Objectives

1. To quantify total protein in the vaginal wall with a Bicinchoninic Acid (BCA) Protein Assay and validate that the protocol is consistent and repeatable.
2. To compare protein concentration between left and right lateral vaginal wall.
3. To quantify protein concentration in the Fibulin-5 wildtype (fbln5 +/-) and heterozygous (fbln5 +/-) vaginal tissue.
4. To quantify the opening angle (mechanical function) of the Fibulin-5 wildtype 5 and heterozygous vaginal tissue pre- and post-elastase digestion.

Methodology

• Female 3-6 month FBLN5 mice at estrus (n=9) were used Tulane IACUC approved.
  - Vaginal tissue (n=3) from the murine reproductive system was extracted from fbln5 +/- mice.
  - Horizontal 2mm rings were trimmed from those samples with the remainder divided into lateral left and right sections and immediately snap frozen before being thawed at room temperature and homogenized.
  - Lysate obtained from the homogenization of the 2mm rings was used to conduct three trials of the BCA Assay to validate protocol consistency.
  - The homogenization process was repeated on the right and left vaginal tissue samples for a single BCA-Assay trial.
  - Vaginal tissue was explanted from fbln5 +/- (n=3) and fbln5 +/- (n=3) animals.
  - Two horizontal 1mm rings were cut from each of the Fbln5 samples with one ring serving as a control equilibrating for 30 minutes in HBSS and the other being treated for 45 minutes with elastase, an enzyme that removes elastic fibers.
  - Images of the rings after treatment were captured with microscope Excelsis-16C camera.
  - Opening Angle: Radial cuts were made to control and treated rings followed by equilibration in HBSS for 30 minutes (Fig 2).
  - Images were taken for quantification of the opening angle with ImageJ software.
  - The remaining Fbln5 tissue samples were separated by the left and right lateral walls with the left being snap frozen for preservation while the right was homogenized for a BCA Assay.

Methodology Continued...

• Pelvic Organ Prolapse (POP) is characterized as the descent of the pelvic organs (bladder, uterus, rectum) into the vaginal canal (Fig 1).
• Vaginal birth and aging are established risk factors, however the underlying mechanism are not fully elucidated.\(^{2,3}\)
• Compositional changes in extracellular matrix protein in vaginal samples from women with POP are reported.\(^{4,5}\)
• Although human tissues are ideal there are many challenges associated with preforming studies on human samples.
• The Fibulin 5 deficient murine readily develops prolapse similar to humans and are commonly used in POP research.\(^{6}\)
• Fibulin 5 is a protein that is important for assembling elastin into an elastic fiber.
• Elastic fibers allow tissue to stretch and recoil.
• There is limited work evaluated on the changes in protein composition and mechanical function of the Fibulin 5 heterozygous model.

Introduction

Quantification of Total Protein and Residual Strain in the Fibulin-5 Wildtype and Haploinsufficient Murine Vagina

Tyra Buckley\(^1\), Gabrielle Clark\(^2\), Kristin Miller\(^2\)

\(^1\)Department of Chemistry, Prairie View A&M University, Prairie View, TX
\(^2\)Department of Biomedical Engineering, Tulane University, New Orleans, LA

Data & Results Continued...

Fig. 7 Opening Angle (OA) Images
- A) Laterally cut 1mm ring of control WT vaginal tissue. (B) Laterally cut 1mm ring of elastase-treated WT vaginal tissue with a significantly decreased angle as compared to the control WT. (D) Laterally cut 1mm ring of elastase-treated HT vaginal tissue with a significantly decreased angle in comparison to the control HT. Data measured as mean +/- SEM.

Conclusion

• This study investigated the validity and consistency of using a Bicinchoninic Acid for the quantification of total protein in fbln5 vaginal tissue.
• The three trials conducted indicate the BCA is a reliable quantification tool, so for future experiments a single trial will be sufficient.
• Significant differences was not observed in comparison between the left and right lateral vaginal wall. This demonstrate that half of the vaginal sample can be used for BCA and the other half for other protein quantification assays.
• There was no significant impact when varying between WT and HT, but the removal of elastic fibers similarly affects both genotypes.
• Digestion of elastin significantly decreased the opening angle (mechanical function) of the vaginal tissue.
• Total protein concentration and opening angle was similar between both genotypes and elastic fibers significantly contributed to the opening angle (mechanical function) in both genotypes.
• Further investigation is needed to quantify elastin protein in both genotypes.

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References

\(^{1}\) Source: https://www.augm.org/prolapss/; \(^{2}\) Mant et al., 1997; \(^{3}\) Hendrix et al., 2002; \(^{4}\) Karam et al., 2007; \(^{5}\) Borenham et al., 2002; \(^{6}\) Drewes et al., 2007; \(^{7}\) Van Dyke et al., 2002.