CROSS-SPECIES ANALYSIS OF FETAL MEMBRANE MECHANICS

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INTRODUCTION

Preterm premature rupture of the fetal membranes (PPROM) complicates 3-4.5% of all pregnancies and is the leading cause of preterm birth (30-40%) [1,2]. PPROM likely occurs via multiple pathologic pathways. A few investigators have suggested that repeated stretching of the fetal membranes induces strain hardening that makes the membranes less elastic and more vulnerable to PPROM [3]. However, more investigation is needed to understand the mechanics of fetal membranes.

We aimed to compare the mechanical and biochemical characteristics of fetal membranes across species—human, porcine, and equine. By comparing such data, we hoped to identify properties that are unique to each form of gestation and properties that are conserved across species.

METHODS

Human placentas were collected from women with singleton pregnancies showing no evidence of infections, delivered via elective cesarean at term before the initiation of labor (Mississippi State University Institutional Review Board approved this study). Porcine reproductive tracts were obtained from a local abattoir and equine tissues were obtained via inter-departmental collaborations at Mississippi State University.

Each placenta was rinsed in Ringer's Lactate supplemented with 1% antibiotics/antimycotics (Sigma-Aldrich St. Louis, MO, USA) to remove blood and debris. Subsequently samples were excised for uniaxial tensile testing, biaxial puncture testing, histology, and biochemistry.

All mechanical testing was performed with samples submerged in phosphate buffered saline (PBS) at 37° C. Uniaxial tensile tests were carried out using the Mach-1 Micromechanical System (Bio Syntech Inc. Laval, Québec, Canada). Samples were mechanically preconditioned and pulled to failure at 50μm/s. Tensile modulus was computed using the slope of the linear region of the stress vs. strain curve from uniaxial testing.

Biaxial failure strength of tissues was determined by the biaxial puncture test using the Mach-1. Samples were mounted between two cylindrical discs with a circular opening of 2 cm DIA and with sandpaper on the grip- ping surfaces. A 3.2 mm DIA spherical probe mounted on the Mach-1 head was used to puncture samples. Failure strength was computed using a previously described method [4].

Tissue thickness was determined using a non-contact surface profiler (Talysurf CLI 2000 Gauge System, Taylor-Hobson Ltd, Leicester UK) to generate profiles of each sample.

Histology was performed on formalin fixed tissue samples embedded in paraffin. Sections were analyzed via H&E and Verhoeff's staining.

Biochemical analysis was performed on lypholized samples digested in papain. Total DNA content was obtained using the DNA Quantitation Flourescent Assay kit (Sigma-Aldrich St. Louis, MO, USA). Total protein content was obtained using the BCA Protein Assay kit (Pierce, Rockford, IL, USA).

RESULTS AND DISCUSSION

All species demonstrate failure of multiple layers (Figure 1). Average failure load for equine tissues was significantly higher than human and porcine (~5-fold and ~7.5-fold, respectively). Tensile modulus for human was significantly higher than porcine or equine. Porcine and equine tensile moduli were similar. Average failure stresses were highest in human tissues, although failure stress was the most closely conserved mechanical property (Table 1).

Biochemical and histological analyses indicate porcine and equine tissues to be less cellular than human. However, total protein content is conserved across species (results not shown).

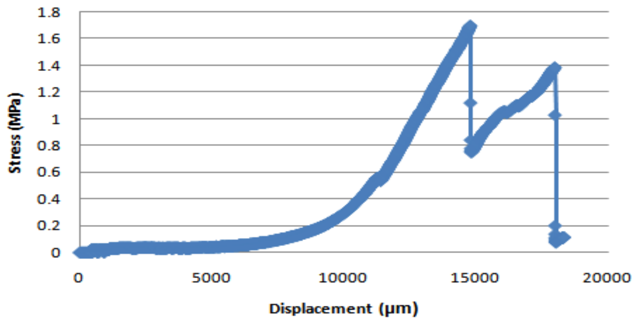

Figure 1: Stress vs. displacement plot of biaxial puncture test. The two failure peaks represent the amnion and chorion layers of human fetal membranes, respectively.

Table 1: Mechanical Results

	Human	Porcine	Equine
Avg. Failure Load (grams-*F*)	303.83	193.19	1559.91
Tensile Modulus (Pa)	2.25E+07	4.14E+06	2.48E+06
Avg. Failure Stress (Pa)	1.67E+06	9.34E+05	1.05E+06

CONCLUSIONS

Average failure load appears closely correlated to mass of the fetus. Furthermore, inherent tissue strength is greater in human than either porcine or equine tissues. We speculate this to be a result of increased physiological membrane stresses in humans due to discoid placentation versus diffuse placentation in porcine and equine tissues.

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