



Linking hyperelastic theoretical models and experimental data of vaginal tissue through histological data



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ARTICLE INFO

Article history:

Accepted 31 October 2018

Keywords:

Vaginal tissue

Biomechanical properties

Histology

Genetic algorithm

Hyperelastic model

ABSTRACT

Mechanical characterization of living tissues and computer-based simulations related to medical issues, has become increasingly important to improve diagnostic processes and treatments evaluation. This work proposes a link between the mechanical testing and the material model predictions through histological data of vaginal tissue. Histological data was used to link tensile testing experiments with material-dependent parameters; the approach was adequate to capture the nonlinear response of ovine vaginal tissue over a large strain range.

The experimental data obtained on a previous study, has two main components: tensile testing and histological analysis of the ovine vaginal tissue. Uniaxial tensile test data and histological data were collected from three sheep groups: virgins, pregnant and parous. The distal part of vaginal wall was selected since it is prone to tears induced by vaginal delivery.

The HGO (Holzapfel-Gasser-Ogden) model parameters were fitted using a stochastic approach, namely the Simple Genetic Algorithm (SGA). The SGA was able to fit the experimental data successfully ($R^2 > 0.986$). The dimensionless coefficient ξ , was highly correlated with histological data. The ratio was seen to increase linearly with increasing collagen content.

Coefficient ξ brings a new way of interpreting and understanding experimental data; it connects the nonlinear mechanical behaviour (tensile test) with tissue's morphology (histology). It can be used as an 'inverse' (approximate) method to estimate the mechanical properties without direct experimental measurements, through basic histology.

In this context, the proposed methodology appears very promising in estimating the response of the tissue via histological information.

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1. Introduction

The use of simulation environments has become increasingly relevant in the exploitation of experimental evidence, to gain deeper insights over many biomechanical problems (Delingette, 1998). Confined to the degree of assumptions and simplifications in the modelling of the material behaviour, the predictions of these boundary-value problems can provide new insights for health issues. For example, through phenomenological material models one can explore relevant biomedical problems such as pelvic floor

dysfunctions (PFD). The pathophysiological nature for PFD is not yet clear. However, it is known to be related to abnormal stretching combined with defective synthesis of the pelvic tissues' constituents (Fenner and Hsu, 2010). Pelvic tissues are soft tissues comprising cells performing essential biochemical functions, while the elastin and collagen in the extracellular matrix (ECM) work as the load bearing constituents (Holzapfel, 2000a, 2000b, 2000c).

Collagen fibres, one of the main components of the extracellular matrix of soft pelvic tissues, are greatly responsible for the anisotropic mechanical behaviour.

The mechanics of pelvic floor soft tissues is strongly influenced by the concentration and structural arrangement of the constituents, dictated by the topographical location and respective function in the pelvic floor cavity (Couri et al., 2012).

Pelvic floor soft tissues undergo mechanical and histological changes during their adaptation to pregnancy. Some researchers

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have found an intimate connection between the tissues' mechanics and histological structure (Urbankova et al., 2018; Ulrich et al., 2014). While collagen is largely responsible for soft tissue tensile strength, elastin makes the tissue more compliant (Fung, 1993). However, these findings still have to be linked with accurate material models. For instance, some first attempts to relate histological data with material properties for vaginal tissue can be found in (De Landsheere et al., 2016; Ulrich et al. 2014). This connection is fundamental to understand the mechanical properties of pelvic tissues, especially in pathophysiological conditions.

The goal of this work is to present the study of the main stages involved in the mechanical characterization of biological soft tissues. Starting from theoretical hyperelastic concepts, the material-dependent parameters are estimated using Genetic Algorithms (GAs). GAs were applied to experimental data for curve fitting and, ultimately to predict the tissues' mechanical behaviour through numerical simulation.

This work presents a strategy to correlate tensile testing experiments, with a given material model, adequate to capture the nonlinear response over a large strain range. The data from longitudinal samples were fit using a simple GA. The fitted material parameters demonstrated a relation with the tissues' microstructure, i.e. collagen and elastin densities. Moreover, the resultant material parameters reflect the particularities of the physiological state associated with pregnancy.

The available (limited) histological information was seen to have a relation with the Holzapfel–Gasser–Ogden model parameters. The adopted approach enables the prediction of tissue mechanics without experimental data, but rather relying on histological and histochemical information.

2. Materials and methods

2.1. Animal model

Experimental stress–stretch curves and histological images from the distal vaginal wall of Swifter sheep, were obtained by Rynkevic et al. (2017). The distal part of vaginal wall was selected since it is prone to tears induced by vaginal delivery. The experimental data was collected from three sheep groups: virgins ($n = 5$; avg. weight = 45 kg, 9 months old), pregnant ($n = 5$; term = 145 days; avg. weight = 65 kg, 3 years old) and parous ($n = 5$; avg. weight = 60 kg, 4 years old). Pregnant sheep had two prior vaginal deliveries and the third was a C-section. Parous sheep were included one year after the third vaginal delivery.

Animals were treated according to a protocol approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine of KU Leuven.

2.2. Experimental data and histological analysis

Uniaxial tensile testing was performed using a vertical Zwick tensiometer (Zwick GmbH & Co, KG, Ulm, Germany). Dog bone shaped samples ($2 \text{ mm} \times 25 \text{ mm}$) were pre-loaded until 0.1 N (at elongation of 5 mm/min) to remove slack from the tissue. A constant elongation rate of 10 mm/min was used to load the specimen to failure, along the longitudinal axis.

For histological analysis, 6- μm slices were stained with Miller's elastic staining and were captured using a Zeiss microscope (Zeiss Axioplan 400, Oberkochen, Germany) at $10 \times$ magnification. An image-stitching algorithm using Matlab (Release 2015[®], The MathWorks, Inc) was applied to combine multiple micrographs from the same stain. The resulting high-resolution images were processed using ImageJ (open platform for scientific image analysis) and a Colour Deconvolution plugin (Caetano et al., 2016). The total content (%) of collagen, elastin, and smooth muscle were quantita-

tively determined from thresholded images, relative to the total area (%) of the stain, using image processing techniques (De Landsheere et al., 2016).

2.3. Hyperelastic materials

Phenomenological approaches to model the mechanical behaviour of soft tissues, describe the material by means of a strain-energy function (SEF or stored-energy) per unit volume (Holzapfel, 2000a, 2000b, 2000c), wherein the inherent complex microstructure is assumed as a homogeneous continuum. The tensile testing data shows nonlinear responses, while the histological data shows the tissue is comprised of collagen and elastin fibres embedded in a matrix of smooth muscle cells (SMCs) and other constituents. As observed by Urbankova et al. (2017), the histological images of the studied ovine vaginal specimens revealed 4 tissue layers. A stratified squamous epithelium, a lamina propria containing a multidirectional network of collagen and elastin fibres, a muscularis containing smooth muscle cells arranged in circular and longitudinal directions (e_1 and e_2 directions in Fig. 1a), and an adventitia which merges with the adventitia of bladder and rectum."

Based on the histological data, the material parameters were reduced to a minimal set, rich enough to allow the comprehension of tissue mechanics in terms of its structure. Therefore, given its mechanical response, the vaginal tissue can be modelled as a fibre reinforced hyperelastic material.

Fig. 1a shows the distribution of smooth muscle (light rose) at a through-thickness cut of the sample. Lamina propria shows the highest level of collagen (in red) among the three layers (as corroborated by Rynkevic et al. (2017)). In lamina propria, smooth muscle (light rose) is surrounded by collagen. The samples were stretched along e_1 -direction, the (observed) preferential direction of collagen fibers in lamina propria (Fig. 1a). Such direction was a reference to mount the specimens in the testing apparatus. In the uniaxial tensile testing conditions, the fibres of all layers tend to align in the stretching direction (e_1) (Calvo et al., 2009). Therefore, a transversely isotropic model (one family of fibres) approximates the material response under the tensile testing conditions.

The deformation gradient \mathbf{F} estimates the 3-dimensional deformation of vaginal tissue, understood as a soft elastic continuum. The right Cauchy–Green tensor $\mathbf{C} = \mathbf{F}^T \mathbf{F}$ measures rotation independent deformation. Moreover, the tensile test results had no evidence of significant volume changes, thus allowing an isochoric approach.

The sample is submitted to uniaxial deformation, considered homogeneous, with stretch λ in the mean fibre direction \mathbf{M} , taken as the e_1 -direction (see Fig. 1b).

Therefore, the matrix (or ground substance) contains the fibres and deforms according to the deformation gradient $[\mathbf{F}] = \text{diag}[\lambda, \lambda^{-\frac{1}{2}}, \lambda^{-\frac{1}{2}}]$ and $\mathbf{M} = [1, 0, 0]^T$, so that the deformed fibre direction, $\mathbf{m} = [\mathbf{F}]\mathbf{M}$ gives $\mathbf{m} = [\lambda, 0, 0]^T$.

The first invariant is $I_1 = \text{tr}\mathbf{C}$ while the fourth invariant is denoted by $I_4 = \mathbf{M} \cdot (\mathbf{C}\mathbf{M})$, which for the deformation state imposed in the samples are reduced to $I_1 = \lambda^2 - 2\lambda^{-1}$ and $I_4 = \lambda^2$.

Under these assumptions and according to the available histological information, the simpler version of the HGO model (Holzapfel et al., 2000) seems adequate to describe the mechanical behaviour of the samples. The strain-energy is postulated as:

$$\Psi = \underbrace{\frac{\mu}{2}(I_1 - 3)}_{\text{matrix}} + \underbrace{\frac{k_1}{2k_2} \left(e^{k_2(I_4 - 1)^2} - 1 \right)}_{\text{collagen fibers}} \quad (1)$$

where μ , k_1 , and k_2 are the constant parameters that define the mechanical response (Abaqus 6.11). While the shear modulus (μ)

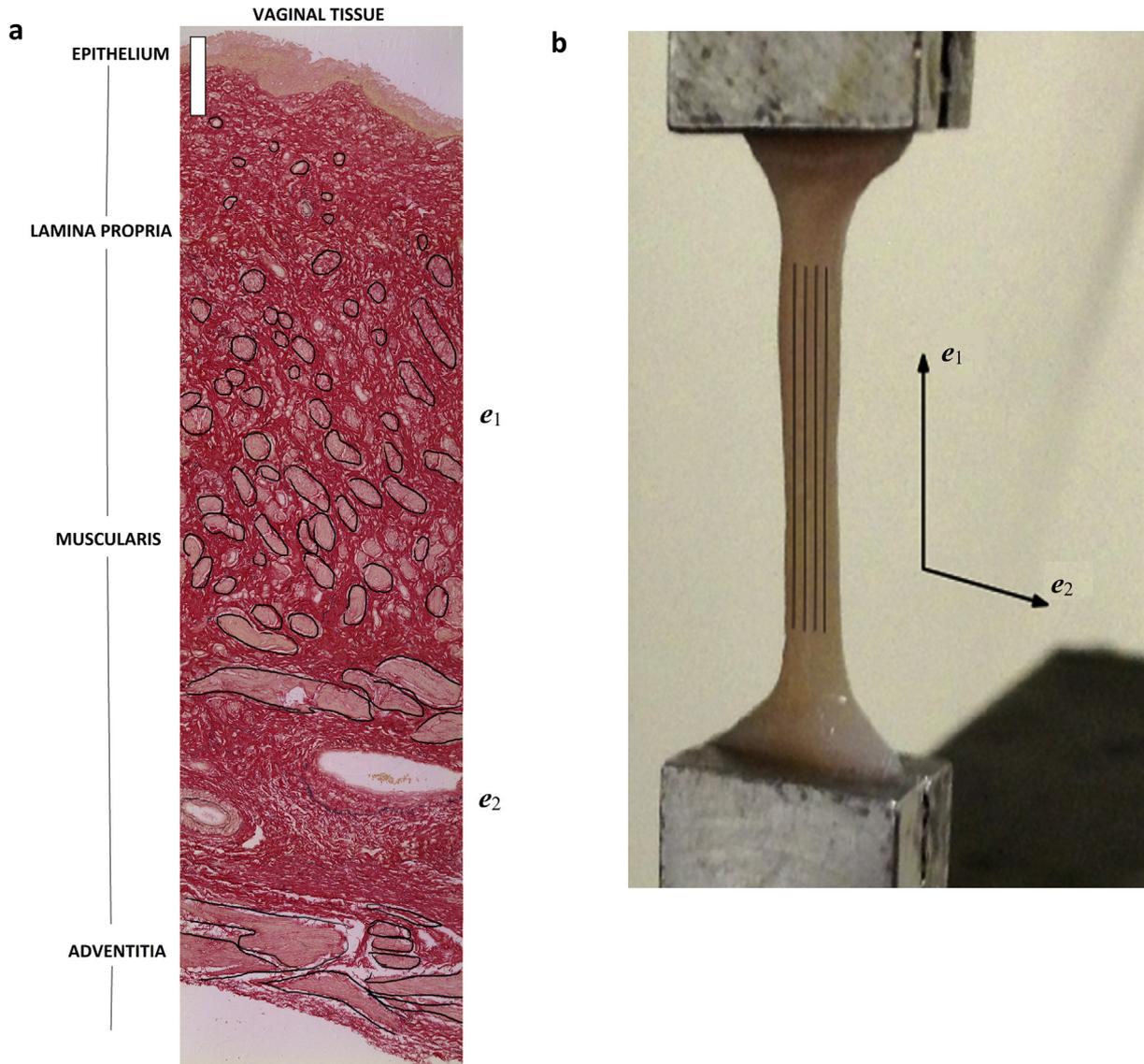


Fig. 1. a – Histological image of the ovine vaginal tissue (distal part), stained with Miller’s Elastica. Staining results: total collagen (red), elastin fibres (jet-black). Scale bar (upper left) for sections – 200 μm . e_1 and e_2 directions correspond to the circular and longitudinal directions of smooth muscle (e_1 direction is normal to the cut section). b – Tensile testing of the dog bone shaped specimen (ovine vaginal tissue) with main fibre orientation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is related to the matrix content; k_1 and k_2 reflect the fibers contribution.

For convenience, we define the dimensionless ratio:

$$\xi = \frac{k_1}{2k_2\mu} \tag{2}$$

as a suitable regulator for the matrix and fibre contributions. It represents the contribution to elastic energy of collagen fibres relative to the matrix content.

The resultant parameters are consistent with the requirements for use in finite element methods, in complex initial boundary-value problems (Drucker, 1959). They are suited to characterize the pelvic floor tissues undergoing large deformations. The Cauchy stresses σ are obtained push-forwarding the second Piola-Kirchhoff stresses $\mathbf{S} = \mathbf{F}^{-1} \frac{\partial \Psi}{\partial \mathbf{F}}$, i.e., $\sigma = \mathbf{F} \mathbf{S} \mathbf{F}^T$.

2.4. Data fitting

The material model presented contains 3 parameters (μ , k_1 and k_2) that must be adjusted to obtain a reliable characterization of

the tissue. Therefore, these 3 parameters were treated as functional coefficients that minimize the error metric, taken to be the sum of the squares of the residuals. For a given set of m experimental points, the error metric is:

$$s = \sum_m \left(\frac{\sigma_{\text{exp}} - \sigma}{\sigma_{\text{exp}}} \right)^2 \tag{3}$$

where residual states for the difference between the observed stresses σ_{exp} and the Cauchy stress provided by the material model σ . By treating such residuals in their dimensionless form, the fitting process became independent on the stress levels observed in the experiments. Thus, the objective function can be expressed as:

$$\arg \min_{\{\mu, k_1, k_2\} \in \mathbb{R}^+} \sum_m \left(\frac{\sigma_{\text{exp}} - \sigma}{\sigma_{\text{exp}}} \right)^2 \tag{4}$$

We ensure material stability (Drucker stability condition) by searching over positive domains (Drucker, 1959) rather than adding restrictions to the optimisation problem. The fast convergence of traditional gradient-based optimizers has the drawback of

requiring objective function derivatives (Khalil et al., 2006), which can become a huge challenge when nonlinearities are present. Gradient-free optimizers such as Genetic Algorithms are a stochastic alternative to select the best parameters guaranteeing local optima solutions. Moreover, due to their metaheuristic nature, they can handle complex and irregular solution spaces, as the ones studied in this work.

Therefore, a simple tournament selection operator, uniform crossover and creep and jump mutations, were employed (Maletta and Pagnotta, 2004). This scheme was used to generate a population of individuals (candidate solutions) under Darwinian principles (David and Goldberg, 1989), and genetic variability controls. That is, if better individuals are not generated, elitism is ensured by keeping the best-fitted individual from the last generation and only generations having less than 10% of similar individuals allow the algorithm to move on. Each candidate solution is evaluated through the fitness function f , defined as:

$$f = \frac{1}{1 + s} \tag{5}$$

Table 1
Control parameters and strategies for the implementation of the simple genetic algorithm.

Number of parameters	3
Population size	40
Selection	Tournament (semifinal)
Crossover	Uniform
Crossover probability	0.6
Mutation probability	0.02
Maximum equal individuals	4
Elite individuals	1
Stopping criterion	51 generations

Table 1 summarizes the implementation of the fitting procedure, and Fig. 2 describes the required pipeline to obtain the material parameters from the data sets. All routines were implemented in Fortran 90.

2.5. Statistical analysis

Statistical analysis was performed to compare mechanical properties and material model parameters of the distal vagina among experimental groups. The fitted values per individual was used for analysis. Quantitative data are represented as mean ± standard error of the mean (±SEM). A statistically significant difference was reported if $p < 0.05$. Kolmogorov-Smirnov tests showed the data follows a normal distribution. One-way ANOVA and post hoc test (Tukey's correction) were carried out for the intergroup comparisons. The level of significance was set to $p < 0.05$.

3. Results

3.1. Individual analysis

The tensile testing results of the vaginal tissue from the virgin group evidences consistent results (Fig. 3). Sheep 5 shows a more compliant mechanical behaviour, consistent with observed collagen and elastin densities (53.21% and 1.86%), Table 2. The reasons behind such disparate mechanical are unknown as the life and hormonal status of the Sheep 5 are the same of the others from virgin group. Nevertheless, Sheep 5 results were not excluded from the study.

The results displaying higher stresses were associated with higher total collagen contents (Fig. 3, Table 2) for all animals, while an increase in elastin content was associated with higher stretches.

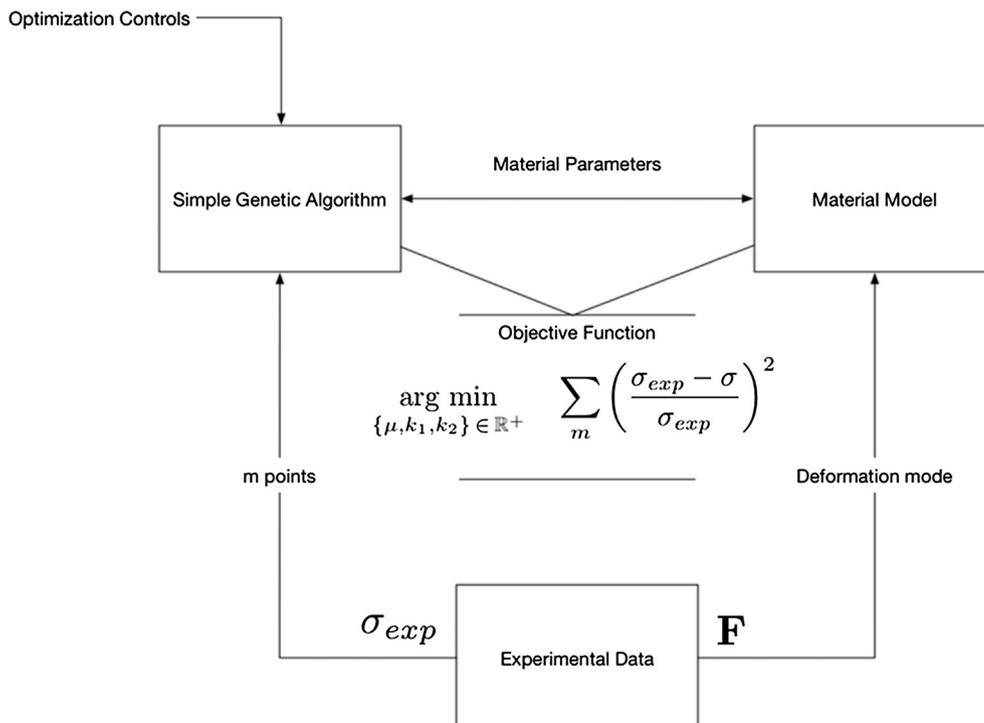


Fig. 2. Graphical representation of the fitting procedure. Each experimental data set contains m points of stretch-stress obtained during tensile testing. The material parameters generated by the simple genetic algorithm are evaluated by the material model through Cauchy stresses response and tested according to the fitness function based on the sum of residuals.

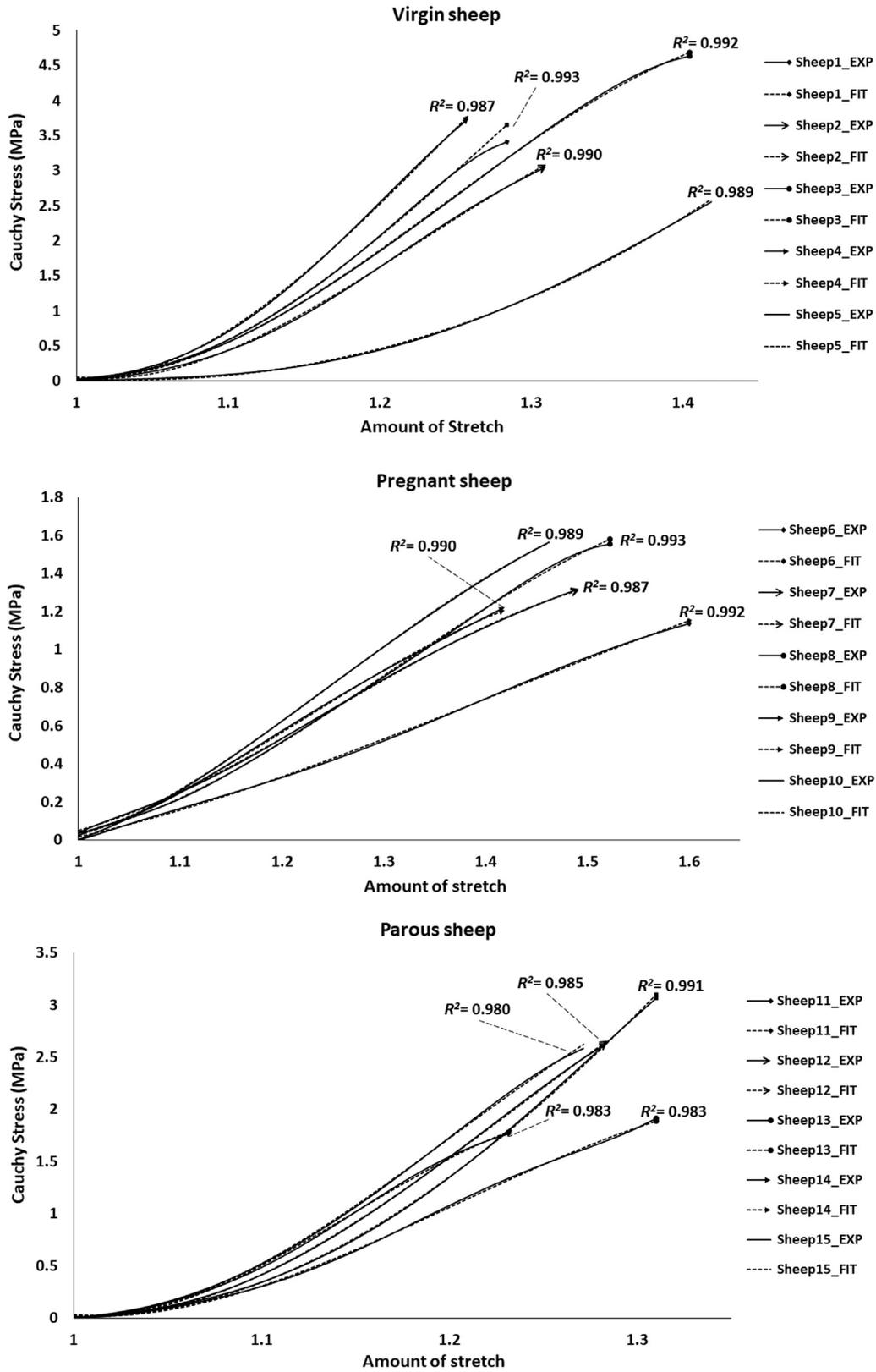


Fig. 3. Mechanical behaviour (experimental and fitting curves) of the vaginal tissue of the virgin, pregnant and parous ewes. Experimental data (solid). fitting data (dashed) and coefficient of determination (R^2).

The HGO model parameters were fitted using a stochastic approach, namely the SGA. In the HGO model, parameter μ is linked with the energy required to deform the tissue matrix.

For highly fibered tissues i.e. vaginal tissues, μ gives a significant contribution for lower stretches (comfort zone (Rynkevic et al., 2017)). However, its influence at higher stretches is

Table 2
Histological component densities and fitting parameters of the virgin, pregnant, and parous ewes. Averaged data is presented as mean (\pm SEM), significant differences among the groups were set to $p < 0.05$. a – virgin vs pregnant, b – pregnant vs parous, c – virgin vs parous.

	Collagen (%)	Elastin (%)	Smooth muscle cells (%)	k_1 (MPa)	k_2	Shear modulus μ (MPa)	$\xi = k_1/(2k_2\mu)$
<i>Virgin (N = 5)</i>							
Sheep 1	58.78	0.656	22.22	1.829	0.781	0.286	4.101
Sheep 2	55.01	0.678	21.55	1.452	0.419	0.492	3.517
Sheep 3	60.60	1.922	24.1	1.400	0.129	1.550	3.501
Sheep 4	58.87	0.517	22.42	2.100	1.065	0.518	1.905
Sheep 5	53.21	1.861	20.08	0.435	0.774	0.195	1.440
Mean \pm SEM	57.29 \pm 1.37 a	1.693 \pm 0.301 a	22.07 \pm 0.84 a	1.443 \pm 0.632 a	0.634 \pm 0.363 a	0.608 \pm 0.544 a	2.893 \pm 1.152 ac
<i>Pregnant (N = 5)</i>							
Sheep 6	43.97	3.85	27.94	0.011	0.197	0.827	0.0350
Sheep 7	46.89	3.55	27.39	0.015	0.066	1.291	0.0856
Sheep 8	46.66	2.77	27.12	0.046	0.207	1.239	0.0894
Sheep 9	46.92	2.96	27.15	0.014	0.056	1.394	0.0885
Sheep 10	49.76	2.87	27.10	0.037	0.091	1.521	0.1346
Mean \pm SEM	46.86 \pm 1.65 b	3.20 \pm 0.21 b	27.40 \pm 0.20 b	0.025 \pm 0.016 a	0.124 \pm 0.073 a	1.254 \pm 0.262 a	0.087 \pm 0.035 ac
<i>Parous (N = 5)</i>							
Sheep 11	53.81	2.59	31.05	1.113	0.919	0.428	1.4133
Sheep 12	50.13	2.45	30.08	1.210	0.726	0.768	1.0845
Sheep 13	48.24	2.53	30.93	0.726	0.581	0.675	0.9261
Sheep 14	49.23	2.01	29.12	1.205	0.890	0.635	1.0647
Sheep 15	50.84	2.27	29.48	1.346	0.697	0.820	1.1777
Mean \pm SEM	50.45 \pm 0.90 c	2.370 \pm 0.10	30.13 \pm 0.29 c	1.120 \pm 0.235 a	0.763 \pm 0.141 a	0.665 \pm 0.151 a	1.133 \pm 0.181 ac

diminished. Parameters k_1 and k_2 regulate the deformation energy associated with fibers. They have a significant (stress) contribution for higher stretches (stress zone (Rynkevic et al., 2017)), due to the exponential part of the HGO strain-energy function (Eq. (1)).

In general, the SGA was able to capture the mechanical behavior of the vaginal tissue (Fig. 3). The 3-dimensional admissible solutions' hyperspace $\{\mu, k_1, k_2\}$ was compatible with numerically stable simulation environment. The SGA was able to fit the experimental data successfully ($R^2 > 0.986$), Fig. 3.

The primer histological differences found between groups were detailed in (Rynkevic et al., 2017). Table 2 contains histological data (collagen and elastin relative densities) and HGO model parameters (Eq. (1)). Paired t -test ($p < 0.05$) was performed. There is a significant difference in ξ virgin and the other groups. There is also a significant difference between virgin and pregnant for the model parameters: μ , k_1 , and k_2 . During pregnancy the vaginal tissue becomes more compliant, with a decrease in total collagen and an increase in elastin content. In contrast, virgin sheep had the stiffest vaginal tissue, with highest total collagen and lowest elastin fibre contents. The dimensionless coefficient ξ , is highly correlated (R^2) with histological data (Fig. 4). The ratio was seen to increase linearly with increasing collagen content. ξ coefficient connects the nonlinear mechanical behaviour (tensile test) with tissue's morphology (histology).

3.2. Group analysis

Fig. 5 shows the typical mechanical behaviour of each sheep group considered, virgin, parous and pregnant. During pregnancy, vaginal tissue becomes highly extensible, when compared to the baseline virgin sheep. This situation is associated with an increase on elastin content (by 1.51%) and a decreased total collagen fraction (by 7.58%), Table 3. There is recovery of the vaginal tissues to levels near their pre-pregnancy state. The curve for parous sheep has the same mechanical behaviour as virgin however, ultimate stress and strain are lower. This mechanical behaviour agrees with histology, as collagen and elastin fractions return levels close to the virgin group.

As described in individual analysis (3.1) the fittings were accurate (high R^2 for all groups) and able to capture the mechanical

behaviour of the vaginal tissue in different reproductive statuses, Fig. 5.

As observed for the individual sheep (3.1), ξ is proportional to the total collagen content (Table 3, Fig. 6). Overall the relations found for individuals (3.1), regarding the link between tissue's morphology and mechanical properties are valid for the group analysis; higher ξ values are linearly associated ($R^2 = 0.982$) with higher total collagen contents.

4. Discussion

The computational simulation of the mechanical behavior of biological systems, is highly dependent on representative constitutive models (Bonet and Wood, 1997). Such models are theoretical abstractions which are assumed to capture the overall behavior of the studied materials under a given set of boundary conditions i.e., under uniaxial or biaxial tension. In the context of the present work, the HGO model parameters are akin to the mechanical properties for 'simpler' constitutive models (ex. Hooke's law). However, due to the hyperelastic mechanical behavior, commonly observed in fibered soft tissues, an intuitive understanding and interpretation of the parameter fittings (from optimization) in relation to the experimental data, is difficult.

The experimental work has two main components, tensile testing, and histological analysis. During a previous investigation (Rynkevic et al., 2017) it was observed a link between the mechanical behavior and tissue's morphology, obtained via histology. There was a consistent relation between local stiffness (Young's moduli in physiological – comfort zone and supra-physiological domains – stress zone) and histological components (elastin, total collagen, and smooth muscle).

For realistic mechanical simulations it is important to rely on robust theoretical models. The nonlinear theory of elasticity in general (Holzapfel, 2000a, 2000b, 2000c), and the HGO model in particular, have been used to model fibrous soft tissues. HGO model is especially suited for tissues composed by two fiber families, such as arteries, pelvic floor tissues, etc.

We present for the first time a relation between HGO coefficients $\{\mu, k_1, k_2\}$, obtained via SGA, $\xi = k_1/(2k_2\mu)$. Through ξ , it was found an agreement between HGO model predictions and experimental data, robust over different kinds of reproductive

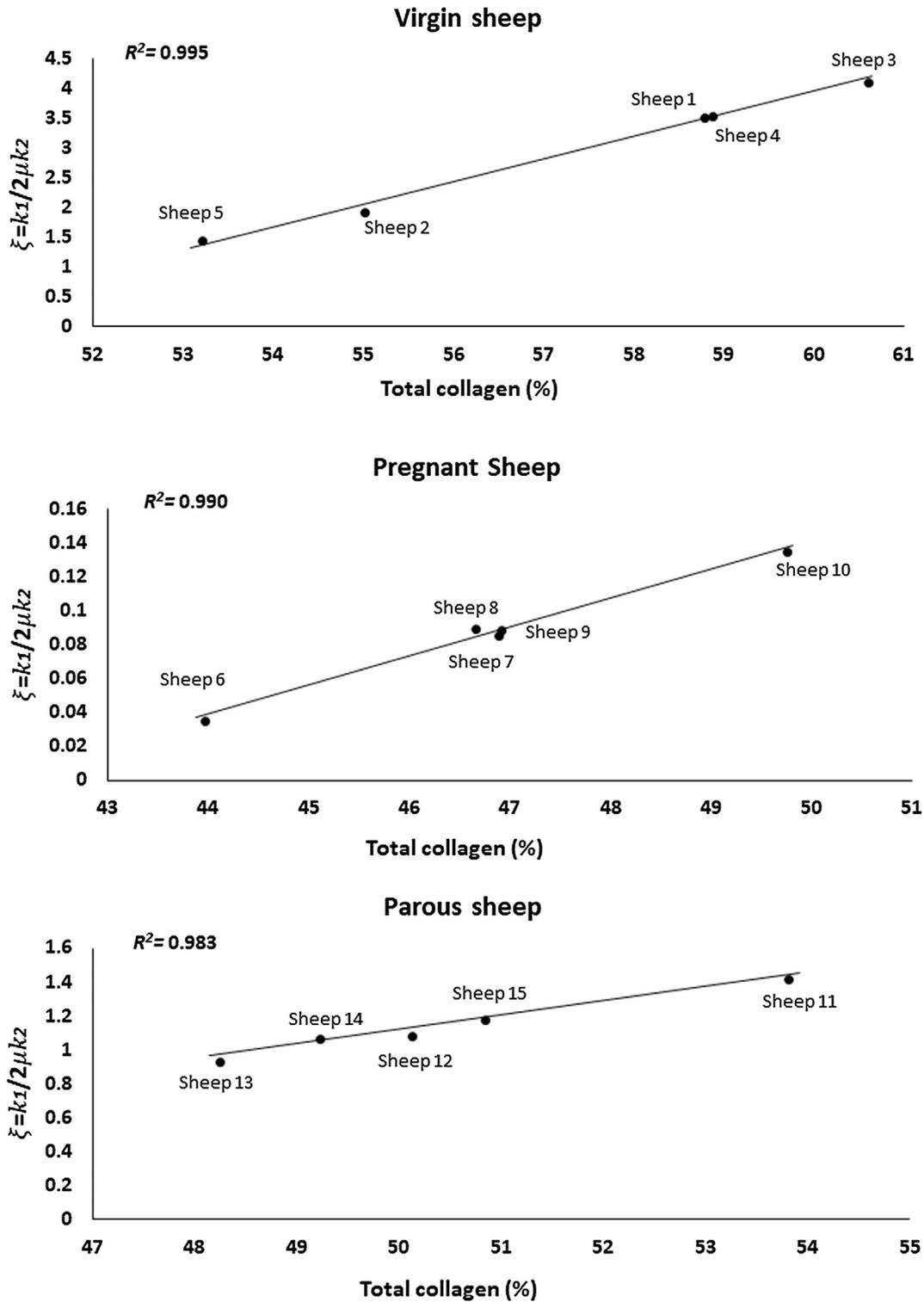


Fig. 4. ξ vs total collagen (%) relationship for virgin, pregnant and parous ewes.

statuses. To establish correlations for all the 3 groups under analysis, it was found necessary to include all parameters of the model via ξ . Other hypotheses involving less parameters were unsuccessfully investigated. The HM (Histologically-Motivated) coefficient, ξ , establishes a link between tissue’s microstructure and the theoretical model results. It was found a consistent correlation between total collagen content and HM coefficient. This correlation is observed at the individual (Fig. 4) and group levels (Fig. 6). More-

over, the same relation was seen in significantly different physiological conditions (virgin, pregnant, etc.).

HM coefficient (ξ) brings a new way of interpreting and understanding experimental data. It can be used as an ‘inverse’ (approximate method) to estimate the mechanical properties without direct experimental measurements, through basic histology. The converse is possible, using correlations shown in Figs. 4 and 6.

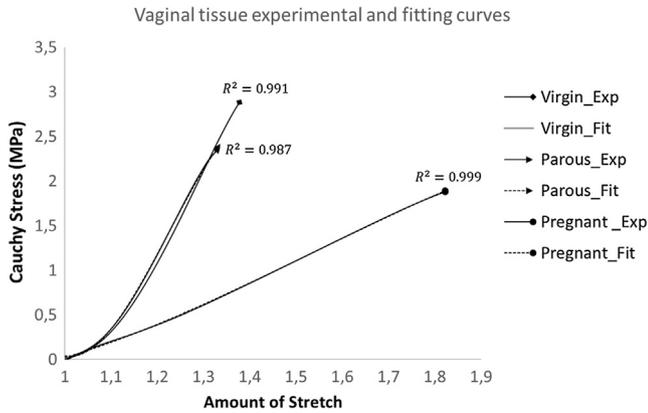


Fig. 5. Mechanical behaviour (experimental and fitting curves) of the vaginal tissue of the virgin, pregnant and parous sheep groups. Experimental data (solid), fitting data (dashed) and coefficient of determination (R^2).

The vaginal tissue undergoes significant remodeling and alterations during pregnancy (Ulrich et al., 2014). Factors such as collagen dispersion, orientation, I:III ratio, crosslinks, and undulation may potentially contribute to these parameters. While immature crosslinks and undulation facilitate tissue compliance (Jackson et al., 2002), collagen type I is largely responsible for tissue' tensile strength. (De Landsheere et al., 2013) found less collagen in pregnant vaginal tissue and no significant difference in collagen type III between pregnant, virgin and parous. To investigate the influence of reproductive status on the material (vaginal tissue) behavior, further research should focus on the use more detailed histological and histochemical data to build relevant material models.

Although biochemical analyses provide precise total collagen and elastin content measurements (Ulrich et al., 2014), morphometric analyses like the one used in this work proved to be an adequate compromise for microstructure evaluation (Caetano et al., 2016; De Landsheere et al., 2016). Using this simple, rapid and a low-cost technology for tissue microstructure evaluation, Rynkevic et al. (2017), Urbankova et al. (2018), and Alperin et al. (2010) found mechanical and histological data agreeing with the results from biochemical analysis (Ulrich et al., (2014)).

In the research it was observed that vaginal tissue from pregnant sheep becomes very extensible, associated with significantly low total collagen and high elastin content. In contrast, virgin sheep had the highest total collagen, which was associated with a high ultimate stress. On the other hand, it contained less elastin fibres, which could explain the higher resistance to stretching (Urbankova et al., 2018). After third delivery, the vagina was stiffer than during pregnancy, but not to the level of stiffness observed in virgins. This mechanical behavior is coherent with the collagen and elastin levels discussed above. These results agree with studies in rats, where the stiffness and strength of vaginal tissues decreased

Table 3
Collagen, elastin and fitting parameters of virgin, pregnant and parous sheep.

	Total collagen (%)	Elastin (%)	k_1 (MPa)	k_2	Shear modulus μ (MPa)	$\xi = k_1/(2 k_2^2 \mu)$
Virgin	54.42	1.693	0.948	0.193	0.647	3.788
Pregnant	46.86	3.201	0.007	0.065	0.995	0.050
Parous	50.45	2.370	0.871	0.290	0.905	1.658

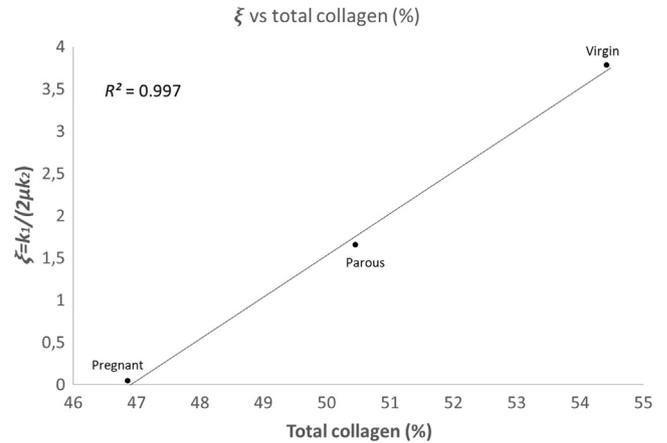


Fig. 6. ξ vs total collagen (%) relationship for virgin, pregnant and parous sheep groups.

during pregnancy (Alperin et al., 2010). The proximal vagina had a higher amount of collagen than distal vagina. A higher total collagen coincided with a higher ultimate stress and Young's modulus.

The HM coefficient may play a significant role on the accurate *in silico* simulation of biological tissues and structures. It enables the estimation of critical mechanical properties hard to obtain through experimentation, or unavailable, as often occurs for the human model. Therefore, given that hyperelasticity theory has been used, nonlinear simulations based on this data, are within reach.

Depending on the tissue and boundary conditions under analysis, one may require the use of more sophisticated models. These may include damage mechanisms (Linka et al., 2018; Balzani et al., 2012), tissue rupture (Gültekin et al., 2018), among others. Since they require information of the protein contents, fluid fractions and fibers orientations, the choice of the histological technique may be relevant (Linka et al., 2017; Nebelung et al., 2017). Further research should address statistical analysis and a combination of tissues and loading conditions in the definition of a histologically-motivated coefficient.

The significance of HM coefficient should be understood within scope of uniaxial loading conditions. The correlation between ξ and total collagen content, may not hold in different loading conditions such as the biaxial or triaxial loading.

The non-dimensional HM coefficient (ξ) was introduced for the first time. ξ establishes a direct link between biology and mechanics by connecting the total collagen content (tissue's microstructure) to the HGO model parameters (nonlinear mechanical behavior). Histological data was seen play the fundamental role of bridging experimental findings and theoretical models.

In this context, the proposed methodology appears very promising in estimating the response of the tissue via histological information.

Acknowledgements

This research has been supported by Fundação para a Ciência e a Tecnologia I.P. (FCT, Portugal) under grants SFRH/BD/96548/2013, IF/00159/2014, SFRH/BD/107860/2015, SFRH/BPD/111846/2015; UROSPHINX - Project 16842, COMPETE2020, through FEDER and FCT; and in part by a grant of the EC in the FP7-framework (Bip-Upy; NMP3-LA-2012-310389).

Conflict of interest statement

The authors do not have to disclose any financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

References

- Abaqus 6.11. Abaqus Documentation, Dassault Systèmes, Providence, RI, USA.
- 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 (9), 1164–1174.
- Balzani, D., Brinkhues, S., Holzapfel, G.A., 2012. Constitutive framework for the modeling of damage in collagenous soft tissues with application to arterial walls. *Comput. Methods Appl. Mech. Eng.* 213, 139–151.
- Bonet, J., Wood, R.D., 1997. *Nonlinear Continuum Mechanics for Finite Element Analysis*. Cambridge University Press, Cambridge.
- Caetano, G.F., Fronza, M., Leite, M.N., Gomes, A., Frade, M.A., 2016. Comparison of collagen content in skin wounds evaluated by biochemical assay and by computer-aided histomorphometric analysis. *Pharmaceut. Biol.* 14, 1–5.
- Calvo, B., Peña, E., Martins, P., Mascarenhas, T., Doblaré, M., Natal Jorge, R.M., Ferreira, A., 2009. On modelling damage process in vaginal tissue. *J. Biomech.* 42, 642–651.
- Couri, B.M., Lenis, A.T., Borazjani, A., Paraiso, M.F.R., Damaser, M.S., 2012. Animal models of female pelvic organ prolapse: lessons learned. *Expert Rev. Obstet. Gynecol.* 7, 249–260.
- David, E., Goldberg, D.E., 1989. *Genetic algorithms in search, optimization, and machine learning*. Addison-Wesley Longman Publishing Co., Inc.
- De Landsheere, L., Brieu, M., Blacher, S., Munaut, C., Nusgens, B., Rubod, C., Noel, A., Foidart, J.M., Nisolle, M., Cosson, M., 2016. Elastin density: Link between histological and biomechanical properties of vaginal tissue in women with pelvic organ prolapse? *Int. Urogynecol. J.* 27, 629–635.
- De Landsheere, L., Munaut, C., Nusgens, B., Maillard, C., Rubod, C., Nisolle, M., Cosson, M., Foidart, J.M., 2013. Histology of the vaginal wall in women with pelvic organ prolapse: a literature review. *Int. Urogynecol. J.* 24, 2011–2020.
- Delingette, H., 1998. Towards realistic soft tissue modeling in medical simulation. *Proc. IEEE, Inst. Electr. Electron. Eng.* 86, 512–523.
- Drucker, D.C., 1959. A definition of a stable inelastic material. *ASME J. Appl. Mech.* 26, 101–195.
- Fenner, D.E., Hsu, Y., 2010. Pathophysiology of the pelvic floor: Basic physiology, effects of ageing, and menopausal changes. *Pelvic Floor Disorders*, 25–32.
- Fung, Y.C., 1993. *Biomechanics: Mechanical Properties of Living Tissues*. Springer, New York.
- Gültekin, O., Dal, H., Holzapfel, G.A., 2018. Numerical aspects of anisotropic failure in soft biological tissues favor energy-based criteria: A rate-dependent anisotropic crack phase-field model. *Comput. Methods Appl. Mech. Eng.* 331, 23–25.
- Holzapfel, D.A., 2000a. *Nonlinear Solid Mechanics*. John Wiley and Sons, Chichester.
- Holzapfel, G.A., Gasser, T.C., Ogden, R.W., 2000. A new constitutive framework for arterial wall mechanics and a comparative study of material models. *J. Elasticity* 61, 1–48.
- Holzapfel, G.A., 2000b. *Biomechanics of Soft Tissue*. Biomech preprint series, Graz, Austria.
- Holzapfel, G.A., 2000c. *Nonlinear Solid Mechanics: A Continuum Approach for Engineering*. John Wiley and Sons Ltd, Chichester, United Kingdom.
- Jackson, S., James, M., Abrams, P., 2002. The effect of oestradiol on vaginal collagen metabolism in postmenopausal women with genuine stress incontinence. *BJOG* 109 (3), 339–344.
- Khalil, A.S., Bouma, B.E., Kaazempur Mofrad, M.R., 2006. A combined FEM/genetic algorithm for vascular soft tissue elasticity estimation. *Cardiovasc. Eng.* 6, 93–102.
- Linka, K., Hillgärtner, M., Itskov, M., 2018. Fatigue of soft fibrous tissues: Multi-scale mechanics and constitutive modeling. *Acta Biomaterialia* 72, 398–410.
- Linka, K., Itskov, M., Truhn, D., Nebelung, S., Thüning, J., 2017. T2 MR imaging vs. computational modeling of human articular cartilage tissue functionality. *J. Mech. Behav. Biomed. Mater.* 74, 477–487.
- Maletta, C., Pagnotta, L., 2004. On the determination of mechanical properties of composite laminates using genetic algorithms. *Int. J. Mech. Mater. Des.* 1, 199–211.
- Nebelung, S., Sondern, B., Oehrl, S., Tingart, M., Rath, B., Pufe, T., Raith, S., Fischer, H., Kuhl, C., Jahr, H., Truhn, D., 2017. Functional mr imaging mapping of human articular cartilage response to loading. *Radiol. J.* 282, 464–474.
- Rynkevic, R., Martins, P., Hympanova, L., Almeida, H., Fernandes, A.A., Deprest, J., 2017. Biomechanical and morphological properties of the multiparous ovine vagina and effect of subsequent pregnancy. *J. Biomech.* 57, 94–102.
- Ulrich, D., Edwards, S.L., Su, K., White, J.F., Ramshaw, J.A., Jenkin, G., Deprest, J., Rosamilia, A., Werkmeister, J.A., Gargett, C.E., 2014. Influence of reproductive status on tissue composition and biomechanical properties of ovine vagina. *PLOS*, 9.
- Urbankova, I., Vdoviakova, K., Rynkevic, R., Sindhvani, N., Deprest, D., Feola, A., Herijgers, P., Krofta, L., Deprest, J., 2017. Comparative Anatomy of the Ovine and Female Pelvis. *Gynecol Obstet Invest.*
- Urbankova, I., Callewaert, G., Blacher, D., Deprest, D., Hympanova, L., Feola, A., De Landsheere, L., Deprest, J., 2018. First delivery and ovariectomy affect biomechanical and structural properties of the vagina in the ovine model. *Int. Urogynecol. J.*