Material properties of mouse cervical tissue in normal gestation

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Abstract

An appropriately timed cervical remodeling process is critical for a healthy delivery, yet little is known about the material property changes of the cervix in pregnancy because obtaining human tissue samples is difficult. Rodent models offer advantages including accurately timed pregnant tissues and genetically altered models. Determining the material properties of the mouse cervix, however, is challenging because of its small size and complex geometry. The aim of this study is to quantify cervical material property changes in a normal mouse pregnancy using a microstructurally-inspired porous fiber composite model. We mechanically test intact, whole, gestation-timed mouse cervix by pulling apart tensioned sutures through its inner canal. To interpret our mechanical testing results, we conduct an inverse finite element analysis, taking into account the combined loading state of the thick-walled cylindrical tissue. We fit the material model to previous osmotic swelling data and load-deformation data from this study using a nonlinear optimization scheme, and validate the model by predicting a separate set of deformation data. Overall, the porous fiber composite model captures the mechanical behavior of the mouse cervix in large deformation. The evolution of cervical material parameters indicates that in a normal

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mouse pregnancy, the cervix begins to soften at day 6 of a 19-day gestation period. The material parameter associated with the collagen fiber stiffness decreases from 3.4 MPa at gestation day 6 to $9.7\text{e-}4$ MPa at gestation day 18, while the ground substance stiffness decreases from $2.6\text{e-}1$ MPa to $7.0\text{e-}4$ MPa.

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1. Introduction

Throughout the course of a pregnancy, the female reproductive tract undergoes significant changes as its various organs synchronously grow and remodel for the safe development and delivery of a baby at term. In particular, the timely
5 remodeling of the uterine cervix from a stiff mechanical barrier into a compliant structure which can dilate for delivery is crucial. Premature cervical remodeling can result in the early dilation of the cervix in the absence of labor, leading to extremely premature births [1]. Along with premature cervical remodeling, the deformation and dilation of the uterine cervix is the final common pathway
10 to many etiologies of preterm birth [2]. Preterm birth (PTB), a leading cause of neonatal death, affects 15 million babies every year [3] where surviving babies often suffer life-long disabilities and congenital disorders. Despite advances in prenatal care and increasing research efforts concentrated on understanding its causes, PTB rates have failed to significantly decrease largely because the
15 physiology of normal and abnormal cervical remodeling in pregnancy remains unclear.

A key gap in knowledge in understanding the deformation and dilation of the cervix in pregnancy, as well as the causes of premature cervical remodeling, is the material characterization of the cervix throughout pregnancy. Since
20 obtaining pregnant human cervical tissue serially throughout pregnancy is near impossible, the obstetric research community has continued to rely on the mouse and rat as animal models for pregnancy. Rodent models offer the advantages of gestation-timed pregnant tissue samples, genetically altered strains of abnor-

mal remodeling, and provide valuable insights into the effects of various clinical
25 treatments on cervical mechanical properties. Additionally, as explained below, the extracellular matrix (ECM) remodeling events of pregnancy are similar between human and mouse cervical tissue [4, 5, 6, 7, 8]. However, it is still unclear if the molecular triggering mechanisms for this remodeling behavior are conserved between human and mice [9] and there are obvious anatomical
30 differences associated with mouse models of pregnancy. Regardless of these differences, there are sufficient similarities in the cervical material composition to study ECM-material property relationships during a mouse pregnancy.

As in the human cervix, the mouse cervix is the cylindrical connective tissue located at the base of the uterus. The internal os is the orifice connecting the
35 bottom of the uterine horns and the top of the cervix and the external os is the portion of the cervix that extends into the vaginal canal (Fig. 1A). Similar to the human cervix, but unlike the rat cervix, the cervix of the mouse has a single inner canal. Dissimilar to the human cervix, the mouse cervix is connected to two separate uterine horns which extend to either side of the mouse abdomen
40 (Fig. 1A). The nonpregnant mouse cervix is approximately 2.2 mm in diameter and 2.8 mm in length. In its 19 day gestation time period in pregnancy, the mouse cervix grows to approximately 4 mm in diameter and 4.4 mm in length. Previous mechanical tests on isolated, whole mouse cervical tissue disregard the contribution of geometry and size. Thus, intrinsic pregnant cervical material
45 properties throughout gestation have not been reported, limiting the translations between studies from mouse to human pregnancy. Therefore, in an effort to determine the mechanical role of the cervix in preventing PTB and to establish a framework to quantify cervical material properties in the pregnant mouse cervix, we mechanically test intact, whole, gestation-time mouse cervical tissue
50 samples and fit a microstructurally-inspired cervical constitutive model to the experimental data using an inverse finite element method. The material model is formulated such that its parameters have a physical interpretation to ECM components known to remodel during pregnancy [2].

In both mouse and human, the changes in the cervical ECM during preg-

nancy are hypothesized to result in the changing tissue mechanical proper-
 ties [4, 5, 6, 7, 8]. The cervical ECM is about 75-80% hydrated, depending on
 the gestational age [10]. It is composed of fibrous collagens types I and III, small
 proteoglycans with sulfated glycosaminoglycan (sGAG) chains, the non-sulfated
 GAG hyaluronic acid (HA), elastin, and other matricellular proteins [2]. Among
 the various ECM components, the cervical collagen properties are hypothesized
 to have a central role in determining the mechanical integrity of the tissue and to
 drastically remodel during pregnancy [11]. Second harmonic generation images
 of the cervical collagen fibers in both mice [12, 13] and humans [13, 14] demon-
 strate similar intrinsic remodeling of the collagen fiber from straight fibers in
 the nonpregnant tissue into undulating fibers in pregnant tissue. Based on
 collagen solubility studies on human [14] and mouse cervical tissue [6, 10], we
 hypothesize that cervical softening during pregnancy is facilitated by the simul-
 taneous breakdown of stronger mature crosslinked collagens and the synthesis of
 weaker immature less crosslinked collagens. To investigate this hypothesis, we
 measured the enzymatic intermolecular immature divalent (crosslinks between
 two collagen molecules) and mature trivalent (crosslinks between three collagen
 molecules) collagen crosslink density as well as corresponding structural ten-
 sile properties of the mouse cervix during pregnancy. In this previous study, we
 found that during a normal mouse pregnancy the collagen crosslinking maturity
 ratio (the content of mature trivalent crosslinks to immature divalent crosslinks)
 decrease with progressing gestation in early softening (mouse gestation days 6 to
 15), but not in late softening (mouse gestation days 15 to 18) [7]. In addition,
 the amount of sGAGs in the cervix does not significantly change throughout
 gestation while the amount of the non-sulfated GAG HA is known to increase
 significantly in late pregnancy [15]. This change in HA content, however, does
 not affect the structural response of the tissue to tensile loading [16].

In their foundational study, Harkness and Harkness mechanically tested the
 tensile properties of nonpregnant and pregnant rat cervix via a ring test [17].
 This study found that in normal pregnancy, the cervical tissue grows and be-
 comes more compliant. Since then, researchers have continued to utilize their

protocol due to its ease of minimal test setup to investigate the functions of various ECM components and the effect of pharmaceutical treatments on the cervical mechanical response [18, 19, 20, 21, 22, 23]. In these studies, structural property relationships – force vs. displacement – are typically reported. Like other
90 collagenous tissues, cervical tissue exhibits complex material behavior including tension-compression nonlinearity, time-dependence, and anisotropy [24, 25]. In addition, the small size and complex geometry challenges the mechanical testing of the rodent cervix as well as the interpretation of the mechanical data. Although structural properties are useful for understanding the relative changes
95 in the mechanical integrity of the tissue within a study, the complex material behavior of the cervix restricts the comparisons that can be made between studies.

In this study, we determine the material property changes of the mouse cervical tissue in a normal pregnancy. We mechanically test whole, intact, gestation-
100 timed mouse cervical tissue from gestation day 6, 12, 15, and 18 samples as well as nonpregnant nulliparous (not previously)tissue. We test the cervical tissue using a ring test with minimal sample preparation and setup similar to previous rodent cervix studies, and develop an analysis framework using finite element modeling. Based on our previous modeling work on human cervical tissue [26]
105 and our knowledge of the key ECM components involved in cervical remodeling [2], we utilize a porous fiber composite material model to describe cervical material response to mechanical loading. This material model accounts for the swelling nature of the negatively charged GAGs, the nonlinear stiffness of the collagen fibers, the stiffness of the ground matrix, and the characteristics of the
110 collagen network. Using an inverse finite element analysis (IFEA) we fit model parameters to the experimental swelling and load-deformation data. In this initial modeling framework, we analyze the equilibrium mechanical data, after the transient material effects have died away. We validate the model’s predictive capability of the equilibrium material response with data not used for fitting.
115 Here, we report the evolution of cervical material parameters throughout pregnancy in an established mouse model of normal remodeling and we discuss these

results in relation to our previous studies of tissue ECM.

2. Materials and Methods

2.1. Mouse cervical samples and tissue preparation

120 All studies were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Research Advisory Committee. The mice used in these studies were generated and maintained as a breeder colony at the University of Texas Southwestern Medical Center (Dallas, TX). Animals were housed under a 12L:12D photoperiod at 22°C. Mice used in this study were from
125 Black 6/129 SvEv strain. Young, nulliparous (never pregnant before) female mice between 3-6 months old were housed overnight with males and checked for vaginal plugs in the morning. The day of plug formation was counted as gestation day 0, with birth occurring in the early morning hours of day 19. Here, we collected samples from nonpregnant (NP) and gestation day 6 (d6), 12 (d12),
130 15 (d15), and 18 (d18) mice. Samples were collected at midday, except for d18, which were collected in the evening of d18, between 6 and 8 PM. Animals were anesthetized with Avertin and then sacrificed via cervical dislocation. Reproductive tracts were collected from the mice and immediately frozen (n=5 per gestation group). Samples were shipped overnight to Columbia University
135 on dry ice and stored at -80°C until testing. We have previously determined that the mechanical properties of cervical tissue are the same between fresh and previously frozen tissue [27].

2.2. Mechanical Testing

To determine the material response to loading of each cervical sample, tensile stress-relaxation tests were conducted. First, frozen reproductive tracts
140 were thawed in phosphate buffered saline (PBS) and the vaginal wall was carefully detached and removed from the anterior and posterior sides of the cervix (Fig. 1A). Next, the uterine horns were disconnected from the superior end of the cervix at the internal os (Fig. 1A). Two surgical sutures (Perma-Hand Silk 2-0,

145 Ethicon) were passed through the cervical canal. Sutures were attached to custom tensile grips and tensioned (Fig. 1E). The tensile grips were positioned in a universal testing machine (Model 5948 MicroTester Instron, 10N load cell, resolution 0.0025N) (Fig. 1B) and samples were immersed in an environmental chamber filled with phosphate buffered saline (PBS) with 2mM ethylenediaminetetraacetic acid (EDTA) protease inhibitor to minimize degradation throughout
150 testing. Two perpendicular CCD cameras (Point Gray Grasshopper, GRAS-50S5M-C 75 mm, f/4 lens) were positioned to track the geometry of the tissue (Fig. 1B,C,D). The tensile grips were adjusted such that the top and bottom sutures were as close together as possible. Cervical samples were allowed to
155 swell to equilibrium for about 2-4 hours, determined by continuously tracking tissue geometry. After confirming swelling equilibrium, samples were subjected to tensile testing. This process ensured that the load-relaxation response was solely due to the tissue relaxing instead of the tissue swelling.

Because of the wide range in sample size and mechanical properties between
160 gestation groups, the stress-relaxation loading regimens were adjusted for each gestation group. All samples were loaded at a grip-to-grip rate of 0.1 mm/s. Nonpregnant and d6 samples were loaded to intervals of 0.5 mm grip-to-grip displacement. Each grip-to-grip displacement interval was held for 30 minutes to allow the load to relax. Gestation day 12 samples were loaded to 0.5 mm
165 grip-to-grip intervals and each interval was held for 60 minutes. Gestation day 15 and 18 samples were initially loaded to 1 mm of grip-to-grip displacement and displaced to subsequent 0.5 mm grip-to-grip intervals. Each grip-to-grip interval was held for 60 minutes of relaxation time. Throughout testing, images were taken to track the deformation of the tissue. All tissue samples were loaded at
170 incremental 0.5 mm intervals until the sample broke. After testing, the CCD cameras were calibrated using a high-resolution ruler (0.4 mm resolution) to determine the pixel to mm ratio.

2.3. Mechanical Test Analysis

We approximated the cervical geometry as a thick walled cylinder with an elliptical cross-section. Figure 2A shows the parameterized geometry of the cylinder with a width $[w]$, length $[l]$, height $[h]$, and thickness $[t]$. In order to account for the compliance of the sutures, the calibrated camera images were used to measure the cervical opening $[co]$, defined as the suture-to-suture distance (Fig. 2D). We used this cervical opening measurement to define the applied deformation to the tissue instead of the grip-to-grip distance from the universal testing machine.

The average geometrical parameters $[w, l, h, t]$ and cervical opening $[co]$ were determined for the undeformed state and at the end of each displacement interval using ImageJ (<http://www.imagej.nih.gov>). To determine the average height $[h]$ of the sample, a $0.2\text{ mm} \times 0.2\text{ mm}$ grid was overlaid on each image of the cervix. Multiple measurements at 0.2 mm intervals were made of the height using the grid as a guide (Fig. 2B). The average height $[h]$ and standard deviation were calculated from these measurements. The same procedure was repeated to measure the length $[l]$ and width $[w]$. The top thickness of the tissue was defined as the distance from the suture to the top edge of the tissue and the bottom thickness of the tissue was defined as the distance from the suture to the bottom edge of the tissue. Using the same $0.2\text{ mm} \times 0.2\text{ mm}$ grid, the top and bottom thickness of the samples were measured to determine the average and standard deviations. From these camera images, we determined the cervical opening $[co]$ vs. width $[w]$, cervical opening $[co]$ vs. length $[l]$, cervical opening $[co]$ vs. height $[h]$, and cervical opening $[co]$ vs. thickness $[t]$ curves for each sample. Using the force data from the universal testing machine, we generated the cervical opening $[co]$ vs. force $[F]$ response of each sample for a total of 5 experimental curves per sample. The experimental dataset for this study is available at the Columbia University Library's digital repository Academic Commons (doi:10.7916/D8DN44QW).

2.4. Porous Fiber Composite Material Model for Cervix

To describe the material behavior of nonpregnant and pregnant mouse cervical tissue, we utilize a porous, fiber composite material model. The porous component accounts for the swelling behavior due to the fluid pressure arising from the charged GAGs, which is balanced by a hyperelastic solid composed of a continuously distributed collagen fiber network embedded in a compressible Neo-Hookean ground substance. This hyperelastic solid model is based on a material model we developed to describe the tension-compression nonlinearity in human cervical tissue [26]. For the mouse tissue, we add a charged porous component to the material to account for the swelling behavior we observe in a previously reported study on pregnant mouse cervical tissue [8]. In this previous study, we determined that the pregnant mouse cervix swelled significantly (30% volume change) from a hyperosmotic solution (2M NaCl) to a physiological solution (0.15M NaCl), but the nonpregnant mouse cervix did not swell significantly (5% volume change). Because this significant swelling in the pregnant samples would introduce a pre-stretch to the soft, remodeled collagen fibers before tensile testing, we account for the swelling in our model.

A three-dimensional map of collagen fiber directionality has not been measured for a mouse cervix throughout pregnancy. The human cervix is known to have a band of circumferentially aligned fibers circling around the inner canal and longitudinally aligned fibers along and parallel to the inner canal and on the outer edges of the tissue [28, 29]. A study on the rat cervix showed a similar trend of circumferential and longitudinal fibers for a nonpregnant cervix [30]. There is evidence of a similar circumferential band of collagen fibers in a mouse cervix in nonpregnant and early gestation day 6 tissue, but a three-dimensional map of collagen fiber directionality has not been shown [12]. **Because these zones of circumferential and longitudinal fibers have not been explicitly measured for the mouse tissue** ~~due to the lack of data~~ and rationalizing that an isotropic fiber distribution represents a homogenization of the circumferentially and longitudinally aligned fibers, we ~~assume~~ **utilized** a randomly distributed collagen fiber network throughout the entire tissue **for this study**.

In this first material modeling attempt for mouse cervical tissue, we investigate the equilibrium material response. We model the passive mechanical properties, ignoring any contributions for possible active contractions, and we assume an affine deformation of the fibers with the whole tissue. Describing the visco- and poroelastic nature of the tissue, collagen fiber directionality, tissue heterogeneity, and the driving mechanisms for material parameter evolution will be a topic of future modeling studies.

In this material model for mouse cervical tissue, the total Cauchy stress (σ) in the material is the additive balance between the fluid pressure arising from the negatively charged GAGs (p) and the stress on the hyperelastic fiber composite solid (σ^s):

$$\sigma = -p\mathbf{I} + \sigma^s = -p\mathbf{I} + \frac{1}{J^s} \frac{\partial \Psi_r^s}{\partial \mathbf{F}^s} (\mathbf{F}^s)^T \quad (1)$$

where $\mathbf{I}$ is the identity matrix, $\mathbf{F}^s$ is the deformation gradient of the solid given by a standard definition derived from the motion of the material from the reference to the deformed configuration [31], $J^s = \det \mathbf{F}^s$ is the Jacobian, and Ψ_r^s is the Helmholtz free energy density of the hyperelastic solid material. Note that the deformation gradient $\mathbf{F}^s$ and the Jacobian J^s are that for the total mixture as well.

We define the reference unstressed state of the cervix at swelling equilibrium in a hypertonic bath solution of 4000 mOsm, where the negative charges of the GAGs are shielded by the excess positive charges leading to a negligible fluid pressure. In this state we assume that $p \approx 0$. This assumption is based on experimental data from cartilage, which has a much higher GAG concentration and thus FCD compared to cervical tissue [32]. **Note that the swelling behavior described here does not compare swelling from an *in-vivo* state, but from a hypertonic solution to a physiological isotonic solution.**

To account for the increase in volume due to swelling pressure induced by GAG fixed charge density (FCD, c^F) of the tissue, we use Donnan equilibrium

theory to describe the charge dependent fluid pressure p [33]:

$$p = RT \left(\sqrt{(c^F)^2 + (2c^*)} - 2c^* \right) \quad (2)$$

where R is the universal gas constant, T is the absolute temperature, c^F is the FCD arising from the negatively charged GAGs, and $2c^*$ is the osmolarity of the environmental bath ($2c^*=4000$ mOsm for 2 M NaCl and $2c^*=300$ mOsm for 0.15M NaCl). The FCD in the deformed configuration is calculated as previously shown in [8]:

$$c^F = \frac{\phi_r^w}{J - 1 + \phi_r^w} c_r^F \quad (3)$$

In this model, we calculate the reference FCD (c_r^F) based on GAG measurements from literature [15] as previously shown [8]. The reference FCD (c_r^F) values for each gestation group are listed in Table 1.

The fiber composite material model for the solid component is presented previously for human cervical tissue [26]. The total Helmholtz free energy density of this solid (Ψ_r^s) is modeled as an additive contribution from a ground substance matrix made up of the proteoglycans and non-fibrous matricellular proteins (Ψ_r^{GS}) and a randomly-oriented, continuously-distributed collagen fiber network (Ψ_r^{COL}):

$$\Psi_r^s(\mathbf{F}^s) = \Psi_r^{GS}(\mathbf{F}^s) + \Psi_r^{COL}(\mathbf{F}^s) \quad (4)$$

The ground substance is modeled as a standard isotropic, compressible Neo-Hookean material:

$$\Psi_r^{GS} = \frac{\mu^{GS}}{2}(I_1 - 3) + \mu^{GS} \ln(J^s) + \frac{\lambda^{GS}}{2}(\ln J^s)^2 \quad (5)$$

where $I_1 = \text{tr} \mathbf{C}$ is the first invariant of the right Cauchy-Green tensor $\mathbf{C} = (\mathbf{F}^s)^T \mathbf{F}^s$ and μ^{GS} and λ^{GS} are the standard lamé constants. This material model can be expressed as functions of Young's modulus E^{GS} and Poisson's ratio ν^{GS} where $E^{GS} = \frac{\mu^{GS}(3\lambda^{GS} + 2\mu^{GS})}{\lambda^{GS} + \mu^{GS}}$ and $\nu^{GS} = \frac{\lambda^{GS}}{2(\lambda^{GS} + \mu^{GS})}$.

The strain energy density function of the randomly and continuously distributed collagen fiber network is given by:

$$\Psi_r^{COL} = \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi H(I_n - 1) \Psi_r^{fiber}(I_n) \sin \phi d\phi d\theta \quad (6)$$

where the heaviside step function H ensures that the fibers only contribute when pulled in tension ($I_n > 1$), $[\theta, \phi]$ are the polar and azimuthal angles in a spherical coordinate system, and Ψ_r^{fiber} is the strain energy density of a collagen fiber bundle. $I_n = \mathbf{n}_o \cdot \mathbf{C} \cdot \mathbf{n}_o$ is the square of the fiber stretch, where $\mathbf{n}_o = \cos \theta \sin \phi \mathbf{e}_1 + \sin \theta \sin \phi \mathbf{e}_2 + \cos \phi \mathbf{e}_3$ in a local Cartesian basis $\{\mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3\}$. Here, we assume a continuous spherical distribution for the collagen fibers of the mouse tissue.

We prescribe a phenomenological power law for the collagen fiber bundle, Ψ_r^{fiber} :

$$\Psi_r^{fiber} = \frac{\xi}{\beta} (I_n - 1)^\beta \quad (7)$$

where ξ represents a fiber stiffness with units of stress and $\beta > 2$ is the dimensionless parameter that controls the shape of the fiber bundle stiffness curve. (Here, the fiber strain energy density is cast in a different form than the model presented for the human cervical tissue [26], hence direct comparison can be made by considering the $1/\beta$ prefactor here.)

In total, there are 3 parameters that determine the total fluid pressure $[\phi_r^w, c_r^F, c^*]$, 2 parameters related to the ground substance $[E^{GS}, \nu^{GS}]$, and 2 parameters related to the collagen fiber network $[\beta, \xi]$. The parameters $[\phi_r^s, c_r^F]$ related to the fluid pressure are calculated based on values from the literature and the bath concentration $[c^*]$ is set by the experimental conditions. This leaves a total of 4 fitting parameters $[E^{GS}, \nu^{GS}, \beta, \xi]$ to be determined in our IFEA.

2.5. Inverse Finite Element Analysis (IFEA)

IFEA was used to determine the best-fit material parameters for each sample. Sample-specific FEA models were generated with the open-sourced software FEBio (v.2.2.6, <http://www.febio.org>), and material parameter optimization was conducted using FEBio coupled with the MATLAB Optimization Toolbox (R2014b, Natick, MA). Material parameters were optimized such that the FEA calculations matched previously reported swelling data [8] and the mechanical

testing data reported in this study. All mechanical test raw data for this IFEA procedure are archived at the Columbia University Library’s digital repository Academic Commons (doi:10.7916/D8DN44QW).

2.5.1. Sample-specific FEA

315 To account for geometry differences, we generated a unique FE model for each cervical sample. The cervix was modeled as a cylinder with an elliptical cross-section with a major diameter $[w]$, minor diameter $[h]$, length $[l]$, and thickness $[t]$ (Fig. 2A). Using symmetry and taking 1/8 of the cylindrical length $[l]$, the model was reduced to a 1/32 model for finite element analysis (Fig. 3A). The
320 three-dimensional geometry for each sample was generated and meshed using hexahedral elements with Cubit (v.13.2, <http://cubit.sandia.gov>) (Average element size (volume) 0.00424 [mm³]; Average number of elements - NP:91, d6:83, d12:111, d15:202, d18:150). The generated geometry was based on the measured dimensions of the tissue in the "slack" position after swelling equilibrium in 0.15M NaCl. In some of the d15 and d18 cervixes, however, we observed
325 that the tissue samples were not deforming during the first stress-relaxation interval. Instead, the sutures were sliding through the inner canal such that the top suture was in contact with the top part of the tissue but the bottom suture was not in contact with the bottom part of the tissue. In these cases,
330 we based the generated geometry on the average measurements between the first two stress-relaxation intervals. The generated geometries for all samples are archived at the Columbia University Library’s digital repository Academic Commons (doi:10.7916/D8DN44QW). Based on our previous investigation of the swelling behavior of the pregnant mouse cervical tissue [8], we considered
335 the unloaded reference configuration when the tissue is equilibrated in 2M NaCl.

The mesh was imported into FEBio and the proposed material model (Section 2.4) was assigned to each element. The suture was modeled as a cylindrical rigid body 0.4 mm in diameter. A sliding contact interface was defined between the suture and the inner canal of the cylinder. Symmetry boundary conditions
340 were assigned in the x and y directions to reflect symmetry (Fig. 3B). One side

of the cross-sectional face was fixed in the z-direction to reflect symmetry, while a sliding-tension-compression contact was defined on the other side with a rigid plane. This contact definition allowed the tissue to compress in the length with progressing deformation (in the negative z-direction) on the same plane.

345 To account for the swelling of the tissue from an unstressed reference state in 2M NaCl to a swollen state in 0.15M NaCl, we conducted a swelling pre-analysis using a single cube element (Fig. 4). In this swelling pre-analysis, we assigned a set of material parameter values to be simulated in the main analysis to a single cube element. In FEBio, the initial bath concentration $2c^*$ was 350 set to 4000 mOsm then decreased to 300 mOsm. This simulated the tissue swelling from a 2M NaCl hypertonic solution to a physiological 0.15M NaCl solution. The swelling volume ratio, $J_{0.15M} = V_{0.15M}/V_{2M}$ was determined for the single element from this pre-analysis and the scaling factor was calculated as $1/(J_{300mOsm})^{1/3}$. Next, the original geometry of each cervical sample was 355 scaled by this factor and imported into the FEA for the main analysis (Fig. 4). This scaling step was conducted in order to simulate the induced collagen fiber pre-stretch after the tissue reached swelling equilibrium in the PBS solution.

Mechanical loading was applied to the meshed geometry in two analysis steps: 1) Swelling from 2M to 0.15M NaCl and 2) tissue deformation via suture displacement (Fig. 4). In the swelling step, the bath concentration $2c^*$ was 360 changed from 4000 to 300 mOsm. In the second step, the sutures were displaced in the positive y direction to simulate the deformation of the tensile experiments reported here (Section 2.2).

2.5.2. Material Parameter Optimization

365 We used two experimental datasets to inform the material parameter optimization: 1.) the swelling dataset $[J_{0.15M}]$ from 2M to 0.15M NaCl (Table 2, [8]) and 2.) the suture displacement (cervical opening $[co]$) versus the measured equilibrium force $[F]$ at each displacement hold of the tensile tests reported here (Fig. 5A and raw dataset doi: 10.7916/D8DN44QW). We informed the 370 model with the known FCD (Table 1) to calculate the osmotic swelling pres-

sure contribution per Eq. 2, and the material parameter values $[E^{GS}, \nu^{GS}, \beta, \xi]$ were fit to the experimental data. The following optimization objective function was used with the `fminsearchbnd` routine in Matlab (R2014b) available on the MathWorks File Exchange,

$$F_{obj} = \frac{(J_{0.15M} - \hat{J}_{0.15M})^2}{J_{0.15M}} + \sum_{i=1}^n \left[\frac{(F_i - \hat{F}_i)^2}{F_i} \right], \quad (8)$$

375 where primed variables were the FEA model prediction. Model validation was tested by comparing the FEA prediction to the experimental data of the geometrical parameters width $[w]$, length $[l]$, height $[h]$, and thickness $[t]$ as a function of cervical opening $[co]$ (Fig. 5B-E and raw dataset doi:10.7916/D8DN44QW).

2.5.3. Statistics

380 The quality of the material model fit to the experimental data was evaluated by calculating the goodness of fit (r^2 value) for the five experimental curves for each sample. Differences in the material property parameters between gestation groups were analyzed using one way analysis of variance (ANOVA). Tukey's honestly significant difference criterion was used with MATLAB's Statistics Toolbox
385 (v8.1.0.604) to determine differences between any two gestation groups. Significant differences were determined for p-values less than 0.05 for all statistical analysis.

3. Results

3.1. Mechanical Testing and Analysis

390 Mouse cervical tissue dramatically softens throughout pregnancy, starting at day 6 of a 19 day gestation period (Fig. 5). By gestation day 18 (d18), the distensibility (i.e. maximum cervical opening sustained by the tissue during stress-relaxation testing) is more than 4 times larger compared to the nonpregnant cervix (Fig. 5, Fig. 6D&F). For nonpregnant and all gestational time-points
395 in pregnancy, the cervix exhibits a force-relaxation response to a ramp-hold in tensile displacement (Fig. 6A-C). This time-dependent response is also seen in

human cervical tissue [25, 24, 34]. After the transient force response dies away, the equilibrium force versus cervical opening is nonlinear (Fig. 5). Samples consistently failed where the thickness of the tissue was minimum rather than at the contact between the tissue and the sutures.

The equilibrium force and deformation response to tensile loading of the intact tissues span a wide range for all samples in the nonpregnant and gestation time point groups (raw dataset, doi:10.7916/D8DN44QW). This range of the data reflects variations in the tissue size among specimens within each group. In addition, because we keep the whole cervical tissue intact, the tensile tests performed here induced a complex combined loading state to the samples. This combined loading condition includes swelling due to osmotic pressure of the GAGs and the combination of unbending and axial tension due to the displacement of the sutures (Fig. 7). The samples begin in their unloaded reference configuration as thick-walled cylindrical structures in 2M NaCl, where the FCD due to the GAGs are shielded. During osmotic loading in physiologic 0.15M NaCl, the samples increase in volume while preserving their thick-walled shape. This swelling is observed experimentally (Table 1, [8]) and in our simulations (Fig. 7).

3.2. Inverse Finite Element Analysis

The degree of tissue unbending during suture displacement is different for each sample because of the variations in the initial geometry. Thus, to interpret our mechanical test results, we performed sample specific finite element analyses to determine material properties. This unbending is evident in the FEA simulations by a gradient of axial stretch (the Y-direction, Fig. 7) through the cross-section from the inner canal to the outer edge of the tissue. This gradient is due to the addition of axial tension at the inner canal and compression at the outer edge due to unbending and axial tensile stretch imposed by the suture displacement. When the cervical tissue sample is cut along its length, the tissue tends to curl up, demonstrating the natural tendency of the tissue to maintain its cylindrical shape. This observation highlights the importance of

incorporating the unbending of the tissue in our mechanical test analysis.

The porous fiber composite material model, captures the whole-specimen mouse cervical multi-axial loading response to tensile displacement (Figs. 8,9 and supplemental figures), where the average best-fit parameters are listed in Table 3 and quality of fits are listed in Table 4. Sample specific best-fit parameters and quality of fits are available as part of the raw dataset (doi:10.7916/D8DN44QW). Changes to the material parameters, and not to the material model, captures the drastic softening that occurs during pregnancy. The osmotic pressure contribution from the charged GAGs captures the swelling behavior from the reference configuration in the 2M NaCl to the physiologic conditions in 0.15M NaCl. The addition of the solid network captures the tensile force-displacement response (Fig. 8&9,A) while also capturing the deformation responses in directions orthogonal to loading (Fig. 8&9,B-E).

We validated our model by predicting the cervical opening $[co]$ vs. width $[w]$, length $[l]$, height $[h]$, and thickness $[t]$ data (Fig. 8&9,B-E). Note here that the height $[h]$ and thickness $[t]$ changes in our model are not independent, since our modeling assumptions requires that $h = co + 2t$. Both measurements of $[h]$ and $[t]$ are included here, however, because experimental measurements of the thickness at the top and bottom of the cervix sample were not always equal. For all of but one sample, the model was able to predict the $[co]$ vs. $[w]$ measurements, demonstrating the ability of our model to capture the combined loading state induced during our test. Only one sample had a negative r^2 value, which is the representative nonpregnant sample shown in Fig. 8. In some of the stiffer nonpregnant and d6 samples, the model underestimated the degree of length deformation $[co]$ vs. $[l]$, indicated by low and negative r^2 values (Table 4). This trend indicates that the tension-compression nonlinearity may be more pronounced in these samples than what the current model is able to capture. This notion is confirmed by better fits for the $[co]$ vs. $[l]$ with higher average r^2 values for the more compliant d12, d15, and d18 samples. The model was able to predict both the cervical opening $[co]$ vs. height $[h]$ and thickness $[t]$ curves. Similar to the $[co]$ vs. $[l]$ curves, the model was able to better predict the

more compliant pregnant d12, d15, and d18 samples, indicated by the higher r^2 values for these samples.

460 3.3. Material Property Changes of the Cervical Tissue with Pregnancy

Throughout the course of a mouse pregnancy, the parameters associated with the stiffness of the collagen fibers $[\xi]$ and the ground substance $[E_{GS}]$ of the cervix decreases dramatically (Table 3, Fig. 10). The fiber stiffness decreases more than three orders of magnitude (average NP: 5.0 [MPa], average d18: 465 9.8e-4 [MPa]), while the stiffness associated with the ground substance $[E_{GS}]$ decreases two orders of magnitude (average NP: 0.12 [MPa], average d18: 7.0e-4 [MPa]) during gestation (Fig. 10). The mouse cervix does not soften within the first 6 days of gestation. The differences in the average collagen fiber stiffness $[\xi]$ between the nonpregnant tissue (NP average $\xi = 5.0$ [MPa]) and gestation 470 day 6 (d6 average $\xi = 3.4$ [MPa]) are negligible, and there is a slight increase in the Young's modulus $[E_{GS}]$ of the ground substance (NP average: 0.12 [MPa], d6 average: 0.26 [MPa]). Statistical analysis concluded that the NP collagen fiber stiffness, $[\xi]$ was significantly stiffer compared to d12, d15, and d18 values ($p < 0.05$).

475 The cervix begins to gradually soften between d6 and d12 of gestation. During this gradual softening, both the collagen fiber stiffness $[\xi]$ and the Young's modulus $[E_{GS}]$ of the ground substance declines by about 16% per gestation day (Table 3, Fig. 10). The mouse cervix begins to accelerate its remodeling between gestation d12 and d15. Within this smaller time frame, the material 480 parameters $[\xi]$ and $[E_{GS}]$ decreases at a rate of about 30% per gestation day. Between gestation d15 and d18, the collagen fiber modulus $[\xi]$ continues to decrease by 30% per day, but the Young's modulus $[E_{GS}]$ of the ground substance slightly increases at about 37% per day. There were no obvious trends in the Poisson's ratio $[\nu_{GS}]$ nor the fiber stiffening parameter $[\beta]$ with progressing 485 gestation (Table 3).

4. Discussion

Quantifying the material property changes of the uterine cervix during pregnancy has been a long-standing challenge because human tissue studies are understandably difficult. Hence, determining the contributions of mechanical forces in premature cervical remodeling and preterm birth remain elusive. Mouse models of pregnancy are an invaluable research tool because of the potential to investigate defined mechanisms of abnormal tissue remodeling. The mechanical testing of the mouse cervix, however, is challenging because of its small volume, natural curvature, and complicated geometry. Thus, studies that distinguish material properties from structural mechanical tests have been scarce. As a result, the utility of the established mouse models of normal and abnormal cervical remodeling have been limited. In the study presented here, we overcome these challenges to gain the full advantage of these mouse models of pregnancy. We present an experimental and modeling framework to quantify the material properties of mouse cervical tissue. As a first step, we investigate the equilibrium material behavior of gestation-timed tissue during normal remodeling.

In a previous mechanical investigation, we demonstrate that mouse cervical tissue swells considerably from a hyperosmotic 2M NaCl solution to physiological 0.15M NaCl by gestation d18, despite insignificant overall changes in the FCD throughout pregnancy (Table 1, [15, 8]). These initial osmotic swelling tests provide information on the interaction of the charged GAGs and the solid matrix, but the level of deformation during these tests are modest, especially in the nonpregnant samples. In the study presented here, a common type of mouse cervix mechanical test with minimal specimen preparation is performed. Whole tissue samples from wild-type mice are pulled in tension via a ring test by pulling apart two surgical sutures through the inner canal while the force on the sutures is measured using custom-made grips attached to a universal material tester (Fig. 1B-E). Tensile tests are performed on cervical samples from gestation-timed mice, and cervical material properties are quantified us-

ing an IFEA framework (Table 3, Fig. 10). The porous fiber composite material model presented here captures the cervical material response to tensile loading (Figs. 8, 9), where the decline of both the stiffness of the ground substance and individual collagen fibers begins at day 6 of a 19 day gestation period (Fig. 10).

520 The *in-vivo* loading state of the human and mouse cervix during pregnancy is multi-axial and heterogeneous, with directionality and magnitude of loading dependent on numerous maternal and fetal anatomical and structural factors [35, 36]. In human pregnancy, the magnitude of loading on the human cervix is dependent on the active and passive loading from the growing contents of the
525 amniotic sac and uterus as well as on the material properties of the amniotic sac, uterus, and cervix. The directionality and pattern of loading is dependent on the placement of the uterus and cervix within the abdomen and the anatomical shape of the uterus and cervix, specifically the patient-specific asymmetry of these organs [35]. A key mechanical function of the cervix in pregnancy is to resist hoop tensile stresses around the inner canal from compressive loads applied
530 by the pressurized amniotic sac. The magnitude of cervical hoop stress and stretch in pregnancy remains to be determined. At parturition we can estimate that the inner canal, which varies considerably from 0.1 cm to 1 cm depending on the woman’s past pregnancies, must soften and stretch to accommodate the
535 fetal head, which on average is approximately 10 cm in diameter.

The *in-vivo* mechanical loading state of the mouse cervix during pregnancy is currently unknown. Drawing on similarities and differences in anatomy between humans and mice, we can hypothesize that the magnitude of loading on the mouse cervix is dependent on litter size/shape and the material properties
540 of the soft tissues supporting the pups. We can also hypothesize that the mouse cervix experiences the similar tensile hoop stresses circling the inner canal during pregnancy from the mechanical load of the pups being pushed longitudinally onto the cervix by the stretched uterus. At parturition the average fetal diameter of a pup at birth is about 8 mm [37], which translates to a circumferential
545 opening of approximately 25 mm. Based on average tissue dimensions measured in our experiments, we estimate the average undeformed circumference

of a pregnant d18 cervix to be about 9 mm. Therefore, we can estimate that the circumferential stretch of the cervix during delivery to be about 2.78. We rationalize that starting with a straight-forward mechanical test that examines the tensile response of the tissue in the circumferential direction interrogates the key mechanical function of the cervix during pregnancy-resisting circumferential hoop stresses to prevent cervical dilation. The tensile tests, as presented here, achieve large deformations at each gestational time point. At gestation d18 the cervical opening reaches a maximum ramp-hold level of 7 mm, which roughly translates to a circumferential opening of approximately 14 mm. In our FEA models, the average of the maximum right Cauchy-Green stretch in the Y-direction was 2.59 (Fig. 7), which is similar to our estimate of the state of *in-vivo* stretch during delivery of 2.78.

4.1. Cervical Remodeling in Pregnancy

The mouse cervix remodels considerably in pregnancy starting at day 6. To accommodate this softening and the drastic distensibility, the material parameters associated with the solid components of the material decline (Fig. 10). Both the modulus associated with the collagen fiber stiffness $[\xi]$ and the Young's modulus of the ground substance $[E_{GS}]$ decrease by multiple orders of magnitude. This evolution of cervical remodeling parameters, particularly of the fiber network, is supported by our previous mechanical and biochemical studies in both human and mouse. We account for swelling in the material model using a charge dependent Donnan contribution to the interstitial pressure. According to the Donnan pressure/GAG relationship, the swelling pressure does not increase significantly with gestation because the total amount of GAGs does not change significantly. Therefore, the increase in swelling must be accommodated by changes to the solid components of the material.

Considering the mode of deformation of the mechanical tests presented here mostly interrogates the tensile properties of the solid network, it follows that the large distensibility achieved by the advanced gestation cervical tissue is due to the remodeling of the solid fiber network. This idea is supported by our

detailed analysis of cervical collagen crosslink types in normal mouse tissue [7], where we see a decline in mature collagen crosslinks starting at gestation d6, but no changes in the collagen crosslinks from d15 to d18. In human cervical tissue
580 we know that the tissue solubility in a dilute solution of acetic acid significantly increases from the nonpregnant state to term, indicating a reduction in stable collagen crosslinks [14]. Therefore, we can conclude that the collagen crosslinks, in part, play a key role in cervical softening during pregnancy. Continued studies are currently underway to understand how the tissue continues to soften in
585 the late stage of gestation (from d15 to d18 in the mouse) while the collagen crosslink content remains the same. To address this issue, we are conducting detailed biochemical analyses and additional mechanical tests to differentiate the contribution of the collagen network apart from other solid ECM components that add to the material response of the solid network (e.g. elastin).

590 4.2. Comparisons to Human Cervical Tissue

The ECM components in the nonpregnant cervix and measured material changes in pregnant tissue are similar between mouse and human tissue [4, 36]. The porous fiber composite material model presented here is an iteration of a material model we developed for the equilibrium material response of *ex-*
595 *vivo* human cervical tissue samples [26], with the addition of charged pores to account for the more pronounced swelling of the mouse tissue. Figure 11 compares the material response to uniaxial tension using the material parameters for human [26, 35] and mouse tissue. Overall, the human tissue is slightly softer than the mouse tissue, for both the nonpregnant and late pregnancy time points.
600 This difference is most likely an artifact of using cut tissue strips for the human tissue tests as opposed to keeping the tissue intact as we do for the mouse tissue. For these previous studies on the mechanical properties of the human cervix, we cut the whole cervix into axial slices (from the internal to the external os) and cut rectangular strips from these slices. **We expect that by** cutting these strips,
605 we interrupt the intact collagen fiber network, which would reasonably reduce the fibers' ability to sustain tension. Despite this slight difference, the overall

decline in material properties from the nonpregnant to the late pregnant tissue is the same order of magnitude. For example, the average collagen fiber stiffness declines from 4.9 [MPa] to $9.7\text{e-}4$ [MPa] for the mouse and from 0.68 [MPa] to $7.4\text{e-}4$ [MPa] for the human. (Note, here we use a material model without a preferentially-aligned collagen network for both human and mouse cervical tissue).

The mouse tissue mechanical data also reflect similar softening trends as seen in *in-vivo* investigations of pregnant human cervical tissue [38, 39, 40]. In a balloon inflation study of the human cervix, material properties soften between early 1st trimester (measured between 7-8 weeks of gestation) and late 3rd trimester (measured between 38-40 weeks of gestation) [39, 40]. The authors use a discrete 3-fiber family composite material model, with a standard exponential-power law to model the collagen fiber constitutive behavior. From early pregnancy to late pregnancy they found a 25 fold decrease in the stiffness modulus associated with the collagen fiber. In the balloon study there was no comparison between nonpregnant and 1st trimester tissue. Therefore, it is unclear from this study if the cervix has remodeled in early pregnancy.

An *in-vivo* aspiration study of the pregnant human cervix shows that the human cervix remodels and softens during pregnancy [38]. The aspiration tool interrogates the end of the cervix protruding into the vaginal canal(external os), and cervical stiffness measurements are taken throughout pregnancy. Aspiration data show that the human cervix begins to remodel and soften as early as the 1st trimester and continues to decline with progressing gestation. In the mouse model of normal cervical remodeling we see tissue softening starting at gestation d6, which roughly translates to the end of a 1st trimester if the gestation days are evenly divided. Unlike the human cervix there is no softening that occurs between the nonpregnant state and the end of the 1st trimester for the mouse. Similar to the human, though, we see substantial remodeling in the 2nd trimester. This mid-pregnancy remodeling has been known to occur in mice [4], and proven here with our quantitative assessment of mouse cervical remodeling. The recent *in-vivo* human data and the material similarities of the mouse cervix

presented here, confirm the utility of the mouse model to investigate the material property changes of the cervix during pregnancy. Regardless of the anatomical differences between mouse and human tissue, mouse tissue achieves the same level of material remodeling and by similar ECM mechanisms as seen in human tissue.

4.3. Porous Fiber Composite Material Model

A porous fiber composite material model with a continuously distributed collagen fiber network is able to describe the evolution of cervical material behavior during pregnancy, with corresponding shifts in the ECM-associated material parameters. The material model is designed to account for the collagenous nature of the tissue and the remodeling of individual collagen fibers and the surrounding ground substance. Key characteristics of the material model are: 1) the swelling due to charged GAGs, 2) the recruitment of off-axis collagen fibers in the direction of tension 3) the uncrimping of the individual collagen fibers, and 4) the nonlinearity of the tension-compression behavior of the tissue. If we attempt to fit a standard non-linear Veronda-Westmann material model to the data, we are able to capture the J-curve of the force vs. cervical opening for both nonpregnant and pregnant d18 tissue but not the nonpregnant cervical opening versus thickness and length changes. This is because the Veronda-Westmann model does not account for the difference in the compressive response compared to tension (Fig. 12). Since the tension-compression nonlinearity of the pregnant d18 tissue is much less pronounced compared to the nonpregnant tissue, the Veronda-Westmann model is able to capture the force and deformation response of the pregnant sample. Next, if we fit a material model with discrete fibers (not continuously distributed) where all of the fibers are in the circumferential direction, we are able to capture the cervical opening vs. force response (Fig. 12). However, the discrete fiber model predicts that with increasing cervical opening, the cylindrical length expands, which was not observed in any of our experiments. If we fit a material model with three orthogonal discrete fibers, the expansion in the cylindrical length is less exaggerated, but still expands with in-

creasing cervical opening. One of the advantages of the continuously distributed fiber model is its ability to capture the non-linearity of the overall Poisson's effect between tension and compression, where the Poisson's ratio is very small in compression and large in tension as seen experimentally in cartilage [41].

4.4. Limitations

In this study, we determine the quantitative changes in the material properties of the mouse cervix in pregnancy. There are, however, limitations to this study. First, the mechanical test conducted in this study imposes a complex loading state on the whole cervix sample, which is not ideal for teasing out contributions from individual ECM components. For a more complete material characterization of the cervical tissue, additional compression, and inflation, and opening angle measurement [42, 43] tests can be conducted. Second, the stretch states reported in this paper have not been verified via local strain measurement methods. In our preliminary studies, we attempted utilizing a non-contact strain measurement system for the mouse tissue. However, we observed from our FEA models that the surface of the cylinder does not stretch significantly before breaking in our NP and d6 samples. In addition, the late pregnant cervix develops a thick mucous layer on the surface of the tissue, which is not easily removed without damaging the delicate tissue. We were unable to stain the tissue reliably with waterproof ink we previously used in human tissue studies [24]. Due to these difficulties, we determined that an overall geometry analysis was a good starting point to determine the material property changes as reported here. SecondThird, we approximate the whole cervix as an ideal cylinder in this study. In reality, the cervix has a complex geometry with folds that exist in the inner canal and various undulations in the length. For this reason, we report the average and standard deviation for each of our geometry measurements to reflect the deviation of the actual geometry from a perfect cylinder. ThirdFourth, damage may be occurring at the higher cervical openings. We are currently conducting load-unload mechanical tests to quantify this and will add this feature to our material model. FourthFifth, the material

response during the hold portion of the force-relaxation test for the advanced gestation mouse cervical tissue and the larger cervical openings do not reach
700 a true equilibrium, even after an hour of holding the displacement. Because the length of the mechanical tests were reaching upwards to 10 to 12 hours, we did not want to the tissue to degrade. Hence, we limited the time of the hold. Finally, we use a phenomenological strain energy density for the collagen fiber in our model. This limits our ability to directly correlate material parameters to
705 the microstructure and properties of the collagen fibers (i.e. collagen crosslinks). In the next iteration of the model, we will incorporate a strain energy density for the fiber which accounts for the gradual recruitment of the crimped collagen fibers for a more physically relevant model [44, 45, 46].

5. Conclusions

710 The experimental and modeling study presented here tackles a long-standing challenge in understanding the progression of material property changes in mouse cervical tissue from nonpregnancy to term gestation. Whole mouse cervical tissue samples are pulled in tension by moving apart two tensioned surgical sutures threaded through the inner canal. Using an IFEA coupled with a porous
715 fiber composite material model, we quantitatively define the remodeling stages in pregnancy in terms of cervical material behavior (Fig. 10). The mechanical testing results reported here confirm previous structural studies that the cervix remodels in stages, with gradual softening occurring between gestation d6 and d12 and accelerated softening occurring after gestation d12. These re-
720 sults provide a framework to tease out the contribution of the cervical ECM in the remodeling events during pregnancy.

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Conflict of interest statement

None.

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Gestation Day	Water volume fraction ϕ_r^w	FCD [mEq/L]
NP	0.74	-10
d6	0.75	-7
d12	0.76	-8
d15	0.81	-7
d18	0.81	-10

Table 1: Reference water volume fraction and fixed charge density (FCD) values used for modeling. Water volume fraction was determined experimentally and FCD was calculated using equation (3) using sGAG values reported in [15]

Gestation Day	Swelling Ratio 2M to 0.15M, $J_{0.15M}$
NP , n= 5	1.05*
d6 , n=1	1.00
d12 , n=5	1.08*
d15 , n=3	1.14
d18 , n=5	1.30*

Table 2: Swelling ratio from 2M to 0.15M NaCl for each gestation group.* values are from [8]. Additional samples were measured for d6 and d15 for this study using the same protocol

Gestation Day	E_{GS} [MPa]	ν_{GS}	β	ξ [MPa]	$\% \Delta E_{GS} / \text{day}$	$\% \Delta \xi / \text{day}$
NP , n= 5	0.12 $\pm$ 0.20	0.25 $\pm$ 0.06	2.9 $\pm$ 0.9	5.0 $\pm$ 3.6	—	—
d6 , n=4	0.26 $\pm$ 0.31	0.24 $\pm$ 0.10	2.2 $\pm$ 0.1	3.4 $\pm$ 2.8	+20%	-5.2%
d12 , n=3	3.8e-3 $\pm$ 4.4e-3	0.34 $\pm$ 0.04	3.0 $\pm$ 0.4	0.22 $^{\dagger} \pm$ 0.32	-16%	-16%
d15 , n=6	3.3e-4 $\pm$ 5.7e-4	0.21 $\pm$ 0.10	3.1 $\pm$ 0.5	1.2e-2 $^{\dagger} \pm$ 2.0e-2	-30%	-32%
d18 , n=4	7.0e-4 $\pm$ 1.8e-4	0.33 $\pm$ 0.07	3.0 $\pm$ 0.1	9.7e-4 $^{\dagger} \pm$ 9.6e-4	+37%	-31%

Table 3: Changes in cervical material parameters throughout pregnancy. Average values $\pm$ standard deviation of the parameters are listed, along with percent change per day of the stiffness measures, E_{GS} and ξ . † indicates statistically significant difference compared to NP values.

Gestation Day	Fit error		Prediction error			
	$J_{0.15M}$	[co vs. F]	[co vs. w]	[co vs. l]	[co vs. h]	[co vs. t]
	% error	r^2	r^2	r^2	r^2	r^2
NP	-2.6±3.0%	0.98±0.02	0.74±0.48	0.18±0.60	0.82±0.20	0.89±0.07
d6	0.070± 0.058%	0.98±0.03	0.79±0.13	0.10±0.79	0.50±0.26	0.54±0.36
d12	-0.87±3.82%	0.95±0.02	0.96±0.02	0.71±0.22	0.97±0.03	0.89±0.13
d15	13.0±6.8%	0.92±0.05	0.95±0.06	0.56±0.38	0.96±0.02	0.88±0.06
d18	5.0 ±3.9%	0.98 ±0.02	0.86 ±0.08	0.66±0.20	0.94 ±0.02	0.75 ±0.09

Table 4: **Average fit and prediction values $\pm$ standard deviation.** The proposed model fit the mechanical response [co vs. F] for all samples (overall average r^2 value: 0.97). For the stiffer NP and d6 samples, the model underpredicted the degree of deformation in the length [l], height [h], and thickness [t] indicating that the tension-compression nonlinearity may be more pronounced in samples than what the current model is able to capture. The higher r^2 values for the width [w] demonstrates the ability of our model to capture the combined loading state during out test.

Figure 1: **Experimental setup for mechanical tensile testing.** A) Diagram of a female mouse reproductive tract. The cervix was detached from the uterus at the cut line, then the vaginal wall was removed. B) Universal mechanical testing machine outfitted with a 10N load cell. Samples were submerged in a PBS bath throughout testing while two perpendicular cameras continuously monitored sample geometry. C) Camera 1 view of the tensile grips and internal os of the cervix. D) Camera 2 length view of the cervix sample. E) Diagram of the custom tensile grips used for mechanical testing.

Figure 2: **Measurements of cervical geometry parameters.** A) Thick-walled cylinder representation of the cervical geometry labeled with measured parameters. B) An example illustrating how the geometry parameters (in this case the height $[h]$) were measured. Using a 0.2mm grid overlay as a guide, multiple measurements were measured for each parameter. The average and standard deviation for $[w, l, h, t]$ were determined. C-D) Camera images illustrating the average width $[w]$, length $[l]$, height $[h]$, and thickness $[t]$. The suture-to-suture distance was also measured to determine cervical opening $[co]$.

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Figure 3: **Finite element analysis geometry and boundary conditions.** A) 1/32 model based on sample measurements used for finite element analysis. B) Boundary conditions to reflect symmetry and contact type used. The cervix was modeled as a porous fiber composite material and the suture was modeled as a rigid body.

Figure 4: **Overview of the finite element analysis.** Top row shows the single element analysis to determine $J_{0.15M}$ for a set of material parameters. Second row shows how the sample specific model was scaled based on $J_{0.15M}$. Bottom row shows the main analysis for the FEA.

Figure 5: **Cervical tissue softens dramatically with progressing gestation.** Plot of cervical opening $[co]$ versus force $[F]$ for whole, intact cervical tissue samples at progressive gestational dates. A representative sample from each group (NP, d6, d12, d15, d18) is shown here.

Figure 6: **Tissue size, compliance and distensibility increases with progressing gestation.** Stress-relaxation response for a representative (A) nonpregnant, (B) gestation day 12, and (C) gestation day 18 samples. Symbols represent the points taken at the end of each ramp-hold to determine experimental curves. Images demonstrate differences in tissue size and deformation for NP (D and G), d12 (E and H), and d18 (F and I) and corresponding cervical opening $[co]$ measurements. For each gestation group, the left image shows the tissue swollen in 0.15M NaCl with no applied load, the middle image shows the tissue halfway throughout the test, and the right most image shows the tissue loaded at the last ramp-hold held by the tissue before yielding/breaking.

Figure 7: **FEA simulation of whole tissue tension tests.** Screenshots of the FEA simulation for nonpregnant (top row), d12 (middle row), and d18 (bottom row) representative samples. Colors contours represent the fiber stretch in the y direction. The first column shows the model in the reference 2M unswollen state. The second column shows the stretch after swelling in 0.15M NaCl. Third and fourth column shows the model midway through the deformation and at the maximum cervical opening held respectively (same cervical opening levels as shown in Figure 6)

Figure 8: **Representative IFEA results for a nonpregnant (NP) cervix.** Symbols represent experimental data points, solid lines represent model fits, and dashed lines represent model predictions for A) cervical opening vs. force, B) cervical opening vs. width, C) cervical opening vs. length, D) cervical opening vs. height, and E) cervical opening vs. top and bottom thickness. Best fit model parameters for this sample are reported as well as the r^2 values for the fit and predictions.

Figure 9: **Representative IFEA results for a pregnant gestation day 18 (d18) cervix.** Symbols represent experimental data points, solid lines represent model fits, and dashed lines represent model predictions for A) cervical opening vs. force, B) cervical opening vs. width, C) cervical opening vs. length, D) cervical opening vs. height, and E) cervical opening vs. top and bottom thickness. Best fit model parameters for this sample are reported as well as the r^2 values for the fit and predictions.

Figure 10: **Collagen fiber stiffness $[\xi]$ and ground substance stiffness $[E_{GS}]$ changes with pregnancy from the IFEA study.** Plot demonstrating the quantitative changes in the material parameters with pregnancy on a log scale. Symbols represent average values and shaded areas represent the range of parameters at gestation time points determined via IFEA.

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Figure 11: **Comparisons between material properties of mouse and human non-pregnant and pregnant cervix.** Material parameters values for nonpregnant and pregnant mouse are based on the average nonpregnant and gestation day 18 values (Table 3). Non-pregnant and pregnant human values from [26, 35]. Inset plot demonstrates that the pregnant mouse tissue has a stiffer response compared to pregnant human tissue.

Figure 12: **Comparisons between proposed model, Veronda-Westmann, and discrete fiber models Neo-Hookean model.** A porous fiber composite model with a continuously distributed fiber network simultaneously captures the force-displacement and deformation data. A Neo-Hookean model is unable to capture the nonlinear force-displacement data and while a Veronda-Westmann model is able to capture the force-displacement data, but overestimates the compressive response (length and thickness) due to the lack of tension-compression nonlinearity response. A material model with discrete fibers arranged circumferentially is able to capture the force-displacement data, but predicts length expansion with increasing deformation. A material model with three orthogonal discrete fibers lessens this length expansion, but still does not capture the experimental data.

1. Measure tensile equilibrium properties of the cervix for a normal mouse pregnancy

2. Fit sample specific inverse finite element model to experimental tensile data

3. Determine the evolution of cervical material properties for a normal mouse pregnancy

Statement of Significance

Accelerated cervical remodeling can lead to extremely premature births. Little is known, however, about the material property changes of the cervix in pregnancy because pregnant human tissue samples are limited. Rodent models overcome this limitation and provide access to gestation-timed samples. Measuring the material property changes of the mouse cervix in pregnancy is challenging due to its small size and complex geometry. Here, we establish a combined experimental and modeling framework. We use this framework to determine the cervical material property changes throughout a normal mouse pregnancy. We present our experimental methods for mechanically testing whole, intact cervical tissue samples. We fit a porous fiber composite material model to the mechanical data and show that the mouse cervix begins to soften at day 6 of a 19-day gestation period.

Figure(s)

A Mouse reproductive tract anatomy

B

E

Figure(s)

A Model geometry **B** Boundary Conditions and materials

Figure(s)

Step 1: Guess $[E, \nu, \alpha, \beta, \xi]$ and assign to cube

Step 2: Run pre-analysis and calculate $J_{0.15M}$

Swell from 2M to 0.15M

Step 3: Scale cylindrical geometry by $(J_{0.15M})^{1/3}$ and assign $[E, \nu, \alpha, \beta, \xi]$ to model

Scale model

Step 4: Run analysis with scaled geometry

Swell
(2M $\rightarrow$ 0.15M)

displace
suture

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)