A QUANTITATIVE HISTOLOGICAL EVALUATION OF THE PUBOVISCERAL ENTHESIS COMPARISON WITH OR WITH SONOGRAPHIC APPEARANCE OF LEVATOR ANI AVULSION.

Hypothesis / aims of study
The pubovisceral enthesis is classified as the connective tissue between levator ani tendon/ligament and the pubic bone (Kim J et al, 2011). The imaging finding of hypoechoicity of the pubovisceral attachment of the pelvic floor has been thought to be due to the literal detachment of the pubovisceral enthesis and has been termed an 'avulsion'. Some groups have suggested (Da Silva et al, 2016) that this imaging finding is not a true anatomical detachment of the pubovisceral muscle from the pelvic sidewall. This study aims to identify whether there is a histopathological difference in the tissue composition in cadavers with or without thelevator ani detachment imaging finding.

Study design, materials and methods
Female cadavers were imaged using 3D-translabial ultrasound using a GE Voluson I with a 5-9MHz electronic probe. The presence or absence of an hypoechoicity of the pubovisceral attachment was identified in accordance with previous described methodology (Dietz et al, 2011). A separate observer, blinded to the USS findings, meticulously dissected each cadaver to visualise the pubovisceral enthesis. The pubovisceral enthesis was excised for further histochemical processing. Samples were stained with Haematoxylin and Eosin and Masson's Trichrome according to standard procedures. Slides were randomly sectioned into three with each enthesis being considered independently and digitally scanned. Quantitative analysis using ImageJ software was conducted to compare tissue composition in samples with or without sonographic pubovisceral enthesis hypoechoicity by a blinded observer. The percentage of collagen and muscle was calculated for each sample. The results were analysed using SPSS v24, IBM, USA.

Results
Fourteen cadavers were imaged, totalling twenty-eight pubovisceral samples. Incomplete data was available for three samples (one missing USS image, two lacking histology specimens). Sonographic pubovisceral enthesis hypoechoicity was seen in four cadavers (30.1%); the defect was bilateral in 1/13 (7.7%) and unilateral in 3/13 (23.1%). No macroscopic anatomical detachment of the puboviseral muscle was seen in any cadaver. ImageJ quantification of Masson’s Trichrome stain identified the proportion of muscle to collagen as 38.8% : 61.2% vs 37.7% : 62.2% for specimens with vs. those without sonographic pubovisceral enthesis hypoechoicity (Kruskal-Wallis p=0.864).

<table>
<thead>
<tr>
<th>Stain type</th>
<th>Mean</th>
<th>SD</th>
<th>min</th>
<th>max</th>
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<tbody>
<tr>
<td>Muscle %</td>
<td>37.7</td>
<td>13.3</td>
<td>15.3</td>
<td>68.0</td>
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<tr>
<td>Collagen %</td>
<td>62.2</td>
<td>13.3</td>
<td>32.0</td>
<td>84.7</td>
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</table>

Table 1: Histological composition of the enthesis tissue using a Masson Trichrome stain in cadavers with no ultrasound avulsion detected on 3D translabial ultrasound (n=20)

<table>
<thead>
<tr>
<th>Stain type</th>
<th>Mean</th>
<th>SD</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle %</td>
<td>38.8</td>
<td>11.1</td>
<td>25.1</td>
<td>53.2</td>
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<tr>
<td>Collagen %</td>
<td>61.2</td>
<td>11.1</td>
<td>46.8</td>
<td>75.0</td>
</tr>
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</table>

Table 2: Histological composition of the enthesis tissue using a Masson Trichrome stain in cadavers with ultrasound avulsion detected on 3D translabial ultrasound (n=5)

Interpretation of results
In this study, there was no evidence of a microscopic tissue avulsion associated with an ultrasound avulsion. The tissue composition relating to the proportion of muscle versus collagen was not significantly different in specimens with or without sonographic pubovisceral enthesis hypoechoicity (avulsion). The pathophysiology of the sonographic pubovisceral enthesis still remains unknown.

Concluding message
Further research is required to investigate this imaging finding, until there is a better understanding of the pathology of this lesion surgical attempts at repair should be avoided.

References
Disclosures

Funding: There was no funding for this study. Clinical Trial: No Subjects: HUMAN Ethics not Req’d: cadaver gave specific consent for research prior to death, when they donated their body for teaching and research. Helsinki: Yes Informed Consent: Yes