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RESEARCH ARTICLE

The effect of consecutive pregnancies on the ovine pelvic soft tissues: Link between biomechanical and histological components

Rita Rynkevic[a,b,c,d], Pedro Martins[a,b,*], Antonio Andre[b], Marco Parente[a,b], Teresa Mascarenhas[e], Henrique Almeida[f], Antonio A. Fernandes[a,b]

[a] University of Porto, Faculty of Engineering, Portugal
[b] INEGI - Institute of Science and Innovation in Mechanical and Industrial Engineering, Portugal,
[c] KU Leuven, Department Development and Regeneration, Biomedical Sciences, Leuven, Belgium
[d] Centre for Surgical Technologies, Group Biomedical Sciences, KU Leuven, Leuven, Belgium
[e] Department of Gynecology and Obstetrics, Centro Hospitalar de São João-EPE, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, Portugal
[f] University of Porto, Faculty of Medicine, Department of Experimental Biology, Portugal

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ABSTRACT

Background: Pelvic organ prolapse, various types of incontinence (urinary incontinence, defecatory dysfunction), chronic cystourethritis, and sexual dysfunctions remain between the most common disorders in urogynecology. Currently, it is believed that the nature and number of births plays a major role in their development. Moreover, after these events, pelvic floor tissues may not recover to their original statuses. The close anatomical relationship among the vaginal wall, bladder and rectum often contribute to the emergence of anatomical-functional failure of adjacent organs and systems.
Basic procedures: The aim of this study was to investigate the effect of consecutive pregnancies on pelvic floor soft tissues, conducting biomechanical and histological analysis. Fifteen Swifter ewes: virgins, parous and pregnant were used. Samples, for uniaxial tension tests and histological analysis, were cut out from fresh tissue. A description of the mechanical properties of native tissue was obtained from the stress-strain curve. Histological samples were stained with Miller's Elastica staining and analyzed using ImageJ software. Collagen, elastin, and smooth muscle contents (%) were analyzed along the full wall thickness of the selected organs. The links between mechanical properties of the soft tissues and histological parameters were analyzed.
Main findings: Mechanically, vaginal wall tissue and cervix of pregnant sheep were more compliant. In contrast, bladder and rectum became stiffer and had the highest total collagen content. Parous sheep rectum and bladder were stiffer, compared to virgin sheep.
Principal conclusions: Tensile strength appears to be linked to total collagen content. Elastin and smooth muscle show a direct influence on tissue compliance.

1. Introduction

The dysfunction of the pelvic floor is understood as a complex of disorders. It affects the ligamentous apparatus and pelvic floor muscles that hold the pelvic organs in a normal position and provide urine and feces containment (Kenton and Mueller, 2006).

Pelvic organ prolapse, various types of incontinence (urinary incontinence, defecatory dysfunction), chronic cystourethritis, sexual dysfunctions remain the most common disorders in urogynecology. Function failure of the pelvic floor muscles and organs has several reasons: age, heredity, childbirth traumatism, birth of a large fetus, severe physical exertion associated with increased intra-abdominal pressure, between others. Currently, it is believed that the nature and number of births plays a major role in this process. This occurs mainly due to damage of the perineal muscles and pelvic diaphragm during childbirth (MacLennan et al., 2000; Karasick and Spettell, 1997). Some authors consider that caesarean section has a protective role for the pelvic floor (Larsson et al., 2009; Lukacz et al., 2006), which seems in contradiction with cases of

* Corresponding author at: INEGI, Campus da FEUP, Rua Dr. Roberto Frias, 400, 4200-465 Porto, Portugal.
E-mail addresses: r.rynkevic@gmail.com (R. Rynkevic), palsm@fe.up.pt (P. Martins), aandre@inegi.up.pt (A. Andre), mparente@fe.up.pt (M. Parente), tqc@sapo.pt (T. Mascarenhas), almeidah@med.up.pt (H. Almeida), aaf@fe.up.pt (A.A. Fernandes).

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prolapse in nulliparous women as reported by Buchsbaum et al., 2006. Moreover, there is no significant difference in the incidence of prolapse in women after vaginal delivery "*per vias naturalis*" and women that underwent caesarean section (Larsson et al., 2009).

Regarding the elastin and collagen contributions for tissue's load bearing, evidence shows that collagen fibers play a dominant role (Urbankova et al. 2018). Collagen is largely responsible for soft tissue tensile strength, while elastin makes the tissue more compliant (Fung, 1993). Factors such as collagen dispersion and orientation, undulation and orientation, type I:III ratio may contribute to the changes occurring during the pregnancy (Ulrich et al., 2014). 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. While collagen type I is largely responsible for tissue' tensile strength (Jackson et al., 2002). Elastin fibers may play an important role in pelvic floor mechanics, however, alone they are unable to change the mechanical properties of the pelvic floor (Rahn et al., 2008).

The close anatomical relationship between the vaginal wall, bladder and rectum often contribute to the emergence of anatomical-functional failure of adjacent organs and systems. The levator ani muscle stabilizes the abdominal and pelvic organs. Urethra and the rectum are mechanically closed by the levator ani muscle; it relaxes at the beginning of urination and defecation.

The aim of this study was to investigate the impact of subsequent pregnancies on pelvic floor soft tissues, through the analysis of the relationship between biomechanical parameters and histological components. This research can contribute to improve the understanding of pelvic floor soft tissues transformation before delivery, both in terms of biomechanics and histology. The study also includes a follow-up of the recovery of pelvic floor soft tissues one year after vaginal parturition, using virgin sheep as baseline model.

Since studies on fresh human pelvic soft tissues are limited, due to shortage of material and ethical concerns, the sheep model was used in these studies. Despite being a quadruped, the pelvic floor tissues and anatomy of sheep are comparable (relatively) in size and structure to humans (Abramowitch et al., 2009). Moreover, the risk factors such as increased intraabdominal pressure, parity or obesity are similar (Abramowitch et al., 2009; Patnaik et al., 2012; Couri et al., 2012; Shepherd, 1992). It is possible to perform vaginal surgery with/without graft insertion (Urbankova et al., 2017a, 2017b). Furthermore, there are some studies regarding the comparative analysis of the ovine and female pelvic floor anatomy (Urbankova et al., 2017a, 2017b).

2. Methods

2.1. Animal model

Three groups of Swifter ewes (virgin, pregnant and parous) were selected: virgins (n = 5; mean weight = 45 kg), pregnant (n = 5; term = 145 days; mean weight = 65 kg) after two prior vaginal deliveries and parous (n = 5; mean weight = 60 kg) at least one year after three vaginal deliveries, were used. Virgin sheep were nine-month-old, while pregnant were three years old and parous four years old. Ewes used in this study were obtained from the Zoötechnical Institute of the KU Leuven. Animals were maintained and treated according to an experimental protocol approved by the Ethics Committee for Animal Experimentation of the KU Leuven Faculty of Medicine. All the animal procedures undertaken as part of the work described in this paper were carried out in accordance with the European regulations for animal use and care (European Directive 2010/63/EU and National Decree-Law 113/2013). Ewes were euthanized by intravenous injection of 10 mL of a mixture of embutramide 200 mg, mebezonium 50 mg and tetracaine hydrochloride 5 mg (T61; Hoechst Marion Roussel, Brussels, Belgium).

2.2. Sample preparation

For this study vaginal wall (distal part), rectum (distal part), bladder, cervix, uterus, levator ani muscle (LAM[1]) and external anal sphincter (EAS[2]) were collected. The distal part of vaginal wall and rectum were selected, since it is prone to tears induced by vaginal delivery.

Excised tissues were divided into samples for biomechanical testing and for histological analyses. The storage time of the soft tissues (until testing) did not exceed 4 h.

For the biomechanical testing vaginal tissue specimens were cut along the longitudinal axis using a dog bone shape punch (2 mm × 30 mm). Samples from rectum, bladder, uterus and cervix were cut in the longitudinal axis using a rectangular shape cutting form (10 mm × 50 mm). External anal sphincter and levator ani muscles were carefully separated from surrounding tissues. Each tissue was then tested as a single sample to preserve its integrity. Prior to measurements tissues were kept in wet (saline soaked) gauze at room temperature (∼20 °C). Each specimen was tested as soon as it was prepared to reduce unnecessary over-exposure to the environment.

For histological analysis vaginal wall, rectum, bladder and uterus (the uterus only from virgin and parous sheep) were fixed in 4% paraformaldehyde during 24 h. Then, specimens were washed in PBS[3] and stored in ethanol. 6-μm tissue slices were stained with Miller's Elastica staining.

2.3. Uniaxial tensile testing

Tensile tests were performed using Zwick tensiometer (Zwick GmbH & Co. KG, Ulm, Germany). Samples were pre-loaded untill 0.1 N using a constant elongation of 5 mm/min. Specimens' width and thickness were taken at three locations along the sample length and averaged, to calculate the stress. After the pre-load specimens were loaded using a constant elongation rate of 10 mm/min until failure. The strain of the analyzed specimens was calculated by dividing the elongation ($\Delta L = L_1 - L_0$) by the clamp-to-clamp length (L_0). The Young's modulus (MPa[4]) was calculated from the stress-strain curve inthe comfort zone, limited to the physiological range (10–20%) of deformation and at the stress zone, in the range of supraphysiological stress (70–80%) (Ozog et al., 2011). The point where these linear segments intersect was defined as the inflection point.

2.4. Histological image processing analysis

Images, showing a complete set of histological components, were analyzed. Separate images were captured at 10× magnification, using Zeiss microscope (Zeiss Axioplan 400, Oberkochen, Germany). Then using a Matlab (Release 2015®, The MathWorks, Inc.) stitching script based on the algorithms available from VLFeat library (Vedaldi and Fulkerson, 2008), individual images were connected into one full thickness image. After stitching, all images were processed using ImageJ (open platform for scientific image analysis) and the Color Deconvolution plugin. Applying a threshold algorithm, RGB images were converted to binary images (Rynkevic et al., 2017). The contents (%) of total collagen, elastin, and smooth

[1] LAM—levator ani muscle.
[2] EAS—external anal sphincter.
[3] PBS—phosphate-buffered saline.
[4] $1 \text{MPa} = 1 \text{N/mm}^2 = 1 \times 10^6$ Pa.

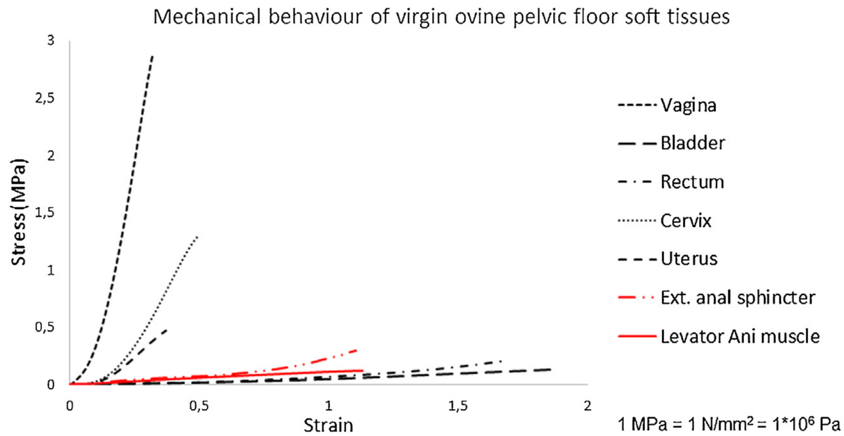

Fig. 1. Mechanical behaviour of virgin sheep pelvic floor soft tissues. Red lines were used for muscles' and black lines were used for the remaining pelvic structures.

muscle were determined from processes images, relative to the total area (%) of the sample.

High-resolution histological images were obtained using image processing techniques, making it easier to analyze the full thickness of the tissues' microstructure (Caetano et al., 2016).

2.5. Statistical analyses

All statistical analyses were conducted using a statistical software package (GraphPad Prism 5, USA). Statistical analyses were performed to investigate possible significant differences in mechanical properties and structural composition among experimental groups. Quantitative data are represented as mean ± standard error of the mean (SEM[5]). Kolmogorov–Smirnov tests showed the data follows a normal distribution, a requirement for ANOVA. 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

For the virgin sheep, the biomechanical properties of the vaginal wall, cervix, uterus, bladder, rectum, and muscles (external anal sphincter and levator ani muscle) differed significantly (Fig. 1). Vaginal wall was stiffer and less extensible than rectal tissue and bladder. Cervix and uterus were less stiff than vagina, but stiffer than bladder and rectal tissue. The muscles could withstand significant deformation compared to the vaginal wall, cervix and uterus, however, much less than rectum and bladder.

Full thickness high quality histologic images of sheep vaginal wall, uterus, bladder and rectal wall are provided in Fig. 2.

To understand the impact of pregnancy and multiple deliveries on pelvic floor soft tissues, each organ was considered separately (Fig. 3). Table 1 summarizes the mechanical behavior and quantitative morphological analysis of distal part of vaginal wall. Pregnant sheep vaginal tissue was more compliant, than of virgin sheep (39.8%; $p < 0.05$). (Table 1). One year after third delivery vagina of parous sheep was less compliant than in the third pregnancy (40.7%; $p < 0.05$). The results of the morphological analysis showed that thickness of vaginal wall changed during the pregnancy (Table 1). Vaginal wall was thinner in pregnant sheep than in virgins (25.5%; $p < 0.05$) and then in parous (33.6%; $p < 0.05$). The vaginal wall of parous sheep became thicker than of virgin (10.9%) ewes.

The total collagen content in vaginal wall was significantly higher in virgin sheep than in pregnant (14.1%; $p < 0.05$) or parous (7.3%; $p < 0.05$). An opposite observation was made for elastin content. Pregnant sheep had a higher amount of elastin fibers than virgin (47.2%; $p < 0.05$) and parous (25.9%, $p < 0.05$). Smooth muscle cells and myofibroblasts content were higher in parous sheep than in pregnant (10.6%; $p < 0.05$) and virgin (26.74%; $p < 0.05$). During the pregnancy, smooth muscle cells content increased, compared to virgin levels (19.45%; $p < 0.05$).

Table 2 represents mechanical behavior of cervical tissue. Biomechanical characteristics of the tissue showed that pregnancy affects the mechanical properties of the cervix. It became more compliant than in virgin (35.9%, $p < 0.05$) and in parous sheep (31.7%; $p < 0.05$).

Mechanical behavior and morphological parameters of virgin and parous sheep uterus are represented in Table 3. Uniaxial tests showed significant difference in mechanical behavior between virgin and parous sheep uterus. Young's moduli in comfort and stress zone differed significantly (70.9%; $p < 0.05$), (55.4%; $p < 0.05$). Elongation increased as well (34.9%; $p < 0.05$). Morphological analysis showed, that parous sheep uterus became thicker (39,6%; $p < 0.05$), it contained less total collagen (30.05%; $p < 0.05$) and more elastin fibers (17.3%; $p < 0.05$) than of virgin sheep.

Pregnant sheep bladder became more rigid. Young's moduli in comfort and stress zones were higher compared to virgin (74.6%; $p < 0.05$) and parous sheep (74.9%; $p < 0.05$) (Table 4). The pregnant sheep bladder became less extensible than virgin's (21.2%; $p < 0.05$) and parous sheep (20.5%; $p < 0.05$). Bladder became significantly thinner during the pregnancy (20.2%; $p < 0.05$). Pregnant sheep bladder conteined more total collagen (34.6%; $p < 0.05$; 13.6%; $p < 0.05$), less elastin (37.6%; $p < 0.05$; 21.6%; $p < 0.05$) and less smooth muscle cells (31.3%; $p < 0.05$; 24.1%; $p < 0.05$) than virgin and parous sheep. However, the total collagen content of parous sheep was higher (24.3%; $p < 0.05$), the elastin fiber content (20.4%) and smooth mucle cell content were lower (10.1% $p < 0.05$) than of virgin sheep.

Significant differences in mechanical properties of the rectum of virgin, pregnant and parous sheep were found (Table 5). Young's moduli of comfort and stress zones were higher in pregnant sheep, compared to virgin (61.9%, $p < 0.05$; 44.1%, $p < 0.05$) and to parous sheep (46.8%, $p < 0.05$; 19.9%, $p < 0.05$), respectively. Parous sheep rectum became stiffer than of virgin (30.5%, $p < 0.05$). However, during the pregnancy rectum became less elastic than of virgin (23.8%, $p < 0.05$) and parous (22.4%, $p < 0.05$).

There were no significant differences in rectal wall thickness. Pregnant sheep rectum conteined more total collagen (24.6%; $p < 0.05$) than virgin and parous (10.7%; $p < 0.05$) sheep. It contained

[5] SEM—standard error of the mean.

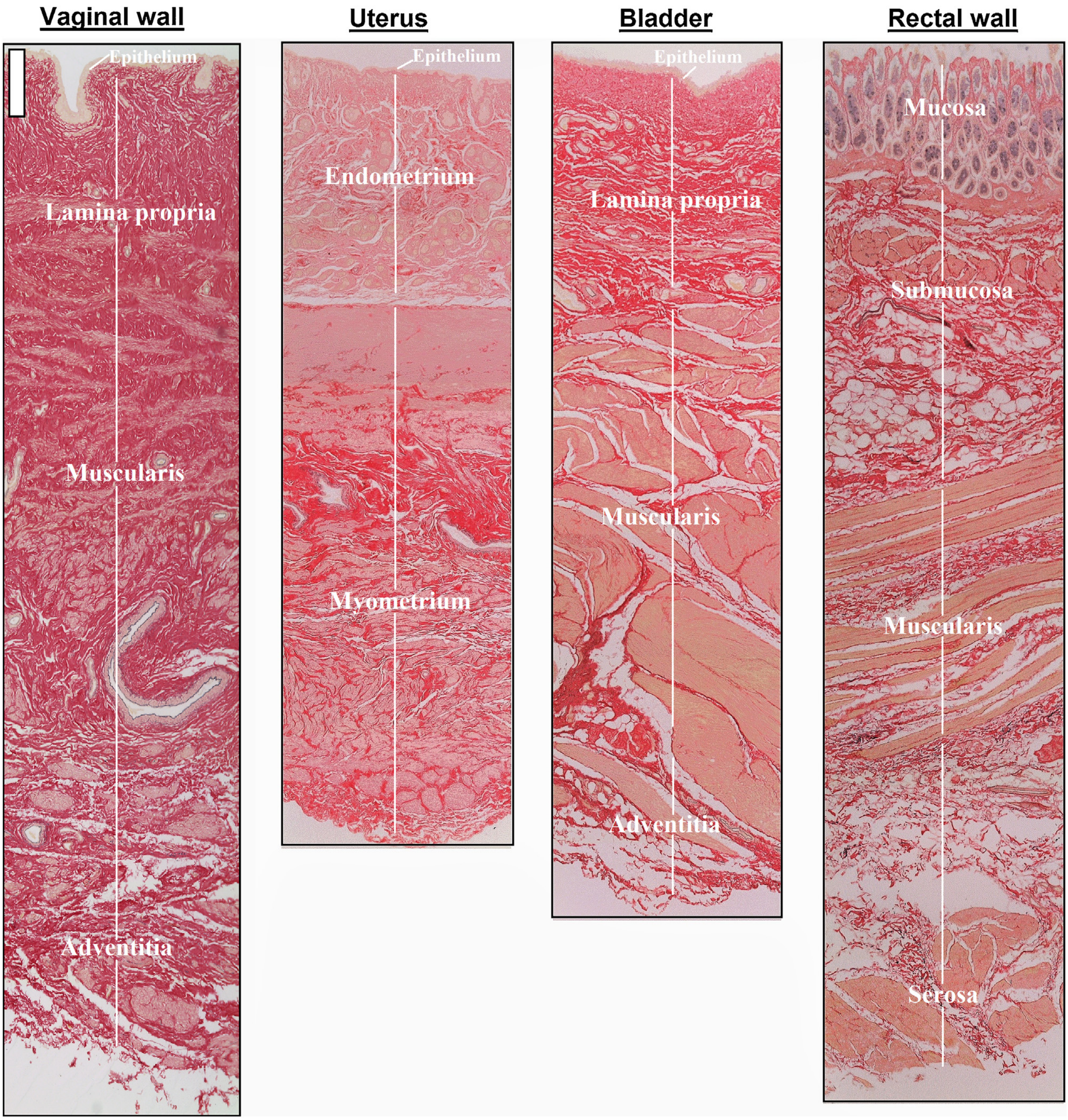

Fig. 2. Histological image of the ovine vaginal wall, uterus, bladder and rectal wall using Miller's Elastica staining. Scale bar (upper left) for sections — 200 μm. Staining results: total collagen (red), elastin fibres (jet-black), smooth muscle cells (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

less elastin fibers than virgin (36.9%, $p < 0.05$) and parous (20.1%, $p < 0.05$) sheep and less smoth muscle cells. Parous sheep total collagen content was higher (15.5%; $p < 0.05$), elastin fiber content was lower (18.7%) than in virgin. Smooth muscle cell content was significantly higher in virgin sheep compared to pregnant (11.2%, $p < 0.05$) and parous (6.8%).

The EAS, surrounds the margin of the anus and helps to control and delay defecation through its contraction. Computed stress-strain curve parameters demonstrate a hyperelastic behavior of EAS (Fig. 4). There was significant difference in the Young's modulus at comfort zone between virgin and pregnant sheep ($p < 0.05$). Pregnant sheep muscle was more compliant (Table 6). However, Young's modulus at stress zone and ultimate stress of parous sheep was significantly higher than of virgin ($p < 0.05$) and pregnant ($p < 0.05$).

During the pregnancy, muscle becomes more compliant (Fig. 4). LAM of parous sheep become significantly stiffer compared to virgin ($p < 0.05$) and pregnant sheep ($p < 0.05$) (Table 6).

4. Discussion

This investigation combines histological analysis of the sheep pelvic floor soft tissue with biomechanical studies. This was achieved by comparing groups of individuals at different life stages, before, during and after pregnancy. The impact of subsequent pregnancies and vaginal deliveries on pelvic floor soft tissues was

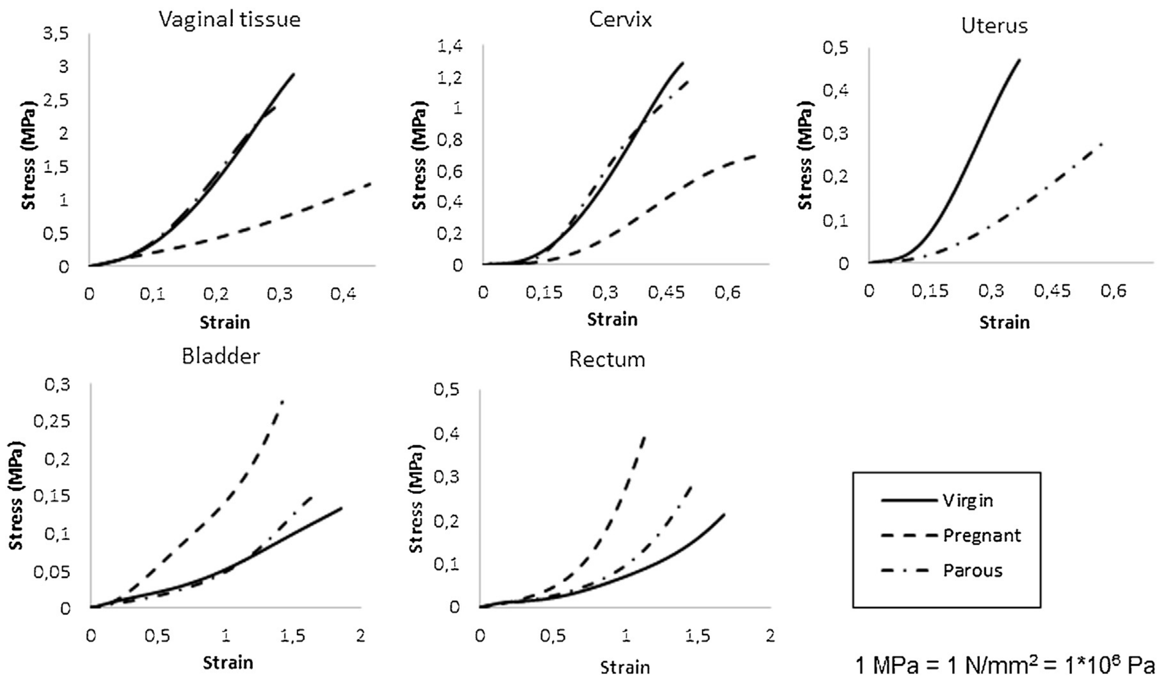

Fig. 3. Representative stress — strain curves showing mechanical behaviour of the pelvic floor soft tissues of virgin (n = 5), parous (n = 5) and pregnant (n = 5) sheep.

Table 1
Mechanical characteristics and morphological analysis of ovine vaginal wall. Data is presented as mean (±SEM), significant differences among the groups were set to p < 0.05: **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin.

Biomechanical properties of ovine distal vagina

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	2.000 ± 0.529	9.738 ± 1.697 **a**	0.1294 ± 0.014	0.3277 ± 0.017 **a**	2.704 ± 0.239 **a**
Pregnant	N = 5	1.759 ± 0.27 **b**	3.879 ± 0.748 **b**	0.1758 ± 0.031	0.4822 ± 0.049 **b**	1.253 ± 0.177 **b**
Parous	N = 5	2.629 ± 0.528	10.58 ± 2.575	0.1181 ± 0.014	0.2958 ± 0.030	2.111 ± 0.342

Morphological analysis of ovine distal vagina

Tissue	Nr	Collagen (%)	Elastin (%)	Smooth muscle (%)	Thickness (mm)
Virgin	N = 5	54.42 ± 0.8644 **a**	1.693 ± 0.3009 **a**	22.07 ± 0.8352 **a**	3.864 ± 0.2541 **a**
Pregnant	N = 5	46.86 ± 1.6513 **b**	3.201 ± 0.2112 **b**	27.40 ± 0.1955 **b**	2.880 ± 0.1039 **b**
Parous	N = 5	50.45 ± 0.8979 **c**	2.370 ± 0.0999	30.13 ± 0.2940 **c**	4.340 ± 0.1374

Table 2
Mechanical characteristics of ovine cervix. Data is presented as mean (±SEM), significant differences among the groups were set to p < 0.05, **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin.

Biomechanical properties of ovine cervix

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate stress (MPa)
Virgin	N = 5	0.2764 ± 0.037	2.031 ± 0.331 **a**	0.2477 ± 0.017 **a**	0.5000 ± 0.017 **a**	1.248 ± 0.116 **a**
Pregnant	N = 5	0.2425 ± 0.029	1.727 ± 0.285 **b**	0.1808 ± 0.012	0.7098 ± 0.048 **b**	0.807 ± 0.059 **b**
Parous	N = 5	0.2620 ± 0.036	2.699 ± 0.331	0.1867 ± 0.008 **c**	0.4644 ± 0.029	1.172 ± 0.104

Table 3
Mechanical characteristics of ovine uterus. Data is presented as mean (±SEM), significant differences among the groups were set to p < 0.05, **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin.

Biomechanical properties of ovine uterus

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	0.3785 ± 0.026 **a**	1.704 ± 0.129 **a**	0.1591 ± 0.011 **a**	0.366 ± 0.015 **a**	0.441 ± 0.027 **a**
Pregnant	N/A	N/A	N/A	N/A	N/A	N/A
Parous	N = 5	0.1048 ± 0.012	0.7603 ± 0.096	0.2936 ± 0.029	0.563 ± 0.054	0.287 ± 0.038

Morphological analysis of ovine uterus

Tissue	Nr	Collagen (%)	Elastin (%)	Smooth muscle (%)	Thickness (mm)
Virgin	N = 5	35.83 ± 2.601 **a**	4.664 ± 0.2881 **a**	55.97 ± 0.8007	1.780 ± 0.06680 **a**
Pregnant	N/A	N/A	N/A	N/A	N/A
Parous	N = 5	25.42 ± 1.556	5.634 ± 0.2780	56.63 ± 0.8171	2.951 ± 0.05119

Table 4
Mechanical characteristics and morphological analysis of ovine bladder. Data is presented as mean (±SEM), significant differences among the groups were set to $p < 0.05$, **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin. .

Biomechanical properties of ovine bladder

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	0.0345 ± 0.007 **a**	0.2185 ± 0.027 **a**	0.5753 ± 0.069	1.783 ± 0.117 **a**	0.1523 ± 0.011 **a**
Pregnant	N = 5	0.1341 ± 0.012 **b**	0.4272 ± 0.030 **b**	0.6148 ± 0.066	1.403 ± 0.055 **b**	0.3140 ± 0.024 **b**
Parous	N = 5	0.0336 ± 0.002	0.1378 ± 0.012	0.6806 ± 0.051	1.727 ± 0.096	0.2047 ± 0.026

Morphological analysis of ovine bladder

Tissue	Nr	Collagen (%)	Elastin (%)	Smooth muscle (%)	Thickness (mm)
Virgin	N = 5	34.20 ± 0.8313 **a**	6.090 ± 0.6831 **a**	53.81 ± 1.250 **a**	2.922 ± 0.1855 **a**
Pregnant	N = 5	52.31 ± 1.8271 **b**	3.798 ± 0.5333 **b**	36.94 ± 1.479 **b**	2.337 ± 0.1105
Parous	N = 5	45.20 ± 0.5299 **c**	4.843 ± 0.2618	48.69 ± 1.794 **c**	2.560 ± 0.0754

Table 5
Mechanical characteristics and morphological analysis of ovine rectum. Data is presented as mean (±SEM), significant differences among the groups were set to $p < 0.05$, **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin.

Biomechanical properties of ovine rectum

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	0.0406 ± 0.0047 **a**	0.2037 ± 0.0190 **a**	0.940 ± 0.055 **a**	1.497 ± 0.126 **a**	0.250 ± 0.027 **a**
Pregnant	N = 5	0.1052 ± 0.0071 **b**	0.3642 ± 0.0332 **b**	0.656 ± 0.062 **b**	1.144 ± 0.094 **b**	0.404 ± 0.029 **b**
Parous	N = 5	0.0559 ± 0.006	0.2920 ± 0.0224 **c**	0.840 ± 0.039	1.474 ± 0.062	0.283 ± 0.014 **c**

Morphological analysis of ovine rectum

Tissue	Nr	Collagen (%)	Elastin (%)	Smooth muscle (%)	Thickness (mm)
Virgin	N = 5	29.71 ± 0.878 **a**	12.36 ± 1.1081 **a**	57.34 ± 0.6928 **a**	2.304 ± 0.1359
Pregnant	N = 5	39.39 ± 2.016 **b**	7.790 ± 0.8203 **b**	50.93 ± 1.233	2.357 ± 0.1207
Parous	N = 5	35.16 ± 1.505 **c**	9.754 ± 0.3816	53.44 ± 3.217	2.165 ± 0.1156

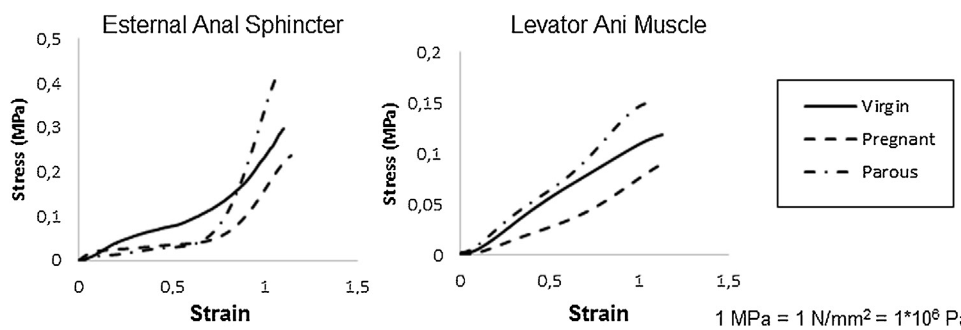

Fig. 4. Representative stress — strain curves showing mechanical behaviour of the pelvic floor muscles (EAS and LAM) of virgin (n = 5), parous (n = 5) and pregnant (n = 5) sheep.

Table 6
Mechanical characteristics of ovine external anal sphincter and levator ani muscle. Data is presented as mean (±SEM), significant differences among the groups were set to $p < 0.05$, **a**—virgin vs pregnant, **b**—pregnant vs parous, **c**—parous vs virgin. .

Biomechanical properties of ovine external anal sphincter

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	0.1629 ± 0.021 **a**	0.3768 ± 0.051 **a**	0.8073 ± 0.081	1.204 ± 0.138	0.2990 ± 0.039
Pregnant	N = 5	0.1069 ± 0.013	0.3456 ± 0.072 **b**	0.6852 ± 0.092	1.289 ± 0.113	0.2664 ± 0.019 **b**
Parous	N = 5	0.1122 ± 0.015	0.5424 ± 0.079 **c**	0.5877 ± 0.033 **c**	1.311 ± 0.117	0.4073 ± 0.026 **c**

Biomechanical properties of ovine levator ani muscle

Tissue	Nr	Young's modulus at comfort zone (MPa)	Young's modulus at stress zone (MPa)	Inflection point	Strain at Ultimate stress	Ultimate Stress (MPa)
Virgin	N = 5	0.1477 ± 0.011 **a**	0.1120 ± 0.007 **a**	0.5486 ± 0.043	1.125 ± 0.043	0.1215 ± 0.013 **a**
Pregnant	N = 5	0.07670 ± 0.01 **b**	0.07841 ± 0.00 **b**	0.4878 ± 0.051	1.037 ± 0.097	0.0917 ± 0.009 **b**
Parous	N = 5	0.1413 ± 0.012	0.1486 ± 0.017 **c**	0.4673 ± 0.041	1.186 ± 0.111	0.1847 ± 0.023 **c**

considered. Comparison between groups showed the effect of pregnancy on vaginal tissues. Image analysis techniques were used for histology analysis.

This research has shown that along the studied reproductive statuses, pelvic floor soft tissue undergoes profound histological and mechanical changes, particularly during pregnancy. Vaginal wall tissue and cervix became very compliant. This was associated with significant decrease in total collagen and a significant increase in elastin and smooth muscle cell content. These results agree with studies in rats, where strength of vaginal tissues decreased during pregnancy (Alperin et al., 2010). External anal sphincter and levator ani muscle also showed compliant behavior. In contrast, bladder and rectum had the highest increase in total collagen, which was associated with a high ultimate stress (Ramshaw et al., 2009). In a sheep the bladder is located inferior to the uterus. During pregnancy the uterus takes up significantly more space and severely limits the expansion of the urinary bladder.

This study has shown that soft tissues from the pelvic floor of parous sheep do not fully recover one year after vaginal deliveries. Parous sheep uterus became compliant and did not return to virgins' level, in agreement with studies by Drewes et al. (2017). Histological analysis confirmed the biomechanical findings. One year after vaginal delivery uterus became more compliant than of virgin sheep. Parous sheep uterus contained less collagen (total) and more elastin fibers and smooth muscle cells. Parous sheep rectum and bladder had higher ultimate stress, compared to virgin sheep. Total collagen content was higher in parous sheep, as expected. There were significant differences between levator ani muscle and external anal sphincter of virgin and parous sheep; they became less compliant one year after vaginal deliveries.

The elastic fibers and smooth muscle cells in the rectum and urinary bladder walls contribute to their distensibility and elasticity. This allows them to stretch and return to their original size and form several times a day (Boron and Boulpaep, 2016).

For both parous and pregnant sheep, the elastin fibres and smooth muscle cell contents of rectum and bladder were significantly decreased. These changes reduced their flexibility and capacity. As a result, this could be related with frequent urination or urinary incontinence, involuntary defecation and haemorrhoids or pelvic organ prolapse. Previous studies on animal models confirm these observations, demonstrating the importance of elastic fibres for maintaining structural and functional integrity of the female pelvic floor (Ferrell, et al., 2009; Liu et al., 2006, 2004).

The current work has some limitations. There was a lack of uterus samples from the pregnant sheep due to birth via c-section. There was no histological analysis of the external anal sphincter, levator ani and cervix due to considerations regarding the integrity of the analyzed specimens. Another limitation of the present work is the lack of an interobserver variability analysis, since the image thresholding required to estimate the relative quantities of tissue components, was semi-automatic. However, other studies comparing this image analysis technique with immunohistochemistry concluded that the former can be used as a simple, rapid, low-cost technology for evaluating histologic features (Caetano et al., 2016).

Basic science may improve clinical outcomes of pelvic floor disorders through advances on the understanding of disease mechanisms, and an improved knowledge about normal physiology.

A database of histology and biomechanical information combined with life history data, with adequate data mining resources may pave the way to produce patient customized prosthetics, with mechanical properties tailored to individual requirements.

5. Conclusions

A comparative biomechanical and histological analysis of pelvic floor soft tissues was carried out. It was observed that pelvic floor soft tissues (vagina, bladder, and rectum) undergo profound histologic and mechanical changes, particularly during pregnancy and do not recover to original levels one year after delivery. A link between the mechanical properties of the vaginal walls, bladder and rectum and their histological components — elastin, total collagen, and smooth muscle was observed. Tensile strength appears to be linked to total collagen fraction and elastin and smooth muscle fraction with tissue compliance.

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