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Cervical consistency index and quantitative cervical texture analysis by ultrasound to predict spontaneous preterm birth

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CERVICAL CONSISTENCY INDEX AND QUANTITATIVE CERVICAL TEXTURE ANALYSIS BY ULTRASOUND TO PREDICT SPONTANEOUS PRETERM BIRTH

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To obtain the degree of "Doctor in Medicine"

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We declare that **Núria Baños López** has, under our supervision, performed the studies presented in the thesis **"Cervical Consistency Index and Quantitative cervical texture analysis by ultrasound to predict spontaneous preterm birth"**. This thesis has been structured following the normative for PhD thesis as a compendium of publications to obtain the degree of **Doctor in Medicine** and the mentioned studies are ready to be presented to a Tribunal.

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PRESENTATION

The present thesis has been structured following the normative for PhD thesis, as a compendium of publications to obtain the dregree of **Doctor in Medicine**. It was approved by the "Comissió de Doctorat de la Facultat de Medicina" on May 15th, 2014. The projects included in this thesis belong to the same research line, which resulted in the publication of four articles in international journals.

Article 1

Baños N, Murillo-Bravo C, Julià C, Migliorelli F, Perez-Moreno A, Ríos J, Gratacos E, Valentin L, Palacio M. Mid-trimester sonographic Cervical Consistency Index to predict spontaneous preterm birth in a low-risk population. Ultrasound Obstet Gynecol. 2017 Mar 31. doi: 10.1002/uog.17482.

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Article 2

Baños N, Julià C, Lorente N, Ferrero S, Cobo T, Gratacos E, Palacio M.Mid-trimester sonographic Cervical Consistency Index to predict spontaneous preterm birth in a high-risk population. American Journal of Perinatology Reports.

Status: under review.

Article 3

Baños N, Perez-Moreno A, Migliorelli F, Triginer L, Cobo T, Bonet-Carne E, Gratacos E, Palacio M. Quantitative Analysis of the Cervical Texture by Ultrasound and Correlation with Gestational Age. Fetal Diagn Ther. 2016 Aug 11.

Status: published. Journal Impact Factor: 2.700, 1st quartile.

Article 4

Baños N, Perez-Moreno A, Julià C, Murillo-Bravo C, Coronado D, Gratacos E, Deprest J, Palacio M. Quantitative analysis of the cervical texture by ultrasound in the mid-pregnancy is associated with spontaneous preterm birth. Ultrasound Obstet Gynecol. 2017 May.

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TABLE OF CONTENTS

1. INTRODUCTION 13

1.1. A public health priority: spontaneous preterm birth 15

1.2. The clinical challenge: prediction of spontaneous preterm birth 16

1.3. The current strategy: measurement of cervical length 18

1.4. The hypothesis: premature cervical remodelling along pregnancy 19

1.5. The ultrasound tools proposed 23

1.5.1 Cervical Consistency Index (CCI) 23

1.5.2 Quantitative analysis of cervical texture (CTx) 24

1.6. Other ultrasound-based methods 26

2. HYPOTHESIS 27

2.1. Main hypothesis 29

2.2. Specific Hypothesis 29

3. OBJECTIVES 31

3.1. Main objective 33

3.2. Specific Objectives 33

4. METHODS 35

4.1. Study 1 37

4.2. Study 2 38

4.3. Study 3 39

4.4. Study 4 40

5. STUDIES 43

5.1. Study 1. Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a low-risk population. 45

5.2. Study 2. Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a high-risk population. 83

5.3. Study 3. Quantitative analysis of the cervical texture by ultrasound and correlation with gestational age. 113

5.4. Study 4. Quantitative analysis of the cervical texture by ultrasound in the mid-pregnancy is associated with spontaneous preterm birth. 125

6. RESULTS 153

6.1. Study 1 155

6.2. Study 2 155

6.3. Study 3 156

6.4. Study 4 157

7. DISCUSSION 159

7.1. Cervical Consistency Index (CCI) 161

7.1.1. Further studies on CCI 163

7.2. Quantitative analysis of cervical texture (CTx) 164

7.2.1. Further studies on CTx 165

7.3. Future steps with the CCI and CTx 165

8. CONCLUSIONS 169

8.1. Study 1 171

8.2. Study 2 171

8.3. Study 3 172

8.4. Study 4 172

9. REFERENCES 173

1. INTRODUCTION

1. INTRODUCTION

1.1. A public health priority: spontaneous preterm birth

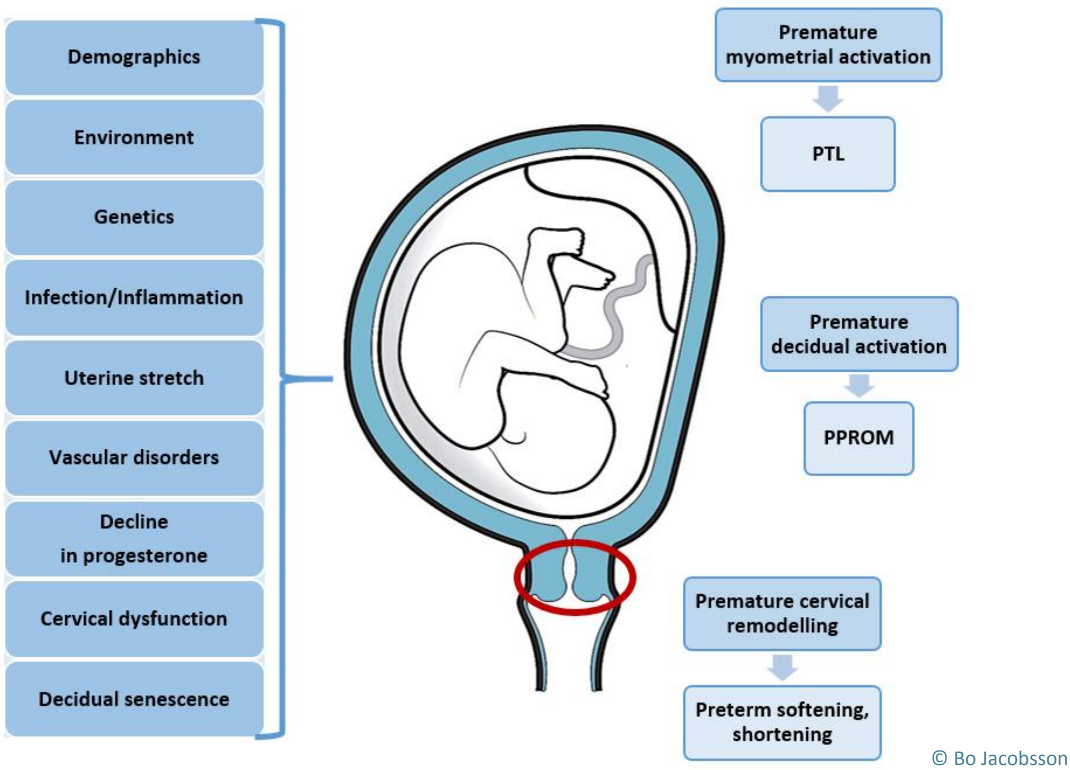
In the field of foetal-maternal medicine, preterm birth (PTB) remains a major contributor to perinatal morbidity and mortality in both developed and developing countries, being the second leading cause of death in children under 5 years of age[1,2]. The rate of spontaneous preterm birth (sPTB) is far from decreasing as desired and still accounts for approximately 5% of all births in European countries and up to 12% in USA and in most developing countries[3]. It affects 13 million infants annually, of whom 35% will die within the first 4 weeks of life from causes directly related to prematurity[4,5]. For the infants who survive, many will face a lifetime of significant disability that will also affect their families and health systems. Preterm birth accounts for 3.1% of all disability-adjusted life years (DALYs) in the Global Burden of Disease, being higher than that of HIV and malaria [6,7]. In fact, neonatal deaths due to prematurity are one of the most important barriers to achieve one of the Millennium Development Goals[8] (MDG) which called for a reduction in the under-5 mortality rate by two thirds between 1990 and 2025. Moreover, according to a study which analysed the evolution in PTB rates from 2000 to 2010, even with an appropriate education and intervention based on current evidence (smoking cessation, single embryo transfers, cervical cerclage, progesterone treatment and avoidance of unnecessary iatrogenic preterm delivery) the incidence of PTB would be reduced by only 5%[5]. The statement that current preventive, predictive and treatment strategies leave 95% of preterm births intractable reinforces the urgent need for further research in this field. It is important to remark that this project focuses on sPTB, which is defined as preterm birth after the spontaneous onset of labour with intact membranes or preterm birth after a premature pre-labour rupture of membranes (PPROM). Spontaneous preterm birth is not a single condition, but a single outcome due to multiple causes[9]. Hence, there is not a single solution, but rather an array of solutions aimed at addressing the heterogeneous biological, clinical, behavioural and social risk factors that result in sPTB[10].

1.2. The clinical challenge: Prediction of sPTB

Spontaneous preterm birth is a heterogeneous syndrome with a multifactorial aetiology. Preterm birth requires cervical ripening, rupture of membranes and uterine contractions, usually caused by multiple and unknown factors with complex interactions[11](**Figure 1**). Therefore, finding a single and powerful predictor of sPTB is an enormous challenge. Regarding the risk factors of sPTB, history of a previous sPTB or late miscarriage ≥ 16 weeks[12-14], Müllerian malformations[15-17] and uterine interventions[18,19] have shown limited utility as stand-alone predictors of sPTB [15,18,20,21].

A history of a previous PTB is the most powerful clinical risk factor, but only 15% of sPTB occur in women with a prior PTB[12]. In addition, this information is missing in nulliparous women, which account for approximately 50% of the population. Regarding other risk factors such as black race[22], smoking during pregnancy[23], bacterial vaginosis[24] or a short interpregnancy interval[25], the causal links are generally unclear[1].

<u>**Figure 1.** Multifactorial aetiology of spontaneous preterm birth syndrome and elements required for spontaneous delivery.</u>



Cervical length (CL) <25 mm measured with transvaginal ultrasound at mid-gestation is a known risk factor for sPTB[26,27]. However, its value for screening a whole pregnant population consisting mainly of women without risk factors for sPTB remains controversial because of the low sensitivity of short CL[28,29] in low-risk women[30,31]. Interestingly, a recent prospective observational cohort study including 9410 nulliparous women with singleton pregnancies, conclude that the low predictive accuracy for sPTB <37 and < 32 weeks of sonographic CL <25 mm between 16^{+0} and 22^{+6} (AUC 0.53 and 0.61, respectively) do not support routine use of these test in such women[32].

Taking into account that, most countries perform screening ultrasound to check for foetal anomalies in all pregnant women during the 2nd trimester of pregnancy, the development of new methods and strategies which could be applied at that time could have a great impact on improving the detection of women at risk. Mid-pregnancy screening ultrasound provides a valuable window of opportunity in which a predictive test could be universally performed at a time point when treatment strategies are more useful. This is the main reason why we have focused our studies between 19^{+0} and 24^{+6} weeks of pregnancy.

It is of note that improvement in the detection rate must walk hand in hand with effective treatments for women identified as being at high-risk. To date, cervical cerclage has proven to be useful in women with a history of sPTB and short CL[33,34]. Despite requiring a surgical procedure in the cervix, and considering the potential complications of this intervention, cervical cerclage placement is generally accepted up to 24 weeks of pregnancy[35]. Several studies also support progesterone treatment in women with a history of sPTB or short CL[36-38], however its efficacy in reducing the rate of sPTB has recently been challenged[27,39,40]. An upcoming meta-analysis of individual patient data, including the data from the OPPTIMUM study[40] will contribute to answering this uncertain situation. The data regarding the use of cervical pessary to prevent sPTB in singleton pregnancies and short CL are even more controversial, with two studies reporting opposite results[41,42]. Further research evaluating cervical pessary in other subgroups of patients (e.g. women with normal CL) is necessary to identify the population which would benefit from their placement.

To sum up, two crucial issues need to be solved if we aim to decrease the rate of sPTB: we must improve the detection of women at risk and find effective treatments for these women. The goal of our project was to increase the identification of women at risk of sPTB by means of cervical assessment by ultrasound. As mentioned previously, the main challenge in trying to predict sPTB is that it is a multifactorial syndrome the aetiology of which is still poorly understood. A multitude of conditions are thought to be responsible for PTB including infection or inflammation[43], uteroplacental ischaemia or haemorrhage, uterine overdistension, cervical insufficiency [44], stress and immunologically-mediated processes[45]. Three main mechanisms responsible for triggering sPTB have been described: premature decidual activation, premature myometrial activation and premature cervical remodelling [46], which intertwine with each other with no established temporal sequence. Nevertheless, since in most cases a spontaneous preterm delivery is preceded by premature cervical remodelling, many efforts have been focused on cervical assessment. However, a combination of techniques assessing cervical properties with methods studying other mechanisms responsible for sPTB syndrome (e.g. foetal fibronectin[47,48]) might be needed to maximize the identification of women at risk.

1.3. The current strategy: measurement of cervical length

To date, the measurement of CL is the most accurate indicator of premature cervical remodelling and sPTB. The shorter the cervix, the higher the risk[26].

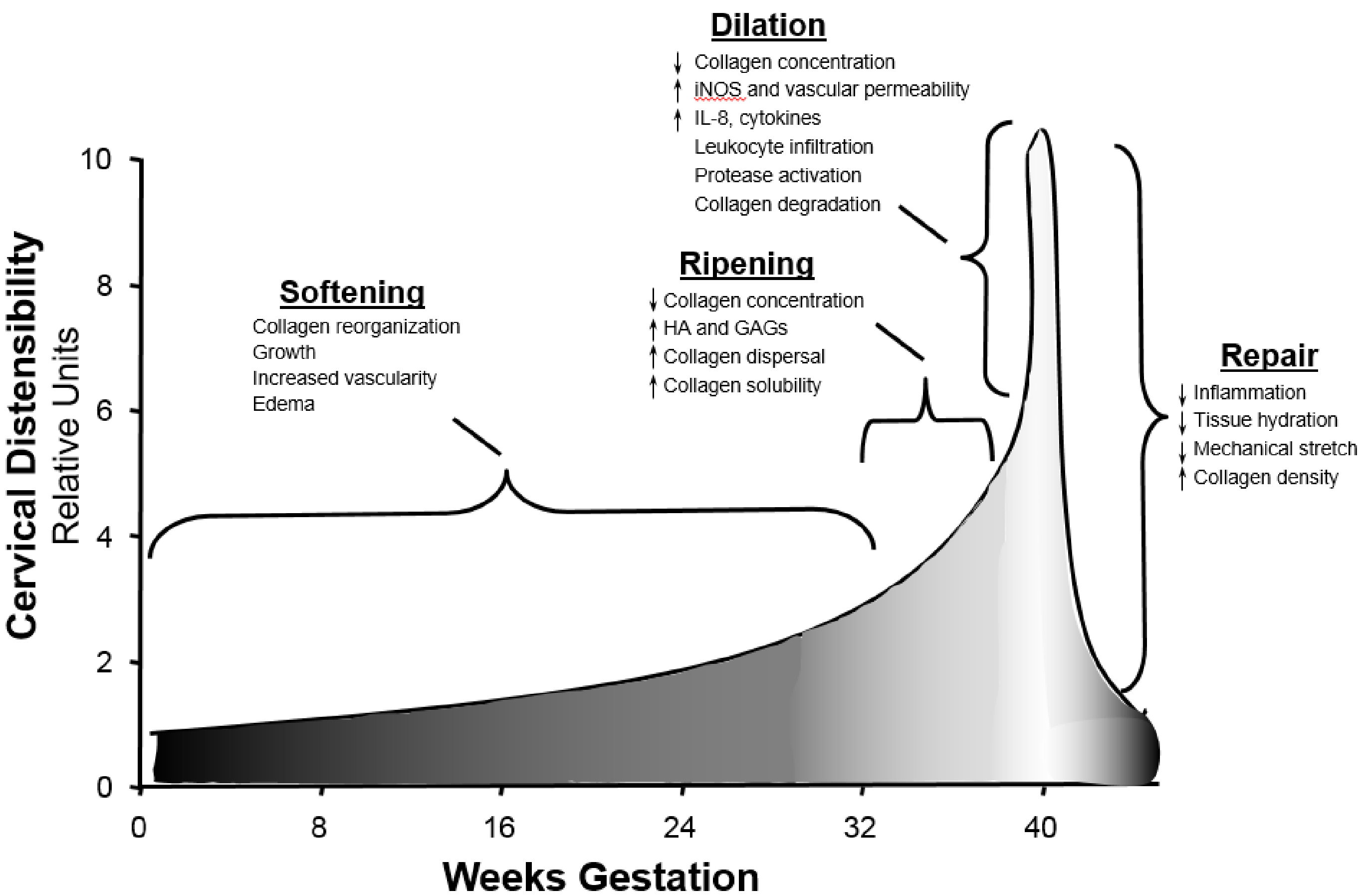
In recent years, CL < 25 mm has become the threshold of significance for the prediction of sPTB. Although the main scientific societies still do not recommend CL screening in low-risk population, they suggest that a policy of universal cervical length screening might be considered taking into account the reduction of sPTB with progesterone treatment in unselected women with a short CL[27]. The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) states that there is currently insufficient evidence to recommend routine cervical length measurements at the mid-trimester in an unselected population.[49] The Society for Maternal-Fetal Medicine (SMFM) and The American College of Obstetrics and Gynecology (ACOG), only recommend routine transvaginal CL screening for women with singleton pregnancy and a history of previous sPTB. CL screening in singleton gestations without prior PTB is not yet universally mandatory, however it is strongly recommended that practitioners implementing CL screening should follow strict guidelines[50].

It is important to highlight that CL presents a limited predictive capacity, which can be explained by the fact that it only describes one of the multidimensional changes that are associated with cervical remodelling which also include cervical softening, cervical funnelling and dilation[51]. In fact, a modest change in CL along pregnancy is associated with an increased cervical softening and cervical volume[52]. Regardless of the particularly poor sensitivity and positive predictive value of CL measurement when assessed in a normal antenatal population[26,53], and also in low[30] and in high-risk pregnancies[54,55], the indication of most treatments is based on these measurement[56]. In addition, there is still no consensus on the cost-effectiveness of universal CL screening followed by progesterone treatment[57,58]. Cost-effectiveness is mainly influenced by three aspects: the prevalence of short CL in a low-risk population, the cost of transvaginal ultrasound and expenses due to prematurity, and the effectiveness of treatments[59]. It is worth mentioning that in cost-effectiveness studies[60,61] the prevalence of a short cervix was higher than that recently published[30,31]. In addition, cost-effectiveness studies have been carried out in the USA, where the rate of sPTB almost doubles that of most European countries[3] and where transvaginal ultrasound costs and lifetime expenses derived from prematurity may differ. Even though the performance of CL is far from optimal, it is currently the gold standard technique, and the results obtained with the two sonographic tools proposed in this work have been compared to CL.

1.4. The hypothesis: Premature cervical remodelling along pregnancy

Cervical remodelling along pregnancy is a chronic process, consisting in microstructural and water concentration changes, which starts during the first trimester and progressively proceeds until term in a normal pregnancy[62]. Four differentiated phases have been described: softening, ripening, dilation and repair (**Figure 2**). Each phase of remodelling is coordinated within an endocrine environment affecting epithelial, stromal, immune and endothelial cell function as well as the composition and structure of the extracellular matrix[63]. Initially there is a progressive softening of the cervix beginning early in pregnancy, due to reorganisation and changes in the collagen fibrillar network, which is responsible for the mechanical functions of the cervix.

Figure 2. Stages of cervical remodelling during pregnancy.



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There is also an increase in the vascularity and oedema of the entire cervix, as well as hypertrophy of the cervical stroma (**Figure 3**) and hypertrophy and hyperplasia of the cervical glands (**Figure 4**).

Figure 3. Changes in the cervical stroma during the remodelling process

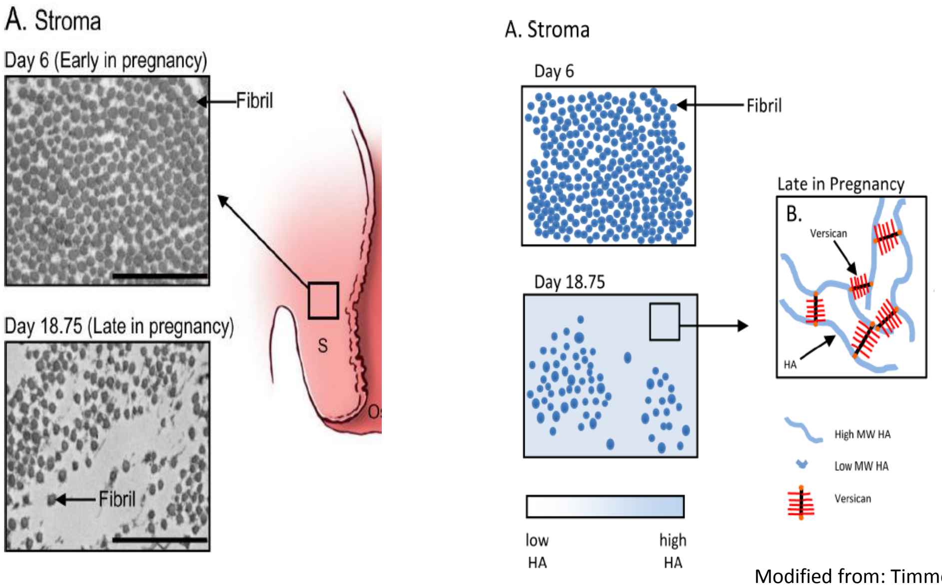

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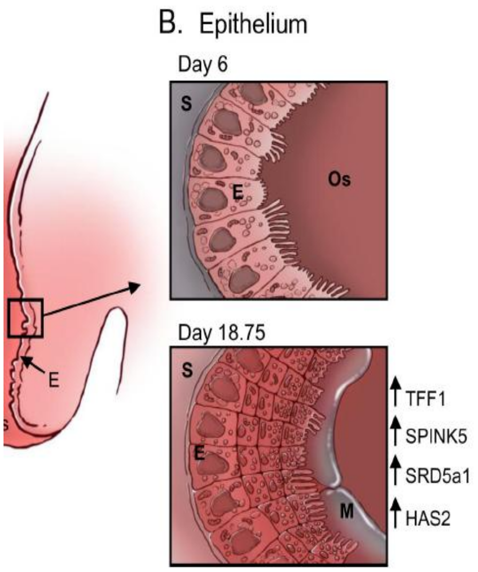
Figure 4. Changes in the cervical glandular zone during the remodelling process



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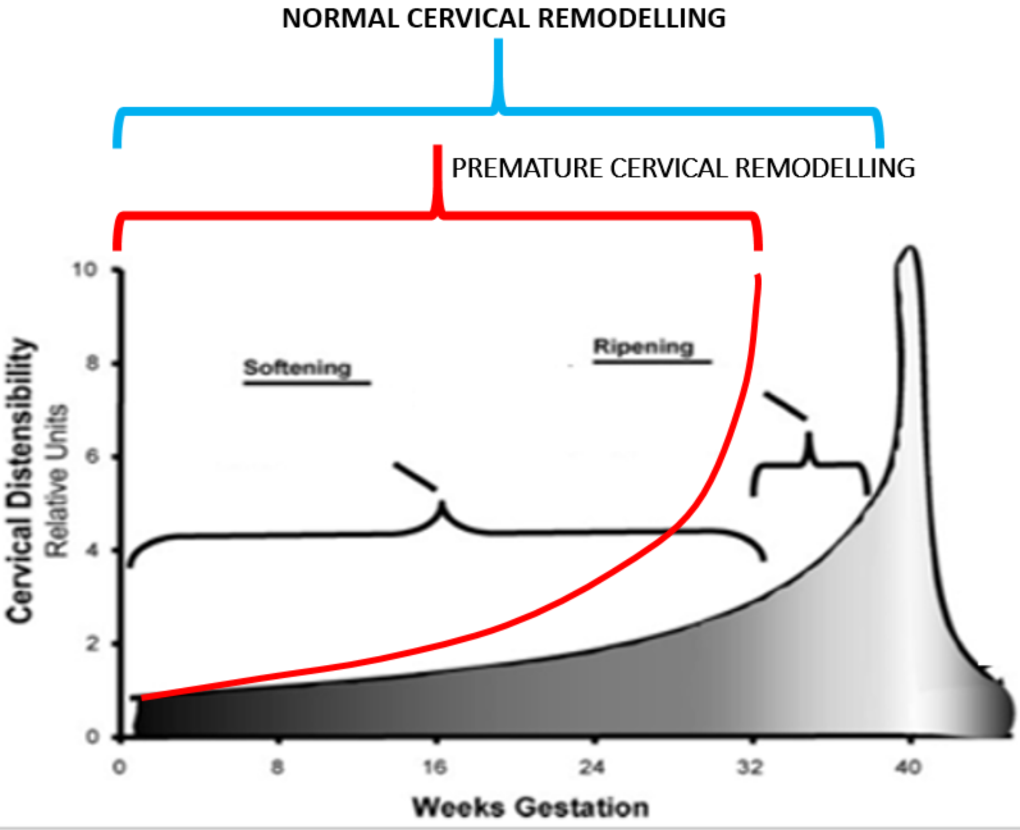
Shortening and dilatation occur as later events, closer to delivery[6]. Appropriate timing in cervical function and composition during gestation are crucial for a successful pregnancy. Therefore, premature cervical remodelling most likely increases the risk of sPTB (**Figure 5**), and objective detection and quantification measurement of its components (i. e. softening and shortening) is required[46].

In this project, we evaluated two different approaches based on transvaginal ultrasound: the cervical consistency index (CCI) and quantitative analysis of cervical texture (CTx).

Figure 5. Normal and premature cervical remodelling.



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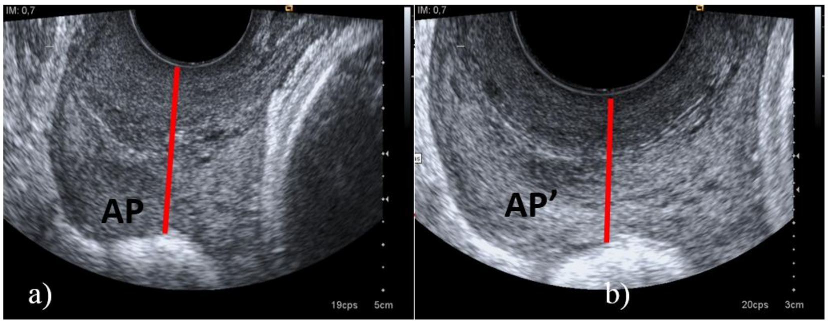
1.5. The ultrasound tools proposed

1.5.1. Cervical Consistency Index (CCI)

The CCI is an ultrasound measurement that aims to estimate cervical softness by measuring maximal tissue compressibility with a vaginal ultrasound probe[64]. To calculate the CCI, two cervical images must be acquired. First, a basal image consisting in a sagittal view of the cervix is obtained in which the cervical canal and the internal and external cervical os are clearly seen without exerting any pressure with the transducer. To acquire the image at maximum compression, pressure is applied softly and progressively on the cervix until achieving maximum compression of the anteroposterior diameter. The CCI is measured by calculating the ratio between the anteroposterior diameter of the uterine cervix at maximum compression and at rest (**Figure 6**).

Figure 6. Cervical consistency index measurement (AP'/AP x 100).



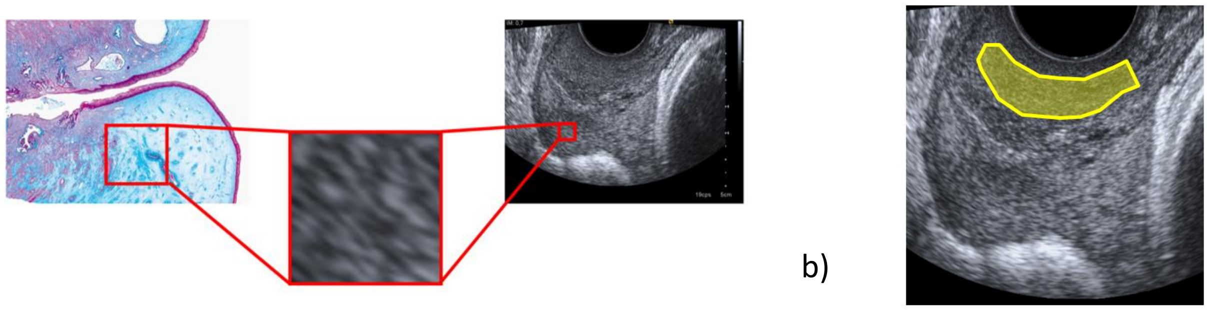
The lower the CCI, the higher the cervical compressibility and cervical softness. The CCI was first described by Parra-Saavedra et al[64]. The main aims of the first study published[64] were: to standardise the new technique, to establish reference ranges from 5 to 36 weeks and to assess the potential value of the CCI to predict sPTB. The authors showed that CCI decreased with advancing gestation and was lower in women who delivered preterm than at term. At all the time intervals during pregnancy (<32, <34 and <37 weeks) the CCI was found to be a much better predictor of sPTB than CL[64]. The promising results of this work, encouraged us to design a study focused on 2nd trimester screening ultrasound to estimate the ability of CCI to predict sPTB and to compare its performance with mid-trimester sonographic CL.

As mentioned above, cervical softening starts in the first trimester and progressively increases as the delivery approaches. Therefore, the assessment of an early phase in the cervical remodelling process such as softening, would allow better identification of women at risk of sPTB who have experienced premature cervical softening compared to term pregnancies. CCI is a measurement that could provide valuable information regarding the biomechanical characteristics of the cervix such as stiffness, without requiring any special equipment or technology.

1.5.2. Quantitative analysis of cervical texture (CTx)

Medicine has continuously improved the lives of humans over time, but these improvements would not have been possible without the help of technology. Multidisciplinary collaboration is needed to transfer knowledge and clinical needs from the clinical to the technological setting. Therefore, the new developing technologies available should be explored in major global health issues such as prematurity. Quantitative texture analysis has been used to extract robust features from ultrasound images of a particular tissue to detect subtle changes in its microstructure and functionality. It has been extensively investigated in fields such as breast cancer[65] and liver diseases[66]. The Local Binary Pattern (LBP) texture-based method used in the two previously cited studies has been widely used for texture classification in the computer vision field. The purpose of applying the LBP method is to characterize microstructural changes of the uterine cervix by characterizing speckle patterns. Encouraging results have been obtained in foetal lung, and successfully translated from the research setting to a clinically applicable tool to predict foetal lung maturity and respiratory distress syndrome.[67-70] However, the feasibility of this method to extract information from cervical ultrasound images has not yet been studied. Each human and foetal tissue has its own characteristics, therefore the progress achieved in other foetal organs (e.g. foetal lung) cannot be directly extrapolated to the cervix. Taking into consideration the scientific evidence supporting the development of changes in the composition of the uterine cervix throughout a normal pregnancy, cervical texture analysis (CTx) aims to identify and quantify the subtle changes in cervical composition and properties which are difficult or impossible to detect in a clinical or routine sonographic examination (**Figure 7a**).

<u>**Figure 7.** a) quantitative analysis of cervical texture by ultrasound and b) region of interest (ROI) delineation for further CTx analysis</u>



To acquire the cervical image for further analysis, a sagittal view of the cervix identifying the internal and external os is obtained. The cervix should be located horizontally on the image and the entire cervical structure visualized. Shadows, saturations, zoom and any post-processing function such as speckle reduction imaging (SRI), smoothing and on-line measurements should be avoided (**Figure 7b**). Gain and harmonics are at the discretion of the physician. Quantitative analysis of cervical texture is an objective and robust method, since a single static ultrasound image is required to perform the analysis, avoiding most of the subjectivity brought by the operator in dynamic measurements. The challenge of this method lies in the development of the technology to extract the features related to the gestational age and to clinical events such as sPTB from an ultrasound image by means of a machine learning process.

1.6. Other ultrasound-based methods

A notable number of techniques are under development to identify premature cervical remodelling, assessing cervical properties such as distensibility and tissue stiffness[71-77], composition and optical characteristics[78-86] in normal pregnancies and also in those complicated with a sPTB.

Regarding ultrasound-based methods, cervical elastography has been the most studied[87-89]. Two different approaches of cervical elastography have been developed for quantitative determination of the physical properties of the pregnant cervix: strain elastography and shear wave elastography. Strain elastography measures the percentage of tissue deformation when oscillatory compression is applied. Low strain values in the internal cervical os are associated with a significantly lower risk of sPTB[89]. However, there are some important technical limitations with elastography[77,88,90], with the main limitation being the inability to standardise the force applied for inducing tissue deformation. On the other hand, the shear wave speed, allows objective characterisation of stiffness because waves travel more slowly in softer tissue[91] and ultrasound imaging can be used to monitor the propagation of the shear wave and measure its speed[77]. Measurements with shear wave elastography are thought to be relatively independent of operator force. A cross-sectional study of women at 11–36 weeks of gestation found a positive correlation between softening and spontaneous preterm delivery, although the results were not statistically significant.[91] Ultrasonic attenuation has also been investigated with the aim of identifying women at risk of sPTB and suggesting that low attenuation may be an additional early marker of sPTB[92]. The methods mentioned above, albeit very promising, are still being investigated and are not yet applicable in clinical practice.

2. HYPOTHESIS

2. HYPOTHESIS

2.1. Main Hypothesis

The diagnostic accuracy of the CCI and CTx by ultrasound to identify women at increased risk of sPTB is better than that of CL.

2.2. Specific Hypothesis

1. Premature cervical remodelling and softening can be better identified with the CCI and with CTx than with CL.
2. The CCI is more accurate than CL in predicting sPTB in low-risk pregnancies during mid-pregnancy screening ultrasound.
3. The CCI is more accurate than CL in predicting sPTB in high-risk asymptomatic pregnancies during mid-pregnancy ultrasound.
4. CTx by ultrasound is able to detect cervical tissue changes along a normal pregnancy related to gestational age and therefore to the physiologic cervical remodelling process.
5. CTx by ultrasound is able to extract features associated with sPTB from the ultrasound images of pregnant women.

3. OBJECTIVES

3. OBJECTIVES

3.1. Main Objective

To improve the identification of women at increased risk of sPTB in low and high sPTB risk asymptomatic pregnancies during mid-gestation with two innovative transvaginal ultrasound techniques, the CCI and CTx.

3.2. Specific Objectives

1. To estimate the ability of a mid-trimester sonographic CCI to predict sPTB in low-risk pregnancies and compare its performance with mid-trimester sonographic CL.
2. To estimate the ability of a mid-trimester sonographic CCI to predict sPTB in high-risk asymptomatic pregnancies and compare its performance with mid-trimester sonographic CL.
3. To evaluate the feasibility of CTx by ultrasound to detect cervical tissue changes along uncomplicated pregnancies.
4. To determine if CTx by ultrasound in the mid-trimester of pregnancy is able to extract features associated with sPTB from the ultrasound images of pregnant women.

4. METHODS

4. METHODS

Each of the studies compiled in this thesis present their own design and methodology which is summarised below. All statistical analyses were performed using STATA/IC 13.0 (StataCorp 4905 Lakeway Drive College Station, Texas, USA) or SPSS 20.0 (IBM Copr, Armonk, NY).

4.1. Study 1.

1. Study Design: Prospective cohort study including women with singleton pregnancies examined during the mid-trimester screening ultrasound at 19^{+0} to 24^{+6} gestational weeks. Both the medical personnel and the women themselves were blinded to the CL and CCI results.
2. Exclusion criteria: a) multiple pregnancy, b) history of sPTB <34 weeks or of miscarriage ≥16 weeks, Müllerian malformation or cervical conization, c) CL < 25 mm, premature rupture of membranes (PPROM), or symptoms of preterm labuor if detected before the routine 2nd trimester scan, and d) treatment to prevent sPTB (progesterone, cervical cerclage or cervical pessary) instituted before the routine 2nd trimester scan.
3. Image acquisition and sonographic measurements: A basal image and an image at maximal compression were obtained for further off-line analysis following an acquisition guideline described in depth in the manuscripts. The CCI (%) was obtained by calculating the ratio between the anteroposterior diameter of the uterine cervix at maximum compression and at rest: (AP'/AP) x 100. CL was measured in millimeters (mm) from the outer to the inner cervical os. To estimate intra- and inter-observer agreement and reliability for CL and CCI measurements, 40 images were randomly selected and off-line measurements were repeated by the same operator and by a second operator.

4. Statistical analysis: Data distribution was assessed using the Shapiro-Wilk test of normality. The statistical significance of differences in continuous data was calculated using the Student's *t*- or Mann-Whitney U-test for normally and non-normally distributed data, respectively. Categorical data were analysed using the Chi-square or Fisher's exact test, as appropriate. A multivariate logistic regression model including CCI and CL as predictive variables was performed to assess which variables were independently associated with sPTB. Receiver operating characteristic (ROC) curves were drawn for CCI, CL and for a logistic regression model including both CCI and CL. Sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) and positive and negative likelihood ratios (LR+ and LR-) were calculated for the optimal cut-off point based on the ROC curve for the 1^{st}, 5^{th} and 10^{th} centiles of CCI and CL and for the combined use of CCI and CL. Intra-observer agreement was expressed as the difference between two measurements obtained by the same observer, and inter-observer agreement was expressed as the difference between two measurements obtained by two different observers.

4.2. Study 2.

1. Study Design: Prospective cohort study including high sPTB risk singleton pregnancies (history of sPTB $<34^{+0}$ weeks or late miscarriage ≥16 weeks, Müllerian malformation or cervical conization, cervical length < 25 mm or previable premature prelabuor rupture of membranes (PPROM) if detected before 2^{nd} trimester routine ultrasound, between 19^{+0} and 24^{+6} gestational age. The medical personnel and the women themselves were blinded to the CCI results.
2. Exclusion criteria: Preterm birth for foetal or maternal indication.
3. Image acquisition and sonographic measurements: The same acquisition protocol and measurements were applied as in Study 1.

4. Statistical analysis: The statistical analyses were the same as those performed in study 1, with the exception of intra- and inter-observer repeatability. The McNemar test was used to compare the diagnostic accuracy of the CCI and CL at certain cut-off points. The relationship between the CCI and CL and preterm birth risk was analysed using logistic regression, and the estimated probability of sPTB by CCI and CL was calculated.

4.3. Study 3.

1. Study Design: Cross-sectional study including singleton pregnancies between 20^{+0} and 41^{+6} weeks of gestation who delivered at term. The medical personnel and the women themselves were blinded to CL and CTx analysis results.
2. Exclusion criteria: Women who received any treatment to prevent sPTB (progesterone or cervical cerclage) and who delivered < 37 weeks.
3. Image acquisition, sonographic measurements and ROI delineation: A sagittal view of the cervix was obtained without exerting any pressure with the transducer. The internal and external os, as well as the cervical canal should be identified and the entire cervical structure visualised, avoiding zoom and using only depth function. The cervix should be located horizontally to the transducer. Calipers, shadows and saturations must also be avoided. Images should be obtained without any post-processing function, such as speckle reduction imaging (SRI), smoothing or on-line measurements. Gain and harmonics were at the discretion of the physician. The region of interest (ROI) was defined as the largest homogeneous region of the middle part of the anterior cervical lip. ROI delineation should avoid the cervical canal, the glandular area and the internal and external cervical os. CL was measured in millimeters (mm) from the outer to the inner cervical os.
4. Feature extraction and statistical analysis: The feature extraction method used was the Local Binary Pattern (LBP). A model of gestational age was developed

according to the following three steps: data splitting, feature transformation, and regression model computation. Demographic characteristics were described as absolute and relative frequencies for qualitative variables and mean and standard deviation for quantitative variables.

4.4. Study 4.

1. Study Design: Single centre, nested case-control study based on a prospective cohort of consecutive singleton pregnancies assessed between 19^{+0} and 24^{+6} weeks.
2. Exclusion criteria: Preterm birth for foetal or maternal indication including induction of labour (IOL) for premature pre-labour rupture of membranes (PPROM) and women receiving any treatment to prevent sPTB such as progesterone, cervical cerclage or cervical pessary.
3. Image acquisition, sonographic measurements and region of interest (ROI) delineation: The acquisition guide, sonographic measurements and ROI delineation were the same as those performed in Study 3.
4. Feature extraction and statistical analysis: The feature extraction method used was the Local Binary Pattern (LBP). A learning algorithm based on two modules, including feature transformation followed by discriminant analysis-regression was computed to obtain the best combination of textural features related to sPTB. Data distribution was assessed according to the Shapiro-Wilk test of normality. The results are expressed as absolute and relative frequencies for qualitative variables and median and interquartile range for quantitative variables. Continuous data were compared with the Mann-Whitney U-test and categorical variables were compared using the Chi-square or Fisher's exact test. The ability of the CTx algorithm to blindly predict sPTB was evaluated using a leave-one- out cross-validation method, obtaining a CTx-based score for each woman. Stepwise multivariate logistic regression analysis was performed to

obtain crude and adjusted odds ratios (ORs). Receiver-operating characteristic (ROC) curves were drawn for the CTx-based score and CL. Sensitivity, specificity and positive and negative likelihood ratios (LR+ and LR-) were calculated for the CTx-based scores and the optimal CL cut-off point based on the ROC curve and for a CL <25 mm.

5. STUDIES

Study 1

Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a low-risk population.

Baños N, Murillo-Bravo C, Julià C, Migliorelli F, Perez-Moreno A, Ríos J, Gratacos E, Valentin L, Palacio M.

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Mid-trimester sonographic Cervical Consistency Index to predict spontaneous preterm birth in a low-risk population

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Study 2

Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a high-risk population.

Baños N, Julià C, Lorente N, Ferrero S, Cobo T, Gratacos E, Palacio M.

American Journal of Perinatology Reports.

Status: under review.

Mid-trimester cervical consistency index and cervical length to predict spontaneous preterm birth in a high-risk population.

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Control ID 1517, Programme ID 309, Poster session II.

Study 3

Quantitative Analysis of the Cervical Texture by Ultrasound and Correlation with Gestational Age.

Baños N, Perez-Moreno A, Migliorelli F, Triginer L, Cobo T, Bonet-Carne E, Gratacos E, Palacio M.

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Quantitative Analysis of the Cervical Texture by Ultrasound and Correlation with Gestational Age

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Key Words
Quantitative ultrasound · Uterine cervix · Image biomarker

Abstract
Objectives: Quantitative texture analysis has been proposed to extract robust features from the ultrasound image to detect subtle changes in the textures of the images. The aim of this study was to evaluate the feasibility of quantitative cervical texture analysis to assess cervical tissue changes throughout pregnancy. ***Methods:*** This was a cross-sectional study including singleton pregnancies between 20.0 and 41.6 weeks of gestation from women who delivered at term. Cervical length was measured, and a selected region of interest in the cervix was delineated. A model to predict gestational age based on features extracted from cervical images was developed following three steps: data splitting, feature transformation, and regression model computation. ***Results:*** Seven hundred images, 30 per gestational week, were included for analysis. There was a strong correlation between the gestational age at which the images were obtained and the estimated gestational age by quantitative analysis of the cervical texture (R = 0.88). ***Discussion:*** This study provides evidence that quantitative analysis of cervical texture can extract features from cervical ultrasound images which correlate with gestational age. Further research is needed to evaluate its applicability as a biomarker of the risk of spontaneous preterm birth, as well as its role in cervical assessment in other clinical situations in which cervical evaluation might be relevant.

Introduction

Cervical ripening is a chronic process, consisting in microstructural and water concentration changes, which starts during the first trimester and progressively proceeds until term in a normal pregnancy. Four differentiated phases have been described: softening, ripening, dilation, and repair. Initially, there is a progressive softening of the cervix beginning early in pregnancy, due to a reorganization of the collagen fibrillar network. There is also an increase in the vascularity and oedema of the entire cervix, a hypertrophy of cervical stroma, and hypertrophy and hyperplasia of the cervical glands. Shortening

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Study 4

Quantitative analysis of the cervical texture by ultrasound in the mid-pregnancy is associated with spontaneous preterm birth.

Baños N, Perez-Moreno A, Julià C, Murillo-Bravo C, Coronado D, Gratacos E, Deprest J, Palacio M.

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Quantitative analysis of the cervical texture by ultrasound in the mid-pregnancy is associated with spontaneous preterm birth

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6. RESULTS

6. RESULTS

6.1. Study 1. Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a low-risk population.

In this study, 532 low sPTB risk women were included for analysis. The maternal baseline characteristics did not significantly differ between women who gave birth at term and those with sPTB. The rate of sPTB <$37^{+0}$and <34^{+0} weeks was 4.1% and 1.3%, respectively, and that of short cervix <25mm and ≤20 mm 0.9% (5/532) and 0.4% (2/532), respectively. The cervix was significantly shorter (median CL 39.8 mm vs. 36.2 mm, p=0.004) and the CCI was significantly lower (median 73.0% vs. 58.1%, p<0.001) in the sPTB group. The area under the ROC curve (AUC) for CCI with regard to predicting sPTB <37+0 weeks was 0.84 (95% CI 0.75-0.93) and that for CL 0.68 (95% CI 0.56-0.81), P = 0.03. The optimal CCI cut-off based on the ROC curve was 64.6% (sensitivity 77.3%, specificity 82.7%) being 37.9 mm (sensitivity72.7%, specificity 61.2%) for CL. The AUC for CCI with regard to predicting sPTB <34+0 weeks was 0.87 (95% CI 0.71-1) and that for CL was 0.71 (0.47-0.94), P=0.25. The optimal CCI cut-off based on the ROC curve was 63.6% (sensitivity 85.7%, specificity 84.0%) and that for CL was 37.9 mm (sensitivity 85.7%, specificity 61.3%). Multivariate logistic regression analysis showed that only CCI was independently associated with sPTB. The AUCs for a model including both CCI and CL to predict sPTB < 37^{+0} and < 34^{+0} weeks, respectively were 0.85 and 0.86. They were not significantly different from the AUCs of CCI alone. The intra-observer intra-class correlation values were >0.90 both for CCI and CL, while the inter-observer ICC values were 0.89 for CCI and 0.90 for CL.

6.2. Study 2. Mid-trimester sonographic cervical consistency index to predict spontaneous preterm birth in a high-risk population.

Eighty-two high sPTB risk women were included in the analysis**.** Regarding the demographic characteristics of the sPTB and term group, women who delivered preterm were older and the gestational age at scan was significantly superior. The rate of sPTB <37^{+0} and <34^{+0}

weeks was 26.8% and 17.1%, respectively. The median CL (mm) was not significantly different between the sPTB and term group and neither was the proportion of short CL (CL < 25 mm or ≤ 20 mm). The CCI (%) was significantly inferior in women who had a preterm delivery compared to the term group. When adjusted for possible confounders (maternal age, gestational age at scan, CL, sPTB risk factors and treatment received) in the multiple regression analysis, the CCI maintained its independent association with sPTB: CCI adjusted OR, 0.91 (95% CI, 0.83-0.99; P=0.03). The AUC of the CCI to predict sPTB $<37^{+0}$ weeks was 0.73 (95% CI, 0.61-0.85) while that of CL was 0.51 (95% CI, 0.35-0.67), P=0.03. The optimal CCI and CL cut-off points to predict sPTB $<37^{+0}$ weeks were 59.4% (sensitivity 72.7%, specificity 63.3%) and 34.0 mm (sensitivity 54.5%, specificity 56.7%), respectively. The AUC of the CCI to predict sPTB $<34^{+0}$ weeks was 0.68 (95% CI, 0.54-0.82), being 0.49 (95% CI, 0.29-0.69), P=0.06 for CL. The optimal CCI and CL cut-off points to predict sPTB $<34^{+0}$ weeks were 59.4% (sensitivity 78.6%, specificity 58.8%) and 29.7 mm (sensitivity 42.9%, specificity 69.1%), respectively.

6.3. Study 3. Quantitative analysis of the cervical texture by ultrasound and correlation with gestational age.

Seven hundred images (30 images per gestational week) were included in the analysis. The feature transformation algorithm obtained was first performed with the features from 189 images, 84 images below 23^{+0} weeks of gestation (bag 1a) and 105 images above 39^{+0} weeks of gestation (bag 1b). A regression model to correlate the features obtained with the gestational age was then evaluated in a large cohort of 511 images between 23^{+0} and 38^{+6} weeks of gestational age. The correlation between the gestational age at which the images were obtained and the estimated gestational age by quantitative analysis of the cervical texture was R=0.88. Cervical length was measured in the same cohort of images, and the correlation between gestational age and cervical length measurements was R = - 0.26.

6.4. Study 4. Quantitative analysis of the cervical texture by ultrasound in the mid-pregnancy and the association with spontaneous preterm birth.

A total of 310 cervical images, 27 sPTB cases and 283 term birth controls were analysed. There were no differences between cases and controls regarding the baseline demographic characteristics, except for the prevalence of sPTB risk factors (history of sPTB, conization or Müllerian malformation). The median CL was comparable. Cases were more likely to have a CL<25 mm than controls: 18.5% (5/27) vs. 0.4% (1/283), P <0.001. Regarding cervical texture analysis, three textural features which explained 95% of the variance within the observed sample were selected by principal component analysis. The median CTx-based score obtained was significantly lower in cases compared to controls. The CTx-based score was independently associated with sPTB after adjusting for candidate confounders (history of sPTB, conization or Müllerian malformation and CL <25 mm): crude OR 0.31 (95% CI 0.17-0.56; P<0.001) vs. adjusted OR 0.37 (95% CI 0.19-0.64; P=0.001). The AUC for the CTx-based score to identify women delivering < 37^{+0} weeks was higher (0.77; 95% CI 0.66-0.87) than for CL (0.60; 95% CI 0.47-0.72), P=0.02. The optimal cut-off point for the CTx-based score based on the ROC curve was -0.68 with a sensitivity 70.4%, specificity 77.7%, LR+ 3.1 and LR- 0.4. Conversely, for the CL the cut-off was 35.5 mm with a sensitivity 44.4%, specificity 68.2%, LR+ 1.4 and LR- 0.8. Using a CL cut-off <25 mm, the sensitivity was 14.8%, the specificity was 98.6%, the LR+ 10.5 and the LR- 0.9.

7. DISCUSSION

7. DISCUSSION

The CCI and CTx measured between 19^{+0} and 24^{+6} weeks of pregnancy have the potential to improve the identification of women at increased risk of sPTB compared to CL.

Even if both of methods are sonographic tools, they represent different approaches with specific acquisition requirements and specific developing and validating processes. For this reason, they are addressed separately in the discussion.

7.1. Cervical Consistency Index (CCI)

The only previously published study on the CCI[64] assessed its predictive capacity in low-risk pregnancies from 5 to 36 weeks of gestation. The two studies regarding the CCI presented in this thesis aimed to assess the diagnostic accuracy of this method focusing on two separate populations and in a narrower gestational age range (from 19^{+0} to 24^{+6}weeks). The populations studied consisted in: a) selected low-risk pregnancies, excluding women with any sPTB risk factors, and b) asymptomatic high-risk pregnancies consisting of women followed in the preterm birth prevention clinic (PBPC) from a single centre. The CCI was measured between 19^{+0} and 24^{+6} weeks' gestation to explore its potential utility as a sPTB screening method during the 2^{nd} trimester screening ultrasound, when prophylactic and therapeutic strategies are still useful. The CCI was analysed separately in low- and high-risk women to determine if it performed differently depending on the population. In both studies, the CCI showed to be superior to CL for predicting sPTB. However, the CCI performed much better when applied to a selected low-risk women compared to those from the high-risk group. The poorer performance in the high-risk population may be due to several reasons: a) the lower number of women included in the high-risk study, b) the heterogeneity within the high-risk population, and c) the interventions and treatments received in the high-risk group, which may potentially interfere with the natural course of the condition.

One of the important findings arising from **study 1** is that we were able to propose an optimal cut-off to select the women at highest risk of sPTB. There are two strategies to choose an optimal cut-off; to magnify the sensitivity and specificity, which can be determined with the ROC curve, or to magnify LR+ while minimizing LR-. Likelihood ratios indicate the magnitude by which a given result raises or lowers the probability of having a condition. A moderate prediction is considered with a LR+ between 5-10 and a LR- between 0.1-0.2, while good predictive evidence is provided with a LR+ >10 and a LR- <0.1[93]. When a method aims to be applied as a screening strategy in a population consisting mainly of women without risk factors, it is also crucial to limit the false positive rate. A CCI cut-off of <60% (10th centile) presented only a 7.8% false positive rate with an improved sensitivity of 54.5% and a moderate LR+ of 7.0 compared to CL cut-off of 25 mm or to the 10th centile of CL (33 mm), which presented a sensitivity of 31.8% and a low LR+ of 3.1. The CCI cut-off of 60% can classify approximately 10% of the population as being at increased risk, and during the mid-trimester screening scan can identify more than half of the women who will present a sPTB.

In **study 2**, we face a different scenario in which we want to maximize the sensitivity in a high-risk population. Although sPTB risk factors do not contribute the same and their overall predictive capacity is poor[55], all women with risk factors are classified as a high sPTB risk pregnancy. Consequently, they undergo a non-negligible number of interventions, ranging from the recommendation of resting, CI surveillance, progesterone treatment or even cervical cerclage. Due to the poor predictive capacity of clinical risk factors, most of the women at real risk of sPTB are not identified, and interventions provide a marginal benefit to those labeled as high-risk. In addition, and to add to the uncertainty, a normal CL (>25 mm) in women with a history of sPTB or PPROM <34 weeks has not demonstrated to be useful in predicting subsequent sPTB[21]. Therefore, taking into account the low negative predictive value of a long cervix in a high-risk population, CCI could be an alternative tool to improve the detection of women at real risk of sPTB among those with a normal CL. An early and sensitive method to select women who will benefit from treatment or those in whom treatment will not be beneficial or even harmful, is greatly needed. As pointed out previously, most women with risk factors including short CL undergo some kind of intervention. Therefore, the evaluation of predictive tools within this context is inevitably subject to this limitation. Unfortunately, we are not able to elucidate if the poor performance of both CCI and CL in high-risk women is due to the bias brought about by interventions. Women from a preterm birth prevention clinic represent a heterogeneous population, since they have different risk factors and receive different interventions also according to the clinical findings. Therefore, efforts should be made to find better tools in this complex scenario, probably combining cervical assessment with methods evaluating other sPTB pathways.

7.1.1 Further studies on the CCI

Despite assessing different populations, the results of the three studies on CCI are consistent and agree on the superiority of the CCI compared to CL. Two main aspects need to be addressed before planning the implementation of the CCI as a screening tool. First, measurement must be better standardized to ensure that the anteroposterior diameter at maximal compression represents the maximum deformability point. To obtain the point of maximum compression is crucial, since a study by Maurer et al. performed under experimental conditions showed that when the maximum compressibility of the cervix was achieved, a variation in the force applied did not result in a significant variation in strain, suggesting the reproducibility and robustness among operators in a real clinical setting[94]. In our study in a low-risk population, intra- and inter-observer repeatability showed good results when measuring the CCI off-line[95]. However, a study evaluating intra- and inter-observer repeatability of the CCI in live measurements is needed. Second, to externally validate the results and especially the proposed cut-off of 60% in a study performing the measurements at the time of examination should also be conducted.

With regard to the high-risk population, a larger sample size would allow the evaluation of the diagnostic accuracy of the CCI: a) stratifying by risk factors to elucidate if CCI performs better in women with a specific risk factors, and b) among women with a normal CL, in which reassurance based on CL measurement is much less than expected.

7.2. Quantitative analysis of cervical texture (CTx)

The two papers assessing CTx compiled in this thesis are preliminary studies to evaluate if CTx can be applied in ultrasound images of the uterine cervix.

In **study 3**, we confirmed the feasibility of CTx to extract features from the ultrasound image related to gestational age and which detect changes among images obtained from weeks 20^{+0} to 41^{+6} of pregnancy. This is an important finding because it shows that changes in images at different gestational ages, and consequently in different stages of cervical remodelling, can be detected using CTx. Therefore, this method could be potentially applied in all those clinical situations in which assessment of cervical composition and characteristics may play an important role.

Study 4 represents a clinical proof of concept showing that the CTx-based score obtained based on the LBP method was associated with a clinical event such as sPTB $<37^{+0}$ weeks. The CTx-based score was significantly lower in women who delivered preterm and in women with a short cervix. An interesting finding is that among the 6 women with a short cervix, the CTx-based score was able to identify the only woman who delivered at term, because she had a significantly higher CTx-based score. A larger sample size would allow confirmation that the CTx-based score is independently associated with sPTB regardless of CL measurement and thereby extend the identification of women at risk among those with a short CL. The improved diagnostic accuracy of the CTx-based score compared to CL (AUC of CCI 0.77 vs. 0.60 for CL) also opens a window of opportunity to potentially apply this method as a sPTB screening tool during the 2^{nd} trimester ultrasound.

7.2.1. Further studies on CTx

The low prevalence of sPTB found in the general population limited the number of sPTB cases included in Study 4**,** thus only allowing the demonstration of an association between the CTx-based score obtained and sPTB. A multicentre study is likely needed to recruit a sufficiently large number of sPTB cases to create a predictive model and to validate it in a new set of images.

As mentioned before, the LBP method aims to characterize microstructural changes of the uterine cervix by characterizing speckle patterns. We hypothesise that changes in speckle patterns identified in the cases might be related to changes in cervical microstructure which are associated with premature cervical remodelling. However, it is not yet possible to interpret the structural changes beyond the changes observed in LBP patterns. To elucidate this question, cervical biopsies showing the structural or histological changes in sPTB cases and their correlation with the CTx-based score would be needed. Unfortunately, cervical biopsies cannot be obtained from pregnant women. Moreover, despite humans and some animals having many similar biochemical processes (i.e. rodents), human anatomy and mechanical forces differ thus, questioning the generalisability of animal models[46].

Finally, the ROI chosen to perform the analysis in the two studies was the largest homogeneous region of the middle part of the anterior cervical lip. However, recent data have shown that location matters when studying the cervix, and that the internal os is significantly more cellular than the external os, and acts as a sphincter[46]. Therefore, other cervical regions which are known to be structural and biomechanically different should also potentially be analysed.

7.3. Future steps with the CCI and CTx

CCI and CTx represent two different ultrasound-based approaches regarding the development of a new sPTB predictive tool. While in **studies 1** and **2** we evaluated the predictive capacity of CCI measurements compared to CL, **studies 3** and **4** represent preliminary steps to create a predictive model based on CTx. Nonetheless, further studies specifically assessing these tools in different populations are needed.

To start with, it would be of interest to analyse images of other gestational ages. Some clinical conditions are present from the beginning of the gestation while others, such as CL shortening, vaginal bleeding, PPROM or preterm labour can appear at different gestational ages and might also require cervical assessment at any time point. Since risk assessment should be continuous along pregnancy, useful tools adjusted for gestational age values which can be applied all along gestation are required.

Multiple pregnancies also carry a substantial risk of PTB and are one of the major contributors to prematurity. Twins account for 15-20% of all PTBs, and the rate of PTB <37 weeks exceeds 50%[93]. CL measurement during mid-pregnancy has also shown limited diagnostic accuracy in this population[93] and has not proven to prolong gestation compared with digital examination in a randomised control trial[96]. Although the mechanisms responsible for sPTB in twin pregnancies may differ from those in singleton pregnancies, the need for better tools to assess the cervix also apply to this subgroup of pregnancies with a preexisting higher risk. However, treatments to reduce sPTB in twin pregnancies, such as progesterone[97,98], cervical cerclage[99] and cervical pessary[100,101] have not proven to be effective so far[102].

Another target population in which cervical assessment would be of great help is in women undergoing induction of labour (IOL). IOL is one of the most common interventions in obstetrics, being performed in approximately 20% of all pregnancies[103]. Its indication is progressively rising, especially due to latest studies reporting improved neonatal outcomes without increasing the risk of cesarean section after IOL from 41+0 weeks' gestation compared with expectant management[104,105]. Many studies have attempted to successfully predict IOL outcome[106-108] in terms of failed IOL, based on demographic characteristics as well as CL measurement performed during mid-pregnancy[109] or prior to IOL[110]. Again, better tools are needed to identify women more likely to experience difficulties in cervical ripening and to thereby enable these women to reach the active phase of labour in an IOL context.

It also remains to be shown if strategies such as progesterone treatment, cervical cerclage and cervical pessary performed to prevent sPTB in women with a short cervix[27,33,41,42] are effective in women identified as having a high-risk of sPTB according to the CCI or CTx results.

The diagnostic accuracy of the CCI and CTx combined with other methods should also be deeply studied. I fact, adding CL measurement to CCI and CTx results did not improve prediction in our studies. Although the addition of quantitative foetal fibronectine to CL did not increase the predictive performance of CL alone in a recent prospective study[32], it might improve prediction in combination with CCI and CTx.

The large number of scenarios in which these tools could be applied have demonstrated their potential impact on the current management of a large number of pregnancies. Cervical assessment is required in a wide range of clinical situations and the main reason why it is not yet universally implemented is the limited performance of the current cervical assessment techniques[32] (i.e. CL). Therefore, it is a healthcare priority to develop sPTB predictive tools with sufficiently improved performance to be used as screening tools.

8. CONCLUSIONS

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8.1. Study 1

1. CCI was superior to sonographic CL for predicting sPTB < 37^{+0} and < 34^{+0} weeks in low-risk women examined between 19^{+0} and 24^{+6} gestational weeks during the 2^{nd} trimester screening ultrasound.
2. CCI, but not CL, was independently associated with sPTB <37^{+0} and <34^{+0} weeks.
3. Combining CL and CCI measurements (both or either below the optimal cut-off) did not improve the predictive capacity of CCI alone.
4. Based on the shape of the ROC curve, the best cut-off for prediciting sPTB <37^{+0} weeks on the basis of the shape of the ROC curve was 64.6%.
5. Based on the best performance in a universal screening scenario, the proposed cut-off point was 60% (10^{th} centile of the CCI).
6. Intra- and inter-observer agreement and reliability for CCI measurement seem sufficient for clinical use and all the intra-class correlation coefficient values were high (>0.90).

8.2. Study 2

1. CCI was superior to sonographic CL for predicting sPTB < 37^{+0} and < 34^{+0} weeks in an asymptomatic high-risk population examined between 19^{+0} and 24^{+6} gestational weeks.
2. The CCI was significantly inferior in women who had a sPTB compared to the term group while CL was not.
3. The diagnostic accuracy of the CCI and CL was limited. Better sPTB predictive tools or a combination of tools are still needed, specifically in high sPTB risk pregnancies.

8.3. Study 3

1. Quantitative analysis of cervical texture can feasibly identify features from cervical ultrasound images related to gestational age.
2. Quantitative analysis of cervical texture is able to identify changes among images from 20^{+0} to 41^{+6} weeks' which might represent the physiological process of cervical remodelling.

8.4. Study 4

1. The CTx-based score obtained after applying the machine learning algorithm, was significantly different between women delivering preterm compared to those delivering at term.
2. The CTx-based score was independently associated with sPTB $<37^{+0}$ weeks, while CL was not.
3. The diagnostic accuracy of the CTx-based score was significantly superior compared to CL.
4. The CTx-based score was able to identify women with a higher risk of sPTB among those with a short CL.

9. REFERENCES

9. REFERENCES

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