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PREGNANCY INDUCED CHANGES OF PELVIC FLOOR SOFT TISSUES: THE RELATIONSHIP BETWEEN BIOMECHANICAL AND HISTOLOGICAL PARAMETERS

Hypothesis / aims of study

The vaginal wall and pelvic floor organs must adapt to pregnancy. This requires pelvic floor tissues to change their biomechanical properties. Nevertheless, epidemiologic studies show that many women fail to recover completely from these events. The aim of this study was to investigate the effect of consecutive pregnancies on pelvic floor soft tissues, conducting biomechanical and histological analyses. In addition, the links between biomechanical and histological parameters were investigated.

Study design, materials and methods

Three groups of Swifter ewes were considered: young post-menarchal (**virgins**) (n=5; avg. age = 9 months), **near term** (n=5; term = 145 days; avg. age= 3 years) and after two prior vaginal deliveries (**pregnant**). For this investigation, vaginal wall (distal part), rectum (distal part), bladder, levator ani muscle and external anal sphincter were collected. Biomechanical and histological analyses were conducted on samples from the excised tissues. The biomechanical properties were estimated through uniaxial tests. The biomechanical properties of the tissues (Young's moduli at comfort and stress zones, inflection point and Strain at Ultimate stress) were obtained from the stress-strain curve. For histological analysis, samples were stained with Miller's Elastica to allow identification and quantification of collagen, elastin, and smooth muscle content distributions. The fraction area of each constituent was measured using ImageJ software [1]. Statistical analysis was performed to study possible variations (on mechanical properties and content distributions) of pelvic floor organs between groups. Using GPower software, the statistical power analysis (to compute required sample size) showed that at least 16 samples for mechanical and 6 for structural analysis were needed to achieve 90% power when alpha error was set to 0.05. Kolmogorov-Smirnov tests showed the data follows a normal distribution, unpaired Student`s t-test was used for intergroup comparisons. The level of significance was set to $p<0.05$.

Results

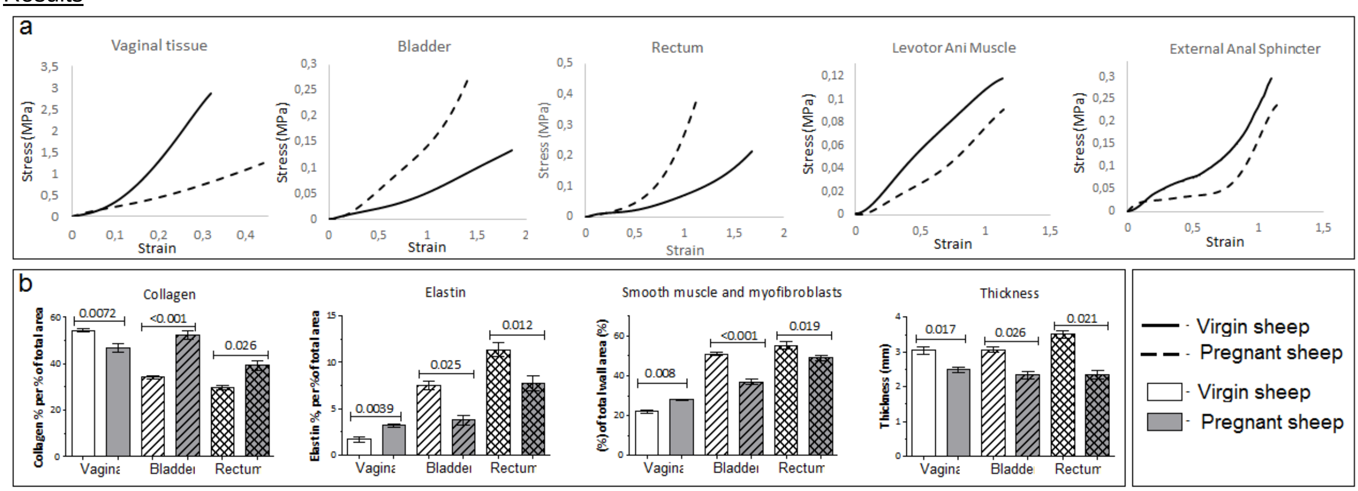

Figure1 **a** - Mechanical behaviour (representative stress-strain curves) of pelvic floor organs of virgin and pregnant sheep. **b**-Vertical column bar graph, (mean with ± SEM) representing total collagen (%), elastin (%) and smooth muscle and myofibroblasts content and thickness (mm). Significant differences between groups are displayed above the bars.

Vaginal tissue of pregnant sheep was more compliant, than of virgin (39.8%; $p<0.05$) (Table 1). The morphological analysis showed that, vaginal wall becomes thinner than virgin ($p<0.05$) during pregnancy (Figure 1b). The total collagen content in vaginal wall was higher in virgin sheep than in pregnant ($p<0.05$). The opposite was observed for the elastin content. Pregnant sheep had a higher amount of elastin fibres ($p<0.05$). During the pregnancy, smooth muscle cell content increased, compared to virgin sheep ($p<0.05$). There was a significant difference in the mechanical behaviour of the bladder during the pregnancy. Pregnant sheep bladder became more rigid. Young's moduli in comfort and stress zones were higher compared to virgin (74.6%; $p<0.05$). The bladder became less extensible (21.2%; $p<0.05$) and thinner during the pregnancy ($p<0.05$). It contained more total collagen ($p<0.05$), less elastin fibres ($p<0.05$) and less smooth muscle cells ($p<0.05$).

The same results were obtained for rectum tissues. Young's moduli of comfort and stress zones were higher in pregnant sheep (61.9%, $p<0.05$; 44.1%, $p<0.05$). Rectum become less extensible than of virgin (23.8%, $p<0.05$). In agreement with the mechanical properties, pregnant sheep rectum contained more total collagen ($p<0.05$), less elastin fibres ($p<0.05$) and smooth muscle cells ($p<0.05$) than virgin. For the external anal sphincter (surrounds the margin of the anus and helps to control and delay defecation through contraction), there were significant differences in Young´s modulus at comfort zone, between virgin and pregnant sheep ($p<0.05$). The urethra and the rectum are mechanically closed by the levator ani muscle; it relaxes at the beginning of urination and defecation. During the pregnancy, the muscle becomes more compliant ($p<0.05$).

Table1. Mechanical characteristics of ovine pelvic floor organs. Data is presented as mean (± SEM), significant differences among the groups were set to $p < 0.05$ (**×**)

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)
Biomechanical properties of distal vagina						
Virgin	N=16	2.000 ± 0.529	9.738 ± 1.697 ×	0.1294 ± 0.014	0.3277 ± 0.017 ×	2.704 ± 0.239 ×
Pregnant	N=16	1.759 ± 0.27	3.879 ± 0.748	0.1758 ± 0.031	0.4822 ± 0.049	1.253 ± 0.177
Biomechanical properties of bladder						
Virgin	N=15	0.0345 ± 0.007 ×	0.2185 ± 0.027 ×	0.5753 ± 0.069	1.783 ± 0.117 ×	0.1523± 0.011 ×
Pregnant	N=15	0.1341 ± 0.012	0.4272 ± 0.030	0.6148 ± 0.066	1.403 ± 0.055	0.3140± 0.024
Biomechanical properties of rectum						
Virgin	N=20	0.0406 ± 0.0047 ×	0.2037 ± 0.0190 ×	0.940 ± 0.055 ×	1.497 ± 0.126 ×	0.250 ± 0.027 ×
Pregnant	N=20	0.1052 ± 0.0071	0.3642 ± 0.0332	0.656 ± 0.062	1.144 ± 0.094	0.404 ± 0.029
Biomechanical properties of external anal sphincter						
Virgin	N=10	0.1629 ± 0.021 ×	0.3768 ± 0.051 ×	0.8073 ± 0.081	1.204 ± 0.138	0.2990 ± 0.039
Pregnant	N=10	0.1069 ± 0.013	0.3456 ± 0.072	0.6852 ± 0.092	1.289 ± 0.113	0.2664 ± 0.019
Biomechanical properties of levator ani muscle						
Virgin	N=10	0.1477 ± 0.011×	0.1120 ± 0.007 ×	0.5486 ± 0.043	1.125 ± 0.043	0.1215 ± 0.013×
Pregnant	N=10	0.07670 ± 0.01	0.07841 ± 0.00	0.4878 ± 0.051	1.037 ± 0.097	0.0917 ± 0.009

Interpretation of results

In this study, it was conducted a biomechanical/histological combined analysis of the sheep pelvic floor soft tissues. The impact of subsequent pregnancies on pelvic floor soft tissues was considered. Due to pregnancy, pelvic floor soft tissue undergoes profound histological and mechanical changes. Vaginal wall tissue become very extensible and compliant. This is associated with significantly low total collagen and high elastin content. External anal sphincter and levator ani muscle also showed compliant behaviour. In contrast, bladder and rectum had the highest total collagen, which was associated with a high ultimate stress.
Elastic fibres and smooth muscle tissue in the walls of the urinary bladder and rectum contribute to its distensibility and elasticity. Results showed, that bladder and rectal walls become thinner. In addition, rectum and bladder elastin fibres and smooth muscle cell content significantly decrease. This consequently reduces their flexibility. As a result, this could be related with frequent urination or urinary incontinence, constipation and haemorrhoids or POP.

Concluding message

It was observed that pelvic floor soft tissue undergoes profound histologic and mechanical changes, particularly during pregnancy and do not recover to its original status one year after. There was a connection observed between mechanical properties of the soft tissues and histological analysis. It was found experimental evidence, that collagen is largely responsible for soft tissue tensile strength and elastin for tissue elasticity.

References

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Disclosures

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