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MICROSTRUCTURAL VAGINAL TISSUE TRANSFORMATIONS IN VIRGIN, PREGNANT AND POSTPARTUM EWES.

Hypothesis / aims of study

Pelvic floor soft tissues and organs undergo changes during pregnancy, labour and are affected by age and hormonal status [1]. However, the degree and nature of this process is not completely understood and characterized.
This study investigates the link between biomechanical tissue behaviour and structural properties. Comprehension of how vaginal tissues' biomechanical properties, its structure and composition change during different reproductive status, may contribute to a better understanding of what is the normal tissue physiology. Morphological analysis could help to identify and describe some parameters influencing the pathophysiology of the pelvic floor. This in turn may assist in the design or improvement of pelvic organ prolapse (POP) reconstruction techniques.
Studies on fresh human vaginal wall samples are limited, due to shortage of material and ethical concerns. We have used the sheep model before because of the pelvic floor anatomy is comparable in size and structure to humans [2]. Also risk factors are similar as in humans, such as increased intraabdominal pressure, parity or obesity.

Study design, materials and methods

Vaginal wall from virgin (n=5), parous (n=5) and pregnant (n=5) Swifter sheep was harvested. Samples, for uniaxial tension test and histopathology, were cut from fresh vaginal tissue (distal region) in the longitudinal axis. Outcome measurements describing the mechanical properties of the native tissue were obtained from stress-strain curves. The explanted tissues for histological analysis were stained with Miller's Elastica staining. Image processing techniques were applied, allowing to work with high-resolution histological images and analyse the full tissue structure. Through thickness/ per layer morphological analysis of the vaginal wall was carried out. Collagen and elastin and contents were estimated. Statistical analyses were performed in order to determine significant differences in mechanical properties and structural composition among experimental groups. Using GPower software, 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.

Results

Virgin sheep vagina was stiffer than that of parous and pregnant sheep. The vaginal wall of pregnant sheep was significantly weaker, than both virgin ($p<0.001$) and parous ($p<0.05$) sheep. This means that pregnant sheep vaginal wall tissue could not withstand the same range of loads as other groups. However, it could sustain significantly higher strains compared with virgin and parous groups ($p<0.001$, $p<0.05$). Postpartum sheep vaginal wall recovers its mechanical properties, however, permanent morphological changes remain.
Morphological analysis showed that pregnant sheep vaginal wall was the thinnest of all groups, in all layers (Table 1). Pregnant sheep total collagen content in vaginal wall was lower than in virgin or parous. Significant difference was obtained in the lamina propria ($p<0.001$, $p<0.05$) and muscularis ($p<0.01$, $p<0.001$), respectively. Vaginal wall of pregnant sheep had higher amount of elastin fibres than virgin sheep (lamina propria, $p<0.001$, muscularis, $p<0.05$, adventitia, $p<0.01$) and parous sheep (lamina propria, $p<0.05$, adventitia, $p<0.01$).
Parous sheep vaginal wall was thicker than virgin and pregnant sheep vagina in all layers. It contained more total collagen than pregnant sheep in the lamina propria ($p>0.05$) and muscularis ($p>0.001$); and significantly less collagen than virgin sheep in lamina propria ($p<0.01$). Parous sheep contained less elastin fibres than pregnant sheep in the lamina propria ($p<0.05$) and adventitia ($p<0.01$) layers. It had more elastin fibres than virgin sheep, however significant difference was obtained only in lamina propria ($p<0.001$).

Interpretation of results

The results show that at different reproductive periods, vaginal tissue undergoes profound changes that influence the mechanical behaviour. Pregnant sheep vaginal tissue cannot withstand high loads, however, it is very extensible and sustains high deformations. This was associated with significantly low total collagen and high elastin content. Virgin sheep vaginal tissue had higher total collagen content relative to total vaginal wall area (%), associated with higher ultimate stress. On the other hand, it contains less elastin fibers, associated with lower stretching. In conclusion, after pregnancy the vaginal wall (distal region) recovers its stiffness, however there are permanent morphological changes.
The vaginal wall major components have been quantified in several studies [3]. However, considering the methods (relatively limited) and techniques (lack of standardization and methods of quantification), used in those studies, it is difficult to compare existing research results [3]. Comparing all layers, virgin sheep vaginal tissue contained higher collagen amount in all layers. While pregnant sheep vaginal tissue contained the highest elastin and lowest total collagen percentage in all layers.

Table1 Mechanical and structural characteristics of ovine distal vaginal wall. Data is presented as mean (± SEM), differences among groups were determined by unpaired t-tests, where: * p <0.05, **p <0.01, *** p <0.001

	Biomechanical properties of ovine vaginal wall				
	Parameters	Low modulus (MPa)	High modulus (MPa)	Strain	Ultimate Stress (MPa)
Tissues	**Virgin (n=16)**	3.69 ± 0.53	12.27± 1.47	0.33 ± 0.02	2.86 ± 0.27
	Pregnant(n=20)	3.58 ± 0.61	7.86 ± 1.99	0.45 ± 0.02	1.38 ± 0.12
	Parous (n=22)	1.87 ± 0.34	9.09 ± 1.15	0.29 ± 0.02	2.11 ± 0.30
P values	**Virgin vs Pregnant**	0,8857	0,1051	0,0014**	<0.0001***
	Virgin vs Parous	0,0048**	0,0933	0,1889	0,0838
	Pregnant vs Parous	0,0214*	0,6034	<0.0001***	0,0247*
	Thickness of the layers (mm ± SEM)				
	Layers	Epithelium	Lamina propria	Muscularis	Adventitia
Tissues	**Virgin (n=10)**	0.06 ± 0.01	1.09 ± 0.11	1.78 ± 0.22	0.72 ± 0.04
	Pregnant (n=10)	0.05 ± 0.01	0.61 ± 0.06	1.44 ± 0.08	0.51 ± 0.04
	Parous (n=10)	0.09 ± 0.01	0.63 ± 0.06	2.51 ± 0.08	0.77 ± 0.06
P values	**Virgin vs Pregnant**	0,0061**	0,0019**	0,1259	0,0021**
	Virgin vs Parous	0,0058**	0,0019**	0,0056**	0,3440
	Pregnant vs Parous	<0.0001***	0,5874	<0.0001***	0,0008***
	Total Collagen (% of total layer thickness ± SEM)				
	Layers	Epithelium	Lamina propria	Muscularis	Adventitia
Tissues	**Virgin (n=10)**	-	66.67 ± 1.27	52.89 ± 2.44	55.78 ± 2.91
	Pregnant(n=10)	-	44.96 ± 2.37	40.93 ± 1.12	48.41 ± 2.87
	Parous (n=10)	-	55.56 ± 1.95	55.41 ± 1.05	48.81 ± 1.79
P values	**Virgin vs Pregnant**	-	0,0001***	0,0019**	0,1184
	Virgin vs Parous	-	0,0031**	0,3776	0,0878
	Pregnant vs Parous	-	0,0127*	<0.0001***	0,91
	Total Elastin (% of total layer thickness ± SEM)				
	Layers	Epithelium	Lamina propria	Muscularis	Adventitia
Tissues	**Virgin (n=10)**	-	0.54 ± 0.19	1.51 ± 0.31	1.91 ± 0.59
	Pregnant(n=10)	-	2.45 ± 0.12	2.87 ± 0.26	4.37 ± 0.22
	Parous (n=10)	-	2.01 ± 0.13	2.37 ± 0.18	3.09 ± 0.13
P values	**Virgin vs Pregnant**	-	<0.0001***	0,0115*	0,0036**
	Virgin vs Parous	-	0,0007***	0,0520	0,0968
	Pregnant vs Parous	-	0,0452*	0,1781	0,0024**

Concluding message

In this study a detailed comparative analysis of the biomechanical properties, histological and morphometric parameters for the distal vaginal wall, among virgin, parous and pregnant ewes was performed. Both biomechanical properties and tissue composition were investigated, with special attention to their possible interdependency. Since elastin has a significant influence on the compliance of soft tissues and collagen is the main “actor” regarding strength, the histological analysis performed in this study justifies the mechanical behaviour observed. In conclusion, our data showed that vaginal tissue undergoes profound changes in tissue composition, during different reproductive stages, that influence the mechanical behaviour, particularly during pregnancy.

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Disclosures

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