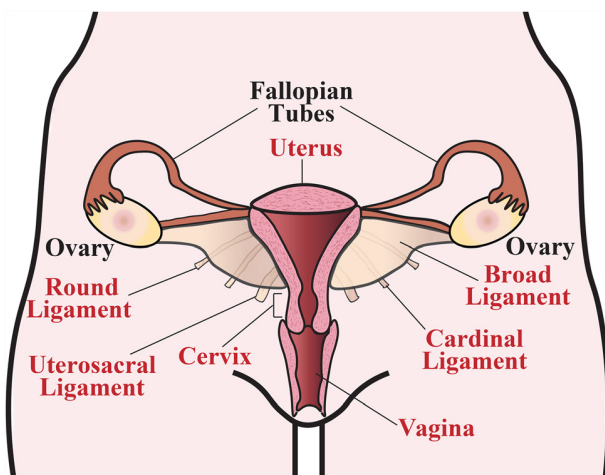


Fig. 1 Female reproductive organs and supportive connective tissues

accepts the fertilized egg via the fallopian tube and implants the egg in the endometrium, which provides nutrients to allow the egg to grow into an embryo. Once the embryo is formed, it attaches to the wall of the uterus, creates the placenta, and develops into a fetus.

The uterus consists of three layers: the endometrium, myometrium, and perimetrium. The endometrium, which is the innermost layer of the uterus, builds during the menstrual cycle to prepare for the implantation of an embryo. If no implantation occurs, the endometrial lining is shed and causes menstrual bleeding. The myometrium is the middle layer of the uterus. It mostly consists of smooth muscle tissue, collagen and elastin fibers. The smooth muscle cells allow the uterus to expand during pregnancy and contract during childbirth, and these cells are assembled in large interwoven bundles within the myometrium [18]. The elastin fibers are present in a spongelike matrix that contains flat sheets, or lamellae [19]. There are two distinct layers within the myometrium: the inner layer, also known as the junctional zone, which contains smooth muscle cells with larger nuclei, and the outer layer which contains smooth muscle cells with smaller nuclei. The elastin content in the inner myometrium layer is lower than in the outer myometrium layer [18]. The perimetrium is the outermost layer of the uterus. Mostly made of loose connective tissue, the perimetrium protects the uterus from friction with other organs in the human body. In the uterine cavity, there are two dense families of muscle and collagen fibers that are mainly oriented in the circumferential direction [20]. The content of muscle fibers increases during pregnancy and decreases with menopause [21].

2.2 Testing Methods and Material Properties

2.2.1 Ex Vivo Studies. *Ex vivo* uterine tissue has been tested in uniaxial tension [22–25], uniaxial compression [23], aspiration [26], and biaxial tension [27]. In one of the first studies to ever measure the material properties of the uterus, Conrad et al. [22] determined the passive stress relaxation of the uterus from pregnant and nonpregnant patients. The study found that uterine tissue from pregnant patients experienced a rate of stress relaxation that was higher than uterine tissue from nonpregnant patients under the same strain levels. Interestingly, the authors did not find that age, parity, or phase of menstrual cycle influence the material behavior of the uterus from nonpregnant patients significantly [22]. Pearsall and Roberts [23] investigated the mechanical behavior of the human myometrium by performing tension and compression tests. Through the use of both test methods, they discovered that the stress increased exponentially with strain and that the stiffness of the myometrium was considerably lower in compression than in tension [23]. More specifically, the outer myometrium was more compliant than the inner myometrium when tested in compression but it was stiffer than the inner myometrium when tested in tension. These studies revealed the anisotropy of the tissue determined by collagen fiber orientation and muscle fiber orientation [23].

In a study by Kauer et al. [26], aspiration tests were performed on excised human uteri, and an inverse finite element method was implemented to characterize the tissue material response. Tests were performed at three physiological locations (the ventral, dorsal, and fundus portions), and the uteri were found to be highly viscoelastic with a mechanical response dependent upon the location of the aspiration. However, Kauer et al. noted that not only the location but also other experimental factors may have influenced the tissue material response.

More recent experimental studies conducted by Manoogian et al. [24,27] investigated the strain rate-dependent mechanical behavior of uterine tissue using uniaxial and biaxial testing methods. Using biaxial testing methods on pregnant porcine tissue, Manoogian et al. [27] found that the uterine tissue exhibited a peak true stress of 500 ± 219 kPa with a corresponding peak true strain of 0.30 ± 0.09 in the circumferential direction and a peak true stress of 320 ± 176 kPa with a corresponding peak true strain

of 0.30 ± 0.09 in the longitudinal direction. By performing uniaxial tension tests on pregnant human uterine tissue, Manoogian et al. determined that the stress–strain behavior of the tissue was nonlinear, with an overall average peak true stress at failure of 656.3 ± 483.9 kPa and a corresponding peak true strain at failure of 0.32 ± 0.112 [24].

Because uterine growth is characterized by increased collagen content, one study analyzed the mechanical behavior of nonpregnant uterine tissue from mice with an induced increase in collagen content [25]. Mondragon et al. found that uterine specimens from mice with increased collagen content had a higher average peak stress (0.078 ± 0.008 MPa) than those from mice with a normal amount of collagen content (0.04 ± 0.01 MPa).

2.2.2 In Vivo Studies. A study conducted by Mizrahi et al. [28] analyzed uterine contractions of pregnant human patients to determine the isotropy and anisotropy of the uterus during labor contractions. By performing a strain rosette analysis, Mizrahi et al. experimentally collected and computationally calculated strains during various stages of labor. Mizrahi et al. found that during the early stages of labor, the uterine muscle was isotropic [28]. As labor progressed, the uterine tissue exhibited anisotropic behavior. Kauer et al. [26] also performed aspiration tests on uteri in vivo. All tissues were tested in the same physiological locations as previously mentioned (the ventral, dorsal, and fundus portions), and in vivo data were compared to ex vivo data. There was a pronounced increase in stiffness for the uterus that was tested in vivo. The largest stretch ratios from in vivo measurements ranged from 1.1 to 1.3 while the largest stretch ratios from ex vivo measurements ranged from 1.2 to 1.45. However, many experimental factors were deemed responsible for the difference between the ex vivo and in vivo material response.

3 Cervix

3.1 Structure and Function. The cervix is a cylindrical and fibrous connective tissue, roughly 3 cm long and 2.5 cm in diameter, that connects the vagina to the uterus [29] (Fig. 1). It is held in place by the cardinal and uterosacral ligaments and is attached to the fetal membranes within the uterus during pregnancy [30]. The cervix serves two primary mechanical functions: first, it must be firm in order to act as a mechanical barrier to the fetus within the uterus during gestation, and second, it must soften and shorten by term pregnancy to allow passage of the fetus [31,32]. In order to accomplish both of these functions, the cervix must undergo a drastic remodeling process over the course of pregnancy [32,33].

The cervix is known to consist of three layers: a thin inner mucosa layer, a thick middle stroma layer, and a thin outer fascia layer [34]. Focus of microstructural and mechanical analysis of the cervix is placed on the stroma layer as it is the load-bearing region of the cervix [35,36]. The tissue of the stroma is made up of a continuous, direction-dependent collagen fiber matrix embedded in a viscous ground substance of negatively charged glycosaminoglycans [37]. This collagen fiber matrix is composed of three layers of type I and type III collagen [31]. The inner and outer layers of the matrix have fibers aligned longitudinally along the axis of the cervical canal, and the middle layer of the matrix has fibers aligned in a circumferential direction around the cervical canal [34].

During pregnancy, the collagen structure within the cervix changes, and this remodeling process occurs in four phases: softening, ripening, dilation, and postpartum repair [38]. Studies have shown that the cervix begins to soften relatively soon after conception by a loss of mature collagen crosslinking while maintaining collagen alignment [32,33,39]. Biochemical components of the cervix are thought to be linked to this softening of the cervix, specifically through the disruption of the collagen fiber network [40–42]. Studies on rats analyzing biochemical influence on mechanical properties have shown an increase in hyaluronan as term pregnancy nears, resulting in increased hydration and

collagen disorganization [38,41]. In a direct comparison of pregnant tissue to nonpregnant tissue from women, hydration levels, collagen extractability, and sulfated glycosaminoglycans were found to increase significantly for pregnant tissue, but the collagen content (percent per dry weight) had no significant change [40]. While these studies provided insight into some of the microstructural changes that occur during pregnancy, the extent to which these factors play a role on preterm birth and other complications is still unknown [43].

3.2 Testing Methods and Material Properties. The cervix has been tested ex vivo via uniaxial, aspiration, and indentation methods and in vivo via aspiration, inflation, and ultrasound methods. A comprehensive review paper written in 2015 by Myers et al. [43] discussed the current state of knowledge of cervical testing and material properties used to distinguish normal and abnormal functions of the cervix. This review indicated that, while there has been a multitude of testing performed both ex vivo and in vivo, each method had its limitations and the understanding of cervical mechanical properties during the remodeling process would benefit from a synthesis of the testing modalities. Hereafter, the main studies that characterized the mechanical behavior of the cervix will be highlighted.

3.2.1 Ex Vivo Studies. Uniaxial testing of the cervix included both uniaxial tension (ring test [40,44,45] and traditional test [36]) and compression (confined [40,46] and unconfined [36,40]). These tests were performed on rat tissue [44–46] as well as on human tissue [36,40] using load-relaxation protocols. The tissues in both tension and compression tests for all the obstetric conditions that were considered displayed a nonlinear stress–strain response. Marked hysteresis and softening due to conditioning were reported, and the response of the tissue was noticeably stiffer in tension than in compression. It was observed that tissue from nonpregnant patients with previous deliveries was more compliant than tissue from those without previous deliveries, but tissue from pregnant patients was one to two orders of magnitude more compliant than tissue from nonpregnant patients with and without previous deliveries [36,40]. In addition, nonpregnant tissue exhibited large stress relaxation in both confined and unconfined compression, and the peak and equilibrium stresses changed by as much as one order of magnitude over time. However, pregnant tissue experienced little relaxation with small changes in peak and equilibrium stresses [40]. In a recent study by Yoshida et al. [45], load-relaxation ring tests were performed on pregnant and nonpregnant rat cervixes. The pregnant tissue was characterized by a very large stress-relaxation compared to the nonpregnant tissue. The cervix stiffness was also observed to vary along its length by Myers et al. where the external os had a stiffer response than the internal os for specimens collected from pregnant patients [40]. Furthermore, the cervix proved to be anisotropic with the loading direction having a large impact on the level of stress experienced by the tissue [36]. When studying mid to term pregnancy in rats, Poellmann et al. found that stiffness decreased as gestational age increased [44]. They also showed that mass and initial circumference of cervix specimens increased with gestation age, and that there was an abrupt increase in extensibility after about 15 days of gestation (midgestation) [44].

Aspiration tests have typically been performed on the cervix in vivo. However, a study by Mazza et al. [47] used aspiration to apply a cyclic suction pressure to cervical tissue of nonpregnant women in vivo as describe below, and then ex vivo one and a half hour post hysterectomy. The aspiration used a pressure-controlled protocol that included two single load–unload tests and two cyclic loading tests with different time intervals between cycles. Comparing the results, no significant difference was found to exist between the stiffness, creep, or rise time of in vivo and ex vivo tissues. However, there was a noticeable difference in the softening of ex vivo tissue imposed by repeated cycles as compared to

in vivo tissue, indicating that ex vivo tissue had a stronger history dependence.

Yao et al. [48] performed displacement-controlled spherical indentation tests on cervixes from pregnant and nonpregnant hysterectomy patients at the midstromal region. The results from the tests indicated that the tissue had a time-dependent response: tissue from nonpregnant patients exhibited much greater relaxation than tissue from pregnant patients. These results were in agreement with the uniaxial test results presented by Myers et al. [40]. Overall, Yao et al. observed that pregnancy, previous vaginal deliveries, age, and specimen location along the axis of the cervix were parameters that would impact the response of cervical tissue. Specifically, nonpregnant tissue was observed to have significantly larger instantaneous and equilibrium shear moduli than pregnant tissue (5.1 ± 3.3 kPa versus 1.0 ± 0.6 kPa and 1.9 ± 0.8 kPa versus 0.47 ± 0.24 kPa, respectively). Furthermore, an increase in the number of previous vaginal deliveries correlated with a stiffer cervix, tissue closer to the internal os was found to be stiffer than tissue closer to the external os, and younger patients were found to have stiffer cervixes than older patients.

3.2.2 In Vivo Studies. Aspiration studies have been performed on nonpregnant tissue [47], pregnant tissue [49], and most recently on both nonpregnant and pregnant tissue [50]. Mazza et al. [47] and Bauer et al. [49] calculated a stiffness parameter that ranged from 0.013 bar/mm to 0.068 bar/mm for cervix tissue from pregnant patients and 0.095 bar/mm to 0.24 bar/mm for cervix tissue from nonpregnant patients. The stiffness of the cervix was shown to not only decrease from nonpregnant to pregnant patients but also with gestation age [49,50]. Badir et al. [50] observed an abrupt decrease in the stiffness between the first and second trimesters of pregnancy. They also found that postpartum patients recovered stiffness to a level comparable to that of early pregnancy after a few weeks. Finally, they noted a history dependence of tissue in nonpregnant patients through a decrease in stiffness between a first and a repeated, second measurement. Because of this cervical history dependence, the study only analyzed the data from the first measurement on tissue from pregnant patients.

Inflation tests were performed on pregnant patients to examine the cervical response of early and term pregnancies [51,52]. An inflatable urethane bag was inserted into the cervical canal and inflated using a pressure control protocol. The results of the testing showed that the average radial stresses ranged from -18.5 to 0 kPa for early pregnant patients and from -4.4 to 0 kPa for term pregnant patients. Furthermore, the circumferential stresses ranged from 0 to 186.6 kPa for early pregnant women and 0 to 390.0 kPa for term pregnant women [52]. A pressure-strain elastic modulus of the cervical tissue from early pregnant women was compared to that of cervical tissue from term pregnant women at the internal os region (41.3 kPa versus 4.23 kPa), middle region (243 kPa versus 5.02 kPa), and external os region (43.7 kPa versus 2.17 kPa) of the cervix [51]. Overall, these studies showed that the stiffness of the cervix decreased by as much as 25 times from early to term pregnancy [51,52]. In addition, the displacements along the cervical canal at term pregnancy were similar, suggesting that the external os and internal os remodel to the same extent [51]. However, one should be careful when interpreting these results. Assumptions made when calculating the mechanical parameters may not have sufficiently accounted for the boundary effects of the tissues connected to the cervix. Furthermore, some patients received various drugs prior to testing that may have caused smooth muscle contractions, and therefore, the study may have measured the active rather than passive properties of the tissue [51].

Finally, some ultrasound techniques have been used recently to investigate the properties of the cervix [43,53–57]. Shear wave elastography has been used to examine the shear modulus of tissue based on the speed of acoustic waves that propagate through the tissue. Carlson et al. [54] analyzed the feasibility of this technique with cervical tissue and found that stiffness decreased with

cervical ripening. Shear wave elastography has also been used to examine the viscoelastic nature of cervical tissue. Peralta et al. [57] applied ultrasound waves over a wide frequency range and found that the Maxwell model may be the best model to use in preliminary assessments of cervical viscoelastic properties [57]. Finally, palpation-type elastography techniques have been used to examine the properties of cervical tissue through manual application of loads. Hernandez-Andrade et al. [55] showed that women with small strain values at the internal os during pregnancy were significantly less likely to experience spontaneous preterm birth, and Molina et al. [53] showed that the internal os and inferior portions of the cervix were stiffer than the external os and superior portions [53,55]. While studies using the palpation technique related measured values of mechanical strain to tissue properties, this measurement technique does not easily provide meaningful data due to the challenge of standardizing the transducer force [43,53,56]. This technique was originally intended for the measurement of relative changes in tissue stiffness to locate malignant tumors, rather than providing absolute quantitative stiffness data [56]. Overall, the ultrasound techniques provide for a noninvasive method of measuring changing cervical biomechanical properties, but further work is needed to properly interpret the results.

4 Vagina

4.1 Structure and Function. The vagina is a soft, elastic, muscular canal that serves as an entryway to the female reproductive organs. The cervix, which protrudes into the vagina, connects the uterus to the vagina and acts as a barrier between the vagina and uterus (Fig. 1). The vagina has several functions including providing lubrication and sensation for sexual activity, pathway for menstrual blood and tissue, and a delivery channel for childbirth. Although there are variations in size, the organ is approximately 7.5 cm along the anterior wall and 9 cm along the posterior wall [1,58].

The vagina is described to have four layers: the epithelium, the subepithelium, the muscularis, and the adventitia [59,60]. These layers consist primarily of smooth muscle, collagen, and elastin. The epithelial layer, which is responsible for protection against infection, and the subepithelium, which is responsible for passive mechanical support of the vagina, are primarily comprised of dense connective tissue of elastin and collagen fibrils with random alignment [59,60]. The muscularis is responsible for the active mechanical response of the vaginal tissue and is comprised mainly of smooth muscle cells that are oriented in the longitudinal loading directions [59,61]. The adventitia, which is connected to the rectum and the bladder to provide additional support, is composed of loose connective tissue containing circular bundles of elastin fibers, nerves, and venous capillaries with random orientation [60,61].

Several studies have shown that the onset of pregnancy, prolapse, and menopause can alter the structure of vaginal tissue [59,61–64]. A study conducted by Downing et al. [64] on the influence of pregnancy and mode of delivery on the elastic fiber architecture and vaginal vault elasticity in rats showed that the tortuosity of elastin fibers decreased when measured at 2 days postpartum compared to virgin vaginal tissue. After 2 weeks postpartum, the measured tortuosity was similar to virgin vaginal tissue, suggesting that the vaginal tissue undergoes structural remodeling to allow for events such as pregnancy and then returns to its prior state [64]. While studying the effect that parity has on the collagen structure in vaginal tissues of *rhesus macaque* monkeys, Feola et al. [63] found a decrease in collagen alignment with parity. It was also discovered that, as pelvic organ support weakened, collagen alignment within the tissue decreased [63]. Comparing vaginal tissue in women with pelvic organ prolapse and women without pelvic organ prolapse, one study found that the smooth muscle content significantly decreased in the vaginal tissue of women with pelvic organ prolapse [62]. The researchers

determined that 61.9% of the surveyed fractional area of nonprolapsed tissue consisted of smooth muscle compared to 41.9% of the surveyed fractional area of prolapsed tissue [62]. Researchers also detected an increase in connective tissue and blood vessels in vaginal tissue of women with pelvic organ prolapse. More specifically, for the surveyed fractional area, the connective tissue occupied 56.8% in prolapsed tissue and 35% in nonprolapsed tissue and the blood vessels constituted 3.4% of prolapsed tissue and 2.2% for nonprolapsed tissue [62]. These results suggested that structural remodeling occurred in the vaginal tissue after the onset of pelvic organ prolapse.

4.2 Testing Methods and Material Properties

4.2.1 Ex Vivo Studies. The most prevalent testing method for vaginal tissue is uniaxial tension [65,66]. Rubod et al. [65] established a new experimental protocol for testing the mechanical properties of the vaginal tissue uniaxially using ewes as animal models. Several experimental conditions including sampling, freezing, preservation conditions, hygrometry, and temperature were studied to determine their influence on the mechanics of vaginal tissue. Rubod et al. found that freezing had no significant impact on the mechanical behavior of the vaginal tissue, and they defined conditions with regard to temperature, hygrometry, and rate of deformation that provided reproducible results. The following year, Rubod et al. [66] conducted preliminary tests using their newly established protocol on human vaginal tissue. Vaginal tissue was excised from women with prolapse during prolapse repair surgery, and from cadavers that had no noticeable form of pelvic floor dysfunction. Percent strain at failure ranged from 19% to 41% for prolapsed tissue and from 20% to 46% for nonprolapsed tissue. Stress at rupture ranged from 2.12 to 6.06 MPa for prolapsed tissue and from 0.82 to 2.62 MPa for nonprolapsed tissue. In this study, Rubod et al. were the first investigators to demonstrate that the vaginal tissue was nonlinear elastic and undergoes large deformations.

Because of the prevalence of pelvic organ prolapse, several studies have been conducted to determine how pelvic organ prolapse affects the mechanical behavior of vaginal tissue [61,66–71]. Ettema et al. [67] conducted slow-rate tension tests with superimposed small amplitude sinusoidal vibrations on prolapsed human vaginal tissue and reported that the elastic modulus was in the range of 7–15 MPa. Rahn et al. [68] conducted a study to determine the influence of pelvic organ prolapse and pregnancy on the stiffness, distensibility, and maximum stress of vaginal tissue from mice. By straining the vaginal tissue to failure, the investigators determined that both prolapse and pregnancy caused increased distensibility, decreased stiffness, and decreased maximal stress at failure [68]. Using Rivlin's model, a study conducted by Jean-Charles et al. [69] showed that vaginal tissue in women with prolapse was significantly stiffer than vaginal tissue in women without prolapse for both the anterior and posterior vaginal walls under small and large deformations. The results from this study suggested that, when pelvic organ prolapse is repaired with autologous vaginal tissue, there may be a higher incidence of recurrence since the vaginal tissue is damaged and more rigid [69]. The authors indicated that the changes in the vaginal tissue due to pregnancy and prolapse contribute to the poor durability of many restorative surgical procedures for prolapse [68]. Considering this, Gilchrist et al. [70] conducted a study to determine a possible correlation between the uniaxial mechanical properties of the vaginal wall and the outcome of anterior vaginal wall suspension with cystocele surgical repair in human patients. Out of 32 patients, 7 experienced failure of the repair upon a 2 to 3-yr follow-up period. However, there was no association between the elastic modulus, which was found to be in the range of 2.5–9.5 MPa, and the clinical outcomes of the repair. These results led the authors to conclude that retropubic scarring and the

properties of the pelvic floor muscles have a higher influence on the success of the repair [70].

Several studies have been conducted to help decipher the influence that menopause has on the mechanical behavior of vaginal tissue [61,70–75]. In 2002, a study conducted by Goh [72] assessed the mechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women using uniaxial tests. This study focused on the elastic properties of the vaginal tissue, noting that vaginal tissue from postmenopausal women had a significantly higher elastic modulus than vaginal tissue from premenopausal women. Specifically, the mean value for the elastic modulus was found to be 11.5 MPa for premenopausal women and 14.35 MPa for postmenopausal women [72]. The authors suggested that the higher elastic modulus seen in postmenopausal women could be due to age since the median age of women in the postmenopausal group was significantly higher than the median age of women in the premenopausal group. However, this study also found that there was very little to no difference in tissue deformation between pre- and postmenopausal women, with tissue from premenopausal women experiencing approximately 30% deformation and from postmenopausal women experiencing approximately 26% deformation [72]. In 2007, a study conducted by Lei et al. [73] analyzed the relationship between menopause and pelvic organ prolapse and their combined effect on the mechanical behavior of vaginal tissue [73]. Lei et al. found that there was a significant difference in the measured mechanical properties, specifically in the Young's modulus, Poisson's ratio, maximum elongation, and maximum stress at failure of the vaginal tissue between premenopausal women with prolapse and without prolapse. For premenopausal women with prolapse, the Young's modulus, Poisson's ratio, maximum elongation, and maximum stress at failure of the vaginal tissue were reported to be 9.45 ± 0.70 MPa, 0.43 ± 0.01 , $1.50 \pm 0.02\%$, and 0.60 ± 0.02 MPa, respectively, and for premenopausal women without prolapse, they were 6.65 ± 1.48 MPa, 0.46 ± 0.01 , $1.68 \pm 0.05\%$, and 0.79 ± 0.05 MPa, respectively [73]. The same significant differences in mechanical properties were reported between postmenopausal women with prolapse and without prolapse [73]. For postmenopausal women with prolapse, the Young's modulus, Poisson's ratio, maximum elongation, and maximum stress at failure of the vaginal tissue were reported to be 12.10 ± 1.10 MPa, 0.39 ± 0.01 , $1.14 \pm 0.05\%$, and 0.27 ± 0.03 MPa, respectively, and for postmenopausal women without prolapse, they were reported to be 10.26 ± 1.10 MPa, 0.42 ± 0.01 , $1.37 \pm 0.04\%$, and 0.42 ± 0.03 MPa, respectively [73]. These changes in mechanical properties for patients with prolapse may suggest that the deterioration of vaginal tissue may lead to the occurrence of prolapse [59].

Age also influences the mechanical behavior of vaginal tissue and is a contributing factor to pelvic organ prolapse. Chantereau et al. [76] conducted a study on the mechanical properties of the pelvic floor tissues from young cadavers and compared the collected data to previous studies [77,78] in order to determine the effect of aging. Using the Rivlin–Mooney model, Chantereau et al. studied the mechanical behavior of the vaginal tissue at small and large deformations. It was concluded that the mechanical behavior of the vagina was significantly different between young and old vaginal tissue, with old vaginal tissue appearing to be stiffer. While analyzing cyclic data of the young tissue, a nonlinear relationship between stress and strain was established and Mullin's effects were observed [76]. Both old and young vaginal tissues exhibited nonlinear stress–strain relations and Mullin's effects [76,77]. Typically, with age comes weight gain, and a recent study conducted by Lopez et al. [79] showed that the stiffness of prolapsed vaginal tissue increased with increasing body mass index. Given the strong correlation between parity and pelvic organ prolapse development, the impact of parity on the mechanical properties of the vagina was recently investigated by Knight et al. [80] in the ewes. By performing tensile tests, the investigators found that the tangent modulus and tensile strength decrease with parity.

While most experiments on the mechanical properties of vaginal tissue were conducted when the tissue was in its passive state, Feola et al. [59] conducted a study to observe the impact of pregnancy and vaginal delivery on the mechanical properties of the rat vagina in both the passive and active states. When testing passive tissues, Feola et al. found that the tangent modulus, which was reported as 25.0 ± 5.1 MPa for virgin rats, significantly decreased by midpregnancy (12.0 ± 7.7 MPa), late pregnancy (7.9 ± 4.0 MPa), and immediately postpartum (8.5 ± 4.7 MPa), but returned to the level of virgin rats by 4 weeks postpartum (30.0 ± 14.0 MPa). The tensile strength, which was reported as 2.1 ± 0.65 for virgin rats, was significantly lower for the late pregnant group (0.95 ± 0.51 MPa) but returned to virgin levels 4 weeks postpartum (3.1 ± 1.7 MPa). The ultimate strain significantly increased in the immediate postpartum group ($24.0 \pm 5.3\%$) compared to virgin animals ($14.0 \pm 4.1\%$) [59]. The maximum contractile force of the vaginal tissue was significantly lower in the immediate postpartum animals compared to virgin animals. In general, the vaginal tissue in pregnant and postpartum animals was more sensitive to the potassium used to activate the smooth muscle cells than the vaginal tissue in virgin animals [59].

Although uniaxial tensile tests are the most prevalent tests to characterize the vaginal tissue, other methods have been employed. One study investigated changes in the rheological behavior of the vagina in women with pelvic organ prolapse using a single-lap, sinusoidal, oscillatory shear testing method [75]. In this study, tissues were excised from premenopausal women without prolapse, premenopausal women with prolapse, postmenopausal women with prolapse, and postmenopausal women with prolapse on hormone treatment therapy. It was found that premenopausal prolapsed tissue had a higher complex modulus under shear deformation than nonprolapsed premenopausal tissue, which was also the least stiff tissue out of all the four participating groups [75]. The higher complex modulus was a result of increases in both the elastic and loss modulus contributions. Vaginal tissue from postmenopausal women with prolapse on hormone therapy exhibited the highest complex modulus. These results suggested that prolapsed tissue had an increased elastic contribution due to changes in its biochemical composition, and that hormones increased the viscous contribution of prolapsed tissue [75].

In addition to measuring the tortuosity of the elastin fibers in the vaginal wall, Downing et al. [64] used a pressure infusion system to measure the stiffness of the vaginal vault of female rats. The goal of the study was to determine the architectural changes in the vagina that lead to changes in the elasticity of the vaginal tissue. Primiparous rats had a higher measured stiffness of the vaginal vault at 2 weeks postpartum than at 2 days postpartum. The vaginal vault of virgin rats was found to be stiffer than that of multiparous rats. These results, in conjunction with the measured tortuosity results, confirmed that the elastin fibers in the vaginal vault may significantly remodel due to pregnancy and parturition, contributing to tissue elasticity [64].

4.2.2 In Vivo Studies. Using an in vivo suction technique, Epstein et al. [81,82] studied the correlation between vaginal stiffness and prolapse in women with and without prolapse [81]. The investigators found that the stiffness and extensibility of vaginal tissue decreased in women with prolapse. Moreover, they determined that the tissue stiffness is inversely related to the distress severity of prolapse [82]. Chuong et al. [83] adopted an in vivo measurement technique to study the viscoelastic properties of prolapsed anterior vaginal wall tissue. In their study, stress-relaxation tests were performed by applying a suction pressure to the anterior vaginal wall tissue, followed by an immediate release. As a control, the same procedure was applied to the suprapubic region, the region above the pelvic bone and below the stomach. Using the Voigt model, the investigators determined the rate of tissue recovery and measured strain energy stored once the tissue reached maximum uplift, the strain energy recovered after vacuum release,

and the strain energy lost over the total loading and unloading cycle. The authors concluded that the anterior vaginal wall tissue was more compliant with higher viscous damping than the suprapubic region, and that the anterior vaginal wall tissue did not store as much recoverable energy upon distention compared to the suprapubic region [83].

5 Pelvic Ligaments

5.1 Structure and Function. Pelvic ligaments such as the uterosacral, cardinal, broad, and round ligaments are crucial to support the pelvic organs such as the uterus, cervix, and vagina in their anatomical positions (Fig. 1). These ligaments suspend the pelvic organs to the pelvic sidewalls, over the levator plate, while allowing them to perform their functions. They are composed of collagen fibers, elastin, smooth muscle cells, adipose cells, nerve fibers, blood vessels, and lymphatics [84–87]. The uterosacral ligament and the cardinal ligament are apical supportive structures of the cervix and upper vagina. The uterosacral ligament, about 12–14 cm long, is a bandlike structure connected distally to the cardinal ligament at the cervix and/or upper part of the vagina and proximally to the vertebral region, between the S2 and the S4 vertebrae, without direct attachment to the sacrum [86]. The cardinal ligament is about 10 cm long. It forms a perivascular sheath at the cervix and attaches the organs laterally to the pelvic sidewalls, with its apex at the first branching of the internal iliac artery [88].

Connected to the cardinal ligament, the broad ligament is a sheetlike structure linking the sides of the uterus laterally to the pelvic sidewalls [89]. It is composed of two layers of peritoneum and serves as a mesentery for the uterus, ovaries, and the uterine tubes. Within the broad ligament, a fibromuscular band, the so-called round ligament, maintains the anteflexion of the uterus. It starts from its relatively broad base at the uterus laterally to the internal inguinal ring and ends in the labia majora [89]. The round ligament is between 10 and 12 cm long.

Pelvic floor disorders, particularly pelvic organ prolapse, are associated with changes of the ligament microstructure. For example, loosely arranged thicker collagen fibers, less dense extracellular matrix [90], and impaired smooth muscle cells [91] are observed in the uterosacral and cardinal ligaments of patients with pelvic organ prolapse. The smooth muscle fraction of the round ligament in women with uterine prolapse significantly decreases compared to that of women without prolapse [84].

5.2 Testing Methods and Material Properties

5.2.1 Ex Vivo Studies. The ex vivo nonlinear mechanical properties of the pelvic ligaments have been investigated via uniaxial tests [76,78,87,92–95] and planar biaxial tests [96,97]. Moalli et al. [93] investigated rat as an animal model for the structural and mechanical properties of the vagina and its supportive tissues. By pulling the rat vagina, the in situ force–displacement curve of the vagina–supportive tissue complex was obtained. The supportive tissue failed at a lower elongation than the vaginal wall tissue. The mean stiffness and energy absorbed at failure of the complex were reported to be 2.9 N/mm and 49.4 J, respectively. The tensile properties of the uterosacral ligament and round ligament collected from female cadavers were studied by Martins et al. [95]. The uterosacral ligament was found to have significantly higher stiffness (14.1 MPa versus 9.1 MPa) and strength (6.3 MPa versus 4.3 MPa) than the round ligament. The uterosacral ligament of nulliparous women was found to have significantly lower stiffness (10.0 MPa versus 15.5 MPa) and strength (4.2 versus 8.2 MPa) compared to the uterosacral ligament of parous women. These findings were attributed to the biomechanical alterations caused by vaginal delivery and adaptation to higher mechanical loads that followed from the increase in pelvic floor laxity and genital hiatus diameter.

A comparison study on the mechanical properties of the uterosacral ligament, round ligament, and broad ligament from female

cadavers was conducted by Rivaux et al. [78]. The mechanical responses of these ligaments were found to be nonlinear elastic. The mean tensile strengths of the uterosacral ligament, round ligament, and broad ligament were reported to be around 4 MPa, 4.1 MPa, and 1.5 MPa, respectively. Using the Mooney–Rivlin model, the uterosacral ligament was found to be the stiffest at both low and high strain levels, and the round ligament was stiffer than the broad ligament. This study confirmed that the uterosacral ligament played an important role in supporting the pelvic organs.

Due to similarities between the histological properties of the cardinal and uterosacral ligaments in swine and humans, and lower cost of swine over nonhuman primates, swine was used as animal model to study the structural and mechanical properties of these ligaments by Tan et al. [87]. The uterosacral ligament was found to be significantly stronger than the cardinal ligament with higher ultimate tensile stress and tangent modulus of the linear region of the stress–strain curve. The mean tangent moduli of the toe region of the stress–strain curve for the left and right cardinal ligaments and uterosacral ligament were 0.503 MPa, 1.154 MPa, and 1.617 MPa, respectively. The mean tangent moduli of the linear region of the stress–strain curve for the left and right cardinal ligaments and uterosacral ligaments were 3.449 MPa, 5.385 MPa, and 29.819 MPa, respectively. The ultimate tensile stresses for the left and right cardinal ligaments and uterosacral ligaments were 0.854 MPa, 1.278 MPa, and 2.767 MPa, respectively. These mechanical properties were highly dependent on the location of the specimens within the uterosacral/cardinal ligament complex relative to the uterus, cervix, vagina, and rectum. The ultimate stress and elastic modulus of the swine ligaments were of the same order of magnitude as those reported in monkeys [94] and cadavers [95]. Some of the observed differences were attributed to the different experimental methods. For example, the Lagrangian strain in the study by Tan et al. [87] was computed by video-tracking the motion of markers attached to the uterosacral and cardinal ligament specimens. This strain measurement method is more accurate than the previously used strain measurement methods [94,95]. Moreover, due to the large size of the swine ligaments, the specimens in the study by Tan et al. [87] had a much larger aspect ratio for uniaxial testing than those used in the aforementioned studies [94,95].

Vardy et al. [94] used the monkey as animal model to study the effect of hormone replacement on the mechanical properties of the uterosacral and round ligaments. Incremental stress–relaxation tests were performed at strains that ranged from 5% to 30%. A tensile test to failure was conducted after conducting stress–relaxation tests on each specimen. For ovariectomy monkeys without treatment, a mean failure stress of 0.6 MPa and a mean tensile modulus of 0.75 MPa at 30% strain were reported for the uterosacral ligament, while a mean failure stress of 2.1 MPa and a mean tensile modulus of 14 MPa at 30% strain were reported for the round ligament. Hormone replacement (using conjugated equine estrogens plus medroxyprogesterone acetate or ethinyl estradiol plus norethindrone acetate) was found to increase the stiffness of the uterosacral ligament but decreased the stiffness of the round ligament. Because the uterosacral ligament is the primary suspensory ligament connecting the cervix and upper vagina to the sacrum, this increase in stiffness may be seen as a necessity for bearing the greater weight of a pregnant woman's uterus, fetus, amniotic fluid, and placenta. The length of the round ligament increased typically during pregnancy and tripled by full term. The decrease in stiffness likely facilitated the increase in length. This study supported the hypothesis that the hormonal status plays an important role in pelvic support, and thus, menopausal status is a risk factor for prolapse. The authors speculated that the hormone replacement therapy could affect the ligament support function.

The aging effects on the tensile properties of the uterosacral, round, and broad ligaments from female cadavers were studied by Chantreau et al. [76]. Using the Mooney–Rivlin model and its c_0 and c_1 model parameters, the uterosacral ligaments and round ligaments from young cadavers were found to be significantly less

stiff than those from old cadavers at both small and large deformations. No significant differences were detected between the model parameters for the young and old broad ligaments. Chantreau et al. concluded that the pelvic floor tissues might naturally become stiffer during aging due to tissue remodeling and stiffer ligaments might no longer be able to stabilize the pelvic floor.

Reay Jones et al. [92] studied the effects of prolapse, vaginal delivery, menopause, and aging on the resilience (the area under the force–displacement curve up to the plastic limit) of the uterosacral ligament using female hysterectomy specimens. Significant decrease in the mean resilience was associated with symptomatic uterovaginal prolapse, vaginal delivery, menopause, and older age. No significant reduction in resilience was detected as the number of deliveries increased. It was speculated that the decrease in the resilience of the pelvic ligaments could lead to the development of symptomatic pelvic organ prolapse.

The biaxial nonlinear elastic and viscoelastic mechanical properties of the uterosacral and cardinal ligament complex were studied in the swine by Becker and De Vita [96] and Tan et al. [97]. The swine ligaments were observed to undergo large biaxial deformations and to be orthotropic. The ligaments were found to be stiffer along their main physiological loading direction. During equibiaxial stress–relaxation tests, the stress in the ligaments was found to decrease by about 70% over 2000 s. This decrease was equal along the main physiological loading direction and the direction perpendicular to it. Higher relaxation was reported at lower equibiaxial displacements [96]. The results of equibiaxial creep tests showed that, over a 120-min period, the mean strain increased by approximately 20–40% in both the main physiological loading direction and the direction perpendicular to it [97]. Moreover, lower creep was produced by the application of higher equibiaxial loads.

5.2.2 In Vivo Studies. The in vivo mechanical properties of the pelvic supportive ligaments have been investigated by tension tests [98,99]. Smith et al. [98] developed a computer-controlled system to measure the in vivo tensile response of the cervix and its supportive ligaments in women with varying uterine support from normal to prolapse. The force was applied in a caudal direction through a tenaculum placed on the cervix. The mean stiffness of the cervix and supportive ligaments was reported to be 0.49 N/mm. Using a similar testing technique, Luo et al. [99] studied the in vivo viscoelastic properties of the uterine suspensory tissue including the uterosacral and cardinal ligaments. Tensile and multiple stress–relaxation tests were performed on patients with prolapse (without prior surgeries). The uterine suspensory tissue was found to be viscoelastic. The mean stiffness, mean energy absorbed during the ramp phase of the test, and mean normalized force (after 60 s) of the uterine suspensory tissue in the first relaxation test were 0.49 N/mm, 0.27 J, and 0.56, respectively. Using the force and displacement of the uterine suspensory tissue from the first relaxation test in a four-cable model, the stiffness of the cardinal ligament and the stiffness of the uterosacral ligament were computed to be 0.2 N/mm and 0.12 N/mm, respectively. Compared with the first relaxation test, the stiffness and normalized force significantly increased while the energy absorbed during the ramp portion of the test significantly decreased in the second and third relaxation tests. It was concluded that the rest time of 60 s between the relaxation tests might not be enough to achieve fully tissue recovery.

6 Constitutive Models

There have been a few attempts to develop constitutive models for the reproductive organs and supportive connective tissues. Human cervical tissue was modeled via linear elastic orthotropic models [100] and isotropic models [48,50]. The assumptions on linear elasticity and isotropy made in these models limited their applicability. Recently, more physiologically relevant models have been proposed to account for the nonlinear elasticity and

anisotropy of cervical tissue [36,52,101]. Myers et al. [101] developed a constitutive model in which the cervix was assumed to be composed of collagen fibers and an isotropic, compressible, neo-Hookean ground substance. The collagen fibers were assumed to be continuously distributed throughout the matrix, and their orientation was defined by an ellipsoidal density function. Liao et al. [52] used a similar approach to model the cervical wall as an isotropic matrix with three families of collagen fibers. In both these models, the viscoelastic contributions of the cervix material response were neglected. Finally, Myers et al. [36] provided a one-dimensional nonlinear rheological model that was formulated by considering the microstructural constituents of the cervix, and the collagen fibers were assumed to be aligned in one single direction.

There are a few published studies on modeling the nonlinear elastic and viscoelastic behavior of vaginal tissue by Jean-Charles et al. [69] and Peña and coauthors [61,71,74]. Jean-Charles et al. [69] selected the Rivlin model due to the low number of parameters that needed to be computed from fitting the nonlinear stress and elongation data [69]. Peña et al. developed constitutive models that captured both the anisotropic elastic and viscoelastic behavior of vaginal tissue. They also modeled the softening behavior of vaginal tissue using the pseudoelasticity theory [74].

The constitutive behavior of the pelvic uterosacral and cardinal ligament complex has been modeled by Becker and De Vita [96]. A three-dimensional constitutive model based on the Pipkin-Rogers integral series was developed to capture the elastic anisotropy, finite strain, and stretch-dependent stress-relaxation behaviors of the ligamentous complex. In the model, the ligaments were assumed to be incompressible and composed of two families of fibers embedded in an isotropic matrix. The model was validated using elastic and viscoelastic biaxial experimental data.

7 Discussion

Testing methods for characterizing the mechanical properties of reproductive organs and supportive structures within the female pelvic floor have been reviewed. These included standard testing techniques such as uniaxial tests, compression tests, and biaxial tests, as well as aspiration tests and ultrasound tests. Figure 2 provides a schematic of the main testing modalities, and Fig. 3

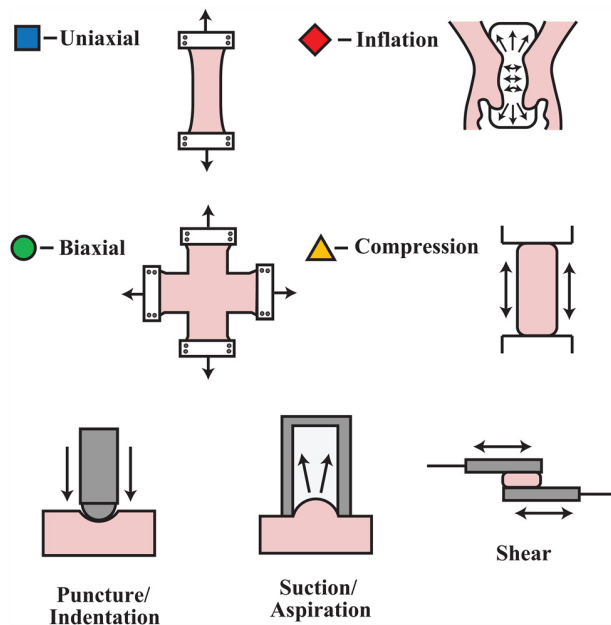


Fig. 2 Testing methods used to determine the mechanical properties of female reproductive organs and supportive connective tissues

presents a range of elastic moduli data organized by organ/tissue. The elastic moduli were obtained from the literature by considering different types of specimens. Because all conditions (i.e., pregnant and nonpregnant, prolapsed and nonprolapsed, human and animal, old and young, and in vivo and ex vivo) were considered, a large variation in the data was observed. The elastic modulus was selected for comparison since this was the most reported mechanical parameter across all test types, organs, and structures. Elastic moduli were reported to be in the range of 0.02–1.4 MPa for the uterus, 2.5–30 MPa for the vagina, 2.17–243 kPa for the cervix, 0.75–29.8 MPa for the uterosacral ligament, 0.5–5.4 MPa for the cardinal ligament, and 9.1–14.0 MPa for the round ligament.

The reproductive organs and supportive connective tissues were all found to be nonlinear elastic or viscoelastic as expected for soft tissues. In addition, each organ and supportive structure was found to have some degree of anisotropy resulting from the multiple layers and collagen fiber/smooth muscle cell alignment in each layer. Due to this inherent material symmetry of the tissues, the use of appropriate protocols and testing methods is important when studying their mechanical response. In many of the studies cited in this review, uniaxial tensile tests were used to measure the mechanical properties. These tests are valuable for obtaining preliminary mechanical data, but they will not exploit the anisotropy of these tissues. Moreover, the uniaxial tests cannot emulate the multi-axial loadings expected to occur in physiological conditions. Therefore, one needs to ensure that at least biaxial tests, whether planar biaxial or inflation-extension tests, are employed when studying the mechanical behavior of these soft tissues. While some work has been done in this regard for the cervix [47–52,102], little to no biaxial testing has been performed on the uterus [27], the vagina [103], and the supportive structures [96,97].

Comparing the results of the different studies was difficult even when the same type of tissue and testing methods were used. Unfortunately, there are no clear guidelines or established protocols for how the mechanical properties of tissues in the female pelvic floor should be tested for any particular testing technique. The variability in testing procedures included sample size, orientation, preload values, preconditioning, load control, displacement control, loading and displacement rates, and more. The mechanical data were undoubtedly affected by the choices made by the investigators due to the nonlinear and viscoelastic nature of the

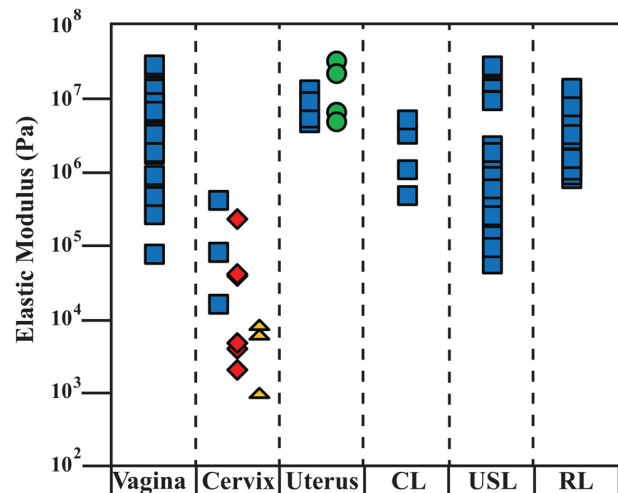


Fig. 3 Mean values of the elastic moduli for the vagina [59,67,68,70–73,79,80], cervix [36,51], uterus [22,23,27], uterosacral ligament (USL) [87,94,95], cardinal ligament (CL) [87], and round ligament (RL) [94,95] determined using testing methods in Fig. 2 as indicated by the symbols. No elastic moduli are reported for the broad ligaments in the literature.

tissues being studied. This makes comparison across studies even more difficult. It introduces significant variability in the data that is added to the inherent biological sample-to-sample variability.

The anatomical location of the tested specimen within the reproductive organs and supportive tissues was not always controlled or reported. Several studies have shown that the mechanical response of the tissue specimens may vary significantly depending upon their anatomical location within the reproductive organs. For example, the stiffness of the cervix varied along its length [40,51,52]. Similarly, the tangent moduli, ultimate tensile strength, and strain at the ultimate tensile strength as well as viscoelastic properties were different for different regions of the swine uterosacral ligament (USL)/cardinal ligament (CL) complex [87,96]. However, not all studies testing the mechanical response of these tissues reported the anatomical region from which the specimens were collected. This information would allow for more accurate comparisons among the published studies and would provide better insight into potential region-to-region variability.

Mechanical tests on human pelvic tissues have shown that mechanical properties will vary based on the subject's age, gravidity, and parity. They were affected not only by whether or not the human subject was pregnant or nonpregnant, but also by the pregnant subject's gestational age. The mechanical properties also changed for patients going through menopause, aging, or suffering from a PFD (Fig. 4). For example, many of the cited studies examined the effect of pregnancy on the mechanical response of the cervix [36,40,44,48–52,102,104] or the effect of menopause on the mechanical response of the vagina [66,72,73,75], but the effects of all the aforementioned subject's conditions have not been investigated for each reproductive organ and connective tissue. In many cases, the anamnesis of human subjects was not reported, making the published results comparing mechanical parameters among many subjects and specimens questionable. Clearly, the availability of human tissue was limited and, in some cases, there could not be control groups for the mechanical tests. However, pertinent information about the subject's medical history should be included in every study since it may influence the interpretation of the reported data.

One of the major challenges for characterizing the mechanics of the female reproductive organs and supportive connective tissues is performing *in vivo* tests. These tests can provide the most physiologically relevant results, but they are often unfeasible and unethical since they can impact the patient's health. Quantifying and, at the very least, estimating *in vivo* loading and deformation is crucial to design meaningful *ex vivo* tests. Toward this end, new methods and devices should be developed to measure the

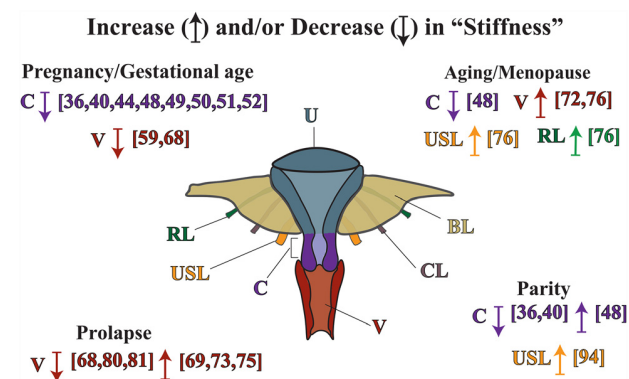


Fig. 4 Effect of pregnancy/gestational age, menopause/aging, parity, and prolapse on the “stiffness” of the reproductive organs and tissues. In the cited studies (numbers in square brackets), different mechanical quantities are used as stiffness measures. Note that U stands for uterus, C for cervix, V for vagina, USL for uterosacral ligament, CL for cardinal ligament, RL for round ligament, and BL for broad ligament.

in vivo mechanical stimuli experienced by the organs and tissues in the pelvis. Some *in vivo* work has been performed on the cervix and supportive ligaments [47,49,50,98,99], but little to no work has been performed on the vagina [83] and uterus [28]. Most mechanical tests are performed *ex vivo* on cadaveric tissue or on tissue collected from hysterectomy patients. However, these acquired tissues are typically afflicted with certain medical conditions, and therefore comparison to healthy tissues is difficult. In general, acquisition of human tissue from the female pelvic floor is troublesome and suitable animal models are needed.

In the recent past, several animal systems have been used to compute the mechanical properties of the female reproductive organs and supportive structures (Fig. 5). These included mice, rats, sheep, swine, and nonhuman primates, as noted in the aforementioned studies. The extent to which the data from the animal tissues can be used to infer the mechanical behavior of the equivalent human tissues remains to be established. However, despite the obvious anatomical differences between the pelvis of quadrupeds and bipeds, recent studies suggested that there were several histological and anatomical similarities between human and non-human pelvic tissues [87,105]. A thorough review by Couri et al. [106] examined the utility of a variety of animal models for investigating pelvic organ prolapse and treatment options. Recently, Knight et al. [80] examined parity and compared the vaginal mechanical properties of ewes to nonhuman primates and rodents, ultimately finding that the ewe was a good model for studying parity and prolapse development. The use of animal models is crucial to advance pelvic mechanics since it allows testing that is otherwise not feasible on human tissue due to ethical constraints. By using animal models, one can determine the mechanical properties of reproductive tissues and supportive connective tissues at varying stages of pregnancy, both *ex vivo* and *in vivo*. The effect of various conditions such as age, gravidity, and parity can be studied in a controlled fashion, circumventing confounding factors that are associated with the use of human tissues.

There are only a few published studies on the development of constitutive models for pelvic floor tissues, and for some organs such as the uterus, no constitutive model has been proposed. This is quite puzzling since, at the same time, there are several computational models of the pelvic floor, as discussed in a recent review by Chanda et al. [16]. Often, simplifying assumptions about the tissues, such as linear elasticity and isotropy, are made that may not be accurate. The relationship between the microstructural and mechanical data needs to be further investigated for many pelvic tissues. The structural constituents (collagen, elastin, smooth

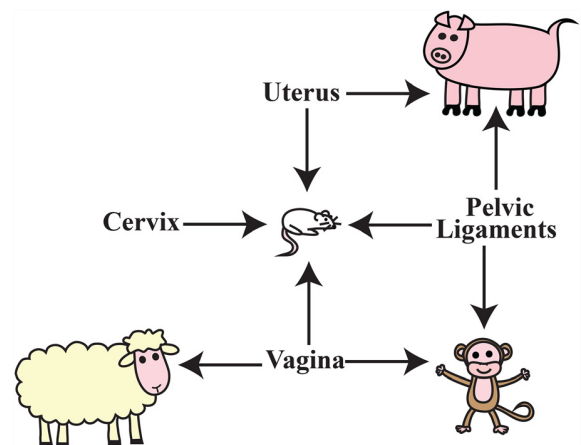


Fig. 5 Animal models used to collect mechanical data on pelvic tissues. Monkeys were used for the vagina and pelvic ligaments [94], swine for the uterus [27] and pelvic ligaments [87,96,97], ewes for the vagina [65,80], and rats/mice for the cervix [44–46], vagina [59,64], uterus [25], and pelvic ligaments [93].

muscle, etc.) of the reproductive organs and supporting tissues are similar among different human and animal subjects (e.g., pregnant versus nonpregnant) but their relative volume fraction, organization, and turnover are different. The difference in the microstructural constituents is likely responsible for the difference in the mechanisms of deformations and forces developed in these organs and tissues. Given the astonishing growth and remodeling of these tissues, constitutive models should be developed within growth and remodeling theoretical frameworks that consider the stress-free configurations of their constituents [107]. Furthermore, it must be noted that the stress-free configurations of tissues that are tested ex vivo are quite different from their in vivo stress-free configurations. This limits the applicability and usefulness of ex vivo experimental data (and constitutive models developed using these data) to describing physiologically relevant material properties of the tissues. A considerable amount of ex vivo and in vivo mechanical testing is still required to develop adequate constitutive laws of all the different organs and structures in the pelvic floor. These models are crucial to fully analyze and predict the mechanics of the pelvis via sophisticated computational models.

The role of smooth muscle cells on the mechanical behavior of the vagina, cervix, uterus, and supportive structures should be examined. Smooth muscle cells cause contraction of pelvic organs and tissues that may be pivotal to their proper physiological function. In a recent study by Vink et al. [108], the content and distribution of smooth muscle cells within the internal and external os were correlated with the cervix contraction, which can ultimately influence the cervix remodeling. Thus, the properties of the pelvic tissues need to be measured both in the active (contracted) and passive (relaxed) states. To date, there is one study that examines the impact of pregnancy on both the active and passive mechanics of the rat vagina [59], but not much else has been done.

Ultimately, a better understanding of the mechanical properties of the reproductive organs and supportive tissues will enable physicians and engineers to propose improved preventative measures, treatment options, and surgical protocols for many different complications and conditions that can arise from pregnancy or PFDs. For example, new exercises or physical therapy programs could be established to prevent PFDs and preterm birth. Better yet, a full understanding of the mechanics led to the development of mechanically based markers to identify risk factors for PFD development and preterm birth. At the least, insight into the mechanics should provide surgeons with some guidance to perform superior surgical procedures using improved mesh materials. The groundwork for women's reproductive and sexual health has been laid by the many studies cited in this review, but clearly, the pelvic floor mechanics field is still in its early stages.

8 Conclusions

Some broad recommendations are offered here based on the reported findings on the mechanics of the female reproductive organs and connective tissues. Progress in pelvic floor mechanics can be accelerated by integrating and comparing experimental protocols and results. The lack of control and consistency in the testing protocols across the cited studies makes the comparison of the results difficult. Researchers in this field should try to design, as much as possible, mechanical tests that use somewhat similar protocols, limiting possible confounding factors.

Due to the shortage of human pelvic tissues, serious efforts should be devoted on the selection and validation of animal models that can be used to test mechanical properties as well as treatment options and surgical protocols. The use of animal models will reduce the variability in mechanical data while providing better control groups, larger sample sizes, and quicker and cheaper data collection. By using animal models, the in vivo and ex vivo experimental data on the active and passive mechanical properties of the tissues should be collected.

Complete sets of active and passive mechanical data can then guide the development of accurate constitutive models that

account for the anisotropy, nonlinearity, and viscoelasticity that are typical of these tissues. Since the reproductive organs and tissues undergo continuous changes during pregnancy, menopause, and aging, the constitutive models should be formulated within growth and remodeling theories that are based on the concept of evolving natural (stress-free) configurations of the tissue constituents. Simplifying assumptions about the material characteristics of these tissues (e.g., isotropy and linear elasticity) can lead to models that curve-fit some data but do not truly describe the constitutive behavior of the tissues. As more mechanical data are collected and as experimental techniques are refined, accurate constitutive models for pelvic tissues should be developed and then implemented in powerful mechanical computation tools.

There is no doubt that the mechanics of female reproductive organs and tissues will have far-reaching implications in the prevention and treatment of PFDs and preterm birth, reducing their health-care costs and improving the quality of life of adult women and neonates. Hopefully, this review will help new investigators to establish a career in pelvic floor biomechanics, an area that has been historically under-researched in spite of the growing public health need.

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