

FACULDADE DE ENGENHARIA DA UNIVERSIDADE DO PORTO

From Experimental Characterization to Simulation: Hyperelastic Modeling of Fetal Membranes and Integration into Pregnancy Scenarios

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Master's in Bioengineering - Specialization in Biomedical Engineering

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Resumo

O parto pré-termo contribui significativamente para a mortalidade perinatal e para problemas de saúde a longo prazo nos recém-nascidos prematuros. A membrana fetal desempenha um papel protetor ao longo da gravidez, mas os mecanismos que conduzem à sua rutura, uma das principais causas do parto pré-termo, permanecem pouco compreendidos. A presente investigação visa colmatar estas lacunas do conhecimento, através de abordagens experimentais e numéricas. Modelos de elementos finitos do ambiente uterino são utilizados para estudar a biomecânica da membrana fetal em diversos cenários relacionados com a gravidez.

Deste modo, os principais objetivos do presente estudo são os seguintes: clarificar a compreensão biomecânica da membrana fetal em diferentes cenários gestacionais; obter os parâmetros hiperelásticos do modelo da membrana fetal, através da utilização de um algoritmo de calibração; e, por fim, determinar a distribuição de tensões e deformações na membrana fetal em diversas circunstâncias associadas com a gravidez.

Para tal, foram realizadas simulações numéricas utilizando um modelo tridimensional do ambiente uterino no terceiro trimestre de gestação. Este modelo engloba o corpo uterino, o colo do útero, a membrana fetal com uma única camada e o abdómen materno. O modelo constitutivo adotado para descrever o comportamento biomecânico do corpo uterino, do colo do útero e do abdómen é do tipo neo-Hookeano, enquanto o comportamento da membrana fetal é representado por um modelo constitutivo hiperelástico anisotrópico. Os parâmetros de material da membrana fetal foram calibrados com recurso a um algoritmo micro-genético.

Com base neste modelo numérico, avaliou-se o efeito de três fatores principais relacionados com a gravidez sobre a membrana fetal: diferentes níveis de pressão intrauterina, rigidezes cervicais distintas e variações entre as superfícies interna e externa da membrana fetal. Os resultados foram analisados tendo em conta a tensão principal máxima, considerando-se três regiões anatomicamente relevantes da membrana fetal: zonas adjacentes à parte central do corpo uterino, ao colo do útero e ao fundus uterino. Para este propósito, foram construídos trajetos específicos em cada uma das regiões anteriormente mencionadas para cada simulação, utilizando o Abaqus Viewer.

A análise dos resultados revelou que a rutura da membrana fetal é mais propícia em situações de pressão intrauterina elevada e rigidez cervical reduzida, uma vez que ambos os cenários resultaram em valores mais altos de tensão principal máxima na membrana fetal, após a realização das simulações. Não foram observadas diferenças significativas entre as superfícies interna e externa da membrana fetal, no que respeita à tensão principal máxima.

Palavras-chave: Membrana fetal, Rutura da membrana, Parto pré-termo, Simulação numérica, Método de elementos finitos, Hiperelasticidade, Modelo constitutivo, Algoritmo genético

Abstract

Preterm birth significantly contributes to perinatal mortality and long-term health issues in preterm infants. The fetal membrane plays a vital protective role throughout pregnancy, but the mechanisms behind its rupture, a key cause of preterm delivery, remain poorly understood. The present research aims to fill these knowledge gaps through experimental and numerical approaches. Finite element models of the uterine environment are used to investigate fetal membrane biomechanics under diverse pregnancy-related scenarios.

Therefore, the primary goals of the current study are as follows: to clarify the biomechanical understanding of the fetal membrane under distinct gestational scenarios; to obtain the hyperelastic parameters for the fetal membrane model, through the use of a calibration algorithm; and, lastly, to determine the distribution of stress and deformation in the fetal membrane across different pregnancy-related circumstances.

To this end, numerical simulations were conducted using a three-dimensional model of the uterine environment in the third trimester of pregnancy. This model encompasses the uterine body, the cervix, a single-layer fetal membrane and the maternal abdomen. The constitutive model used to represent the biomechanical behavior of the uterine body, cervix and abdomen is neo-Hookean, whereas the fetal membrane is characterized by an anisotropic hyperelastic constitutive model. The material parameters for the fetal membrane were calibrated using a micro-genetic algorithm.

Using this numerical model, the influence of three key pregnancy-related factors on the fetal membrane was evaluated: varying intrauterine pressures, distinct cervical stiffness values and differences between the inner and outer surfaces of the fetal membrane. The results were analyzed in terms of maximum principal stress, with a focus on three anatomically relevant regions of the fetal membrane: areas adjacent to the central part of the uterine body, the cervix and the fundus. For this purpose, specific paths were constructed within each of these regions for every simulation, using the Abaqus Viewer.

The analysis of the results indicated that fetal membrane rupture is more likely to occur under conditions of elevated intrauterine pressure and reduced cervical stiffness, as both scenarios resulted in higher maximum principal stress values in the fetal membrane, after performing the simulations. No significant differences were observed between the inner and outer surfaces of the fetal membrane in terms of maximum principal stress.

Keywords: Fetal membrane, Rupture of the membrane, Preterm birth, Numerical simulation, Finite element method, Hyperelasticity, Constitutive model, Genetic Algorithm

UN Sustainable Development Goals

When undertaking a master's dissertation project, it becomes essential to reflect on how the work aligns with the Sustainable Development Goals (SDGs) and how its impact can be maximized. The SDGs represent a global framework comprising 17 interconnected objectives, adopted by the United Nations in 2015, which set a clear direction to address the most pressing challenges facing our society, including climate change, environmental degradation, poverty, peace, inequality and justice.

In Table 1, a set of guidelines is presented, designed to support the reflection on how the project aligns with the SDGs. These guidelines include the identification of relevant SDGs, the specification of concrete targets, the assessment of the project's contribution to sustainable development and the identification of performance indicators and metrics.

The specific SDGs addressed in the present investigation are named as follows:

SDG 3 Ensure access to quality healthcare and promote well-being for all, at all ages

SDG 17 Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development

Table 1: Guidelines for analyzing the alignment of the project with the SDGs.

SGD	Target	Contribution	Performance Indicators and Metrics
3	3.1	By 2030, reduce the global maternal mortality ratio to less than 70 deaths per 100 000 live births.	Maternal mortality ratio; Proportion of births attended by skilled health personnel
	3.2	By 2030, end preventable deaths of newborns, with all countries aiming to reduce neonatal mortality to at least 12 per 1000 live births.	Neonatal mortality rate
	3.9.d	Strengthen the capacity of all countries, particularly developing countries, for early warning, risk reduction and management of national and global health risks.	Capacity for International Health Regulations (IHR) and health emergency preparedness
17	17.17	Aiming to raise awareness, inform, implement, monitor and evaluate the business sector's contribution to the SDGs.	Number of collaborative initiatives; Number of academic-industry partnerships; Joint research outputs

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Mariana Querido

“In the middle of every difficulty lies opportunity.”

Albert Einstein

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Abbreviations

CAE	Computer-Aided Engineering
CT	Computed Tomography
ECM	Extracellular Matrix
FEA	Finite Element Analysis
FEUP	Faculdade de Engenharia da Universidade do Porto
HCA	Histologic Chorioamnionitis
HGO	Holzapfel-Gasser-Ogden
INEGI	Instituto de Ciência e Inovação em Engenharia Mecânica e Engenharia Industrial
IUP	Intrauterine Pressure
MRI	Magnetic Resonance Imaging
ODB	Output Database
PFM	Pelvic Floor Muscle
PPROM	Preterm Pre-labor Rupture of the Fetal Membrane
PTB	Preterm Birth
ROS	Reactive Oxygen Species
SDG	Sustainable Development Goal
SMC	Smooth Muscle Cell
UMAT	User-defined Material subroutine
UN	United Nations

Chapter 1

Introduction

At term, several dynamic events take place to facilitate childbirth, including the remodeling and softening of the cervix, the rupture of the fetal membranes and the initiation of uterine contractions [1]. The normal rupture of the membranes is an integrant and essential part of the vaginal delivery process [2]. However, if this rupture occurs prematurely, it can lead to serious health complications, not only for the mother but also for the fetus [3, 4]. It is estimated that approximately 3% of pregnancies worldwide suffer premature rupture of the membranes before 37 weeks of gestation, a condition known as preterm pre-labor rupture of the fetal membranes (PPROM) [3, 5]. PPRM is a major contributor to preterm birth and it is closely related to increased rates of perinatal morbidity and mortality [6–8].

1.1 Fetal membrane considerations

The fetal membrane, also known as the amniotic sac, is composed of layered biological structures that surround and protect the fetus throughout the course of pregnancy, acting as both a mechanical and immunological barrier [6, 7]. This membrane undergoes several complex microstructural changes during pregnancy, including a gradual weakening of its mechanical properties as labor approaches and an intense process of growth and deformation to adapt to distinct stages of pregnancy [9], which leads to its natural rupture [3, 10].

Additionally, fetal membranes are vital biological tissues that play an integral role in the complex anatomy of pregnancy, which includes the uterine body and the cervix [11]. The geometry and configuration of the pregnant uterus are influenced by a variety of factors, such as the position of the fetus within the uterus, the size and shape of the uterine body, the location of the placenta and the presence of any structural anomalies, among other considerations [11].

The aforementioned membranes consist of two primary layers, called the amnion and the chorion, each with distinct microstructural characteristics [12]. The amnion is the mechanically dominant layer and its tensile response is largely determined by randomly oriented collagen fibers

[13]. The chorion is mechanically less dominant, exhibiting greater flexibility and deformability under load [13].

In addition, a maternal layer, referred to as the decidua, lies adjacent to the chorion and maintains a close anatomical relationship with it [13]. This spatial proximity suggests that structural or functional alterations in the decidua may be associated with changes in the chorioamniotic membrane [13]. The development of both the chorion and decidua occurs in a synchronized manner from the early stages of implantation, with the smooth chorion eventually fusing with the decidua [13].

Consequently, accessing the geometry of the uterus during pregnancy is essential for determining how certain pregnancy-related scenarios - such as varying intrauterine pressures and differences in cervical stiffness - can affect both maternal and fetal health. Moreover, it enables the study of their impact on the fetal membrane, offering valuable insights into the mechanisms underlying its rupture at term and preterm [11].

Imaging techniques, including magnetic resonance imaging (MRI) and ultrasound, are invaluable in assessing different pregnancy-related conditions [6, 14]. These imaging methods complement each other by providing detailed views of fetal anatomy, monitoring growth, assessing amniotic fluid levels and identifying potential abnormalities, among other diagnostic functions [6, 14].

1.2 Context

Preterm birth occurs before the 37th gestational week and is a significant contributor to perinatal mortality, being associated with numerous long-term health complications in surviving preterm infants. The fetal membrane plays a crucial role in protecting the fetus throughout pregnancy and preterm pre-labor rupture of membranes is identified as a primary factor leading to preterm delivery. Despite its importance, the mechanisms underlying fetal membrane rupture remain poorly understood, motivating the current research to address critical gaps in knowledge through both experimental and numerical perspectives.

In order to investigate the biomechanical behavior of fetal membranes across diverse pregnancy conditions, finite element models of the uterine environment can be employed. The application of appropriate constitutive models is essential for accurately representing the mechanical properties of each component within the model.

1.3 Main objectives

The primary goal of the current investigation is to elucidate the biomechanical behavior of the fetal membrane under the influence of various physiological scenarios typically observed during pregnancy, with particular emphasis on the third trimester. A hyperelastic constitutive model - commonly applied to biological tissues - will be employed to represent the mechanical response

of the fetal membrane. For this purpose, the hyperelastic parameters of the membrane model must be determined using an optimization algorithm.

Through the application of finite element analysis, the project will assess the distribution of stress and deformation across the fetal membrane tissue in response to varying mechanical stimuli representative of pregnancy conditions, contributing to a deeper understanding of membrane integrity and its vulnerabilities. The finite element model utilized herein characterizes the uterine environment, encompassing the uterine body, cervix, fetal membrane and abdomen.

Therefore, this research ultimately seeks to improve the current understanding of the biomechanical processes underlying fetal membrane failure, with the intention of supporting clinicians in making informed decisions related to labor and pregnancy management.

1.4 Dissertation structure

The present report, prepared within the scope of the second-year dissertation project for the Master's in Bioengineering, is systematically divided into five chapters. Chapter 1 serves as the introduction, providing an overview of the structure and function of the fetal membranes and the significance of modeling these tissues under different pregnancy-related scenarios. It also presents the background and motivation for the study and defines the main objectives of the project, establishing its scientific and clinical relevance.

Chapter 2 offers a thorough literature review, detailing the current state of knowledge regarding uterine anatomy, the mechanics of fetal membranes and the physiological processes associated with childbirth, with an emphasis on preterm birth and its related complications. Furthermore, it discusses models already developed by research groups, in order to characterize the mechanical properties of biological tissues, including hyperelastic, anisotropic and viscoelastic models. In addition, this chapter addresses key concepts and provides essential definitions regarding the mechanical characterization of the aforementioned tissues.

Chapter 3 outlines the methodology adopted, regarding experimental data selection, finite element modeling, material parameter optimization and simulation setup for analyzing the uterine environment mechanical response, namely the amniotic sac, under diverse pregnancy-related conditions.

Additionally, Chapter 4 includes the results and discussion of the numerical simulations conducted using the uterine environment finite element model, focusing on the distribution of maximum principal stress and considering various pregnancy-related conditions.

Lastly, Chapter 5 presents the main conclusions drawn from the current dissertation and outlines directions for future work.

Chapter 2

Literature Review

The current chapter examines the fetal membranes, focusing on their structural characteristics, functional roles and contributions throughout gestation, while also addressing uterine anatomy and the process of childbirth. It further explores the primary causes and resulting complications associated with preterm birth, namely PPRM. In addition, it presents an in-depth review of previously developed constitutive models that provide valuable insights into the mechanical behavior of soft biological tissues, with particular emphasis on anisotropic, hyperelastic and viscoelastic formulations. The chapter also offers precise definitions of key concepts relevant to this research, including soft tissue characterization, constitutive modeling, isotropic and anisotropic hyperelasticity, user-defined material subroutines, genetic algorithms and other foundational terms. Furthermore, it references a previously proposed micro-genetic algorithm designed specifically for the optimization of material parameters.

2.1 Mechanics of normal labor and preterm birth

Pregnancy is a necessary biological process that ensures the propagation of all mammalian species [15]. In the case of human beings, a normal term pregnancy is defined as one that reaches between 37 and 40 weeks of gestation [15]. Deliveries occurring before 37 weeks are classified as preterm or premature, while those extending beyond 41 weeks are labeled post-term pregnancies [15].

On the other hand, parity has a notable impact on measurements of lower uterine segment thickness and cervical length, reflecting potential long-term mechanical and structural changes resulting from pregnancy-related remodeling events [11]. A multiparous patient - defined as a woman who has experienced one or more previous pregnancies resulting in live births - exhibits distinct patterns in these parameters compared to a nulliparous woman [11].

In multiparous patients, a greater rate of thinning in the lower uterine segment has been observed, suggesting two possible underlying mechanisms [11]. First, the mechanical load imposed

by the amniotic sac contents may shift more rapidly toward the lower portion of the uterus in subsequent pregnancies [11]. Second, the uterine tissue itself may experience a reduction in stiffness, becoming softer with each successive pregnancy [11].

Regarding the cervix in multiparous women, the evidence is less conclusive [11]. Although it remains uncertain whether the cervix consistently becomes mechanically softer with each additional pregnancy, some studies indicate that women with a history of prior vaginal deliveries tend to have softer cervixes compared to nulliparous women [11]. This finding highlights the need for further research to establish a clearer understanding of the cervical changes associated with parity [11].

In order for a pregnancy to reach full term successfully, a coordinated interplay must occur among three primary mechanical structures: the uterus, the cervix and the fetal membrane [15]. These compartments work in a complex and interdependent manner, forming a symbiotic relationship to support the growing fetus until it is ready for delivery [15].

Throughout the progression of pregnancy, the uterus - the main muscular organ consisting predominantly of smooth muscle, blood vessels and extracellular matrix (ECM) - undergoes considerable growth and remodeling, while maintaining a quiescent and relaxed state in its smooth muscle [15]. Such state is vital for the accommodation of the fetus as it develops [15]. Simultaneously, the fetal membrane must enlarge to keep pace with the developing fetus, remaining intact to protect the growing baby [15]. The cervix, a cylindrical structure located at the base of the uterus, plays an equally crucial role [15]. Initially firm and rigid to keep the uterus closed, the cervix must undergo gradual remodeling over time, transitioning from a stiff and supportive structure to one that is soft, flexible and compliant, allowing the eventual delivery process of the fetus at term [15].

Toward the end of pregnancy, a remarkable transition occurs and the uterus starts contracting rhythmically in order to initiate labor, the fetal membrane ruptures and the cervix dilates, collectively establishing the conditions necessary for a safe and efficient delivery [15]. Despite the importance of these transitions, the understanding of how each compartment changes during pregnancy and synchronizes to achieve the process of childbirth, known as parturition, remains unclear and incomplete [15]. This knowledge gap is particularly pronounced in humans, where restricted access to tissue samples during pregnancy presents challenges for research [15].

As highlighted in the preceding discussion, in typical and successful vaginal deliveries, the process follows a predictable sequence: (1) cervical ripening, (2) uterine contractions and (3) rupture of the fetal membrane [1, 13]. However, in cases of preterm labor, the sequence can vary - for example, it may involve early cervical ripening, often due to cervical insufficiency, preterm premature rupture of the fetal membrane and the early onset of uterine contractions [15]. These divergences may lead to preterm birth (PTB), a condition with global incidence rates rising from 9.8% in 2000 to 10.6% in 2014 [15, 16]. PTB poses significant health risks to surviving infants, who are at increased risk for several short-term morbidities [17, 18] and long-term health complications [13, 19, 20].

Currently, there are no highly accurate methods for the prediction of these adverse outcomes, nor are there universally effective treatments for preventing them [15]. Although several ap-

proaches are employed to reduce PTB, namely cervical cerclage, progesterone therapy, antibiotic prophylaxis and anti-inflammatory treatments, none have proven fully effective across all cases [21]. Furthermore, a complete characterization of the underlying biomechanical processes by which women initiate full-term labor remains elusive [13]. Since these phenomena are inherently mechanical in nature, advancing our understanding of the biomechanical responses of the uterus, cervix and fetal membranes during labor is crucial to better predict and manage labor onset [13, 22].

2.2 Anatomy, physiology and biomechanics of the human uterus

In terms of anatomical structure, the uterus consists in a thick-walled, muscular organ with a remarkable capacity for expansion to accommodate fetal development throughout gestation [23]. Anatomically, it is positioned distally in continuity with the vagina and laterally connected to the uterine (fallopian) tubes [23].

As illustrated in Figure 2.1, the uterus is typically divided into four main regions [23]:

- **Fundus:** the uppermost portion of the uterus, located above the point of entry of the uterine tubes;
- **Body (Corpus):** the central part of the uterus and the most common site for blastocyst implantation;
- **Isthmus:** the transitional zone between the uterine body and the cervix, characterized by a narrowing of the uterine wall;
- **Cervix:** the lower, cylindrical portion of the uterus that connects it to the vaginal canal. The cervix differs both structurally and functionally from the uterine body.

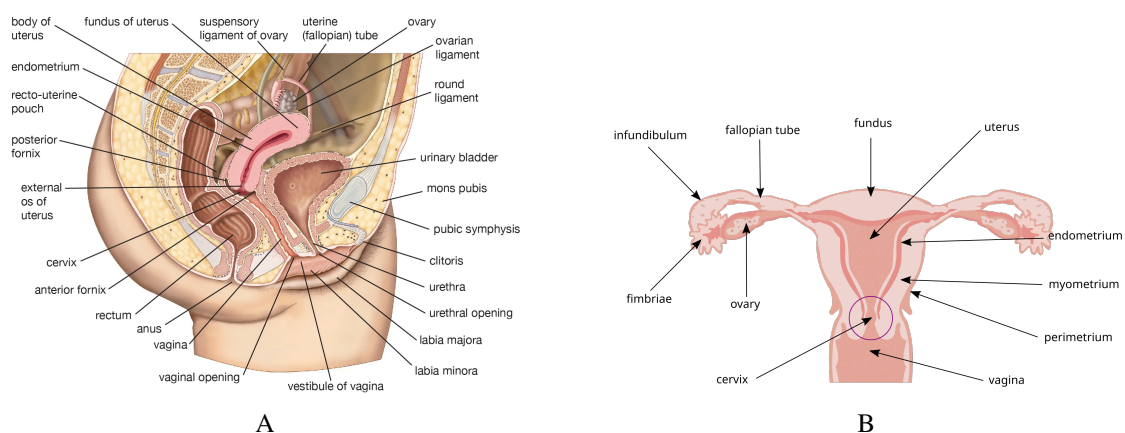


Figure 2.1: Anatomical structure of the human uterus: (A) sagittal cut and (B) frontal cut. Authorized, available at [24, 25].

The position of the uterus can vary depending on the degree of bladder distension [23]. In a typical adult female, the uterus is described as [23]:

- **Anteverted:** tilted forward relative to the axis of the vagina;
- **Anteflexed:** bent forward in relation to the cervix.

As a result of this orientation, the uterus normally resides immediately posterosuperior to the urinary bladder and anterior to the rectum [23].

The proper biomechanical functioning of the uterus is required for the successful execution of human reproduction [26]. These functions include facilitating embryo transport to the implantation site, remodeling the uterine walls to accommodate the placenta, protecting the developing fetus during gestation, generating contractile forces to ensure a safe parturition and a postpartum recovery and subsequently returning to a nonpregnant state to renew the menstrual cycle [26].

To fulfill its remarkably complex and varied roles, the uterus is intricately structured with dynamic and contractile layers of muscle and tissue, responsive to a range of chemical, hormonal, electrical and mechanical signals [26]. Nevertheless, the intricate interplay between these highly active biological signaling mechanisms and uterine biomechanical function are not yet completely understood, particularly in the context of both normal reproductive processes and pathological conditions, namely adenomyosis, endometriosis, infertility and preterm labor [26].

Research in animal models has provided valuable insights into the structural and functional aspects of the uterus, especially throughout pregnancy [26]. In human studies, advancements in medical imaging techniques, such as ultrasound and MRI, have been combined with computational engineering approaches to characterize the uterine structure and function in vivo [26].

The uterine wall itself is composed of three distinct layers, according to Figure 2.2: (1) the endometrium, a mucosal inner layer, (2) the myometrium, a thick middle layer composed predominantly of smooth muscle tissue and (3) the perimetrium, a thin outer layer [13, 27].

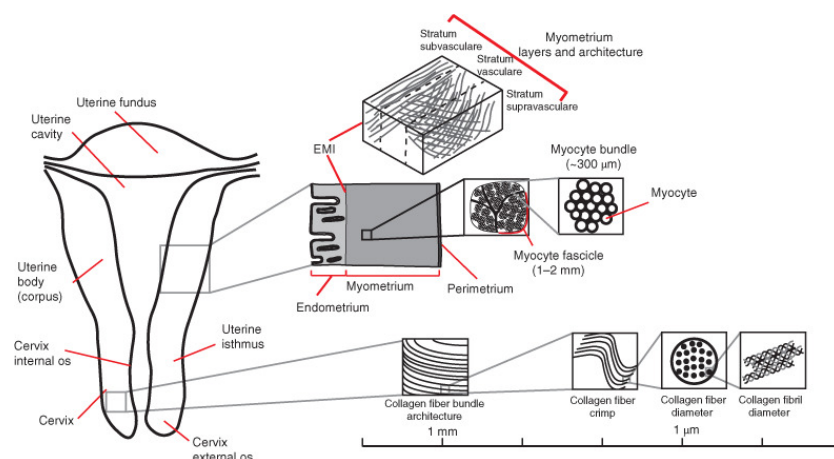


Figure 2.2: Anatomical representation of the human uterus, illustrating its three principal histological layers: the endometrium, the myometrium and the perimetrium. Authorized, available at [26].

The endometrium-myometrium interface, often referred to as the junctional zone, plays a pivotal role in normal reproductive function and presents significant implications for the development of uterine pathologies [13, 27].

The myometrium, the muscle layer of the uterus, consists primarily of smooth muscle cells (SMCs) embedded within an ECM of collagen fibers [28]. This layer plays a critical role in generating the contractions necessary for labor [28]. Throughout gestation, the myometrium produces spontaneous, irregular and long-lasting contractions, which are relatively low in intensity and serve to maintain uterine tone [28]. However, as pregnancy reaches full term, the myometrium transitions into an activated state, generating strong and high-frequency contractions essential for labor [28]. This increase in myometrial activity at normal term labor results from a dual mechanism: the physiological release from pregnancy-related inhibitory effects on the myometrium as well as the effect of pro-contractile stimuli [28].

The myometrium undergoes four distinct phases of activity throughout pregnancy and postpartum [28]. During most of gestation, it remains in a quiescent state (Phase 0), where contractility is limited and controlled [28]. As labor approaches, the myometrium progresses to the activation phase (Phase 1), followed by the stimulation phase (Phase 2) [28]. During activation and stimulation, the myometrium undergoes various biochemical changes that enhance and sustain its increased contractile ability [28]. These changes include altered levels of hormones, production of uterotonic agents and contraction-associated proteins as well as the formation of gap junctions between myometrial cells, allowing for coordinated contractions [28]. The remodeling of the ECM supports these adaptations by fostering structural connectivity and promoting the effective transmission of contractile forces [28]. Following the delivery of the fetus and placenta, the uterus enters the involution phase (Phase 3), during which it gradually returns to its nonpregnant state [28].

Throughout the activation and stimulation phases of labor, the myometrium experiences notable anatomical transformations, leading to a clear distinction between the upper and lower segments of the uterus, in terms of shape and molecular environment [28]. The upper uterine segment (near the fundus) becomes more robust, both in thickness and contractile capability, while the lower uterine segment (near the cervix) becomes thinner and more compliant [28]. A recent study, involving two-dimensional ultrasound analysis on pregnant women in the third stage of labor, has confirmed this morphological difference, showing a pronounced thickening of the myometrial fibers in the upper segment and a corresponding thinning in the lower segment [28].

This anatomical division has important implications for the mechanism of labor [28]. The thickened upper segment is capable of generating powerful contractions, compressing the upper uterine cavity and propelling the fetus downward toward the relaxed lower segment, thereby facilitating its descent through the birth canal [28]. Notably, the previously mentioned study found that women experiencing a normal third stage of labor tended to have more defined thickening in the upper uterine segment and thinning in the lower segment [28]. In contrast, women with prolonged or abnormal third-stage labor exhibited less differentiation between the uterine segments, with the lower segment failing to thin effectively [28].

The findings also suggest that an abnormal third stage of labor, such as in cases of preterm labor, may involve the absence of a clear distinction in myometrial thickness between the upper and lower segments [28]. This lack of segmental differentiation could result in less efficient contractions, complicating the labor process [28]. Thus, the coordination between upper segment thickening and lower segment thinning appears to be critical for a smooth and effective labor, underscoring the complex structural and functional adaptations of the myometrium that enable normal childbirth [28].

2.2.1 Uterine body

The uterine body consists of a highly specialized and complex organ composed of billions of SMCs embedded within dense connective tissue, which collectively enable it to generate substantial force, required for childbirth [13, 27]. Functionally, these SMCs generate and transmit electrical impulses, known as action potentials, which spread across the uterine wall, simultaneously facilitating communication that coordinates contractions [13, 27]. In a typical vaginal delivery, these action potentials become synchronized, producing a unified and propulsive force as the uterine muscles contract [13, 27]. This contraction activity, in concert with the gradual dilation of the cervix, culminates in the expulsion of the fetus from the uterine cavity [13, 27].

The contractile activity of the uterus, represented by these electrical signals, can be directly measured in laboratory settings using electrodes attached to the uterine muscle in animal models [27]. Similarly, in clinical practice, surface electrodes placed on the abdominal skin are used to non-invasively capture uterine electrical activity for obstetric monitoring [27]. This electrical activity reflects the intensity and pattern of uterine contractions and is essential in evaluating labor progress [27].

Coordinated uterine contractions are vital for a successful vaginal birth [27]. Toward the end of pregnancy, the gentle contractions occurring during gestation gradually become stronger and more regular, eventually leading to the active labor phase [27]. In normal labor, these regular contractions, together with cervical dilation, facilitate the expulsion of the fetus and placenta from the uterus [27]. Clinicians closely monitor these contractions to ensure they are effective enough to progress labor but not overly intense, as excessive contractions could compromise fetal oxygenation [27].

Despite the established understanding of uterine activity, the precise mechanisms by which these contractions bring about labor progression remain only partially understood [27]. To support labor management, clinicians must assess uterine activity to make timely intervention decisions, if necessary [27]. Techniques available for measuring uterine contractions in clinical settings include manual palpation, tocodynamometry and electrohysterography, which provide valuable data for monitoring and guiding labor [27].

2.2.2 Cervix

The cervix, located at the base of the uterus, is composed of epithelial and stromal layers [13]. It plays a critical mechanical role between the uterine body and the vagina by remaining closed throughout most of gestation, thereby supporting the increasing mechanical loads exerted by the developing fetus [13]. Although progressive softening of the cervical tissue naturally occurs during pregnancy in preparation for labor, premature cervical softening can lead to an elevated risk of PTB [13, 15]. Consequently, the cervix undergoes a complex remodeling process during gestation, characterized by the reorganization and realignment of collagen fibrils and alterations in the ECM [13].

It has been proposed that the cervix functions passively in response to uterine contractions at the onset of labor [13]. Nonetheless, several studies have explored the potential for active cervical contractions during both pregnancy and parturition [13]. Smooth muscle fibers account for approximately 10% to 45% of the cervical stroma, in addition to collagen and ECM components [1, 13]. These fibers may contribute to active biomechanical responses within the cervix and play a role in its remodeling [13]. Furthermore, anatomical studies have identified both longitudinal and circumferentially oriented collagen fibers within cervical tissue: the former may facilitate cervical dilation through contraction, while the latter may function to maintain cervical closure [13].

More recent studies indicate that the cervical collagen network is highly heterogeneous, featuring interwoven collagen zones that vary in structure from the internal os (the opening of the cervix that connects to the uterine cavity) to the external os (the opening of the cervix that connects to the vaginal canal) [1], both of which are depicted in Figure 2.3.

In addition, the strength and mechanical properties of this collagen network are influenced by the degree and type of crosslinks between collagen fibrils. Recent findings reveal that the internal os displays greater heterogeneity in collagen crosslinking than the external os [1]. These structural distinctions likely correspond to functional differences between the internal and external os, underscoring the principle that "location matters", when studying cervical anatomy and function [1].

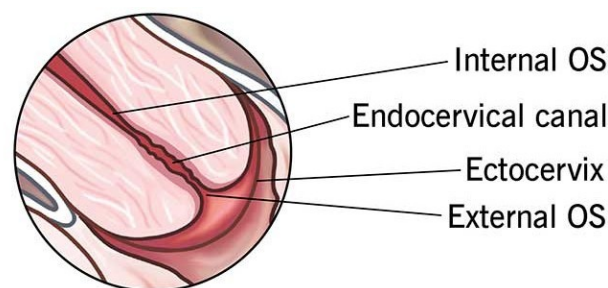


Figure 2.3: Anatomical illustration of the cervix, which connects the uterus to the vagina, highlighting both the internal os and the external os. Authorized, available at [29].

2.2.2.1 Cervical Ripening

Cervical ripening, also known as cervical remodeling, refers to the progressive changes and subsequent recovery of cervical structure and function throughout pregnancy, encompassing four overlapping phases: an initial, prolonged softening phase; a more rapid phase of intense softening and increasing flexibility near term (ripening); active dilation immediately before birth; and, finally, the postpartum repair [1]. This remodeling process has been studied mainly in rodent models due to challenges in obtaining tissue samples from pregnant women [1]. In rodents, the softening phase involves an increase in collagen solubility (indicating reduced crosslinking), along with a decrease in enzymes that form collagen crosslinks [1]. Studies also show that the ratio of immature (weaker) to mature (stronger) collagen crosslinks decreases during early softening, suggesting that mature crosslinked collagen fibers are replaced by immature, less rigid fibers, thereby increasing tissue compliance [1]. Beyond changes in collagen crosslinking, softening likely includes modifications to other ECM proteins that regulate collagen assembly and cell-matrix interactions, such as thrombospondin-2 and tenascin-C [1].

The ripening phase follows softening, resulting in an exceptionally flexible tissue capable of significant dilation to facilitate fetal passage [1]. Research indicates that this phase is marked by (1) increased collagen fiber diameter and increased spacing between fibers, along with a shift from straight to wavy collagen fibers, (2) elevated production of hyaluronic acid, which hydrates tissue and presumably enhances compliance and (3) the recruitment or activation of immune cells that release matrix metalloproteinases, enzymes that degrade ECM components [1]. However, recent studies indicate that neither hyaluronic acid synthesis nor immune cell activation are essential for cervical ripening in mice, suggesting species-specific differences in the underlying mechanisms [1].

The influence of pregnancy hormones on cervical remodeling remains unclear, particularly in light of these differences between rodents and humans [1]. Unlike humans, who are bipedal with a single uterus, rodents are quadrupedal and possess two uteri [1]. These anatomical and physiological variations call into question the direct applicability of rodent models to human cervical physiology [1].

2.2.3 Fetal membrane

The fetal membranes are a robust, collagen-rich bilayer comprising the amnion and the chorion, which are connected to the maternal decidua capsularis [3, 30]. This bilayer constitutes the innermost tissue layer of the intrauterine cavity and forms the core of the fetal-maternal interface [3, 30]. During pregnancy, this structure must withstand deformations caused by internal pressure and fetal movements [12]. Rupture of the fetal membrane is a natural event throughout term labor, but it can have serious consequences when it occurs prematurely [12]. The condition known as PPRM affects approximately 3% of all pregnancies worldwide and is associated with an increased risk of perinatal morbidity and mortality [12].

Moreover, premature rupture following fetal surgery - referred to as iatrogenic PPROM - poses a major challenge to the advancement of this field, occurring in about 30% of procedures [12]. It has also been established that the separation of the chorioamniotic membrane leads to PPROM in 63% of cases following surgery [12]. In this sense, developing strategies to prevent premature rupture of the fetal membrane requires a deeper understanding of its mechanical behavior [12].

2.2.3.1 Fetal membrane constitution

The amnion forms the inner layer of the fetal membrane, which directly faces and surrounds the amniotic fluid, while the chorion constitutes the outer layer contacting the uterine wall [31, 32]. The amnion is a nonlinear viscoelastic material, known for being the mechanically dominant component of the fetal membrane, where thin and randomly oriented collagen fibers dominate the material response to loading [3, 10]. As a result, the amnion is thinner, stiffer and stronger compared to the chorion, which is more compliant and has greater elasticity and extensibility [4, 12, 33]. At term, the thickness of the amnion layer in fetal membranes falls within the range of $100\text{ }\mu\text{m}$ [12].

Scanning electron microscopy (SEM) images of a fetal membrane sample are presented in Figure 2.4. These images reveal a crimped collagen fiber network, randomly oriented within the membrane plane, without any preferential alignment [12].

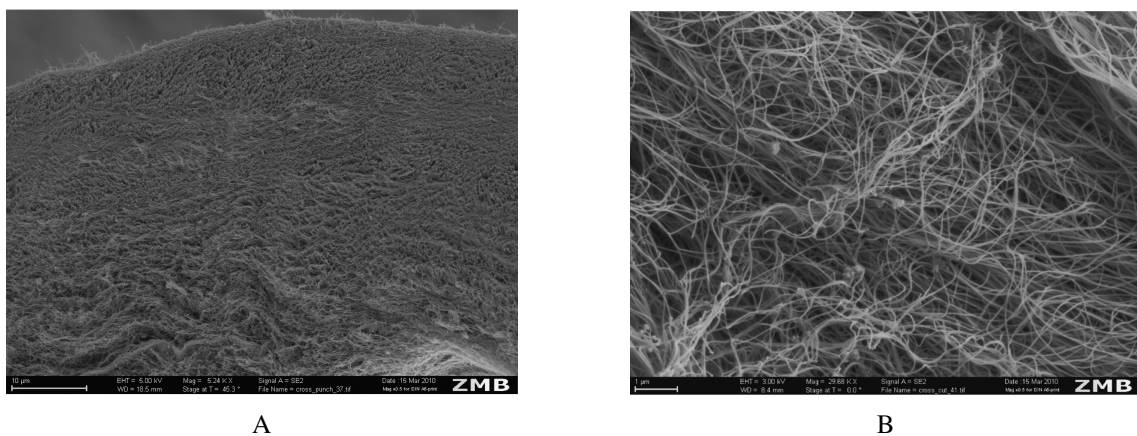


Figure 2.4: Scanning electron microscopy images of a human fetal membrane sample. (A) Cross-sectional view and (B) top view display an irregular arrangement of individual collagen fibers lacking a defined directional pattern. Authorized, available at [12].

Throughout the gestation and labor process, the amnion undergoes significant chemical and mechanical changes, whereas the chorion remains largely unaffected [10]. These two layers are separated by a spongy layer that contains proteoglycans, glycoproteins and type III collagen [2, 34, 35]. This interface provides a structural foundation that enables the amnion to slide smoothly over the underlying chorion [2, 34, 35]. However, the spongy layer is mechanically very weak, making it challenging to test its properties directly.

In typical anatomical illustrations, such as Figure 2.5, these layers are depicted enveloping the fetus within the uterus, providing clear insight into their spatial relationships and functional roles in the context of prenatal development [2, 3, 12].

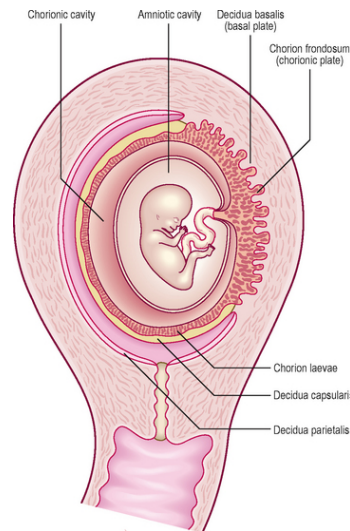


Figure 2.5: Anatomical structures of a developing embryo within the uterus. The two primary fetal membrane layers, the amnion and chorion, as well as the maternal decidua layer, are visible. Authorized, available at [36].

Beyond the amnion and chorion, there is another layer often taken into consideration, also illustrated in Figure 2.5, called the decidua. This maternal tissue layer is closely connected to the chorion and plays an essential role in the mechanics of the fetal membrane [37]. The decidua results from the differentiation of the uterine endometrium under the influence of progesterone [38].

It is known that this layer shares a close anatomical relationship with the chorion, forming the chorio-decidual layer, which might suggest that any modifications in the decidua can be associated with changes in the chorioamnion membrane structure [2, 13]. From the earliest phase of implantation, the development of the decidua layer and the chorion progresses in tandem, eventually leading to the fusion of the smooth chorion with the decidua [39].

It is also noteworthy that, during the later stages of gestation, a region of altered structural morphology develops within the fetal membranes overlying the cervix [8, 13]. This area, commonly referred to as the "weak zone", exhibits diminished mechanical strength [8, 13]. It has been proposed that the rupture of the fetal membranes is initiated in this specific region [8, 13].

A more detailed illustration of the feto-maternal interface is provided below, in Figure 2.6. It is perceptible that the fetal membrane, which lines the intrauterine cavity, consists of two main components: the amnion and the chorion, as discussed earlier [30]. The amnion membrane is composed of amnion epithelial cells (depicted in blue), the stroma (shown by green lines) and amnion mesenchymal cells (represented in light purple) [30]. The chorion membrane, on the other hand, includes chorion mesenchymal cells (shown in dark purple), the stroma (also indicated with green lines) and chorion trophoblast cells (depicted in yellow) [30]. It is also noticeable that

fetal membranes are interconnected with the maternal decidua, forming the crucial fetal-maternal interface [30].

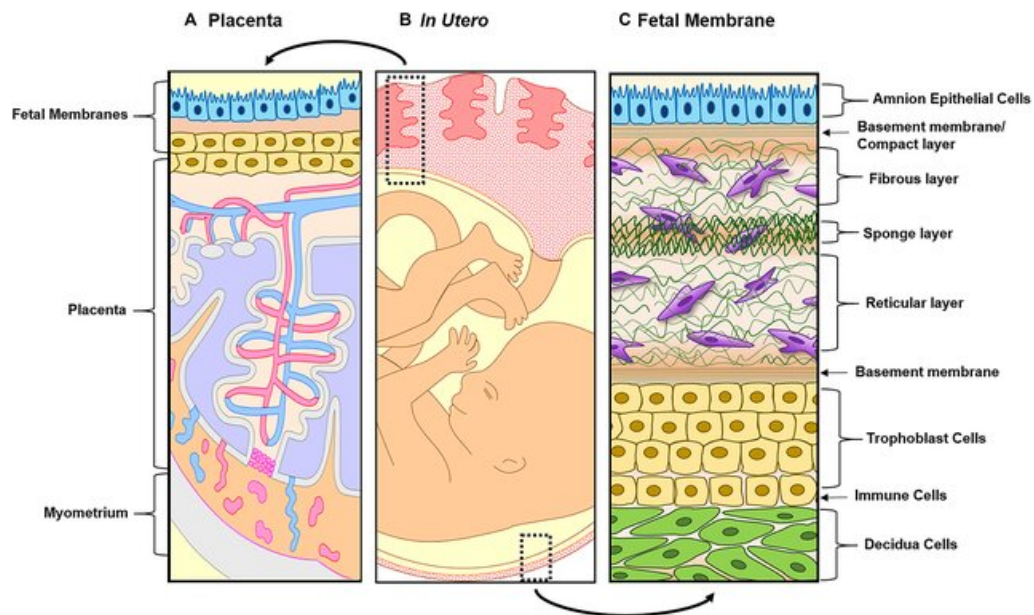


Figure 2.6: The feto-maternal interface throughout gestation. Authorized, available at [30].

2.2.3.2 The role of fetal membranes throughout gestation, at term and in preterm labor

One of the primary goals of the fetal membranes during gestation is to act as a protective barrier, isolating the fetus from various maternal risk factors, such as infections, environmental toxins and behavioral hazards that could trigger a fetal inflammatory response [30]. This inflammatory reaction, if not properly contained, may result in adverse pregnancy outcomes [30].

The amnion, in particular, plays a central role in this barrier function [30]. Its key responsibilities include: (1) facilitating fetal growth and maintaining the volume of amniotic fluid, (2) enduring mechanical stresses and deformations within the uterine environment, (3) maintaining the integrity of the amnion's epithelial layer, (4) monitoring environmental conditions and responding to potential insults, such as infections or oxidative stress and (5) undergoing continuous cellular and collagen remodeling to preserve the ECM [30]. All of these functions are vital for maintaining the structural integrity of the fetal membranes throughout pregnancy [30].

In contrast, the chorion assumes a pivotal role in regulating the immune environment at the choriodecidual interface, which represents the boundary between the fetal membranes and the maternal tissues [30]. The chorion helps to modulate immune responses by producing anti-inflammatory hormones and cytokines that mitigate maternal immune cell infiltration [30]. Additionally, the chorion acts as a major source of endocrine signaling, producing hormones such as progesterone, which is crucial for maintaining pregnancy [30]. It also releases paracrine factors, which further support immune homeostasis at the choriodecidual interface during gestation [30].

Furthermore, the fetal membranes serve as a crucial communication hub between the mother and the developing fetus [30]. This is demonstrated by their involvement in maintaining immune balance and regulating the transport of nutrients, water and pharmaceuticals during pregnancy [30]. Ensuring proper inflammatory regulation and immune homeostasis at the interface between the fetal membranes and maternal decidua is vital for preventing dysfunction in the membranes [30].

One of the most important regulators of inflammatory balance during pregnancy is progesterone, a key anti-inflammatory hormone [30]. Progesterone supports immune homeostasis by acting through a locally inducible negative feedback system at the feto-maternal interface [30]. Both the placenta and the cells within the fetal membranes produce progesterone throughout gestation, facilitating endocrine signaling across maternal and fetal tissues [30].

Progesterone signaling serves as a critical role in maintaining the structural integrity of the amnion epithelium, which is responsible for maintaining the barrier that encloses amniotic fluid within the intrauterine cavity [30]. This hormone induces mesenchymal cells within the collagen layers of the fetal membranes to undergo mesenchymal-to-epithelial transition, a cellular transformation that helps repair weak areas in the epithelial barrier [30]. In addition, progesterone inhibits the activity of several pro-inflammatory cytokines, which can otherwise degrade the collagen matrix within the fetal membranes, weakening their structure [30].

Moreover, water and nutrient transport are fundamental processes during pregnancy, ensuring the normal growth and development of the fetus [30]. Aquaporins, proteins embedded in the plasma membrane, exist in more than eleven different isoforms, with their expression levels in endometrial and fetal-derived cells changing during implantation [30]. These proteins not only play roles in regulating myometrial contractions and cervical ripening, but also contribute significantly to various functions at the feto-maternal interface [30]. Specifically, aquaporins facilitate: (1) the transport of water across the placenta to support placental development, (2) the regulation of amniotic fluid volume and (3) the overall survival, growth and development of the embryo and fetus [30]. Additionally, research suggests that fetal membrane cells may express proteins involved in the transport of amino acids, fatty acids and vitamins, similar to those found in the placenta, indicating that fetal membranes could also be involved in nutrient transport to the fetus during pregnancy [30].

Recent research has uncovered the presence of specific transport proteins within fetal membrane cells and in vesicles derived from these cells, such as exosomes, i.e., extracellular vesicles measuring between 30 and 160 nm [30]. These proteins, which play key roles in transporting various molecules, including maternal drugs, between maternal and fetal systems, challenge the traditional view that only the placenta performs these functions [30]. The aforementioned proteins are predominantly localized in the chorion, with comparatively lower expression levels observed in the amnion [30].

Such findings suggest that the fetal membranes function similarly to the placenta in terms of molecule transport, expanding our knowledge of intrauterine drug transportation [30]. As a result, this improved understanding could lead to new therapeutic approaches, allowing for safer and

more effective drug delivery during pregnancy by targeting these membrane pathways [30].

2.2.3.3 Contribution of fetal membranes to labor induction

While the placenta is essential for fetal survival throughout gestation and at term, it does not significantly participate in the signaling mechanisms that induce labor, as its uninterrupted function remains critical until delivery [30]. In contrast, the fetal membranes play a pivotal role in initiating labor through multiple signaling pathways that shift the immune environment to a pro-inflammatory state [30].

Initial research, beginning with Keirse et al. in 1976 and confirmed by Duchesne et al. in 1978, suggested that the primary function of the membranes in the onset of labor was the production of prostaglandins [30]. These lipid autacoids, derived from arachidonic acid, are synthesized in response to local tissue damage or infection and are known to regulate inflammation, vascular flow and the induction of labor [30]. In fact, prostaglandins are crucial mediators of parturition, affecting collagen degradation, myometrial contraction and cervical ripening [30]. Elevated levels of these lipids in amniotic fluid are observed during both term and preterm labor, likely due to reduced metabolism, increased production or alterations in receptor expression [30]. However, prostaglandins represent only one component of the broader signaling pathways activated by the fetal membranes to facilitate delivery [30].

As gestation approaches term, the growing fetus increases its aerobic respiration and metabolic demands [30]. This leads to a rise in reactive oxygen species (ROS) within the amniotic cavity, a condition exacerbated by low levels of maternal and fetal antioxidants, like selenium, copper, zinc, vitamin C and vitamin E [30]. The resultant oxidative stress triggers cellular damage in fetal membrane cells, altering telomeres and activating a protein kinase pathway, which promotes cellular senescence, a process that generally aligns with fetal maturation at term [30]. In addition, intrauterine oxidative stress and cytokines further weaken the fetal membranes by promoting dysfunctional cellular transitions, increasing epithelial-to-mesenchymal transition and decreasing mechanical resistance to rupture [30].

Furthermore, the transmission of cellular senescence leads to the propagation of inflammatory signals from fetal to maternal tissues, which may occur through two distinct mechanisms: diffusion via microfractures in the fetal membranes or through exosomes released from fetal membrane cells [30]. Microfractures have been noted to be more prevalent and pronounced in both term and preterm membranes, while exosomes are capable of transporting biologically active molecules that influence labor signaling [30].

In conclusion, as labor approaches, the fetal membranes undergo processes of senescence and structural weakening, producing potent pro-inflammatory signals that communicate the fetus's readiness for labor and delivery to the maternal side [30].

2.2.3.4 Fetal membrane pathologies associated with preterm birth

Labor induction pathways can be triggered physiologically at term or pathologically in the context of PTB [30]. PTB, defined as delivery before the 37th week of gestation, is a major clinical concern that may occur either spontaneously or as a result of medical intervention [30]. Spontaneous PTB is further classified into two primary categories: those preceded by preterm labor with intact membranes and those preceded by PPRM [30]. Among cases of spontaneous PTB, PPRM accounts for approximately 40% and affects about 3% of all pregnancies [30]. PPRM is defined as the rupture of fetal membranes prior to the onset of labor and before 37 weeks of gestation [30].

Rupture of the fetal membranes can be caused by either physical stretching or oxidative stress, both of which biomechanically weaken the collagen structure, leading to a mechanical failure of the membranes [30]. This rupture, regardless of gestational timing, is associated with significant risks, including increased susceptibility to intraamniotic infections and a range of adverse fetal outcomes [30]. Complications for the fetus can include hypoxia, pulmonary hypoplasia, respiratory distress syndrome, neonatal sepsis, umbilical cord prolapse, necrotizing enterocolitis, neuromuscular impairments, cerebral palsy, developmental delays and, in severe cases, fetal mortality [30].

The precise mechanisms and causes underlying spontaneous PTB and PPRM remain only partially understood [30]. Research indicates that spontaneous PTB may involve minimal DNA damage and activation of cellular pathways, without leading to premature cellular senescence [30]. In contrast, PPRM is often associated with elevated levels of oxidative stress and inflammation, characterized by increased ROS, thrombin and pro-inflammatory cytokines [30]. These factors activate several fetal membrane signaling pathways, which subsequently induce cellular senescence, epithelial-to-mesenchymal transitions and the formation of microfractures within the membrane structure [30]. Additionally, paracrine and endocrine signaling via exosomes from the fetal membranes plays a role in the premature initiation of labor, as these vesicles carry biochemical signals capable of influencing distant cells and tissues [30].

Further investigation is essential to elucidate the complex interactions and distinct roles of these pathways in both term and pathological preterm labor [30]. A more thorough understanding of these mechanisms may clarify the differences between the physiological processes of term parturition and the pathological onset of preterm labor, as well as identify potential targets for intervention to prevent adverse outcomes associated with PTB [30].

As illustrated in Figure 2.7, the intrauterine cavity (depicted in pink) is surrounded by maternal and fetal tissue layers that collectively form the feto-maternal interface [30]. This interface includes the maternal decidua (shown in green) and the fetal membranes, consisting of the amnion (blue) and chorion (yellow) layers [30].

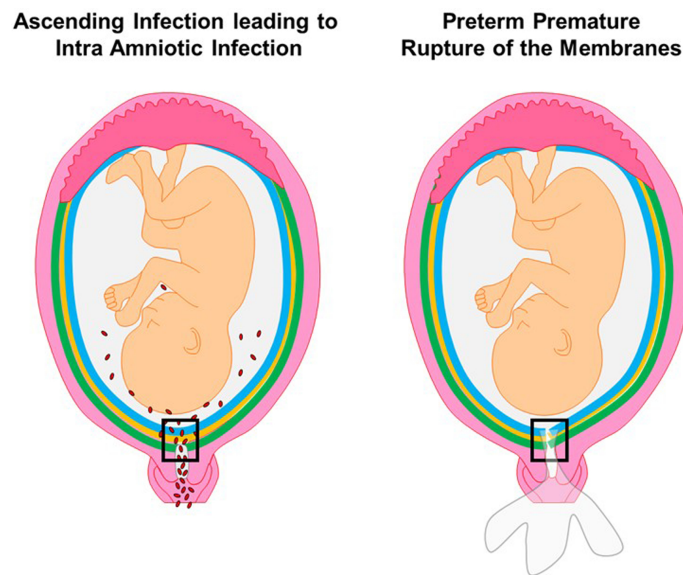


Figure 2.7: Schematic of the feto-maternal interface illustrating adverse pregnancy outcomes. Authorized, available at [30].

In certain pregnancy complications, particularly those involving ascending infections from the vaginal canal (e.g., bacterial and viral infections, indicated by red dots in Figure 2.7), infectious agents penetrate the decidua and subsequently reach the fetal membrane layers, leading to a condition known as chorioamnionitis [30]. Chorioamnionitis elicits an immune response characterized by the infiltration of immune cells, namely neutrophils, which migrate across the feto-maternal interface [30]. This immune cell infiltration results in the release of high levels of inflammatory mediators, causing substantial local inflammation [30]. As the infection advances, pathogens that reach the amniotic cavity itself can cause intraamniotic infection, which has been closely associated with an increased risk of PTB [30].

In cases of infection-driven PTB, PPRM is frequently observed [30]. This takes place when the fetal membranes are compromised by infection-induced cellular damage or oxidative stress, leading to degradation of collagen fibers within the membrane structure [30]. The resulting weakening and eventual rupture of the membranes facilitate the onset of labor well before term, underscoring the severe impact of infections and inflammatory responses at the feto-maternal interface on pregnancy outcomes [30].

Histologic chorioamnionitis (HCA) is a relatively prevalent pregnancy complication that can give rise to serious adverse outcomes for both mother and infant [30]. Clinically, HCA is distinguished by a range of maternal and fetal symptoms, including maternal fever, tenderness of the uterine fundus, maternal tachycardia exceeding 100 beats per minute, fetal tachycardia above 160 beats per minute and the presence of purulent or foul-smelling amniotic fluid [30]. Increasingly, HCA has been associated with PTB, with an estimated 40-70% of HCA-related births occurring with complications such as PPRM or spontaneous PTB [30].

HCA is frequently linked to intraamniotic infections caused by polymicrobial bacterial agents, including species such as Group B Streptococcus, *Mycoplasma pneumoniae*, *Ureaplasma*, Gard-

nerella vaginalis, Escherichia coli and Bacteroides [30]. In addition to these infections, HCA involves a cascade in which chemokines create a gradient that promotes the migration of immune cells, predominantly neutrophils, from maternal circulation or the decidua capsularis into the fetal membranes [30]. These chemokines, in conjunction with bacterial antigens, activate the neutrophils, prompting them to release pro-inflammatory cytokines, which then stimulate prostaglandins and collagen-degrading enzymes (e.g., matrix metalloproteinases) [30]. These activated neutrophils migrate through the fetal membranes from the chorion to the amnion, releasing cytokines and enzymes that compromise membrane integrity [30].

Simultaneously, neutrophils within the collagen matrix of the fetal membranes produce neutrophil extracellular traps (NETs), which are web-like structures composed of DNA fibers, histones and antimicrobial proteins designed to entrap pathogens [30]. While these NETs serve to immobilize bacteria, they can also initiate NETosis, a unique form of cell death associated with NET formation that facilitates collagen degradation through the cleavage and remodeling of laminin [30]. The release of pro-inflammatory cytokines, coupled with NET formation, ultimately weakens the fetal membranes, making them more susceptible to premature rupture [30].

The relatively high incidence of HCA, especially compared to the rarer occurrence of placentitis, characterized by immune cell infiltration of the placental tissue, highlights the significance of studying fetal membranes in understanding the onset of PTB [30].

Gestational diseases such as pre-eclampsia and gestational diabetes significantly impact maternal health, often leading to adverse outcomes by inducing systemic stress, which includes elevated ROS levels and a downregulation of NADPH oxidases [30]. Pre-eclampsia, a leading cause of maternal and fetal morbidity and mortality, is defined by the onset of stage 2 hypertension after 20 weeks of gestation in a woman with previously normal blood pressure [30]. This condition, which also serves as a primary indicator for systemic inflammation, has been strongly linked to abnormal placental function and vascular stress [30].

Gestational diabetes, marked by a diagnosis of diabetes mellitus for the first time during pregnancy, is recognized as a risk factor for developing pre-eclampsia [30]. Although the complete pathophysiology of pre-eclampsia remains under investigation, research suggests that systemic maternal endothelial dysfunction may play a central role, originating from factors such as improper placental development, preexisting maternal conditions (e.g. obesity, advanced maternal age, insulin resistance, chronic hypertension) and the inflammatory responses activated by normal pregnancy [30]. These factors may collectively contribute to oxidative stress, inflammation and vascular dysfunction in the maternal system [30]. Although gestational diseases primarily impact the placenta, their associated systemic inflammation, particularly in cases of pre-eclampsia, may also compromise fetal membrane function, thereby increasing the risk of preterm rupture of membranes [30].

Consequently, investigating the biological function of fetal membranes and their cellular layers under normal pregnancy conditions could offer insights into the mechanisms by which fetal membranes contribute to gestational health and how they may play a role in the onset of PTB [30]. A deeper understanding of these processes is anticipated to open new avenues for diagnosing and

treating adverse pregnancy outcomes [30].

2.3 Previous studies on the biomechanical characterization of fetal membranes

There are still several unknowns regarding the mechanical response and microstructure of the fetal membranes [3]. Despite their importance, only a limited number of studies have been conducted to thoroughly understand and characterize their biomechanics [2, 3, 12].

One of the earliest contributions came from Oxlund et al. (1990), who developed a technique to collect uniaxial data that provided insights into the mechanical properties of the fetal membranes [40]. Later, Helmig et al. (1993) explored how these mechanical properties change as pregnancy progresses, especially during the later stages and labor, concluding that significant alterations occur in both the amnion and chorion layers [41].

Oyen et al. (2004) explored the time-dependent behavior of soft tissues, particularly the amnion layer, studying how it differs from the chorion in terms of failure properties [33]. They analyzed the puncture failure of the intact chorioamnion membrane, as well as the individual amnion and chorion layers when separated, through biaxial mechanical testing [33]. Furthering their investigation, Oyen and colleagues also performed uniaxial and planar biaxial mechanical tests to determine the nonlinear and time-dependent mechanical response of the amnion layer [42].

In more recent work, Verbruggen et al. (2017) developed linear elastic finite element models of the fetal membranes to determine the mechanical role of both the amnion and chorion layers [8]. Their models also simulated the uterine environment, helping to shed light on the behavior of the membranes in the context of term gestation and in cases of PPROM [8]. Nevertheless, the mechanical response of the chorioamnion, especially the amnion layer, exhibits a strongly nonlinear response, which limits the applicability of the findings from Verbruggen's study [40, 42]. Therefore, these studies collectively contribute to a more comprehensive understanding of the complex mechanical performance of fetal membranes, though much remains to be discovered [2, 3].

Moreover, the separation of the fetal membrane has been observed in puncture experiments [12]. Consistent separation between the amnion and chorion was likewise observed during uniaxial tensile tests conducted on the intact fetal membrane, as shown in Figure 2.8 [12]. In these tests, the separation is attributed to the distinct contraction behavior of the two layers - an effect related to Poisson's phenomenon - with the amnion exhibiting significantly greater lateral contraction [12].

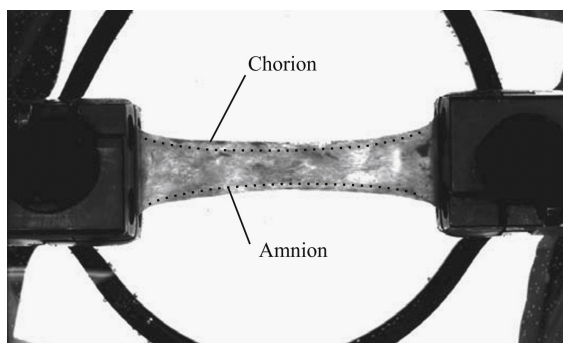


Figure 2.8: Fetal membrane sample during a uniaxial tensile test. The constituent layers of the fetal membrane begin to separate beyond a certain level of deformation. The dotted line indicates the boundary of the amnion layer after separation. Authorized, available at [12].

The unusually pronounced contraction of the amnion under tensile loading had not been previously reported [12]. This observation prompted a series of tensile tests on human amnion aimed at quantitatively characterizing its lateral contraction behavior [12]. The experimental results, combined with considerations of the mechanisms of fiber rotation, stretching and buckling within a random network, led to the selection of a constitutive model formulation proposed by Buerzle and Mazza [12]. The model parameters were computed to simultaneously fit both the tension-stretch and contraction-elongation curves obtained from the tensile tests [12]. Ultimately, the model response to equibiaxial tension was compared with corresponding data from prior experimental studies [12].

This compilation of mechanical tests and models highlights the complex material properties of fetal membranes, emphasizing the need for the development of a layered model that accurately represents their structural and mechanical functionality [2]. To fully understand the intricate and complex interactions among the fetal membranes and also the cervix and the uterus at term or preterm, it is crucial to develop an accurate model of the fetal membrane, especially due to the amnion's nonlinear and anisotropic behavior, which largely influences the membrane's mechanical response [2].

This work was already developed by Fidalgo et al. (2024a) [2] and it introduces a multilayer biomechanical finite element model of the fetal membrane that accounts for its physiological behavior within the uterine environment. By treating the amnion as an anisotropic nonlinear material, this model offers a significant improvement over the existing ones [2].

The study performed by Fidalgo et al. (2024b) [3] aimed to investigate the mechanical response of the fetal membrane under different physiological conditions throughout pregnancy, using a numerical inflation setup of the membrane. Specifically, the primary goal was to analyze whether certain conditions may contribute to PPROM, based on the outcomes of the numerical simulations [3].

The validation of the model was achieved by comparing its inflation apex displacement with data from an inflation mechanical dataset [43] and by evaluating the computed maximum principal

stress values in both the amnion and chorion layers against the respective physiological ranges reported in the literature [2].

2.4 Model developed by Fidalgo et al. (2024)

The research conducted by Fidalgo et al. (2024a) [2] was structured into three main components: (1) the mechanical characterization of the amnion, chorion and decidua layers; (2) the development of a multilayer fetal membrane model and (3) the establishment of numerical simulations.

In part (1), a non-linear constitutive model was introduced to characterize the mechanical behavior of the amnion, along with the determination of the associated material parameters [2, 3]. The mechanical properties of the chorion and decidua were described using linear elastic models [2, 3]. In part (2), a multilayer fetal membrane model was developed, integrating the findings from part (1) and combining the amnion, chorion and decidua layers [2, 3]. Lastly, in part (3), numerical simulations were designed to analyze four distinct physiological scenarios utilizing the multilayer fetal membrane model [2, 3].

These scenarios included varying contact conditions between the amnion and chorion, normal and abnormal intrauterine pressures, spanning the 30th to 40th gestational weeks, variations in the thicknesses of the amnion and chorion layers and different collagen fiber orientations within the amnion layer [2, 3].

2.4.1 Formulation of the amnion constitutive model

The mechanical behavior of the amnion layer was modeled using a constitutive formulation designed to capture the effects of stretching, rotation and buckling of its thin, randomly oriented collagen fibers [2, 3, 12, 44]. Collagen fibers significantly influence the amnion's mechanical response, as highlighted in earlier studies [2, 3]. Under uniaxial stress, these fibers exhibit reorientation at low strain levels, with the elastin matrix primarily responsible for resisting deformation [2, 3, 7]. In contrast, under biaxial mechanical testing, the fibers contribute to low deformation resistance without substantial reorientation [2–4].

To model this behavior, a modified version of the Buerzle-Mazza formulation was employed [2, 3, 12]. This modification was developed by Mauri and Ehret [45] and further adapted by Fidalgo et al. (2024a) [2] to describe the amnion's mechanical response. Further details regarding the material model employed can be found in [2].

Eight parameters were identified in the description of the constitutive model, including μ_0 , q , m_2 , m_3 , m_4 , m_5 , N and ϕ [2, 3]. The values for these parameters were determined based on the work of Fidalgo et al. (2024a) [2], in which the constitutive model was presented and validated. The respective parameters are provided in Table 2.1.

Table 2.1: Parameters employed in the formulation of the constitutive model for the mechanical behavior of the fetal membrane; all parameters are dimensionless, except for μ_0 [2, 3].

Parameter	Description	Value
μ_0	Material constant expressed in units of stress	2.4 MPa
q	Dimensionless parameter that regulates the degree of nonlinearity in the moduli corresponding to the various stress responses of the composite structure	2.96
m_2	Model parameter	0.00228
m_3	Model parameter	41.12
m_4	Model parameter	1.27
m_5	Model parameter	0.463
N	Number of fiber families included in the model	32
ϕ	Slight off-plane inclination	0°

2.4.2 Characterization of the chorion and decidua

In contrast to the amnion, which plays a vital role in maintaining the integrity and mechanical resilience of the fetal membrane against external forces, the chorion and decidua are relatively less significant in terms of mechanical properties [2, 3]. Their primary function is to protect the fetus, rather than to provide direct mechanical support or resistance [2, 3]. As a result, these layers were modeled as elastic, linear materials, with their respective properties outlined in Table 2.2 [2, 3].

Table 2.2: Elastic linear properties of the chorion and decidua layers, including Young's modulus and Poisson's ratio [2, 3, 8, 40, 46, 47].

Layer	Young's modulus, E [MPa]	Poisson's ratio, ν
Chorion	1	0.4–0.5
Decidua	1	0.4–0.5

It was assumed that the chorion and decidua exhibit similar mechanical characteristics, given that the smooth chorion fuses with the decidua during pregnancy, indicating a potential mechanical similarity between the two layers [2, 3].

2.4.3 Numerical setup for the inflation mechanical test

The biomechanical behavior of the fetal membrane under various physiological conditions was analyzed using a finite element model, illustrated in Figure 2.9, designed to capture the inflation response of the amnion-chorion-decidua complex [2, 3]. This numerical model was developed based on the inflation mechanical test outlined by Samimi et al. (2023) [2, 3, 43].

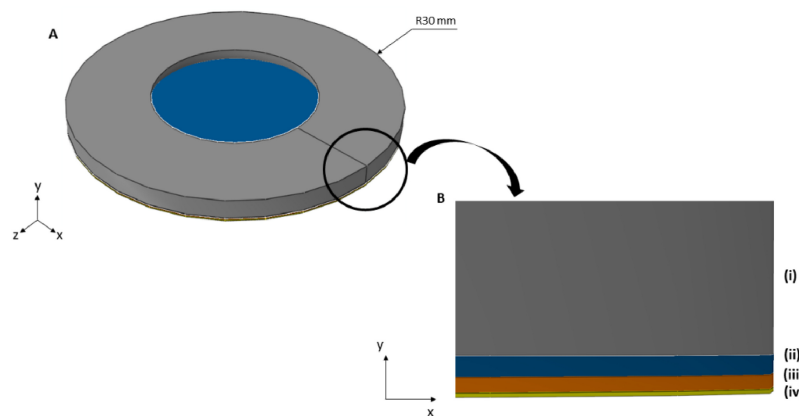


Figure 2.9: Finite element representation of the inflation mechanical test utilizing a multilayer fetal membrane model. A: three-dimensional depiction of the entire model; B: close-up view highlighting the components (grey: clamping ring (i); blue: decidua layer (ii); orange: chorion layer (iii); yellow: amnion layer (iv)). Authorized, available at [2, 3].

The finite element model consists of four primary components: (i) a clamping ring, (ii) a segment of the decidua, (iii) the chorion and (iv) the amnion [2, 3]. The thicknesses of the decidua, chorion and amnion were determined using optical coherence tomography experimental measurements, which averaged the initial thicknesses of eighteen peri-placental c-section membrane samples [2, 3], as reported by Samimi et al. (2023) [43]. The attributed thickness values for each layer are summarized in Table 2.3.

Table 2.3: Thickness values assigned to the amnion, chorion and decidua layers, based on averaged optical coherence tomography measurements from peri-placental fetal membrane samples [2, 43].

Layer	Thickness [mm]
Amnion	0.062
Chorion	0.217
Decidua	0.311

The amnion measured 60 mm in diameter and 0.062 mm in thickness [2, 3]. Its mechanical behavior was modeled using a modified version of the Buerzle-Mazza constitutive model, with the corresponding parameters provided in Table 2.1 [2, 3]. The amnion mesh consisted of 3422 eight-node brick elements (C3D8) and 7034 nodes [2, 3].

The chorion and decidua also had a diameter of 60 mm, with the chorion having a thickness of 0.217 mm and the decidua a thickness of 0.311 mm [2, 3]. Both layers were modeled as elastic linear materials, with the material properties defined in Table 2.2 [2, 3]. The finite element meshes for the chorion and decidua consisted of 3422 C3D8 elements and 7034 nodes [2, 3].

On the other hand, the clamping ring was modeled as a rigid body, with an inner diameter of 30 mm and an outer diameter of 60 mm [2, 3].

In addition, the contact condition between the amnion and chorion was initially modeled as surface-to-surface contact with a friction coefficient of 0.5, simulating the interconnecting spongy

layer between these two structures [2, 3]. The chorion and decidua were connected using a tie constraint to represent the fusion process that occurs between these layers during gestation [2, 3]. The contact between the decidua and the clamping ring was also defined as surface-to-surface contact, but with a high friction coefficient value to prevent slippage between the mentioned structures, in accordance with the experimental setup [2, 3].

Regarding the boundary conditions, all nodes along the perimeter (i.e., border nodes) of the three layers were constrained in all directions, while the clamping ring was fully fixed [2, 3].

2.4.4 Validation of the multilayer fetal membrane model against experimental data

The evolution of apex displacement, d , in the multilayer finite element model - which incorporates an anisotropic and elastic representation of the amnion - is illustrated in Figure 2.10, alongside the experimental results reported by Samimi et al. [2, 43]. The experimental curve was derived by averaging the apex displacements measured in eighteen c-section fetal membrane samples collected from the peri-placental region [2]. In order to facilitate comparison, a data interpolation procedure was applied to align the measurements onto a common pressure axis [2]. The resulting pressure range shared across all samples extends approximately from 1 kPa to 10 kPa [2].

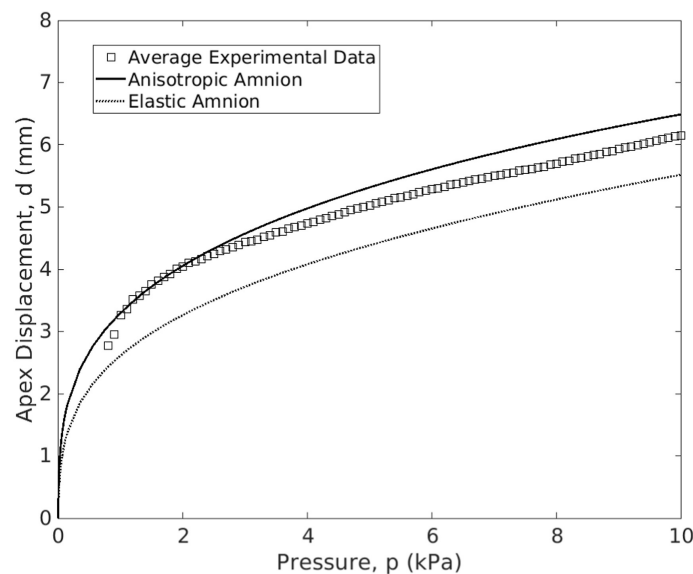


Figure 2.10: Comparison between the multilayer fetal membrane model - featuring an anisotropic and elastic representation of the amnion - and experimental data reported by Samimi et al. (2023). The elastic behavior of the amnion is defined according to the characterization by Verbruggen et al. (2017). Authorized, available at [2].

It is evident from Figure 2.10 that, in all cases, the apex displacement increases more rapidly during the initial stage of the inflation simulation [2]. This stage corresponds to the pre-inflation phase, in which the membrane is in a slack state [2]. As the internal pressure, p , rises, the curves

begin to plateau, indicating increased mechanical resistance from the membrane [2]. Consequently, the rate of change in apex displacement diminishes throughout the course of the simulation [2].

At lower pressure levels (below 2 kPa), the apex displacement predicted by the multilayer model with the anisotropic amnion closely aligns with the experimental data [2]. In contrast, the model incorporating an elastic amnion yields significantly lower displacement values in this range [2]. For higher pressures, the relative error at 10 kPa between the simulation and experimental data is calculated as 5.0% for the anisotropic amnion model and 11.0% for the elastic amnion model [2].

Additionally, the standard deviation was determined to assess the variability of the experimental data in relation to the mean displacement curve [2]. A value of 1.764 was obtained, indicating a moderate dispersion of data points around the average trend [2].

2.4.5 Numerical simulations

Following the establishment of the multilayer model, various physiological conditions were investigated within the framework of biaxial extension [3]. In experimental procedures, it is common to perform a pre-inflation step to slightly stretch the membrane sample, thereby eliminating excess slack [3]. This step ensures a repeatable and consistent definition of the reference configuration for experimental analysis [3, 48].

In order to replicate this process, the numerical simulations of the inflation mechanical test were divided into two main steps: (1) pre-inflation and (2) inflation-to-failure [3]. During the pre-inflation stage, a pressure of 0.15 kPa was applied to bring the membrane to a pre-inflated state [3]. In the subsequent inflation stage, the pressure was gradually increased up to 20 kPa, except in cases where different intrauterine pressure values were specifically analyzed [3]. Both pressure loads were sequentially applied to the lower surface of the amnion layer, aligned with the positive y-axis direction, as indicated in Figure 2.9 [3].

Indeed, these simulations were designed to evaluate the mechanical response of the multilayer fetal membrane under various physiological conditions in a biaxial inflation setup [3]. The effects of several parameters, including contact conditions, intrauterine pressures, variations in amnion and chorion thicknesses and off-plane collagen fiber angles, were systematically analyzed [3].

2.4.6 Findings reported by Fidalgo et al. (2024)

The present section presents and discusses the outcomes detailed by Fidalgo et al. (2024b) [3] for all physiological conditions, with a particular emphasis on the distribution of maximum principal stress, thickness variations at the apex region of the amnion and chorion layers and the overall displacement at the apex of the fetal membrane.

2.4.6.1 Control Model

Regarding the control model, during the initial phase of the inflation simulation - corresponding to the pre-inflation stage - the apex displacement increases rapidly, as the membrane is initially in a slack state [2, 3]. As pressure rises, the displacement curve begins to level off, reflecting the increased resistance of the membrane [2, 3].

By the end of the mechanical test, the amnion layer experiences a maximum principal stress of 4156 kPa at the apex region, whereas the chorion reaches only 219 kPa (see Figure 2.11) [2, 3]. It is noteworthy that throughout the inflation simulation, the amnion consistently undergoes higher maximum principal stresses compared to the chorion as pressure increases [2, 3].

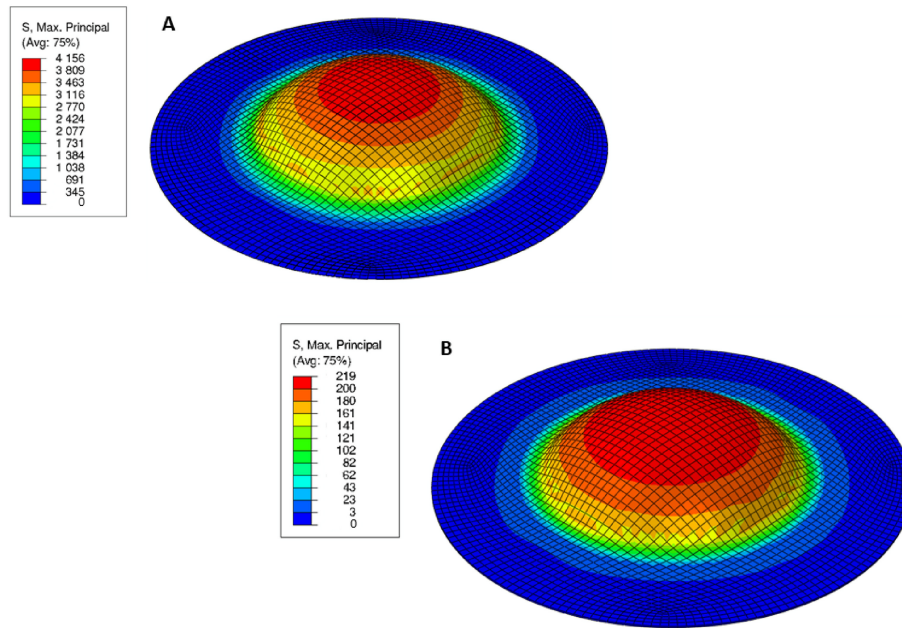


Figure 2.11: Distribution of the maximum principal stress in (A) the amnion layer and (B) the chorion layer at the conclusion of the inflation mechanical test, expressed in kilopascals (kPa). Authorized, available at [2, 3].

2.4.6.2 Study of the effect of varying contact conditions at the amnion-chorion interface

In the amnion layer (Figure 2.12A), the maximum principal stress values increase when either frictional or tied contact is considered, as compared to the frictionless condition [3]. Conversely, in the chorion layer, the stress values exhibit the opposite trend, as illustrated in Figure 2.12B [3]. Furthermore, no notable differences in stress values are observed between the frictional and tied contact scenarios in either layer [3]. When compared to the control model, the order of magnitude of the stress values in both layers remains consistent across all conditions [3].

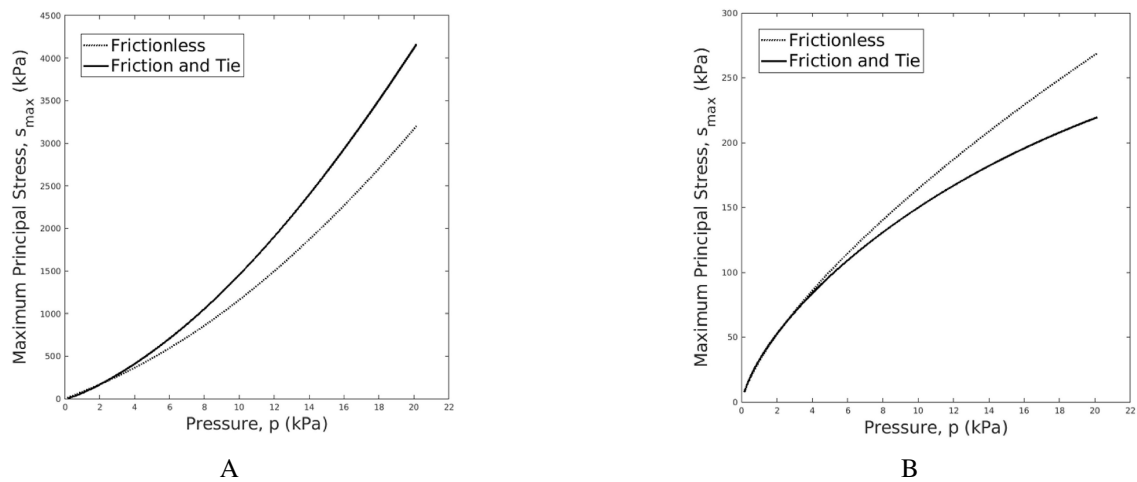


Figure 2.12: Evolution of maximum principal stress at the apex region of (A) the amnion and (B) the chorion layers under varying contact conditions. Authorized, available at [3].

2.4.6.3 Study of the effect of typical and atypical intrauterine pressures throughout gestation

The maximum principal stress values at the apex region of the amnion and chorion layers were analyzed for different gestational weeks, as shown in Figure 2.13 [3]. The findings indicate that the amnion consistently experiences higher maximum principal stresses compared to the chorion across all gestational weeks, with the disparity becoming more pronounced near term (40th week) [3].

Additionally, the stress values in the amnion layer exhibit a more substantial increase over the gestational period, whereas the stress values in the chorion remain relatively stable [3]. Notably, the application of abnormally high pressure during the inflation simulation results in increased maximum principal stresses in both layers and further amplifies the difference in stress levels between them [3].

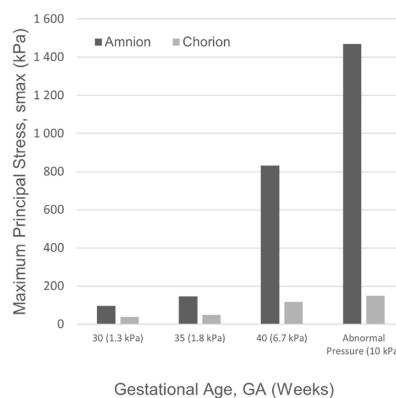


Figure 2.13: Maximum principal stress distribution at the apex region of the amnion and chorion layers under normal and abnormally high pressures across various gestational weeks. Authorized, available at [3].

2.4.6.4 Study of the effect of amnion and chorion thickness variations

The impact of varying the thickness of the amnion and chorion layers on the maximum principal stress at their apex regions is presented in Figure 2.14 [3]. A notable increase in maximum principal stress is observed in the amnion layer as its thickness decreases, while the chorion layer shows relatively stable stress values across different thickness combinations [3]. Compared to the control model, all simulated scenarios demonstrate that increasing the thickness of either the amnion or chorion results in a reduction in maximum principal stress [3].

Conversely, decreasing the thickness leads to elevated stress levels [3]. The lowest maximum principal stress values are observed when both the amnion and chorion are set to their maximum thicknesses [3]. Lastly, it is fundamental to emphasize that alterations in the thickness of one layer influence the stress distribution in the other, underscoring their mechanical interaction [3].

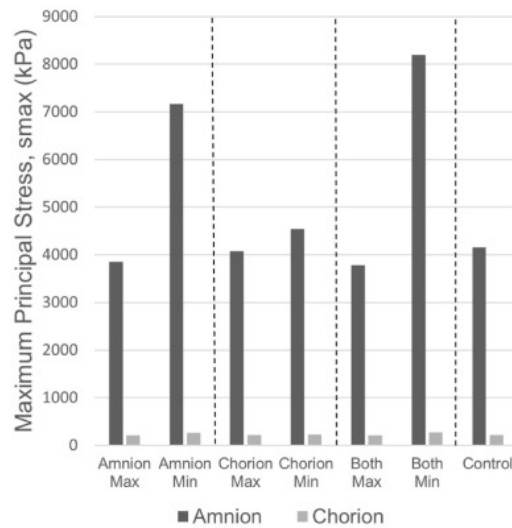


Figure 2.14: Maximum principal stress values at the apex region of the amnion and chorion layers for various thickness configurations. Authorized, available at [3].

2.4.6.5 Study of the effect of the off-plane angle variation

The apex displacement of the fetal membrane is observed to increase as the off-plane angle in the amnion layer is set to higher values, as depicted in Figure 2.15 [3]. The progression of the maximum principal stress curves at the apex region of the amnion displays distinct behaviors depending on the off-plane angle setting [3]. When the angle is set to 0° or 10° , the stress curves show a continuous increase throughout the simulation [3].

In contrast, for angles of 20° or 30° , the stress remains negligible at lower pressures but exhibits a sharp rise as the pressure becomes larger (Figure 2.16A) [3]. The maximum principal stress in the amnion layer increases notably when the angle changes from 0° to 10° and from 30° to 20° [3].

Regarding the chorion layer, the maximum principal stress at the membrane's apex consistently rises with an increase in the off-plane angle (Figure 2.16B) [3].

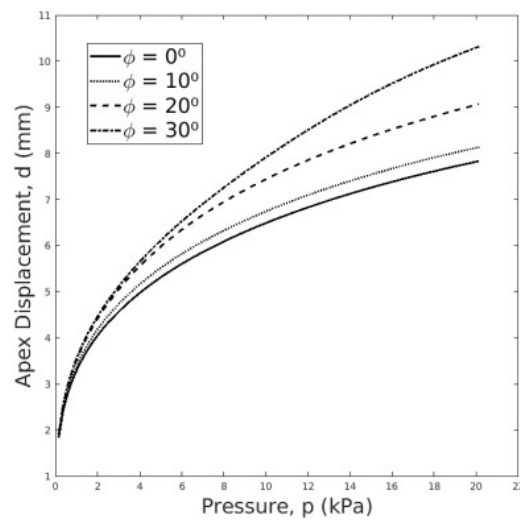


Figure 2.15: Apex displacement of the fetal membrane throughout the inflation test for varying off-plane angles. Authorized, available at [3].

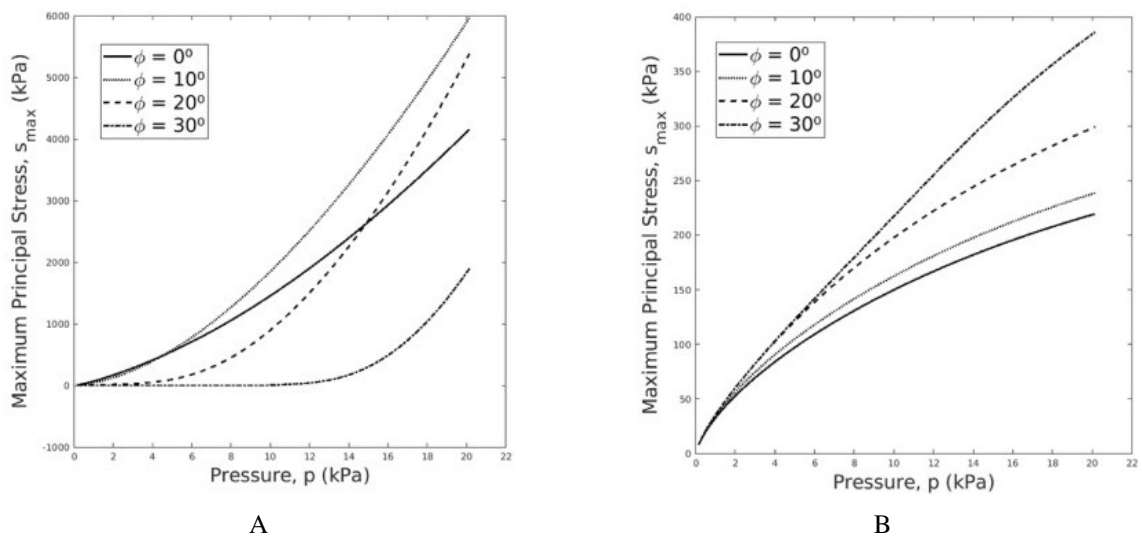


Figure 2.16: Evolution of maximum principal stress at the apex region of (A) the amnion and (B) the chorion for various off-plane angles. Authorized, available at [3].

In a similar manner to the analysis of maximum principal stress, the trends associated with thickness variation at the apex region of the amnion layer for 0° and 10° off-plane angles display distinct patterns compared to those for 20° and 30° angles, as shown in Figure 2.17A [3]. By the end of the inflation simulation, the smallest reduction in thickness for the amnion is observed at 0° , whereas the largest reduction is recorded at 30° , closely followed by the 20° angle [3]. In the chorion layer, the reduction in thickness becomes progressively more significant as the off-plane angle increases, which is evidenced in Figure 2.17B [3].

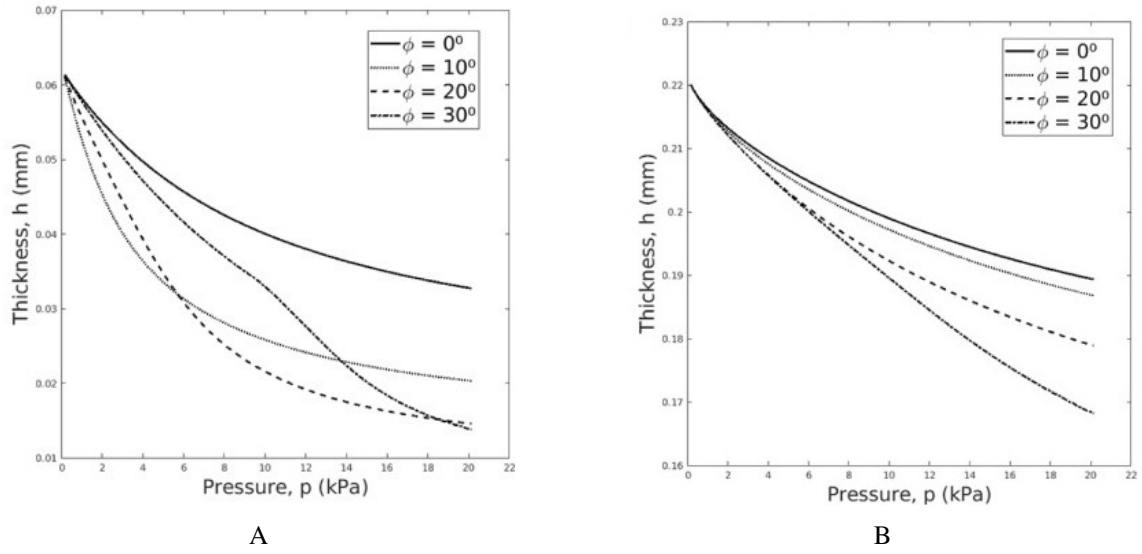


Figure 2.17: Thickness changes in (A) the amnion and (B) chorion layers for varying off-plane angles. Authorized, available at [3].

To conclude, the study conducted by Fidalgo et al. (2024b) [3] highlights several key factors that may contribute to the onset of PPROM throughout pregnancy. These include: (1) friction between the amnion and chorion layers, (2) abnormally high intrauterine pressures, (3) reduced thicknesses of the amnion and chorion and (4) the arrangement of collagen fibers in the amnion layer [3]. A deeper understanding of how these factors influence fetal membrane biomechanics and pregnancy outcomes is essential for improving clinical practices [3].

Moreover, the layered model proposed in the mentioned study can serve as a foundation for more comprehensive numerical models of the gravid uterus, encompassing the fetal membrane, uterus and cervix [3]. Such models could provide valuable insights into the complex biomechanical interactions between these structures [3]. Their clinical application may lead to improved diagnostic and preventive strategies for conditions, such as spontaneous PTB, cervical funneling, cervical insufficiency and PPROM, among others, which profoundly affect pregnancy outcomes and maternal-fetal health in the short and long term [3].

2.5 Parametrized numerical representation of the gravid uterine body and cervix developed by Fidalgo et al.

A fully parametrized numerical model of the gravid uterine body and cervix, presented in Figure 2.18, was established by Fidalgo et al. (2024c) [13], based on the methodology proposed by Louwagie et al. (2021) [11]. Owing to its parametrized nature, the geometric model allows for the generation of patient-specific anatomies through the adjustment of key dimensional parameters [13]. The model represents a mid-third-trimester gravid uterus and cervix [13], constructed using average anatomical measurements obtained from a cohort of patients as reported by Louwagie et al. (2021) [11]. It encompasses four primary anatomical components: the uterine body, the cervix,

the multilayer fetal membrane (comprising the amnion, chorion and a portion of the decidua) and the abdominal region [13].

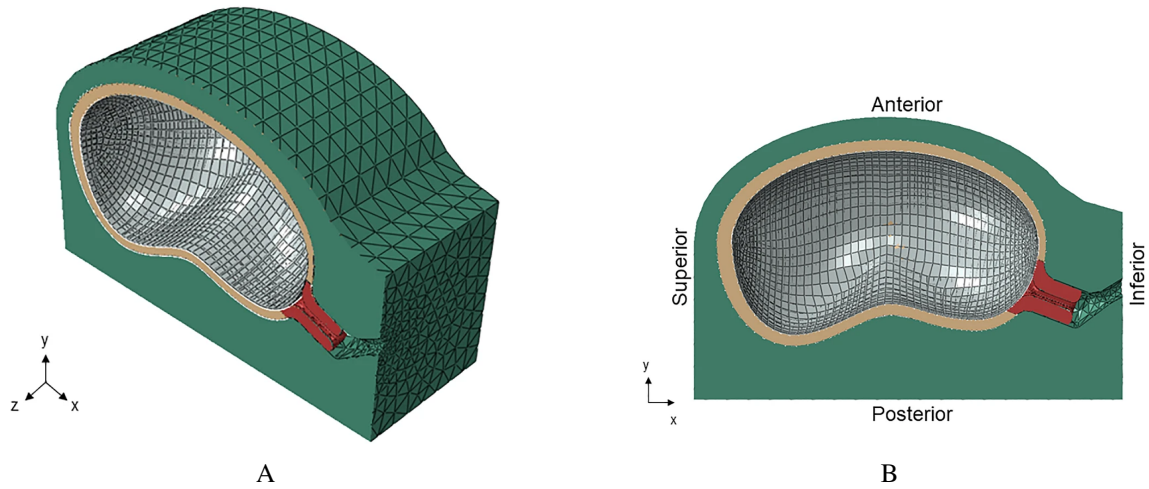


Figure 2.18: Parametrized finite element model of the gravid uterine body and cervix, consisting of four distinct anatomical structures (A): the uterine body (brown), cervix (red), multilayer fetal membrane (gray) and abdominal wall (green). The anatomical orientations - anterior, posterior, superior and inferior - are also indicated in (B). Authorized, available at [13].

Further details concerning the finite element characterization and mechanical properties of each component of the model, as well as the contact interactions, loading conditions and boundary constraints, are thoroughly described in [13].

2.6 Soft tissue characterization

Soft tissue characterization involves determining the material properties that define the behavior of biological soft tissues [49]. Understanding these properties is essential for gaining insight into the natural mechanical responses of these tissues and for effectively applying their unique characteristics in biomedical applications [49]. Among the various types of properties, mechanical properties are the most extensively studied [49]. These include parameters commonly used in engineering, such as [49]:

- **Young's modulus:** quantifies stiffness under tension or compression;
- **Shear modulus:** measures resistance to shear deformation;
- **Complex modulus:** the stress-to-strain ratio under dynamic loading;
- **Poisson's ratio:** the ratio of transverse to axial strain;
- **Yield strength:** the stress at which permanent deformation begins;
- **Ultimate strength:** the maximum stress the material can endure before failure.

Besides, it is pertinent to emphasize that several viscoelastic properties associated with biological soft tissues are discussed in subsection 2.6.7, presented later in this report.

Characterizing the mechanical properties of soft tissues plays a crucial role in medical research and clinical applications, as it provides a deeper understanding of tissue mechanics [49]. However, due to their small size and delicate structure, soft tissues are much more fragile than other biological tissues, which makes mechanical testing particularly challenging [49]. To overcome limitations in the availability of human samples, such tests are frequently conducted on cadaveric animal tissues, which serve as suitable substitutes [49].

An alternative approach to experimental testing is the development of three-dimensional computational models, which allow for the non-invasive investigation of soft tissue mechanics [49]. These models can be constructed using imaging techniques, namely MRI or CT (Computed Tomography) scans, combined with advanced numerical methods like finite element analysis [49]. This strategy not only preserves the integrity of biological specimens but also provides valuable insights into tissue behavior without the ethical and practical constraints associated with physical testing [49].

2.6.1 Mechanical behavior of soft tissues

Elasticity is a fundamental material property that describes a material's ability to return to its original shape after being deformed by an external load [50]. In an elastic material, the stress-strain relationship is reversible, meaning the loading and unloading paths on the stress-strain curve are identical [50]. Furthermore, the stress at any given moment depends solely on the current strain, independent of the deformation history [50]. In the case of linear elasticity, this relationship follows Hooke's law, where stress is directly proportional to strain [50].

Hyperelastic materials represent a class of ideally elastic materials characterized by a nonlinear stress-strain response [50]. Unlike linear elastic materials, their behavior is defined by a strain energy density function, which quantifies the internal energy stored in the material due to deformation [50]. Despite the nonlinearity, these materials remain fully elastic, as they can recover their original shape upon unloading [50].

Viscoelasticity refers to the time-dependent mechanical behavior of materials that exhibit both elastic (spring-like) and viscous (dashpot-like) characteristics [50]. In viscoelastic materials, the stress-strain response is influenced not only by the magnitude of deformation but also by the rate and duration of loading [50]. This implies that their mechanical behavior changes over time under the influence of stress or strain (external loading) [50]. This behavior gives rise to key phenomena such as [50]:

- **Stress relaxation:** when a viscoelastic material is subjected to a constant strain, the internal stress required to maintain that deformation gradually decreases over time due to the material's time-dependent behavior;

- **Creep:** when a constant stress is applied to a viscoelastic material, it continues to deform progressively over time, illustrating the material's tendency to undergo time-dependent strain;
- **Hysteresis:** energy dissipation that occurs due to a phase lag between stress and strain during cyclic loading. In other words, the loading and unloading curves of a viscoelastic material do not coincide, resulting in a loss of mechanical energy.

2.6.2 Constitutive models

Constitutive models are mathematical frameworks that characterize the behavior of materials under various mechanical and/or thermal loading conditions [51]. They define the stress-strain relationships necessary to formulate the governing equations of a system, in conjunction with the fundamental conservation laws and kinematic constraints [51]. Additionally, a failure criterion must be incorporated to detect the onset of material failure and to model its progression accurately [51].

To ensure the constitutive model properly represents the real material behavior, it is essential to calibrate the material parameters by fitting the model's numerical response to experimental data [51]. This process involves minimizing the discrepancy between simulated and experimental stress-strain curves, for example [51]. Parameter calibration can be carried out through manual tuning, which is feasible for models with a limited number of parameters, or through optimization algorithms, which are more suitable for complex models with larger parameter spaces [51].

2.6.3 Isotropic hyperelasticity

Isotropy refers to the condition in which a material exhibits identical mechanical properties in all directions [52]. In such cases, the material's response to stress or strain is independent of the direction in which it is applied [52].

2.6.3.1 Neo-Hookean constitutive model

The Neo-Hookean constitutive model consists in a hyperelastic formulation commonly employed to characterize the mechanical response of rubber-like materials subjected to large deformations [53]. It stands for a simplified version of the Mooney-Rivlin model and is derived from the principles of statistical thermodynamics governing the behavior of cross-linked polymer networks [53]. The elastic strain energy density function can be defined as [53]:

$$W = c_{10} (\bar{I}_1 - 3) + \frac{1}{D_1} (J - 1)^2 \quad (2.1)$$

In this expression, $c_{10} = \frac{\mu_0}{2}$ corresponds to the stiffness coefficient (material parameter) [13], where μ_0 denotes the initial shear modulus [53]. The parameters D_1 and J represent the incompressibility coefficient and the volume ratio, respectively [53]. The model is formulated based on

the first invariant of the right Cauchy-green strain tensor, $\bar{I}_1$ [53]. Under the assumption of material incompressibility, $J = 1$, and thus the volumetric contribution represented by the second term vanishes [53].

2.6.4 Anisotropic hyperelasticity

Anisotropy characterizes materials whose mechanical properties differ depending on the direction of loading [52]. This directional dependence is particularly evident in biological soft tissues, which demonstrate anisotropic behavior due to their intricate and heterogeneous microstructural organization [52].

2.6.4.1 Holzapfel-Gasser-Ogden constitutive model

The Holzapfel-Gasser-Ogden (HGO) constitutive model is designed to represent the anisotropic mechanical properties of hyperelastic materials, particularly those found in soft biological tissues [54]. As an anisotropic hyperelastic framework, it captures both the non-linear elastic response and the directional dependence of material behavior [54]. The model's strain energy function integrates contributions from both the isotropic matrix - representing the ground substance - and the anisotropic reinforcing fibers [54]. Further details and equations pertaining to the aforementioned model are provided in subsection 2.6.6.

2.6.5 User-defined material subroutine (UMAT)

While Abaqus offers an extensive library of built-in constitutive models for simulating a wide range of material behaviors, certain advanced or highly specific material laws may not be available by default [55]. To address this limitation, user-defined material subroutines, particularly the UMAT subroutine, enable the implementation of custom constitutive models tailored to complex material responses [55].

The UMAT subroutine is written in the Fortran programming language and allows users to define material behavior by specifying the stress-strain relationship beyond the capabilities of standard Abaqus models [55]. Within UMAT, the user is responsible for computing the Cauchy stress tensor (STRESS) and the material Jacobian matrix (DDSDDE) [55]. In continuum mechanics, the Cauchy stress tensor defines the state of stress at a point within a material in its deformed configuration and the Jacobian matrix represents the derivative of stress with respect to strain [55]. When a UMAT subroutine is used, it is responsible for calculating and updating the Cauchy stress tensor and the Jacobian matrix at each integration point within a finite element [55]. The accurate definition of the Jacobian matrix is essential, as it ensures the numerical stability and convergence of the finite element solution [55].

UMATs are widely used in advanced simulations for a variety of applications, including [55]:

- Complex constitutive modeling, such as non-linear elasticity, time-dependent behavior and anisotropy;

- Material damage and failure analysis, incorporating continuum damage mechanics or failure criteria into the simulation framework;
- Special material phenomena, including viscoelasticity, plasticity and thermomechanical coupling, particularly in high-temperature environments.

2.6.6 Plane stress formulation based on the HGO constitutive model

A UMAT subroutine was implemented with the HGO constitutive model, incorporating two fiber families [56]. In order to account for the plane stress condition, the model was adapted following the methodology proposed by Prot et al. (1983) [57]. Additionally, the viscoelastic component introduced by Vila Pouca et al. (2018) [58] was integrated, resulting in a visco-hyperelastic constitutive model suitable for finite element implementation [56] (the viscoelastic component of the model is described in greater detail in subsection 2.6.7).

The isochoric component of the strain energy function, $\bar{\Psi}$, for the HGO constitutive model is defined as follows [56]:

$$\bar{\Psi} = \frac{c_1}{2} (\bar{I}_1 - 3) + \frac{k_1}{2k_2} \sum_{i=4,6} \{ \exp [k_2 \langle E_\alpha \rangle^2] - 1 \} \quad (2.2)$$

where

$$\langle E_\alpha \rangle = \kappa (I_1 - 3) + (1 - 3\kappa)(I_i - 1), \quad i = 4, 6 \quad (2.3)$$

Within this framework, c_1 and $k_1 > 0$ represent stress-like material parameters (units: MPa), while $k_2 > 0$ is a dimensionless material parameter [56, 59]. When $k_1 = 0$, the strain energy function (Equation 2.2) simplifies to the form of the Neo-Hookean constitutive model [56].

The constitutive model formulation assumes that the collagen fibers within each fiber family are dispersed around a mean preferred orientation [56]. The dispersion is characterized by the parameter κ , where $0 \leq \kappa \leq 1/3$ [56]. A value of $\kappa = 0$ corresponds to perfectly aligned fibers (no dispersion), while $\kappa = 1/3$ indicates a completely random fiber orientation [56].

In membrane elements, a plane stress state can be achieved by invoking the incompressibility condition, which enables the expression of the out-of-plane strain component as a function of the in-plane strain components [57, 59]. Under this incompressibility assumption, the C_{33} component of the right Cauchy-green deformation tensor can be defined in terms of its in-plane constituents [59]:

$$C_{33} = (C_{11}C_{22} - C_{12}^2)^{-1} \quad (2.4)$$

Within the context of a plane stress formulation, the second Piola-Kirchhoff stress tensor is computed as [59]:

$$\mathbf{S} = 2 \sum_{\substack{i=1 \\ i \neq 3}}^5 \frac{\partial U}{\partial I_i} \frac{\partial I_i}{\partial \mathbf{C}} + p \mathbf{C}^{-1} \quad (2.5)$$

The scalar p is determined by enforcing the condition that the S_{33} stress component is zero, a valid approximation for thin membranes (Equation 2.6) [59]:

$$p = -2 \sum_{\substack{i=1 \\ i \neq 3}}^5 \frac{\partial U}{\partial I_i} \frac{\partial I_i}{\partial C_{33}} C_{33} \quad (2.6)$$

The material elasticity tensor can then be evaluated using the following expression [59]:

$$\mathbb{C} = 4 \sum_{\substack{i,j=1 \\ i,j \neq 3}}^5 \frac{\partial^2 U}{\partial I_i \partial I_j} \frac{\partial I_i}{\partial \mathbf{C}} \otimes \frac{\partial I_j}{\partial \mathbf{C}} + 2 \mathbf{C}^{-1} \otimes \frac{\partial p}{\partial \mathbf{C}} + 2p \frac{\partial \mathbf{C}^{-1}}{\partial \mathbf{C}} \quad (2.7)$$

For the implementation of the UMAT in Abaqus, it is necessary to determine both the Cauchy stress tensor and the tangent stiffness matrix [56]. These quantities are obtained via the push-forward operation applied to the second Piola-Kirchhoff stress tensor and the material elasticity tensor, described in Equations 2.5 and 2.7, respectively [56]. The detailed procedure for computing these variables under plane stress conditions is thoroughly described by Prot et al. (1983) [57].

Prior to applying the push-forward operation to derive the second Piola-Kirchhoff stress tensor and the elasticity tensor in the spatial configuration, the viscoelastic contribution, implemented in a decoupled manner as described by Vila Pouca et al. (2018) [58], was incorporated [59]. It is noteworthy that the author accounts for the volumetric component of the strain-energy function. Nevertheless, due to the assumption of total incompressibility inherent to the plane stress condition, the volumetric contribution is omitted in this specific implementation [59].

2.6.7 Viscoelastic constitutive formulation based on the generalized Maxwell model

Given the critical role of viscoelastic behavior in accurately characterizing the biomechanics of biological tissues, the constitutive model adopted in the study conducted by Moura et al. (2022) [59] is the viscoelastic formulation developed by Vila Pouca et al. (2018) [58], based on the framework proposed by Holzapfel (2000) [60]. This model was implemented in the Abaqus software via a UMAT subroutine [59].

To represent the viscoelastic contribution, a generalized Maxwell model was employed [59]. This approach incorporates a dissipative potential into the strain energy function (Equation 2.8), thereby capturing the material's non-equilibrium response [59]:

$$U_M(C, \Gamma_1, \dots, \Gamma_m) = U_{vol}(J) + U_{iso} + \sum_{\alpha=1}^m \gamma_{\alpha}(\bar{\mathbf{C}}, \Gamma_{\alpha}) \quad (2.8)$$

In this equation, the configurational free energy, γ_{α} , is expressed as a function of the modified right Cauchy-Green strain tensor, $\bar{\mathbf{C}}$, and a set of internal strain-like variables, defined by Γ_{α}

[59]. These internal variables are introduced to represent the time-dependent behavior of the material, including both relaxation and creep phenomena, through $\alpha = 1, \dots, i$ viscoelastic processes [59].

Accordingly, the second Piola-Kirchhoff stress tensor is decomposed into an equilibrium (elastic) component and a non-equilibrium (viscoelastic) component, reflecting the combined elastic-viscoelastic material response, as expressed in Equation 2.9 [59]:

$$\mathbf{S} = \mathbf{S}_J^\infty + \mathbf{S}_m^\infty + \mathbf{S}_f^\infty + \sum_{\alpha=1}^i \mathbf{Q}_\alpha \quad (2.9)$$

The variables $\mathbf{S}_J^\infty$, $\mathbf{S}_m^\infty$ and $\mathbf{S}_f^\infty$ represent the volumetric, matrix and fiber contributions of the second Piola-Kirchhoff stress tensor, respectively [59]. The isochoric non-equilibrium stress tensors, $\mathbf{Q}_\alpha$, are defined according to Equation 2.10 and their solution can be established through the application of convolution integrals [59]:

$$\mathbf{Q}_\alpha = \int_{t=0^+}^{t=T} \exp \left[-\frac{(T-t)}{\tau_\alpha} \right] \beta_\alpha \dot{\mathbf{S}}_\alpha dt \quad (2.10)$$

The parameters τ_α and β_α denote the relaxation time and the free-energy coefficient, respectively, which characterize the viscoelastic behavior, as described by the generalized Maxwell model composed of α parallel elements [59].

The material elasticity tensor can be formulated in a decoupled manner as [59]:

$$\mathbb{C} = \mathbb{C}_J^\infty + \mathbb{C}_m^\infty + \mathbb{C}_f^\infty + \sum_{\alpha=1}^m (\mathbb{C}_m^\alpha + \mathbb{C}_f^\alpha) \quad (2.11)$$

Here, $\mathbb{C}_J^\infty$, $\mathbb{C}_m^\infty$ and $\mathbb{C}_f^\infty$ correspond to the volumetric, matrix and fiber contributions, respectively [59]. The contributions of the matrix and fibers to the viscous component are characterized by [59]:

$$\mathbb{C}_{m,f}^\alpha = 2 \frac{\partial \mathbf{Q}_{m,f}^\alpha}{\partial \mathbf{C}} \quad (2.12)$$

2.6.8 Damage model

To evaluate the extent of tissue damage under mechanical loading, damage models are commonly employed [61]. These models quantify tissue degradation by associating damage with strain levels that surpass the tissue's inherent maximum capacity [61]. When strain exceeds these thresholds, the tissue is considered damaged and the model captures this progression through damage variables [61].

Within a constitutive damage model, the strain energy function is defined as follows [61]:

$$U = (1 - D_m)U_I^0 + (1 - D_f)U_f^0 + U_J \quad (2.13)$$

where D_m and D_f represent the damage variables for the matrix and fiber components, respectively [61]. These variables are bounded between 0, representing an undamaged state, and 1,

indicating the maximum amount of damage [61]. U_I^0 and U_f^0 describe the effective strain energy terms corresponding to an idealized undamaged material, while $(1 - D_m)$ and $(1 - D_f)$ are known as the reduction factors [61], as outlined by Simo (1987) [62].

The function U_J represents the energy term responsible for enforcing the quasi-incompressibility condition [61]. This ensures that the deformation remains nearly volume-preserving, as required for the accurate representation of the tissue's mechanical behavior [61].

The relationship between the damage variables and the material's properties is governed by the following polynomial equation [61], established by Peña et al. (2008) [63]:

$$D_k = \begin{cases} 0 & \text{if } \Xi_{kt} < \Xi_{\min}^0 \\ \xi^2 [1 - \beta_k(\xi^2 - 1)] & \text{if } \Xi_{\min}^0 \leq \Xi_{kt} \leq \Xi_{\max}^0 \\ 1 & \text{if } \Xi_{kt} > \Xi_{\max}^0 \end{cases} \quad (2.14)$$

In this context, the variable $\xi = \frac{(\Xi_{kt} - \Xi_{\min}^0)}{(\Xi_{\max}^0 - \Xi_{\min}^0)}$ is dimensionless, while $\Xi_{\min}^0$ and $\Xi_{\max}^0$ correspond to the variables associated with the strain energy at the onset of damage and at complete failure, respectively [61, 63]. Additionally, $\beta_k \in [-1, 1]$, $k = [m, f]$ represent the exponential parameters [61, 63]. This specific formulation characterizes an anisotropic damage mechanism, reflecting the material's inevitable failure [61]. Moreover, it enables the regulation of both the onset of damage and the rate at which it evolves as energy accumulates [61].

2.6.8.1 Application to pelvic floor damage during childbirth

A practical application of this damage model is in assessing the mechanical effects on the pelvic floor muscles during vaginal delivery [61]. Finite element simulations reveal how tissue damage evolves in response to the vertical displacement of the fetal head [61]. As shown in Figure 2.19, there is a progressive increase in total fiber damage in the pelvic floor muscles as the fetus descends through the birth canal [61].

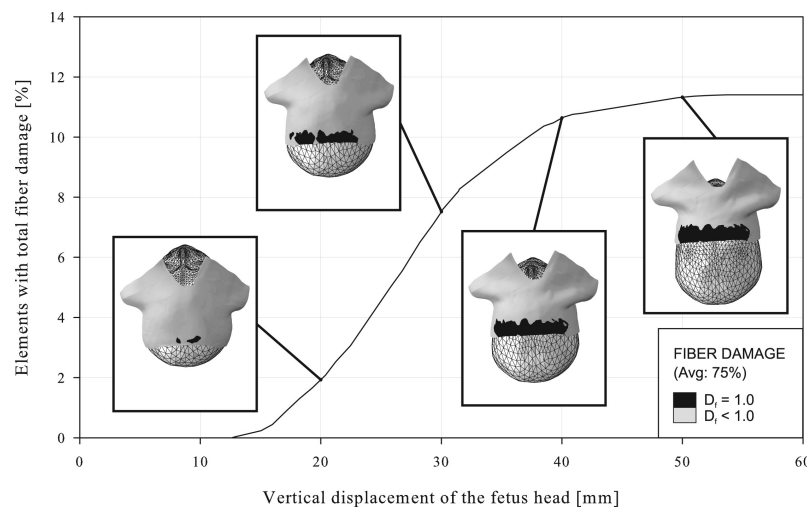


Figure 2.19: Evolution of fiber damage in finite elements in relation to fetal descent. Contour plots of total fiber damage are shown for selected stages along the simulation curve, corresponding to vertical displacements of the fetal head at 20 mm, 30 mm, 40 mm and 50 mm. Authorized, available at [61].

The findings reported by Oliveira et al. (2016) [61] demonstrate that vaginal delivery can cause substantial injury to the pelvic floor tissues, particularly during the phase of maximum fetal head extension. This highlights the clinical relevance of biomechanical modeling in understanding and potentially mitigating childbirth-related injuries [61].

2.7 Genetic algorithm

A genetic algorithm consists in an advanced computational method used to identify approximate solutions for optimization and search problems [64]. It belongs to the broader category of evolutionary algorithms and draws inspiration from biological evolution, incorporating mechanisms such as inheritance, mutation and natural selection, as proposed by Darwinian theory [64]. Through these biologically inspired processes, genetic algorithms aim to determine the most suitable model or solution that effectively captures or predicts patterns within data [64]. They are particularly well-suited for tasks involving regression-based prediction, where discovering optimal relationships between variables is essential [64].

The concept of genetic algorithms was initially introduced by Holland in 1975 [64]. In this approach, each potential solution to a problem is encoded as a sequence of genes, commonly referred to as a chromosome [64]. A collection of these chromosomes constitutes a population and at any given point in time, this population is identified as a generation [64].

Indeed, in the context of genetic algorithms, several foundational terms are used to describe the structural components and processes involved in the optimization cycle [64]. A concise glossary of key terminology commonly applied in genetic algorithm methodologies is presented in Table 2.4 [64].

Table 2.4: Glossary of basic terms in genetic algorithms [64].

Term	Definition
Gene	Represents a property or variable within a solution.
Chromosome (or organ)	A sequence of genes; a complete solution composed of multiple variables.
Population	A collection of chromosomes used in a single generation.
Parents	Selected chromosomes that contribute genetic material to generate the next generation.
Reproduction	The process of generating new chromosomes by applying crossover and mutation operations to parent chromosomes.

Once the objective function is established, an initial population is randomly generated [64]. Each chromosome within this population is evaluated and assigned a fitness score based on its performance relative to the objective [64]. If the desired criteria are not met, the algorithm proceeds through iterative generational cycles to enhance the quality of solutions [64]. In each cycle, the genetic algorithm employs three primary operators to produce a new generation [64]:

- 1. Selection operators:** Responsible for choosing individuals from the current population to serve as parents for the next generation, based on their fitness.
- 2. Crossover (recombination) operators:** Combine genetic information from selected parents to generate offspring.
- 3. Mutation operators:** Introduce random alterations to the genetic makeup of offspring to maintain genetic diversity and explore new areas of the solution space.

In order to initiate the process, chromosomes are constructed from the variables present in the problem domain [64]. The algorithm then generates an initial, diverse population of chromosomes to ensure broad exploration of the solution space [64]. Each chromosome is evaluated using a fitness function and those with higher performance are more likely to be selected for survival and reproduction, whereas less fit chromosomes are eliminated over successive iterations [64].

Subsequently, a new generation is formed through processes such as crossover and mutation, where selected parent chromosomes are recombined and altered to produce offspring [64]. This iterative procedure yields successive generations that increasingly approximate optimal solutions [64].

The algorithm continues this cycle until a predefined termination criterion is met. These termination conditions typically include [64]:

- Reaching a specified number of generations;
- Identifying an optimal or sufficiently good solution;
- Observing convergence or stagnation in population diversity;

- Manual termination based on user or expert inspection.

Owing to the inherent stochastic characteristics of genetic algorithms, the final solutions obtained across multiple executions of the algorithm may vary slightly [64]. Although these outcomes are generally similar and lie within a close range, the observed differences arise from the internal variability of the algorithm's mechanisms, such as random selection, crossover and mutation processes [64]. This variability is an expected aspect of the algorithm's probabilistic nature and contributes to its exploratory capacity in complex solution spaces [64].

2.7.1 Micro-genetic algorithm developed by Vila Pouca et al. for material parameter calibration

A micro-genetic algorithm was implemented by Vila Pouca et al. (2018) [58] using Matlab, integrated with Python and Abaqus, to calibrate the parameters of the material model. This algorithm begins by generating a random population composed of five individuals, where each individual represents a distinct set of material parameters [65]. The fitness of each individual is evaluated through a predefined objective function, with the fittest individual being preserved and carried over to the subsequent generation [65]. The remaining individuals undergo a tournament selection process, through which the selected individuals - deemed the "parents" - participate in a crossover mechanism to produce the next generation [65].

Following this, the algorithm assesses the diversity of the population [65]. If a lack of diversity is detected, the population is reinitialized randomly, while still retaining the fittest individual [65]. This iterative procedure continues until the algorithm converges [65]. In this study, the stopping criterion was defined as achieving a normalized root mean squared error of less than 0.03 between the experimental and numerical results [65]. The objective (evaluation) function employed is expressed by the following equation [65]:

$$\mathbf{F}_e = \sqrt{\sum_{i=1}^n \frac{(P_i^e - P_i^\Psi)^2}{n-2}} \quad (2.15)$$

Here, n denotes the number of experimental data points considered [65]. The terms P_i^e and P_i^Ψ correspond to the engineering stresses in the direction of the applied stretch, obtained from the experimental tests and the finite element simulations, respectively [65]. To compute P_i^Ψ , a compressive simulation was configured in Abaqus to replicate the mechanical behavior observed in the experimental tests [65]. This simulation is executed for each individual using its specific set of material parameters prior to fitness evaluation [65].

Chapter 3

Methodology

This chapter presents the methodological framework adopted in this study, encompassing the main computational tools employed, as well as the experimental and numerical approaches applied to the biomechanical modeling and simulation of the uterine environment. The characterization of the amniotic sac through inflation mechanical tests is described, along with the detailed finite element modeling strategy, including mesh definition, element types, interaction properties, loading conditions and boundary specifications, among other relevant aspects. A convergence analysis of the amniotic sac is also performed, aiming to achieve a balance between mesh quality and computational cost.

Furthermore, an automatic calibration process of the hyperelastic material parameters assigned to the amniotic sac is described, using a micro-genetic algorithm that minimizes the discrepancy between the selected experimental curve and the numerical response simulated in Abaqus. The numerical simulations carried out are also outlined, including a description of the uterine environment model adopted to study the biomechanical behavior of the fetal membrane under different pregnancy states. Finally, the procedures used to extract biomechanical data from the simulation results are presented, employing path construction in Abaqus to enable stress analysis across three anatomical regions of the fetal membrane.

3.1 Software tools

The numerical simulations carried out in this work are based on the finite element method (FEM), implemented through the use of advanced commercial software, such as Abaqus. This software suite offers a comprehensive environment for the modeling, management, monitoring and visualization of complex engineering problems. While Abaqus includes a broad library of built-in material models, particularly for conventional structural materials, it lacks many of the constitutive models required to accurately represent the complex mechanical behavior of biological tissues.

Consequently, to simulate the specific biomechanical response of these tissues, it becomes necessary to define a user-specific material model. This is achieved by implementing a custom constitutive formulation that describes the stress-strain relationship of the material through a user subroutine written in the Fortran programming language. In Abaqus, such user-defined material behavior is incorporated via the UMAT subroutine, which allows full control over the material's constitutive response and is supported by extensive documentation within the Abaqus environment.

In this sense, this approach enables the incorporation of experimentally calibrated models that account for the specific microstructural and physiological features of biological tissues, including fiber orientation, layer-specific properties and damage mechanisms. In the context of fetal membrane modeling, this allows for the integration of hyperelastic and damage formulations that are not otherwise supported by the native capabilities of Abaqus. As such, the adoption of user subroutines is essential for advancing the biomechanical understanding of fetal membrane under distinct pregnancy-related scenarios.

Ultimately, the micro-genetic algorithm used in the current project and proposed by Vila Pouca et al. (2018) [58] was implemented in Matlab with Python integration. This computational framework, in conjunction with Abaqus, enables the calibration of hyperelastic material model parameters.

3.2 Selection of the undamaged experimental curve for parameter optimization

In order to optimize the parameters of the constitutive model, it was first necessary to identify experimental curves that accurately reflect the mechanical behavior of the amniotic sac in the absence of tissue damage.

For this purpose, experimental data obtained by Samimi et al. (2023) [43] were used, derived from inflation tests in which pressure (kPa) versus apex displacement (mm) curves exhibiting damage were recorded.

The undamaged curves were constructed in Microsoft Excel using various polynomial approximations fitted to the damaged experimental curves. Several polynomial degrees (ranging from 2 to 6) were tested, with the final choice based on three main criteria:

- The generation of a continuous and smooth curve, devoid of discontinuities or abrupt variations, which aligns with the expected mechanical response of an undamaged material;
- The use of the lowest possible polynomial degree, to ensure mathematical simplicity and to reduce the risk of overfitting, without compromising the quality of the fit;
- The accurate representation of the initial behavior of the curve, characterized by a gradual increase in pressure with respect to apex displacement. This ensures that the constitutive

model can adequately capture the mechanical response of the tissue during the early stages of inflation, a phase that is particularly sensitive and critical for proper model calibration.

The coefficient of determination, R^2 , was employed as a primary metric to assess the goodness of fit. It is important to note that, despite the precautions taken during the curve construction process, a certain degree of error is inherently associated with curve fitting.

The selection of the most appropriate curve was carried out through direct observation, leading to the identification of a candidate curve that met the previously established criteria. Therefore, this curve is believed to most accurately represent the undamaged mechanical behavior.

It is critical to point out that, despite multiple attempts, constructing undamaged curves proved to be a challenging task, particularly when using lower-degree polynomials, which tended to introduce discontinuities or unrealistic behaviors.

The curve selected as the most representative for use in the parameter optimization of the constitutive model, along with its respective polynomial equation, is depicted in Figure 3.1.

After selecting the undamaged experimental curve, the corresponding pairs of x and y coordinates, representing apex displacement and pressure, respectively, were extracted. These data points will serve as reference inputs for the optimization of the constitutive model parameters.

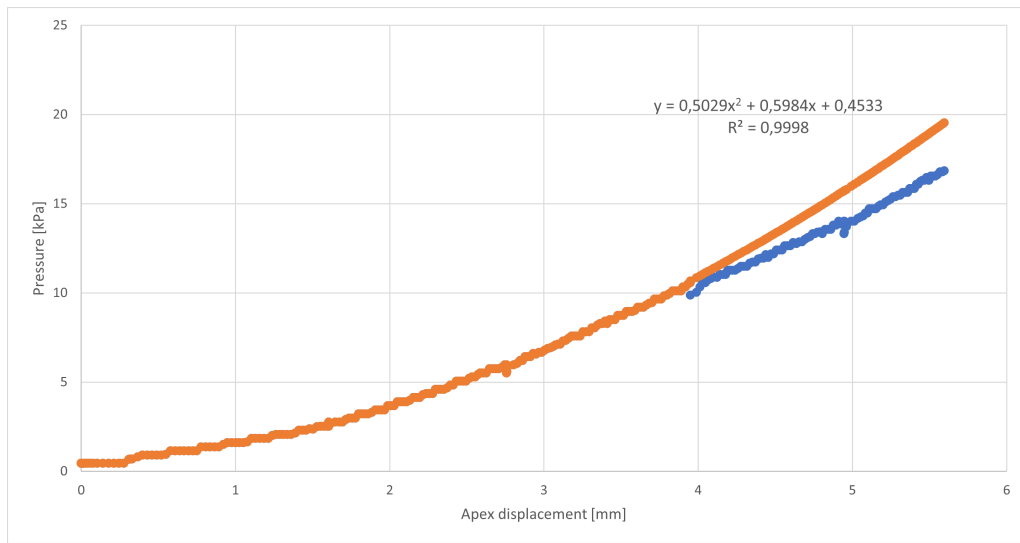


Figure 3.1: Graphical representation comparing the experimental curve exhibiting damage (blue) with the polynomial approximation representing the undamaged mechanical response (orange). The undamaged curve was obtained through a second-degree polynomial fit ($y = 0.5029x^2 + 0.5984x + 0.4533$) with a coefficient of determination $R^2 = 0.9998$.

3.3 Inflation mechanical test

After determining the best experimental curve without damage, a finite element model was developed in Abaqus software for the inflation mechanical test, including the amniotic sac and a clamping ring. The clamping ring holds the edges of the amniotic sac sample, which is subjected to controlled pressure on one of its faces, causing deformation outward in a dome shape.

The clamping ring was modeled as a discrete rigid body, with an internal diameter of 30 mm and an external diameter of 60 mm. Additionally, a fillet edge was implemented to prevent cutting or tearing of the membrane during the mechanical testing. The amniotic sac, with a diameter of 60 mm, was considered as a single layer, without distinguishing between the individual layers of the fetal membrane, namely the amnion, chorion and decidua. Therefore, its thickness was defined as the sum of the average initial thicknesses of eighteen peri-placental membrane samples obtained from cesarean sections, as reported by Samimi et al. (2023) [43]. These same samples were previously used to characterize the individual layer thicknesses in the multilayer fetal membrane model developed by Fidalgo et al. (2024a) [2], according to the values presented in Table 2.3. Based on this approach, the final thickness assigned to the amniotic sac in the model was 0.59 mm.

3.3.1 Amniotic sac mechanical properties

Regarding the mechanical properties assigned to the amniotic sac for the purpose of mesh sensitivity analysis, it was modeled with a Young's modulus of 4 MPa. This value enables the simulation of a mechanical response with sufficient resistance to the deformation induced by inflation. Although the literature reports a range of rupture values due to the application of different experimental methodologies, commonly accepted failure criteria for the amnion tend to converge around 4 MPa [8]. This convergence further supports the choice of this value.

Furthermore, a Poisson's ratio of 0.4 was also adopted, assuming a nearly incompressible behavior, which is characteristic of linear, elastic and isotropic biological tissues [8]. It is also important to note that the mentioned parameters were selected based on the values reported by Verbruggen et al. (2017) [8], in order to ensure consistency with previous studies and to maintain biomechanical realism in the simulation.

3.3.2 Contact interactions, loads and boundary conditions

Concerning the contact conditions, the interaction between the amniotic sac and the clamping ring was modeled using a surface to surface contact approach with tangential behavior. In order to characterize frictional resistance, the penalty method was employed, incorporating a high friction coefficient (0.9) to accurately represent the resistance to relative sliding between the contacting surfaces.

With respect to the loading conditions implemented in the numerical model, a ramping inflation pressure of 20 kPa was applied across the inferior surface of the amniotic sac, in the positive direction of the y-axis.

In terms of boundary conditions, the clamping ring was rigidly constrained and fixed in all directions through the definition of a reference point, whose inclusion is mandatory in the Abaqus software for bodies defined as discrete rigid bodies. This reference point facilitates the application of boundary conditions to the ring, ensuring its behavior as a non-deformable entity throughout the entire simulation.

The assembly, which consists of the amniotic sac and the clamping ring, is presented in Figure 3.2. Also depicted are the loads applied in the positive direction of the y-axis, acting on the lower surface of the amniotic sac, along with the boundary condition imposed at the reference point, which ensures that the clamping ring remains fully restrained in all degrees of freedom.

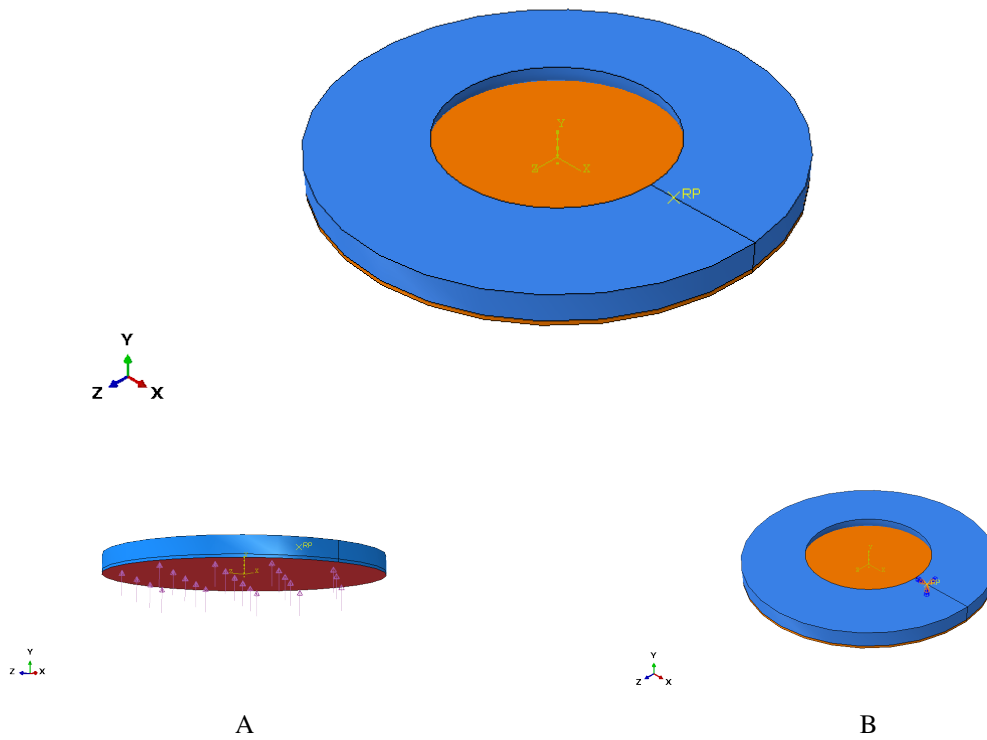


Figure 3.2: Representation of the inflation mechanical test setup, comprising the clamping ring (blue) and the amniotic sac (orange). Also visible are (A) the loads applied in the positive direction of the y-axis and (B) the boundary condition imposed at the reference point located on the clamping ring.

3.3.3 Element type and mesh definition

In the finite element model developed using the Abaqus software, the amniotic sac was discretized using a three-dimensional element with a hybrid formulation, selected to more accurately represent the nearly incompressible mechanical behavior characteristic of biological tissues. The element type adopted was C3D8H - a linear hexahedral element with 8 nodes, hybrid formulation and constant pressure.

The choice of a hybrid formulation is particularly appropriate given the nearly incompressible nature of the current amniotic sac model, as indicated by the high Poisson's ratio (0.4). Under such conditions, conventional finite elements are susceptible to numerical issues such as volumetric locking. Hybrid elements overcome this limitation by treating pressure as an independent variable, thereby enhancing numerical stability and accuracy under incompressible or quasi-incompressible conditions.

The decision to use hexahedral elements was further motivated by their superior numerical performance, namely enhanced accuracy and computational efficiency, when modeling regular geometries, which is a relevant consideration given the circular shape of the amniotic sac within the limits defined by the clamping ring.

The clamping ring was modeled as a discrete rigid body, as required by Abaqus for entities that are assumed to undergo no deformation during the simulation. Although a discrete rigid body does not experience strain or displacement, Abaqus still requires the assignment of a mesh to its geometry.

Accordingly, the clamping ring was defined using the R3D4 element type, which represents a four-node, three-dimensional bilinear quadrilateral rigid element. In this case, since the mesh quality has no impact on the mechanical behavior of the ring, a default element size of 4.2 mm was employed without the need for further refinement. This approach satisfies software requirements while avoiding unnecessary increases in computational cost. The discretization adopted for the clamping ring is illustrated in Figure 3.3, which shows the mesh configuration used in the present study.

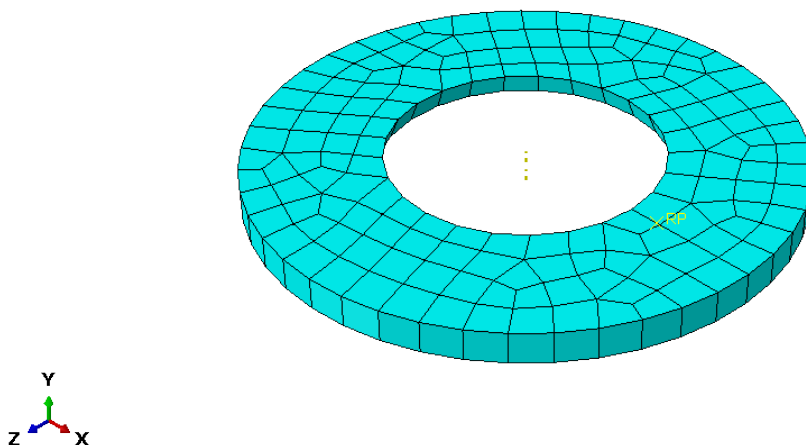


Figure 3.3: Finite element mesh of the clamping ring using R3D4 elements with a default element size of 4.2 mm. The mesh satisfies the software constraints for rigid body definitions without impacting the mechanical behavior of the system.

As a result, a mesh convergence analysis was not performed for the clamping ring, since its deformation and internal stress are not evaluated in the simulation. The convergence study was conducted exclusively for the amniotic sac, where mesh sensitivity directly affects the accuracy of the numerical predictions and the fidelity of the model's mechanical response.

3.3.4 Mesh convergence analysis

Mesh convergence analysis, also referred to as discretization sensitivity analysis, is a fundamental step in verifying the reliability and accuracy of results obtained through finite element simulations.

This analysis aims to ensure that the numerical results are not overly dependent on the mesh size used, thereby confirming that the model faithfully represents the physical behavior of the system under study.

In finite element analysis, the physical domain of the problem is discretized into finite elements. The accuracy of the results largely depends on the density and quality of the mesh. A mesh that is too coarse may lead to significant errors, whereas an excessively refined mesh, although more accurate, can substantially increase computation time and demand greater computational resources.

In this investigation, the mesh convergence analysis of the amniotic sac involved performing multiple simulations using meshes with varying global element sizes and monitoring the evolution of the results. This was done by analyzing and comparing the maximum principal stress across the different simulations and by plotting a graph of the maximum principal stress at the apex (in MPa) against the number of finite elements.

Convergence is considered achieved when the results stabilize, i.e., when the differences between successive mesh refinements become negligible. At this point, the mesh is deemed sufficiently accurate to represent the problem with an acceptable level of precision.

The data corresponding to each simulation, including the global element size, the total number of elements and nodes, the total simulation runtime (in minutes) - which is available in the .dat file of the respective simulation - and the value of the maximum principal stress recorded at the apex of the geometry, are presented in Table 3.1. The apex refers to the highest or most protruding point of the amniotic sac inflation test, which is critical for stress analysis as it typically experiences the maximum deformation and loading.

Table 3.1: Mesh convergence data detailing the relationship between element size, mesh density, simulation time and maximum principal stress at the apex.

Element Size (mm)	No. of Elements	No. of Nodes	Max Principal Stress (MPa)	Runtime (min)
0.9	4320	8852	0.520634	17.85
1.0	3422	7034	0.520623	13.77
1.1	2876	5926	0.522094	11.1
1.2	2420	5000	0.524576	10.97
1.3	2058	4264	0.525468	7.50
1.4	1786	3708	0.526179	5.90
1.5	1558	3244	0.527142	4.97
1.6	1282	2684	0.528283	4.43
1.7	1228	2570	0.528225	4.40
1.8	1038	2182	0.531391	3.72
1.9	988	2076	0.529856	3.28
2.0	880	1856	0.530663	2.38

The relationship between the maximum principal stress (MPa) and the number of finite elements is illustrated in Figure 3.4. This representation constitutes a critical component of the mesh

sensitivity analysis, as it enables the evaluation of the influence of discretization on the numerical results.

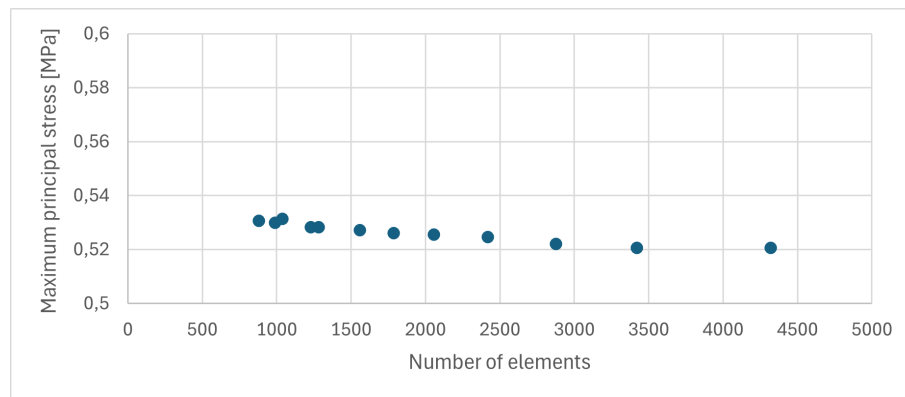


Figure 3.4: Mesh convergence analysis: plot of maximum principal stress at the apex of the amniotic sac versus the number of finite elements.

As visible in Figure 3.4, the graph depicts how the maximum principal stress values progressively stabilize as the mesh is refined, indicating convergence of the solution. In particular, convergence is observed when the element size reaches 1.1 mm, as further mesh refinements (i.e., 1.0 mm and 0.9 mm) result in negligible variations in stress values. Given this stabilization, along with the significantly higher computational cost associated with finer meshes, the element size of 1.1 mm was selected for future simulations, as it provides an optimal balance between numerical accuracy and computational efficiency.

The finite element mesh of the amniotic sac applied in this study, corresponding to an element size of 1.1 mm, is presented in Figure 3.5. This mesh configuration consists of 2876 elements and 5926 nodes and was selected based on the results of the mesh convergence analysis.

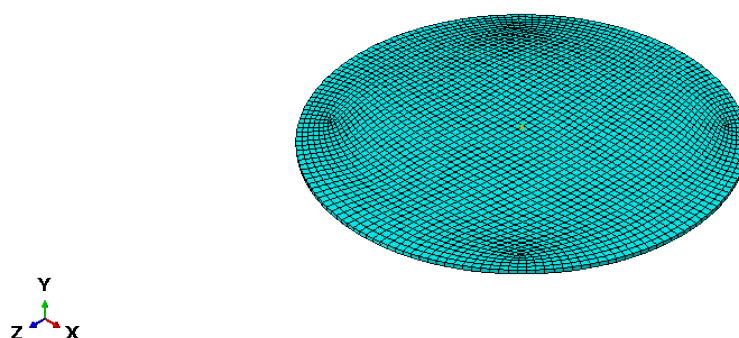


Figure 3.5: Finite element mesh of the amniotic sac corresponding to an element size of 1.1 mm, composed of 2876 elements and 5926 nodes.

In summary, mesh convergence analysis is a crucial step in the development of robust and reliable numerical models. In Abaqus, as in any finite element software, careful execution of

this analysis ensures that the results obtained are both physically and numerically valid, thereby reinforcing the credibility of the study and the conclusions drawn from it.

3.3.5 Assessment of mesh layering across the thickness direction

The impact of using different through-thickness discretizations was evaluated by considering 2 and 3 elements along the vertical direction, corresponding, respectively, to 2 and 3 finite element layers across the thickness of the amniotic sac.

The finite element meshes generated with element sizes of 1.0 mm and 1.1 mm, both corresponding to 2 elements through the thickness, are displayed in Figure 3.6. The mesh with an element size of 1.0 mm consisted of 6844 finite elements and 10551 nodes. The total simulation runtime was around 15.93 minutes and the maximum principal stress recorded at the apex of the geometry was 0.551772 MPa.

In contrast, the configuration considering an element size of 1.1 mm resulted in a mesh composed of 5752 finite elements and 8889 nodes. The total simulation runtime was approximately 14.23 minutes, with the maximum principal stress recorded at the apex of the geometry reaching 0.552343 MPa.

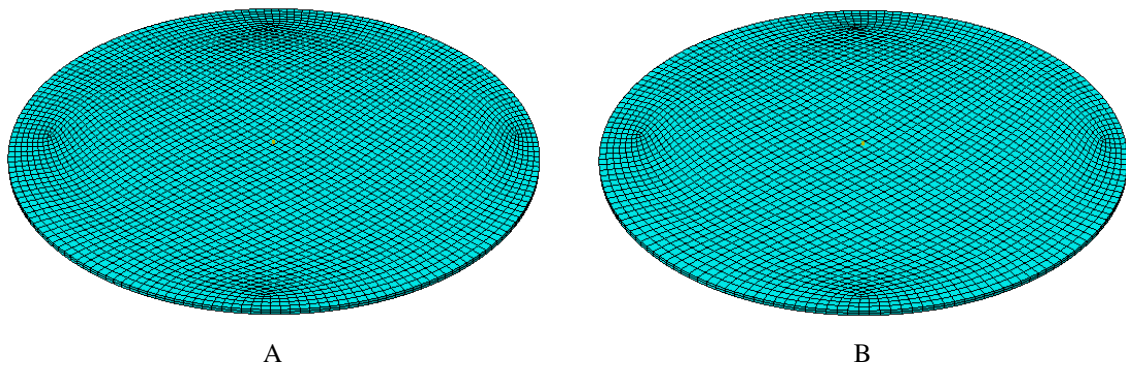


Figure 3.6: Finite element meshes with 2 vertical layers across the thickness direction of the amniotic sac, using element sizes of (A) 1.0 mm and (B) 1.1 mm.

In turn, the finite element meshes with 3 layers along the thickness direction, also maintaining element sizes of 1.0 mm and 1.1 mm, are illustrated in Figure 3.7. The configuration with an element size of 1.0 mm resulted in a total of 10266 elements and 14068 nodes. The total simulation time was approximately 24.7 minutes and the maximum principal stress recorded at the apex of the geometry was 0.563466 MPa.

The mesh generated with an element size of 1.1 mm consisted of 8628 elements and 11852 nodes. In this case, the total simulation runtime was close to 18.8 minutes and the maximum principal stress at the apex was found to be 0.561796 MPa.

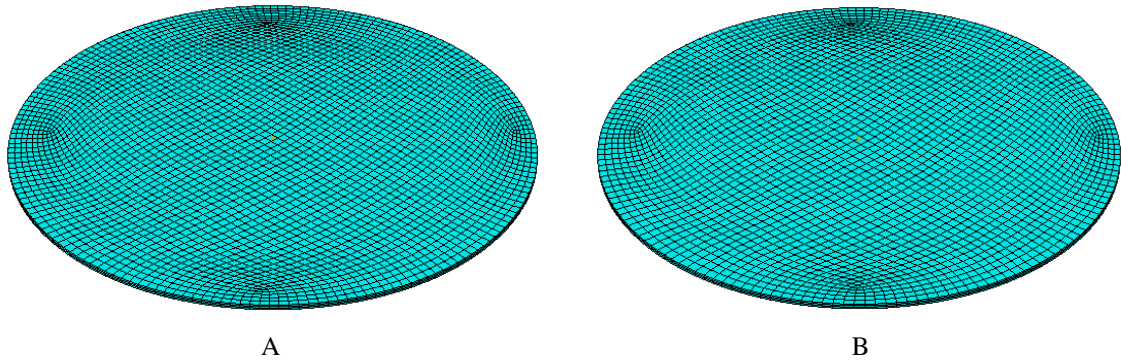


Figure 3.7: Finite element meshes with 3 layers across the thickness direction of the amniotic sac, for two element sizes: (A) 1.0 mm and (B) 1.1 mm.

Although this refined discretization provides a more detailed representation of the geometry, it significantly increases the computational time and resource demands. For this reason, only an exploratory simulation was carried out and a systematic mesh sensitivity analysis in the thickness direction was not pursued. The variations in maximum principal stress observed across the simulations, considering one, two and three elements through the thickness, as well as the two different element sizes, are thoroughly analyzed and discussed in Chapter 4.

3.4 Automatic optimization of hyperelastic material parameters for the amniotic sac

In this study, a previously implemented micro-genetic algorithm, originally developed by Vila Pouca et al. (2018) [58], was employed to perform material parameter calibration for a user-defined constitutive model. The code, written in Matlab and designed to interface with Abaqus through Python scripts and Fortran user subroutines, executes an optimization process that minimizes the normalized least-squares error between experimental and numerically simulated mechanical responses (objective function). The numerical simulations automatically performed by the algorithm during the calibration process are based on a mechanical inflation test. For further information on the general operation of the micro-genetic algorithm, refer to subsection 2.7.1 in the literature review chapter.

The algorithm is based on the micro-genetic approach, which differs from classical genetic algorithms by operating on small population sizes and incorporating rapid convergence and restart strategies. This makes the algorithm particularly well-suited for computationally expensive evaluations, such as finite element simulations involving user-defined material models.

The constitutive model applied, in this case, to the amniotic sac incorporates the HGO formulation to accurately represent the anisotropic hyperelastic behavior characteristic of soft biological tissues. To account for time-dependent mechanical responses, the model is further extended with a generalized Maxwell framework, as described by Holzapfel and Gasser, which captures

the viscoelastic behavior. The parameters of the resulting anisotropic viscohyperelastic model include: c_{10} , which characterizes the isotropic ground matrix contribution; k_{11} and k_{12} , which define the anisotropic fiber reinforcement; and the viscoelastic parameters β_α and τ_α , with $\alpha = m, f$, referring to the matrix and fiber contributions, respectively. In the present study, however, the viscoelastic behavior was not considered. Accordingly, the parameters β_α and τ_α were set to zero.

3.4.1 Main script

The `main.m` script serves as the central control file for the optimization process. In this script, several key parameters were initialized to control the behavior and convergence criteria of the algorithm. The number of variables to be optimized was set to $nvar = 3$, corresponding to the three material parameters of the HGO model: c_{10} , k_{11} and k_{12} . The population size for each generation was defined as $npop = 5$ and the desired numerical precision for each parameter was specified by $p = [6; 6; 6]$, indicating retention of six decimal places.

The optimization search space was constrained by parameter-specific lower and upper bounds: $dom1 = [0.001, 2.0]$ for c_{10} , $dom2 = [0.001, 1.0]$ for k_{11} and, finally, $dom3 = [0.01, 1.0]$ for k_{12} . These limits were concatenated into a single matrix, dom , to define the full parameter domain. The initial population was randomly generated by setting $h_0 = 'yes'$.

Moreover, the micro-genetic algorithm was executed only once ($nloops = 1$), with a maximum of 200 generations per run ($ger_max = 200$). To ensure adequate convergence, the algorithm was allowed up to 3000 consecutive iterations without significant improvement ($conv_iter = 3000$) and a tight convergence tolerance of 1×10^{-10} was imposed to enhance precision in the final result. To mitigate premature convergence, a minimum of 40 restarts ($nrestart = 40$) was allowed. Elitism was enabled ($elitismo = 'y'$) to retain the best-performing individual in each generation and the objective of the optimization was set to minimization ($obj = 'min'$).

3.4.2 Fibers

A family with randomly oriented fibers (conferring anisotropic characteristics to the material) was considered. All fiber direction vectors were normalized to unit length. A total of 2876 fiber vectors were assigned to the fiber family - matching the number of elements in the mesh used for the analysis - to ensure consistency between the fiber distribution and the finite element discretization.

3.4.3 Material definition

Initially, the material properties assigned to the amniotic sac were defined in the `parameters.inp` file. This file is automatically updated by the `subs_param.py` script, which replaces placeholder values with specific parameter values read from a separate `par.txt` file, as the optimization process progresses.

In the main Abaqus input file, the amniotic sac is associated with a user-defined material, labeled $m1$, through the `*USER MATERIAL` command. This material definition includes 25

constants that describe the isotropic, anisotropic and viscoelastic behavior of both the matrix and the embedded fiber components.

The selected values for the hyperelastic strain energy coefficients utilized in the constitutive model are summarized in Table 3.2. The parameters subject to optimization were initially set to 1.0, corresponding to the parameters that need to be changed throughout the calibration process, ensuring that the *subs_param.py* script can replace them during each iteration of the process.

The remaining fiber parameters, namely k_{21} and k_{22} , as well as the viscoelastic parameters, were assigned negligible values, meaning their contribution to the mechanical response is minimal and intentionally disregarded. In particular, since the viscoelastic behavior was not considered in this study, as previously mentioned, the corresponding parameters were explicitly set to zero.

Furthermore, to maintain consistency with the assumption of material incompressibility, the bulk modulus parameter D_1 , which appears in the volumetric part of the strain energy function, was fixed at $D_1 = 1 \times 10^{-5} \text{ MPa}^{-1}$.

Table 3.2: Initial material parameters used for the amniotic sac model (consistent units). Parameters subject to optimization are initialized at 1.0; k_{12} is a dimensionless parameter.

Material Parameters			
$D_1 = 1 \times 10^{-5} \text{ MPa}^{-1}$	$c_{10} = 1.0 \text{ MPa}$	$k_{11} = 1.0 \text{ MPa}$	$k_{12} = 1.0$

3.4.4 Numerical curve

To extract the numerical curve corresponding to the pressure-apex displacement response of the amniotic sac, the post-processing functionalities of the Abaqus/CAE Viewer environment were employed. A custom Python script, *get_rpt.py*, was developed to automate the retrieval and export of displacement data from the simulation output database (ODB) file.

It is important to note that the *get_rpt.py* script was derived from the *abaqus.rpy* replay file, which is automatically generated by Abaqus after executing the input file and subsequently launching the Viewer module. This replay file records all GUI actions that are performed (like opening an ODB, creating plots, extracting data, etc.) in Python syntax.

The *get_rpt.py* script begins by identifying the ODB file associated with the numerical simulation, by concatenating the current working directory with the file name. It then opens this ODB file in the Abaqus Viewer and configures the visualization parameters, including the activation of contour plots on the deformed configuration. At this stage, it is essential to ensure that the results are displayed using an averaging threshold set to 100%, in order to obtain consistent nodal values across the domain.

The main objective of this script is to extract the vertical displacement (U2) of the central point of the amniotic sac, referred to as the apex. For this purpose, the node set labeled *PART-1-1.APEX* is specified and the displacement in the U2 direction is retrieved using the *xyPlot.xyDataListFromField* function. The extracted data is then plotted and processed as an XY curve, which initially represents an apex displacement vs time relationship.

To convert the apex displacement-time data into the desired pressure-apex displacement format, the script first swaps the axes and then multiplies the time values by a factor of 20. This scaling factor corresponds to the internal pressure applied in the simulation (20 kPa), thereby associating each vertical displacement value with the constant pressure magnitude of the inflation test. The resulting curve, which approximates the pressure-displacement response at the apex node, is stored as an XY plot in the session.

Finally, the numerical data is written to a text file named `report.rpt` using the `session.writeXYReport` function. This file contains the coordinates of the numerical curve and can be directly compared with the experimental data to optimize material parameters accordingly.

The graph representing the pressure-apex displacement response obtained from the initial `report.rpt` file, generated through the Abaqus/CAE Viewer, is presented in Figure 3.8. This curve corresponds to the numerical simulation performed using the initial material parameter values summarized in Table 3.2. The initial numerical curve is composed of 101 discrete coordinate points.

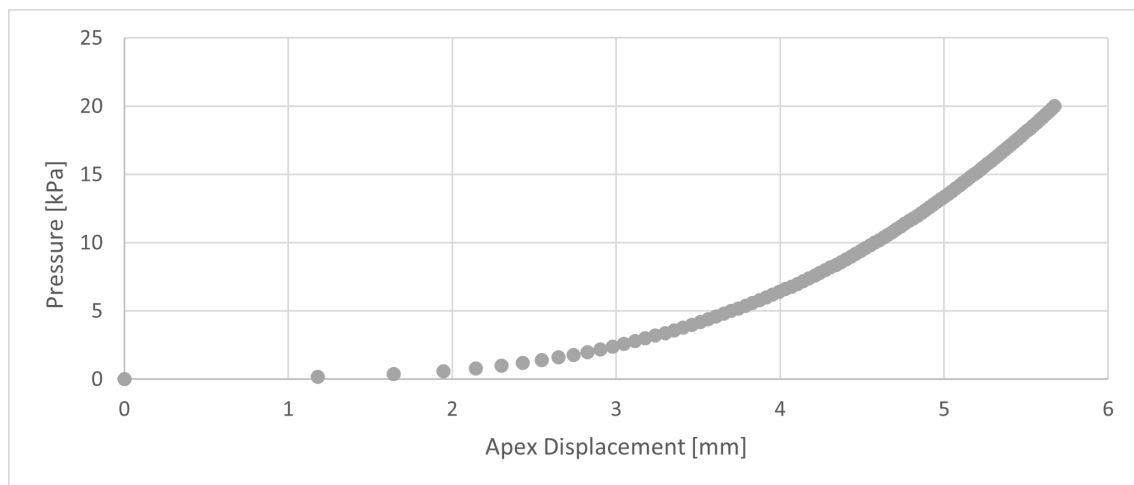


Figure 3.8: Numerical pressure-apex displacement curve obtained from Abaqus simulation using the initial hyperelastic parameter values. The curve consists of 101 coordinate points.

3.4.5 Experimental curve

To enable the optimization of the material parameters, it is essential that the number of points in the numerical curve matches that of the experimental curve. Therefore, the experimental pressure-apex displacement data were processed to obtain 101 coordinate points, uniformly spaced in terms of displacement. These data were derived from the experimental curve shown in Figure 3.1, after removing their initial segments (corresponding to non-zero initial pressure values) and translating each curve to begin at the origin, (0,0).

Subsequently, a linear interpolation technique was applied to generate the uniformly spaced points. The process, implemented using a Python script, presented in Appendix A.1, involved reading the original data, generating 101 equally spaced displacement values within the range of

the dataset, and linearly interpolating the corresponding pressure values. The interpolated dataset was then saved to a new file, named *experimental.txt*, and plotted for comparison against the original data, as shown in Figure 3.9.

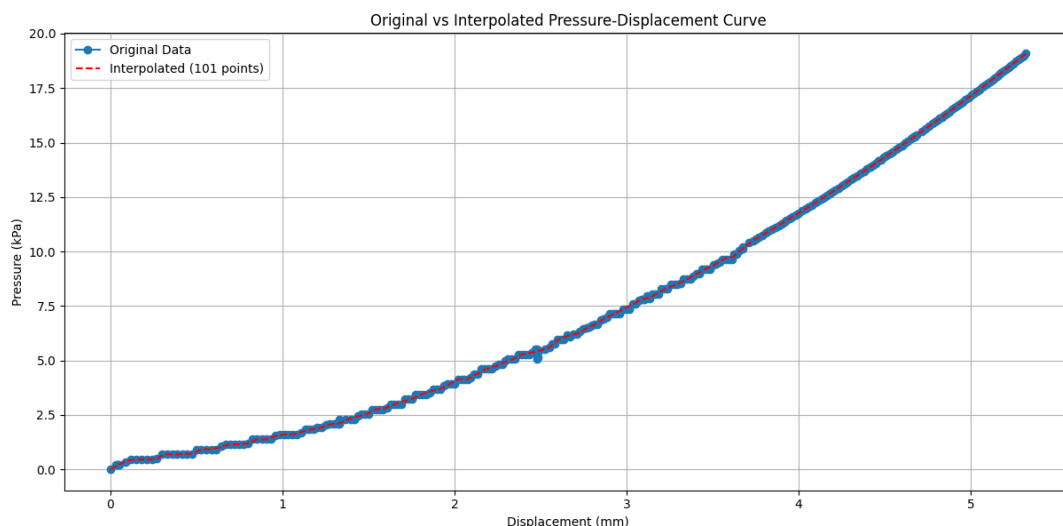


Figure 3.9: Comparison between the original and interpolated experimental pressure-displacement curves. The interpolated curve contains 101 uniformly spaced points used for parameter optimization.

This preprocessing step ensures that the experimental curve is defined with the same resolution as the numerical one, thus enabling a direct, point-by-point comparison during the optimization procedure.

The subsequent calibration of the material parameters within the constitutive model aims to minimize the discrepancy between the numerical and experimental responses, thereby enhancing the model's ability to replicate the mechanical behavior of the amniotic sac.

3.5 Numerical simulations

The numerical simulations were based on a simplified computational model of the full-term uterine environment, specifically representative of the third trimester of pregnancy. This model, developed by Fidalgo et al., is a reduced version of the more complex and parametrized model previously employed to characterize the intrauterine domain, as described in [13] (the parametrized complex model is represented in section 2.5, Chapter 2). The model used in the present study also incorporates key anatomical structures, including the cervix, uterine body, the fetal membrane as a unified structure - without distinguishing between the amnion, chorion and decidua - and the maternal abdominal wall.

Several physiological scenarios associated with late gestation were investigated to evaluate their influence on the mechanical response and potential rupture of the fetal membrane. These scenarios included: (1) variation in cervical stiffness, (2) different magnitudes of intrauterine pressure and (3) distinctions between the outer and inner surfaces of the fetal membrane.

The effect of varying intrauterine pressure was examined across three anatomical regions of the fetal membrane, namely those corresponding to the central part of the uterine body, the fundus and the cervix. Simulations were conducted using three different intrauterine pressures: 0.00867 MPa, 0.005 MPa and 0.015 MPa. These values were selected in accordance with those reported by Fidalgo et al. (2024c) [13]. For all cases involving pressure variation, the cervical stiffness parameter was held constant at 0.020 MPa [13].

To assess the influence of cervical stiffness, simulations focused primarily on the region of the fetal membrane adjacent to the cervix, under a constant intrauterine pressure of 0.00867 MPa. This pressure corresponds to typical values observed during labor contractions. Three distinct stiffness values (c_{10}) were assigned to the cervical tissue: 0.186 MPa (stiff cervix), representative of the stiffness typically observed in non-pregnant human cervical tissue [13], 0.070 MPa (mid-cervical stiffness) and 0.020 MPa (soft cervix), to evaluate their impact on the deformation and stress distribution in the membrane.

Additionally, the model was employed to explore potential differences in the mechanical behavior between the outer and inner surfaces of the fetal membrane. This analysis was performed in each of the three aforementioned uterine regions (central part of the corpus; cervix and fundus), under an intrauterine pressure of 0.00867 MPa and a constant cervical stiffness value of 0.020 MPa. Therefore, in total, five simulation cases were carried out as part of this study.

All numerical simulations were executed on a virtual machine environment. This computational setup was necessary to enable the integration of the UMAT subroutine, which incorporates the anisotropic hyperelastic HGO constitutive formulation specifically applied to model the mechanical behavior of the amniotic sac.

3.5.1 Mechanical properties and constitutive models of the uterine environment components

The uterine body, cervix and abdominal wall were modeled using an isotropic Neo-Hookean constitutive formulation. In contrast, the fetal membrane was represented using the anisotropic hyperelastic HGO model, whose parameters were previously optimized via the micro-genetic algorithm, proposed by Vila Pouca et al. (2018) [58], as detailed earlier. The mechanical parameters employed for each structural component of the uterine model and the corresponding material constitutive model are summarized in Table 3.3.

For the fetal membrane, the optimized hyperelastic parameters were introduced into the numerical simulations through the inclusion of a separate file, `parameters.inp`, referenced within the Abaqus main input file. This ensured that the calibrated parameters were correctly assigned to the user-defined material behavior of the fetal membrane.

Table 3.3: Mechanical properties and constitutive models assigned to each component of the uterine environment. For the fetal membrane, the HGO model parameters (i.e., c_{10} , k_{11} and k_{12}) were obtained through calibration and the respective values are presented in section 4.2, Chapter 4.

Component	Constitutive Model	Material Parameters
Uterine body	Neo-Hookean	$c_{10} = 0.030$ MPa, $D_1 = 1 \times 10^{-5}$ MPa ⁻¹ [13]
Cervix	Neo-Hookean	$c_{10} = 0.020$ MPa (soft cervix), $D_1 = 2.48$ MPa ⁻¹ [13]
Fetal membrane	HGO	Parameters obtained through calibration (see section 4.2)
Abdomen	Neo-Hookean	$c_{10} = 0.005$ MPa, $D_1 = 1 \times 10^{-5}$ MPa ⁻¹ [13]

3.5.2 Finite element mesh specifications for each component of the uterine environment

Regarding the finite element discretization, the uterine body was modeled using 6333 linear hybrid hexahedral elements of type C3D8H and 8536 nodes. The cervix mesh consisted of 7600 C3D8H elements and 8840 nodes. The fetal membrane, modeled as a single-layer structure, was discretized with 2609 linear hexahedral elements of the same type and 7833 nodes. Finally, the abdominal wall was meshed using 27836 hybrid tetrahedral elements of type C3D4H and 5887 nodes.

In order to ensure the correct implementation of the UMAT subroutine, 2609 fiber vectors were assigned to represent the family of randomly oriented fibers, thereby matching the total number of elements in the fetal membrane mesh.

It is important to note that the use of hybrid finite elements was essential for all components modeled with hyperelastic materials. This choice is particularly critical when dealing with quasi-incompressible behavior, as hybrid elements are specifically designed to avoid volumetric locking. Moreover, in the absence of hybrid elements, the Abaqus solver does not allow the simulations to proceed due to numerical instabilities associated with incompressibility constraints.

3.5.3 Loads, contact interactions and boundary conditions

In the numerical model implemented by Fidalgo et al. (2024c) [13], a fluid cavity was incorporated within the uterine body to simulate the amniotic fluid. Within this cavity, a gradually increasing intrauterine pressure (ramp loading) was applied to replicate physiological loading conditions [13]. The fluid density was defined as 1.025 kg/m^3 and the bulk modulus was set to 2200 MPa [13] - both values corresponding to the mechanical properties of blood plasma, as reported by Orczyk-Pawilowicz et al. (2016) [66]. The reference value for intrauterine pressure was chosen to replicate the peak pressure observed during uterine contractions in labor and was set at 0.00867 MPa.

All contact interactions between the anatomical components - namely, the uterine body and abdomen, uterine body and cervix, cervix and abdomen, fetal membrane and cervix and, lastly, uterine body and fetal membrane - were modeled using tie constraints. These constraints ensured perfect bonding between the interacting surfaces, thereby eliminating any possibility of relative displacement or detachment between the respective components.

To ensure numerical stability and prevent rigid body motion, two primary boundary conditions were imposed:

- **Posterior fixation of the abdomen:** An ENCASTRE boundary condition was applied to the posterior region of the abdominal structure. This condition fully constrained all translational and rotational degrees of freedom, effectively anchoring the model in space.
- **Axial displacement restriction at the superior abdominal surface:** A displacement boundary condition was applied to the superior region of the abdomen, specifically constraining movement in the axial (longitudinal) direction, i.e., degree of freedom 1. This constraint was implemented to mitigate nonphysical vertical displacements, while still allowing physiologically realistic deformation in the remaining degrees of freedom.

3.5.4 Path construction in the ODB file using Abaqus

To evaluate the maximum principal stress as a function of normalized distance in different regions of the fetal membrane - specifically, those adjacent to the central part of the uterine body (also named corpus) and to the cervix and fundus - it was necessary to construct paths in each of the three anatomical regions for every simulation. Paths were created both on the inner and outer surfaces of the fetal membrane in each region. The selected paths corresponding to the internal and external surfaces of the membrane are illustrated in Figures 3.10 and 3.11, respectively.

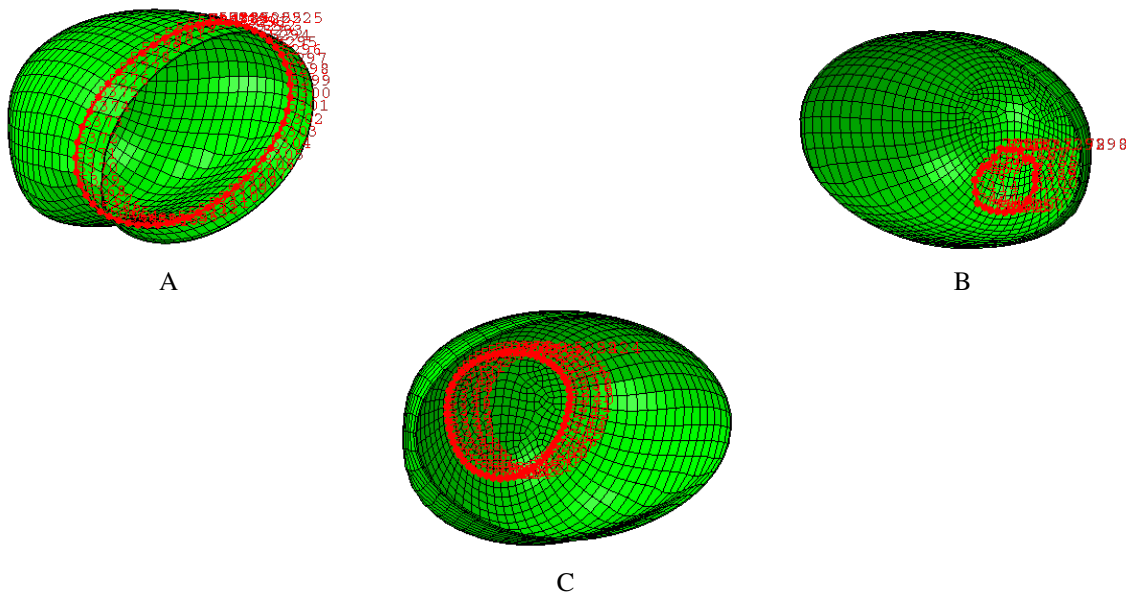


Figure 3.10: Paths defined on the inner surface of the fetal membrane in the regions corresponding to the (A) central part of the uterine body, (B) cervix and (C) fundus.

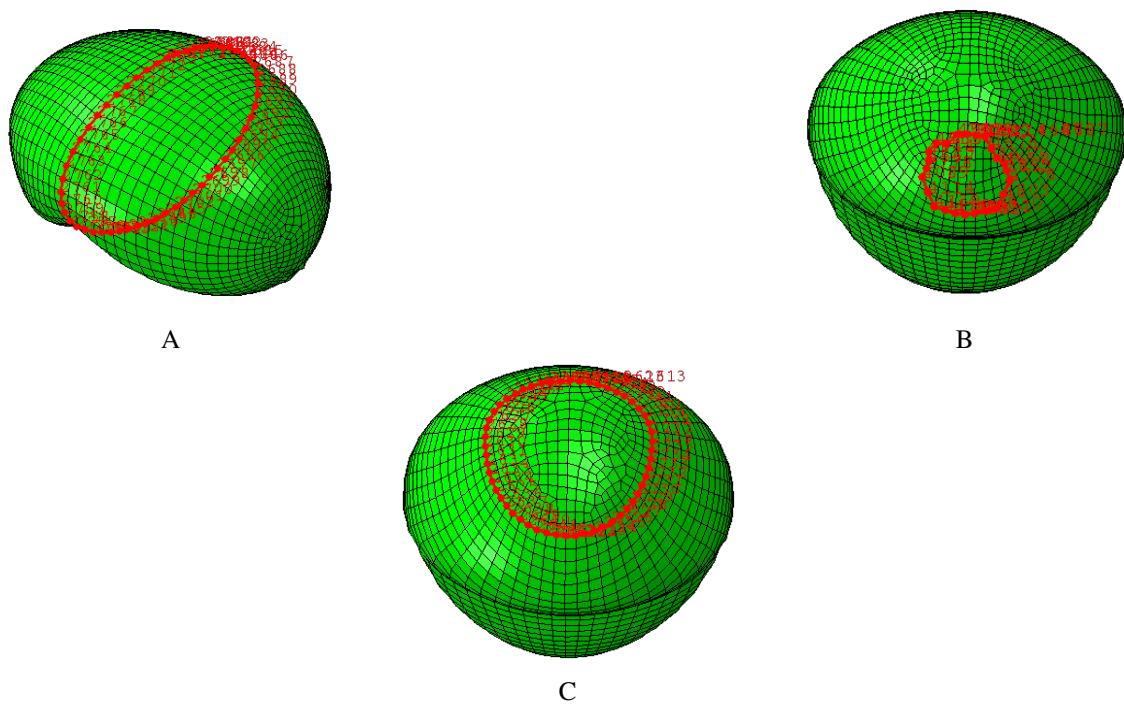


Figure 3.11: Paths defined on the outer surface of the fetal membrane in the regions corresponding to the (A) central part of the uterine body, (B) cervix and (C) fundus.

It is fundamental to emphasize that the same node sets were consistently used to define each path across all simulations, ensuring methodological consistency. The specific node IDs employed to define the paths for both the inner and outer surfaces, depending on the anatomical region of the fetal membrane considered are presented in Table 3.4.

Table 3.4: Node IDs used to define paths on the inner and outer surfaces of the fetal membrane for each anatomical region. The same nodes were maintained consistently across all simulations to ensure comparability.

Region	Nodes on inner surface	Nodes on outer surface
Central part of the corpus	5225, 5288:5313:1, 5226, 5359:5384:1, 5225	2614, 2677:2702:1, 2615, 2748:2773:1, 2614
Cervix	7298, 7293, 7287, 7288, 7269, 5463, 7387:7441:27, 7442, 7443, 5444, 6748:6750:1, 6725, 6700, 5389, 7178, 7255, 7177, 7167, 7173, 5450, 7298	4687, 4682, 4676, 4677, 4658, 2852, 4776:4830:27, 4831, 4832, 2833, 4137:4139:1, 4114, 4089, 2778, 4567, 4644, 4566, 4556, 4562, 2839, 4687
Fundus	5224, 5229:5254:1, 5223, 5333:5358:1, 5224	2613, 2618:2643:1, 2612, 2722:2747:1, 2613

Chapter 4

Results analysis and discussion

The present chapter describes and compares the results related to the evaluation of the maximum principal stress in the amniotic sac, considering finite element meshes constructed with one, two and three layers in the vertical direction, based on an inflation mechanical test. Additionally, the outcomes of the hyperelastic material parameter optimization for the amniotic sac - conducted using the HGO constitutive model and the respective micro-genetic algorithm - are reported and analyzed. Finally, the results of numerical simulations assessing the maximum principal stress behavior under various pregnancy-related scenarios are examined. These simulations are based on a previously established numerical model of the uterine environment, incorporating the uterine body, cervix, fetal membrane and abdomen.

4.1 Comparison of through-thickness discretizations

In order to assess the influence of through-thickness discretization on the stress distribution within the amniotic sac, based on an inflation mechanical test performed in Abaqus, finite element simulations were conducted using 1, 2 and 3 elements along the thickness direction. For each discretization, two element size values were considered: 1.0 mm and 1.1 mm. The maximum principal stress distribution at the end of the inflation simulations is shown in Figures 4.1 and 4.2, corresponding to the simulations with element sizes of 1.0 mm and 1.1 mm, respectively.

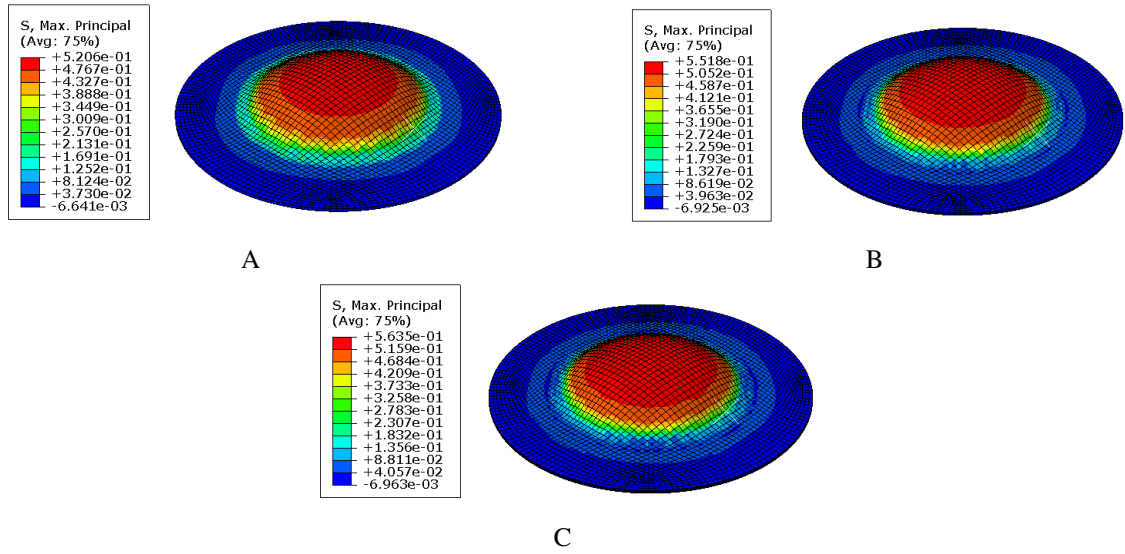


Figure 4.1: Maximum principal stress distribution in the amniotic sac for (A) 1, (B) 2 and (C) 3 elements across the thickness direction, considering a mesh element size of 1.0 mm (units: MPa).

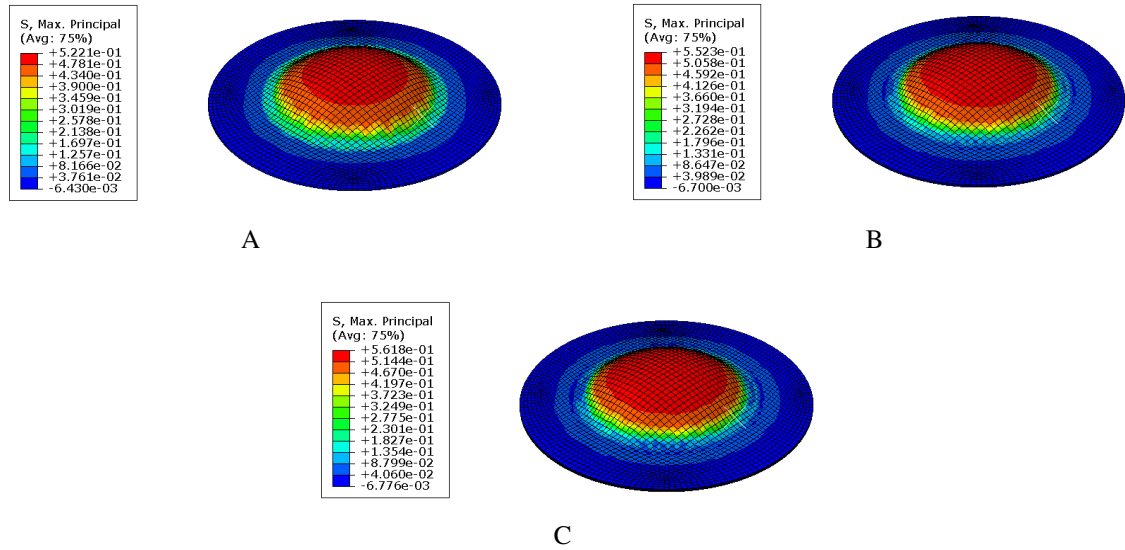


Figure 4.2: Maximum principal stress distribution in the amniotic sac for (A) 1, (B) 2 and (C) 3 elements across the thickness direction, considering a mesh element size of 1.1 mm (units: MPa).

It is evident from Figures 4.1 and 4.2 that the overall stress distribution remains consistent across all configurations (i.e., coherent spatial pattern of maximum principal stress), with the highest stress values localized at the apex of the deformed geometry, which is in agreement with the expected response under inflation. However, a slight increase in the spatial smoothness of the stress contours is observed as the number of through-thickness elements increases.

When comparing the simulations conducted with a 1.0 mm element size (Figure 4.1), the use of only one element through the thickness (Figure 4.1A) yielded a peak stress of approximately

0.520623 MPa. With two and three elements in the vertical direction (Figures 4.1B and 4.1C), the maximum principal stress increased to 0.551772 MPa and 0.563466 MPa, respectively.

This progressive rise in peak stress values suggests that increasing the number of layers improves the resolution of the stress gradients through the thickness, enabling a more accurate representation of the local mechanical response, especially near the apex where stress concentrations develop.

A similar trend is perceptible in the results corresponding to the 1.1 mm element size (Figure 4.2). The simulation with a single through-thickness element (Figure 4.2A) predicted a maximum principal stress of 0.522094 MPa, which increased to 0.552343 MPa and 0.561796 MPa for the two and three-layer configurations (Figures 4.2B and 4.2C, respectively). The magnitude of stress variation between successive refinements is comparable to the 1.0 mm mesh case, reinforcing the conclusion that through-thickness discretization has a noticeable, though moderate, effect on the predicted stress field.

It is noteworthy that despite a 10% coarsening in element size from 1.0 mm to 1.1 mm, the changes in maximum principal stress values are minimal for equivalent through-thickness discretizations. This indicates that the simulation results are not overly sensitive to small variations in in-plane mesh density, as long as the through-thickness resolution is adequately captured.

Nevertheless, increasing the number of layers across the thickness is associated with a significant rise in computational cost. As reported in the methodology chapter (subsection 3.3.5), the simulation time increased substantially when increasing the number of elements across the thickness from one to three. Given that the variations in stress magnitude and spatial distribution across the different discretizations are relatively minor, employing a single element through the thickness appears to be sufficient to capture the essential mechanical behavior in this context.

To conclude, while increased vertical discretization leads to improved resolution of the stress field, the marginal gain in accuracy does not justify the significantly higher computational cost for the current objective. As such, the single-layer configuration offers a practical balance between computational efficiency and numerical fidelity for the simulations regarding mesh sensitivity analysis conducted using an inflation mechanical test.

4.2 Optimization of hyperelastic parameters using a micro-genetic algorithm

The calibration of the hyperelastic parameters of the HGO model was carried out using a micro-genetic algorithm. Among the five individuals generated throughout the optimization process, individual *xr1* emerged as the most optimal, demonstrating the highest fitness score and providing the best set of parameters. The optimized parameters obtained from this individual were as follows: $c_{10} = 1.702129$ MPa, $k_{11} = 0.052110$ MPa and $k_{12} = 0.490805$ (dimensionless parameter). In order to ensure incompressibility, property D_1 , relative to the volumetric contribution of the strain energy function, was assigned a fixed value of $D_1 = 1 \times 10^{-5}$ MPa⁻¹, as previously stated.

These parameters were subsequently used to define the mechanical behavior of the fetal membrane in the numerical simulations performed.

A comparison between the numerical response curve obtained after the optimization of the hyperelastic parameters and the interpolated experimental curve is displayed in Figure 4.3. This comparison was essential to evaluate the accuracy of the calibration process.

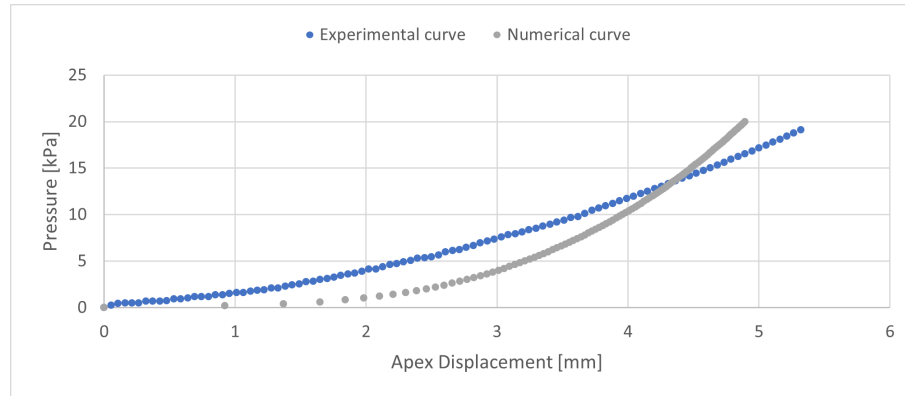


Figure 4.3: Comparison between the numerical response (simulated with optimized parameters) and the experimental curve of the pressure-apex displacement behavior of the amniotic sac.

As previously mentioned, the goal of the genetic algorithm was to approximate the numerical response curve to the experimental data as closely as possible. However, as can be observed in Figure 4.3, the calibrated numerical response does not fully match the experimental behavior. Notable discrepancies remain, indicating that the optimization process did not yield a fully satisfactory result. A normalized relative error of 31.83 % was obtained between the numerical and experimental curves (the script responsible for the calculation of the normalized error is provided in Appendix A.2).

It is also visible from Figure 4.3 that the experimental data exhibits a stiffer mechanical response compared to the numerical curve generated using the calibrated hyperelastic parameters, up to approximately 14 kPa. Specifically, for the same applied pressure, the corresponding apex displacement predicted by the numerical model is higher than that measured experimentally, indicating a lower structural rigidity in the simulated response.

A subsequent attempt was made to manually adjust the parameters in an effort to improve the fit between the curves. Nonetheless, the numerical behavior obtained through this manual approach, using Abaqus software, closely resembled the response achieved through automatic calibration. Thus, for consistency and objectivity in the subsequent pregnancy scenario simulations and based on the outcomes observed in the manual optimization process, only the results from the automatic optimization were considered.

4.2.1 Comparison between optimized numerical curve and average experimental data reported by Samimi et al. (2023)

A comparison between the numerical response obtained using the optimized hyperelastic parameters and the average experimental data reported by Samimi et al. (2023) [43] is presented in Figure 4.4 (the latter set of data is also illustrated in Figure 2.10 of subsection 2.4.4), both describing the apex displacement induced by the pressure to which the amniotic sac is subjected. It is important to note that the constitutive models employed in both cases are inherently different. While the numerical response was simulated using a HGO hyperelastic model, the experimental results stated by Samimi et al. (2023) [43] were interpreted through a distinct modeling framework and under different experimental assumptions.

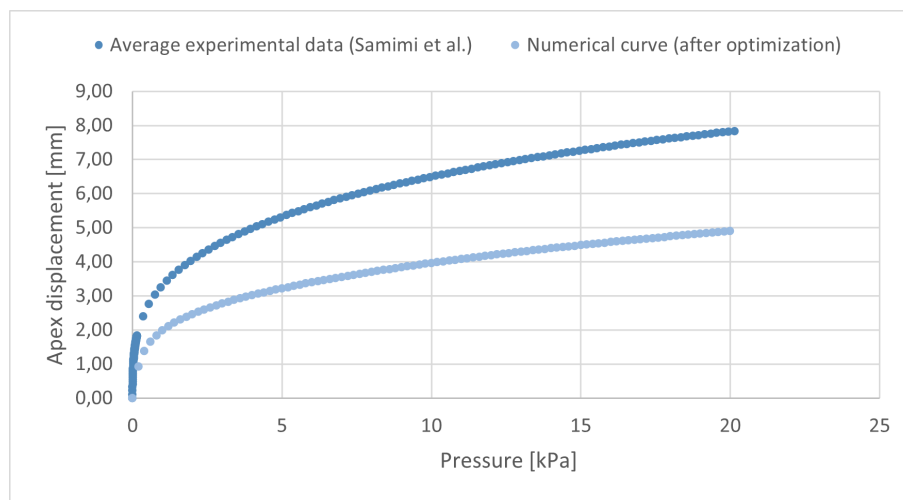


Figure 4.4: Comparison between the numerical response (simulated using the optimized parameters) and the average experimental curve reported by Samimi et al. (2023) [43], both describing the apex displacement-pressure behavior of the amniotic sac.

As shown in Figure 4.4, the numerical model demonstrates a mechanical behavior that is qualitatively similar to the average experimental response. Specifically, both curves exhibit a comparable overall trend in the apex displacement-pressure relationship, suggesting that the general structural response of the amniotic sac is adequately captured. However, a quantitative discrepancy remains: for the same applied pressure, the apex displacement predicted by the numerical simulation is consistently lower than the values observed in the average experimental data. This suggests that the material numerical response defined by the optimized hyperelastic parameters exhibits a stiffer mechanical behavior when compared to the average experimental curve.

Furthermore, it is critical to point out that the goal of the optimization process was not to replicate the average experimental response, but rather to determine the set of parameters that best fit a particular experimental dataset - specifically, one of the individual curves documented by Samimi et al. [43]. These individual experimental curves, which represent the mechanical behavior of different c-section amniotic sac samples from distinct gestational weeks, were collectively used to derive the average experimental response presented in Figure 4.4. Accordingly, the experimental

curve employed in the calibration procedure of the current study corresponds to a subset of the data contributing to the average, ensuring consistency in the comparison while also justifying the observed discrepancies.

Given this context, some degree of deviation between the optimized numerical curve and the averaged experimental trend is both expected and acceptable within the scope of this work.

4.2.2 Limitations of the automatic optimization procedure

Despite extensive efforts to automatically and accurately optimize the hyperelastic material parameters of the amniotic sac, following the methodology described in section 3.4, the automated optimization process did not perform as expected. One of the likely contributing factors was the lack of convergence in some simulation increments, which may have hindered the optimization algorithm's ability to evaluate the objective function consistently. Specifically, it was observed that from generation 1 onward, the individual with the highest merit showed no further variation in the hyperelastic parameters. This stagnation indicated a failure in the evolutionary process.

Beyond this, additional limitations should be noted:

- **Unequal prioritization of parameters:** greater emphasis was placed on optimizing the parameter c_{10} , which corresponds to the isotropic stiffness parameter of the matrix, than on the anisotropic fiber-related parameters, k_{11} and k_{12} . This likely skewed the objective function's sensitivity and may have limited the model's ability to fully represent the directional mechanical behavior of the membrane.
- **Original purpose of the algorithm:** the micro-genetic algorithm was initially implemented to calibrate pelvic floor muscle tissue, which differs significantly in structure and mechanical properties from the fetal membrane. As a result, specific modifications tailored to the intrinsic nature of the amniotic sac were not incorporated.
- **Algorithmic simplifications due to time constraints:** adjustments to the optimization framework, such as expanding the search space, refining fitness functions or introducing constraints specific to fetal tissue mechanics, were not made, due to project time limitations. This may have compromised the accuracy of the final calibration.
- **Limited parameter space and incomplete modeling:** the material behavior was modeled as purely hyperelastic, excluding time-dependent (viscoelastic) effects known to be relevant in fetal membranes.

4.3 Numerical simulations

The current section illustrates and analyzes the results of numerical simulations, focusing on the distribution of maximum principal stress within the fetal membrane across various pregnancy-related scenarios.

4.3.1 Analysis of the influence of intrauterine pressure on fetal membrane stress distribution

The maximum principal stress magnitudes and their spatial distributions within the anterior and posterior regions of the fetal membrane, as induced by varying intrauterine pressures (0.005 MPa, 0.00867 MPa and 0.015 MPa), under a constant cervical stiffness of 0.020 MPa, are illustrated in Figures 4.5, 4.6 and 4.7.

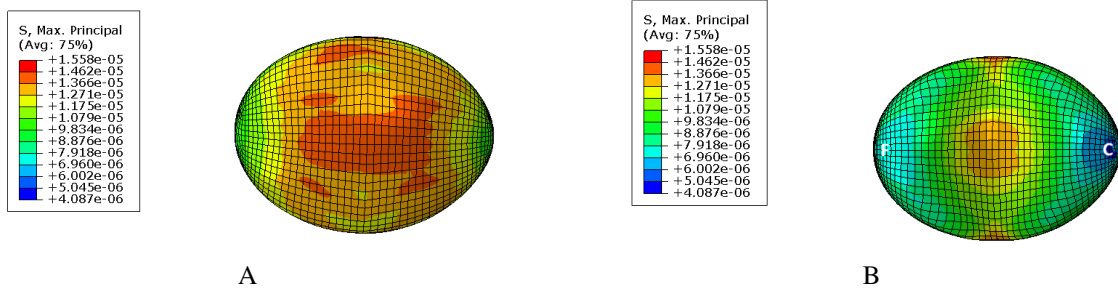


Figure 4.5: Maximum principal stress distribution (units: MPa) in the (A) anterior and (B) posterior regions of the amniotic sac, considering an intrauterine pressure of 0.005 MPa; C: refers to the fetal membrane region adjacent to the cervix; F: refers to the fetal membrane region adjacent to the fundus.

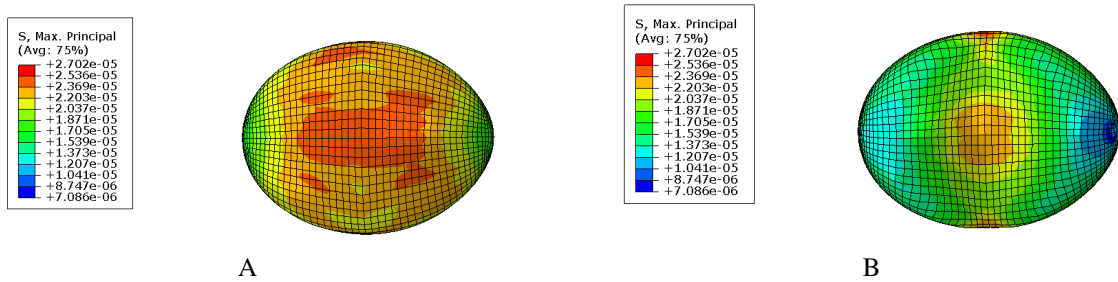


Figure 4.6: Maximum principal stress distribution (units: MPa) in the (A) anterior and (B) posterior regions of the amniotic sac, considering an intrauterine pressure of 0.00867 MPa.

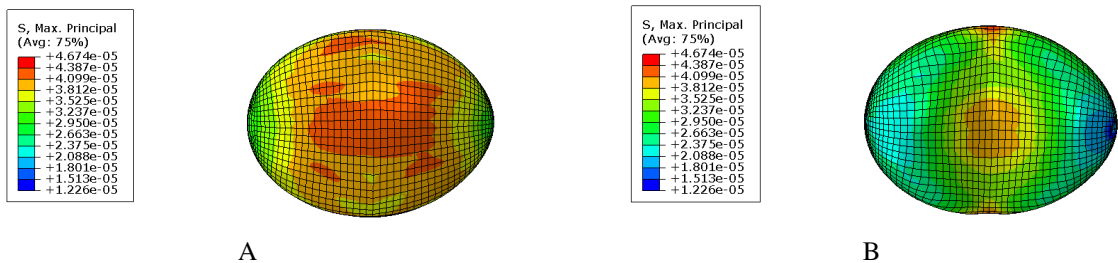


Figure 4.7: Maximum principal stress distribution (units: MPa) in the (A) anterior and (B) posterior regions of the amniotic sac, considering an intrauterine pressure of 0.015 MPa.

The simulation results reveal a clear dependence of the maximum principal stress distribution within the fetal membrane on the magnitude of intrauterine pressure (IUP). A progressive increase in IUP from 0.005 MPa to 0.015 MPa results in a corresponding increase in the maximum principal stress experienced by the amniotic sac, as shown in Figures 4.5, 4.6 and 4.7.

At the lowest IUP value of 0.005 MPa (Figure 4.5), the stress distribution is relatively moderate, with peak values remaining within the range of approximately 1.46×10^{-5} MPa to 1.56×10^{-5} MPa. As the pressure increases to 0.00867 MPa (Figure 4.6), the magnitude of the high-stress regions expand, particularly within the anterior region. This trend becomes even more pronounced at 0.015 MPa (Figure 4.7), where the maximum principal stress exceeds 4.0×10^{-5} MPa in the anterior region, indicating a substantial rise in membrane loading.

This behavior is in agreement with biomechanical expectations. As IUP increases, the membrane is subjected to greater tensile loads, particularly in areas where curvature and structural constraints promote stress concentration. The anterior region consistently exhibits higher stress magnitudes compared to the posterior region across all tested pressures, which may be attributed to geometric asymmetries or mechanical heterogeneity in the uterine environment.

Moreover, the plots that provide a comparative view of the spatial variation of maximum principal stress along normalized paths in three distinct regions of the fetal membrane - adjacent to the mid-region of the uterine body, cervix and fundus - under increasing IUP are depicted in Figures 4.8, 4.9 and 4.10. In this case, the paths were constructed on the outer surface of the fetal membrane, according to the methodology outlined in subsection 3.5.4, Chapter 3.

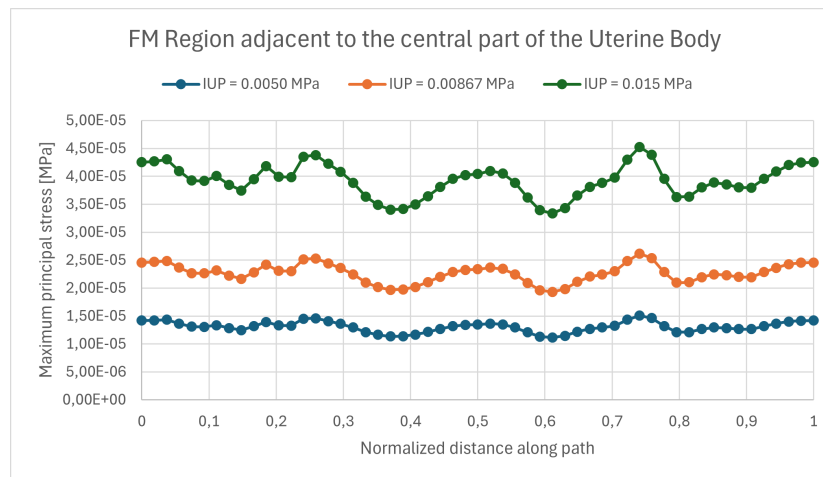


Figure 4.8: Plot of maximum principal stress (units: MPa) versus normalized distance along the path, measured on the outer surface of the fetal membrane in the region adjacent to the central part of the uterine corpus, for different IUPs; FM: abbreviation for Fetal Membrane.

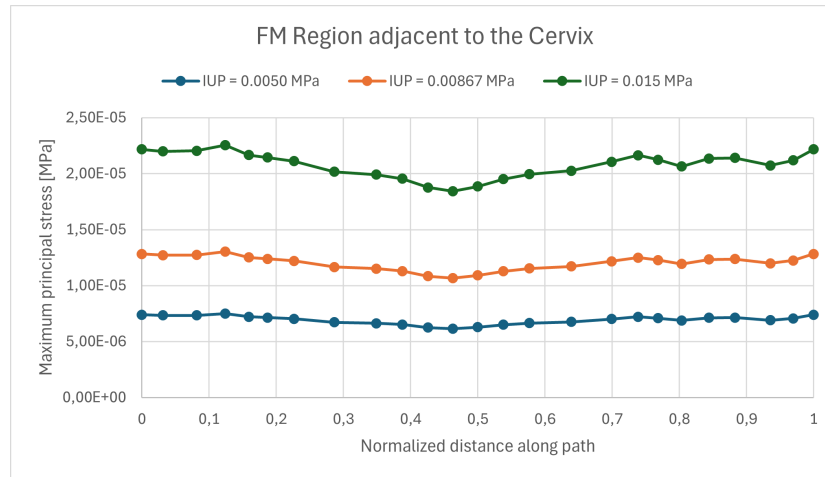


Figure 4.9: Plot of maximum principal stress (units: MPa) versus normalized distance along the path, measured on the outer surface of the fetal membrane in the region adjacent to the cervix, for different IUPs; FM: abbreviation for Fetal Membrane.

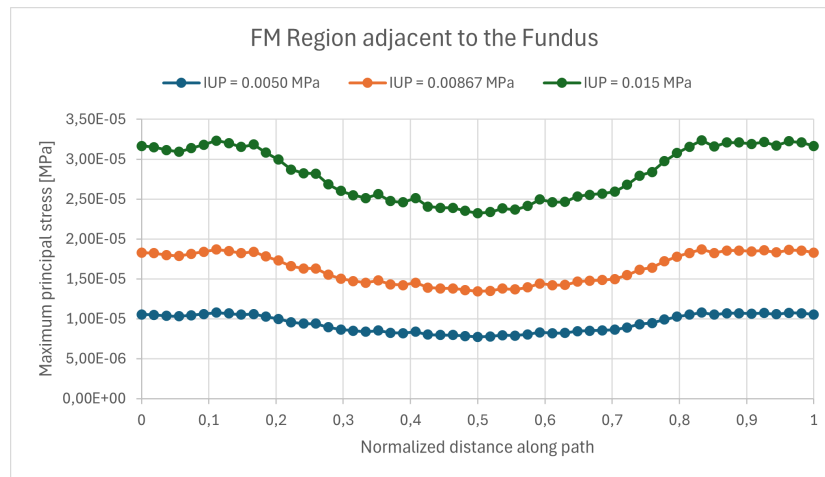


Figure 4.10: Plot of maximum principal stress (units: MPa) versus normalized distance along the path, measured on the outer surface of the fetal membrane in the region adjacent to the fundus, for different IUPs; FM: abbreviation for Fetal Membrane.

Indeed, in all the anatomical regions considered, an increase in IUP is consistently accompanied by a rise in maximum principal stress, indicating that the stress levels in these areas are strongly influenced by IUP, as was to be expected.

To better contextualize the specific area being analyzed in the plot shown in Figure 4.8, it is important to note that the normalized distance values within the intervals $[0; 0.2]$ and $[0.8; 1]$ correspond to the anterior region of the fetal membrane adjacent to the central portion of the uterine corpus. In contrast, the values ranging between 0.2 and 0.8 represent the lateral and posterior regions of the fetal membrane adjacent to the midsection of the uterine body.

Among the three regions, the fetal membrane segment adjacent to the intermediate part of the uterine body (Figure 4.8) exhibits the highest overall stress levels along the entire normalized

path for all IUP conditions considered. This area also demonstrates greater variability in stress, suggesting a more heterogeneous mechanical environment or higher sensitivity to curvature and geometric constraints. Furthermore, it can be inferred that the maximum principal stress values are particularly elevated in the anterior region, as well as in certain portions of the lateral and posterior regions of the fetal membrane adjacent to the mid zone of the uterine corpus (as defined by the path under analysis).

The cervix-adjacent region of the fetal membrane (Figure 4.9) presents the lowest overall stress levels with minimal variation, indicating a more compliant structure compared to the other analyzed regions - that is, one capable of accommodating deformations with relative ease and offering limited mechanical resistance.

The region of the fetal membrane adjacent to the fundus (Figure 4.10) demonstrates an intermediate and relatively uniform stress profile along the analyzed path, characterized by modest peak values and a subtle U-shaped distribution, likely influenced by the local geometric curvature near the fundal dome.

Overall, the three numerical simulations - each corresponding to a distinct IUP value - confirm that increasing IUP leads to a significant rise in maximum principal stress within the fetal membrane, particularly in the anterior region, as well as in specific portions of the lateral and posterior regions adjacent to the midsection of the uterine body. This trend aligns with clinical observations suggesting that elevated IUP can contribute to membrane weakening and eventual rupture, as reported by Verbruggen et al. (2017) [8] and Fidalgo et al. (2014c) [13]. These results underscore the critical mechanical role of IUP in maintaining fetal membrane integrity and provide valuable insight into potential biomechanical pathways involved in preterm birth and membrane rupture mechanisms.

4.3.2 Investigation of the impact of cervical stiffness variability

Another analysis was conducted to investigate the effect of varying cervical stiffness (c_{10}) on the mechanical behavior of the fetal membrane. For this purpose, three distinct values of cervical stiffness were considered: 0.020 MPa (representing a soft cervix), 0.070 MPa (intermediate cervical stiffness) and 0.186 MPa (representing a stiff cervix). In all three simulations included in this subsection, the intrauterine pressure was kept constant at 0.00867 MPa.

The maximum principal stress distribution across the fetal membrane for each cervical stiffness scenario is presented in Figures 4.11, 4.12, and 4.13, with particular emphasis on the region of the fetal membrane adjacent to the cervix (lower region of the fetal membrane).

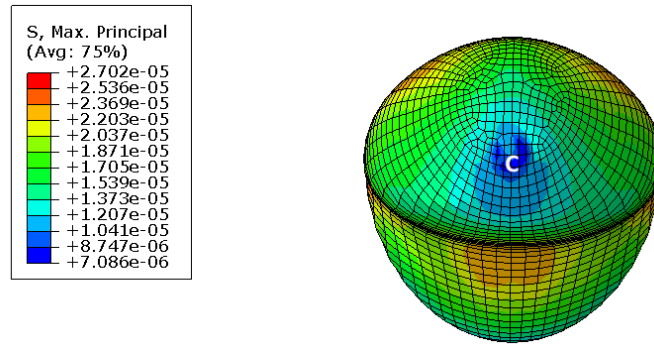


Figure 4.11: Maximum principal stress distribution (units: MPa) mainly obtained in the fetal membrane region adjacent to the cervix, considering a cervical stiffness of 0.020 MPa; C: refers to the fetal membrane region adjacent to the cervix.

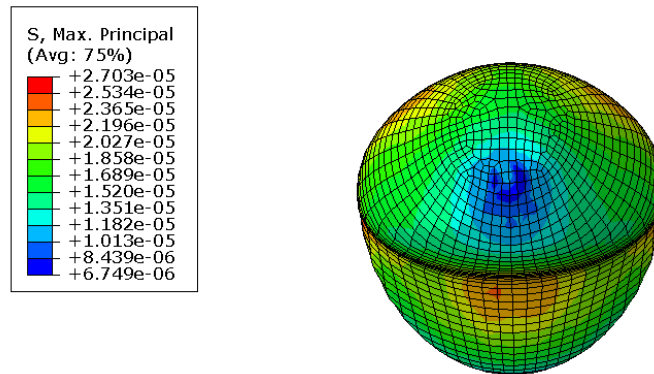


Figure 4.12: Maximum principal stress distribution (units: MPa) mainly obtained in the fetal membrane region adjacent to the cervix, considering a cervical stiffness of 0.070 MPa.

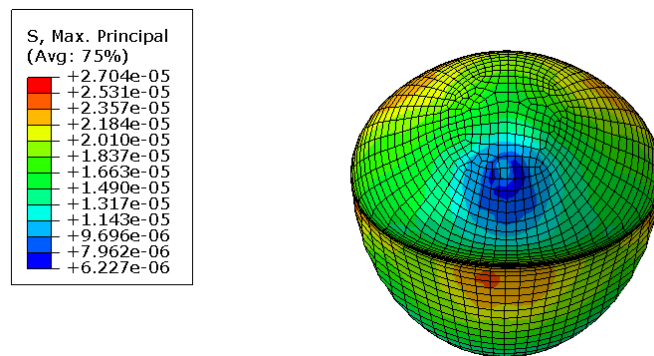


Figure 4.13: Maximum principal stress distribution (units: MPa) mainly obtained in the fetal membrane region adjacent to the cervix, considering a cervical stiffness of 0.186 MPa.

In addition, graphs depicting the maximum principal stress as a function of normalized distance along a predefined path were generated for both the inner and outer surfaces of the fetal membrane, specifically in the region adjacent to the cervix, as visible in Figures 4.14 and 4.15. In

this context, the paths for each considered cervical stiffness were defined in accordance with the methodology outlined in subsection 3.5.4 of Chapter 3.

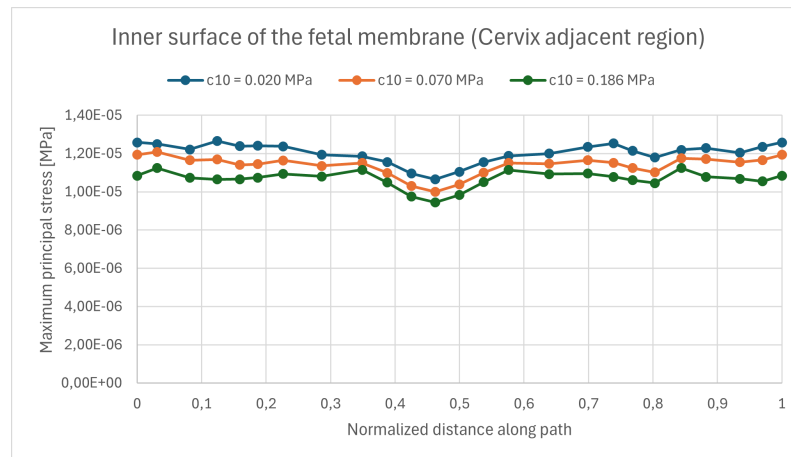


Figure 4.14: Maximum principal stress distribution (units: MPa) versus normalized distance along the path on the inner surface of the fetal membrane, focusing on the region adjacent to the cervix, for each considered cervical stiffness.

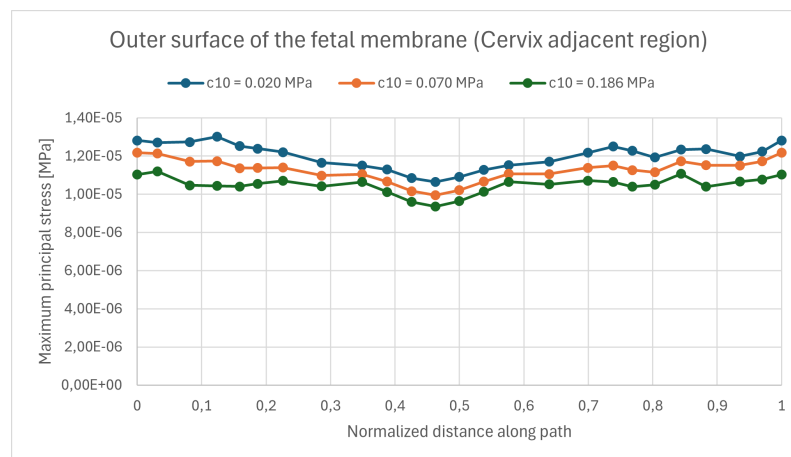


Figure 4.15: Maximum principal stress distribution (units: MPa) versus normalized distance along the path on the outer surface of the fetal membrane, focusing on the region adjacent to the cervix, for each considered cervical stiffness.

A comparative analysis of the influence of cervical stiffness on the mechanical behavior of the fetal membrane, namely in the region adjacent to the cervix, can be drawn from Figures 4.11 to 4.15. This evaluation is especially pertinent within the context of cervical remodeling during pregnancy, a physiological process in which the cervix evolves from a closed, rigid structure into a softer, more compliant one to facilitate childbirth [13, 67].

A clear trend is observed in Figures 4.11 to 4.13: with increasing cervical stiffness, there is a noticeable reduction in the magnitude of stress within the fetal membrane area adjacent to the

cervix. Conversely, the variations in maximum principal stress across the remaining regions are comparatively minor.

In the soft cervix case (Figure 4.11), the fetal membrane region adjacent to the cervix experiences higher and more localized principal stress values, suggesting that reduced cervical support leads to greater mechanical loading on the adjacent region of the membrane. Hence, having a soft cervix in the final stages of pregnancy is crucial, as this condition increases the maximum principal stress in the region of the fetal membrane adjacent to the cervix, thereby promoting rupture of the membrane and enabling the baby's passage during parturition. In this context, a soft and compliant cervix is able to shorten and dilate effectively, thereby facilitating the delivery process [13].

On the other hand, this observation aligns with the physiological condition known as cervical insufficiency, in which a weakened cervix is unable to maintain closure during gestation, often leading to excessive deformation of the fetal membrane in the early stages of pregnancy and an increased risk of preterm birth [13, 68]. This condition is more prevalent in multiparous women, as the cervix tends to become progressively softer with each successive pregnancy [11].

In contrast, the stiff cervix condition (Figure 4.13) exhibits a more uniform and reduced stress distribution across the fetal membrane region adjacent to the cervix. This suggests that increased cervical stiffness enhances structural stability and mechanical resistance, thereby limiting stress accumulation in the membrane. Thus, throughout pregnancy, it is essential to maintain a firmer and stiff cervix, which results in lower stress values in the fetal membrane region adjacent to the cervix, thereby ensuring effective cervical closure [13].

However, while a stiff cervix may alleviate membrane stress, it can also hinder cervical dilation during labor, potentially leading to unsuccessful induction and complications during delivery, as noted in clinical studies [13].

A quantitative support for these observations is provided in Figures 4.14 and 4.15, which display plots of the maximum principal stress along a normalized path on both the inner and outer surfaces of the fetal membrane near the cervix. In both surfaces, the soft cervix condition consistently exhibits the highest stress values throughout the entire path. The intermediate stiffness case (0.070 MPa) shows moderate stress levels, while the stiff cervix condition corresponds to the lowest stress values. These findings align with the expectation that a progressively softer and more dilated cervix leads to an increased mechanical load on the adjacent region of the fetal membrane and, consequently, facilitates fetal membrane rupture, thereby promoting the passage of the baby during delivery process [13].

Moreover, several studies have indicated that the region of the fetal membrane overlying the cervix - commonly referred to as the "weak zone" - becomes increasingly susceptible to rupture as gestation approaches term [8, 13]. Researchers hypothesize that this area serves as the initial site of membrane rupture, acting as a critical trigger for the onset of labor [8, 13]. This region is characterized by a significant collagen remodeling and increased levels of apoptosis (programmed cell death), which collectively compromise its structural integrity, rendering it weaker than other regions of the fetal membrane [69].

4.3.3 Comparative analysis of the inner and outer surfaces of the fetal membrane in terms of maximum principal stress

In order to compare the mechanical behavior at the inner and outer surfaces of the fetal membrane, a simulation was conducted using an intrauterine pressure of 0.00867 MPa and a cervical stiffness fixed at 0.020 MPa. Within this simulation framework, specific paths were defined in the ODB file generated by Abaqus, corresponding to the anatomical regions of the central part of the uterine body, cervix and fundus. These paths enabled the evaluation of the maximum principal stress along both the inner (facing the amniotic cavity) and outer (facing the maternal tissues) surfaces of the fetal membrane in each of the aforementioned regions.

The resulting plots of the maximum principal stress (in MPa) as a function of the normalized distance along each defined path is displayed in Figures 4.16, 4.17 and 4.18. The graphs allow for a direct comparison between the inner and outer surfaces of the fetal membrane in the regions adjacent to the central part of the uterine body, cervix and fundus, respectively. Accordingly, the paths corresponding to each surface were established based on the methodological approach detailed in subsection 3.5.4 of Chapter 3.

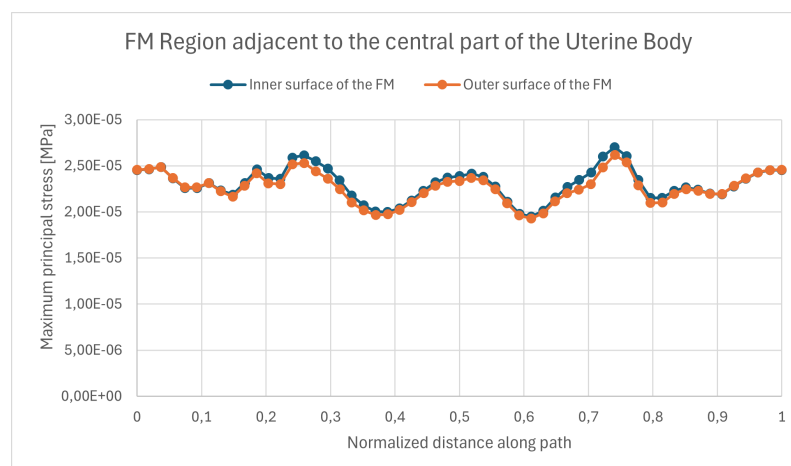


Figure 4.16: Variation of maximum principal stress (units: MPa) along the normalized path length for both the inner and outer surfaces of the fetal membrane in the region adjacent to the intermediate zone of the uterine body. Here, the normalized distance intervals [0; 0.2] and [0.8; 1] represent the anterior region of the fetal membrane adjacent to the central portion of the uterine corpus. In contrast, the values ranging between 0.2 and 0.8 correspond to the lateral and posterior regions of the fetal membrane adjacent to the midsection of the uterine body; FM: abbreviation for Fetal Membrane.

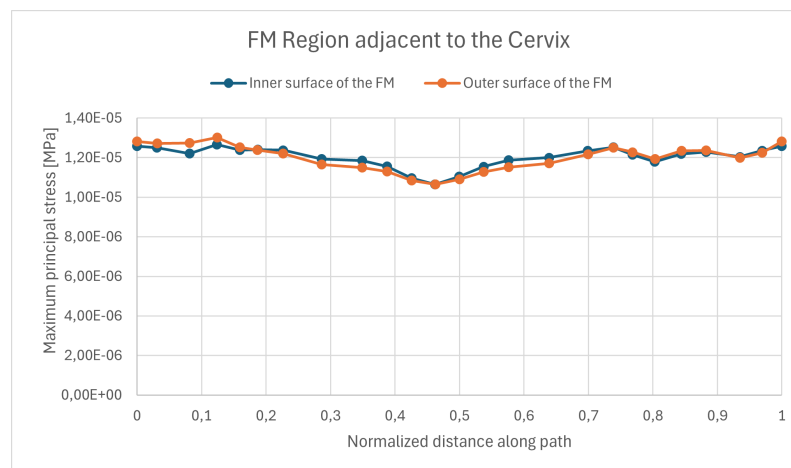


Figure 4.17: Variation of maximum principal stress (units: MPa) along the normalized path length for both the inner and outer surfaces of the fetal membrane in the region adjacent to the cervix; FM: abbreviation for Fetal Membrane.

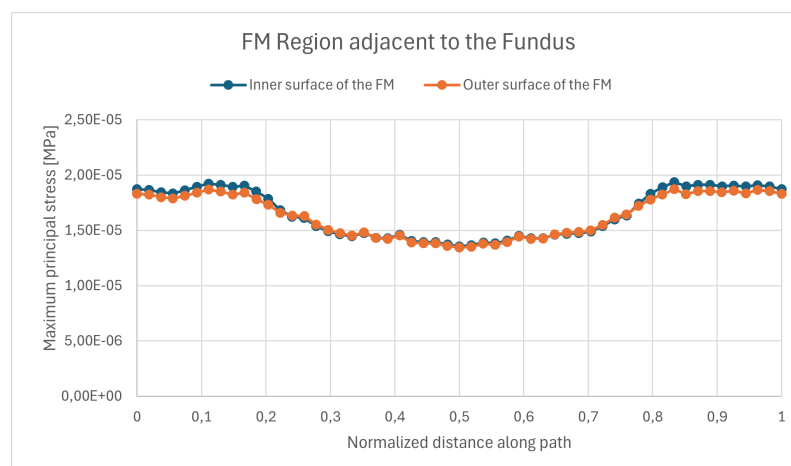


Figure 4.18: Variation of maximum principal stress (units: MPa) along the normalized path length for both the inner and outer surfaces of the fetal membrane in the region adjacent to the fundus; FM: abbreviation for Fetal Membrane.

The comparative analysis presented in Figures 4.16, 4.17 and 4.18 reveals that the differences in maximum principal stress between the inner and outer surfaces of the fetal membrane are generally minimal across all anatomical regions. In the membrane region adjacent to the mid part of the uterine body and the fundus, the inner surface displays slightly higher stress values than the outer surface, particularly along curved areas of the evaluated path. Additionally, in the region of the fetal membrane adjacent to the cervix, the inner surface also shows marginally higher stress values for normalized distance values approximately between 0.2 and 0.7, whereas for the initial segment of the path - between 0 and approximately 0.15 - the outer surface exhibits slightly higher stress levels compared to the inner one.

These findings indicate a largely uniform stress distribution across the thickness of the fetal membrane, with only minor, surface-dependent variations. Furthermore, the results confirm that the region of the fetal membrane adjacent to the central portion of the uterine corpus consistently experiences the highest maximum principal stress values among all evaluated regions. In contrast, the cervix-adjacent region of the fetal membrane remains the least stressed, displaying the lowest maximum principal stress levels along the analyzed path.

Chapter 5

Final Remarks and Future Work

5.1 Final Remarks

The primary objective of the present dissertation project was to investigate the impact of distinct pregnancy-related conditions on the fetal membrane, by employing a hyperelastic constitutive model capable of characterizing its mechanical behavior.

The numerical and computational analyses conducted in this study indicate that both intrauterine pressure and cervical stiffness significantly influence the mechanical behavior of the fetal membrane. Increased intrauterine pressure leads to higher maximum principal stress values within the membrane, while greater cervical stiffness contributes to a reduction in stress magnitude, particularly near the cervix.

In addition, throughout pregnancy, it is essential for the cervix to remain relatively stiff in order to maintain its closure, thereby ensuring lower stress levels in the region of the fetal membrane adjacent to the cervix and preventing premature rupture. Conversely, in the final stages of gestation, cervical softening becomes crucial, as it facilitates increased stress in the adjacent fetal membrane region, promoting membrane rupture and enabling the fetal expulsion during delivery. The clinical concern arises when the cervix becomes soft prematurely, particularly in the early stages of pregnancy, which may lead to cervical insufficiency and, consequently, an increased risk of preterm birth.

Moreover, comparative assessments between the inner and outer surfaces of the fetal membrane reveal minimal variations in maximum principal stress across the membrane thickness, suggesting an overall uniform stress distribution between the outer and inner surfaces. Collectively, these results offer meaningful insights into the biomechanical factors affecting fetal membrane integrity and reinforce the hypothesis that cervical softening, in conjunction with elevated intrauterine pressure, contributes to increased membrane stress and a potential risk of fetal membrane rupture.

It can also be concluded that numerical simulations represent a powerful tool for studying the biomechanics of the fetal membrane, offering several notable advantages. As a non-invasive technique, it enables the analysis of complex physiological processes without posing any risk to the patient. Furthermore, it allows for the efficient storage and processing of large volumes of data and provides a rapid means of evaluating various physiological scenarios. For these reasons, numerical modeling stands out as a promising approach that is expected to yield increasingly accurate and clinically relevant results as computational technologies continue to evolve.

However, it is important to acknowledge the limitations inherent to numerical simulations. In order to make the simulations computationally feasible, certain simplifications and assumptions are often required. These may lead to discrepancies between the simulated outcomes and actual physiological behavior. It is, therefore, the role of ongoing technological development to provide increasingly advanced tools and modeling techniques, with the aim of minimizing such discrepancies and bringing numerical predictions ever closer to real biological responses.

5.2 Project Limitations

The present study is subject to several limitations: (1) the viscoelastic behavior of the amniotic sac was not incorporated during the calibration of material parameters and therefore not accounted for in the numerical simulations of the intrauterine environment; (2) the multilayer structure of the fetal membrane was not included in the simulations. According to the study by Verbruggen et al. (2017) [8], representing the chorion and amnion as a single layer leads to a markedly different mechanical response when compared to a more anatomically accurate bilayer configuration. This simplification results in a significant underestimation of the stress experienced by the amnion (mechanically dominant layer) and consequently yields a lower predicted risk of membrane rupture, potentially compromising the reliability of biomechanical assessments [8]; (3) uterine contractions, particularly those occurring in the uterine body and cervix, were not modeled; and (4) the material parameter calibration process presents limitations that resulted in a significant discrepancy between the experimental and numerical curves, as discussed in detail in subsection 4.2.2.

5.3 Future Work

As a continuation of the work developed in the present dissertation and considering it as a foundation for future research, several avenues can be explored to enhance the physiological realism and predictive capabilities of the current model.

Firstly, in this study, particularly in the numerical simulations, an electro-chemo-mechanical model could have been considered for the uterine body and cervix, capable of simulating a sequence of intrauterine muscular contractions and evaluating their effect on the fetal membranes. Furthermore, at this stage, it would be both relevant and challenging to implement a multilayer

fetal membrane model. In such a model, the hyperelastic parameters obtained for the HGO constitutive law would be assigned to the amnion, the mechanically dominant layer, whereas the chorion and decidua - being mechanically less dominant - would be modeled as linear elastic materials.

In addition, it would be pertinent to conduct numerical analyses that incorporate the viscoelastic behavior of biological tissues, particularly that of the amniotic sac. Given the fact that, in the current study, viscoelastic parameters were intentionally set to zero, integrating viscoelasticity into the micro-genetic algorithm used for parameter optimization represents a key advancement, as it enables the evaluation of time-dependent mechanical responses, thereby moving closer to more realistic and physiologically accurate simulations.

The incorporation of a damage model would also be a valuable enhancement, as it would allow the study of biomechanical behavior at a more complex level. Such a model would make it possible to simulate the progressive degradation of the fetal membrane structure, offering a deeper understanding of the mechanisms leading to rupture.

Following the implementation of a damage model, it would also be possible to investigate the physiological conditions that may promote preterm rupture of the fetal membranes, using a constitutive damage model capable of characterizing the mechanical behavior of these tissues under a range of clinically relevant scenarios.

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Appendix A

A.1 Python code for experimental curve interpolation

```
1 import numpy as np
2 import matplotlib.pyplot as plt
3
4 # Read data from the original file
5 data = np.loadtxt('experimental_initial.txt')
6
7 # Separate the columns
8 displacement = data[:, 0]
9 pressure = data[:, 1]
10
11 # Generate 101 equally spaced points in displacement
12 displacement_uniform = np.linspace(displacement.min(), displacement.max(),
13                                     101)
14
15 # Linear interpolation of pressure values
16 pressure_uniform = np.interp(displacement_uniform, displacement, pressure)
17
18 # Combine the interpolated data
19 interpolated_data = np.column_stack((displacement_uniform,
20                                     pressure_uniform))
21
22 # Save the interpolated data to a new file
23 np.savetxt('experimental_interpolated.txt', interpolated_data, fmt='%.6f',
24            comments='')
25
26 # Plotting
27 plt.figure(figsize=(8, 6))
28 plt.plot(displacement, pressure, 'o-', label='Original Data')
29 plt.plot(displacement_uniform, pressure_uniform, 'r--', label='
    Interpolated (101 points)')
30 plt.xlabel('Displacement (mm)')
31 plt.ylabel('Pressure (kPa)')
```

```

29 plt.title('Original vs Interpolated Pressure-Displacement Curve')
30 plt.legend()
31 plt.grid(True)
32 plt.tight_layout()
33 plt.show()

```

A.2 Python code for computing the normalized relative error between the experimental and numerical curves

```

1 import numpy as np
2
3 def load_curve(filename):
4     # Load 2-column data: column 0 = displacement (x), column 1 = pressure
5     # (y)
6     data = np.loadtxt(filename)
7     return data[:, 0], data[:, 1]
8
9 def compute_normalized_error(y_exp, y_num):
10     # Ensure arrays are same size
11     if y_exp.shape != y_num.shape:
12         raise ValueError("Pressure arrays must be the same shape to
13         compute error.")
14     # Compute normalized L2 error
15     error = np.linalg.norm(y_exp - y_num) / np.linalg.norm(y_exp)
16     return error
17
18 def main():
19     _, p_exp = load_curve("experimental_curve.txt")
20     _, p_num = load_curve("numerical_curve.rpt")
21
22     error = compute_normalized_error(p_exp, p_num)*100
23     print(f"Normalized L2 Error: {error:.6f}%")
24
25 if __name__ == "__main__":
26     main()

```