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Inflation and rupture of vaginal tissue

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Around 80% of women experience vaginal tears during labour when the diameter of the vagina must increase to allow the passage of a full-term baby. Current techniques for evaluating vaginal tears are qualitative and often lead to an incorrect diagnosis and inadequate treatment, severely compromising the quality of life of women. In order to characterize the failure properties of the vaginal tissue, whole vaginal tracts from rats (n = 18)were subjected to free-extension inflation tests until rupture using a custombuilt experimental set-up. The resulting deformations were measured using the digital image correlation technique. Overall, the strain and changes in curvature in the hoop direction were significantly larger relative to the axial direction. At a failure pressure of 110 ± 23 kPa (mean \pm s.d.), the hoop and axial stresses were computed to be 970 \pm 340 kPa and 490 \pm 170 kPa, respectively. Moreover, at such pressure, the hoop and axial strains were found to be 12.8 \pm 4.4% and 6.4 \pm 3.7%, respectively. Rupture of the vaginal specimens always occurred in the hoop direction by tearing along the axial direction. This knowledge about the rupture properties of the vaginal tissue will be crucial for the development of clinical approaches for preventing and mitigating vaginal tearing and the associated short- and long-term traumatic conditions.

1. Introduction

Roughly 4 million women give birth each year in the USA with 2.7 million women undergoing vaginal deliveries [1]. Unfortunately, adverse events during childbirth are very common with up to 80% of all vaginal deliveries resulting in some degree of laceration to the vagina and surrounding tissues [2,3]. The lesions vary from a small tear that is likely to be of no harm to extensive lacerations that involve the muscles of the pelvic floor and lead to long-term faecal and urinary incontinence and sexual dysfunction [4]. In addition to pain, the lacerations have damaging and lasting psychological effects on the mothers including post-traumatic stress disorder, depression, anxiety, poor perception of body image and feelings of embarrassment [5]. Risk factors for tears (e.g. episiotomy, baby weight over 4 kg, forceps delivery and prolonged second stage of labour) have been investigated [6,7]. But, surprisingly, although trauma starts at the vaginal wall as the fetus passes through during delivery, very little is known about the mechanical properties of the vagina.

The vagina is a fibromuscular tubular organ that is primarily composed of three layers: the tunica mucosa, the tunica muscularis and the tunica adventitia [8]. The tunica mucosa is made of a keratinized stratified squamous epithelium and a thick lamina propria of dense connective tissue. The tunica muscularis is composed of two indistinguishable layers of smooth muscle cells that are oriented in the hoop and axial directions. The tunica adventitia consists primarily of a thick layer of loose connective tissue. The mechanical properties of the vaginal tissue have been characterized primarily by testing strips of vaginal tissue via uniaxial tensile tests as discussed in our review article [9]. Overall, the vagina was found to be nonlinear elastic, exhibiting the typical nonlinear stress–strain response of soft tissues with an initial nonlinear region, the

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so-called *toe-region*, that transitions into a linear region. In addition, the vagina displayed a viscoelastic behaviour with the stress decreasing over time in response to constant strain.

Biaxial tests, which are more physiologically relevant than uniaxial tests, have also been performed to quantify the mechanical properties of the vaginal wall [10-12]. The inflation-extension biaxial tests by Miller's group revealed the anisotropy and axial coupling of the vagina [10]. More recently, we quantified the anisotropy of the vaginal tissue by performing planar biaxial tests while also inducing the contraction of the tissue chemically and electrically [11]. In both Miller's study [10] and our study [11], the vagina was stretched up to sub-failure loads and, for this reason, the loading conditions that lead to tearing were not investigated. Recently, we provided the first characterization of the tear behaviour of the vagina under planar biaxial loading [12]. However, in this study, the tears were pre-imposed and the formation of a tear was not studied. Thus, despite the current body of work, new mechanical studies are needed to determine how the vaginal wall tears.

In the present study, we provide the first mechanical characterization of the vagina up to tearing. Towards this end, rat vaginas are inflated while allowing their free extension in the axial direction using a custom-built experimental set-up. The digital image correlation method is employed to measure the strain field of the vagina under inflation. The findings of this study will help in advancing our limited knowledge of the tear formation mechanisms of the vaginal tissue.

2. Methods

The current study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech. A total of 18 three-month-old, virgin female Sprague– Dawley rats were used for this study. Rats were sacrificed by decapitation, frozen at -20° C and allowed to thaw over 2–3 days at 4°C before the vaginal tracts were isolated. The vaginal specimens were hydrated with phosphate-buffered saline (PBS, pH 7.4, Fisher Scientific, USA) and frozen at -20° C until mechanical experiments were performed.

Prior to testing, vaginal tissue was thawed in PBS. Images of the vaginas were collected under a dissection microscope using a CMOS camera (Thorlabs Inc., Newton, NJ). Measurements of the initial inner radii and thicknesses were performed with ImageJ (NIH, Bethesda, MD). The mean and standard deviation (s.d.) of these measurements are reported in table 1. Each specimen was mounted onto two stainless steel dispensing needles as schematically presented in figure 1. In a horizontal position, nitrile rubber o-rings were placed onto each dispensing needle and glued in place. Specimens were then carefully pulled over the dispensing needles and o-rings, tied down with a nylon thread and the ends wrapped with Teflon tape to ensure a tight seal. The mounting design was oriented vertically for testing to allow the upper needle to freely extend relative to the fixed lower needle as the vagina was inflated. Following mounting, the axial length between the clamped ends of each specimen was measured via calipers (accuracy ± 0.1 mm, Mitutoyo Absolute Low Force Calipers Series 573, Japan). The mean and s.d. of these measurements are also reported in table 1. Specimens were then dyed blue with an aqueous methylene blue solution (1% w/v) and speckled with an aerosol fast dry gloss white paint. This created a high contrast speckle pattern on the specimen surface for non-contact strain measurements [13]. Throughout the preparation process, the specimens were hydrated with PBS.

Table 1. Mean (\pm s.d.) of geometrical quantities for n = 18 vaginal specimens prior to mechanical testing.

	inner radius (mm)	thickness (mm)	length (mm)
mean	2.58	0.29	11.9
s.d.	0.06	0.07	1.1

Testing was performed using a custom-built inflation setup (figure 2). The specimen was immersed in a bath made of acrylic glass and connected via plastic tubing to a 345 kPa pressure transducer (accuracy $\pm 0.03\%$, Omega Engineering Inc., Norwalk, CT) and a computer controlled syringe pump (accuracy ±1%, New Era Pump Systems Inc., Farmingdale, NY). The pressure transducer was interfaced with the computer via an NI myDAQ (accuracy $\pm 0.5\% + 20$ mV, National Instruments, Austin, TX). The pump provided precision control over the volume and flow rate of PBS infused into the specimen while the resulting pressures were measured by the pressure transducer and recorded by a custom Matlab script (MathWorks[®], Natick, MA). Preliminary tests were performed to determine the experimental parameters adequate for this study. The weight of the upper dispensing needle assembly was found to be 0.0259 ± 0.0003 N. The flow rate, pre-load, preconditioning amplitude and number of cycles were selected to be 4.7 ml min⁻¹, 1.4 kPa, 100% increase in initial volume and 20 cycles, respectively. These parameters provided a consistent reference configuration, relatively low pressures throughout preconditioning (less than 10% of the average rupture pressure) and an infusion similar to other soft tissue inflation studies [14,15].

For testing, the specimens were first mounted into the water bath such that the dorsal side of the vaginal tissue was visible. The water bath was then filled with PBS at room temperature (approx. 20°C). Specimens were pre-loaded to a pressure of 1.4 kPa, established as the reference configuration for each test, and preconditioned from the reference configuration to a 100% increase in volume and back at a flow rate of 4.7 ml min⁻¹ for 20 cycles. The specimens were then unloaded, allowed to recover for 600 s, pre-loaded again to 1.4 kPa, and then inflated until rupture at a flow rate of 4.7 ml min⁻¹. Pressure and volume data were collected throughout each test up to rupture. High-resolution $(2448 \times 2048 \text{ pixels})$ images of the specimens were captured at 20 Hz during testing via two CMOS cameras (Basler ace acA2440-75 um, Basler, Inc., Exton, PA) equipped with c-mount lenses (Xenoplan 2.8/50, Schneider Optics Inc., Hauppauge, NY). Non-contact strain measurements were performed with a 3D-DIC system (Vic-3D 8, Correlated Solutions, Columbia, SC) that was calibrated via imaging of a $9 \times$ 9 mm² plastic grid with 3 mm spacing.

Normal local Lagrangian strains in the hoop and axial directions were obtained for each specimen. The average values of these strains were calculated over a smaller circular region of interest at the centre of each specimen, resulting in a single average normal Lagrangian strain in the hoop direction and a single average normal Lagrangian strain in the axial direction for every image recorded during testing. The average normal Lagrangian strains will be referred to simply as 'strain' along the hoop and axial directions hereafter. Local curvature data were also collected and average curvatures in the hoop and axial direction were calculated in the same manner as the strain data.

Normal stresses in the hoop and axial directions were calculated from the recorded pressure data by assuming that the inflated vaginal tissue represented a closed cylindrical pressure vessel. The hoop stress, $\sigma_{\theta\thetar}$ and axial stress, σ_{zzr} are given by



Figure 1. Schematic of the vagina mounted onto two needles for free-extension inflation tests. (a) Three-dimensional representation. (b) Two-dimensional representation of the transverse cross section. (Online version in colour.)



Figure 2. Schematic of the experimental set-up for testing vaginal tissue. A vaginal specimen is mounted to the set up within a water bath and connected to a pressure transducer and computer controlled syringe pump via tubing. Two lights illuminate the specimen surface and two cameras record images of the specimen surface during testing. (Online version in colour.)



Figure 3. Pressure versus volume data collected from n = 18 vaginal specimens. Data from different specimens are denoted using different colours and labelled with different letters, as denoted by the legend. The rupture point is marked by circular endpoints on each curve. (Online version in colour.)

the following equations:

$$\sigma_{\theta\theta} = \frac{2Pr_i^2}{2r_i t + t^2} \tag{2.1}$$

and

$$\sigma_{zz} = \frac{Pr_i^2}{2r_i t + t^2} - \frac{F}{\pi(r_o^2 - r_i^2)},$$
 (2.2)

where P is the internal pressure, r_i , r_o and t are the inner radius, the outer radius, and the thickness of the vagina, respectively,

and F is the compressive axial load due to the weight of the upper dispensing needle assembly. The normal stresses will be referred to simply as 'stress' along the hoop and axial directions hereafter.

All statistical analysis was performed in a statistical software package (SPSS 25.0; IBM Corp, Armonk, NY). Curvatures in the hoop and axial directions at the beginning of testing and at rupture were compared with a two-way repeated measures ANOVA. None of the assumptions for this analysis were violated. The hoop and axial stresses at rupture were compared via paired t-tests. The data contained one outlier and was not normally distributed as determined by the Shapiro-Wilk's test of normality (p = 0.003). A log 10 transformation was performed to correct for these violations. The hoop and axial strains at rupture were also compared via paired t-tests. There were no violations of the assumptions for this test. The hoop and axial strains were further compared at normalized volumes of 2, 4 and 6 with a two-way repeated measures ANOVA. These normalized volumes were obtained by dividing the volume data by the initial volume based on the geometry of each specimen (table 1). No outliers were found, but the axial strain data at normalized volumes of 2 and 4 were not normally distributed (p = 0.008 and p = 0.041, respectively). A log ₁₀ transformation was performed to correct for these violations. The ANOVA revealed a significant interaction between factors, so simple main effects were examined via one-way repeated measures ANOVAs with a Greenhouse-Geisser correction when appropriate. Finally, a paired t-test was performed to compare the hoop and axial stresses at 2% strain. This strain was selected since it was the largest strain achieved by all the specimens in both directions at rupture. There were no outliers in the stress data, but the data were not normally distributed (p = 0.026). A



Figure 4. Representative strain maps of a vaginal specimen under a pressure of 0 kPa, 62.7 kPa and 141 kPa. (*a*) Average hoop curvatures were 0.187, 0.165 and 0.146 1/mm and average hoop strains were 0, 0.084 and 0.134. (*b*) Average axial curvatures were 0.072, 0.065 and 0.058 1/mm and average axial strains were 0, 0.032 and 0.037. (Online version in colour.)

Table 2. Mean (\pm s.d.) of maximum values for pressure, volume, stresses, and strains recorded from inflation tests of n = 18 vaginal specimens.

	pressure (kPa)	volume (ml)	hoop stress (kPa)	axial stress (kPa)	hoop strain	axial strain
mean	110	2.18	970	490	0.128	0.064
s.d.	23	0.41	340	170	0.044	0.037

square root transform was performed to correct for the violation. In all cases where transforming data were necessary, the statistical tests were carried out for both the original and transformed data, and statistically significant differences were found with and without transformation. Therefore, results of the statistical tests for the original data will be reported. Statistical significance was set to p < 0.05.

3. Results

The pressure–volume relationship for vaginal tissue up to rupture was predominately linear with a slight deviation observed for one specimen (figure 3). The average slope of the linear region was 53 ± 6 kPa ml⁻¹. Accounting for the infusion rate of 4.7 ml min⁻¹, the average pressure rate was estimated to be 4 ± 0.5 kPa s⁻¹. At very low volumes and pressures, a nonlinear relationship was consistently observed. The average pressure and average volume at rupture computed from the n = 18 specimens are reported in table 2.

Strain maps over the surface of a representative specimen displaying the increase in strain in the hoop and axial directions at increasing values of the pressure are reported in figure 4. The strain in both directions was inhomogeneous and the strain in the hoop direction was of larger magnitude.

The expansion of the vagina from the reference state to the deformed state just before rupture can also be observed. As the pressure increased, the specimen appeared to maintain its cylindrical shape up to the point of failure. This observation was further quantified by calculating the average curvature of the surface in the hoop and axial directions. As the pressure increased, curvature in the hoop direction decreased while curvature in the axial direction remained somewhat constant (figure 5). On average, the hoop curvature in the reference configuration was 0.177 ± 0.020 1/mm compared to the axial curvature of 0.060 \pm 0.026 1/mm. This difference was statistically significant ($F_{1.17} = 390$, p <0.0005). At rupture, the hoop curvature of 0.140 ± 0.017 1/ mm was significantly smaller compared to the hoop curvature in the reference configuration ($F_{1,17} = 245$, p < 0.0005). The axial curvature at rupture was 0.062 \pm 0.015 1/mm which was not statistically significantly different from the axial curvature in the reference configuration ($F_{1.17} = 0.139$, p = 0.74). However, the hoop curvature at rupture was still statistically significantly larger than the axial curvature $(F_{1.17} = 372, p < 0.0005).$

In both the hoop and axial directions, there was a nonlinear increase in pressure with strain (figure 6). In the initial nonlinear region, the pressure-strain behaviour in the two



Figure 5. Pressure versus curvature data in the axial direction (solid line) and hoop direction (dashed line) collected from n = 18 vaginal specimens. Data from the same specimen are reported using the same letter and lines of the same colour, as denoted by the legend. The rupture point is marked by circular endpoints on each curve. (Online version in colour.)

directions was quite similar, but the overall behaviour in the two directions differed with increasing pressures and strains. Some irregularities were seen in the pressure–strain curves such as for specimens l and p in figure 6. These specimens experienced larger axial strains relative to the hoop strains up to roughly 9% strain for the same pressures. Specimens, such as n and o, also experienced large axial strains relative to the majority of specimens, but the hoop strains for n and o remained larger than the axial for n and o up to rupture. The observations of the pressure–strain curves for vaginal tissue indicate far more variability for the tissue response in the axial direction.

The stress-strain relationship for vaginal tissue was nonlinear in the hoop and axial directions, with the characteristic toe regions often followed by linear regions for strain values above 10% (figure 7). The average maximum stress and strain values in the hoop and axial directions are reported in table 2. The maximum stress in the hoop direction was statistically significantly larger than the maximum stress in the axial direction ($t_{17} = 12.053$, p < 0.0005). This was expected since these stresses were computed from equations (2.1) and (2.2). The maximum hoop strain was also statistically significantly larger than the maximum axial strain ($t_{17} = 5.473$, p <0.0005). On average, the hoop strains were twice those of the axial strains at rupture. Despite the variation among vaginal specimens, the tissue response appeared to be stiffer in the axial direction. When comparing the average hoop and axial stresses at 2% strain, which was the maximum strain achieved by all specimens in both directions, the axial stress was found to be statistically significantly larger ($t_{17} = 2.597$, p = 0.019) (figure 8). Furthermore, hoop and axial strains were compared at normalized volumes up to 6 to evaluate the strain behaviour in the two directions beyond 2% strain (figure 9). The normalized volume of 6 was selected as it was the maximum achieved across all specimens before rupture. The hoop strain was significantly larger than the axial strain at a normalized volume of 2 ($F_{1,17} = 21.1$, p < 0.0005), 4 ($F_{1.17} = 30.6$, p < 0.0005) and 6 ($F_{1.17} = 30.9$, p < 0.0005).

The vaginal tissue specimens consistently ruptured in regions that were located away from the clamped ends. The rupture always resulted in the formation of a tear oriented



Figure 6. Pressure versus strain data in the axial direction (solid line) and hoop direction (dashed line) collected from n = 18 vaginal specimens. Data from the same specimen are reported using the same letter and lines of the same colour, as denoted by the legend. The rupture point is marked by circular endpoints on each curve. (Online version in colour.)

along the axial direction of the vagina, indicating that the large stress in the hoop direction caused the rupture of the vagina. Failure predominately occurred at random locations on the ventral side of the vaginal specimens, which was not exposed to the cameras. However, the rupture of one specimen occurred within the field of view of the cameras (figure 10). The progression of strains in the hoop and axial directions are shown at two intermediary time points of the inflation test, followed by the point just prior to rupture and finally the point just after rupture. Strains in the hoop direction grew significantly in magnitude in the near region of the rupture site up to 150% strain. This is in stark contrast to the axial strains in the same region. The axial strains increased a relatively small amount, and the largest axial strains of 26% were found in other regions of the specimen surface away from the rupture site.

4. Discussion

Although several studies have characterized the behaviour of vaginal tissue up to failure by means of uniaxial tensile tests [16–20], our study is the first one to characterize the rupture properties of vaginal tissue under a biaxial state of stress. Because the goal of our study was to induce tears in the vaginal wall, we designed an experimental set-up that allowed free extension of the rat vagina in the axial direction under inflation. Unlike planar biaxial testing, our inflation tests did not require clamping via hooks or cruciform specimens that induce complex boundary conditions and limit testing of the tissue to sub-failure loads. Furthermore, we preserved the near cylindrical in vivo structure of the vagina during testing. Consistent with the findings of uniaxial tests, vaginal tissue was observed to be anisotropic; the tissue was, on average, stiffer in the axial direction than the hoop direction. The maximum stress and strain of 970 kPa and 12.8%, respectively, were comparable to the values reported in other studies on rat vaginal tissue [16,17]. Notably, tearing always occurred away from the clamped ends and the tears were predominately located in the ventral region with major axes oriented along the axial direction. Interestingly, Rubod et al. [20] found that



Figure 7. (*a*) Axial stress versus axial strain data (solid lines) and (*b*) hoop stress versus hoop strain (dashed lines) collected from n = 18 vaginal specimens. Data from the same specimen are reported using the same letter and lines of the same colour, as denoted by the legend. The rupture point is marked by circular endpoints on each curve. (Online version in colour.)

the strength of cadaveric vaginal tissue was significantly greater in the posterior (dorsal for the rat) region compared with the anterior (ventral for the rat) region.

We found that the pressure-volume response was remarkably consistent across all the tested specimens (figure 3). Pressure-volume data have been previously reported by Alperin et al. [21], who conducted in vivo inflation tests of the vagina in anaesthetized rats. The volume of water that was infused into a catheter balloon placed in the vagina was incrementally increased and the resulting pressure after a 4 min long interval were measured. The authors reported a nonlinear pressure-volume curve for low volumes that transitioned into a linear curve at larger volumes. These results are comparable with our pressure-volume data, but the slope of our linear region at 53 kPa ml⁻¹ was roughly double the slope in their linear region at 26 kPa ml^{-1} . This discrepancy could be explained by a difference in inflation protocol: a continuous infusion of PBS in our study versus an incremental infusion of water in the cited study. An incremental infusion is likely to induce viscoelastic effects. Other crucial differences are the volume of infused water, which was much higher in our study (max volume: 2.18 ml versus 1 ml), and the fact that the vagina was isolated from the pelvic floor in our ex vivo tests.

Curvature data showed a significant decrease in hoop curvature and little change in axial curvature (figure 5),



Figure 8. Stress data in the axial direction (solid colour, 126 ± 105 kPa) and hoop direction (striped colour, 61.1 ± 19.3 kPa) at 2% strain. This strain value was selected since it was the largest strain achieved by all the tested vaginal specimens (n = 18) in both the hoop and axial directions (*p < 0.05). (Online version in colour.)

indicating that the vaginal tissue experienced a radial expansion while maintaining a somewhat cylindrical geometry. Hoop strains were observed to be significantly larger than axial strains (figure 9), and the average hoop strains were typically twice the magnitude of the average axial strains throughout testing. These results affirmed that the tissue behaved similar to a closed cylindrical pressure vessel. The stress-strain response of the tissue was highly nonlinear, anisotropic, and displayed larger variability in the axial direction relative to the hoop direction (figures 7 and 8). It is well known that the collagen micro-structure plays a significant role in the gross mechanical response of vaginal tissue. Although the composition of vaginal tissue has been established, very few studies have investigated the organization of the collagen fibres [22]. In combination with our previous study [12], the mechanical behaviour of the vaginal tissue in this study suggests a preferential orientation of collagen fibres in the axial direction.

For consistency, the field of view of the cameras used for strain measurement was restricted to the dorsal side of the rat vaginas. The dorsal side was of interest for this study as clinical cases of vaginal tearing are frequently present in this region. Yet, rupture typically occurred outside this field of view near the ventral side of the rat vaginas. When captured, the strains, especially in the hoop direction, were found to be much higher in the regions that were closer to the tears (figure 10). Very recently, Genovese *et al.* [23,24] have developed a new optical method to quantify full surface strain fields on tubular soft tissues with a 360° DIC system. The implementation of such method could significantly improve our current set-up since it would enable us to characterize the tear formation and propagation in vaginal tissue.

Because tearing of the vaginal tissue occurs primarily during delivery, a future experimental study should investigate how pregnancy alters the inflation and rupture properties of the vagina. In the study by Alperin *et al.* [21], the vaginas from pregnant rats were found to be much more distensible than those from virgin rats by analysing pressure–volume data. This is in agreement with other findings from uniaxial tests [17]. Interestingly, Alperin *et al.* [21] discussed the possibility of permanent alterations to the vaginal tissue from pregnancy and delivery being more



Figure 9. Strain data in the axial direction (solid colour) and hoop direction (striped colour) at three values of the normalized volume collected from n = 18 vaginal specimens. Hoop strains were 0.040 ± 0.008 , 0.067 ± 0.016 , and 0.094 ± 0.026 . Axial strains were 0.023 ± 0.011 , 0.036 ± 0.018 , and 0.049 ± 0.026 (***p < 0.0005). (Online version in colour.)

pronounced in the hoop direction, but the compliance of the pregnant cervix made the results of their *in vivo* inflation tests difficult to interpret. Such compliance prevented the catheter balloon to remain within the vagina during inflation. Our experimental set-up was created to characterize the inflation response of the vagina alone, without considering the contribution of other organs (e.g. cervix) and supportive structures, in order to better simulate *in vivo* tearing. However, we recognize that the rupture mechanisms of the rat vagina in our tests may not be the same as the natural rupture mechanism in women.

Translating our findings on the rupture pressures, stresses and strains of the vagina from rats to humans is challenging since there are no published studies that directly compare the structure, composition and mechanics of the vagina between the two species. Yet, rat models have been extensively used to study the mechanical behaviour of the vagina [11,17,21,25] since histological data have indicated that there are similarities between the rat and human vaginas [25]. Indeed, the vaginal wall in rats also consists of three structural layers, the tunica mucosa, the tunica muscularis and the tunica adventitia, and the constituents of these layers are similar to those in humans. Given these similarities, we believe that the rat model may provide critical insights into the mechanical factors that contribute to vaginal tearing in humans. Future studies should be conducted to identify similarities and differences between the rat and human vaginal microstructure to better interpret the mechanical data that are collected in rats and enhance their usefulness. These studies will be especially important to translate prevention



Figure 10. Strain map from a representative vaginal specimen at four pressure values: 80.9, 102, 106 and 105 kPa. Average strain values are also reported above each image. Arrows indicate the location of tearing. (Online version in colour.)

and treatment methods for vaginal tears into successful clinical practice.

5. Conclusions

This experimental study has presented the first characterization of the rupture properties of the vaginal tissues using the rat as an animal model. Toward this end, an inflation testing apparatus that allowed free extension of the vagina in the axial direction was custom-made, and the digital image correlation method for non-contact strain measurement was employed. The vagina was found to be a highly nonlinear elastic and anisotropic material, with the hoop strain being significantly larger than the axial strain. Rupture of the vagina consistently occurred in the hoop direction by tearing along the axial direction. The findings of this study have the potential to lead to a more refined and systematic approach to the measurement, imaging and treatment of vaginal tears following delivery.

Ethics. The proposed study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech.

Data accessibility. This article has no additional data.

Authors' contributions. R.D. and S.A. conceived the study; R.D. and J.M. designed the experiments; J.M. and C.C. performed the experiments; R.D., J.M. and C.C. analysed the data and wrote the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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References

- Martin JA, Hamilton BE, Osterman MJK, Driscoll AK, Drake P. 2018 Births: final data for 2016. *Natl. Vital Stat. Rep.* 67, 1–55.
- 2. Samuelsson E, Ladfors L, Lindblom BG, Hagberg H. 2002 A prospective observational study on tears

during vaginal delivery: occurrences and risk factors. *Acta Obstet. Gynecol. Scand.* **81**, 44–49. (doi:10. 1046/j.0001-6349.2001.10182.x)

3. Hopkins LM, Caughey AB, Glidden DV, Laros RK. 2005 Racial/ethnic differences in perineal, vaginal

and cervical lacerations. *Am. J. Obstet. Gynecol.* **193**, 455–459. (doi:10.1016/j.ajog.2004. 12.007)

 Phillips C, Monga A. 2005 Childbirth and the pelvic floor: 'the gynaecological consequences'. *Rev.* *Gynaecol. Pract.* **5**, 15–22. (doi:10.1016/j.rigp.2004. 09.002)

- Skinner EM, Dietz HP. 2015 Psychological and somatic sequelae of traumatic vaginal delivery: a literature review. *Aust. N. Z. J. Obstet. Gynaecol.* 55, 309–314. (doi:10.1111/ajo.2015.55.issue-4)
- Christianson LM, Bovbjerg VE, McDavitt EC, Hullfish KL. 2003 Risk factors for perineal injury during delivery. Am. J. Obstet. Gynecol. 189, 255–260. (doi:10.1067/mob.2003.547)
- Fernando RJ, Sultan AH. 2004 Risk factors and management of obstetric perineal injury. *Obstet. Gynaecol. Reprod. Med.* 14, 320–326.
- Krstic RV 2013 Human microscopic anatomy: an atlas for students of medicine and biology. Berlin, Germany: Springer Science & Business Media.
- Baah-Dwomoh A, McGuire J, Tan T, De Vita R. 2016 Mechanical properties of female reproductive organs and supporting connective tissues: a review of the current state of knowledge. *Appl. Mech. Rev.* 68, 060801. (doi:10.1115/1.4034442)
- Robison KM, Conway CK, Desrosiers L, Knoepp LR, Miller KS. 2017 Biaxial mechanical assessment of the murine vaginal wall using extension – inflation testing. *J. Biomech. Eng.* **139**, 104504. (doi:10.1115/ 1.4037559)
- Huntington A, Rizzuto E, Abramowitch S, Del Prete Z, De Vita R. 2019 Anisotropy of the passive and active rat vagina under biaxial loading. *Ann. Biomed. Eng.* 47, 272–281. (doi:10.1007/s10439-018-02117-9)
- 12. McGuire J, Abramowitch S, Maiti S, De Vita R. 2019 Swine vagina under biaxial loading: an investigation

of large deformations and tears. *J. Biomech. Eng.* **141**, 041003. (doi:10.1115/1.4042437)

- Lionello G, Sirieix C, Baleani M. 2014 An effective procedure to create a speckle pattern on biological soft tissue for digital image correlation measurements. *J. Mech. Behav. Biomed. Mater.* 39, 1–8. (doi:10.1016/j.jmbbm.2014.07.007)
- Kim J-H, Avril S, Duprey A, Favre J-P. 2012 Experimental characterization of rupture in human aortic aneurysms using a full-field measurement technique. *J. Biomech. Model. Mechanobiol.* 11, 841–853. (doi:10.1007/s10237-011-0356-5)
- Marra SP, Kennedy FE, Kinkaid JN, Fillinger MF. 2006 Elastic and rupture properties of porcine aortic tissue measured using inflation testing. *Cardiovasc. Eng.* 6, 123–131. (doi:10.1007/s10558-006-9021-5)
- Alperin M, Feola A, Meyn L, Duerr R, Abramowitch S, Moalli P. 2010 Collagen scaffold: a treatment for simulated maternal birth injury in the rat model. *Am. J. Obstet. Gynecol.* **202**, 589.e1–589.e8. (doi:10.1016/j.ajog.2010.04.003)
- Feola A, Moalli PA, Alperin M, Duerr R, Gandley RE, Abramowitch SD. 2011 Impact of pregnancy and vaginal delivery on the passive and active mechanics of the rat vagina. *Ann. Biomed. Eng.* 39, 549-558. (doi:10.1007/s10439-010-0153-9)
- Rubod C, Boukerrou M, Brieu M, Dubois P, Cosson M. 2007 Biomechanical properties of vaginal tissue. Part 1: new experimental protocol. *J. Urol.* **178**, 320-325. (doi:10.1016/j.juro.2007.03.040)
- 19. Rubod C, Boukerrou M, Brieu M, Jean-Charles C, Dubois P, Cosson M. 2008 Biomechanical properties

of vaginal tissue: preliminary results. *Int. Urogynecol. J.* **19**, 811–816. (doi:10.1007/s00192-007-0533-3)

- Rubod C, Brieu M, Cosson M, Rivaux G, Jean-Charles C, de Landsheere L, Gabriel B. 2012 Biomechanical properties of human pelvic organs. Urology 79, 968.e17-968.e22. (doi:10.1016/j. urology.2011.11.010)
- Alperin M, Feola A, Duerr R, Moalli P, Abramowitch S. 2010 Pregnancy-and delivery-induced biomechanical changes in rat vagina persist postpartum. *Int. Urogynecol. J.* 21, 1169–1174. (doi:10.1007/s00192-010-1149-6)
- Sridharan I, Ma Y, Kim T, Kobak W, Rotmensch J, Wang R. 2012 Structural and mechanical profiles of native collagen fibers in vaginal wall connective tissues. *Biomaterials* 33, 1520–1527. (doi:10.1016/ j.biomaterials.2011.11.005)
- Genovese K, Lee YU, Humphrey JD. 2011 Novel optical system for *in vitro* quantification of full surface strain fields in small arteries: I. Theory and design. *Comput. Methods Biomech. Biomed. Engin.* 14, 213–225. (doi:10.1080/10255842.2010.545823)
- Genovese K, Cortese L, Rossi M, Amodio D. 2016 A 360-deg digital image correlation system for materials testing. *Opt. Lasers Eng.* **82**, 127–134. (doi:10.1016/j.optlaseng.2016.02.015)
- Moalli PA, Howden NS, Lowder JL, Navarro J, Debes KM, Abramowitch SD, Woo SLY. 2005 A rat model to study the structural properties of the vagina and its supportive tissues. *Am. J. Obstet. Gynecol.* **192**, 80–88. (doi:10.1016/j.ajog.2004.07.008)

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