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Impact of Pregnancy and Vaginal Delivery on the Passive and Active Mechanics of the Rat Vagina

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Abstract

Remodeling of vaginal extracellular matrix and smooth muscle likely plays a critical role in reducing the risk of maternal injury during vaginal delivery by altering the mechanical properties to increase distension and reduce stress. Long-Evans rats were divided into five groups to examine the passive mechanical and active contractile properties throughout pregnancy and postpartum: virgin ($n = 17$), mid-pregnant (Day 14–16, $n = 12$), late-pregnant (Day 20–22, $n = 14$), immediate postpartum (0–2 h after delivery, $n = 14$), and 4 week postpartum ($n = 15$). Longitudinal sections of vaginal tissue were loaded to failure uniaxially for passive mechanical or active contractile properties were examined. For passive mechanics, the tangent modulus decreased 45% by mid-pregnancy and immediately postpartum ($p < 0.001$). The ultimate strain continuously increased up to 43% higher than virgin animals ($p = 0.007$) in the immediate postpartum group. For active mechanics, the maximal contractile force was 36–56% lower through immediate postpartum animals, and was significantly more sensitive to K^+ throughout pregnancy and postpartum ($p = 0.003$). The changes observed in the passive and active properties of the rat vagina are consistent with what would be expected from a tissue that is remodeling to maximize its ability to distend at the time of vaginal delivery to facilitate passage of the fetus with minimal injury.

Keywords

Contractility; Smooth muscle; Mechanics; Prolapse

INTRODUCTION

Pelvic organ prolapse is a common condition defined as the loss of vaginal support to the pelvic organs and their descent into the vaginal canal. Surgical treatment of prolapse incurs an annual cost of over a billion dollars a year in the United States alone.^{9,20} Prolapse affects as many as half of women over the age of 50,^{9,16,20} with parity being the largest risk factor.^{14,16} However, the mechanism by which parity increases susceptibility to prolapse is still unclear.⁸

When properly supported the vagina supports the urethra, bladder, uterus, and rectum.² The vagina has four layers along its cross-section. The epithelial layer is the first line of defense against infection. The subepithelium is a dense collagen layer that provides a large degree of the passive mechanical integrity to the vagina. The vaginal muscularis immediately beneath the vaginal subepithelium provides active support to the vagina.⁷ The adventitia is a loose connective tissue layer shared with the bladder anteriorly and the rectum posteriorly.² While it is clear that each of these sublayers likely undergoes remodeling to afford passage of the fetus,^{13,24} to date little has been done to quantify the adaptations that mediate increased vaginal distensibility at the time of delivery and subsequent events that allow recovery to the non-pregnant state during the postpartum period.

In mechanical terms, remodeling likely impacts (1) the passive mechanics of the vagina by altering the composition and organization of the fibrillar extracellular matrix²¹ and (2) the active mechanics by altering the amount, organization, and contractile ability (reactivity) of the smooth muscle. As collagen is the primary component of the fibrillar matrix contributing to the vagina's physical stability and smooth muscle is primarily responsible for short-term changes in geometry through contraction and relaxation, it is likely that remodeling of both vaginal collagen and smooth muscle play a critical role in mediating maternal adaptations in preparation for vaginal delivery.

In our previous work, we have shown similarities between the rodent model and human vaginal cross-section and supportive tissues which was used to quantitate the adaptations of the entire vagina-supportive tissue complex (VSTC) measured collectively from mid-pregnancy up to the time of delivery.^{13,15} We found that stiffness and failure load significantly decreased while maximum elongation increased in pregnant animals. Complete recovery of the VSTC's structural properties was observed 4 weeks after vaginal delivery.

In this study, we aim to focus on the changes specific to the vagina by examining both passive (extracellular matrix) and active (smooth muscle) mechanical properties. We define vaginal remodeling in terms of its mechanical function by investigating how the passive mechanical and active contractile properties adapt throughout pregnancy and recover postpartum using the rat model. The findings of our previous work suggest that the rat is capable of successfully adapting to meet the mechanical demand of labor and delivery resulting in minimal tissue injury to both its passive and active components. Thus, based on our previous studies of the VSTC, we hypothesize that the passive properties will adapt during pregnancy to increase the ability of vaginal tissue to distend at the time of delivery and that the active properties will display a concomitant decrease in the contractile force generated by the tissue in response to K^+ stimulation. We further hypothesize that the tissue will rapidly remodel to pre-pregnancy levels of mechanical function (passive and active properties) by 4 weeks postpartum. Data obtained from our study are important to establish a baseline understanding of the normal delivery process such that future studies can aim to infer the impact of injury when the mechanical demand exceeds these adaptations. In addition, quantitative data on progressive alterations in vaginal tissue mechanics in pregnancy to the time of delivery is critical for the development of accurate finite element models aimed at better understanding the birthing process.

MATERIALS AND METHODS

Animals

A total of 71 3-month-old female Long-Evans rats (Harlan Laboratories, Indianapolis, IN) were utilized in this study, which was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Animals were housed with a 12 h alternating light–dark cycle and provided free access to food and water *ad libitum*. Rats were divided

into five separate groups: virgin ($n = 17$), mid-pregnant (Day 14–16, $n = 12$), late-pregnant (Day 20–22, $n = 14$), immediate postpartum (0–2 h after delivery, $n = 14$), and 4 weeks postpartum ($n = 14$). Pregnancy status of the animals was checked daily and confirmed by the presence of a seminal plug. The first appearance of the plug was considered day 1 of gestation. To obtain the immediate postpartum group, rats were examined every 2 h over a 48 h period during late gestation and euthanized immediately after delivery. The number of fetuses, when applicable, was recorded for each rat along with the maternal length and weight. Total vaginal length (TVL) and genital hiatus (GH) measurements were taken using a measuring device marked in millimeters and used to note changes throughout pregnancy and after delivery.

PASSIVE MECHANICAL PROPERTIES

For evaluation of the passive mechanical properties, virgin ($n = 8$), mid-pregnant ($n = 7$), late-pregnant ($n = 7$), immediate postpartum ($n = 8$), and 4 week postpartum ($n = 6$) rats were dissected to expose the pelvic anatomy immediately after they were euthanized. The vagina, cervix, and uterine horns were isolated from the surrounding tissue and placed in saline soaked gauze and frozen at -20°C , which has been previously shown to have minimal effects on the passive biomechanical properties of the vagina and other tissues.^{18,22} Prior to testing, each specimen was allowed to thaw at room temperature and then was further dissected to prepare for mechanical testing. The uterine horns and cervix were removed and the vagina was cut along its length at the urethra. Periurethral tissue was removed and not included in any of the samples tested. The specimen was placed in custom designed soft tissue clamps to form a clamp-vagina-clamp complex and was cut to form a dog-boned shape (Fig. 1). This was done to achieve a minimal aspect ratio of 5 (length to width ratio) that would ensure a uniform stress and strain distribution along the mid-substance of the tissue. This is essential to obtain reliable and repeatable results that are representative of the tensile behavior of the tissue when performing a uniaxial tensile testing protocol.^{1,19}

The cross-sectional geometry and area measurements were made using the non-contact laser micrometer with an accuracy of 0.1 mm^2 .^{1,23} Measurements were taken at three locations along the length of the sample (distal, mid, and proximal) and averaged together. This average cross-sectional area (CSA) value was used to calculate the lagrangian stress, which is defined as the force divided by the initial, unloaded CSA. Subsequently, two contrast markers were placed on the luminal surface of the vagina near the mid-line, roughly 5 mm apart. A camera system (Keyence CV-2600) and motion analysis software (Spicatek, Inc. Maui, HI) were used to track these markers and calculate strain. Strain was defined as the change in distance between the two markers divided by the original marker distance measured immediately after the application of a preload. Throughout tissue preparation the samples were kept moist with 0.9% saline.

For testing, the clamp-tissue-clamp construct was submerged in a 37°C physiological saline bath with the distal clamp (introitus) attached to the base of a InstronTM testing machine (Instron 5565) and the proximal clamp (proximal vagina) attached to a load cell (Honeywell Model 31; 50 lbs), which was rigidly fixed to the cross-head of the machine. The tissue was subjected to a small 0.1 N preload and aligned to the loading axis of the testing system. The initial clamp-to-clamp distance was measured and the specimen was then allowed to equilibrate in the bath for 30 min in a slacked position. For the load to failure test, the preload (0.1 N) was once again applied followed by 10 cycles of preconditioning to 7% of the previously measured clamp-to-clamp distance (7% clamp-to-clamp strain was previously determined to be approximately 4% tissue strain and within the toe region of the stress-strain curve) at an elongation rate of 10 mm/min. The tissue was then loaded to failure and a

load–elongation curve was obtained. The corresponding stress–strain curve was then computed utilizing the CSA and tissue strain measurements made via our optical system. The parameters describing the mechanical properties of the tissue were then obtained from this curve. The tangent modulus, an indicator of tissue stiffness on a per unit basis, was the maximum slope of the stress–strain curve recorded over a 1% interval of strain. The tensile strength was defined as the maximum stress achieved at failure and the ultimate strain was the strain corresponding to the tensile strength. The strain energy density, or tissue toughness on a per unit basis, was calculated from the area underneath the stress–strain curve until failure.

ACTIVE PROPERTIES

To characterize the active properties of the vagina, virgin ($n = 9$), pregnant (mid $n = 5$ and late $n = 7$), and postpartum (immediate $n = 6$ and 4 week $n = 8$) animals were dissected to isolate the vagina immediately following euthanasia. Vaginas were cut longitudinally along the urethra, which was removed as described for the passive properties. During dissection, tissues were continuously moistened with 0.9% saline. The vagina was oriented along its longitudinal axis clips were placed on the distal vagina at the edge of the introitus attached to the bottom of a 20 mL organ bath, and on the distal edge of the cervix and secured to a force transducer (0.2 N capacity). The tissue was bubbled by 95% air balanced with 5% CO₂ in PSS HEPES (37 °C, pH 7.4). Contractile responses were monitored with a pressure transducer (Transbridge 4M, World Precision Instruments) and recorded using Chart software on a PowerLab™ system (sampling at 40 Hz, AD Instruments). Each strip was adjusted to a tension of 3 mN and then allowed to equilibrate for at least 60 min, and then washed three times. All washes during this study were performed using the PSS HEPES solution.⁶

Potassium Response

To measure the amount of contractile force generated by the vaginal strips, the tissue was subjected to seven varying concentrations of potassium (K⁺: 5.88, 20, 30, 40, 50, 80, and 124 mM). The force generated at 124 mM of K⁺ was defined as the maximum force response of the tissue to potassium. The forces generated were normalized to this maximum force response and a line was fitted to the linear portion of the dose–response curve and utilized to calculate the effective concentration for 50 and 100% of the tissues response. The K⁺ dose required to achieve 50% of the maximum response (EC₅₀) correlates to the sensitivity of smooth muscle to K⁺, and the EC₁₀₀ correlates to the lowest dose of potassium that resulted in 100% of the maximum contraction in the tissue sample. The EC₁₀₀ is not necessarily the maximum dose given, and therefore along with the EC₅₀ can help identify overall shifts in the potassium response curve between each group. The tissue was given 15 min to equilibrate at each K⁺ dose when the load was recorded. The sample was then washed three times and given 15–20 min to recover.

Smooth Muscle Contribution with Elongation

Next we wanted to examine how elongation affected the contractile properties of smooth muscle within the vagina. The tissue was contracted with the potassium concentration required to invoke the maximum contractile force response from the tissue in all groups and while contracted, was elongated in 5 mm increments until a maximum force of 0.2 N was achieved. The resulting force (load)–displacement graph represents the total force resulting from both the activated smooth muscle and passive elongation of the extracellular matrix components. The tissue was then returned to zero elongation and washed three times. A calcium free + papaverine buffer was applied to inhibit smooth muscle contraction and 15–20 min was allowed to ensure the sample was completely inactivated. The sample was

again elongated in 5 mm increments until a maximum force of 0.2 N was achieved. The resulting force (load)–displacement curve was referred to as the passive curve, which represents the force resulting from passive elongation of the tissue with no active smooth muscle contribution. The maximum displacement required to generate 0.2 N of force was recorded for each specimen for both the activated and inactivated states of the smooth muscle. To estimate the force generated by the smooth muscle component of the vagina during elongation, the passive curve values were subtracted from the active curve to create a curve called the “total force minus passive” curve, which was fit with an exponential in the form of:

$$F = C * e^{B * x}, \quad (1)$$

where F represents the force generated per unit volume of tissue (mN/mm^3) and x represents the displacement (mm) to which each tissue was elongated. C (mN/mm^3) and B ($1/\text{mm}$) are the fit parameters. The parameter C represents the basal force at 0 mm of elongation. In the case of the total force–passive curve, it should be similar to the force response at 80 mM of K^+ for each group from the potassium response portion of this study as the testing conditions are similar. Parameter B represents the nonlinearity of each curve, or how sharply the force increases during extension. A larger value for B relates to a more nonlinear force response (sharper increase) to the applied elongation.

The wet weight of each vaginal sample was recorded immediately after testing. To measure the volume of the tissue, the length (L) was measured using digital calipers (Mitutoyo Corp.), while the CSA along the vagina was calculated after testing using the non-contact laser micrometer method described above. The length and CSA measurements were utilized to calculate the tissue volume of each specimen ($L \times \text{CSA}$). All generated forces were normalized to tissue volume to account for changes in tissue size and muscle quantity.

Statistics

A power analysis on the preliminary data between the virgin and mid-pregnant animals was performed to assess the number of animals required to observe significant differences. To achieve a power of at least 80%, a minimum of five animals per group were needed to detect a 50% change in the tangent modulus, tensile strength, contractile force, EC_{50} , EC_{100} , maximum displacement, as well as parameter C . Passive mechanical properties were normally distributed and compared using a one-way ANOVA with a Sidak *post hoc* ($p = 0.05$), and presented as mean $\pm$ standard deviations. The active properties, on the other hand, were not normally distributed and therefore were compared using the non-parametric Kruskal–Wallis and Mann–Whitney *post hoc* tests where appropriate ($p = 0.05$), and are presented as median (interquartile range). All data analysis was done using a statistical software package (12.0 SPSS Inc, Chicago, IL).

RESULTS

As expected, the average weights of the animals increased from virgin 221 g to late-pregnant 303 g (Table 1). TVL and GH values continuously increased from virgin to mid- and late-pregnant animals. These parameters began decreasing immediately postpartum (0–2 h). At 4 weeks postpartum, the animal weights were decreased from the early postpartum period but elevated by 13% with respect to virgin animals (Table 1). The vaginal weight and volume from each tested sample for the active properties did not differ significantly between the groups ($p = 0.4$ and $p = 0.5$, respectively, Table 1).

PASSIVE PROPERTIES

All stress–strain curves were nonlinear with the characteristic toe, linear, and failure regions typical for soft tissues. All specimens failed within the central region of the tissue. The vaginal samples from mid-pregnant, late-pregnant, and 0–2 h postpartum animals were considerably less nonlinear compared to all other time points (Fig. 2). This is reflected by the 53–68% decrease in the tangent modulus from longitudinal sections of the vagina during pregnancy and immediately postpartum ($p < 0.001$; Table 2). By 4 weeks postpartum, the tangent modulus recovered to virgin levels (30.0 ± 13.7 MPa, $p = 1.0$). The strain level at which the maximum tangent modulus was found was significantly different between virgin ($12 \pm 3\%$) and the 0–2 h postpartum group ($20 \pm 5\%$, $p = 0.04$). No significant differences in the strain values corresponding to the maximum tangent modulus were observed for the mid-pregnant ($14 \pm 3.5\%$, $p = 0.95$), late-pregnant ($16 \pm 7.4\%$, $p = 0.8$), or immediate postpartum ($18 \pm 6.5\%$, $p = 0.27$) groups. The tensile strength followed a similar trend, decreasing 47% in mid-pregnant ($p = 0.04$) and 55% in late-pregnant ($p = 0.01$) compared to virgin animals. However, unlike the tangent modulus, a significant difference was not detected in the tensile strength between virgin and immediate postpartum ($p = 0.1$) groups with complete return to virgin levels by 4 weeks postpartum (Table 2, $p = 1.0$).

The ultimate strain increased from 33 to 77% during pregnancy compared to virgin animals (Table 2). The ultimate strain was largest in the immediate postpartum group ($24 \pm 5\%$) where it was found to be significantly higher than in virgin animals ($14 \pm 5\%$, $p = 0.007$); indicating that tissue changes allowing for maximal distension are present at the time of delivery. There were no significant differences in strain energy density between each group ($p = 0.25$; Table 2).

ACTIVE PROPERTIES

Response to Potassium

The normalized force response (mN/mm^3) of each tissue to varying doses of K^+ (mM) is illustrated in Fig. 3. For the contractile properties, we observed a 56% decrease in contractile force (load) generated by mid-pregnancy ($p = 0.02$); however, only a 36% decrease by late-pregnancy ($p = 0.3$) compared to virgin animals. This may reflect some change in the distribution, quantity, or phenotype of the smooth muscle during pregnancy, because immediately postpartum (0–2 h) animals again had a 54% decreased capacity to generate force in response to potassium compared to virgin animals ($p = 0.05$, Table 3). Sensitivity of longitudinal vaginal strips to K^+ increased (lower EC_{50}) in mid-pregnancy and immediate postpartum period compared to virgin animals ($p = 0.002$ and $p = 0.04$, respectively). Late-pregnant rats had EC_{50} and EC_{100} values that fell between virgin and mid-pregnant animals. At 4 weeks postpartum, the contractile force response returned to virgin levels ($p = 0.4$, Table 3), but the EC_{50} and EC_{100} remained 17 and 24% lower than virgin animals ($p = 0.04$ and $p = 0.002$, respectively) suggesting that the heightened sensitivity to K^+ is maintained long term.

Smooth Muscle Contribution with Elongation

The maximum displacement achieved in virgin animals following the application of 0.2 N of force was 2.5 (1.0) mm. The tissue distended 29% more during mid-pregnancy ($p = 0.004$), 29% in late-pregnancy ($p = 0.003$), and 17% in the 0–2 h postpartum animals ($p = 0.05$). By 4 weeks postpartum, the tissue was less distensible returning to virgin values (Table 3, $p = 0.7$). From the total force–passive curve (Fig. 4), we observed that the basal force prior to elongation (parameter C) followed a trend similar to the contractile force (Table 4). Virgin animals had a 53 and 54% larger baseline force generated compared to mid-pregnant ($p = 0.007$) and 0–2 h postpartum ($p = 0.03$) rats (Table 4). Finally, the total force–passive curve

showed significant differences in the B parameter, or nonlinearity, between mid- and late-pregnant to virgin animals ($p = 0.03$ and $p = 0.008$, respectively).

DISCUSSION

The vagina must undergo significant changes during pregnancy to allow passage of a fetus at the time of delivery. Although clinically it is understood that these maternal adaptations occur, this study sought to quantify how these adaptations are manifested in terms of the mechanical function of the vagina. The data confirmed clinical impressions by demonstrating significant changes in the passive mechanical properties and contractility of the vagina throughout pregnancy and into the postpartum period. The critical findings in this study include the significant changes in the passive (tangent modulus and ultimate strain) and active (contractile force and sensitivity to K^+) mechanical properties during pregnancy and ability to recover by 4 weeks postpartum.

As we hypothesized, the increase in vaginal distensibility results from changes in both the passive mechanical and active contractile properties. In terms of the passive properties, a conceptual figure depicts the changes observed in the tensile behavior of longitudinal tissue samples throughout pregnancy and during the postpartum period (Fig. 5). While the changes in tangent modulus, tensile strength, and ultimate strain match our expected results, the finding that the strain energy density remained relatively constant, despite all of these changes in these parameters, is rather surprising. By definition the strain energy is the mechanical energy needed to deform a sample and is a measure of toughness of the material. Thus, the required strain energy to fail vaginal tissue longitudinally appears to be the same throughout all stages of pregnancy and postpartum. Whether there is a physiological basis for this result, e.g., remodeling is driven under the condition that the strain energy density must be maintained, or it is simply coincidental warrants further investigation.

We observed a significant amount of variability in the passive mechanical properties in the 4 week postpartum group. This was largely due to two samples whose values were substantially higher than others in the group relative to virgin controls. While it is unclear why this was observed, one possible explanation was that these rats sustained some degree of vaginal injury resulting in a grossly unobservable, but mechanically measurable change resulting in stronger, stiffer, and tougher tissue (i.e., scar). Complimentary histological and biochemical studies are needed to explore the structural and compositional mechanisms that govern these functional adaptations.

The active smooth muscle properties were significantly altered throughout pregnancy in preparation for vaginal delivery. The 56% decrease in the contractile force generated during mid-pregnancy confirms the previous changes observed by us in smooth muscle phenotype of the vagina.⁵ Interestingly, there was a slight increase in the contractile force during late-pregnancy, and may reflect previous findings which illustrated that between mid-pregnancy and late-pregnancy there is an increase in the percentage of smooth muscle cells that assume the contractile (vs. synthetic) phenotype.⁵

While previous studies have shown the phenotypes of smooth muscle cells 4 weeks postpartum are similar to virgin animals, we found them to have a persistently lower EC_{50} and EC_{100} . This is interesting because the contractile force generated by potassium was not significantly different between virgin and 4 week postpartum animals ($p = 0.4$). This may reflect a more longterm effect on the responsiveness or sensitivity of vaginal tissue to stimuli postpartum, which may be a sign of injury to the smooth muscle portion of the vagina.¹¹ However, due to the general reaction of smooth muscle to increasing doses of potassium, our next step will be to examine the effects and response of the vagina to specific

nerve mediated responses via adrenergic, cholinergic, and electric force stimulation. Examining the total force–passive curve parameters allowed us to gain insight into the role of vaginal smooth muscle changes during elongation. After the initial contraction (parameter *C*), we found that the degree of nonlinearity (parameter *B*) decreased during pregnancy.

Some important limitations of this study should be noted. First, it utilized only 4 week postpartum animals to examine any long-term effects due to pregnancy and vaginal delivery; however, we felt it was a reasonable time point to examine the recovery of the passive and active properties of the vagina postpartum. A second limitation was that, despite our efforts to consistently time and test each animal within a specific period immediately following euthanasia, the degree of variation within the contractility data was found to be larger than expected. This is consistent with previously published studies on vaginal tissue, indicating a natural variation in the contractile ability of the vagina between specimens.^{10,12} As with all animal studies there are limitations to the use of an animal model. Previous research has shown the similarities between the rodent model and women in terms of connective tissue support and vagina.^{2,13,15} However, a more detailed comparison between rodents and humans mechanical properties cannot be made without obtaining tissue from patients throughout gestation and following delivery. As this has many ethical issues, we must accept and understand the limits of our animal models. Finally, this study was limited to understanding the passive and active mechanical properties of longitudinal sections of vaginal tissue. Uniaxial tensile testing has proven to be an invaluable tool for understanding how mechanical properties change during pregnancy and postpartum, yet has a few limitation. A zero-strain state is difficult to define during any mechanical test. For the present study, we utilized a small preload value (0.1 N) in an attempt to minimize the strain caused to the tissue prior to the load to failure protocol. Also the function of the vagina is more complex as it is not loaded in a single direction *in vivo*. Although these results are consistent with studies of the VSTC, previous *in vivo* studies from our group have shown that the vaginal distensibility does not recover postpartum indicating that understanding the coupled interactions of the circumferential and longitudinal fibers within the vaginal wall via biaxial testing is an important next step for characterizing the mechanical properties of the vagina.³ We are currently exploring biaxial testing as a tool for quantitating maternal tissue adaptations during pregnancy.

This study examined how the passive and active longitudinal mechanical properties of the vagina change due to pregnancy related maternal adaptations and vaginal delivery. We found both passive and active properties are significantly altered during pregnancy likely as a mechanism to increase vaginal distensibility and reduce the risk of a birth injury to the mother and fetus. Most of these parameters were found to recover to pre-pregnant values by 4 weeks postpartum illustrating minimal effect of vaginal delivery on the active and passive properties of longitudinal samples in the rodent model. Future studies will aim to examine how the biomechanical properties are altered utilizing a biaxial testing protocol and will correlate these changes to collagen fiber alignment and matrix composition in order to gain a better understanding of how vaginal tissue prepares for delivery and recovers postpartum. This will also allow us to understand the coupled interactions between the fibers within the longitudinal and circumferential directions. In addition, we will examine the effects of a simulated birth injury, which has been previously used to examine incontinence and changes in vaginal properties,^{4,17} on the passive and active mechanical properties of the vagina to understand how the recovery process is compromised by birth injury.

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FIGURE 1.

Illustrating the method for dividing the tissue for the uniaxial tensile test. (a) Represents the vagina as a tube (not drawn to scale) with the urethra at the 12 o'clock position. The dashed line indicates where the vagina was cut so the vagina would create a square piece of tissue (b). The urethra was removed (dashed line) and the sample was placed into custom design soft tissue clamps (c). The tissue was cut into a dog-boned (dashed lines) shape to create an adequate aspect ratio (not drawn to scale). (d) Depicts a sample tissue clamped after it was dog-boned and the strain markers placed on the mid-substance.

FIGURE 2.

Representative stress–strain curves of the passive mechanical response from virgin, mid- and late-pregnant, immediate (0–2 h) and 4 week postpartum animals.

FIGURE 3.

Force normalized to the volume of tissue at varying potassium doses. The † represents an overall p value between the groups ($p = 0.01$, Table 3).

FIGURE 4.

Total force minus passive data points illustrating the smooth muscle contribution to the load generated. Each sample was fit with an exponential: where the coefficient (C) represents the basal force at 0 mm of elongation, and the exponent (B) relates to the nonlinearity of the curve (Table 4).

FIGURE 5.

The vagina undergoes significant changes in its passive mechanical properties throughout pregnancy. (a) Illustrates how the tensile strength and tangent modulus (stiffness) change throughout pregnancy and recover postpartum. (b) Indicates how the ultimate strain (%) continuously increases during pregnancy until delivery. While these mechanical properties were altered during pregnancy, the strain energy density was found to not be significantly different between groups (c).

TABLE 1

Baseline characteristics (weight, length, total vaginal length (TVL), and genital hiatus (GH)) of rodents including the tissue weight and volume of longitudinal vaginal sections used during the contractile assay

	Weight (g)	Length (cm)	TVL (cm)	GH (cm)	Tissue weight (g)	Tissue volume (mm ³)
Virgin	221 (11.2)	29.0 (0.5)	2.1 (0.2)	0.5 (0.1)	0.25 (0.1)	51.8 (30.4)
Mid-pregnant	246 (18.5)	29.5 (0.5)	2.5 (0.1)	0.5 (0.1)	0.24 (0.06)	69.4 (4.4)
Late-pregnant	303 (13.7)	29.0 (0.8)	3 (0.1)	0.6 (0.25)	0.3 (0.03)	68.2 (33.4)
0-2 h postpartum	250 (36.7)	29.0 (0.8)	3 (0.2)	0.7 (0.15)	0.33 (0.06)	77.7 (12.5)
4 week postpartum	243 (11.1)	30.0 (1.0)	2 (0.2)	0.5 (0.1)	0.28 (0.06)	58.8 (25.6)
Overall <i>p</i> value	0.003	0.3	<0.001	0.001	0.4	0.5
Virgin vs. mid-pregnant	0.3	N/A	0.002	0.4	N/A	N/A
Virgin vs. late-pregnant	0.001	N/A	<0.001	0.008	N/A	N/A
Virgin vs. 2 h postpartum	0.1	N/A	0.004	0.003	N/A	N/A
Virgin vs. 4 week postpartum	0.3	N/A	0.4	0.7	N/A	N/A

Data represented as median (interquartile range).

Passive mechanical properties and cross-sectional area measurements of longitudinal vaginal sections subjected to a uniaxial tensile test

TABLE 2

	Tangent modulus (MPa)	Tensile strength (MPa)	Strain (%)	Strain energy density (MPa)	Cross-sectional area (mm ²)
Virgin (<i>n</i> = 8)	25.0 ± 5.1	2.1 ± 0.65	14.0 ± 4.1	0.12 ± 0.06	1.8 ± 0.59
Mid-pregnant (<i>n</i> = 7)	12.0 ± 7.7	1.1 ± 0.47	18.0 ± 5.6	0.08 ± 0.04	2.6 ± 0.69
Late-pregnant (<i>n</i> = 7)	7.9 ± 4.0	0.95 ± 0.51	21.0 ± 6.9	0.09 ± 0.06	2.8 ± 0.83
0–2 h postpartum (<i>n</i> = 8)	8.5 ± 4.7	1.3 ± 0.46	24.0 ± 5.3	0.12 ± 0.04	1.9 ± 0.73
4 week postpartum (<i>n</i> = 6)	30.0 ± 14.0	3.1 ± 1.7	20.0 ± 7.1	0.25 ± 0.18	2.2 ± 1.3
Overall <i>p</i> value	<0.001	0.001	0.01	0.2	0.1
Virgin vs. mid-pregnant	0.003	0.04	0.7	N/A	N/A
Virgin vs. late-pregnant	<0.001	0.01	0.2	N/A	N/A
Virgin vs. 2 h postpartum	<0.001	0.09	0.007	N/A	N/A
Virgin vs. 4 week postpartum	1.0	1.0	0.9	N/A	N/A

Data is represented as mean ± SD.

Active properties of longitudinal vaginal sections

TABLE 3

	Contractile force (mN/mm ³)	EC ₅₀ (mM)	EC ₁₀₀ (mM)	Max. displacement (mm)
Virgin (<i>n</i> = 9)	0.18 (0.07)	40 (4.3)	74 (5.3)	2.5 (1.0)
Mid-pregnant (<i>n</i> = 5)	0.076 (0.009)	15 (9.2)	34 (9.6)	3.5 (1.5)
Late-pregnant (<i>n</i> = 7)	0.11 (0.09)	28 (12)	71 (20.9)	3.5 (1.0)
0–2 h postpartum (<i>n</i> = 6)	0.063 (0.1)	24 (7.2)	68 (16.3)	3.0 (1.4)
4 week postpartum (<i>n</i> = 8)	0.21 (0.07)	34 (7.3)	57 (10.5)	2.0 (0.5)
Overall <i>p</i> value	0.01	0.003	0.002	0.001
Virgin vs. mid-pregnant	0.02	0.002	0.001	0.004
Virgin vs. late-pregnant	0.3	0.04	0.4	0.003
Virgin vs. 2 h postpartum	0.05	0.04	0.1	0.05
Virgin vs. 4 week postpartum	0.4	0.04	0.002	0.7

The data is presented as medians (interquartile range).

TABLE 4

The total force–passive curve was fit with an exponential equation resulted in basal force (C , mN/mm³) and exponent (B , 1/mm)

	C (mN/mm ³)	B (1/mm)
Virgin ($n = 9$)	0.26 (0.04)	0.82 (0.6)
Mid-pregnant ($n = 5$)	0.17 (0.04)	0.38 (0.3)
Late-pregnant ($n = 7$)	0.26 (0.04)	0.35 (0.1)
0–2 h Postpartum ($n = 6$)	0.13 (0.08)	0.49 (0.3)
4 week Postpartum ($n = 8$)	0.30 (0.1)	0.74 (0.3)
Overall p value	0.006	0.03
Virgin vs. mid-pregnant	0.007	0.03
Virgin vs. late-pregnant	0.3	0.008
Virgin vs. 2 h postpartum	0.03	0.09
Virgin vs. 4 week postpartum	0.7	0.7

The data for each group is represented as median (interquartile range).