Towards a diagnostic device - mechanical characterization of the uterine cervix in pregnancy

Author(s):
Badir, Sabrina

Publication Date:
2015

Permanent Link:
https://doi.org/10.3929/ethz-a-010385341

Rights / License:
In Copyright - Non-Commercial Use Permitted
TOWARDS A DIAGNOSTIC DEVICE –
MECHANICAL CHARACTERIZATION OF
THE UTERINE CERVIX IN PREGNANCY

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES OF ETH ZURICH
(Dr. sc. ETH Zurich)

presented by

SABRINA BADIR
MSc ETH HMS
born on 21.09.1985
citizen of Waldkirch (SG)

accepted on the recommendation of

Prof. Dr. Edoardo Mazza, examiner
Prof. Dr. med. Michael Bajka, co-examiner
Prof. Dr. Kristin Myers, co-examiner

2015
Abstract

Preterm parturition is a pathological event that is defined as childbirth before completion of 37 weeks of pregnancy. According to the World Health Organization about 15 million babies are born preterm every year, which is more than every 10th baby. Infants born prematurely have an increased risk of mortality and morbidities, thus leading to high economic costs. Due to the multi-factorial causes that lead to preterm delivery, women at risk are clinically difficult to identify. A short uterine cervix measured by trans-vaginal ultrasound has become an important criterion for detecting women at risk. However, the measurement of cervical length is not sufficient to identify the majority of women who are truly at risk.

The present work aims at a better understanding of the evolution of the mechanical properties of human uterine cervix throughout pregnancy. The main focus was on the development of a new measurement procedure, based on the aspiration method, compatible with clinical routine. This allowed performing different clinical studies on the cervix of pregnant and non-pregnant women. The new procedure applies a progressive negative pressure on the cervical tissue thus pulling the tissue into the aspiration tube while limiting the deformation to a maximum level. The higher the stiffness of the tissue, the larger the negative pressure (called $p_{cl}$) required to reach the pre-defined displacement.

A first study on non-pregnant women was performed in order to develop an appropriate measurement protocol for the new procedure. Based on these measurements, simple model equations for cervical tissue in non-pregnant and pregnant state were determined. These models were used in a parametric study using finite element analysis to analyze the un-
Abstract

certainties associated with the measurement procedure. Based on these
findings, the protocol was optimized, thus contributing significantly to the
reliability of the measurements. Safety, short duration and ease-of-use of
the measurement procedure allowing for collection of a large number of
data are the most important advantages of the new instrument. The limi-
tations in the mechanical characterization of cervical tissue are related to
time-dependent, history-dependent and anisotropic properties. Moreover,
aspiration measurements characterize only a local portion of the cervix
and are not representative for the whole organ.

Within the same study additional 50 pregnant women were recruited to
assess the feasibility of providing quantitative information on the evolution
of the mechanical properties of the cervix in normal pregnancy. Aspiration
measurements were performed at each of the eight routine consultations
and at the regular post-partum visit. At the same visit, the cervical length
was assessed by ultrasound. For the first time information was obtained
about mechanical characteristics of the ecto-cervix in pregnancy. The
study findings demonstrate that cervical changes (i.e. cervical softening)
start with the initiation of pregnancy and well-before cervical shortening is
observed. The cervix transforms continuously to a lower level of stiffness
within the first two trimesters and then stabilizes at a low level in the
third trimester. After delivery the cervical stiffness reaches a similar level
as in early pregnancy and finally recovers to the non-pregnant state.

Other quantitative methods were proposed for the assessment of the in-
vivo mechanical properties of the cervix, namely quasi-static elastography
and maximum deformability using ultrasound. The results of the stud-
ies reported in literature are contradictory. In fact, elastography indi-

cates very modest changes in the course of pregnancy, whereas maximum
deformability, similar to aspiration, shows a strong decrease in stiffness,
which starts early in pregnancy and continues until delivery. A mechanical
analysis was performed to rationalize these inconsistent findings obtained
with the different quasi-static procedures. Two approaches were used for
the present investigation: (i) quasi-static elastography was conducted on
ultrasound phantoms with known mechanical properties to evaluate the
effectiveness of the compression standardization procedure applied in pre-
vious studies, and (ii) a data analysis was conducted to investigate the
agreement of biomechanical changes quantified by aspiration and maximum deformability measurements over the course of gestation. The results clearly illustrate that the loading standardization procedure applied on the phantoms leads to repeatable strain values, however for different forces. The same is true for cervical tissue, thus quasi-static elastography cannot detect cervical softening in pregnancy. The comparison of maximum deformability and aspiration measurements demonstrated a general agreement of the procedures.

The usefulness of aspiration and maximum deformability measurements for identifying women at risk of preterm delivery is currently assessed in the clinical study SOFTCERVIX. SOFTCERVIX is an international clinical study carried out to collect biomechanical and clinical data from 1000 subjects at mid-pregnancy and to evaluate the predictive value of each technique. Aspiration and maximum deformability measurements performed so far are in line with the mean values of previous studies for the same gestational age. The study procedure will now be incorporated into standard clinical practice at the hospitals which will facilitate the recruitment in the future. Completion of the study is expected by December 2016.

The quantification of pharmacologically induced cervical softening (using misoprostol as medication) is another identified field of application for the developed instrument and biomechanical measurement procedure. In a pilot study aspiration measurements were performed prior intrauterine contraceptive placement (with administered misoprostol) and repeated at the regular follow-up consultation. The results demonstrate that our biomechanical procedure is able to characterize pharmacologically induced cervical changes. Misoprostol was shown to have a detectable softening effect on cervical tissue at first insertion of an intra-uterine contraceptive.

A large number of in-vivo aspiration measurements were performed and analyzed in this thesis to provide fundamental knowledge and clinically relevant information. Insight into the unique mechanical behavior of cervical tissue was gained and novel approaches have been defined to improve identification of women at risk of preterm delivery.
ZUSAMMENFASSUNG


Zusammenfassung

webes ist, desto größer ist der benötigte negative Druck (genannt $p_{cl}$) um das Gewebe zu verformen.


Zur Beurteilung der mechanischen Eigenschaften des Gebärmutterhalses in-vivo wurden weitere quantitative Methoden vorgeschlagen: die quasi-


Das Quantifizieren von pharmakologisch induzierter Gewebeerweichung
Zusammenfassung


Zahlreiche in-vivo Aspirationsmessungen wurden durchgeführt und in dieser Arbeit analysiert um Grundlagenwissen und klinisch relevante Informationen zu erarbeiten. Einblicke in das einzigartige mechanische Verhalten des Gebärmuttermhalsgewebes wurden gewonnen und neue Ansätze konnten definiert werden, um die Identifizierung von Frauen mit Risiko einer Frühgeburt zu verbessern.
Acknowledgments

The research related to this dissertation has been conducted at the Institute of Mechanical Systems at ETH Zurich from 2011 to 2014. I’m very thankful to all people who contributed to this work. In particular, I would like to express my gratitude to:

Prof. Dr. Edoardo Mazza, my supervisor, who supported and guided me through this thesis; he gave me the opportunity to build the bridges between science, engineering and health care. I am very thankful for his confidence in my work, his availability at any time, the discussion during which he shared his valuable experience and innovative ideas. Moreover, I am thankful for his careful review of this thesis;

Prof. Dr. med. Michael Bajka, my co-supervisor, who supported me through this thesis; he gave me the opportunity to work very close to his daily clinical work, provided me with precious feedback, ideas and inputs for the project and the thesis. I’ve appreciated his confidence and interest in my work and the unconditional presence during these years. I am thankful for his careful review of this thesis;

Prof. Dr. Kristin Myers, my co-supervisor, for the thorough review of my thesis as well as for the valuable corrections;

Ueli Marti, electrician at the IMES, for his amazing support in developing the aspiration device;

Prof. Dr. med. Roland Zimmermann, Dr. med. Katharina
Acknowledgments

Quack Lötscher, Dr. med. Kathrin Rohling, medical doctors at the University Hospital of Zurich, for such a straight-forward collaboration in the SOFTCERVIX study;

Prof. Dr. med. Jan Deprest, Dr. med. Jute Richter, MD Sander Galjaard, MD Geertje Callewaert, MD Julio Jimenez and Nikhil Sindhwani, medical doctors and PhD student at the University Hospital of Leuven (BE), for such a straight-forward collaboration in the SOFTCERVIX study;

Dr. med. David Scheiner, medical doctor at the University Hospital of Zurich, for presenting me to Prof. Dr. Bashiri and the great time we had in Israel, for providing me insight in gynecologic surgery and for such a straight-forward collaboration during the clinical study;

Arabella Mauri, Michela Perrini, Laura Bernardi, Johannes Weickenmeier, Manfred Maurer, Raoul Hopf, Dr. Alex Ehret, Nikolaos Karathanasopoulos, Marco Pensalfini, and Manuel Zündel, the Experimental Continuum Mechanics Group, for the good spirit, and the famous soup lunches in the LEE N-floor kitchen;

Robert Ernst, Andreas Lamprecht and Ivo Leibacher, the Mechanics and Experimental Dynamic Group, for the Mechanics I&II episodes;

Dr. Barbara Röhrnbauer, Dr. Thomas Schwarz and Dr. Johannes Hengstler for the exhausting muscle pump sessions;

Jean-Claude Tomasina, mechanics at the IMES, who provided support in manufacturing mechanical parts for the aspiration device;

Gabriela Squindo, Beate Fonfe and Dr. Stephan Kaufmann, for the administrative and IT support;

Dr. Jose Txema Mateos, for his great support in generating MPM images at the Center for Microscopy and Image Analysis;
Christian Pfister, Fiber Optic P. & P. AG, for his enduring and uncomplicated support during the development and maintenance of the aspiration device;

Laura Frese, Post Doc at Regenerative Medicine Institute, for her introduction in biochemical assays of the ECM;

Petra Mathieu-Minnig, Mahendraraja Krishnar and Attila Bitai, the team at USZ sterilization unit, for their professional support concerning the hygienic procedure of the aspiration device;

Dr. Lisette Paratore, Dirk Smolinski, Dr. Annette Widmann and Dr. med. Gabriela Senti, the team at CTC Zurich, for the support in creating the complete study documentation and generation of eCRF in Secutrial at University Hospital of Zurich;

Martin Kenner, supervisor at Electro Suisse, for the examination of electrical security in the aspiration device;

Günther Schnell, microbiologist at Qualis Laboratorium, for his validation of the cleaning and sterilization procedure of the aspiration device;


Zudem geht mein Dank an meine Freunde: Melissa Caflisch, Olivier Greder, Anna Vichery, Yvonne Ascherberg, Andrea Fuchs und Stephanie Kochbeck.

Sabrina Badir
Zurich, January 2015
# Contents

Abstract III  
Zusammenfassung VII  
Acknowledgments XI  

## 1 Introduction  
1.1 Background of preterm birth ........................................... 1  
1.1.1 Prediction .......................................................... 2  
1.1.2 Prevention ......................................................... 3  
1.2 Biomechanical modeling and measurement techniques ........... 3  
1.3 Thesis thread .......................................................... 5  
1.3.1 Objectives .......................................................... 5  
1.3.2 Structure ............................................................ 5  

## 2 The human uterine cervix  
2.1 Anatomy ................................................................. 7  
2.2 Microstructure ........................................................ 8  
2.2.1 Collagen ............................................................. 9  
2.2.2 Glycosaminoglycans and proteoglycans ......................... 10  
2.3 Contraception .......................................................... 10  
2.4 The role of the cervix in pregnancy ............................... 11  
2.4.1 Interventions to prevent preterm birth ....................... 13  

XV
3.7 Conclusion ........................................ 54

4 In-vivo characterization of the uterine cervix in pregnancy 57
4.1 Introduction .................................. 57
4.2 Methods ...................................... 60
   4.2.1 Subjects .................................. 60
   4.2.2 Measurements ............................... 61
   4.2.3 Statistical analysis ......................... 61
4.3 Results ...................................... 62
4.4 Discussion ................................... 66
4.5 Conclusion ................................... 68

5 Elastography procedures for determination of cervical stiffness 71
5.1 Introduction .................................. 71
5.2 Methods ...................................... 73
   5.2.1 Quasi-static elastography ................. 73
      Phantom manufacturing ....................... 73
      Phantom characterization ..................... 74
      Elastography measurements .................. 74
   5.2.2 Data analysis of biomechanical procedures 76
5.3 Results ...................................... 76
   5.3.1 Quasi-static elastography ................. 76
   5.3.2 Data analysis of biomechanical procedures 78
5.4 Discussion ................................... 79
5.5 Conclusion ................................... 83

6 Biomechanics-based prediction of preterm birth 85
6.1 Introduction .................................. 85
6.2 Study preparation ............................. 85
   6.2.1 Clinical investigation plan ............... 86
      Data management .............................. 86
      Adverse events management .................. 86
      Study team .................................. 87
   6.2.2 Investigator brochure ..................... 87
6.3 Study protocol ................................ 89
## 9 Conclusions

9.1 Contribution of the present work ................................ 115
9.2 Outlook ......................................................................... 117
  9.2.1 Fundamental research ............................................. 117
  9.2.2 Clinical diagnosis and research ................................. 118

### Bibliography

121

### Appendix

141

A Aspiration tubes ................................................................. 141
  A.i Prototype 1.0 .............................................................. 142
  A.ii Prototype 2.0 .............................................................. 143
B Data management ................................................................. 144
1.1 Background of preterm birth

Spontaneous preterm birth (sPTB) is a pathological event that is defined as childbirth before completion of 37 weeks of pregnancy. According to Blencowe et al. (2012), approximately 15 million babies are born preterm every year, which is more than every 10th baby. 80% of these babies are delivered spontaneously while 20% are medically induced. The classification of preterm birth (PTB) is based on gestational age and divided into early (< 34 weeks) and moderate/late (> 34 weeks) (Goldenberg et al., 2008). Infants born prematurely have an increased risk of mortality and a variety of short term (e.g. respiratory complications, infections) and long-term morbidities (e.g. cognitive problems, cardiovascular diseases). The greatest risk for mortality and morbidity is for those children born at a very early gestational age. However, moderate and late preterm infants (about to 70% of all preterm infants) are still at risk to experience complications. The economic cost of PTB is high in terms of health care but also regarding special educational care in the later life (Blencowe et al., 2012; Lawn et al., 2013).
1. Introduction

Despite intense research that was conducted to identify women at risk, sPTB remains an unsolved problem in prenatal diagnosis.

1.1.1 Prediction

Research involved the assessment of different parameters such as maternal demographic characteristics (Goldenberg et al., 1998), biomarkers in the maternal blood (Ashoor et al., 2010; Beta et al., 2011) or in the vagina (Leitich et al., 2003), digital cervical palpation (Rozenberg et al., 2005) and cervical length measurement by trans-vaginal ultrasound (Iams et al., 1996). The study findings have shown that ethnicity, a history of prior sPTB, smoking, body-mass index, vaginal infection or an increased level of fetal fibronectin are associated with an increased risk of sPTB (Goldenberg et al., 2008). Particular interest has since risen in cervical morphology. Many large clinical studies evaluated predictive capabilities of the cervical length parameter for different populations, i.e. high-risk (Berghella and Mackeen, 2011), symptomatic (Tsoi et al., 2003), routine populations at different gestational ages (Greco et al., 2011; Heath et al., 1998; To et al., 2006) and including demographics, obstetric history and digital palpation (Celik et al., 2008; Reiter et al., 2012; To et al., 2006). The study results led to the agreement that a short cervix is the best available indicator of an impending sPTB (Berghella et al., 2007).

The measurement of the cervical length by trans-vaginal ultrasound is nowadays considered the gold standard method to detect women at risk (Iams, 2014). The most commonly applied criterion for diagnosis of sPTB is cervical length of less than 25mm in mid-trimester between 18 and 24 weeks of pregnancy (ACOG, 2012). Clinical evaluations have shown that the detection rate of this criterion (sensitivity) is about 37% for a false-positive rate of 8% (92% specificity) (Heath et al., 1998; Iams et al., 1996). This parameter can be further associated with obstetric history and maternal characteristics to improve sensitivity to 69% for a specificity of 90% (Celik et al., 2008; To et al., 2006). However, the measurement of cervical length is not sufficient to identify the majority of women who are truly at risk. So far too many costly resources have been applied on only supposedly endangered subjects, while premature births in low-risk women with a normal cervical length are still not recognized on time.
This is why new diagnostic tools are needed to early and reliably detect women at risk of sPTB (Feltovich and House, 2014).

1.1.2 PREVENTION

Pharmacological and structural approaches that are applied on the cervix are common therapies to prolong pregnancy. Cerclage and pessary devices fall in the latter category and aim at mechanically help the supportive function of the cervix to close the uterus at the distal end (see Chapter 2). Evidence indicates that providing cervical support in prevention of sPTB might be relevant for women with a short cervix or a history of previous sPTB (Iams, 2014; Romero et al., 2014). Progesterone is an important hormone to maintain pregnancy and the use of progesterone supplementation was shown to prevent sPTB. Driven by the remarkable 50% reduction of preterm delivery after vaginal progesterone application (Hassan et al., 2011) and cost-effectiveness analyzes by Werner et al. (2011), universal trans-vaginal screening with prescribed progesterone for short cervices is widely recommended by professional societies (ACOG and RCOG). However, this approach requires large numbers of staff well-trained in ultrasonography. Moreover, this carries a considerable risk that women with a cervical length at the borderline of the defined cut-off value will be subjected to unneeded therapy (Iams, 2014). Hence, early and reliable identification of pregnancies at risk using a simple diagnostic method is the key to allow existing and future interventions to increase the success rate of therapies (Feltovich and House, 2014; Greco et al., 2011; Nicolaides, 2011).

1.2 BIOMECHANICAL MODELING AND MEASUREMENT TECHNIQUES

Given the important functional role of the cervix in pregnancy and the relevance of cervical morphology in clinical diagnosis and intervention of sPTB, prenatal research currently focuses on the cervix using two dedicated approaches. With the introduction of biomechanical modeling and new measurement techniques this research aims at improve the under-
1. Introduction

standing of physiological and pathological processes in the human cervix. The acquired knowledge might be the key to reliably predict and prevent sPTB.

_Biomechanical modeling_ is a powerful tool to analyze the process of cervical deformation and quantify factors contributing to cervical dysfunction (House et al., 2012; Mahmoud et al., 2013). The research group at MIT (House and Socrate, 2006; Myers et al., 2010; Paskaleva, 2007) elaborated a complex finite element model based on anatomical data of a pregnant women and a constitutive model formulation representing the mechanical behavior of human cervical tissue. They identified essential factors governing the opening of the cervix which includes material properties and geometry of the cervix, uterine loading, material properties and adhesion of the fetal membrane. House et al. (2012) used an anatomically accurate numerical model of the uterus to study the deformation response of the cervix after application of fundal pressure to the system. The magnitude of fundal pressure was estimated in the subject by a vaginal pressure catheter placed into the vaginal canal close to the cervix. Using a simplified version of the constitutive model proposed by this group, the deformation response of the cervix was acquired from ultrasound images and matched with inverse FE analysis to estimate cervical mechanical parameters for the first time in-vivo. House et al. (2012) concluded that a patient-specific inverse FE model is an excellent tool to study the involved mechanisms but not appropriate to be applied in the routine prenatal care. However, they suggested a simplified methodology, which was not further explained in the publication, for clinical practice which could estimate a “cervical tissue compliance index” based on dynamic changes in the cervix measured with ultrasound.

Existing knowledge of microstructural and mechanical properties in the human cervix is mostly available from ex-vivo experiments on tissue harvest from non-pregnant and pregnant women at term. New measurement techniques have been developed to evaluate physical properties of the uterine cervix in-vivo. These techniques quantify mechanical (see Chapter 3, Chapter 5), optical or electrical properties (see Chapter 4) of the cervical stroma (Carlson et al., 2014; Fruscalzo and Schmitz, 2012; Gennisson et al., 2011; Hee et al., 2014, 2013; Liao et al., 2014; Maul et al.,
2005; McFarlin et al., 2010; O’Connell et al., 2000; Parra-Saavedra et al., 2011) and aim at detecting critical cervical parameters such as microstructural organization or softening of the cervix (Feltovich and House, 2014). Promising preliminary clinical results were recently obtained by determining mechanical properties of cervical tissue using dynamic elastography (Carlson et al., 2014; Gennisson et al., 2011), other ultrasound based procedures (Fruscalzo and Schmitz, 2012; Parra-Saavedra et al., 2011) and aspiration, see Chapter 4 and Chapter 5.

1.3 THESIS THREAD

1.3.1 OBJECTIVES

This thesis provides a novel method based on the aspiration technique for mechanical characterization of the uterine cervix during routine pregnancy consultations. The present work aims at contributing to the challenging open questions in prenatal research. The following objectives intent to improve current knowledge about the cervix in pregnancy:

- Quantification of cervical changes in terms of cervical softening during pregnancy.
- Evaluation of cervical stiffness in mid-pregnancy as a novel predictor of sPTB.

1.3.2 STRUCTURE

The structure of this thesis is along the lines of different projects conducted to achieve the defined objectives. Chapter 2 introduces the anatomy of the human uterine cervix and its physiological role in pregnancy. Chapter 3 presents a novel in-vivo measurement procedure used to characterize the mechanical behavior of the human uterine cervix. This chapter also covers measurements performed on non-pregnant women in order to develop an appropriate protocol. The corresponding inverse analysis to determine representative model equations for cervical tissue from
1. Introduction

non-pregnant and pregnant conditions has been elaborated. The finite element based parametric study, focusing on the uncertainties related to the experiments, is presented and the advantages and disadvantages of this novel procedure are discussed. Chapter 4 presents a prospective clinical study to describe the evolution of ecto-cervical stiffness in normal pregnancy. In Chapter 5 a mechanical analysis is presented to rationalize results from available ultrasound based methods for quantitative determination of mechanical properties of the cervix during pregnancy. The results provide insight into the limitations of quasi-static ultrasound procedures and the possibility to predict sPTB. Based on the promising findings presented in Chapter 4 and Chapter 5, the clinical multicenter study SOFTCERVIX is presented in Chapter 6, aiming at improving the prediction of sPTB using biomechanical parameters. In this chapter information about the study preparation process, the corresponding study protocol and the current state of the study is provided. Chapter 7 presents a preliminary study that combines in-vivo aspiration measurements and corresponding microstructural analysis in order to rationalize different biomechanical behaviors. Chapter 8 represents another identified field of application for the novel measurement procedure. A clinical study is presented using the novel procedure to quantify pharmacologically induced cervical softening in women prior to the placement of an intra-uterine contraceptive. Chapter 9 summarizes the main findings of this thesis and gives an outlook for future research.
CHAPTER TWO

THE HUMAN UTERINE CERVIX

2.1 ANATOMY

The uterus is located inside the pelvis and is supported by pelvic floor muscles and several ligaments. The cervix is the cylindrically-shaped part of the uterus that protrudes into the vaginal canal, see Figure 2.1. The cervical opening into the vagina is called the external os. The external and internal os are connected by the cervical canal which builds a link between the uterine cavity and the vagina. The vaginally accessible part of the cervix is called ecto-cervix and is approximately 35mm long and has a diameter of 25mm. The cervical wall is around 10mm thick. These values vary significantly during pregnancy. The ecto-cervix is covered with squamous epithelium (thickness $\leq 5\text{mm}$) whereas the cervical canal and the internal os close to the uterine cavity are lined by columnar epithelium. The transition from squamous to columnar epithelium is called squamo-columnar junction. This junction is sometimes visible on the ecto-cervix. Ecto-cervical areas covered by columnar epithelium are more vulnerable and prone to injuries (Danforth, 1983; Ludmir, 2000), see Figure 2.1.
2. The human uterine cervix

![Diagram of the uterine cervix](image)

**Figure 2.1 Uterine cervix:** The uterine body and uterine cervix are illustrated, top. The part protruding into the vaginal canal is called ecto-cervix. The ecto-cervix is shown through the vaginal canal with help of a speculum to dilate the vaginal canal. The dark red part in the middle of the ecto-cervix close to the cervical canal is the squamo-columnar junction, bottom. The pictures are reprinted with permission from Primal Pictures.

### 2.2 Microstructure

Compared to the uterine wall, that is mainly composed of muscular tissue, the cervix consists of collagenous tissue. Fibroblast cells secrete an extracellular matrix (ECM) that mainly contains fibrillar collagen, glycosaminoglycans and proteoglycans.
2.2.1 \textbf{COLLAGEN}

In collagenous tissues, the collagen structure provides the tissue with its stiffness and strength properties. The majority of collagen types in the cervical stroma consists of fibrillar collagen fibers (type I and III). A collagen fibril is formed by a string of multiple collagen molecules. Assembly of these fibrils involves initially immature divalent cross-linking such as dihydroxylysino norleucine (DHLNL) and subsequently mature trivalent intermolecular cross-links such as deoxypyridinoline (DPD) and pyridinoline (PYD). The cross-link formation is regulated by the enzyme lysyl oxidase (LOX) (Frantz et al., 2010; Fratzl, 2008). Another cross-link group is the non-reducible advanced glycation cross-links such as pentosidine (PEN) that increases during aging and in hyperglycemic environments (Brownlee, 1995). The most abundant collagen cross-link found in the cervical tissue is PYD followed by DHLNL and DPD. PEN is only present in smaller amounts and more abundant in the ecto-cervix than in the endocervix (Zork et al., 2014). Fiber architecture of the human uterine cervix was investigated using X-ray diffraction (Aspden, 1988) and MRI diffusion tensor imaging (Weiss et al., 2006). Two to three distinct zones of fiber alignment were found. These zones are defined in radial direction ($r$) from the cervical canal towards the cervical border.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{collagen_fiber_orientation.png}
\caption{Collagen fiber orientation in the uterine cervix: Collagen fibers point in axial ($a$) and circumferential ($\phi$) direction in cervical stroma. This illustration is based on the figure in Aspden (1988).}
\end{figure}
X-ray diffraction showed that along the cervical canal and in the outer zone, the fibers are predominantly oriented in axial \((a)\) direction, whereas the fibers in the middle zone point in circumferential \((\varphi)\) direction (Aspden, 1988), see Figure 2.2. In MRI diffusion tensor imaging, the main collagen fiber direction close to the cervical canal was found to be in axial \((a)\) direction, while the outer zone showed main fiber alignment in circumferential \((\varphi)\) direction (Weiss et al., 2006).

### 2.2.2 Glycosaminoglycans and Proteoglycans

Overall, tissue integrity and load bearing function of cervical tissue is determined by a network of interactions among collagens, glycosaminoglycans (GAGs) and proteoglycans. GAGs are polysaccharides located in the ECM and affect hydration as well as swelling of tissue by binding large amounts of water due to the negatively charged molecules. The negative charges are important for the electrostatic binding of GAG to other molecules, such as collagen (Akgul et al., 2012; Uldbjerg and Malmstrom, 1991). There are sulfated and non-sulfated GAGs in the ECM. The former include chondroitin sulfate (CS) and dermatan sulfate (DS) in the cervical tissue. They link to a core protein, thus forming proteoglycans such as decorin, biglycan, fibromodulin, osteomodulin, aspirin and versican. Hyaluronan (HA) is a non-sulfated GAG that only exists as free glycosaminoglycan and also attracts large amounts of water molecules (Akgul et al., 2012; Uldbjerg and Malmstrom, 1991).

### 2.3 Contraception

The pathway of a sperm targeting an ovum first passes the cervical canal before it reaches the uterine cavity and uterine tubes. To suppress the fertilization of the ovum, different types of contraception exist, such as intra-uterine contraceptives, condoms, contraceptive pills and many others. The principle of the hormonal intra-uterine contraceptive (IUC) is introduced here and the relevance of cervical consistency for the placement of the contraceptive is later discussed in Chapter 8. The hormonal
IUC (LNG IUS) is a small T-shaped piece of plastic that is inserted transvaginally into the uterine cavity to prevent pregnancy, see Figure 2.3. The contraceptive is coated with the hormone levonorgestrel, a type of progesterone, which is released into the uterine cavity. Only small amounts of hormone reach the systemic circulation after a few weeks (Sitruk-Ware and Inki, 2005; Tang et al., 2007). The primary mechanism of action is to act locally and hormonally prevent the ovum implantation. This includes several contraceptive effects such as cervical mucus thickening and suppression of endometrium growth (Caron, 2007; Searle, 2014). Every 5 years the IUC has to be replaced, after a decline of 50% of the initial release rate.

Figure 2.3 Hormonal intra-uterine contraceptive (LNG IUS): Implanted contraceptive in the uterine cavity (frontal view), left. T-shaped piece of plastic, right. The pictures are reprinted with permission of Prof. Michael Bajka and Manfred Maurer.

2.4 The role of the cervix in pregnancy

The cervix is a dynamic organ that changes its mechanical properties through a remodeling process that influences the ECM composition (see
next sections). This property allows the cervix to have two opposed functions in pregnancy, temporally separated from one another. During pregnancy the uterine cervix, supported by the surrounding muscles and ligaments, resists the imposed gravitational forces and intra-uterine pressure to allow fetal development in the uterine cavity in a progesterone dominated hormonal environment, see Figure 2.4.

Close to term, the concentration of estrogen (also a steroid hormone as progesterone) increases and antagonizes the pregnancy maintaining progesterone. This leads to an increase of oxytocin (hormone) and prostaglandin (hormone-like lipid molecule) (Silbernagl and Despopoulos, 2003). As a consequence, uterine contractions are induced and final cervical softening takes place (House and Socrate, 2006).

The level of cervical consistency must be appropriately coordinated with the initiation of uterine contractions at term. Large deformations of cervical tissue follow by increasing the diameter of the cervical canal up to 10cm. This cervical opening allows the passage of the child into the vaginal canal, see Figure 2.5.

Abnormal cervical remodeling leads to significant complications. Early
cervical remodeling reduces the capability of the cervix to resist the imposed loading and might lead to changes in the cervical morphology, thus increasing the risk of a spontaneous preterm birth (sPTB). On the other end, insufficient cervical remodeling could lead to post-term pregnancies that need cervical ripening agents (prostaglandins or anti-progesterone agents) to induce delivery (Lukoschus et al., 2003; Treacy et al., 2006).

Figure 2.5 Morphological changes throughout pregnancy: During pregnancy the uterine cervix is firm and closed to resist the imposed gravitational forces and uterine pressure, thus allowing fetal development. Close to term the internal os starts to dilate. This process reduces the closed part of the cervix. With initiation of uterine contractions the cervix dilates allowing the passage of the child into the vaginal canal.

2.4.1 INTERVENTIONS TO PREVENT PRETERM BIRTH

Women at risk of sPTB are treated to prolong the pregnancy. Two common strategies include structural and pharmacological approaches that are applied on the uterine cervix.

CERVICAL CERCLAGE

Cervical cerclage is a structural treatment to support the mechanical strength of the uterine cervix to prevent preterm cervical opening. In this surgical procedure sutures are used to close the cervix, see Figure 2.6.
2. The human uterine cervix

Typically, it is placed in the second trimester and the sutures are removed at term. There is evidence that women with a history of a sPTB, a short cervix or carrying more than one baby might benefit from this procedure (Alfirevic et al., 2012; Romero et al., 2014). However, most clinical trials reported adverse effects related with the procedure. The most dramatic event is the higher rate of maternal infection followed by the use of tocolytics to suppress contractions (Romero et al., 2014; Smith et al., 2009).

![Figure 2.6 Cervical cerclage: It consists of a suture (green) close to the internal os. The picture is reprinted with permission of Prof. Michael Bajka.](image)

CERVICAL PESSARY

Pessaries, usually used for pelvic organ prolapse, are available in a wide range of shapes and sizes. In case of a risk of preterm birth, some versions of a ring-like pessary are used to encompass the cervix similar to a cerclage, see Figure 2.7. A pessary is also recommended for women with a history of sPTB or short cervical length. In contrast to conventional cervical cerclage, cervical pessary offers a safe and simple alternative (Dharan and Ludmir, 2009).
The role of the cervix in pregnancy

PROGESTERONE

Progesterone is a pregnancy maintaining hormone. The vaginal administration of progesterone to prevent sPTB is motivated by the fact that progesterone is able to inhibit prostaglandin production and reduce uterine contractions. The use of progesterone for preventing preterm birth appears promising and is already widely applied vaginally on the uterine cervix in women at risk of sPTB (Romero et al., 2014; Smith et al., 2009).

Figure 2.7 Cervical pessary: A pessary ring closes the cervix (Dharan and Ludmir, 2009). The picture is reprinted with permission from Elsevier.
2. The human uterine cervix

2.5 Cervical Remodeling in Pregnancy

Given the complications that result from abnormal cervical remodeling, underlying molecular mechanism of cervical remodeling, cervical mechanical properties and microstructural composition throughout pregnancy were investigated. The investigation of the cervical remodeling process in humans is hampered due to the challenges of obtaining sufficient tissue samples throughout pregnancy. Animal models overcome this limitation. Advantages of the animal model, especially in mice, are numerous and include i) the ability to obtain timed cervical tissue at all time points in pregnancy, ii) the fact that the tissue is obtained within a short time interval (duration of pregnancy is 19 days), iii) the availability of mice with target mutations, iv) the fact that progesterone function loss results in the onset of labor in humans and mice and v) the evidence that mouse models show similar molecular processes during pregnancy as occurring in humans. Thus, advances in the understanding of cervical remodeling in mice provide also relevant insight into human cervical biology (Mahendroo, 2012).

2.5.1 Normal Cervical Remodeling

Recent studies on cervical tissue of mice have shown that cervical remodeling leads to changes in the ECM constituents in four stages during pregnancy and delivery (Word et al., 2007). First, the cervix gradually softens with measurable differences detectable around gestation day 12 of the mouse gestation period. This softening stage is characterized with a decline in collagen cross-link density, tissue growth, and increased vascularity (Akins et al., 2011; Read et al., 2007; Timmons et al., 2010). Second, the cervix becomes even more compliant before delivery around day 18, characterized by a progressive decline of collagen cross-link density, an increase in HA and water content (Akgul et al., 2012; Akins et al., 2011; Holt et al., 2011). Third, at delivery (day 19), the collagen network becomes further dispersed as the cervix dilates for delivery. Fourth, postpartum, repair mechanisms transform the cervix back to the non-pregnant state within one day (Word et al., 2007). These changes in the ECM correlate with the cervical stiffness (Read et al., 2007; Timmons et al., 2014;
Yoshida et al., 2014b), see Section 2.6.

2.5.2 Premature Cervical Remodeling

The mechanisms of premature preterm remodeling were studied on two murine models (Holt et al., 2011; Mahendroo, 2012). The first model (RU486) served as a hormonal withdrawal (non-infection) model. This model includes administration of the progesterone receptor antagonist mifepristone. Administration of mifepristone induces clinically preterm cervical remodeling and preterm birth. The second model reproduces the infection-induced preterm birth. Animals are treated vaginally with endotoxin lipopolysaccharide (LPS) found in the outer membrane of bacteria. This molecule triggers a pro-inflammatory response in the uterus similar to infection. Ascending vaginal infection into the uterine cavity is a well-known risk factor that might cause preterm birth (Goldenberg et al., 2008).

In both models, Akgul et al. (2012) identified similar changes in GAG content and GAG composition compared to normal remodeling, but the regulation of these processes seemed to differ between term and preterm birth. It was shown that different genes (Has 1 and Has 2) that encode the enzymes responsible for HA synthesis were activated. Has 2 gene expression was only found in term models. This expression is regulated by estrogen, while Has 1 gene expression in preterm birth models acts independent of estrogen (Akgul et al., 2012).

2.5.3 The Role of Estrogen

The gene Has 2 encodes enzymes responsible for HA synthesis during normal cervical ripening and is regulated by estrogen. This finding suggests that estrogen might play an important role in up-regulation of cervical HA at term. In fact, progesterone treatment in term mice suppressed the increase of HA content. Decreased local progesterone levels at term might allow the increase in cervical HA responsible for final remodeling of cervical tissue prior to the onset of contractions (Akgul et al., 2012).
2. The human uterine cervix

2.5.4 THE ROLE OF PROSTAGLANDIN

In clinics, beside mifepristone, misoprostol (prostaglandin) is used to induce cervical softening in both non-pregnant and pregnant women (Lukoschus et al., 2003; Radulovic et al., 2007). In Timmons et al. (2014) it was shown that an increase of local prostaglandin concentration is not needed for cervical remodeling in term and mifepristone-mediated premature ripening murine models. However, application of prostaglandins leads to a similar decline of tissue stiffness between mice treated with misoprostol or LPS. In infection-induced premature cervical ripening (LPS), the local prostaglandins concentration increases being necessary for the remodeling. In-vitro studies of human cervical tissue have shown that prostaglandin influences glycosaminoglycan synthesis (Carbonne et al., 2000; Uldbjerg et al., 1983) and collagen metabolism (Uldbjerg et al., 1983), thus inducing cervical remodeling.

2.5.5 THE ROLE OF PROGESTERONE

Vaginal progesterone has been reported to reduce the rate of sPTB in a high risk population. The biological mechanism for the protective effect of progesterone has been identified in Gonzalez et al. (2013). LPS mice models were treated with progesterone to investigate the capability to inhibit preterm delivery and the related cervical remodeling processes. Pretreatment with progesterone inhibited preterm delivery in LPS-treated mice. In these mice neither cervical remodeling induced by LPS nor inflammatory cell infiltration was found. Apparently progesterone acts as an anti-inflammatory agent on cervical tissue. This observation was also confirmed on a tissue stiffness level. LPS and progesterone treated mice had significantly stiffer tissue compared to mice treated only with LPS (Gonzalez et al., 2013).

The direct inhibitory action of progesterone during cervical remodeling is supported by the in-vitro experiments on human cervical fibroblasts by Carbonne et al. (2000). The results have shown that successive incubation of cervical fibroblasts with progesterone and prostaglandin led to an inhibition of prostaglandin induced GAG synthesis. This inhibitory mechanism is well known in obstetrics. At term the prostaglandin in-
duced activation of uterine contractions is regulated by local estradiol and progesterone concentrations (Abel and Baird, 1980).

2.5.6 SUMMARY

The described mice studies provided insight into cervical remodeling processes. The five most important aspects are shortly summarized. i) Cervical remodeling can occur due to different mechanisms, ii) premature cervical remodeling can not simply be considered as an acceleration of normal cervical remodeling, iii) initial cervical softening is promoted by decrease of enzymatic collagen cross-links and later followed by an increase in GAG and water content, iv) infection-induced cervical remodeling promoted by prostaglandin is inhibited by progesterone and v) progesterone and estrogen play an important role in suppressing or supporting cervical remodeling, respectively. However, it has to be proven yet that the same processes occur in humans.

2.6 MECHANICAL CHARACTERISTICS OF THE CERVIX IN PREGNANCY

2.6.1 EX-VIVO MECHANICAL PROPERTIES

Ex-vivo cervical tissue taken from hysterectomy subjects was studied to formulate a constitutive material model and to better understand the relationship between changes in ECM and the corresponding material characteristics (Fernandez et al., 2013; Myers et al., 2008, 2010, 2009; Yao et al., 2014). The investigations focused on different modes of deformation tailored to specific kinematic configurations. Uniaxial compression and tension stress-relaxation experiments along relevant anatomical directions (axial and circumferential) have demonstrated the complex non-linear, time-dependent and anisotropic material behavior of cervical tissue as well as asymmetric large-strain response in tension and compression. Material response was found to differ, depending on the obstetric history. Pregnant tissue (P) is by one order of magnitude softer than non-pregnant (NP) tissue. Furthermore, non-pregnant tissue of women with previous
vaginal delivery was shown to be stiffer than pregnant tissue, but non-
significantly softer than tissue from women who never had a vaginal de-
livery (Myers et al., 2008, 2010). Biochemical analysis of these samples
revealed that significant increases were measured for total GAG, HA and
collagen solubility, however no change in the total collagen content was
found (Myers et al., 2009).

The reported time-dependent stress relaxation response is attributed to
the ECM, including fluid-independent unraveling of the collagen cross-
link network and fluid-dependent draining of pressurized interstitial fluid.
These time-dependent mechanisms were further studied using two exper-
imental setups. First, direct measurements of the permeability of cervical
tissue based on Darcy’s law were performed to identify the poro-elastic
contribution to the time-dependent deformation mechanisms of the tissue.
An order of magnitude difference between the mean permeability of NP
and P tissues was found (Fernandez et al., 2013). Second, the indentation
method was used to measure the compressive time-dependent material
characteristics of cervical tissue slices with different thicknesses. Visco-
elastic material parameters were quantified using the inverse FE method.
A significant difference in the instantaneous and equilibrium shear mod-
ulus between NP and P samples was found, consistent with the findings
from uniaxial compression tests (Yao et al., 2014).

2.6.2 In-vivo loading and geometric conditions

The loading conditions in the cervix are complex and play an important
role during pregnancy. They can be categorized into active and passive
loading. Active loading is defined as the action of the myometrium (mus-
cle tissue in the uterine wall) called “uterine contraction”. In the finite
element model of Paskaleva (2007), contractions were simulated and the
effect on the cervix was studied. Close to the internal os the cervix is in
a state of tension with significant stresses close to the uterine cavity due
to contractions. However, the external part of the cervix is not affected.
Passive loading describes loading in absence of contractions. As the fetus
grows, increased intra-uterine pressure is applied on the cervix. As indi-
cated in Paskaleva (2007), fetal membrane adhesion and its mechanical
properties might have a significant influence on the distribution and the
magnitude of stresses acting on the cervix. In case of no adhesion, high stress concentrations are observed in a narrow area of the internal os. Lowering the cervical stiffness together with increasing the intra-uterine pressure results in the dilation of the internal os and a gradual bulging of the membrane into the cervical canal. In case of adhesion of the membrane to the cervix and uterine wall, the stresses are distributed over the whole area of the uterus. The effect of cervical length and cervical canal diameter were also investigated under intra-uterine pressure. The major difference among varying cervical canal diameters consists of the dimension of the affected area of stress concentration. In case of a very small cervical canal (4mm diameter) the stresses are confined to a very narrow ring at the internal os, while with a larger diameter (12mm), a substantially bigger part of the internal os is subjected to high stresses. Changing cervical length has no significant effects on the absolute values of stresses in the cervix. However the percentage of cervical stroma experiencing high stresses varies significantly. For the case of a very short cervix (15mm) almost the whole organ is affected, while the affected regions decrease with increasing length (Paskaleva, 2007).
CHAPTER THREE

ASPIRATION DEVICE AND MEASUREMENT PROTOCOL

3.1 Introduction

The development of diagnostic tools to evaluate the risk of spontaneous preterm birth (sPTB) requires further understanding of the changes in the mechanical properties during gestation. In finite element simulations of pregnancy and birth, constitutive models describing the mechanical behavior are needed to investigate the influence of cervical deformation (House et al., 2013), including the effects of interventions on the cervix conducted on patients at risk (House et al., 2013; Mancuso et al., 2010). The knowledge of the mechanical behavior of the cervix is required to study the interaction between fetal membrane and cervix in advanced gestation (House et al., 2013; Paskaleva, 2007) and for the investigation of vaginal delivery-related damage of the pelvic floor (Cosson et al., 2013; Lepage et al., 2014). Mechanical tests were performed ex-vivo on human cervical tissue in order to provide constitutive model equations and parameters describing cervical tissue which are needed to perform advanced FE simulations (Fernandez et al., 2013; Myers et al., 2010; Yao et al., 2014) as
well as to assess the correlation of stiffness parameters with microstructural components (Myers et al., 2008, 2009), see Chapter 2. To some extent, ex-vivo experiments achieve tight control of kinematic and kinetic boundary conditions, however they are performed on non-physiological configurations. Very limited quantitative data are available to describe the in-vivo mechanics of cervical tissue during gestation due to technical and ethical problems related to this kind of experiments. However, quantitative local in-vivo measurement of the mechanical properties of cervical tissue has the potential to describe the softening process throughout pregnancy and provide accurate tissue classification for early detection of a risk of preterm birth. To this end, an in-vivo measurement procedure is introduced to determine cervical stiffness. Finite element calculations were performed to analyze the main sources of measurement uncertainties and to determine constitutive model equations from an inverse analysis of the data from non-pregnant and pregnant subjects. Parts of this chapter, including paragraphs of text, figures and tables are published in Badir et al. (2013a).

### 3.2 Aspiration method - previous work

The instrumentation for tissue aspiration was developed by Kauer (2001) and modified by Nava (2007) to characterize the in-vivo mechanical behavior of soft tissues. The working principle of this technique is based on the pipette aspiration technique (Aoki et al., 1997) consisting of the application of a user-defined pressure profile to the underlying tissue with a cylindrical probe. The applied pressure is a relative pressure difference, i.e. the atmospheric pressure \((p_{at})\) is reduced to a lower pressure level \((p_{at_r})\) and the difference results in the applied pressure, usually referred to as negative pressure \(p_{neg}\):

\[
p_{neg} = p_{at} - p_{at_r}
\]  

(3.1)
Aspiration method - previous work

Figure 3.1 Side-view profile of deforming tissue: In this image the deformation of vaginal tissue is illustrated.

A camera inside the probe captures the side-view profile of the deforming tissue, see Figure 3.1. After post-processing of the images, the pressure-displacement behavior is obtained. There are two possible approaches to analyze the recorded data. The first approach is based on solving the so-called “inverse problem” by iterative comparison of measured and calculated tissue response using the FE method. At the end of this procedure, constitutive model parameters describing the tissue behavior for a specific model formulation are obtained. The second approach consists in the definition of scalar mechanical parameters that are extracted directly from the experimental data. This procedure provides limited information about the mechanical behavior of the investigated tissue, however the calculation of scalar parameters is straightforward and allows to compare the mechanical behavior of different subjects or the correlation with microstructural components. Both approaches have successfully been applied in clinical studies on the liver (Hollenstein et al., 2013; Mazza et al., 2007; Nava, 2007). For female
pelvic organs so far only scalar parameters were extracted from aspiration measurements on the vaginal wall (Roehrbauer, 2013) and in preliminary investigations on the uterine cervix (Bauer et al., 2009; Mazza et al., 2006).

Based on these preliminary clinical studies, the feasibility and safety of this procedure for future applications on the cervix was proven (Mazza et al., 2006). The dimensions of the external and internal diameter of the aspiration tube were successively reduced to a final external diameter of 15mm and internal diameter (aspirator opening) of 8mm. The miniaturized device allowed for simple placement on the ecto-cervix but also reduced the amount of tissue involved, increasing the locality of the measurement. An average tissue displacement of 3 to 4mm with an aspirator opening of 8mm was measured during these preliminary measurements on the cervix. First results on pregnant subjects showed a decrease of cervical stiffness with gestational age. Consequently the applied pressure level ($p_{\text{neg}}$) was adapted for different pregnancy stages (Bauer et al., 2009).

3.3 Device and measurement procedure

The encouraging results of the feasibility evaluation of aspiration measurements on the cervix led to the development of a novel procedure for measurements on pregnant subjects. To this end, the existing aspiration device was redesigned and the measurement procedure was adapted. The main idea was to apply a progressive negative pressure ($p_{\text{neg}}$) with the aspiration tube on the cervical tissue but limit the deformation to a maximum level. The higher the stiffness of the tissue, the larger the negative pressure $p_{\text{neg}}$ required to reach the pre-defined displacement of 4mm. Thus, the force controlled end-point ($p_{\text{neg}}$) from previous aspiration devices was changed to a displacement controlled end-point (Sarvazyan, 2011). The introduction of a displacement controlled endpoint aimed at increasing safety of the application by limiting the magnitude of deformation, but also simplifying trans-vaginal application and reducing duration of the measurement. For the first time the new device allowed for the integration of aspiration measurements into clinical practice.
Device and measurement procedure

In the next paragraphs the developed prototypes 1.0 and 2.0 are presented, followed by a detailed description of the measurement procedure.

### 3.3.1 Prototype 1.0

The instrument illustrated in Figure 3.2, consists of a circular tube with inner diameter of 8mm and external diameter of 12mm connected to a peristaltic pump used to generate a progressive negative pressure $p_{neg}$ inside the tube. Air is extracted through a thin pipe called evacuation pipe. The evacuation pipe has a diameter of 1mm and is set back from the aspirator tip surface by 4mm.

![Prototype 1.0 of the aspiration device](image)

**Figure 3.2** Prototype 1.0 of the aspiration device: a) Trolley with peristaltic pump, monitor, aspiration tube and pedals; b) aspiration tube with circular opening at one extremity; c) close up view of the aspiration tip: the integrated camera, LED and pressure sensor are placed within the tube.

Two pressure sensors with precision of 1mbar are integrated into the system: one is located at the aspiration tip (pressure sensor 1, $p_{neg,c}$) and the other (pressure sensor 2, $p_{neg,n}$) in the control unit. A CCD-minicamera with image quality of 120'000 pixels (see Figure 3.3) and a LED for illumination, both pointing at the aspiration opening and thus tissue surface,
are integrated into the tip of the tube. The whole device is mounted on a trolley for easy transportation and placement in the doctor’s practice. The probe is designed such that the thin evacuation pipe and the cylinder in contact with the subject can be disassembled, as shown in Appendix A, for cleaning and sterilization following standard autoclave procedure according to Swissmedic (2005). A schematic illustration of the aspiration system is shown in Figure 3.4. Technical drawings of the aspiration tube are shown in the Appendix A and device specifications are summarized in Table 3.1.

Figure 3.3 Camera image: The camera offers an image quality of 120’000 pixels on the image area and provides a top view of the tissue deformation.
### Device and measurement procedure

<table>
<thead>
<tr>
<th>Unit</th>
<th>Type</th>
<th>Manufacturer</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCD Camera</td>
<td>IntroSpicio 115</td>
<td>Medigus</td>
<td>S-video output</td>
</tr>
<tr>
<td>Foot pedal</td>
<td>Pneumatic</td>
<td>Camozzi</td>
<td>2+/-8 mbar</td>
</tr>
<tr>
<td>Foot pedal</td>
<td>Electrical</td>
<td>Marquardt</td>
<td>PN: 2410.0401</td>
</tr>
<tr>
<td>Peristaltic pump</td>
<td>401U/D1</td>
<td>Watson-Marlow</td>
<td>5 rollers, max rpm: 200</td>
</tr>
<tr>
<td>Valve</td>
<td>VDW11-5G</td>
<td>SMC</td>
<td>-</td>
</tr>
<tr>
<td>Tubing for pump</td>
<td>Bioprene</td>
<td>Watson-Marlow/</td>
<td>ID:0.8mm D:2.4mm</td>
</tr>
<tr>
<td>Tubing for system</td>
<td>Silicone</td>
<td>Maag Technik</td>
<td>ID:2mm D:5mm</td>
</tr>
<tr>
<td>Pressure sensor</td>
<td>MC-104</td>
<td>Keller</td>
<td>+/- 480 mbar, absolute</td>
</tr>
<tr>
<td>Control board</td>
<td>Bascom AVR</td>
<td>MCS</td>
<td>V1.11.8</td>
</tr>
<tr>
<td>Body assembly</td>
<td>-</td>
<td>Fiber Optics</td>
<td>-</td>
</tr>
<tr>
<td>Adhesive</td>
<td>KE 180</td>
<td>Astorit</td>
<td>Epoxy resin</td>
</tr>
</tbody>
</table>

**Table 3.1 Prototype 1.0: Device specification.**

![Diagram](image)

**Figure 3.4 Aspiration system:** Schematic illustration with all components.
3. Aspiration device and measurement protocol

3.3.2 Prototype 2.0

For the clinical study presented in Chapter 6, Prototype 1.0 was re-designed to fulfill all requirements for this clinical study and at the same time to reduce manufacturing costs for four aspiration stations, see Figure 3.5 and Table 3.2. All electronic parts, namely LED, camera and pressure sensor, were removed from the front part of the aspiration tube and relocated to the control unit of the device. This solution allowed a massive reduction of costs. After removing all electronic parts, the aspiration tube remained a simple metal housing which allowed for the autoclave sterilization procedure. Trial runs with different adhesives ensured compatibility with high temperatures (134°C) during sterilization of the new setup. Factors such as concentration of the detergent solution, duration of exposure and temperature attacked the anodic oxide layer of the aluminium and changed the appearance of the aspirator handle surface. Thus it was decided to treat the aluminium surface with ematal instead of anodic oxide. The camera at the tip of the aspiration tube was substituted by a fiber optic bundle (endoscope). The optical setup includes a stack of lenses in front of the image bundle, which is made of fused quartz glass fibers offering an image quality of 30’000 pixels on the image area, see Figure 3.6. The endoscope is introduced into the aspiration tube and connected with a 2m medical cable to the non-sterile CCD camera in the control unit, which transmits the image to the monitor screen. The field of view captures the aspiration opening and tissue surface, as in prototype 1.0. The focus plane is set at the edge of the tip (adjustable). Since the endoscope is physically separated by a glass plate from the tissue or any liquid and does not need sterilization, every aspiration station is equipped with only one endoscope. Illumination is provided by glass fibers that are integrated in the aspiration tube and connected to the LED in the control unit. Disposable air filters are used to separate the sterile aspiration tube from the non-sterile control unit. The latter is an aluminium box including all electronical parts (see Table 3.2). Temperature and humidity differences between the vaginal canal and the outside during the application of the aspiration device lead to condensation at the glass plate, which deteriorates the endoscopic view. Thus a heating device is used to warm the scope to body temperature prior to application.
## Device and measurement procedure

<table>
<thead>
<tr>
<th>Unit</th>
<th>Type</th>
<th>Manufacturer</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCD Camera</td>
<td>TV7181</td>
<td>ABUS</td>
<td>S-video output</td>
</tr>
<tr>
<td>Foot pedal</td>
<td>Electrical</td>
<td>Marquardt</td>
<td>PN: 2410.0401</td>
</tr>
<tr>
<td>Pump motor</td>
<td>Step motor</td>
<td>Marquardt</td>
<td>-</td>
</tr>
<tr>
<td>Perist. pump head</td>
<td>120U/114 DV</td>
<td>Watson-Marlow</td>
<td>5 rolls, max rpm: 200</td>
</tr>
<tr>
<td>Valve</td>
<td>VDW11-5G</td>
<td>SMC</td>
<td>-</td>
</tr>
<tr>
<td>Tubing for pump</td>
<td>Bioprene</td>
<td>Watson-Marlow/Maag Technik</td>
<td>ID: 0.8mm D: 2.4mm</td>
</tr>
<tr>
<td>Tubing for system</td>
<td>Silicone</td>
<td>Maag Technik</td>
<td>ID: 2mm D: 5mm</td>
</tr>
<tr>
<td>Pressure sensor</td>
<td>MPX5100</td>
<td>Freescale</td>
<td>0-1 bar, differential</td>
</tr>
<tr>
<td>Control board</td>
<td>Bascom AVR</td>
<td>MCS</td>
<td>V1.11.8</td>
</tr>
<tr>
<td>Body assembly</td>
<td>-</td>
<td>Fiber Optics</td>
<td>-</td>
</tr>
<tr>
<td>Adhesive</td>
<td>Araldit Standard</td>
<td>Huntsman</td>
<td>Epoxy resin</td>
</tr>
<tr>
<td>LED</td>
<td>Alustar</td>
<td>LEDXON</td>
<td>3W, 700mA, cold white</td>
</tr>
<tr>
<td>Power supply</td>
<td>ECM60UT32</td>
<td>XP Power</td>
<td>Medical approved</td>
</tr>
<tr>
<td>Monitor</td>
<td>FA1046</td>
<td>Lilliput</td>
<td>S-video output</td>
</tr>
<tr>
<td>Trolley</td>
<td>-</td>
<td>Schmitz AG</td>
<td>PN: 233.606</td>
</tr>
<tr>
<td>Air filter</td>
<td>-</td>
<td>Thermofisher</td>
<td>X200 25MM</td>
</tr>
<tr>
<td>Heating device</td>
<td>-</td>
<td>2MEDICAL</td>
<td>ClearView</td>
</tr>
</tbody>
</table>

Table 3.2 **Prototype 2.0**: Device specification.
3. Aspiration device and measurement protocol

Figure 3.5 **Prototype 2.0 of the aspiration device**: a) Trolley with peristaltic pump, monitor, aspiration tube and pedal are visible; b) aspiration tube with circular opening at one extremity; c) close up view of aspiration tip: glass plate covering the endoscope, glass fibers for illumination and evacuation and cavity pressure pipes are placed within the tube. The pictures were reprinted with permission of Manfred Maurer.
Aspiration measurements are performed on the vaginally accessible ectocervix (see Chapter 2). For this purpose, a speculum is used to dilate the vaginal canal and obtain access to the organ. Visual monitoring via the built-in camera allows placing the tip of the aspiration device as gently as possible orthogonally on the anterior lip of the cervix on the squamous epithelium (and not on the columnar epithelium to prevent bleeding), while the peristaltic pump is running. The suction procedure starts as soon as tight closure with the tissue is reached. The camera feedback allows not only to guide safe intra-vaginal placement of the instrument but also to monitor cervical tissue deformation during the aspiration. However, the camera captures the top-view deformation of the tissue and is not used to quantify tissue deformation as in previous versions (Hollenstein et al., 2013; Mazza et al., 2007; Nava, 2007). The measurement cycle proceeds as follows: the pressure ($p_{at}$) in the tube is progressively reduced by extraction of air through the evacuation pipe. Cervical tissue is sucked into the aspiration tube through the circular opening until the tissue vault reaches and closes the evacuation pipe at 4 mm peak extension, see Figure 3.7 (a safe and well-defined magnitude of tissue displacement based
on preliminary studies). This corresponds to a threshold difference of pressure values detected by the pressure sensors 1 \( (p_{neg_c}) \) in the aspirator cavity and the pressure sensor 2 \( (p_{neg_p}) \) in the evacuation pipe \( (\Delta p_{neg_p} = \Delta 10\text{mbar}) \). A digital display allows the investigator to monitor the continuously increasing negative pressure \( p_{neg} \) until closure (indicated by a light and acoustic signal). The negative pressure \( p_{neg} \) required to deform the tissue up to 4mm is named closing pressure \( p_{cl} \) [mbar] and is the sole scalar mechanical parameter of the experiment. \( p_{cl} \) is a direct measure of tissue stiffness. The corresponding pressure history is recorded as a text file on a memory stick for data storage and quality checks. The pressure is then immediately and completely reversed to atmospheric conditions \( (p_{at}) \). At this moment, the tip of the device is detached from the cervix. Depending on the stiffness of the investigated organ the duration of the experiment varies, with faster deformation of softer tissue for the same prescribed pressure history. The loading ramp was chosen such that the duration of the experiment does not exceed one minute but sufficiently slow to measure quasi-static response of the tissue. Note that visco-elastic effects affect the outcome, see Section 3.5.2. The whole procedure is controlled by the physician using two foot pedals (in prototype 2.0 only one pedal with same functions). One serves as a start button and the second pedal as the emergency stop. The physician can interrupt the suction procedure by using the latter. The pressure sensors are zeroed after every measurement. However, to ensure that pressure sensors provide long term consistency of readings and the peristaltic pump a meaningful loading ramp, the setup is regularly tested using a silicone block of known consistency and an aspirator holder as shown in Figure 3.8.
Device and measurement procedure

Figure 3.7 Measurement procedure: The aspirator is placed at 12 o’clock position on the ecto-cervix, left. Cervical tissue is sucked into the cavity formed by the tube and the tissue forms a nearly half spherical cup until it touches and closes the thin evacuation pipe, right.

Figure 3.8 Calibration set-up: The aspiration tube is mounted into the aspirator holder and positioned in contact with the silicone block.
3. Aspiration device and measurement protocol

3.3.4 Cleaning procedure for prototype 2.0

The used aspiration tube\(^1\) is disconnected from the control unit and the endoscope is removed. The tube is cleaned with detergent on the surfaces, in the interior of the tube and finally flushed with a syringe. The pre-cleaned aspiration tube is connected to the sterilization tray with two silicone tubes providing safe positioning of the device. The metal tray (see Figure 3.9) with an identification tag allows integration of this device into the daily working process of the sterilization unit. In the sterilization unit the tray is connected by the two luer-locks to the washer-disinfection machine before the cleaning process starts. The device undergoes, as specified in Swissmedic (2005), different cycles of cleaning with deionized water at different temperatures with and without detergent for 10 minutes. Subsequently the device is thermally disinfected for 10 minutes. Finally the device is dried for 20 minutes. A first check is performed to assess if the device is completely dry and clean before the whole tray is wrapped in sterile paper. A sterilization cycle of 18 minutes at 134\(^\circ\)C finishes the preparation of the device for re-use.

Figure 3.9  Metal tray for sterilization: Individual parts of the aspiration tube are mounted in the metal tray.

\(^1\)The cleaning procedure for prototype 1.0 deviates slightly due to different design and is not further described in detail
3.4 Methods

3.4.1 In-vivo measurements

50 non-pregnant women were asked to take part in the study and provided informed consent for the investigations (ethical approval by KEK Zurich StV0272007 and following amendments). Measurements on all but 10 subject were performed in one single individual session as follows: A first measurement was performed, the tube was detached from the measurement position. After a few seconds, the tube was reattached to perform the second measurement at the same position. Repeated measurements at 3-4 hour intervals were performed on 10 of the non-pregnant women, in order to evaluate the reproducibility of the aspiration test. On 10 subjects, measurements were performed at different locations on the ecto-cervix (12, 3, 6, 9 o’clock).

3.4.2 Finite element simulations

An axisymmetric finite element (FE) model was implemented to analyze the aspiration measurement into the commercial FE software Abaqus (Abaqus, 2010). Dimensions and non-central placement of the aspirator (thus avoiding the cervical canal) ensured rather homogenous tissue at the measurement site, thus justifying an axisymmetric approach (Figure 3.10). The aspirator was modeled as a rigid body in order to simulate the aspirator being pushed onto the cervix with an applied force of CF=0.2N in axial direction. Contact between tissue surface and aspirator was considered as hard in normal direction with sliding being allowed in tangential direction. Cervical tissue is generally wet and slippery, but in most cases some portion of fluid was removed in order to improve conditions for the measurement. Therefore, the contact between aspirator (master surface) and tissue (slave surface) in the reference calculation was initially assumed to have a friction coefficient of $\mu=0.1$, as reported in Nava (2007). The suction load was modeled as a pressure load, over the free surface of the tissue within the aspirator opening. The lateral boundary of the tissue
was modeled as free, and a vertical constraint was imposed at the bottom. The material for the cervix was assumed to be isotropic, homogeneous and elastic. A reduced polynomial form n=1 (Neo-Hook, NH) was implemented as the material formulation, whereas the constitutive parameters were identified via an inverse analysis of aspiration measurements (average $p_{cl}$ value for the non-pregnant cervix of 32kPa (320mbar) for an apex displacement of 4mm). This simple model formulation was selected in order to obtain a unique set of constitutive model parameters from the inverse analysis.

In order to study the effect of the material nonlinearity on the investigated model, a second set of simulations were performed with a reduced polynomial form (n=2, RP2) as an extended material description. The constitutive parameters for RP2 were defined as follows: The RP2 material description was fitted to the uniaxial stress-strain response of non-pregnant cervical tissue reported in Myers et al. (2010). Using the resulting parameters to simulate the aspiration experiment of non-pregnant subjects, an apex displacement of 4mm for 320mbar could not be obtained. Thus, parameters $C_{10}$ and $C_{20}$ had to be adapted. Their absolute values were reduced in order to reach 4mm apex displacement for 320mbar of negative pressure. However, to keep consistent with the overall nonlinear stiffening behavior of the tissue at moderate strains (15%) - as seen in the stress-strain response of uniaxial tension, which served as a basis for initial parameter estimation, the ratio of $C_{20}$ to $C_{10}$ was kept constant. This resulted in a new set of $C_{10}$ and $C_{20}$. The bulk modulus ($K$) was set to 1000kPa to represent high resistance to volume change. The mesh was refined to smaller element size near the aspirator opening to account for high strain gradient in that region. The final model had approximately 6000 elements. Six-node quadratic axisymmetric triangle elements with displacement-pressure hybrid formulation (CAX6H) were used to discretize the tissue portion.

---

2A fully incompressible formulation led to convergence problems
Aspiration experiments were simulated using an axisymmetric model: FE mesh, dimensions, boundary conditions and loads (contact force $CF$, $p_{\text{neg}}$, friction coefficient $\mu$) are indicated, left. Non-central placement of the aspirator, right.

Hyperelastic materials are described in terms of a strain energy potential $W$, which represents the stored strain energy density of the material as a function of deformation. The Neo-Hookean (NH) is the simplest form.
of the so called “reduced polynomial functions” (Holzapfel, 2006). The
compressible form of NH is defined as

\[ W = C_{10}(I_1 - 3) + D_1(J - 1)^2 \]  (3.2)

where \( C_{10} \) and \( D_1 \) are the material parameters. The reduced polynomial
formulation of second order (RP2) is defined as

\[ W = C_{10}(I_1 - 3) + C_{20}(I_1 - 3)^2 + D_1(J - 1)^2 + D_2(J - 1)^4 \]  (3.3)

where \( C_{10} \), \( C_{20} \), \( D_1 \) and \( D_2 \) are the material parameters. \( C_{j0} \) characterize
the distortional response of the material whereas \( D_j \) are the material
parameters accounting for its dilatation. \( I_1 \) depends on the principal
stretch \( \lambda_i (i=1,2,3) \) as

\[ I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \]  (3.4)

In \( \overline{I}_1 \) only the distortional response of the material (Holzapfel, 2006) is
considered. It is defined as

\[ \overline{I}_1 = J^{-2/3}I_1 \]  (3.5)

while \( J \) is a measure for the volumetric change. It is defined as

\[ J = \lambda_1\lambda_2\lambda_3 \]  (3.6)

This finite element model applied both NH and RP2 formulations was
used to perform parametric studies to investigate the influences of different
parameters and measurement conditions on the measured value of closure
pressure.
3.5 Results

3.5.1 In-vivo Measurements

Measurement position

In order to evaluate the dependence of the $p_{cl}$ on the placement of the aspirator on the cervix, measurements were performed at different positions (12, 3, 6, 9 o’clock). Variability among subjects is lowest for 12 o’clock position. Frequently, larger compressive forces were needed to obtain a tight contact between the aspirator and tissue for the two lateral positions, 3 and 9 o’clock. Additionally, the cervix is typically inclined towards the posterior vaginal wall, often hampering access to the posterior lip at 6 o’clock. This assessment led to the decision to perform aspiration measurements on the anterior lip of the cervix at the 12 o’clock position, see Figure 3.10 and Figure 3.11.

![Figure 3.10 and Figure 3.11: Closure pressure values measured at different positions: 12, 3, 6 and 9 o’clock. The diagram reports mean value and the corresponding standard deviation.](image-url)
3. Aspiration device and measurement protocol

REPEATED MEASUREMENTS

Repeated measurements on each of the 10 subjects at 3-4 hours intervals indicate a standard deviation of approximately 15% (see Figure 3.12) of the reference value (first measurement) which is smaller than the observed inter-subject variability (38%).

![Figure 3.12 Reproducibility of measurements at 3-4 hours interval: The values were normalized with respect to the first measurement in the morning.](image)

In Figure 3.13 the value of $p_{cl}$ for the second measurement versus the first measurement immediately preceding first measurement is plotted (as described in Section 3.4.1) with each cross indicating a measurement pair of a single subject. The black line indicates the locus of identical first and second measurement. The distribution of the measurement points clearly shows a decrease in $p_{cl}$ for the second measurement. This preconditioning effect is related to residual tissue deformation after disconnecting the aspirator.
Figure 3.13 **Repeated measurements**: First and second $p_{cl}$ value are reported as black crosses ($p_{cl1}$ and $p_{cl2}$, 50 measurement pairs). The distribution points clearly show that the second measurement is lower than the first one.

### 3.5.2 Finite element simulations

Analysis of the deformation state in the cervical tissue during aspiration tests has shown that the tissue can be interrogated down to a depth of 10mm. The state of deformation is multi-axial, with regions subjected to large elongation as well as large compression in radial ($r$), axial ($a$) and circumferential ($\varphi$) directions of the organ, see Figure 3.14 a) and b). The major contribution to the overall deformation during this test is the axial extension, while physiological loading is expected to elongate the cervix mainly in circumferential direction. Positive strains in this direction are present in the aspiration experiment, but they are confined to the tissue surface.
3. Aspiration device and measurement protocol

Figure 3.14  Mode of deformation during the aspiration test: a) Non-central placement of the aspiration tube with respect to the cervix, left. Deformed tissue at 4mm peak extension with axial and side-view, right. b) Logarithmic strain during the aspiration test in x-direction and y-direction.
Based on the in-vivo measurements, an inverse FE analysis was performed to determine corresponding constitutive model parameters for the non-pregnant cervix and the cervix in the course of pregnancy, fitting the mean values of $p_{cl}$ obtained for the non-pregnant state (NP), first (T1), second (T2) and third (T3) trimester (for pregnant subjects, see Chapter 4), respectively. Table 3.3 reports resulting parameters for the NH and RP2 model formulations.

<table>
<thead>
<tr>
<th>Group</th>
<th>NP</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH: $C_{10}$ [kPa]</td>
<td>3.920</td>
<td>1.895</td>
<td>0.950</td>
<td>0.650</td>
</tr>
<tr>
<td>RP2: $C_{10}$ [kPa]</td>
<td>0.130</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RP2: $C_{20}$ [kPa]</td>
<td>2.800</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.3 Constitutive model parameters: The parameters were determined for non-pregnant state (NP), first (T1), second (T2) and third (T3) trimester (for pregnant subjects, see Chapter 4).

Figure 3.15 illustrates the uniaxial response for the linear (NH, $C_{10} = 3.92$ kPa) and nonlinear (RP2, $C_{10} = 0.13$ kPa, $C_{20} = 2.8$ kPa) material formulation in comparison to experimental results in uniaxial tension configuration of Myers et al. (2010). Good agreement for pregnant and non-pregnant data is found with the data from Myers et al. (2010) for moderate strains.

The reported constitutive parameters for the non-pregnant state were used for the following parameter study, varying thickness and radius of the cervical tissue portion in the model, boundary conditions at the outer surface, friction coefficient between aspirator and cervix, force of aspirator on the tissue, tissue inhomogeneity (including an epithelium layer) as well as model formulation (including visco-elastic effects). The reference set of conditions is reported in Section 3.4.2.
3. Aspiration device and measurement protocol

Figure 3.15  Uniaxial stress-strain behavior for non-pregnant (NP) and pregnant in the third trimester (P). The uniaxial stress-strain response of aspiration data obtained with the NH material description (green) and RP2 (blue) are shown along with ex-vivo experimental results (uniaxial tension configuration, dashed black) of Myers et al. (2010) for NP and P (pregnant at term). The NP data of Myers et al. (2010) was fitted using RP2 (red), but the obtained parameters were too stiff for the aspiration simulations. The response for the adapted parameter is shown in blue.

Tissue dimensions and boundary conditions

In order to estimate the influence of tissue geometry on the simulated $p_{cl}$ needed to reach an apex displacement of 4mm, the radius and thickness of the tissue portion were varied from 10 to 8mm and 20 to 40mm respectively. Increasing the thickness from 20 to 40mm or decreasing the radius from 10 to 8mm varied the closure pressure by $\leq 1\%$. Similarly, changing the boundary conditions at the outer tissue surface to fully constraint did not influence the closure pressure significantly ($\leq 1\%$).

Friction between aspirator and cervical tissue

The friction coefficient between aspirator and cervical tissue has been changed from frictionless ($\mu=0$, i.e. free sliding) to a friction coefficient of $\mu=1$. The resulting changes in closure pressure are shown in Figure 3.16. The results indicate that the measurement outcome is affected by the unknown friction interaction at the contact between aspirator and cervix.
Results

Even though low friction is expected since the tissue was observed to be moist in-vivo, the present results have shown that the closure pressure could be up to 12% smaller or 8% higher than expected from the reference calculation. For higher friction coefficients, a deviation of the NH and RP2 material formulation can be observed with the NH needing higher closure pressure to reach 4mm displacement.

![Displacement in x](image1)

![Pcl/Pcl reference](image2)

**Figure 3.16 Effect of the friction coefficient:** radial displacement for the case of $\mu = 0.1$, left. Dependence of the closure pressure on the variation of the friction coefficient $\mu$ for both material formulations, right. The cross corresponds to the reference computation ($\mu = 0.1$).

**Contact force**

The force applied by the gynecologist to ensure a tight contact between tissue and device causes a pre-deformation with an initial elevation of the tissue into the tube. The corresponding reduction of closure pressure was calculated for forces up to 1N (estimation from measurements on phantoms). As shown in Figure 3.17, the value of $p_{cl}$ decreases approximately 9% for NH formulation and only by 2% for RP2 formulation. The results show that differences in initial tissue elevation are almost compensated by material stiffening at larger strains. As reported in Section 3.3.3, the as-
3. Aspiration device and measurement protocol

Aspiration tube is placed on the tissue while the peristaltic pump is running. This is clearly the key advantage to reduce this influence.

![Displacement in y](image)

**Figure 3.17 Effect of the contact force:** the contact force causes a pre-deformation with an initial elevation of the tissue into the tube (CF=1N in this case), left. Change of $p_{cl}$ for a variation of the contact force from 0 to 1N, right. The cross corresponds to the reference computation.

### Tissue inhomogeneity

The aspirator is in direct contact with the squamous epithelium layer (thickness typically ≤ 0.5mm). In order to study its influence on the outcome of aspiration measurements, a parameteric study was performed by introducing an additional thin layer (thickness: 0.5mm) between aspirator and cervix (Figure 3.18) and gradually softening its response (decreasing $C_{10}$) with respect to the cervical tissue. This epithelium layer was represented as membrane elements (MAX2) with their nodes tied and prescribed to follow the displacement of the underlying elements. The results demonstrate that the closure pressure decreases only up to 4% in case of a surface layer with very soft response (Figure 3.18).
Figure 3.18 Influence of tissue inhomogeneity: the coefficient $C_{10}^{epithelium}$ is decreased and $p_{cl}$ is reported. The cross corresponds to the reference computation.

**Strain rate**

The duration of an aspiration measurement depends on the tissue stiffness since loading is applied as a continuous (ramp like) pressure reduction: the stiffer the material the longer the time to reach the pressure to deform the tissue up to 4mm and vice versa. Time-dependent deformation was observed in cervical tissue in ex-vivo experiments (Fernandez et al., 2013; Myers et al., 2010; Yao et al., 2014), see Chapter 2. Consequently, the effective tissue stiffness (measured as $p_{cl}$) depends on the deformation rate (i.e. material stiffening is observed with increasing strain rate). The time-dependence in a visco-elastic material can be described by using time dependent coefficients in the strain energy potential. To describe the time dependency, prony series are used for the present evaluation with
3. Aspiration device and measurement protocol

the coefficients $g_1$ (weighting constant) and $\tau_1$ (relaxation time constant) applied to $C_{10}$ from NH formulation. This results in the formulation

$$C_{10}(t) = C_{10}^0[1 - g_1(1 - \exp(-t/\tau_1))]$$

(3.7)

where $C_{10}^0$ describes the instantaneous response and $g_1$ and $\tau_1$ characterize the relaxation behavior.

For this analysis, the constitutive parameter $C_{10}$ obtained from non-pregnant cervical tissue is used for the material formulation of a stiff cervix ($C_{10}^{0\text{stiff}}=3.92\text{kPa}$). The corresponding $p_{cl}$ is called $p_{cl\text{stiff}}$. The amount of tissue relaxation was defined by $g_1=0.5$ (Yao et al., 2014) and the relaxation time was varied to study its influence on $p_{cl}$. Numerical problems were encountered during the simulations and therefore the edge radius of the aspirator was increased to 0.5mm in order to reach the convergence. However, this geometrical change affected the closure pressure to reach 4mm displacement for time-independent material behavior, leading to a value of $p_{cl}=270\text{mbar}$ instead of $p_{cl}=320\text{mbar}$.

![Figure 3.19 Influence of $\tau_1$ on $p_{cl\text{stiff}}$ and $p_{cl\text{soft}}$: Both $p_{cl}$ are nonlinearly increasing with increasing $\tau_1$ from 135mbar to a value 270mbar and from 67.5mbar to 135mbar, respectively.](image)
Figure 3.19 shows that $p_{cl stiff}$ is nonlinearly increasing with increasing $\tau_1$. After a sharp increase for small relaxation times up to 100s, the closure pressure stabilizes. In order to estimate the effect of different strain rates on the ability of the aspiration technique to differentiate between a soft and stiff cervix, a constitutive parameter representative for a soft cervix is defined as

$$C^{0}_{10 soft} = \frac{C^{0}_{10 stiff}}{2}$$  \hspace{1cm} (3.8)

The corresponding closure pressure $p_{cl soft}$ for time independent material behavior for the soft cervix ($C^{0}_{10 soft}=1.96\text{kPa}$) is 135mbar. Performing the same analysis as before, the closure pressure increases with increasing $\tau_1$, and stabilizes thereafter (Figure 3.19).

![Graph showing the relationship between closure pressure and relaxation time](image)

**Figure 3.20** The relaxation time constant was varied from $\tau_1=0.01\text{s}$ to $\tau_1=1000\text{s}$ to study different time scales of tissue relaxation. The results indicate no change in $p_{cl ratio}$ for very small ($<1\text{s}$) and very large ($>500\text{s}$) relaxation times. The minimum ratio of 1.72 is observed for a relaxation time of 7s. This means that the stiffness of the soft cervix is overestimated by 14% (or the stiffness of the stiff cervix underestimated by 14%) in this extreme case.
To quantify the effect of different strain rates on the soft and stiff cervix, the \( p_{cl\text{ratio}} \) between \( p_{cl\text{stiff}} \) and \( p_{cl\text{soft}} \) is calculated as \( p_{cl\text{ratio}} = \frac{p_{cl\text{stiff}}}{p_{cl\text{soft}}} \). The evolution of this ratio with varying relaxation time values is illustrated in Figure 3.20. This diagram indicates that for very small (\(< 1\) s) and very large (\(> 500\) s) relaxation times the ratio of closure pressure \( p_{cl\text{ratio}} \), and thus stiffness, is similar (value of 2, i.e. identical for the ratio of stiffness assuming a non time dependent material behavior). The minimum ratio of 1.72 is observed for a relaxation time of 7s. So the stiffness of the soft cervix is overestimated by 14% (or the stiffness of the stiff cervix underestimated by 14%) in this extreme case. However, even in this extreme case, it seems that the aspiration experiment can adequately distinguish between soft and stiff tissue. Note that for most relaxation times, the ratio of stiff to soft cervix stays above 1.9, allowing for easy discrimination of the two tissues. Considering the characteristic time constants (\(\tau_1=6.8\) s with \(g_1=0.89\) and \(\tau_2=82\) s with \(g_2=0.54\)) published in Yao et al. (2014) \(\tau_{average}=47\) s might be taken here to represent the average relaxation time value for the non-pregnant cervix. This time constant leads to a ratio of 1.86 (-7 %) in the simulation of stiff and soft cervix aspiration, again showing a relative modest effect of visco-elasticity on the discriminative power of the measurement procedure.

3.6 DISCUSSION

Direct measurements of in-vivo cervical tissue stiffness were performed using the aspiration technique. The novel setup was optimized for transvaginal measurement at routine gynecologic consultations. The latest development of the instrument and protocol has considerably enhanced ease of use during routine examinations and reduced the duration of intra-vaginal aspiration measurements remarkably. The essential new features finally enabled to perform over 1100 measurements on human cervices. The feasibility of the proposed protocol and its application during routine consultations has confirmed that none of the measurements caused pain, bleedings or discomfort, this being a major achievement for the potential future clinical application of this technique. As compared to previous
versions of the aspiration device (Bauer et al., 2009; Hollenstein et al., 2013; Nava, 2007), the introduction of the displacement controlled end-point of the experiment increased the inherent safety and reliability of the measurement procedure. The main purpose of this instrument was to apply it in clinical practice as a potential diagnostic tool in future and to easily collect a large number of measurements. These characteristics led to significant drawbacks in providing relevant information for the biomechanical characterization of the cervix. The interrogated tissue portion at the clinically accessible ecto-cervix is very local. This biomechanical characterization of the ecto-cervix might be relevant for prediction of pre-term delivery, but no conclusion can be drawn about the mechanical properties of the whole organ. Furthermore, the state of loading during the aspiration experiment is physiologic in terms of duration (quasi-static) and in terms of magnitude (large strains), but not in terms of direction (axial instead of circumferential elongation).

Another clear limitation of the current measurement procedure is the lack of information of the full pressure-deformation history. An output data couple of \( p_{cl}/4\text{mm} \) apex displacement corresponds to only one single deformed state, thus it loses the information about non-linearity, time-dependency of the tissue behavior (Fernandez et al., 2013; Myers et al., 2010; Yao et al., 2014), and tissue anisotropy (Myers et al., 2010; Weiss et al., 2006). Previous aspiration devices (Bauer et al., 2009; Hollenstein et al., 2013; Nava, 2007) used image analysis to extract the deformed profile of the tissue and quantify tissue displacement associated with prescribed pressure profiles. This procedure was significantly more time consuming, but yielded information on the full pressure-deformation history of a measurement, thus allowing for a more reliable description of the material based on whole stress-strain curves. Additionally, the time dependent pressure-deformation relationship could be extracted, thus providing input for the time-dependent mechanical behavior of the tissue and the choice of material model to describe the tissue.

In fact, since in aspiration measurements only one single point in the deformation response of the tissue (corresponding to a pair of \( p_{cl} \) and \( 4\text{mm} \) apex displacement) is known, almost any arbitrary strain energy formulation can be fitted to match this point. However, to properly
3. Aspiration device and measurement protocol

form complex, higher order models, possibly anisotropic and depending on several invariants of the deformation tensor, other experiments have to be performed, in which the deformation modes are tailored to specific kinematic configurations, allowing for model parameter extraction. The aspiration experiment cannot deliver such data, and thus it cannot form the basis for more detailed material modeling.

This is reflected in the modeling approach chosen in the inverse FE analysis performed in this study. A simple NH material was chosen to fit the corresponding pair of $p_{cl}$ and apex displacement for non-pregnant and pregnant in the first, second and third trimester. This simplified approach however still allows for a qualitative or semi-quantitative evaluation of the softening behavior of the uterine cervix in the course of gestation, reflected in the decrease of the single material parameter $C_{10}$ from NP to T3.

As a second step, the model complexity was increased by adding a second term (and thus a second parameter) to the material formulation to account for the nonlinearity of cervical tissue. However, material parameters were not chosen arbitrarily to just again fit the single point of $p_{cl}$ and 4mm apex displacement, but the ratio of $C_{20}$ and $C_{10}$ were kept based on Myers et al. (2010) and scaled for non-pregnant tissue. These two material model approaches then served as an informed basis to evaluate possible uncertainties in the aspiration measurements in terms of the influence of several parameters and model properties on the response of the modeled cervix-aspirator system.

The friction coefficient and initial tissue geometry after aspirator application (i.e. the deformation induced by the contact force between tube and cervix) are critical factors affecting measurement outcomes. However, the results for reduced polynomial material description (RP2) have shown that the material nonlinearity almost compensates for these influences.

Myers et al. (2010) performed uniaxial tension experiments on cervix samples along the circumferential direction of hysterectomy specimens from non-pregnant and pregnant women at term. The corresponding stress responses are of similar magnitude as the presented results, in particular for moderate strains, as shown in Figure 3.15. Comparison of the results should consider that the data correspond to different i) configurations, ii) strain rates, iii) different tissue location within the organ (thus different
Discussion

microstructure) and iv) tissue states. There are no comparable material model parameters specifically for cervical tissue in non-pregnant or pregnant state. The present values of $C_{10}$ (NH) are in the range of the values reported for adjacent tissue in the uterus (Kauer, 2001). The values of $C_{10}$ from intra-operative measurements on the uterus are considerably lower ($\geq 5$ times) when compared to the values reported by Paccini et al. (2005) from indentation experiments performed on the uterus after hysterectomy. A few cervical stiffness parameters are reported in literature based on elastography (Carlson et al., 2014; Gennisson et al., 2011; Hee et al., 2013) and an FE inverse analysis approach (House et al., 2012). A direct comparison of these parameters is difficult due to the different procedures and assumptions. An analysis of the Young’s moduli ($E$) shows that the values are different, but in the same order of magnitude for $E = 6C_{10}$ reported here. In Table 3.4 the obtained Young’s moduli for the different procedures are summarized.

An experimental verification of the variability associated with the measurement procedure is very difficult. The repeatability of measurements on synthetic materials is excellent ($\leq 1\%$ scatter in $p_{cl}$ measurements on a silicone elastomer block, data not shown), but this does not reflect the procedural problems associated with intra-vaginal measurements on the same subject, at the same position. The non-elastic nature of the tissue behavior caused a permanent deformation after the first measurement, affecting the closure pressure in the subsequent immediately following test. However, the variability (15% standard deviation) observed in repeated measurements on the same organ is much lower than the standard deviation between different subjects (ranging between 38% and 50% in the different groups). This result is in agreement with expectations based on the FE analysis, in which the measurement uncertainties are in the range of 15%. Note also that observations of variability in cervical length measurements indicate similiar results ($\approx 20\%$)(Berghella et al., 2007).
3. Aspiration device and measurement protocol

<table>
<thead>
<tr>
<th>Publications</th>
<th>Young’s modulus [kPa]</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badir et al., 2012</td>
<td>NP: 24</td>
<td>aspiration, in-vivo</td>
</tr>
<tr>
<td></td>
<td>T1: 12</td>
<td>ecto-cervix, anterior lip</td>
</tr>
<tr>
<td></td>
<td>T2: 5.7</td>
<td>from inverse problem</td>
</tr>
<tr>
<td></td>
<td>T3: 4</td>
<td>E=6C_{10}</td>
</tr>
<tr>
<td>Hee et al., 2013</td>
<td>mid-term: 80</td>
<td>quasi-static US with elastomer cap, in-vivo</td>
</tr>
<tr>
<td></td>
<td>full-term: 30</td>
<td>ecto-cervix, anterior lip</td>
</tr>
<tr>
<td></td>
<td></td>
<td>assumption: σ_{cap}=σ_{cervix}</td>
</tr>
<tr>
<td>Carlson et al., 2014</td>
<td>NP: 35</td>
<td>dynamic US, ex-vivo, anterior lip</td>
</tr>
<tr>
<td></td>
<td>pregnant: 13</td>
<td>assumption: E=3\cdot c^2</td>
</tr>
<tr>
<td>Gennisson et al., 2011</td>
<td>NP: 19</td>
<td>dynamic US, in-vivo, ectocervix, anterior lip</td>
</tr>
<tr>
<td></td>
<td>P: 7</td>
<td>assumption: E=3\cdot c^2</td>
</tr>
<tr>
<td>House et al., 2012</td>
<td>P (24 weeks): 6.7</td>
<td>fundal pressure, MRI/US data, from inverse problem, small strain, for collagen</td>
</tr>
</tbody>
</table>

Table 3.4 The determined Young’s moduli $E$ from in-vivo aspiration measurements, in-vivo and ex-vivo dynamic elastography measurements and an FE inverse analysis approach are all in the same order of magnitude.

3.7 Conclusion

A novel procedure for intra-vaginal assessment of the mechanical properties of the uterine cervix was developed. Protocol definition was complemented by finite element analysis of possible uncertainties associated with the measurement procedure. Friction and contact force between aspirator tube and cervical tissue were identified as the main influential factors. The main characteristics of this instrument is to provide rele-
vant mechanical information for clinical practice. However, parameters of model equations were determined as representative of the resistance to deformation of non-pregnant and pregnant cervical tissue. Despite evident limitations on time and history dependence, but also the very local nature of the measurement, the proposed model might be useful for semi-quantitative evaluation of cervical stiffness changes in pregnancy or to benchmark proposed model equations for cervix simulations.
CHAPTER FOUR

IN-VIVO CHARACTERIZATION OF THE UTERINE CERVIX IN PREGNANCY

4.1 Introduction

A firm and closed uterine cervix is essential to allow fetal development throughout pregnancy. At term, between 38 and 42 weeks of gestation, dramatic changes in cervical consistency accompanied by uterine contractions lead to extensive radial opening of the cervix, allowing the passage of the child into the vaginal canal. The most impressive performance of the cervix is the reversibility of these changes leading back to a closed shape within a few hours after delivery. These changes in mechanical properties are related to modifications of the extracellular matrix of cervical tissue, in particular, to the organization of collagen fibers and glycosaminoglycan concentration (Myers et al., 2008; Read et al., 2007; Timmons et al., 2010). Although knowledge of the physiological mechanisms determining timely effacement and opening of the cervix is progressing (Badir et al., 2013b; House et al., 2012; House and Socrate, 2006; Parra-Saavedra et al., 2011), the understanding of the clinically relevant pathological preterm ripening of the cervix leading to spontaneous preterm birth (sPTB) (House
et al., 2013; House and Socrate, 2006; Romero et al., 2006; Slattery and Morrison, 2002) is only beginning, see Chapter 2.

New non-invasive measurement procedures, which were lately reviewed by Feltovich et al. (2012), assess physical properties of the cervix by quantifying its microstructure, hydration level or elasticity. However, the following approaches, except for few cases, so far could not substantiate clinical relevance. McFarlin et al. (2010) quantified the reduction of ultrasound amplitude due to absorption and scattering (defined as ultrasonic attenuation) in cervical tissue of pregnant women. First evaluation on humans found only weak correlations, indicating a decrease in the ultrasonic attenuation coefficient with gestational age. Previous studies on rats have shown a significant decrease in attenuation coefficient as pregnancy progressed, which was correlated with the tissue water content (McFarlin et al., 2006). The authors suggested to embark in a longitudinal study with larger sample size to assess the decrease of attenuation coefficient in every women individually. Two groups (Kuwata et al., 2010; Tekesin et al., 2005) evaluated echogenicity in the uterine cervix using ultrasound grey-scale histograms. It was hypothesized that biochemical changes influence scattering properties of the tissue which could affect textural appearance of ultrasound images. Kuwata et al. (2010) found a positive correlation between anterior-posterior gray-level difference and cervical consistency determined by digital palpation. However, the diagnostic relevance to predict sPTB was not evaluated. In Tekesin et al. (2005), gray-level evaluation combined with a fetal fibronectin test was found to improve prediction of preterm delivery. Extensive clinical evaluation (Stein et al., 2011) has shown poor inter- and intra-observer variability of this procedure. This finding led the authors to the conclusion that this method should not be applied in clinical practice. O’Connell et al. (2000) and later Jokhi et al. (2009) proposed an instrument to measure the in-vivo impedance of the cervix, which was shown to be inversely related to the hydration of tissue. These measurements demonstrated a reduced resistivity in pregnant women. However, the overall performance of this procedure and the resulting correlations were so weak that the authors did not recommend this technique to be used for clinical purposes, either. Hornung et al. (2011) presented a procedure based on spectroscopy with
frequency domain in near infrared range to detect changes in absorption and scattering coefficients of cervical tissue. These coefficients are sensitive to light absorbing molecules found in the blood and light scatterers found in cellular and extracellular components. The study results demonstrated only weak correlation between optical properties and gestational age. The authors reported an ongoing clinical study evaluating the predictive capabilities of this technique to detect women at risk and suggested to combine this procedure with the collascope. The collascope is a device that delivers excitation light to the tissue with specific wavelength and collects the fluorescence emission from the tissue. This light-induced autofluorescence is attributed to pyridinoline, the major cross-link protein in collagen fibers. The intensity of this fluorescence signal significantly decreased after 25 weeks of gestation, suggesting a significant decrease in collagen cross-links quantity. In fact, corresponding increase in the signal intensity post-partum was reported and associated with the reorganization of the collagen structure (Maul et al., 2003, 2005; Schlembach et al., 2009). However, the predictive capabilities of this technique to identify women at risk have so far not been evaluated.

Among these recent attempts, elastography was applied to measure the deformability of cervical tissue. This approach could become a promising method to quantify and detect relevant cervical changes in pregnancy. Interestingly, inconsistent result in terms of correlation between softness and gestational age were found (Gennisson et al., 2011; Hernandez-Andrade et al., 2013, 2014b; Molina et al., 2012; Parra-Saavedra et al., 2011), see Chapter 5 for a detailed analysis of this approach.

In today’s clinical practice cervical consistency is a relevant parameter for the prediction of timely cervical opening in the final stage of pregnancy. This parameter is part of the Bishop Score (Bishop, 1964), which determines cervical consistency qualitatively by digital palpation of the ecto-cervix as an additional information beside cervical morphology (Laughon et al., 2012). These findings support the expectation that an objective measurement of the mechanical response of the ecto-cervix might provide relevant information on the progressive maturation of the cervix during pregnancy. To evaluate the feasibility of providing quantitative information on the evolution of the mechanical properties of the ecto-cervix in

61
normal pregnancy, a pilot study was performed, on 50 pregnant women and 50 non-pregnant women (results for this group were already reported in Chapter 3) using the aspiration device. The hypotheses of the present investigation were as follows: 1) softening of cervical stroma in normal pregnancies is detectable by aspiration measurements, 2) there is a progressive softening during pregnancy, 3) this softening is more pronounced in late pregnancy and 4) the tissue recovers its stiffness post-partum. Parts of this chapter, including paragraphs of text, figures and tables are published in Badir et al. (2013b).

4.2 Methods

4.2.1 Subjects

This longitudinal study (Ethical approval by KEK Zurich StV02/2007 and later amendments) included women with a healthy singleton pregnancy. All pregnant women presenting after September 2010 at their first pregnancy consultation at the physician’s private office were invited to take part in the study. Non-inclusion criteria were communication problems, multiples, prior surgery on the cervix (e.g. conisation or cerclage), and not treated pre-malignant or malignant changes on the cervix. In January 2012, 50 subjects (Tables 4.1 and 4.2) had agreed by signing the informed written consent and the patient information. Exclusion criteria from the study were continuing bleedings, severe genital infections, loss of pregnancy, placenta praevia, preterm cervical ripening and rupture of the membranes. Aspiration measurements were performed at each of the eight routine consultations, usually scheduled at intervals of three to six weeks and also at the regular post-partum visit, after six to sixteen weeks. At the same visit, cervical length was assessed by ultrasound as the linear distance between internal and external os (Heath et al., 1998). As a reference, measurements were performed on 50 non-pregnant women (see Chapter 3 and Tables 4.1 and 4.2).
Subjects

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects included</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Measurement sets completed</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>Age at years: mean +/- SD (range)</td>
<td>30 +/- 4 (20-35)</td>
<td>31 +/- 6 (20-42)</td>
</tr>
<tr>
<td>Nulli-parous: number (%)</td>
<td>16 (38%)</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>Multi-parous: number (%)</td>
<td>26 (62%)</td>
<td>28 (56%)</td>
</tr>
</tbody>
</table>

Table 4.1 Demographics of the study population: Aged-matched (p = 0.36) non-pregnant and pregnant subjects. Forty-two pregnancies could be followed until term delivery. There were three exclusions following the study criteria (preterm ripening, preterm delivery, and placenta previa) and five drop outs due to non-medical reasons.

Pregnant subjects

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery at weeks: mean +/- SD (range)</td>
<td>39 +/- 2 (38+0 -41+0)</td>
</tr>
<tr>
<td>Cesaren section: number (%)</td>
<td>20 (48%)</td>
</tr>
<tr>
<td>Vaginal birth: number (%)</td>
<td>22 (52%)</td>
</tr>
<tr>
<td>Healthy newborn: number (%)</td>
<td>42 (100%)</td>
</tr>
<tr>
<td>Newborn adaptation problems: number (%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Table 4.2 Pregnancy outcome: Delivery and newborn outcome for the forty-two pregnant women with completed measurement sets.

4.2.2 Measurements

Prototype 1.0 was used in this study with the specific measurement protocol as reported in Section 3.3.3. To reduce uncertainties related to the placement of the aspiration device on the tissue, all measurements were performed by the same gynecologist, well-trained in aspiration measurements. All ultrasonographic measurements of the cervical length (Heath
et al., 1998) were performed using the usual trans-vaginal route (probe RIC 5-9-D, Voluson® E8, GE Healthcare, Zipf, Austria).

4.2.3 Statistical analysis

Statistical analysis was performed with the statistical computing environment R: A Language and Environment for Statistical Computing (Open Source Software 2012). Data were pooled and divided into sub-groups according to the trimenons and post-partum. A linear model was applied to compare nulli-parous with parous subjects under consideration of the factor age. Wilcoxon rank-sum test was used to compare differences between the non-pregnant reference group and the pregnant group. For the comparison of values of the same pregnant subjects in trimenons and post-partum, a linear mixed model was applied. Model assumptions were checked by residual analysis (QQ-Plot) and post hoc tests were Bonferroni corrected.

4.3 Results

In this study, 448 measurements for non-pregnant and pregnant subjects were performed. No related pain or bleeding or any other negative outcome due to the application of the device has been reported in any of the aspiration experiments. From the 50 pregnant women taking part in the study, three had to be excluded following the study criteria for preterm birth, preterm cervical ripening and placenta praevia. Five additional drop-outs occurred due to non-medical reasons. The records of these eight subjects were completely removed from the analysis. The other 42 considered pregnancies ended with deliveries at term between week 37 and 41.

Collective results of the closure pressure \( p_{cl} \) of all sub-groups are reported in Figure 4.1 and Table 4.3.
Results

<table>
<thead>
<tr>
<th>Group</th>
<th>μ ± SD[mbar]</th>
<th>NP</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>320 ± 120</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1</td>
<td>153 ± 78</td>
<td>p&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T2</td>
<td>74 ± 33</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T3</td>
<td>53 ± 26</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PP</td>
<td>150 ± 70</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.3 Statistical evaluation of closure pressure $p_{cl}$ for reference group and all pregnant sub-groups: Data are divided into reference group and sub-groups in pregnant patients: non-pregnant (NP), first (T1), second (T2) and third trimester (T3), and postpartum (PP). Mean ($\mu$) and standard deviation (SD) are presented for all groups. NS: non-significant ($p \geq 0.05$).

Figure 4.1 Collective results of closure pressure $p_{cl}$ of the reference group and during gestation: Closure pressure of non-pregnant (NP, left) and pregnant women during pregnancy (months 2-9) and post-partum (PP, right) are shown as vertical bars. Crosses indicate cervical length (CL) and the values refer to the second vertical axis on the right. For all values, means and standard deviations are reported.
As reported in Table 4.3, the difference between the non-pregnant reference group and the first trimester sub-group is large, with a mean value in early pregnancy less than half the corresponding value of the non-pregnant group. Significant differences are also found between the first and second trimester. Although the mean value of $p_{cl}$ further decreases in later pregnancy, differences between second and third trimester are statistically not significant. The mean value of $p_{cl}$ for the post-partum sub-group is three times larger than in the third trimester reaching a level similar to early pregnancy. However, the value is still significantly lower in post-partum group than in the non-pregnant reference group. Dividing the non-pregnant group into nulli-parous and parous subjects with previous vaginal delivery reveals that $p_{cl}$ in both groups cannot be distinguished ($p=0.5$). This finding indicates that cervical stiffness recovers completely to an indistinguishable non-pregnant state after the first delivery (Figure 4.2).

In Figure 4.3 the individual histories normalized with respect to the first measurement throughout gestation are shown. One case was part of the present investigation with so called dystocia: for this patient (and only for this one) $p_{cl}$ increased during gestation instead of decreasing, as shown in this figure. This cervix was not able to open at delivery leading to a cesarean section.

No correlation is observed between $p_{cl}$ values at any stage of pregnancy and time to delivery for the whole data set. However separating spontaneous deliveries from cesarean sections (mostly elective) indicate a relationship between cervical stiffness and delivery week, see Figure 4.4.

Cervical length (CL) values shown in Figure 4.1 are in line with values for normal pregnancy reported in previous studies. CL was found to decrease at the second month of the third trimester, leading to a statistically significant difference between second and third trimesters (Berghella et al., 2007). No correlation is found between $p_{cl}$ values and CL (Figure 4.5).
Figure 4.2  Non-pregnant subjects: $p_{cl}$ in nulli-parous and parous subjects cannot be distinguished ($p=0.5$). This finding indicates that cervical stiffness recovers completely to an indistinguishable non-pregnant state after first delivery.

Figure 4.3  Subject specific history of normalized $p_{cl}$ (with first value in pregnancy $p_{cl\text{initial}}$) throughout gestation: Cervical softening is observed in every individual subject, except for one subject (dashed line). This cervix was not able to open at delivery leading to a cesarean section.
Figure 4.4 Relationship between $p_{cl}$ in the 5th month of pregnancy and week of delivery: There is no correlation between ecto-cervical stiffness in the mid-pregnancy and delivery week. However separating spontaneous deliveries from cesarean sections indicate a relationship between cervical stiffness and week of delivery.

Figure 4.5 Relationship between $p_{cl}$ and CL in the 5th month of pregnancy: There is no correlation between $p_{cl}$ and CL.
4.4 DISCUSSION

The aspiration technique allowed quantitative evaluation of the stiffness of the ecto-cervix during pregnancy. The feasibility of aspiration as a safe, well-tolerated complementary examination method during routine consultations in pregnancy has been demonstrated in this study. The data reported in Figure 4.1 confirm hypothesis 1, cervical softening during pregnancy can be detected with aspiration measurements on the ecto-cervix. Cervical tissue in early pregnancy is firm, but already much softer when compared to non-pregnant women. The pressure needed to deform cervical tissue in the third trimester, is significantly smaller (about five times) than in early pregnancy. This result confirms our hypothesis 2 that there is progressive softening throughout pregnancy. In line with these findings, Myers et al. (2010) have shown in ex-vivo mechanical tests that cervical tissue at term was one order of magnitude softer than tissue of non-pregnant women. Microstructural explanations for the tissue softening were investigated in Myers et al. (2009) with light microscopy. Collagen fibers were well organized and densely packed in the cervix of non-pregnant women, whereas at term the structure changed to a more fragmented fibrous network with increased inter-fibrous spaces. Decrease of stiffness during gestation is confirmed, but the observed time history of stiffness reduction during gestation was not anticipated. In line with the morphological rearrangements in the cervix, no or only small reduction of its stiffness was expected in early pregnancy, whereas a more significant decrease was found in the second part of the third trimester (hypothesis 3). On the contrary, our data present a more pronounced decrease in stiffness in the first two trimesters followed by a stabilization of stiffness at low values in the third trimester. Post-partum stiffness increases again to values similar as in early pregnancy, thus confirming hypothesis 4.

The discrepancy between initially expected and observed progression of the ecto-cervix stiffness deserves further consideration. The hypothesis was based on the fact that cervical softening is required to allow cervical deformation in preparation for birth. These deformations (demonstrated by the significant decrease of CL values, Figure 4.1) develop starting from the internal os and concern mainly the endo-cervix (Berghella et al., 2007; Myers et al., 2008, 2010). In this phase the ecto-cervix remains sufficiently
stiff to avoid dilation, which finally occurs in the hours immediately before delivery. The final reduction of stiffness at the external os required for parturition could not be observed in our last measurement since this test took place up to one week before delivery.

In order to rationalize the observed reduction in stiffness in the first and second trimester, available information on biochemical and histological changes of the cervix was evaluated. Results of the light-induced fluorescence (LIF) measurements on rats (Maul et al., 2003, 2005) suggested that there is a gradual decrease in mature collagen cross-links during the second part of pregnancy (from day 13 to day 21, delivery is at day 22). The final softening occurs with dramatic decrease in cross-links immediately before delivery. The observation of decreased collagen cross-links at mid-pregnancy is also supported by the corresponding mechanical tests on cervical tissue of rats (Schlembach et al., 2009). Yoshida et al. (2014a) provided recently clear evidence that the gradual cervical tissue softening also takes place in mice pregnancy starting with day 12 of the 18 days pregnancy. They found a strong correlation between tissue softening and the breakdown of mature collagen cross-links. Further cervical softening in mice was associated with increased tissue hydration and related collagen dispersion (Read et al., 2007). These results support the hypothesis that tissue alterations are not limited to the final stage of pregnancy.

LIF measurements in humans (Maul et al., 2005) showed a continuous decrease in collagen cross-links from the second trimester (week 25) to delivery. Our measurements indicate lower stiffness in the third trimester as compared to the second trimester, but this difference is not statistically significant. On the contrary, significant decrease of stiffness is observed from the non-pregnant to the first and from the first to second trimester. So it seems that early cervical remodeling starting with initiation of pregnancy is only observed in human pregnancy and cannot be detected by measuring the changes in mature collagen cross-links.

Our observations might be interpreted as follows: progressive softening in the first two trimesters is an indication of a continuous transformation of the ecto-cervix, which stabilizes at a low level of stiffness sufficient to preserve pregnancy in the third trimester. In line with these findings, mechanical assessment of the human cervix at term, but before delivery,
showed that tissue in the proximity of the external os is stiffer than at the internal os (Myers et al., 2008). Cervical changes during pregnancy are associated with a loosening of the connective tissue (Read et al., 2007) which causes softening of the endo-cervix leading to the observed shortening of the cervix. Therefore, methods for the evaluation of the local physical properties (i.e. stiffness, collagen cross-links, resistivity, optical properties) at the ecto-cervix will not detect these changes of the endo-cervix. This fact is confirmed by our results, with a stable stiffness of the ecto-cervix on the third trimester despite a significant shortening of CL.

4.5 Conclusion

The relevance of measurements on the clinically easily accessible site, the ecto-cervix, for early prediction of sPTB is an open question. Palpation of the ecto-cervix is considered to be a relevant complement of CL measurements for improving prediction of sPTB (Reiter et al., 2012). The aspiration technique used in the present work provides a means for a more objective assessment of stiffness of the ecto-cervix. Future studies will evaluate the usefulness of aspiration measurements to identify women at risk of sPTB, in conjunction with corresponding cervical length measurement. The clinical multicenter study SOFTCERVIX (see Chapter 6) aims at comparing aspiration measurements performed by different investigators and evaluate possible correlations with time-point of delivery. This will allow determining the usefulness of the aspiration method in identifying women at risk of sPTB.
CHAPTER
FIVE

ELASTOGRAPHY PROCEDURES FOR
DETERMINATION OF CERVICAL
STIFFNESS

5.1 Introduction

Medical ultrasonic imaging is widely used in obstetrics for the visualization of the uterine cervix and surrounding tissue. Acoustic reflections allow the construction of an image representing the morphology of the organ, however lacks information about tissue stiffness (Bamber, 2013). Additional to cervical morphology, measuring the stiffness of the cervix might be useful in the prediction of spontaneous preterm birth (sPTB). Recently, a number of clinical studies have addressed this topic, proposing quantitative methods for the assessment of the mechanical properties of the cervix. Quasi-static elastography (Hernandez-Andrade et al., 2013, 2014b; Molina et al., 2012), maximum deformability using ultrasound (Fruscalzo and Schmitz, 2012; Parra-Saavedra et al., 2011) and aspiration tests (Badir et al., 2013a,b) have been applied for this purpose. The results of these studies are contradictory in that elastography indicates very
5. Elastography procedures for determination of cervical stiffness

modest changes in the course of pregnancy, whereas aspiration and maximum deformability show a strong decrease in stiffness, which starts early in pregnancy and continues until delivery. In fact, one main motivation of the present investigation is that the results in Hernandez-Andrade et al. (2013) and Molina et al. (2012) are in contrast with the findings presented in Chapter 4 and we identified the need to rationalize this difference.

Quasi-static elastography was initially introduced to differentiate malignant tumors and normal tissue by quantifying local tissue deformability (Ophir et al., 1999). Elastography measurements are performed as follows: A force is applied to the tissue by the ultrasound probe and the corresponding displacement or velocity field is obtained using image analysis algorithms that track the position of specific particles during their motion. Local strains are calculated from the displacement gradient and displayed in a colored image called “elastogram”, indicating regions of large and small deformations, which can be a measure of local relative stiffness within the organ. Depending on the system, the force is applied by a hand-held probe, the breathing movement of the patient or arterial pulsation is used to generate tissue motion (Bamber, 2013).

Thomas et al. (2007) published the first elastography measurements on pregnant cervices and calculated an elasticity tissue quotient (TQ). These results demonstrated no correlation of the TQ with the duration of pregnancy. Similar findings were reported by (Hernandez-Andrade et al., 2013) and by (Molina et al., 2012). In both studies, pregnant subjects were included. Slow loading cycles were applied to obtain the strain maps. Since the applied force cannot be measured in current ultrasound systems, different standardization procedures were proposed aiming at a repeatable loading of the cervix in different measurements. Molina et al. (2012) controlled the procedure by limiting the probe displacement up to one centimeter. Hernandez-Andrade et al. (2013) used the provided pressure scale on the ultrasound monitor of their equipment to control compression. The question of how to standardize elastography measurements on the cervix has been addressed in recent publications (Feltovich et al., 2012; Feltovich and Hall, 2013). Hee et al. (2013) manufactured a soft elastomer to be applied on the vaginal probe and used it to provide a reference.
Ultrasound measurements of the maximum deformability of the cervix were first introduced by Parra-Saavedra et al. (2011) and later by Fruscalzo and Schmitz (2012). This trans-vaginal ultrasound based procedure does not use the elastography strain maps to determine cervical consistency. The ratio of the anterior-posterior distance before \( (AP) \) and after compression \( (AP') \) is calculated \( (CCI = AP'/AP) \) and quantifies the maximum deformability of the cervix (for more details about this procedure, see Section 6.3.3). The obtained results using this procedure are in line with those obtained with aspiration measurements reported in Section 4.3 on the pregnant ecto-cervix, indicating a progressive decrease of stiffness during gestation. This indicates that biomechanical characterization might contribute to the detection of increased risk of sPTB, while clinical studies using quasi-static elastography could so far not show a potential for diagnosis. The objective of the present study is to rationalize the inconsistent findings obtained with the different quasi-static procedures based on a simple mechanical analysis. Parts of this chapter, including paragraphs of text and figures are published in Badir et al. (2014a)

## 5.2 Methods

Two approaches were used for the present investigation: (i) quasi-static elastography was conducted on phantoms with known mechanical properties, to evaluate the effectiveness of the compression standardization procedure proposed in Hernandez-Andrade et al. (2013) and (ii) data analysis was conducted to investigate the agreement of biomechanical changes quantified by aspiration (ASP) and maximum deformability measurements (CCI) over the course of gestation.

### 5.2.1 Quasi-static Elastography

**Phantom Manufacturing**

Two tissue-mimicking ultrasound phantoms (Hall et al., 1997) were manufactured using agar powder hydrated in boiling water. The stiffness of the phantoms was controlled by the amount of powder (0.005g/ml,
5. Elastography procedures for determination of cervical stiffness

0.001g/ml) mixed into the solution. While the gel solution was liquid, Metamucil fibers were added to increase the absorption and scattering characteristics for ultrasound imaging. The solution was poured into a cylindrical container with 80 millimeters inner diameter. Gelation occurred at room temperature overnight. A thin plastic film covered the containers to assure hydrated surfaces.

**PHANTOM CHARACTERIZATION**

The phantoms were mechanically characterized prior to elastography measurements using an indentation setup. The indenter tip with diameter of 25 millimeters (similar dimensions as ultrasound probe) was positioned centrally on the phantoms with hydrogel applied to reduce friction at the interface. The tip was indented up to 15 millimeters into the samples with a strain rate of 0.1%/s. The corresponding load-displacement curve was measured with a load cell (Stentor II, Andilog Industries, France, 50N). The stiffness ratio between the soft and stiff phantom was determined from the load-displacement curve. The forces obtained at 7mm (estimated displacement during the elastography experiment) are 1N and 1.4N for the soft and stiff phantom, respectively. The ratio of the forces (1.4) is proportional to the stiffness ratio (Figure 5.1).

**ELASTOGRAPHY MEASUREMENTS**

The experimental setup for elastography measurements is shown in Figure 5.2. It consisted of an ultrasound machine equipped with elastography software, a transvaginal probe (Hitachi 7MHz, Hitachi VISION Preirus, Hitachi Medical Corporation, Tokyo, Japan) and a balance (Grundig Küchenwaage KW 5040). The measurements were performed according to the protocol of Hernandez-Andrade et al. (2013) on the two reference phantoms. The phantoms were positioned on the balance to measure the applied force during the measurement, thus providing the information which is missing in all elastography equipments. Slow cyclic motion (constant amplitude and frequency) by the hand-held transducer was applied to the phantoms (Figure 5.3) and the level of compression standardized according to the pressure bar, as done in Hernandez-Andrade et al. (2013).
Elastography images were recorded and synchronized with the recordings of measured force magnitude.

Figure 5.1 Load-displacement curves for the soft (grey) and stiff (black) phantom: The forces obtained at 7mm (estimated displacement during the elastography experiment) are 1N and 1.4N for the soft and stiff phantom, respectively. The ratio of the forces (1.4) is proportional to the stiffness ratio.

Figure 5.2 Experimental setup for elastography measurements on phantoms using Hitachi VISION Preirus: Hitachi ultrasound machine and test bench, left. Elastography measurement on a phantom placed on the balance, right. The pictures are reprinted with permission of Manfred Maurer.
5.2.2 Data analysis of biomechanical procedures

$p_{cl}$ is a measure of tissue stiffness, whereas CCI quantifies tissue compliance. These two mechanical descriptors (stiffness and compliance) are inversely related, allowing for the following analysis: A linear fitting curve (see Figure 5.4) was determined for CCI mean values as a function of gestational age (Parra-Saavedra et al., 2011). Based on this fit, the corresponding compressive strain ($\epsilon = 1 - CCI$) and tissue compliance were calculated. The inverse was used for prediction of the corresponding $p_{cl}$ evolution.

5.3 Results

5.3.1 Quasi-static elastography

Using the “pressure bar” provided by the elastography software for standardization of the loading cycles (a “pressure” value of 3 was obtained), the same magnitude of deformation was induced in the soft and the stiff phantom, as reflected by the elastograms in Figure 5.5 (red indicates high strain and blue indicates low strain). Note that due to the difference in
**Figure 5.4 CCI trend line:** CCI mean values (black crosses) are from Parra-Saavedra et al. (2011). A linear trend line is determined for the dependence of CCI on gestational age.

![CCI trend line](image)

**Figure 5.5 Elastogram images:** soft phantom, left; stiff phantom, right.

stiffness (stiff/soft ≈ 1.4) the level of applied force measured by the balance was different. The average applied force was 136 ± 6g for the stiff phantom and 81 ± 5g for the soft phantom, i.e. a ratio of 1.6 ± 0.3, which is in line with the stiffness ratio (1.4). These results clearly illustrate that the loading standardization procedure lead to repeatable strain values, however for different loading forces. Two materials with different stiffness were interrogated with elastography, but since the applied force cannot be standardized, this approach could not discriminate between stiff and soft. The same is true for cervical tissue, thus this approach cannot detect
5. Elastography procedures for determination of cervical stiffness

Figure 5.6 Prediction of $p_{cl}$: $p_{cl}$ mean values and standard deviation from the study results reported in Section 4.3. The predicted evolution of $p_{cl_{pred}}$ during gestation (black crosses) is based on the CCI trend line in dependence of gestational age.

cervical softening in pregnancy.

5.3.2 DATA ANALYSIS OF BIOMECHANICAL PROCEDURES

Based on the evolution of CCI values, the expected time history of stiffness can be estimated as $s(t) \sim \frac{1}{\varepsilon(t)}$ since the strain $\varepsilon(t)$ is proportional to the compliance, i.e. the inverse of stiffness. Starting from the measured value of $p_{cl}$ at the beginning of pregnancy, the decrease in $p_{cl}$ can be estimated based on the decrease in stiffness as predicted by the evolution of CCI values. The predicted $p_{cl_{pred}}$ values are within the measured range of $p_{cl}$ obtained with aspiration tests during pregnancy, demonstrating a general agreement of the procedures, see Figure 5.6. This analysis has shown that CCI, a measure of tissue compliance, decreases linearly during pregnancy while $p_{cl}$, a measure of tissue stiffness follows a nonlinear decrease throughout pregnancy.
5.4 Discussion

Evidence from histological and clinical studies (Myers et al., 2008, 2010, 2009; Read et al., 2007) indicates that the cervix softens during pregnancy. The magnitude and timeline describing this softening behavior as reported for CCI measurements (Parra-Saavedra et al., 2011) agree with the findings of aspiration measurements. This agreement holds true despite limitations of each method: Aspiration is conducted at the ectocervix and depends on tissue properties on a very local scale. The CCI procedure on the other hand relates to a global measure of bulk properties of the cervix and no force sensor is available to standardize the procedure, for more details see Badir et al. (2014a).

The proposed protocol in Parra-Saavedra et al. (2011) applies a criterion that is based on the nonlinear nature of the mechanical response of cervical tissue. It defines the maximum deformation of the cervix until no more compression can be achieved, hence the material response reaches the highly nonlinear stiff region of the stress-strain relationship. As qualitatively illustrated in Figure 5.7, a variation in the same magnitude of force ($\Delta CF^+$) as in the lower region of the curve ($\Delta CF^-$) does not result in a significant variation in compressive stretch ($\Delta \lambda$) allowing high levels of intra-observer and inter-observer reproducibility and a differentiation between a soft ($\Delta CF_{soft}^+$) and stiff cervix ($\Delta CF_{stiff}^+$).

The findings reported in Section 4.3 and in Parra-Saavedra et al. (2011) about cervical changes in pregnancy seem contradicted by the data from quasi-static elastography (Hernandez-Andrade et al., 2013; Molina et al., 2012). Two tissue mimicking phantoms with different stiffness provided a basis to rationalize this discrepancy, demonstrating that despite an identical "pressure" value, the loading cycles applied different forces. Since quasi-static elastography is based on a purely kinematic measure, i.e. signals are proportional to strain or strain rate, no direct, quantitative measures of biomechanical properties can be obtained. The guide for the examiner when performing elastography measurements is called “pressure bar”, and this might suggest a standardized force being applied. But this is clearly misleading. In the phantom measurements, consistent values of the “pressure bar” correspond to identical elastograms but different forces for the two phantoms. In order to assess the stiffness of a material,
5. Elastography procedures for determination of cervical stiffness

Figure 5.7  Force-deformation relationship for a stiff and a soft cervix: Reaching the highly nonlinear stiff region of each material response, a variation in force does not result in a significant variation in strain.

a measure of deformation has to be combined with a measure of force. In this sense, the term “elastogram” is also misleading, in that it does not provide an absolute elasticity or stiffness measure, but is rather a representation of the relative compliance as seen from kinematic data. Thus quasi-static elastography as described in Hernandez-Andrade et al. (2013) and Molina et al. (2012) does not allow for a quantitative assessment of absolute values of cervical stiffness and its softening in pregnancy.

In contrast, dynamic elastography might allow to determine absolute values of cervical stiffness. One common working principle is based on mechanical excitation applied by a localized acoustic radiation force and the imaging of the resulting shear wave speed using the same ultrasound transducer (pushing and imaging mode, respectively). Acoustic radiation force is a phenomenon associated with the propagation of acoustic waves in attenuating tissue. Attenuation in soft tissues it dominated by absorption. With increasing acoustic frequencies, the tissue does not respond
fast enough to the transitions between positive and negative pressures, thus its motion gets out of phase with the acoustic wave, and energy is stored in the tissue. This leads to an increase in tissue temperature and the creation of a shear wave propagating with cylindrical symmetry a few millimeters away from the pushing-beam’s focus. The shear deformation is along the ultrasound imaging beam allowing to measure the small particle motion (tens of $\mu$m) and detect its time of arrival at lateral positions, see Figure 5.8. The shear wave velocity can then be calculated. For a complete final image, shear wave speed maps from all the pushing lines are combined (Bamber, 2013; Bercoff et al., 2004; Cosgrove et al., 2013; Gennisson et al., 2013).

In general, the stiffer the tissue, the greater a shear wave speed as it travels through the tissue. Thus, the velocity of a shear wave is directly linked to the mechanical properties of the tissue (under assumption of isotropy, elasticity and homogeneity):

\[
G = \rho c^2
\]  

(5.1)

where $G$ is the shear modulus, $c$ is the speed of the shear wave and $\rho$ the density of the tissue. For biological tissue with its high water content,
5. Elastography procedures for determination of cervical stiffness

\[ \rho = 1000 \text{kg/m}^3 \] is assumed.

Under the assumption that the bulk modulus \((K)\) is much larger than the shear modulus \(G\), the Young’s modulus \((E)\) of the tissue can be quantitatively estimated as

\[ E = 3G \quad (5.2) \]

Recently, two ultrasound equipment manufacturers have released commercial implementations of this method, both of which use local radiation force excitations. Siemens Medical Solutions has implemented a version of shear wave elastography on the ACUSON S2000 ultrasound scanner and SuperSonic Imagine released the Aixplorer ultrasound scanner.

Carlson et al. (2014) and Gennisson et al. (2011) performed initial measurements on the human cervix using ACUSON S2000 and Aixplorer, respectively. Both proved feasibility of this technique to detect cervical softening and by that supporting aspiration and maximum deformability data. In Carlson et al. (2014) hysterectomy specimens were investigated, a subset of which were softened by misoprostol. Measurements were performed longitudinally along the cervical canal on the anterior and posterior part of the cervix. The results have shown that shear wave speed increases monotonically from distal to proximal along the cervix. The shear wave speed properties seemed to vary in the anterior compared to the posterior cervix and have shown significant differences between non-pregnant vs. softened cervical tissue. Similar results were found in Gennisson et al. (2011). Transvaginal measurements were performed on the cervix of healthy and preterm labor subjects. The Young’s modulus \((E)\) of the cervix (see equation 5.2) among subjects with preterm labor has shown to be smaller compared to healthy subjects. In the latest clinical study using dynamic elastography, Hernandez-Andrade et al. (2014a) interrogated pregnant subjects and demonstrated continuous cervical softening of the entire organ.

As summarized in Section 3.6, the outcome of transient elastography procedures is in line with results from aspiration, clearly confirming the potential of these techniques to study cervical softening in pregnancy. The strong advantage of dynamic elastography is that mechanical excitation is operator-independent, leading to more reproducible data. Note however that the contact of the transducer with the tissue influences the
outcome. According to the manufacturer information, the reported penetration depth is about 3-8cm which clearly allows for the interrogation of the whole cervix. The ability of interrogating the whole organ in combination with the operator-independent mechanical excitation could be a distinct advantage over the maximum deformability and aspiration measurements. However, there might be problems related with this technique on the human cervix. The cervix is certainly smaller than the liver or female breast, for which a successful application of dynamic elastography has been shown. This indicates that the assumption of a semi-infinite tissue block is no longer valid for the cervix and boundary conditions as well as tissue inhomogeneity could lead to systematic influences and thus limitations of this technique (Carlson et al., 2014; Gennisson et al., 2013; Palmeri et al., 2013). Small displacements are applied to the tissue but the expected amount of deformation has never been reported. The relevance of these data for the prediction of sPTB need to be determined in the future. Moreover, more data are needed to evaluate the importance of the applied mode of deformation (shear deformation perpendicular to the axis of the cervix) for the biomechanical characterization of the uterine cervix.

5.5 Conclusion

Quasi-static elastography is easy to perform and provides a real-time image on the local strains within the tissue. But this methodology does not provide useful information about tissue stiffness due to the lack of a force sensor (Bamber, 2013; Gennisson et al., 2013; Mazza et al., 2014). In contrast, the results obtained from aspiration measurements and maximum deformability measurements have shown the feasibility to quantify cervical softening during pregnancy. Similar trends are observed in measurements performed with dynamic elastography, which has the strong advantage of being quantitative compared to quasi-static elastography.
6.1 Introduction

The novel measurement procedure presented in Chapter 3 successfully quantified progressive softening of the human uterine cervix in pregnancy. This finding, along with observations from ultrasound based procedure by Parra-Saavedra et al. (2011) indicate that measuring the stiffness of the cervix might be useful in early prediction of spontaneous preterm birth (sPTB). To verify this hypothesis, an international clinical multicenter study (SOFTCERVIX) is carried out to assess biomechanical and clinical data at mid-pregnancy and evaluate predictive value of each technique. Substantial efforts in study protocol preparation and tools development were required to allow for this clinical study to be realized.

6.2 Study Preparation

The dedicated aspiration device described in Section 3.3.2 evaluates the predictive capability of cervical stiffness to identify women at risk of sPTB.
Due to the potential diagnostic relevance of this procedure, this device is considered as a medical device class I\(^1\) according to Medizinprodukteverordnung (MepV). In Switzerland, compliance with standard EN ISO 14155 (Standards, 2011) is mandatory for clinical trials of medical devices. The following paragraphs provide important aspects of the procedure in line with standard EN ISO 14155 (Standards, 2011), which finally helped to obtain approval for the clinical study by the Cantonale Ethics Committee and Swissmedic.

6.2.1 **CLINICAL INVESTIGATION PLAN**

In contrast to basic research study protocols the clinical investigation plan of SOFTCERVIX has to address the following critical aspects: data management, adverse events management and study team to ensure the quality of the data and the safety of the study participants.

**DATA MANAGEMENT**

In this study, investigators and monitors are in charge of the data management. Investigators generate and edit electronic case report forms (eCRF) to collect study relevant data, while monitors review data completeness and correctness. Both groups use a dedicated internet-based secure database, called SecuTrial\(^{©}\), which offers EN ISO 14155 (Standards, 2011) compliance for each assignment. An illustration of the data collection tool is found in the Appendix B.

**ADVERSE EVENTS MANAGEMENT**

Adverse events (AE) are medical incidents or unintended injuries in subjects whether or not related to the medical device. Adverse events have

\(^{1}\)Medical devices are categorized in four classes (I, IIa, IIb and III) based on the rules in 93/42/EEC (Standards, 1993) annex IX to rate the vulnerability of the human body and consider the potential risk in the context of the technical aspects of the medical device. Applying these rules, the aspiration device is categorized into class I since it is used temporarily (duration of application is less than 5 minutes, annex IX 1.2) as an invasive device (the device is applied inside the body, but uses a natural orifice (trans-vaginally) annex IX 1.2).
to be recorded in the eCRF and serious adverse events that cause injury associated with the biomechanical measurements have to be reported to the Ethics Committee and to Swissmedic. For this clinical trial, the following events are categorized as serious AE if they appear within 7 days after application of the instrument:

- Contractions
- Rupture of fetal membrane
- Preterm delivery

**STUDY TEAM**

The clinical study is managed by a so called “sponsor”. Dr. David Scheiner from University Hospital Zurich is the sponsor in this study since he is a qualified GCP sponsor-investigator and is familiar with the aspiration technique. Dr. Scheiner carries the responsibility of the trial and needs to be in close contact with all involved parties, i.e. to their representatives, Prof. Dr. Zimmermann principal investigator at University Hospital Zurich, and Prof. Dr. Deprest, the said at University Hospital Leuven. By Swiss law, every study team member is obliged to follow Good Clinical Practice (GCP) guidelines, EN ISO 14155 (Standards, 2011). Therefore, the whole study team had to undergo GCP training to learn about requirements regarding GCP to ensure subject safety and standardization of the trial.

**6.2.2 INVESTIGATOR BROCHURE**

The investigator’s brochure complements the clinical investigation plan with detailed explanation about the technical aspects of the aspiration device, its application and sterilization procedure. The required risk assessment according to EN ISO 14971 (Standards, 2009) is summarized and actions taken to minimize the identified risks are highlighted. The following adverse events were identified, which might be associated with use and misuse of the aspiration device:

- **Cervical tissue bleeding** is related to performance of aspiration measurement with placement of the instrument on the columnar
epithelium (instead of the squamous epithelium), which is fragile and might cause superficial bleeding. This event is not considered dangerous, since cervical bleeding occurs not only during gynecologic routine examination, but also spontaneously in normal pregnancy, usually followed by spontaneous resolution, without any consequences. Only if necessary, bleedings arising from the cervix might be coped by application of slight pressure through a soft swab for one minute. Successful prevention of bleeding has been achieved with proper device design (avoiding sharp edges), regular device calibration and endoscope/camera monitoring of instrument placement on the ecto-cervix. Training of the investigators aim at teaching safe placement of the device to avoid vulnerable areas. Note that no bleeding was reported in any the numerous measurements.

- **Cervical tissue injury** is related to misuse of the device. It occurs when the investigator applies pulling forces to extract the device from the vagina before vacuum is released. Training places a focus on the importance of releasing the vacuum before extracting the instrument.

- **Infections** The cleaning, disinfection and sterilization are performed according to the investigator brochure. The corresponding procedure has to be applied after each measurement. Efficacy of the cleaning, disinfection and sterilization procedure was verified by Qualis Laboratorium (validation test nr: 13 2773). The validation procedure consisted of the following steps: First, the device was contaminated with heparinised blood and microorganisms (enterococcus faecium) and subsequently cleaned and disinfected. Second, the microbial reduction was measured and checked to be within the acceptance range. Third, the cleaned and disinfected device was contaminated with heat-resistant bacteria (geobacillus stearothermophilus) and underwent the subsequent steam sterilization. Forth, the device was incubated at 60°C for 14 days and was examined for evidence of microbial growth. The device was found to be sterile, which means free from all living microorganisms.

- **Electrical shock** All parts in contact with subjects are totally
isolated from the control unit to avoid any risks of an electrical shock. The device is designed according medical safety regulations and is approved by Electro Suisse (validation test nr: MK-13-016). The protocol included the inspection of safety-labeling, as well as the device’s compliance with the investigator brochure. Moreover, protective conductor resistance measurements were performed to ensure that all parts are secured to the protective conductor terminal. After that, leakage current was measured. The leakage current is the sum of all possible leakage currents which could flow over the user or patient in the event of an interrupted protective earth conductor. Finally, insulation resistance was measured to find potential insulation faults.

6.3 STUDY PROTOCOL

The protocol of the clinical multicenter study is presented in the following paragraphs.

6.3.1 STUDY DESIGN

This study is a cross-sectional study with biomechanical measurements on pregnant women in mid-pregnancy consultation. Every participant is invited at 12 weeks of pregnancy for only one measurement session between 18+0 - 22+0 weeks of pregnancy. The outcome of pregnancy is assessed in absence of the subject (Table 6.1). During the visit, information for completing the eCRF including personal data, prior obstetric/gynecologic history and current characteristics of pregnancy is acquired. To avoid bias such as the treatment paradoxon, decisions for any clinical treatment is conducted by another medical doctor.
6. Biomechanics-based prediction of preterm birth

<table>
<thead>
<tr>
<th>Prescreening</th>
<th>Screening</th>
<th>Examination</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study invitation</td>
<td>Informed consent</td>
<td>Measurements</td>
<td>Delivery information</td>
</tr>
<tr>
<td>Study information</td>
<td>Start eCRF</td>
<td>Adverse effects</td>
<td>Withdrawal</td>
</tr>
<tr>
<td>Inclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1 Phases of the study.

6.3.2 SUBJECTS

This study includes all eligible pregnant women presenting at their mid pregnancy consultation (18+0 - 22+0 weeks of pregnancy). The presence of any of the following criteria leads to exclusion of the subject:

- Communication problems: Patient information and informed consent are available in German, French, English and Dutch. Patients that do not understand one of these languages have to be excluded
- Missing consent: signed informed consent after being informed is a prerequisite for enrollment
- Age ≤ 18
- Active bleeding, PROM
- Active genital infection
- Known carrier of HIV or hepatitis B/C
- Placenta praevia: placenta is placed on the lower part of the uterus close to the internal os
- Muellerian anomalies: malformation of uterus that carry a significant potential to worsen pregnancy outcome
- Cerclage: surgical procedure to close cervix by stitches
- Known non-compliance, drug or alcohol abuse
Pregnancies are divided into high and low risk with respect to their risk of sPTB.
The following criteria apply for subjects admitted to high risk group:

- Higher order (not singleton)
- History of sPTB $\leq 34$ weeks of pregnancy
- History of surgical cervical treatment
- Cervical length $\leq 25$mm

6.3.3 MEASUREMENTS

The biomechanical data (aspiration and maximum deformability) and cervical length data are collected. Feedback from subjects on pain or discomfort related to the measurements is requested for both biomechanical measurements and recorded as VAS (visual analogue scale ranging from 0-10).

ASPIRATION

Aspiration measurements are performed based on the previously described procedure in Section 3.3.3. This procedure requires specific training. Accordingly, physicians of each center were trained for the forthcoming aspiration measurements. The training phase was initially performed on an aspiration simulator in the laboratory at ETH Zurich, see Figure 6.1. This phase finished with a validation procedure using elastomer-cervices of known consistency. Under the supervision of Prof. Bajka and Dr. Scheiner all teams were introduced to the in-vivo measurements and were provided with all background information. After this second phase, the aspiration equipment was transferred to the hospitals and installed for study initiation. The local offices for cleaning and sterilization were informed and introduced to the device. After initial sterilization trials the device was inventoried and labelled accordingly.
6. Biomechanics-based prediction of preterm birth

Figure 6.1 Aspiration training: Training setup in the laboratory using an aspiration simulator with elastomer cervixes of known consistency. The pictures are reprinted with permission of Manfred Maurer.

MAXIMUM DEFORMABILITY

The method proposed in Parra-Saavedra et al. (2011) is applied to determine the maximum tissue deformability, in the form of the cervical consistency index CCI. Intra-vaginal ultrasound measurements are performed before aspiration measurements. An ultrasound image of the cervix is obtained by the standard procedure, the second image is captured after compression is applied with the transducer, until no further deformation is observed. The CCI is calculated as the ratio of the anterior-posterior distance before and after compression application (Figure 6.2). Performance of CCI measurements and image analysis for extraction of the required geometrical features is performed according to the instructions of Dr. Miguel Parra Saavedra. In his first visit he introduced Prof. Bajka and the project team to his procedure and verified the correctness of our protocol for CCI measurement. Although the measurement principle is quite simple, there are three difficulties related to the protocol that might affect the reproducibility of the measurements. First, the physicians is required to reach the defined state of maximum compression of the cervix. There is a certain doubt that the physicians apply sufficient compression due to their fear to impair the organ. Second, during the
Study protocol

acquisition of the first image according to the standard procedure, special care has to be taken that no pre-deformation of the cervix is induced.

Third, the proposed measuring procedure, as shown in Figure 6.2, allows some variability on how to draw the lines.

![Figure 6.2 Evaluation of the CCI value](image_url)

**Figure 6.2 Evaluation of the CCI value:** Shift cervical length line (green) to cervical canal in both images (blue), the uncompressed (left) and compressed (right). Draw a perpendicular line (pink) through the mid-point of the blue line until cervical boarder. Calculate the ratio between the length of both pink lines (AP'/AP). This ratio is called CCI. The pictures are reprinted with permission of Prof. Michael Bajka.

### 6.3.4 Study Objectives and Hypotheses

The study compares three different methods for early prediction of preterm delivery: cervical length, maximum deformability, and ecto-cervix aspiration.

The following objectives were defined:

- Quantify the cervical tissue stiffness by aspiration measurements in women with preterm and term deliveries.
6. Biomechanics-based prediction of preterm birth

- Quantify the cervical consistency index (CCI) in women with preterm and term deliveries.
- Compare results of both methods with the findings of cervical length data.
- Evaluate the predictive capabilities of aspiration measurements, maximum deformability and cervical length, possibly associated with other existing indices (personal characteristics, prior obstetric/gynecologic history, birth characteristics).
- Quantify the inter-observer variability in aspiration and maximum deformability measurements.
- Quantify the correlation between aspiration and maximum deformability measurements.

The following hypotheses were defined:

- Stiffness from aspiration and maximum deformability measurements will be significantly lower in women with preterm delivery.
- Stiffness from aspiration and maximum deformability measurements are correlated.
- Aspiration and/or maximum deformability measurements at mid-pregnancy provide at least the same diagnostic power (sensitivity, specificity, for pre-term and early pre-term) than corresponding cervical length (CL) measurements.
- Biomechanical data combined with cervical length data and other existing indices improve prediction of sPTB.

6.3.5 Statistics

Statistical analysis includes calculation of minima and maxima, means, standard deviations and distributions. Descriptive analysis and regression analysis are performed and correlations as well as predictive capabilities are evaluated (level of significance 0.05). Sample size calculation was performed based on the variability of previous results (aspiration and maximum deformability measurements). The mean value for second trimester $p_{cl}$ is $77 \text{mbar} \pm 39 \text{mbar}$ (standard deviation of 50% ) according to the
mean values reported in Chapter 4. In that study only healthy pregnant women were included and a preterm delivery was excluded. The mean value for women with preterm delivery is expected to be about 30% lower as CCI measurements have shown. The estimated mean value for women with sPTB is $p_{cl}=54\text{mbar} \pm 27\text{mbar}$. The calculated effect size is 0.69. With $\alpha=0.05$ and power of 0.95, 50 subjects in each group are needed to measure a significant difference. Considering a total preterm delivery rate of 5% (in Europe 5-9%) about 1000 measurement is set as the endpoint of this study ($\approx 950$ term deliveries and $\approx 50$ preterm deliveries $\leq 37$ weeks). Depending on the findings (level of cervical stiffness in each group and rate of early pre-term deliveries) that number of subjects might enable differentiation between early pre-term ($\leq 34$ weeks) and pre-term ($\leq 37$ weeks).

### 6.3.6 Timeline

Approximately 500 subjects at USZ/VOL and 500 subjects at KUL are needed to obtain the calculated sample size. The expected duration of the clinical trial is 2.5 years (December 2016). Hence, USZ/VOL and KUL each have to recruit individually at least 14 subjects per month. The annually expected mid-pregnancy patients in the hospital ambulatory of USZ and KUL are 3000 each, while VOL reports 250 patients per year.

### 6.4 Current state of the study

This study was approved by the ethics committee (KEK ZH Nr. 2013-0244) in November 4th 2013 and by Swissmedic (notification 2013-MD-0036) in December 30th 2013. The study was initiated at the private office in Volketswil (VOL) and the University Hospital Leuven (KUL) in April 2014. Due to problems with the infrastructure, the study at the University Hospital Zurich (USZ) started a few months later in August 2014 so that the first measurement were performed in October. The first recruitment phase between April and October has demonstrated that the recruitment in hospitals is slower compared to the private office, probably due to the doctor-patient relationship. So far 50 aspiration and
maximum deformability measurements have been performed (KUL: n=12, USZ: n=3 and VOL: n=35). The data collected are in line with the mean values of previous studies for the same gestational age, for both maximum deformability (CCI) (new: 0.71± 11% versus old: 0.60 ± 10%) and aspiration (ASP) (new: 82 ± 29mbar versus old: 74 ± 33mbar). The values for each site are illustrated in Figure 6.3.

![Figure 6.3 Preliminary results from aspiration (ASP) and maximum deformability (CCI) measurements:](image)

The data are in line with the mean values of previous studies for the same gestational age, for both CCI and ASP. Abbreviations: University Hospital Leuven (KUL), University Hospital Zurich (USZ), private office Prof. Bajka Volketswil (VOL).
Figure 6.4 illustrates the ASP and CCI values with each cross indicating a measurement pair of a single subject.

**Figure 6.4** **ASP and CCI value for each subject:** Each cross indicates a measurement pair of a single subject.

### 6.5 Outlook

The study procedure is about to be incorporated into standard clinical practice in the hospitals. This clearly will facilitate the recruitment in the following months. Completion of the study is expected for December 2016. The investigations aim to evaluate the hypothesis that cervical softness in early pregnancy correlate with the risk of sPTB. A soft cervix might be detected prior to cervical shortening which provides more time to intervene. This finding would be a strong rationale for therapeutic approaches to increase cervical stiffness in order to delay premature shortening and opening. For clinical practise these findings could complement cervical length measurements and support selection of subjects for therapy to prevent sPTB.
CERVICAL STIFFNESS AND THE UNDERLYING MICROSTRUCTURE

7.1 INTRODUCTION

Extensive mechanical and biochemical testing was performed ex-vivo on samples of mice and human cervical tissue in previous studies. These analyzes indicated that collagen cross-linking, glycosaminoglycans and water content determine cervical stiffness, see Section 2.5. A variety of in-vivo methods have been presented for quantitative determination of cervical stiffness, see Section 5.1. Knowledge of the underlying microstructural key components determining the in-vivo ecto-cervical stiffness and correlation with data obtained by biomechanical measurements is relevant towards better understanding of the observed physiological processes transforming the ecto-cervix from a stiff to a soft state. Such an analysis is required also for aspiration measurements.

The reported preliminary investigation aimed to determine the closure pressure of human ecto-cervix in-vivo as well as the underlying microstructural ECM components from biopsies taken in close proximity to the measurement location. Microstructural analysis was performed with the goal
7. Cervical stiffness and the underlying microstructure

to allow a classification of cervical tissue samples which helps rationalizing differences in the biomechanical behavior. For this purpose aspiration measurements were performed on hysterectomy subjects undergoing surgery. Corresponding cervical biopsies were obtained after extraction of the uterus.

7.2 METHODS

This study was conducted according to the ethical approval KEK: StV 09/2010/2013. 5 post-menopausal subjects undergoing hysterectomy surgery due to pelvic organ prolapse were recruited for this study.

7.2.1 PROCEDURE

Measurements (as described in Section 3.3.3) took place in the operation room by one surgeon, before any surgery related interventions. After extraction of the uterus, one cylindrical sample (radius: $\approx 4\text{mm}$, depth: $\approx 10\text{mm}$) in close proximity to the previously investigated cervical tissue was obtained using a disposable biopsy punch. Tissue samples were snap-frozen in liquid nitrogen and stored at $-80^\circ\text{C}$ until the campaign was finished.

7.2.2 MICROSTRUCTURAL INVESTIGATIONS

Biochemical assays were performed to quantify hydroxypyridinoline (HYP), sulfated glycosaminoglycans (sGAG) and deoxyribonucleic acid (DNA). Additionally, the amount of collagen cross-links (PYD and DPD) was determined. Total collagen content can be measured by performing a standard hydroxyproline (HYP) spectrophotometric assay. Hydroxyproline is an amino acid molecule found in collagen. It exists in a 7.46:1 ratio with collagen (Oxlund et al., 2010). Therefore the obtained hydroxyproline amount provides the total collagen content. GAG and DNA content determination is also based on a spectrophotometric assay. The experiments were performed under the guidance of Dr. Laura Frese, Post doc in the lab of regenerative medicine (ReMedi) at USZ.
Methods

Tissue hydration

The wet weight of cervical tissue samples was determined before lyophilization (freeze-drying). The samples were lyophilized overnight. The dry weight of the samples was determined to calculate tissue hydration by subtracting the dry weight from wet weight and normalized by the wet weight.

Sample digestion

An average dry weight of 20 µg (wet weight of 80 µg) was used to perform the biochemical assays. DNA, GAG and HYP were determined from the same sample. The dry tissue samples were incubated with papain digestion buffer overnight in a large water bath. After incubation the samples were cooled down and then stored at 4°C until further analysis.

Hydroxyproline assay

Standards for the HYP assay were prepared using HYP stock solution (hydroxyproline, Sigma H5534) and NaOH-buffer. The samples and standards were autoclaved for 10 minutes at 120°C (whole process 55 minutes). Samples were hydrolyzed to release the hydroxyproline from the peptide link. After hydrolyzation, the chromophore were formed using Chloramine-T and aldehyde/perchloric acid solution. After incubation for another 15 minutes at 65°C, samples and standards were then pipetted into a 96 wells plate. The spectrophotometer measured the absorbency of the samples at a wavelength of 550nm. This protocol is a modified version of the protocol provided by Huszar et al. (1980).

Glycosaminoglycan assay

Standards for the sGAG assay were prepared using GAG stock solution (chondroitin sulfate from shark cartilage, Sigma C4348) and papain digestion buffer. Samples and standards were then pipetted into a 96 wells plate. Total sulfated GAG content was measured using dimethyl methylene blue (DMMB) solution. The spectrophotometer measured the absorbency of the samples at a wavelength of 540 and 595nm. This is a
7. Cervical stiffness and the underlying microstructure

modified protocol described by Farndale et al. (1986)

Deoxyribonucleic Acid Assay

Standards for the deoxyribonucleic acid (DNA) assay were prepared using DNA stock solution (calf thymus DNA, Sigma) and tris-EDTA (TE)-buffer. Samples and standards were then pipetted into black 96 wells plate. Total DNA content was measured as an indicator for cell number using Hoechst dye solution (Cesarone et al., 1979). The spectrophotometer measured the absorbency of the samples at a wavelength of 355 and 460nm.

The content of HYP, DNA and sGAG in each sample was normalized by tissue dry weight to calculate the concentration.

Collagen Cross-link Analysis

Measurement of the pyridinoline (PYD) and deoxypyridinoline (DPD) molecules was performed at the ENDONET laboratory, Basel, Switzerland. An average wet weight of 80 µg per sample was sent to the lab to perform the analysis. Cross-links were determined by High Performance Liquid Chromatography (HPLC). The tissues were hydrolyzed and after dilution the mixture was loaded on an extraction tube. After a washing step, the molecules PYD and DPD were eluted from the extraction tube. The separation of PYD and DPD on the HPLC system was based on the ion-exchange chromatography on a reversed phase cartridge with isocratic elution. Detection and quantification is reached by utilising their natural fluorescence.

7.3 Results and Discussion

Cervical stiffness has been assessed in all 5 post-menopausal subjects, Caucasians, from 57 to 77 years of age, presenting a moderate (m) to total (t) pelvic organ prolapse. The $p_{el}$ mean value of all measurements is 469 ± 122 mbar ($\approx 25\%$ variability). Goh (2002) and Roehrbauer (2013) found an increased vaginal wall stiffness in post-menopausal prolapse subjects
with lack of estrogen which is in line with the findings of Chantereau et al. (2014) and those of this study. Compared to our reference pre-menopausal control group (320±120mbar, ≈38% variability) reported in Section 4.3, the obtained mean value is about 150mbar higher than expected. Within the investigated population, a large difference in $p_{cl}$ (2.6 times smaller) is found for the 57 years old subject with moderate prolapse ($p_{cl}=205$mbar) compared to the remaining four subjects (average of 535mbar, min: 428mbar to max 600mbar). Despite the large difference in cervical stiffness, no evident corresponding explanation was found at microstructural level (Table 7.1).

From the evaluation of all data, it could be hypothesized that the number of total enzymatic collagen cross-link content (DPD and PYD) is related to the stage of prolapse and tissue stiffness since subjects with total prolapse show an average of 6107pmol/ml while subjects with moderate prolapse show twice as much cross-link concentration (13069pmol/ml). Conversely, subjects with total prolapse have shown rather high stiffness values which would not be expected based on the low crosslink concentration in their tissue. The high stiffness values could be explained by the following observation: both subjects with total prolapse had the cervix protruding through the vaginal opening which probably led to keratinized cervical epithelium and possibly pre-deformation of cervical tissue, thus leading to higher resistance to deformation at the ecto-cervix.

The measured level of hydration, GAG concentration and PYD as the most abundant enzymatic crosslink are in line with data previously reported for non-pregnant pre-menopausal cervical tissue (Myers et al., 2008; Zork et al., 2014). The reported collagen concentration (mean value: 38.9%) is lower than the data of Myers et al. (2008) (mean value: 77%). This finding is in line with reported data by Wong et al. (2003) which indicate that collagen concentration in the cervix of prolapse subjects is lower than in healthy non-pregnant subjects. In Zork et al. (2014) a measure of collagen cross-link density was proposed as the ratio of the amount of collagen cross-link to collagen content of the sample. Comparison to the present data shows that the average collagen density in this group (0.0002mol/mol) is smaller than the previously reported 0.1mol/mol. However it should be noted that this comparison is based on
two different samples, i.e. colorimetric assay (total collagen content) and HPLC (collagen crosslinks).

Another explanation for the increased cervical stiffness in the study population could be the level of non-reducible collagen crosslinks which was not measured in this study. From skin studies (Calleja-Agius and Brincat, 2012; Pawlaczyk et al., 2013), it is known that increased tissue stiffness with aging, independent of estrogen levels, is due to increased amount of non-reducible collagen crosslinking, see Chapter 2. This type of crosslink increases stiffness but also brittleness of tissue due to the highly packed collagenous network. Similar findings were reported in bone studies, where an increase of bone fragility (brittleness) due to lack of estrogen was associated with high level of non-reducible collagen crosslinks (Saito and Marumo, 2010; Willet et al., 2014).

It should be noted that pelvic organ prolapse subjects are not the most suitable study population for analysis of cervix biomechanics. Most of the subjects are post-menopausal which cannot be assumed to be representative for the pre-menopausal state of the cervix due to their lack of estrogen. Moreover, pelvic organ prolapse is a pathologic condition that has influence on the cervical collagen metabolism, morphology and anatomy.

This preliminary investigation indicates the following priorities for a future study aiming at rationalizing cervical tissue biomechanics based on tissue microstructure:

- Identify possibilities to obtain younger cervical tissue from hysterectomy or other surgical procedures
- Include only pre-menopausal subjects
- Extend collagen cross-link analysis by quantifying the amount of non-reducible collagen crosslinks
- Perform cross-link and hydroxyproline analysis on the same sample to enable determination of sample specific cross-link density
- Increase the number of samples to allow proper statistical analysis
Table 7.1 Microstructural components and cervical stiffness for the investigated population: Age in years; number of vaginal deliveries (VD); stage of pelvic organ prolapse (i.e. moderate (m) or total (t)); hydration level (H); collagen concentration (C), GAG (G), DNA (D) are in percentage of dry weight; concentration of DPD and PYD cross-links; cervical stiffness $p_{el}$. 

<table>
<thead>
<tr>
<th>Age</th>
<th>VD</th>
<th>POP</th>
<th>H[%]</th>
<th>C[%]</th>
<th>G[%]</th>
<th>D[%]</th>
<th>D/PYD[pmol/ml]</th>
<th>$P_{el}$[mbar]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>71</td>
<td>2</td>
<td>m</td>
<td>71.1</td>
<td>31.8</td>
<td>2.6</td>
<td>10.0</td>
<td>2303/11925</td>
</tr>
<tr>
<td>NP2</td>
<td>65</td>
<td>3</td>
<td>t</td>
<td>80.5</td>
<td>25.1</td>
<td>1.9</td>
<td>14.6</td>
<td>939/5413</td>
</tr>
<tr>
<td>NP3</td>
<td>57</td>
<td>3</td>
<td>m</td>
<td>75.4</td>
<td>48.0</td>
<td>1.2</td>
<td>10.0</td>
<td>3021/9285</td>
</tr>
<tr>
<td>NP4</td>
<td>77</td>
<td>2</td>
<td>t</td>
<td>74.9</td>
<td>41.7</td>
<td>0.8</td>
<td>7.4</td>
<td>1234/4629</td>
</tr>
<tr>
<td>NP5</td>
<td>62</td>
<td>1</td>
<td>m</td>
<td>78.2</td>
<td>47.9</td>
<td>0.7</td>
<td>12.4</td>
<td>3109/9564</td>
</tr>
<tr>
<td>mean</td>
<td>66.4</td>
<td>2.2</td>
<td></td>
<td>76.0</td>
<td>38.9</td>
<td>1.4</td>
<td>10.9</td>
<td>2121/8163</td>
</tr>
</tbody>
</table>
CHAPTER EIGHT

OBJECTIVE ASSESSMENT OF CERVICAL CONSISTENCY AFTER ADMINISTRATION OF MISOPROSTOL FOR INTRA-UTERINE CONTRACEPTIVE INSERTION

8.1 Introduction

Intra-uterine contraceptives (IUC) belong to the most widely used family planning methods worldwide (UnitedNations, 2009) with more than 180 million users worldwide (Darney and Speroff, 2010). Modern IUCs are reversible, long-acting, safe and cost-effective (Winner et al., 2012). However, IUC uptake varies significantly across the world. Women living in China constitute about 70% of the global users, while 13.6% of European and only 1.7% of U.S. women practising contraception use an IUC (UnitedNations, 2009). Fear of pain during insertion is one of the well-known reasons that may prevent women from choosing an IUC as their method
8. Objective assessment of cervical consistency after administration of misoprostol for intra-uterine contraceptive insertion

of choice (Allen et al., 2009; Hubacher et al., 2006). For the vast majority, IUC insertion does not cause severe pain, but around 10% of (multi-)para (P) (Heikinheimo et al., 2010) and 20% of nulli-para (0P) (Marions et al., 2011) women report this procedure as very painful. It may, therefore seem surprising that the need for pain relief during IUC insertion is still under debate and no generally accepted concept exists.

In obstetrics and gynecology, positive results from the application of misoprostol for cervical softening (CS) have led to common “off-label” usage, such as induction of labor (Lukoschus et al., 2003), postpartum hemorrhage control (Blum et al., 2007) and abortion care (Ngai et al., 2000; Oppegaard et al., 2006; Radulovic et al., 2007), as well as easing cervical dilation prior to hysteroscopy (Choksuchat et al., 2006; El-Rafaey et al., 1994; Ngai et al., 2001; Oppegaard et al., 2008).

Cervical consistency (CC) might be reduced by misoprostol triggered changes in the tissue microstructure (Shi et al., 2000), like disorganization of the collagenous network (Fittkow et al., 2005) and an increase in GAG synthesis (Hertelendy and Zakár, 2004; Norman et al., 1993).

Based on these promising results, misoprostol was further evaluated for pain reduction in IUC insertion (Allen et al., 2009; Black et al., 2012), especially exploring the possible advantage for nulli-para (0P), who generally present a narrow and un-stretched cervix (Farmer and Webb, 2003; Kaislasuo et al., 2014; Lathrop et al., 2013). Indeed, some studies found the use of misoprostol leads to an easier IUC passage through the cervical canal (Li et al., 2005; Lotke et al., 2013; Sääv et al., 2007; Scavuzzi et al., 2013) and greater cervical dilation (Scavuzzi et al., 2013), but several other studies could not support the advantages of misoprostol administration, neither from the doctor’s judgment regarding ease of insertion (Dijkhuizen et al., 2011; Espey et al., 2014; Heikinheimo et al., 2010; Lathrop et al., 2013) nor from the patient’s judgment on pain experience (Espey et al., 2014; Heikinheimo et al., 2010). In a large review, Gemzell-Danielsson et al. (2013) found no conclusive evidence to prove that prophylactic pharmacological intervention reduces pain on IUC insertion. This conflicting data, doubting the effectiveness of misoprostol, might arise from a lack of i) an adequate drug administration route (Li et al., 2005; Lotke et al., 2013; Sääv et al., 2007; Tang et al., 2007),
drug dosage (Crane, 2008; Dijkhuizen et al., 2011; Fiala et al., 2007) and the time interval between application and intervention (Dijkhuizen et al., 2011; Heikinheimo et al., 2010; Li et al., 2011), ii) standardized ease of insertion assessment (Sääv et al., 2007; Swenson et al., 2012), iii) standardized discomfort assessment (Castro et al., 2014; Heikinheimo et al., 2010; Scavuzzi et al., 2013) and iv) standardized protocol for IUC insertion (Bahamondes et al., 2014; Sääv et al., 2007). Another explanation may be that CS is so slight that the beneficial effect can barely be identified by the providers nor can it be felt by the women. Some may, therefore, assume that the supposed CS effect of misoprostol is non-existent. Despite the lack of clear evidence, health care providers frequently provide misoprostol in the belief reducing pain during IUC insertion (Ward et al., 2011). However, so far no study has ever assessed CS in a quantitative and objective manner, verifying or rejecting the hypothesis that misoprostol may cause CS.

In the present study, it was focused on acquiring objective data to measure the situation-related CC using the aspiration method (ASP). This study is aimed at objective quantification of CC in misoprostol users prior to IUC insertion and at follow-up consultation to evaluate the influence of misoprostol on cervical priming. Parts of this chapter, including paragraphs of text and figures are published Badir et al. (2014b).

8.2 METHODS

8.2.1 SUBJECTS

From July 2013 all women presenting for IUC placement at the private office were invited to take part in the study. We focused on the 52mg-levonorgestrel intra-uterine system LNG IUS (Mirena®, Bayer Healthcare, Germany), since in this unit more than 90% of the women chose an LNG IUS as their IUC. Non-inclusion criteria were communication problems, prior surgery on the cervix, untreated premalignant or malignant changes on the cervix, contraindications to using a LNG IUS, and the use of misoprostol or non-steroidal anti-inflammatory drugs. By October 2014, 40 women were included. 40 Caucasians aged from 22 to 49 years
8. Objective assessment of cervical consistency after administration of misoprostol for intra-uterine contraceptive insertion

(mean: 35 years), mostly parous subjects. 25 underwent a first LNG IUS insertion and 15 for the consecutive LNG IUS insertion (exchange). Participants received 200µg of misoprostol combined with 75mg of diclofenac in a single tablet orally (Arthrotec forte 75/200®, Pfizer, USA) 6 to 12 hours before insertion in “off label” use.

8.2.2 Measurements

Directly before LNG IUS insertion, aspiration measurements were performed, as described previously in Section 3.3.3 to measure ecto-cervical stiffness as a quantitative measure for CC. Aspiration measurements were repeated at regular follow up consultations including a sonographic IUC position check around 6 weeks after insertion.

8.3 Statistics

Statistical analysis was performed with the statistical computing environment R: A Language and Environment for Statistical Computing, Open Source Software, 2012. The Wilcoxon rank-sum test was used to compare differences between the non-pregnant control group (Section 4.3) and the two groups in this study, both prior to IUC placement (first and consecutive insertion) and at follow-up consultation. For comparison of values of the same subjects at insertion and at follow-up, the Wilcoxon signed-rank test was applied.

8.4 Results

CC was successfully assessed in all subjects. The primary outcome was the $p_{cl}$ value (i.e. CC) at insertion after misoprostol intake. We found average values for $p_{cl}$ of 290 mbar ± 138mbar (mean ± SD) at insertion and 324mbar ± 138mbar at follow-up showing no significant difference when assessing the group as a whole. However, when we divided the study population into first and consecutive LNG IUS insertion, we found 230mbar ± 93mbar in the first LNG IUS insertion group ($n = 25$), and $p_{cl}$ of 396mbar ± 90mbar in the consecutive LNG IUS insertion group.
Results

\((n = 15)\). The comparison with control subjects \((n = 50)\) reported in Section 4.3 \((320\text{mbar} \pm 120\text{mbar})\) revealed that \(p_{cl}\) at insertion was significantly lower \((p = 0.005)\) in the first LNG IUS insertions indicating a reduction in CC. In contrast, the comparison between control subjects and consecutive LNG IUS insertions demonstrated a higher \(p_{cl}\), but did not reach statistical significance \((p = 0.08)\), see Figure 8.1 and Figure 8.2.

![Graph showing closure pressure \(p_{cl}\) for control and consecutive insertions with significance levels marked.](attachment:image.png)

**Figure 8.1** Results of closure pressure \(p_{cl}\) of the control group (Section 4.3) and subjects at insertion and at follow-up: first IUC insertion, left; consecutive IUC insertion, right. For all values, means and standard deviations and p-values are reported; significant results are indicated by * \((p < 0.05)\).

Additionally, comparison of \(p_{cl}\) values at insertion between first and consecutive insertions showed a significant difference \((p \leq 0.001)\). Pre and post-comparison of \(p_{cl}\) at insertion and at follow-up demonstrated a differentiated behavior in cervical consistency change in the two groups. In the first LNG IUS insertion group, CC increased significantly \((p \leq 0.001)\) to a similar level \((308\text{mbar} \pm 105\text{mbar})\) at follow-up as the expected from
8. Objective assessment of cervical consistency after administration of misoprostol for intra-uterine contraceptive insertion

control group (320mbar ± 120mbar) while in the consecutive IUS insertion group cervical consistency decreased significantly to the level of the control group (p = 0.03). Closure pressure values obtained at follow-up are not statistically different from the control group (first insertion, p = 0.6 and consecutive insertion, p = 0.7). The same non-significant finding is revealed in the comparison between $p_{cl}$ values between follow-up after first and consecutive LNG IUS placement (p = 0.5), see Figure 8.1.

![Figure 8.2 Pre and post-comparison of closure pressure $p_{cl}$ of each subject individually at insertion and at follow-up: first IUC insertion, left; consecutive IUC insertion, right.]

8.5 Discussion

For the first time, cervical consistency was quantitatively assessed in misoprostol users prior to IUC insertion, showing that i) aspiration is able to detect pharmacologically induced cervical changes, and ii) misoprostol has a detectable softening effect on cervical tissue at first insertion. We compared measurements taken from women at their first LNG IUS insertion with their measurements taken around 6 weeks later (when there were no
effects from the previous misoprostol administration). It was found that CC was significantly lower initially with misoprostol but after 6 weeks CC recovered to the reference stiffness values of non-pregnant women.

In contrast, the group with an LNG IUS exchange did not show a decrease of CS caused by misoprostol. CC was not significantly different, at insertion and at follow up some weeks later, and was comparable to the reference cohort. These results are in line with Heikinheimo et al. (2010).

In their study misoprostol did not have an effect on the ease of insertion in subjects having a consecutive insertion of an LNG IUS. Thus, we assume that prostaglandin induced CS is blocked in IUS exchange subjects by locally released LNG leading to non-significant change in mechanical properties of the cervix after misoprostol administration as discussed in Section 2.5.

In our study, 200\(\mu\)g misoprostol and 75mg diclofenac was given orally 6-12 hours prior to insertion of the LNG IUS. The oral administration route was chosen for practical reasons combined with a smaller dose of misoprostol, as recommended by Sääv et al. (2007), to lower the incidence of uterine cramps. Nonetheless, diclofenac was co-administered, as suggested by Gemzell-Danielsson et al. (2013), to manage prostaglandin-induced side effects. In previous IUC insertion studies misoprostol was administered bucally (Edelman et al., 2011; Lathrop et al., 2013), sub-lingually (Espey et al., 2014; Heikinheimo et al., 2010; Kaislasuo et al., 2014; Sääv et al., 2007) or vaginally (Dijkhuizen et al., 2011; Li et al., 2005; Scavuzzi et al., 2013). In contrast to our protocol the dosage of 400\(\mu\)g administered in the above studies was significantly higher and the time lag between misoprostol intake and insertion was within 1 to 4 hours significantly shorter (Dijkhuizen et al., 2011; Edelman et al., 2011; Espey et al., 2014; Heikinheimo et al., 2010; Lathrop et al., 2013). We decided for a longer priming interval based on the recommendations of different authors (Choksuchat et al., 2006; Crane, 2008; Li et al., 2005, 2011; Sääv et al., 2007). Li et al. (2011) critically addressed the importance of the time interval (three hours are not enough) to induce significant CS and to obtain the benefit of misoprostol for insertion. Since we measured a significant CS after misoprostol intake in the first LNG IUS insertions, our observation supports the importance of a priming interval of 6 to 12
8. Objective assessment of cervical consistency after administration of misoprostol for intra-uterine contraceptive insertion

hours. However, this study was not designed to answer this question. The exact role of diclofenac remains unclear, but we believe that the very low rate of misoprostol induced uterine cramps (5%) in this study is the result of the diclofenac effect, as assumed in Bahamondes et al. (2014). A limitation of this study is the lack of sub-analysis of the misoprostol effect on nulli-parous (0P) versus (multi)-parous (P) due to the small number of nulli-parous subjects, and the restriction to only one typically used IUC. Another possible limitation to this study is that it was not conducted as a blind randomized controlled trial.

8.6 Conclusion

The usefulness of aspiration measurements in non-pregnant women prior and after IUC insertion has been demonstrated. This method allowed simple and quantitative CC assessment to evaluate the effect of misoprostol on the cervical tissue. Our results are indicative for misoprostol induced CS in subjects for first IUC insertion. As a clinical consequence, based on our results, we could suggest a differentiated misoprostol administration policy, i.e. women undergoing first LNG IUS insertion might benefit from 200 \( \mu \)g oral misoprostol administration 6-12 hours prior to insertion (evidence of CS), whilst women undergoing LNG IUS exchanges might not benefit from (lack of CS). The clinical value of this finding should be substantiated, and further aspiration measurements could be helpful in searching for the ideal candidate, appropriate route, dosis and interval of misoprostol intake prior to IUC insertion.
CHAPTER
NINE

CONCLUSIONS

This thesis aimed at contributing to the present challenging questions in prenatal research where the human uterine cervix plays an important role. The focus was on the development of a new measurement procedure compatible with clinical routine to perform studies on the cervix in pregnant and non-pregnant state. Numerous aspiration measurements (≥1100) were performed and analyzed to provide fundamental knowledge and clinically relevant information. Insight into the fascinating mechanical behavior of cervical tissue was gained and novel approaches have been defined to investigate cervical characteristics to improve the identification of women at risk of a spontaneous preterm birth (sPTB).

9.1 Contribution of the present work

Aspiration device and measurement protocol A novel measurement procedure based on the aspiration technique was developed for intravaginal assessment of cervical tissue stiffness. The latest optimization of the procedure and instrument significantly contributed to the practicality and reliability of the measurements during routine consultation. Safety, short duration and ease-of-use of the measurement procedure allowing to
9. Conclusions

Collect a large number of data were the most important features of the instrument, leading to evident limitations in the mechanical characterization of cervical tissue. The obtained parameters of model equations are representative of the resistance to deformation of non-pregnant and pregnant cervical tissue but do not inform about time and history characteristics of the cervix. Moreover, the measurements were performed at the ecto-cervix on a very local portion of tissue, and the mechanical behavior of this location might not be representative for the organ. Despite these limitations the provided model parameters of ecto-cervical tissue might be qualitatively useful for biomechanical simulations investigating the evolution of cervical stiffness, or its components, during pregnancy and related conditions leading to preterm cervical dilation.

**In-vivo characterization of the uterine cervix in pregnancy** For the first time, the important mechanical characteristics of the cervix in pregnancy were investigated. The study findings demonstrate that cervical changes (measured as cervical softening) start with initiation of pregnancy and well-before cervical shortening is observed. Cervical changes are relevant to prepare the reproductive tract for delivery. However, early abnormal cervical softening might lead to the inability to maintain pregnancy until term, since the cervix is unable to sustain increasing uterine pressure. The usefulness of this biomechanical method in identifying women at risk of sPTB based on this hypothesis is currently assessed in the clinical multicenter study SOFTCERVIX.

**Elastography procedures for determination of cervical stiffness** Two tissue mimicking phantoms with different stiffness measured with a commercially available ultrasound machine provided a basis to rationalize contradictory results obtained by quasi-static elastography measurements compared to aspiration test performed during gestation. It was demonstrated that quasi-static elastography as previously proposed does not allow to distinguish between a stiff and soft cervix. On the other hand, the maximum deformability approach can deliver an assessment of cervical consistency in pregnancy, which is in line with the aspiration data.

**Objective assessment of cervical consistency after administration of misoprostol for intra-uterine contraceptive insertion** The quantification of pharmacologically induced cervical softening is another
identified field of application for the developed instrument and biomechanical measurement procedure. This pilot study allowed for the first time to successfully assess cervical stiffness in subjects who were given misoprostol prior to intrauterine contraceptive insertion. The results have shown that the aspiration device is able to detect quantitatively pharmacologically induced cervical changes and misoprostol has a detectable softening effect on cervical tissue at first intrauterine LNG IUS insertion.

9.2 OUTLOOK

9.2.1 FUNDAMENTAL RESEARCH

Cervical stiffness and the underlying microstructure The quantitative link between ECM composition and cervical stiffness, as well as the evolution in pregnancy remains an important open question. The preliminary analysis of the microstructural components leading to differences in the in-vivo biomechanical behavior indicated criterions for planning future studies. In particular, in order to avoid bias, subjects with total pelvic organ prolapse and protruding cervix through the vaginal opening should not be included in future investigations. This leads to the problem of obtaining biopsies after biomechanical measurements. Thus, in-vivo methods such as the Collascope (Maul et al., 2003) could be helpful to quantify microstructural components.

Biomechanical simulations and constitutive modeling A constitutive model based on aspiration data to represent the response of the ecto-cervix failed to describe the mechanical behavior of the bulk organ. Consequently, more information is needed to predict cervical deformation under physiological loading. Ex-vivo experiments (Fernandez et al., 2013; Myers et al., 2008, 2010, 2009; Yao et al., 2014) have been performed tailored to specific kinematic configurations to inform constitutive model equations to be implemented in advanced FE models. Biomechanical analysis using a 3-D finite element model of pregnancy with appropriate boundary conditions (including contact conditions) and a corresponding constitutive model capturing the most important mechanical features of
the cervix (e.g. fiber orientation) represent essential tools for better understanding of physiology and pathology of cervical function. The performance of the cervix in pregnancy depends on multiple factors such as mechanical loading, structural support and mechanical properties of the whole reproductive tract. The circumstances leading to premature cervical deformations and cervical opening are still unclear. In-vivo experimental procedures measuring mechanical and microstructural properties of the cervix are needed to describe changes in pregnancy. The provided model parameters of ecto-cervical tissue might be useful for determination of evolution of response of specific ECM components during pregnancy. In the future, this set of parameters could be extended by parameters representative of the resistance to deformation of subjects with abnormal cervical remodeling (e.g. preterm and post-term delivery), as a result of the on-going clinical studies.

9.2.2 CLINICAL DIAGNOSIS AND RESEARCH

Biomechanics-based prediction of preterm birth The relevance of aspiration measurements on the clinically easily accessible site, the ecto-cervix, for early prediction of sPTB is an open question. The usefulness of this approach for diagnostics in combination with maximum deformability measurements is currently assessed in the international multicenter clinical study SOFTCERVIX. The stiffness of the uterine cervix in 1000 subjects at mid-pregnancy will be measured using prototype 2.0 and compared between subject groups with term and preterm delivery. The goal of this study is to demonstrate that biomechanical evaluation (aspiration and/or maximum deformability) helps to improve the prediction of sPTB.

Further development of the instrument The current device is a prototype and has to undergo the required development phase in order to transform into a cost-effective medical product ready for a wider clinical use. This involves: i) the review of the present design including existing technical features, ii) the consideration of additional features to improve usability and eliminate misuse faults, iii) the miniaturization and redesign of some components, iv) the simplification of the intra-vaginal application procedure, and v) the definition of materials and manufacturing processes
Outlook
to allow for industrialization. Currently, every aspiration measurement is monitored via the built in camera. Our collaborators’ feedback revealed that the visual feedback is not as necessary as assumed. Thus the camera feature needs to be reconsidered for the next version of this device. A repeatable and standardized aspirator placement on the cervix is a substantial task for a successful measurement. The current procedure is reliable, however not sufficient for use by untrained physicians and needs further development. Current trends in medical industry are towards disposable devices. Our experience has clearly illustrated that the sterilization procedure after every application is time-consuming and costly. Future design optimization of the components should consider these aspects and finally lead to a disposable device. The revised device concept shall be developed in close cooperation with medical doctors to ensure that practical needs for application are met.

Assessment of in-vivo biomechanical properties of the cervix
During this research work other fields of application for this device were identified and could soon turn into new investigations with high relevance for clinical practice, as follows:

- **At delivery:** Cervical tissue at delivery is expected to be significantly softer as compared to the present observation in the third trimester. A future clinical study could quantitatively characterize this final softening in the hours preceding delivery. Additionally to this investigation, the effect of misoprostol and its optimal dosis could be studied to allow a successful induction of labor.

- **Prior to IUC insertion:** Misoprostol is given to women undergoing IUC placement in order to facilitate insertion and reduce the related pain. The benefit of this medication is under debate and more investigation is needed to answer this open question. Aspiration measurements could be helpful in searching for an appropriate administration of misoprostol to optimize its benefit.

Amniocentesis is a procedure performed in early pregnancy in prenatal diagnosis to detect fetal genetic disorders. Fetoscopy is an endoscopic procedure to allow necessary medical interventions on the fetus during pregnancy. For both purposes the fetal membrane is punctured. Two
9. Conclusions

studies during these procedures are suggested:

- Puncturing the fetal membrane bears the risk of inducing preterm delivery. Performing aspiration measurements on the cervix at the time of amniocentesis/fetoscopy could help identifying women at risk of iatrogenic induced preterm delivery.

- Studying the relationship between amniotic fluid biomarkers (e.g. inflammatory cytokines as markers for up-regulation of uterine activity) and cervical stiffness might link downstream changes in the cervix with etiology.
Bibliography


Bibliography


Bibliography


Bibliography


128


Bibliography


Bibliography


Bibliography


Bibliography


Bibliography


Bibliography


Bibliography


Appendix

A  Aspiration tubes

Both aspiration tubes were manufactured by Fiberoptic P&P AG. Technical data is provided in the following drawings.
Appendix

A.1 Prototype 1.0

Figure A.1 Dimensions of the aspiration tube.
Aspiration tubes

Figure A.2 Components of the aspiration tube: The individual parts are the aspiration tube (aluminium and stainless steel), cylinder (aluminium) and evacuation pipe (stainless steel).

A.ii Prototype 2.0

Figure A.3 Dimensions of the aspiration tube.
Appendix

Figure A.4  Components of the aspiration tube: The individual parts are the aspiration tube (aluminium and stainless steel) and cap (polyether ketone).

B  Data Management

The electronic case report form is accessed from the login page http://www.usz.ch/non_cms/zkf/DM/DCdp.html. The next step is to select the customer area USZ (2) and enter login access information (user-ID, password). After successful login the welcome page is displayed. In Figure B.5 the different levels of the data collection tool are shown.
LEVEL 1: Overview - Patients and Visits

<table>
<thead>
<tr>
<th>Patient</th>
<th>Σ SDV</th>
<th>Screening</th>
<th>Measurement</th>
<th>Follow-up</th>
<th>STUDY END</th>
<th>ADVERSE EVENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>eux320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fpy297</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LEVEL 2: Overview patient eux320

Planned visits
- Screening: 03.06.14
- Measurement: 03.06.14
- Follow-up: 31.10.14

LEVEL 3: Screening folder

Planned visits
- Screening: 03.06.14
- Measurement: 03.06.14
- Follow-up: 31.10.14

LEVEL 4: Screening form personal characteristics

Demographic Data

- Date of birth: 10.10.1986
- Age at time of signature of informed consent (calculated): 27 Jahre 7 Monate 24 Tage

Ethnic Origin

- Caucasian
- Asian
- African
- Other

If OTHER: Please specify.

Figure B.5 Data collection tool.