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Material properties of mouse cervical tissue in normal gestation

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ABSTRACT

An appropriately timed cervical remodeling process is critical for a healthy delivery, yet little is known about the material property changes of the cervix in pregnancy because obtaining human tissue samples is difficult. Rodent models offer advantages including accurately timed pregnant tissues and genetically altered models. Determining the material properties of the mouse cervix, however, is challenging because of its small size and complex geometry. The aim of this study is to quantify cervical material property changes in a normal mouse pregnancy using a microstructurally-inspired porous fiber composite model. We mechanically test intact, whole, gestation-timed mouse cervix by pulling apart tensioned sutures through its inner canal. To interpret our mechanical testing results, we conduct an inverse finite element analysis, taking into account the combined loading state of the thick-walled cylindrical tissue. We fit the material model to previous osmotic swelling data and load-deformation data from this study using a non-linear optimization scheme, and validate the model by predicting a separate set of deformation data. Overall, the proposed porous fiber composite model captures the mechanical behavior of the mouse cervix in large deformation. The evolution of cervical material parameters indicates that in a normal mouse pregnancy, the cervix begins to soften between day 6 and day 12 of a 19-day gestation period. The material parameter associated with the collagen fiber stiffness decreases from 3.4 MPa at gestation day 6 to 9.7e−4 MPa at gestation day 18, while the ground substance stiffness decreases from 2.6e−1 MPa to 7.0e−4 MPa.

Statement of Significance

Accelerated cervical remodeling can lead to extremely premature births. Little is known, however, about the material property changes of the cervix in pregnancy because pregnant human tissue samples are limited. Rodent models overcome this limitation and provide access to gestation-timed samples. Measuring the material property changes of the mouse cervix in pregnancy is challenging due to its small size and complex geometry. Here, we establish a combined experimental and modeling framework. We use this framework to determine the cervical material property changes throughout a normal mouse pregnancy. We present our experimental methods for mechanically testing whole, intact cervical tissue samples. We fit a porous fiber composite material model to the mechanical data and show that the mouse cervix begins to soften between day 6 and day 12 of a 19-day gestation period.

1. Introduction

Throughout the course of a pregnancy, the female reproductive tract undergoes significant changes as its various organs synchronously grow and remodel for the safe development and delivery of a baby at term. In particular, the timely remodeling of the uterine cervix from a stiff mechanical barrier into a compliant structure which can dilate for delivery is crucial. Premature cervical remodeling can result in the early dilation of the cervix in the absence of labor, leading to extremely premature births [1]. Along with premature cervical remodeling, the deformation and dilation of the uterine cervix is the final common pathway to many etiologies of preterm birth [2]. Preterm birth (PTB), a leading cause of neonatal death, affects 15 million babies every year [3] where surviving babies often suffer life-long disabilities and congenital disorders. Despite advances in prenatal care and increasing research

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efforts concentrated on understanding its causes, PTB rates have failed to significantly decrease largely because the physiology of normal and abnormal cervical remodeling in pregnancy remains unclear.

A key gap in knowledge in understanding the deformation and dilation of the cervix in pregnancy, as well as the causes of premature cervical remodeling, is the material characterization of the cervix throughout pregnancy. Since obtaining pregnant human cervical tissue serially throughout pregnancy is near impossible, the obstetric research community has continued to rely on the mouse and rat as animal models for pregnancy. Rodent models offer the advantages of gestation-timed pregnant tissue samples, genetically altered strains of abnormal remodeling, and provide valuable insights into the effects of various clinical treatments on cervical mechanical properties. Additionally, as explained below, the extracellular matrix (ECM) remodeling events of pregnancy are similar between human and mouse cervical tissue [4–8]. However, it is still unclear if the molecular triggering mechanisms for this remodeling behavior are conserved between human and mice [9] and there are obvious anatomical differences associated with mouse models of pregnancy. Regardless of these differences, there are sufficient similarities in the cervical material composition to study ECM-material property relationships during a mouse pregnancy.

As in the human cervix, the mouse cervix is the cylindrical connective tissue located at the base of the uterus. The internal os is the orifice connecting the bottom of the uterine horns and the top of the cervix and the external os is the portion of the cervix that extends into the vaginal canal (Fig. 1A). Similar to the human cervix, but unlike the rat cervix, the cervix of the mouse has a single inner canal. Dissimilar to the human cervix, the mouse cervix is connected to two separate uterine horns which extend to either side of the mouse abdomen (Fig. 1A). The nonpregnant mouse cervix is approximately 2.2 mm in diameter and 2.8 mm in length. In its 19 day gestation time period in pregnancy, the mouse cervix grows to approximately 4 mm in diameter and 4.4 mm in length. Previous mechanical tests on isolated, whole mouse cervical tissue typically disregard the contribution of geometry and size. Thus, intrinsic pregnant cervical material properties throughout gestation have not been reported, limiting the translations between studies from mouse to human pregnancy. Therefore, in an effort to determine the mechanical role of the cervix in preventing PTB and to establish a framework to quantify cervical material properties in the pregnant mouse cervix, we mechanically test intact, whole, gestation-time mouse cervical tissue samples and fit a microstructurally-inspired cervical constitutive model to the experimental data using an inverse finite element method. The material model is formulated such that its parameters have a physical interpretation to ECM components known to remodel during pregnancy [2].

In both mouse and human, the changes in the cervical ECM during pregnancy are hypothesized to result in the changing tissue mechanical properties [4–8]. The cervical ECM is about 75–80% hydrated, depending on the gestational age [10]. It is composed of fibrous collagens types I and III, small proteoglycans with sulfated glycosaminoglycan (sGAG) chains, the non-sulfated GAG hyaluronic acid (HA), elastin, and other matricellular proteins [2]. Among the various ECM components, the cervical collagen properties are hypothesized to have a central role in determining the mechanical integrity of the tissue and to drastically remodel during pregnancy [11]. Second harmonic generation images of the cervical collagen fibers in both mice [12,13] and humans [13,14] demonstrate similar intrinsic remodeling of the collagen fiber from straight fibers in the nonpregnant tissue into undulating fibers in pregnant tissue. Based on collagen solubility studies on human [14] and mouse cervical tissue [6,10], we hypothesize that cervical softening during pregnancy is facilitated by the simultaneous breakdown of stronger mature crosslinked collagens and the synthesis of weaker immature less crosslinked collagens. To investigate this hypothesis, we measured the enzymatic intermolecular immature divalent (crosslinks between two collagen molecules) and mature trivalent (crosslinks between three collagen molecules) collagen crosslink densities as well as corresponding structural tensile properties of the mouse cervix during pregnancy. In this previous study, we found that during a normal mouse pregnancy the collagen crosslinking maturity ratio (the content of mature trivalent crosslinks to immature divalent crosslinks) decrease with progressing gestation in early softening (mouse gestation days 6–15), but not in late softening (mouse gestation days 15–18) [7]. In addition, the amount of sGAGs in the cervix does not significantly change throughout gestation while the amount of the non-sulfated GAG HA is known to increase significantly in late pregnancy [15]. This change in HA content, however, does not affect the structural response of the tissue to tensile loading [16].

In their foundational study, Harkness and Harkness mechanically tested the tensile properties of nonpregnant and pregnant

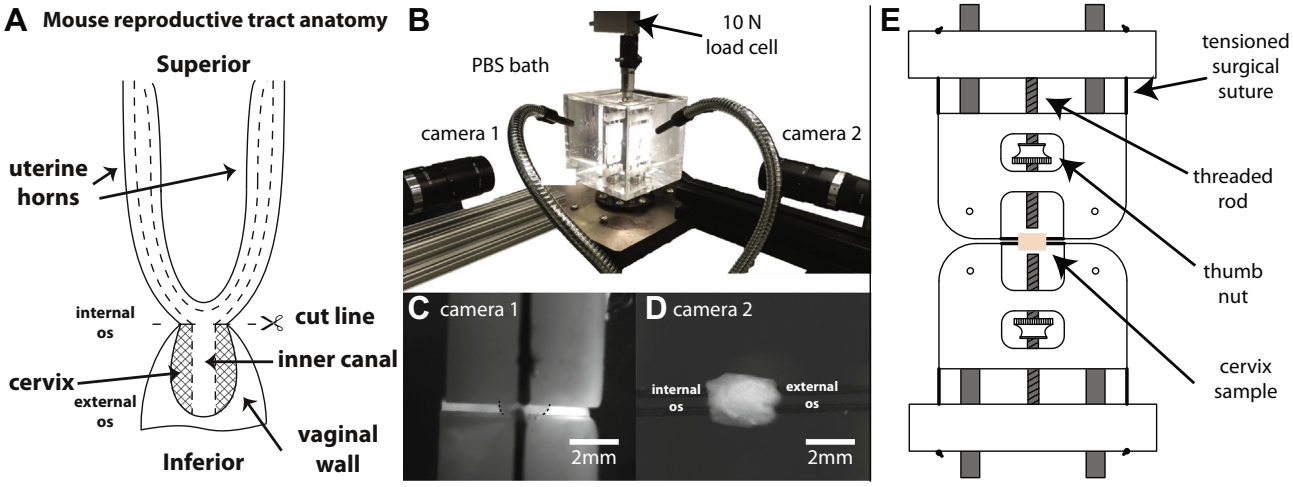

Fig. 1. Experimental setup for mechanical tensile testing. A) Diagram of a female mouse reproductive tract. The cervix was detached from the uterus at the cut line, then the vaginal wall was removed. B) Universal mechanical testing machine outfitted with a 10 N load cell. Samples were submerged in a PBS bath throughout testing while two perpendicular cameras continuously monitored sample geometry. C) Camera 1 view of the tensile grips and internal os of the cervix. D) Camera 2 length view of the cervix sample. E) Diagram of the custom tensile grips used for mechanical testing.